Sodium Pump Activity in Arteries of Rats with Goldblatt Hypertension

HENRY W. OVERBECK, M.D., PH.D., AND DON E. GRISSETTE, M.S.

SUMMARY Several laboratories have reported evidence suggesting abnormalities in the activity of the sarcolemmal sodium pump in vascular smooth muscle in hypertension. The present experiments were designed to investigate the relationship of such changes to the status of the renin-angiotensin-aldosterone system and body fluid volumes. We assessed sodium pump activity in vitro in sodium-loaded tail artery and thoracic aorta freshly excised from rats with chronic one-kidney, one clip, and two-kidney, one clip hypertension, and from appropriate normotensive control rats. $^{86}$Rb uptake in the absence (total uptake) and presence of 1.0 mM ouabain (ouabain-insensitive uptake) was measured, and ouabain-sensitive uptake (nmole/mg dry weight/18 min) was calculated. There were increases in plasma renin activity in the two-kidney, one clip rats only. In the hypertensive rats there were significant increases (up to +60%) in the ouabain-sensitive and total $^{86}$Rb uptakes in both tail artery and aorta. The magnitude of increases in arterial tissue uptakes in the two forms of Goldblatt hypertension, and in one-kidney, one clip hypertensive rats given 0.9% saline to drink for 2 to 3 days before sacrifice, were similar. Further sodium loading of aortas from normotensive control rats did not increase their uptake. The results of this study provide no evidence for decreases in sodium pump activity, instead indicating that there are increases in the activity of the pump in the sarcolemmas of arterial smooth muscle studied in vitro. These increases in pump activity do not appear to be related to altered activity of the renin-angiotensin-aldosterone system, to changes in body fluid volumes, or to increases in intracellular concentrations of sodium. Increases in numbers or concentration of sarcolemmal pump molecules or in their turnover rate may be involved. However, in vitro $^{86}$Rb uptake by tail artery and aorta may not reflect the status of sodium pump activity in resistance vessels in vivo. (Hypertension 4: 132-139, 1982)

KEY WORDS • arterial hypertension • tail artery • aorta • Na,K-ATPase • volume-expanded hypertension • renin-dependent hypertension • plasma volume • blood volume • extracellular fluid volume • intracellular sodium

There is evidence that the contractile state of vascular smooth muscle, and, hence, vascular resistance, is in part a function of the operation of the electrogenic sodium pump of the sarcolemma.1 Several observations suggest that abnormalities in the function of the sodium pump occur in hypertension; such changes may contribute to the abnormal state of the peripheral resistance. Both increases and decreases in sodium pump activity have been reported in various forms of hypertension. Decreases in pump activity might account in part for the increased contractile state of vascular smooth muscle in hypertension, whereas increases in pump activity might reflect compensatory mechanisms.

The underlying basis of these changes in pump activity in hypertension is not understood, despite suggestions that the changes may reflect alterations in intracellular sodium concentrations, or may result from humoral factors released in the presence of volume-expansion. It is also possible that such changes may be related to activity of the renin-angiotensin-aldosterone system in hypertension.

It was the purpose of the present investigation to pursue these possibilities further. We chose Goldblatt hypertension in rats as our model, because there is evidence that the chronic one-kidney, one clip form is volume-dependent, whereas the chronic two-kidney, one clip form is renin-dependent. Furthermore, there...
is evidence suggesting that these forms of hypertension may be accompanied by altered pump activity in vascular smooth muscle.11,14

Methods

Using methods similar to those we have previously reported,4 we assessed vascular sodium pump activity by measuring 42Rb uptake in vitro in ventral tail artery and thoracic aorta freshly excised from four groups of rats: 1) one-kidney, one clip hypertensives (1KH); 2) one-kidney, sham-clipped normotensive controls (1KC); 3) two-kidney, one clip hypertensives (2KH); and 4) two-kidney, sham-clipped normotensive controls (2KC).

Male Sprague-Dawley rats, body weights 150–170 g, underwent unilateral nephrectomy and constriction of the contralateral (left) renal artery with an i.d. 0.38 mm silver clip (1KH), unilateral nephrectomy and sham clipping (1KC), constriction of the left renal artery with an i.d. 0.38 mm silver clip (2KH), or sham clipping (2KC). Rats were maintained on standard rat chow (Na+0.39%, K+ 0.96%) and water ad libitum for 3 to 4 weeks of sustained hypertension (systolic blood pressure greater than 150 mm Hg) in the hypertensive rats, and at an equal time interval in the control rats, we obtained arterial tissue. In other rats similarly prepared, we measured body fluid volumes and plasma renin activity. Serum creatinine (autoanalyzer) and hematocrit were measured in all rats, and serum Na+ and K+ (flame photometer) in some.

Arterial tissue for 42Rb uptake was obtained from rats of the four groups in rotation. Each rat was anesthetized with pentobarbital (75 mg/kg i.p.). His ventral tail artery, and then the ascending thoracic aorta, and then the descending thoracic aorta, were trimmed of adventitia and blood and opened longitudinally. Body weights and conscious tail systolic blood pressures by the cuff method (Natsume Tail Manometer System) were measured in all rats weekly. After 3 to 4 weeks of sustained hypertension (systolic pressure greater than 150 mm Hg) in the hypertensive rats, and at an equal time interval in the control rats, we obtained arterial tissue. In other rats similarly prepared, we measured body fluid volumes and plasma renin activity. Serum creatinine (autoanalyzer) and hematocrit were measured in all rats, and serum Na+ and K+ (flame photometer) in some.

To measure 42Rb uptake, we immediately placed these opened arteries in K+-free Krebs-Henseleit solution (NaHCO3, 27.2 mM; NaCl, 118.0 mM; KH2PO4, 1.0 mM; KCl, 4.8 mM; MgSO4·7H2O, 1.2 mM; CaCl2·2H2O, 1.25 mM; and glucose 11.1 mM) at 27° bubbled with 95% O2, 5% CO2 (pH 7.4). After 40 minutes incubation, the arterial tissue was dried at 100° for 24 hours and also weighed. 42Rb uptake was calculated as nmole/mg of wet weight/18 min and also as nmole/mg of dry weight/18 min. Ouabain-sensitive uptake was calculated as the difference between the 42Rb uptake without (total uptake) and with ouabain (ouabain-insensitive uptake). Each rat was necropsied, and heart weight/body weight, kidney weight/body weight, and adrenal weight/body weight were calculated. The unpaired Student’s t test was used to compare results in hypertensive and corresponding control groups. The null hypothesis was rejected at p ≤ 0.05.

To ensure that the 1 KH rats had ample sodium intake, we placed a subgroup (n = 16) of these rats on 0.9% saline drinking water for 48 to 72 hours before arterial tissue was obtained. For controls we used 1KC (n = 10) and unoperated (n = 6) rats. Three of the uninephrectomized and three of the unoperated rats also received saline for drinking water. Measurements of 42Rb uptake, serum creatinine, sodium, potassium, hematocrit, and heart weight/body weight in these rats were as described above.

Because we found evidence for greater uptakes in tissue from hypertensive rats, we investigated the possibility that intracellular sodium concentrations were rate-limiting in our measurements of 42Rb uptake in tissue from control rats. Thus, we tested the effect of further tissue sodium loading in an additional 28 unoperated male rats (body weight, 413 ± 3 g) maintained on the same stock diet. Aortas were obtained from pentobarbital-anesthetized rats, as described above. We used a paired experimental design: each freshly dissected aorta was divided in half, half was loaded for 10 and half for 60 minutes. Methods used for the 10-minute sodium loading were identical to those described above. K+-free Krebs-Henseleit solution was also used for the prolonged sodium loading, but the temperature was maintained at 37° to further promote sodium-potassium interchange. In sodium-loaded thoracic aorta segments from nine of these rats, we measured total 42Rb uptake, and in sodium-loaded tissue from another nine rats we measured ouabain-insensitive uptake, using procedures identical to those described above. We used Student’s t test for paired replicates to compare uptakes in arterial segments loaded for 10 minutes with those in the corresponding segments loaded for 60 minutes.

In the remaining 10 rats, we used lithium-substitution procedures similar to those developed by Friedman et al.28 to estimate intracellular concentrations of Na+ in freshly dissected thoracic aorta sodium-loaded for 10 or 60 minutes, as described above. Again, we used a paired experimental design, dividing each artery in half. Then, the sodium-loaded arterial tissue was incubated at 0–2° for 40 minutes in a medium (LiCl, 120.0 mM; CaCl2, 2.0 mM; MgCl2, 1.0mM; KCl, 5.0 mM; and 20 mM Heps buffer adjusted to pH 7.6 with Tris base) designed to replace extracellular sodium in the tissue with lithium, thereby allowing estimation of intracellular sodium concentra-
tion [Na\(^+\)]. The tissues were digested for 1 week in 0.75 N H\(_2\)NO\(_3\), and ion concentrations estimated by atomic absorption spectrophotometry. Assuming that all measured Na\(^+\) was from the intracellular components, we calculated [Na\(^+\)]\(_i\) as nmole/mg dry weight. We used Student's \(t\) test for paired replicates to compare [Na\(^+\)]\(_i\) in arterial segments sodium-loaded for 10 minutes with those in the corresponding segments loaded for 60 minutes.

In other rats with chronic Goldblatt hypertension and appropriate age- and weight-matched normotensive control rats, we estimated body fluid volumes by indicator-dilution, using methods similar to those we have reported: plasma volume by \(^{131}\)I-labelled human serum albumin (Mallinckrodt) and extracellular fluid volume by \(^{35}\)S(Na\(_2\)SO\(_4\), New England Nuclear). Volumes were measured at the same time of day in each group to control for diurnal variation. All rats had been maintained on standard rat chow and water ad libitum. With the rat under ether anesthesia, both kidneys were excised by flank incision. Then approximately 0.5μCi \(^{131}\)I-labelled human serum albumin and 0.05μCi Na\(_2\)SO\(_4\) were injected intravenously in 100 μl saline, with flush of 300 μl saline. For standards, identical injections were made into volumetric flasks and diluted. To facilitate complete mixing of the labels, the rats were allowed to recover from anesthesia. Then, exactly 1 hour after the isotopes were administered, the rats were decapitated and trunk blood collected for hematocrit and isotope concentrations. \(^{131}\)I was measured in 100 μl aliquots of plasma and standards on a crystal scintillation counter; plasma volume and total blood volume (hematocrit corrected for 2% trapped plasma) were calculated. Plasma proteins were then precipitated with ice-cold 10% trichloroacetic acid. \(^{35}\)S was measured in 50 μl aliquots of deproteinated plasma and standards on a liquid scintillation counter; extracellular fluid volume was thereby calculated. Later counting of these deproteinated specimens on a crystal scintillation counter revealed that less than 3% of the \(^{131}\)I remained, verifying that most albumin had been removed and also verifying that dissociation of the \(^{131}\)I from the albumin was less than 3%.

Calculated body fluid volumes were plotted against body weight. Regression lines and 95% confidence intervals were plotted for values in the control normotensive rats.

Finally, plasma renin activity was measured by radioimmunoassay (Angiotensin I Immutope Kit, E.R. Squibb and Sons, Inc.) in 16 1KH, 9 1KC, 9 2KH, and 9 2KC. Unanesthetized rats were decapitated, and trunk blood was collected for the first 3 seconds.

**Results**

Mean values (±SEM) of final body weight, tail systolic blood pressure (averaged over the period of hypertension in the Goldblatt rats and over the entire postoperative period in the control normotensive rats), heart weight/body weight, kidney weight/body weight, adrenal weight/body weight, hematocrit, serum creatinine, K\(^+\) and Na\(^+\), and plasma renin activity are presented in table 1. Significant hypertension developed in the clipped rats accompanied by increases in heart weight/body weight, small increases in serum creatinine (in no rat reported was serum creatinine >1.4 mg%), and some impairment of body growth. Additionally, in the 2KH there were increases in the weight of the untouched kidney and the adrenals, as well as decreases in serum K\(^+\). Compensatory hypertrophy of the remaining kidney was similar in 1KH and 1KC with no evidence for adrenal hypertrophy. Plasma renin activity was significantly elevated in the 2KH but not in the 1KH rats.

Measured body fluid volumes plotted against body weight are presented in figures 1-3. Values in 1KC and 2KC were not significantly different. Therefore, regression and 95% confidence intervals in these figures were plotted for pooled values in these control rats. It may be seen that body fluid volumes in most hypertensive rats fell within these 95% confidence intervals. Attempting to remove the complicating covariable, body weight, we additionally compared body fluid volumes in the eight 2KH and eight weight-matched control rats (body weights 240 ± 15 and 255 ± 7 g respectively; \(p > 0.2\)). There were no significant differences in plasma volume (\(p > 0.2\)), total blood volume (\(p > 0.1\)), or extracellular fluid volume (\(p > 0.1\)). We also compared the slope and intercept of the regression line calculated for 1KH or 2KH with that calculated for the normotensive control rats, and found no significant differences (\(p > 0.1\)). These data,
TABLE 1. Body Weight, Blood Pressure, Organ Weight/Body Weight, PRA, Serum Creatinine (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>1KH</th>
<th>p</th>
<th>1KC</th>
<th>p</th>
<th>2KH</th>
<th>p</th>
<th>2KC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>330.4 ± 12.1</td>
<td>&lt; 0.01</td>
<td>390.6 ± 3.4</td>
<td>&lt; 0.01</td>
<td>308.9 ± 10.8</td>
<td>&lt; 0.001</td>
<td>408.5 ± 6.8</td>
<td>&lt; 0.001</td>
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<tr>
<td>Systolic blood</td>
<td>190.6 ± 4.1</td>
<td>&lt; 0.001</td>
<td>115.3 ± 2.5</td>
<td>&lt; 0.001</td>
<td>185.0 ± 2.5</td>
<td>&lt; 0.001</td>
<td>120.6 ± 1.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td></td>
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<td></td>
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<tr>
<td>Heart wt/body wt</td>
<td>46.9 ± 1.7</td>
<td>&lt; 0.001</td>
<td>27.4 ± 0.3</td>
<td>&lt; 0.001</td>
<td>43.4 ± 1.3</td>
<td>&lt; 0.001</td>
<td>26.4 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(g/100 g × 10^3)</td>
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<td></td>
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<tr>
<td>L. kidney wt/body</td>
<td>52.2 ± 2.1</td>
<td>&gt; 0.1</td>
<td>56.4 ± 1.7</td>
<td>&gt; 0.4</td>
<td>34.8 ± 1.7</td>
<td>&gt; 0.1</td>
<td>36.3 ± 1.1</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>wt (g/100 g × 10^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>R. kidney wt/body</td>
<td>1.84 ± 0.13</td>
<td>&lt; 0.05</td>
<td>1.45 ± 0.06</td>
<td>&lt; 0.001</td>
<td>2.11 ± 0.11</td>
<td>&lt; 0.001</td>
<td>1.42 ± 0.08</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>wt (g/100 g × 10^3)</td>
<td>(n = 11)</td>
<td></td>
<td>(n = 7)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>Adrenal wt/body</td>
<td>5.41 ± 0.92</td>
<td>&gt; 0.2</td>
<td>3.72 ± 0.52</td>
<td>&gt; 0.01</td>
<td>10.47 ± 1.22</td>
<td>&lt; 0.001</td>
<td>2.97 ± 0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>wt (g/100 g × 10^3)</td>
<td>(n = 16)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>35.7 ± 1.0</td>
<td>&lt; 0.01</td>
<td>40.4 ± 0.9</td>
<td>&gt; 0.1</td>
<td>44.0 ± 0.8</td>
<td>&gt; 0.1</td>
<td>42.6 ± 0.6</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>Hematocrit (vol%)</td>
<td>0.94 ± 0.04</td>
<td>&lt; 0.02</td>
<td>0.79 ± 0.03</td>
<td>&lt; 0.01</td>
<td>0.89 ± 0.04</td>
<td>&lt; 0.01</td>
<td>0.74 ± 0.02</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>4.29 ± 0.13</td>
<td>&gt; 0.5</td>
<td>4.30 ± 0.12</td>
<td>&gt; 0.01</td>
<td>3.75 ± 0.09</td>
<td>&lt; 0.01</td>
<td>4.27 ± 0.15</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(mg%)</td>
<td>(n = 12)</td>
<td></td>
<td>(n = 10)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Serum K⁺ (mEq/liter)</td>
<td>51.7 ± 1.7</td>
<td>&gt; 0.5</td>
<td>51.2 ± 2.2</td>
<td>&gt; 0.2</td>
<td>145.4 ± 1.5</td>
<td>&gt; 0.1</td>
<td>147.9 ± 1.3</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>(mEq/liter)</td>
<td>(n = 12)</td>
<td></td>
<td>(n = 10)</td>
<td></td>
<td>(n = 10)</td>
<td></td>
<td>(n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 1KH = one-kidney, one clip hypertensive rats; 1KC = one-kidney controls; 2KH = two-kidney, one clip hypertensive rats; and 2KC = two-kidney controls.

Student's t test was used to determine the probability factor.

therefore, provide no evidence that altered body fluid volumes accompanied these forms and this stage of hypertension in our rats. Hematocrits in the four groups of rats in which body fluid volumes were measured did not differ significantly (M ± SEM: 44.8 ± 0.7 in 1KC plus 2KC; 43.4 ± 1.0 in 1KH; and 46.3 ± 1.1 in 2KH).

Table 2 presents measured in vitro ^86Rb uptake by tail artery and aorta. As indicated, Student's t test revealed that the total and ouabain-sensitive components were elevated in both arteries in both forms of Goldblatt hypertension. In 2KH, percentage increases in the ouabain-sensitive uptake by tail artery (48%) and aorta (20%) were similar to those in 1KH (59% and 22%, respectively). In the thoracic aorta, but not the tail artery, there were also elevations of the ouabain-insensitive component in the hypertensive rats. Calculations made using wet weights gave similar results.

Similar measurements were made in arterial tissues excised from 16 1KH rats that had received 0.9% saline as drinking water for 48 to 72 hours before
TABLE 2. **Rb Uptakes, nmol/mg dry wt/18 min (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>1KH p</th>
<th>1KC</th>
<th>2KH p</th>
<th>2KC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rats</td>
<td>19</td>
<td>13</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Total uptake (T)</td>
<td>30.11 ± 2.38</td>
<td>&lt;0.02</td>
<td>21.41 ± 1.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ouabain-insensitive uptake (I)</td>
<td>5.76 ± 0.25</td>
<td>&gt;0.2</td>
<td>6.11 ± 0.18</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Ouabain-sensitive uptake (T-I)</td>
<td>24.35 ± 2.40</td>
<td>&lt;0.02</td>
<td>15.30 ± 1.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rats</td>
<td>18</td>
<td>14</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Total uptake (T)</td>
<td>48.21 ± 1.78</td>
<td>&lt;0.01</td>
<td>38.30 ± 2.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ouabain-insensitive uptake (I)</td>
<td>6.78 ± 0.25</td>
<td>&lt;0.001</td>
<td>4.68 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ouabain-sensitive uptake (T-I)</td>
<td>41.43 ± 1.70</td>
<td>&lt;0.02</td>
<td>33.44 ± 2.34</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Abbreviations: 1KH = one-kidney, one clip hypertensive rats; 1KC = one-kidney controls; 2KH = two-kidney, one clip hypertensive rats; and 2KC = two-kidney controls.

Student's t test was used to determine the probability factor.

sacrifice and in tissues from 16 normotensive control rats, 10 of which had been uninephrectomized. **Rb uptakes by thoracic aorta and tail artery from uninephrectomized control rats did not significantly differ from those of unoperated rats. Similarly, uptakes in control rats on saline did not differ from those receiving water. Again, the ouabain-sensitive **Rb uptakes by tail artery and aorta excised from the hypertensive rats were increased by 54% and 21%, respectively. Mean values (nmole/mg dry wt/18 min ± SEM) were, for tail artery: total uptake, 34.28 ± 2.23 (hypertensive), 24.24 ± 1.24 (control), p < 0.001; ouabain-insensitive uptake, 5.59 ± 0.25 (hypertensive), 5.65 ± 0.21 (control), p > 0.5; ouabain-sensitive uptake, 28.69 ± 2.18 (hypertensive), 18.59 ± 1.12 (control), p < 0.001. For thoracic aorta, values were: total uptake, 42.63 ± 2.23 (hypertensive), 34.12 ± 1.21 (control), p < 0.001; ouabain-insensitive uptake, 6.11 ± 0.18 (hypertensive), 4.01 ± 0.16 (control), p < 0.001; ouabain-sensitive uptake, 36.52 ± 1.20 (hypertensive), 30.11 ± 1.19 (control), p < 0.001.

Finally, table 3 presents the results of our study of the effect of prolonged sodium loading on **Rb uptake and estimated [Na+] in arteries from normotensive control rats. Compared to 10-minute sodium loading at 0-2° (as in table 2), prolonged loading of freshly excised aorta at 37° increased [Na+] in rats by 32% (p < 0.01). Despite these changes in tissue ionic contents, no significant changes were induced in total **Rb uptake.

Discussion

The results of this study provide evidence that the ouabain-sensitive component of **Rb uptake is elevated in tail artery and thoracic aorta freshly excised from rats with chronic one-kidney, one clip and two-kidney, one clip renovascular hypertension. The ouabain-sensitive component of **Rb uptake by tissues reflects activity of the cell membrane sodium pump.** In tail artery and aorta the major cellular element is smooth muscle, so the increases we observed in **Rb uptake probably reflect elevated activity of the sar-

TABLE 3. **Rb Uptake, [Na+] in Thoracic Aorta after 10 or 60 Minutes of Sodium Loading (mean ± SEM)

<table>
<thead>
<tr>
<th>Intracellular ions (nmole/mg dry wt)</th>
<th>10-min loading</th>
<th>p</th>
<th>60-min loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>[Na+]</td>
<td>55.0 ± 3.3</td>
<td>&lt;0.01</td>
<td>72.7 ± 3.4</td>
</tr>
<tr>
<td>**Rb uptake (nmole/mg dry wt/18 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rate</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total uptake</td>
<td>39.5 ± 3.4</td>
<td>&gt;0.1</td>
<td>29.8 ± 2.3</td>
</tr>
<tr>
<td>Ouabain-insensitive uptake</td>
<td>6.3 ± 0.4</td>
<td>&gt;0.1</td>
<td>5.2 ± 0.6</td>
</tr>
</tbody>
</table>

Student's t test for paired replicates.
colemmal sodium pump of vascular smooth muscle per unit tissue weight. In contrast to the ouabain-sensitive component, the ouabain-insensitive component of $^{86}$Rb uptake represents nonspecific Rb binding and Rb uptake by interstitial fluid, as well as passive movement of Rb into cells. Thus, we may not cite the increases we observed in ouabain-insensitive uptake in aorta in our hypertensive rats as evidence for increased sarcolemmal permeability to K+ in these rats.

As expected, plasma renin activity was elevated only in the 2KH rats, accompanied by other suggestive evidence for activation of the renin-angiotensin-aldosterone system (increased adrenal weight/body weight, decreased serum K+). However, the magnitude of increase in pump activity was similar in the two forms of chronic Goldblatt hypertension, as well as in the 1KH rats given saline to drink. Although not measured in these latter rats, plasma renin activity was probably reduced below normal and body fluid volumes expanded. Thus, the increases in pump activity do not appear to be related to changes in the renin-angiotensin-aldosterone system.

We detected no measurable changes in plasma volume, total blood volume, or extracellular fluid volume in either form of Goldblatt hypertension in our rats. Thus, our data suggest that the increases in pump activity we observed are also unrelated to changes in body fluid volumes. Other laboratories similarly fail to find volume changes in chronic one-kidney, one clip hypertension. However, most investigators report that this form of Goldblatt hypertension is volume-expanded. Two-kidney, one clip hypertension in rats, in contrast, is felt by most investigators not to be volume-dependent; however, elevations in exchangeable sodium have also been reported in the chronic state of this form of Goldblatt hypertension.

Recently we observed similar increases in activity of the sodium pump in tail artery and aorta excised from Dahl salt-sensitive rats, where we documented volume-expansion accompanying high NaCl intake. We concluded that our findings in these in vitro studies failed to support the hypothesis that suppressed activity of the sodium pump in vascular smooth muscle characterizes volume-expanded forms of hypertension in general. Similarly, if Goldblatt hypertension in rats is, in fact, accompanied by volume expansion, our in vitro findings again do not support this hypothesis, nor do they support the more recent suggestion by Haddy's laboratory limiting the hypothesis to non-genetic, low-renin forms of volume-expanded hypertension.

In this regard, the increases in vascular sodium pump activity we are reporting here in the 1KH rats are in conflict with preliminary reports from Haddy's laboratory. These investigators observed significant decreases in the ouabain-sensitive component of $^{86}$Rb uptake in tail artery freshly excised from rats with this form of chronic hypertension. Their measurements were made in rats prepared similarly to ours and in tissues studied under in vitro conditions virtually identical to ours. However, it is possible that their rats had hypertension of greater severity with deterioration of renal function; uremia is known to be accompanied by depression of the sodium pump in a variety of tissues. We can offer no other explanation for this striking disagreement.

The increases we are here reporting in sodium pump activity in tail artery and aorta excised from the 2KH rats are apparently in contrast with an observation made several years ago in our laboratory in the same form of hypertension. In these 2KH rats, which also were not uremic, we found attenuated in vivo hindlimb vasodilator responses to local intraarterial infusions of K+ because K+ evokes vasodilation by stimulating the sodium pump, attenuated responses suggested decreased pump activity. It is unlikely that these attenuated vasodilator responses we observed in vivo could be explained by increases in wall/lumen ratio in the hypertensive rats; increases in wall/lumen would elevate, rather than decrease, responses to vasodilator agents. Furthermore, the attenuated response to K+ appeared to be specific, because we did not find attenuated responses to other vasodilators in hypertensives.

According to evidence recently reviewed by Fleming, it seems unlikely that our 5-minute infusions of K+ had effects on transmitter release by nerve terminals. Therefore, the results of our earlier experiments probably reflected decreased pump activity in vivo in the smooth muscle cells of resistance vessels in rats with Goldblatt hypertension.

These contrasting results of our two studies, one in vivo and the other in vitro, add additional weight to our suggestion that in vitro measurement of near-maximal activity of the sodium pump in aorta and tail artery, even if freshly excised and rapidly processed, may not represent levels of pump activity present in vivo in vascular smooth muscle of resistance vessels. If there is a circulating digitalis-like pump inhibitor in hypertension, in cardiovascular tissue of rats, a species relatively insensitive to digitalis, it is likely that this inhibitor dissociates extremely rapidly from the pump molecule in vitro.

However, this rapid dissociation would not, by itself, explain the increases in pump activity we and others observed in arterial tissue excised from hypertensive rats and studied in vitro. It has been suggested that these increases may represent a compensatory response to elevated sarcolemmal permeability to sodium, increasing [Na+]. However, in arterial tissue excised from Dahl rats we estimated intracellular sodium concentrations and concluded that elevated [Na+], in tissue from the salt-sensitive rats could not explain their increased sodium pump activity. In the present study, similar measurements of arterial [Na+] lead us to similar conclusions. Increasing intracellular sodium concentration in aortic tissue from the control normotensive rats did not elevate their $^{86}$Rb uptake. Thus, the lower uptakes by the control tissue cannot be explained by a lower [Na+], and one cannot say that the tissues from the hypertensive rats were just behaving like sodium-loaded tissue from normotensive control rats.
More extensive studies of the relationship between intracellular sodium content and pump activity in vascular smooth muscle in hypertension are under way in our laboratories. In these in vitro studies, Dr. T.A. Brock (unpublished observations) has found a sigmoid relationship between tissue [Na+]i and ouabain-sensitive Rb uptake, with maximal uptakes greater than about 30 nmoles/mg dry weight. Of greatest interest, the curve in tissue from hypertensive rats (DOCA-salt or one-kidney Goldblatt [Na+]i greater than about 30 nmoles/mg dry weight) is significantly shifted toward the Rb-uptake axis. This indicates that tissues from these rats have higher levels of sodium pump activity for any level of [Na+]i than those of control rats.

Thus, it is likely that the increased vascular sodium pump activity in our rats with Goldblatt hypertension, in our Dahl rats, and in Dr. Brock’s rats with chronic DOCA-salt or Goldblatt hypertension, is not the immediate result of elevated [Na+]i. A more likely explanation is that the elevated pump activity reflects more sarcolemma per unit tissue weight, more pump molecules per unit sarcolemma, or a faster turnover rate of a normal complement of pump molecules, not, apparently, related to [Na+]i, [K+]o, or to activity of the renin-angiotensin-aldosterone system.

Sodium pump activity in vascular tissue is expressed in terms of tissue weight. Tissue composition in hypertension may be altered by hyperplasia. Therefore, it is possible that the increases we and others are reporting simply reflect increased amounts of sarcolemma per unit tissue weight, more pump molecules per unit sarcolemma, or the turnover rate per molecule, remains constant. In contrast, this is an unlikely possibility if the vascular smooth muscle undergoes only hypertrophy, because the ratio of cell surface area to cell volume would decrease, rather than increase.

However, we favor another explanation for the increases we and others have observed in sodium pump activity in vascular tissue studied in vitro. If there is an increased number of pump molecules per unit sarcolemma or the turnover rate per molecule, remains constant. In contrast, this is an unlikely possibility if the vascular smooth muscle undergoes only hypertrophy, because the ratio of cell surface area to cell volume would decrease, rather than increase.

However, we favor another explanation for the increases we and others have observed in sodium pump activity in vascular tissue studied in vitro. If there is a circulating digitalis-like pump inhibitor in hypertension, as increasing evidence indicates, it may be that this inhibitor induces the formation of new pump molecules in the sarcolemma of cardiovascular cells. In this regard, it has been reported that chronic administration of digitalis increases myocardial Na,K-ATPase activity. Induced de novo synthesis of pump molecules in the sarcolemma of vascular smooth muscle cells would account for the increased pump activity observed in vitro after the inhibitor rapidly dissociates from the pump molecules.

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