Effects of High Potassium or Low Sodium Diet on Vascular $\text{Na}^+,$ $\text{K}^+$-ATPase Activity and Blood Pressure in Young Spontaneously Hypertensive Rats

A. LOUISE SUGDEN, BARBARA L. BEAN, AND JAMES A. STRAW

SUMMARY These studies were designed to investigate whether the antihypertensive effects of high potassium or low sodium diets are related to changes in vascular $\text{Na}^+,$ $\text{K}^+$-adenosine triphosphatase (ATPase) activity. Vascular $\text{Na}^+,$ $\text{K}^+$-ATPase was measured as ouabain-sensitive rubidium uptake in aorta incubated in buffer or plasma from spontaneously hypertensive rats (SHR) fed either a high potassium, a low sodium, or a normal diet for 2 weeks. The high potassium diet significantly increased $\text{Na}^+,$ $\text{K}^+$-ATPase activity, whereas the low sodium diet significantly decreased activity. There was no evidence of a ouabainlike factor in plasma. The increased pump activity on the high potassium diet appeared to be due to an increase in maximum activity ($V_{\text{max}}$) of the enzyme, rather than to an increased affinity for potassium. Potentially, an increase in $\text{Na}^+,$ $\text{K}^+$-ATPase activity could contribute to the antihypertensive effect of potassium by hyperpolarizing the cell membrane. The decrease in vascular $\text{Na}^+,$ $\text{K}^+$-ATPase activity on a low sodium diet probably is unrelated to its depressor effect, but it may be a homeostatic mechanism for maintaining sodium balance in the animal.

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KEY WORDS • hypertension • sodium • potassium • water balance • spontaneously hypertensive rats • electrolyte balance

RECENTLY, there has been considerable interest in the role of $\text{Na}^+,$ $\text{K}^+$-adenosine triphosphatase (ATPase) in the control of vascular tone. Inhibition of this enzyme increases, whereas stimulation is thought to decrease, vascular tone. The finding that a ouabainlike factor is present in the plasma of volume-expanded forms of experimental hypertension has led to the suggestion that inhibition of $\text{Na}^+,$ $\text{K}^+$-ATPase in hypertensive patients may contribute to the development of high blood pressure. Factors that modify the activity of the enzyme or the synthesis of the ouabainlike factor would therefore be expected to have an important effect on the development of high blood pressure.

The present study examined the effects of a high potassium or low sodium diet on $\text{Na}^+,$ $\text{K}^+$-ATPase activity in the aorta of SHR. The changes seen are discussed with respect to the effects of these two diets on blood pressure.

Materials and Methods

Animals

In all studies, female SHR (Charles River Laboratories, Wilmington, MA, USA) were used. They were 7 weeks old at the start of the study and were housed individually in a 12-hour light/12-hour dark cycle (lights on at 0600) at a temperature of 22°C (72°F).

Diet

Basal diet (TD 82383) was obtained from Teklad (Madison, WI, USA) and was supplemented with Na and K ions in the form of NaCl and KCl to achieve the
required concentrations. Groups of rats were matched for body weight and blood pressure, fed a normal diet (0.4% Na, 0.4% K) for 1 week, and then fed either a normal diet, a high K diet (2.0% K, 0.4% Na), or a low Na diet (0.4% K, 0.02% Na) for 2 weeks. The rats were allowed to drink demineralized water ad libitum.

Blood Pressure Measurement

Blood pressure was measured by the tail cuff method in restrained, unanesthetized rats using a photoelectric detector (Innovators and Instrumentation, Landing, NJ, USA). A series of measurements of blood pressure were taken for each rat, and when the values had stabilized the lowest three were averaged to give the final value of blood pressure. The average standard error of these three readings taken from a sample of 20 rats was 1.6 mm Hg.

Measurement of Na⁺,K⁺-ATPase Activity by Ouabain-Sensitive 86Rb Uptake

Plasma Crossover Studies

Ouabain-sensitive 86Rb uptake was measured in segments of aorta by a modification of the method of Overbeck and Grissette. To determine if plasma from the SHR contained a ouabainlike factor and if this factor was modified by the high K or low Na diet, ouabain-sensitive 86Rb uptake was measured in aortic segments incubated in buffer, the rat's own plasma, and plasma from a paired treated or control rat.

Each rat was anesthetized with ether, the abdomen was opened by a midline incision, and the abdominal aorta was exposed. Approximately 5 ml of blood was withdrawn from the abdominal aorta into a Vacutainer coated with ammonium heparin then centrifuged (2000 rpm for 15 minutes at 4°C) to obtain plasma. Immediately after a blood sample was obtained, the aorta was gently and rapidly excised and placed in ice-cold Krebs buffer (NaHCO₃, 27.2 mM; NaCl, 117.0 mM; NaH₂PO₄·H₂O, 1.0 mM; KCl, 5.0 mM; MgSO₄·7H₂O, 1.2 mM; CaCl₂·2H₂O, 1.25 mM; glucose, 11.1 mM) that had previously been aerated with 95% O₂, 5% CO₂.

Each aorta was cut longitudinally and then divided into three segments (top, middle, and bottom) of approximately equal length. The top segment included the aortic arch, and the bottom segment extended as far as the bifurcation into the iliac arteries. The segments of aorta were incubated for 18 minutes at 37°C in aerated (95% O₂, 5% CO₂) Krebs buffer (composition the same as Krebs buffer, but no KCl was added) at 4°C for 40 minutes to load the cells with Na. Paired segments of aorta were incubated for 18 minutes at 37°C in aerated (95% O₂, 5% CO₂) Krebs buffer (top segment), the rat's own plasma (middle segment), or plasma from a paired treated or control rat (bottom segment; 0.5 ml total volume) to which trace amounts of 86RbCl (0.05 mM) had been added. One of the paired segments was incubated with ouabain (1.0 mM) to measure ouabain-insensitive uptake, and the other was incubated without ouabain to give total uptake of 86Rb. After the 18-minute incubation, tissues were washed rapidly three times with ice-cold Krebs, then blotted dry and weighed. Ouabain-sensitive 86Rb uptake was calculated as the difference between Rb uptake in the absence (total uptake) or in the presence (ouabain-insensitive uptake) of ouabain.

Kinetic Studies

To determine the Kₘ and maximum activity (Vₘₐₓ) for Na⁺,K⁺-ATPase in control rats and rats on a high K diet, the aortas were divided into four segments. Ouabain-sensitive 86Rb uptake was then measured in the top, upper middle, lower middle, and bottom segments of aorta incubated in Krebs buffer containing either 15 mM, 5 mM, 2.5 mM, or 1.0 mM K. A Latin square design (Table 1) was used to ensure that differences in aortic segments did not contribute to differences observed at any one K concentration. Since there were eight rats in each group, Rats 5 through 8 followed the same pattern as Rats 1 through 4. Thus, there were two values for each box in the Latin square.

The kinetic constants Kₘ and Vₘₐₓ were determined for each of the four segments using the LIGAND program, and the Student's t test was used to test the differences between the means obtained in aortas from control rats and rats fed a high K diet.

Results

Body Weight, Food Intake, and Plasma Potassium

Body weight and food intake were not significantly different between treated and control rats throughout treatment. Previous experiments from this laboratory have shown that plasma K is increased by approximately 0.5 mEq/L in rats fed a high K diet but is unchanged in rats fed a low Na diet (unpublished observations).

Blood Pressure

Blood pressure was significantly decreased after 3 days of treatment with a high K diet and remained lower than control for the rest of the treatment period (Figure 1). In rats fed a low Na diet, blood pressure

<table>
<thead>
<tr>
<th>Table 1. Determination of Kₘ and Vₘₐₓ: Latin Square Design</th>
<th>Segment number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat number</td>
<td>Segment</td>
</tr>
<tr>
<td>1 and 5</td>
<td></td>
</tr>
<tr>
<td>2 and 6</td>
<td></td>
</tr>
<tr>
<td>3 and 7</td>
<td></td>
</tr>
<tr>
<td>4 and 8</td>
<td></td>
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</tbody>
</table>

Numbers within the square refer to the concentration (mM) of K, in which segments of aorta from control and treated rats were incubated (see text for details).

Segment T refers to the top segment of the aorta including the aortic arch; segment M1, the upper middle segment; segment M2, the lower middle segment; and segment B, the lower abdominal segment of the aorta down to the bifurcation into the iliac artery.
VASCULAR NA⁺,K⁺-ATPASE ACTIVITY IN SHR/Sugden et al.

Figure 1. Blood pressure of SHR fed a high K diet. After a control week on a normal diet, 9-week-old female SHR were fed either a normal or a high K diet for 2 weeks. Each point represents the mean ± SEM of 14 to 16 animals. Single (p < 0.05), double (p < 0.02), and triple asterisks (p < 0.01) indicate significant difference between groups using Student's t test.

Figure 2. Blood pressure of SHR fed a low Na diet. After a control week on normal diet, 9-week-old female SHR were fed either a normal or a low Na diet for 2 weeks. Each point represents the mean ± SEM of 14 to 16 animals. Single (p < 0.05) and triple asterisks (p < 0.01) indicate significant difference between groups using Student's t test.

Figure 3. The effect of a high K diet on vascular Na⁺,K⁺-ATPase. Na⁺,K⁺-ATPase activity (measured as ouabain-sensitive ⁸⁶Rb uptake) was determined in top, middle, and bottom segments of aorta from female SHR fed either a normal or a high K diet for 2 weeks. The top segment was incubated in buffer (5 mM K⁺), the middle segment was incubated in the rat's own plasma, and the bottom segment was incubated in plasma from a paired treated or control rat. Numbers in parentheses represent the number of animals in the group. Analysis of variance showed a highly significant difference (p < 0.005) in Na⁺,K⁺-ATPase activity between high K and control groups.

was significantly decreased after 1 week and remained lower than control for the rest of the experiment (Figure 2).

Plasma Crossover and Kinetic Studies

Ouabain-sensitive ⁸⁶Rb uptake was significantly increased (p < 0.005, by analysis of variance) in the aortas of rats fed a high K diet (Figure 3). An increased Na⁺,K⁺-ATPase activity was seen in the presence of buffer (top segment), the rats' own plasma (middle segment), or plasma from a paired treated or control rat (bottom segment).

In rats fed the low Na diet, ouabain-sensitive ⁸⁶Rb uptake was significantly decreased (p < 0.002, by analysis of variance) in segments of aorta. A decrease in activity was seen when the segments were incubated in buffer, in the rats’ own plasma, or in plasma from a paired treated or control rat (Figure 4). Thus, addition of plasma to the tissue had no effect on ⁸⁶Rb uptake in rats fed either a normal, a high K, or a low Na diet (see Figures 3 and 4).

Ouabain-insensitive ⁸⁶Rb uptake was not significantly different (by analysis of variance) compared with control values in both the high K and the low Na studies (Table 2). V\textsubscript{max} was significantly increased in the rats on a high K diet (Table 3).

Discussion

Ouabain-sensitive ⁸⁶Rb uptake, a measure of membrane-bound Na⁺,K⁺-ATPase activity, was significantly increased in segments of aorta from rats fed a high K diet and significantly decreased in segments of aorta from rats fed a low Na diet. The finding that the high K diet increased Na⁺,K⁺-ATPase activity supports the work of Hayslett et al., who found an increase in the number of Na⁺,K⁺ pump sites, measured using [H]ouabain binding, in the kidney after high K
treatment. Our results, like theirs, also suggest that the change was due to an increase in $V_{\text{max}}$ of the enzyme rather than to an increased affinity for K. It is possible that an increase in plasma K or extracellular fluid K may be the important factor initiating the increased pump activity in vascular smooth muscle.\(^3\)

In both the high K and the low Na study, Na\(^+\),K\(^+\)-ATPase activity differed along the length of the aorta. The activity of the bottom segment appeared slightly higher than that in the other two segments, but this difference only reached significance in the high K study. This finding may be related to the presence of collateral blood vessels at the lower end of the aorta. In the low Na study, these collaterals were trimmed more carefully and the difference in Na\(^+\),K\(^+\)-ATPase activity along the length of the aorta did not reach statistical significance.

Differences in control values of ouabain-sensitive $^8$Rb uptake were also apparent between the two studies. This difference may be because rats in the second study were a little older and were purchased 6 months after those in the first study. Perhaps a more likely explanation is that the Na\(^+\),K\(^+\)-ATPase activity in the two experiments falls at two extremes of a normal range. Support for this explanation can be found in the kinetic study, in which values of ouabain-sensitive $^8$Rb uptake fell between these two extremes.

The increase in vascular Na\(^+\),K\(^+\)-ATPase activity on a high K diet may be related to the antihypertensive action of K. Several mechanisms may be responsible.

### Table 2. Ouabain-Insensitive $^8$Rb Uptake During High K and Low Na Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Top</th>
<th>Middle</th>
<th>Bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>High K study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>347±73 (11)</td>
<td>435±123 (11)</td>
<td>384±69 (11)</td>
</tr>
<tr>
<td>High K</td>
<td>693±230 (10)</td>
<td>470±132 (11)</td>
<td>242±48 (12)</td>
</tr>
<tr>
<td>Low Na study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>518±159 (14)</td>
<td>791±204 (14)</td>
<td>464±104 (15)</td>
</tr>
<tr>
<td>Low Na</td>
<td>289±70 (13)</td>
<td>520±196 (14)</td>
<td>344±54 (11)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers in parentheses represent the number of animals in each group. Analysis of variance showed there were no significant differences between any of the groups or segments tested.

Ouabain-insensitive Rb uptake was measured in top, middle, and bottom segments of aorta from SHR fed either a high K or a normal diet for 2 weeks. The top segment of aorta was incubated in Krebs (5 mM K), the middle segment of aorta was incubated in the rat's own plasma, and the bottom segment was incubated in plasma from a paired treated or control rat.

### Table 3. Effect of a High K Diet on $K_{\text{m}}$ and $V_{\text{max}}$

<table>
<thead>
<tr>
<th>Group</th>
<th>$K_{\text{m}}$ (mM)</th>
<th>$V_{\text{max}}$ (pmol $^8$Rb/mg wet wt/18 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2±0.54 (4)</td>
<td>3130±210 (4)</td>
</tr>
<tr>
<td>High K</td>
<td>2.7±1.2 (4)</td>
<td>5670±1021* (4)</td>
</tr>
</tbody>
</table>

Values are means ± SEM for each segment. Numbers in parentheses represent the number of aortic segments. Na\(^+\),K\(^+\)-ATPase activity was measured in top, upper middle, lower middle, and bottom segments of aorta (according to the Latin Square design; see Table 1) from SHR (8 per group) fed either a high K or a normal diet for 2 weeks.

* $p<0.05$, compared with control (using Student's $t$ test).

First, an increase in vascular Na\(^+\),K\(^+\)-ATPase would hyperpolarize the smooth muscle membrane.\(^2\),\(^10\),\(^11\)

Such a hypothesis has been used to explain the acute vasodilator action of K in isolated, perfused vascular preparations.\(^10\),\(^11\)

Second, an increase in ouabain-dependent norepinephrine reuptake at adrenergic nerve terminals\(^12\) may occur, causing a decreased sympathetic tone in the vasculature. This mechanism of action of K was suggested recently by Sato and Fujita\(^13\) in deoxycorticosterone acetate–salt rats. Third, it has been suggested that Na\(^+\),K\(^+\)-ATPase establishes Na gradients across the cell membrane that influence Na\(^+\)-Ca\(^2+\) exchange.\(^14\),\(^15\)

An increase in Na\(^+\),K\(^+\)-ATPase activity would increase efflux of Na from the cell, thereby increasing the gradient of Na across the membrane. The resulting effect would be an increase in the driving force for Ca efflux, a reduction in intracellular Ca, and a decrease in smooth muscle tone. However, Na\(^+\)-Ca\(^2+\) exchange has not been found in all blood vessels and has not been studied in aorta from SHR. Thus, further studies are needed to clarify this hypothesis. In
addition, although the aorta is a good example of vascular tissue, smooth muscle tone in the aorta may not reflect the tone of the resistance vessels,\(^6\) and therefore the relationship between Na\(^{+}\),K\(^{+}\)-ATPase activity in the aorta and blood pressure changes should be treated with caution.

Low dietary Na decreased Na\(^{+}\),K\(^{+}\)-ATPase activity (see Figure 4). This finding confirms the studies of Swales and colleagues.\(^{17,18}\) According to the Na\(^{+}\)-Ca\(^{2+}\) exchange hypothesis, inhibition of the pump normally is associated with increases in intracellular Na and Ca and an increase in vascular tone. A possible explanation for the decrease in Na\(^{+}\),K\(^{+}\)-ATPase activity is that in rats fed low Na (0.02\% Na), intracellular Na, which is normally high in the SHR,\(^{19,20}\) may decrease to normal levels (i.e., to those seen in the Wistar-Kyoto rat). This decrease would lead to a reduction in the need to pump Na out of the cell, and Na\(^{+}\),K\(^{+}\)-ATPase activity thus may be reduced to compensate for the reduced level of intracellular Na. Measurement of intracellular Na should resolve this question.

The finding that a low Na diet had opposite effects on Na\(^{+}\),K\(^{+}\)-ATPase as compared with the high K diet suggests that a combination of low Na and high K may not be additive in their effects on blood pressure. We tested the effects of this combination in SHR and found that the hypertensive effects of the individual diets were negated in that the combination high K, low Na diet did not lower blood pressure.\(^{21}\) Although aortic Na\(^{+}\),K\(^{+}\)-ATPase activity was not measured in rats on the combined diet, these findings suggest that changes in vascular Na\(^{+}\),K\(^{+}\)-ATPase may have a causal role in the maintenance of high blood pressure in SHR.

The results of the present experiments are in contrast with the hypothesis of De Wardener et al.,\(^{22}\) that a low Na diet reduces the amount of a circulating plasma inhibitor of Na\(^{+}\),K\(^{+}\)-ATPase and thus increases the activity of this enzyme. The results reported here for both high K and low Na experiments indicate that no ouabainlike factor is present in plasma from SHR. Other investigators\(^{17,18}\) have also failed to detect a ouabainlike factor. Most animal studies investigating this hypothesis of De Wardener et al.\(^{22}\) that a low Na diet reduces the amount of a circulating plasma inhibitor have been performed in volume-expanded, high-K, low-Na experiments indicate that no ouabainlike factor is present in plasma from SHR. Low dietary Na decreased Na\(^{+}\),K\(^{+}\)-ATPase activity on a low Na diet as compared with the high K diet.\(^{21}\) Although aortic Na\(^{+}\),K\(^{+}\)-ATPase activity was not measured in rats on the combined diet, these findings suggest that changes in vascular Na\(^{+}\),K\(^{+}\)-ATPase may have a causal role in the maintenance of high blood pressure in SHR.

In conclusion, the differences in \(^{86}\)Rb uptake in the aortas of SHR after high K and low Na treatment suggest that there may be differences in the mechanism of the antihypertensive effects of these two diets. The decrease in Na\(^{+}\),K\(^{+}\)-ATPase activity on a low Na diet probably is not involved in the depressor effect of low Na. However, an increase in vascular Na\(^{+}\),K\(^{+}\)-ATPase activity in rats fed high K may decrease smooth muscle tone in a number of ways and thus may contribute to the decrease in blood pressure seen with a high K diet.

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