Determinants of Salt Sensitivity in Black and White Normotensive and Hypertensive Women

Jackson T. Wright, Jr, Mahboob Rahman, Antonio Scarpa, Marjan Fatholahi, Valerie Griffin, Rachel Jean-Baptiste, Monir Islam, Moustafa Eissa, Suzanne White, Janice G. Douglas

Abstract—Salt sensitivity (SS) has been linked to human hypertension. We examined ethnic differences in the relation between SS; erythrocyte sodium ([Na\(^+\)], calcium ([Ca\(^{2+}\)], potassium ([K\(^+\)], and magnesium ([Mg\(^{2+}\)]) and sodium pump activity in African-American (AA) and white women. In a crossover protocol, similar numbers of normotensive, hypertensive, AA, and white women were randomized to 7 days of a 20 meq/d and a >200 meq/d salt diet (n=199). After an overnight inpatient stay, group differences in supine blood pressure (BP), heart rate, erythrocyte cations, and sodium pump activity were measured. The prevalence of SS (53.5% vs 51%) and salt resistance (26.3% vs 30.0%) was similar in both races. Greater mean BP increase with salt loading was seen in AA vs white hypertensives but not between the normotensive women. In hypertensives, increase in mean arterial pressure was 12.6 vs 8.2 mm Hg in AAs vs whites, respectively (P<0.01), and for systolic BP, it was 23 vs 14.8 mm Hg (P<0.01). Higher [Na\(^+\)] and [Ca\(^{2+}\)] were noted in SS and salt-intermediate AA than in the corresponding white subjects. [Na\(^+\)], [Ca\(^{2+}\)], and the ratios of [Na\(^+\)] to [K\(^+\)], and of [Ca\(^{2+}\)] to [Mg\(^{2+}\)] were positively correlated with salt responsiveness in AA but not in white women. Sodium pump activity was similar between groups, although the change in maximal activity trended to vary inversely with SS in AA. In closely matched AA and white women, the prevalence of SS is similarly high in both races, although the magnitude of BP increase is greater in AA hypertensives. In AA but not in whites, SS is positively associated with [Na\(^+\)], [Ca\(^{2+}\)], and the ratios of [Na\(^+\)] to [K\(^+\), and of [Ca\(^{2+}\)] to [Mg\(^{2+}\)]. (Hypertension. 2003;42:1087-1092.)

Key Words: sodium □ hypertension, sodium dependent □ sodium, dietary □ race □ ethnicity □ ions □ sodium pump

Essential hypertension continues to be a major cause of morbidity and mortality in industrialized populations of the world and one for which there is no known cause. In the United States, the prevalence of hypertension increases with age, and at about age 55, the prevalence becomes greater in women versus men.1 More than half of white and three fourths of African-American (AA) women will develop hypertension by age 65 to 74 years. Acute blood pressure (BP) elevation with increasing salt intake (salt sensitivity [SS]) is commonly reported in large segments of the population, especially in those with renal disease, diabetes, obesity, hypertension, and older age and in AA.2,3 BP sensitivity to salt might also predict chronic BP elevation, and normotensives with this trait are more likely to develop hypertension.2,4 However, the pathophysiology of SS and its progression to hypertension remain poorly understood. This is further complicated by the significant heterogeneity in methods of defining SS.5–8,20 Increased intracellular sodium ([Na\(^+\)]) assessed primarily in circulating blood cells, is one of the most consistently reported abnormalities of cation metabolism in essential hypertension, although a link between intracellular cation metabolism and salt-induced elevation of BP has not been established. A number of epidemiologic studies have documented a direct correlation between [Na\(^+\)] and BP in AA but not in non-AA.9–15 Racial differences in several membrane sodium-transport systems have also been reported.14–16 Na,K-ATPase (sodium pump) is a principal regulator of [Na\(^+\)]. Lower sodium pump activity has been reported in AA versus other populations, and greater racial differences have been reported in women compared with men.13,16–19 A higher [Na\(^+\)] secondary to depressed sodium pump activity will activate Na\(^+\)/Ca\(^{2+}\) exchange and calcium/calmodulin signaling pathways in smooth muscle cells, thereby increasing vascular smooth muscle reactivity and tone by enhancing vascular responsiveness to vasoactive agonists.16

In this study, we examined whether there are ethnic differences in the relation between sodium pump activity, [Na\(^+\)], and other intracellular cations with BP responsiveness

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to a salt challenge. We hypothesized that BP responsiveness was related to $[Na^+]$, and $[Ca^{2+}]$, in both AA and white hypertensives and normotensives. This was examined in a matched, healthy, nonobese population of normotensive and hypertensive, AA and white, postmenopausal women.

### Methods

The study consisted of a 2-period crossover protocol, wherein 199 postmenopausal women (similar distribution of hypertensives, normotensives, whites, and AA) were randomized to receive 7 days of a $<20$ meq/d and a $>200$ meq/d salt diet in random order.

### Subjects

The study population was healthy and within 30% of ideal body weight. Patients were excluded or discontinued for a systolic BP $>115$ mm Hg at any visit, SBP $>180$ or DBP $>110$ on any 2 visits, symptomatic hypertension, clinical evidence of secondary hypertension, or history of excess alcohol or recreational drug use. The protocol was approved by the University Hospitals of Cleveland’s Institutional Review Board, and written, informed consent was obtained from all participants.

All antihypertensive medications were discontinued for at least 1 week before starting the dietary phases. Participants on antihypertensive medications with longer-acting effects on salt homeostasis had their BP controlled with a calcium channel blocker for 4 weeks before washout.

### Dietary Protocol

Each participant was randomized to receive 7 days of either a low- or a high-salt diet initially. The alternative diet was prescribed during a second 7-day period. The diets were provided by the General Clinical Research Center (GCRC). Dietary adherence was verified by 24-hour urinary sodium excretion. On the morning after an overnight stay in the GCRC, supine BPs and heart rates were measured every 15 minutes over a 4-hour period with a DinaMapp vital signs monitor. Participants with a $\geq10$-mm Hg increase in mean arterial pressure (MAP) at the end of the high-salt phase compared with the low-salt phase were classified as SS, whereas those with $<5$-mm Hg increase were classified as salt resistant. Those with a 5- to 10-mm Hg difference were classified as salt intermediate.$^{3,21,22}$

### Measurement of Erythrocyte (RBC) Total Content of Na, K, Ca, and Mg

Cations were measured in washed RBCs by atomic absorbance spectrophotometry on a Perkin-Elmer instrument (model 3100). Cation content was calibrated against aliquots of standards and normalized for hematocrit and per $10^6$ RBCs.

### Measurement of Na,K-ATPase Pump Activity

The rate of $^{86}$Rb uptake for 30 minutes normalized to $10^6$ RBCs was determined in the presence and absence of ouabain and reported as the difference between these values. Monensin (sodium ionophore) was added to measure maximal activity of the pump.$^{24}$

### Statistical Analyses

All statistical analyses were done with an SPSS software package. A 2-tailed Student t test (or Wilcoxon signed-rank test for nonnormally distributed data) was used to compare 2 groups. A value of $P<0.05$ was considered significant. ANOVA with adjustment for multiple comparisons was used for comparing $>1$ group.

### Results

Table 1 shows the demographic and baseline data of the 199 participants in the study. Unlike many previous comparisons in AA and whites, the population was similarly matched for socioeconomic status as well as clinical parameters. Baseline (seated) BPs, renal function (estimated by Cockcroft-Gault creatinine clearance), prevalence of estrogen replacement,
versus the low-salt diet were similar in normotensive AA and white participants, respectively \( (P=0.932) \). However, in the hypertensive participants, the mean MAP response was 12.6 versus 8.2 mm Hg in AA versus whites, respectively \( (P<0.01) \), and for SBP, it was 23 versus 14.8 mm Hg \( (P<0.01) \). Although the traditional definition of SS has focused on changes in MAP, we were impressed by the large increments of SBP in both groups. Overall, SBP increased 18.3±12.7 mm Hg in AA and 13.7±12.2 mm Hg in whites \( (P<0.01, \text{Table 2}) \).

Because a major focus of this study was on the relation between ions and responsiveness to salt, we assessed the comparative ionic responses to salt challenge. In general, elevated \( \text{Na}^+ \) and \( \text{Ca}^{2+} \), were associated with salt responsiveness in AA but not in white women (Table 3). SS and salt-intermediate AA displayed higher \( \text{Na}^+_i \) than did the corresponding white subjects. Similarly, \( \text{Ca}^{2+} \), was significantly higher in the AA SS and salt-intermediate groups. Neither \( \text{Na}^+ \), nor \( \text{Ca}^{2+} \), differed significantly in the salt-resistant AA group or in either white group. In addition, on the low-salt diet, no difference in intracellular cation level was noted by ethnicity or salt responsiveness. We also evaluated the ratio of \( \text{Na}^+ \) to \( \text{K}^+ \), because they are modulated in parallel by differences in Na,K-ATPase, and of \( \text{Ca}^{2+} \), to \( \text{Mg}^{2+} \), because they have been intimately linked as modulators of vascular reactivity. In comparing the \( \text{Na}^+/-\text{K}^+ \), and \( \text{Ca}^{2+/-}\text{Mg}^{2+} \), ratios, SS AA \((n=53)\) had significantly higher ratios when compared with whites \((n=53)\) but only on the high-salt diet (Figure). By contrast, salt-resistant subjects \((n=26 \text{ for AA and } n=30 \text{ for whites})\) did not display significant increments in these ratios when challenged with a high-salt diet. No significant correlation between change in MAP and \( \text{Na}^+/-\text{K}^+ \), \( \text{Ca}^{2+/-}\text{Mg}^{2+} \), ratio during the high-salt phase was noted in AA and white SS participants.

Table 4 reports the activity of the sodium pump, which was assessed both under basal conditions on the low-salt diet and with a monensin challenge on the high-salt diet to define maximal activity. Marked variability in sodium pump activity and number of pumps was found in all subgroups. Although no racial difference in basal activity was noted, SS AA displayed an \( \approx 14\% \) \( (P=0.30) \) lower maximal sodium pump activity.

### Table 2. Mean (±SD) BP Response to Salt

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>MAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>16.0±12.6</td>
<td>6.0±7.4</td>
<td>8.2±7.9</td>
</tr>
<tr>
<td>AA</td>
<td>18.3±12.7*</td>
<td>6.5±8.0</td>
<td>10.5±8.9</td>
</tr>
<tr>
<td>White</td>
<td>13.7±12.2</td>
<td>5.4±6.6</td>
<td>8.2±7.9</td>
</tr>
<tr>
<td>SS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>26.9±9.2</td>
<td>11.9±6.1</td>
<td>16.9±5.8</td>
</tr>
<tr>
<td>White</td>
<td>22.5±7.3</td>
<td>10.0±3.5</td>
<td>14.2±3.5</td>
</tr>
<tr>
<td>SI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>15.2±4.0</td>
<td>3.9±2.8</td>
<td>7.7±1.5</td>
</tr>
<tr>
<td>White</td>
<td>12.5±4.2</td>
<td>4.5±2.9</td>
<td>7.2±1.3</td>
</tr>
<tr>
<td>SR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>3.1±6.4</td>
<td>−2.4±4.4</td>
<td>−0.56±4.3</td>
</tr>
<tr>
<td>White</td>
<td>−0.40±8.0</td>
<td>−2.0±5.3</td>
<td>−1.5±5.6</td>
</tr>
<tr>
<td>NTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>13.4±11.9</td>
<td>5.7±7.9</td>
<td>8.3±8.5</td>
</tr>
<tr>
<td>White</td>
<td>12.7±12.0</td>
<td>5.9±6.0</td>
<td>8.1±7.7</td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>23.0±11.7†</td>
<td>7.3±8.1</td>
<td>12.6±8.9†</td>
</tr>
<tr>
<td>White</td>
<td>14.8±12.4</td>
<td>4.9±7.3</td>
<td>8.2±8.2</td>
</tr>
</tbody>
</table>

Mean BP responsiveness to the salt diets by race, hypertensive status, and SS status. SI indicates salt-intermediate; SR, salt resistant; NTN, normotensive; and HTN, hypertensive. *\( P<0.05 \), †\( P<0.01 \), Student \( t \) comparisons by race.
TABLE 4. Sodium Pump Activity and Number by Race and SS Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS</th>
<th>Salt Intermediate</th>
<th>Salt Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal activity, mean±SD</td>
<td>0.04±0.05</td>
<td>0.05±0.05</td>
<td>0.04±0.03</td>
</tr>
<tr>
<td>Maximal activity, mean±SD</td>
<td>0.26±0.11</td>
<td>0.26±0.16</td>
<td>0.26±0.30</td>
</tr>
<tr>
<td>Maximal – basal activity, mean±SD</td>
<td>0.22±0.12</td>
<td>0.22±0.17</td>
<td>0.27±0.22</td>
</tr>
<tr>
<td>Basal pump No., median±SD</td>
<td>375±574</td>
<td>368±428</td>
<td>360±317</td>
</tr>
<tr>
<td>Maximal pump No., median±SD</td>
<td>336±385</td>
<td>394±921</td>
<td>356±307</td>
</tr>
</tbody>
</table>

Basal sodium pump activity and pump number were measured after a low-salt diet. Maximal pump activity was measured after monensin challenge, and pump number was measured after a high-salt diet.

*P=0.057, comparisons by race within SS status (ANOVA).

Intracellular cation ratio in AA and white postmenopausal SS (A) and salt-resistant (B) women after 1-week ingestion of a 20 meq (low) vs 200 meq (high) salt diet. *P<0.05, AA vs white.

activity. In comparing the change in sodium pump activity (activity after a monensin challenge minus basal values), there was a trend (P=0.14) toward a smaller increment in sodium pump activity in SS AA, whereas salt-resistant AA tended toward a greater (P=0.06) increment in activity versus salt-resistant whites.

Discussion

The present study recruited almost 200 carefully matched AA and white normotensive and hypertensive women to assess SS and its relation to intracellular ions. An important aspect of these studies was the relatively high socioeconomic status of the study population and lack of major risk factors for BP responsiveness to salt (obesity, diabetes, and renal insufficiency). Yet despite the lack of these risk factors and using a rigorous definition of SS, we observed a similarly high prevalence of SS in both AA and white subjects (54% and 51%, respectively). Although the definition of SS was somewhat more rigorous than that in previous protocols, the study population was older (mean age, 56 years), had a higher prevalence of hypertensives (50%), and was exclusively female. These characteristics are associated with a higher prevalence of SS.26-27

A second equally important aspect of these studies was the magnitude of BP elevation, especially SBP elevation with a salt challenge. Changes ranged from a mean of 23 mm Hg in hypertensive AA to 13 to 15 mm Hg in all other groups. Although previous studies of SS have focused on MAP and shown only modest changes, this study as well as several recent studies have shown the greatest effect of a salt challenge to be on SBP.26 Recent analyses of data from the Framingham and Multiple Risk Factor Intervention Trial have emphasized the importance of SBP as a risk factor for cardiovascular and renal diseases.28-30 In this study, hypertensive AA women experienced a 23-mm Hg increment in SBP, and normotensive AA and white, as well as hypertensive white women, experienced a 13- to 15-mm Hg increment. Thus, excessive salt ingestion is an important modifiable risk factor for elevations in SBP in both AA and white postmenopausal women.

Our original hypothesis was that higher Na⁺, in AA was mechanistically related to a greater prevalence of SS in AA. This remains a possibility, because we observed that SS and salt-intermediate AA had a significantly higher Na⁺, and Na⁺,K⁺, ratio than did the corresponding white groups. In addition, the ratio of Ca²⁺ to Mg²⁺ was significantly higher in SS AA compared with SS whites when challenged with a high-salt diet. These intracellular ions are suggested to contribute to increased vascular resistance, which has been reported to accompany a salt challenge in SS hypertension.31

Na⁺, K⁺, Ca²⁺, and Mg²⁺ have maintained a central role in investigations into the pathophysiology of hypertension.32 Human studies on the role of cations in BP regulation have focused on circulating blood cells, and these cells are hypothesized to be a surrogate for other cells of the body (e.g., vascular smooth muscle cells, transporting epithelial cells, and cardiac myocytes) and permit assessment of cation regulation. A number of studies, dating back to the early...
1950s, have reported Na\(^+\), to average \(\approx 30\%\) higher in AA than in whites.\(^9\)\(^{,\,}32\) Excess Na\(^+\), has not been suggested to directly affect vascular reactivity but rather to stimulate activity of the Na\(^+\)/Ca\(^{2+}\) exchanger and induce higher Ca\(^{2+}\).

The link between Ca\(^{2+}\) and SS has long been suggested.\(^33\)\(^,\,\)34 Our studies confirm this hypothesis, because we demonstrated increased Ca\(^{2+}\), in SS and salt-intermediate AA women compared with whites. These differences were not present in salt-resistant subjects. Increased Ca\(^{2+}\), enhances vascular reactivity to a variety of vasoactive agonists, and increased levels have been reported in hypertensives when compared with normotensives.\(^9\)\(^,\,\)32\(^,\,\)34 In one early study, Oshima et al.\(^35\) reported that an elevation in MAP with a salt challenge was positively correlated with a change in free Ca\(^{2+}\), in the lymphocytes of 12 subjects with essential hypertension. Previous studies focusing on total Ca\(^{2+}\), stores have demonstrated greater Ca\(^{2+}\), pools in a variety of cell types from AA, despite lower levels of resting Ca\(^{2+}\), in the RBCs.\(^39\) During the high-salt challenge, we observed a changers, which have been described in muscle cells as well as in RBCs.\(^39\) During the high-salt challenge, we observed a significant increase in Ca\(^{2+}\), in SS AA compared with SS white subjects. SS was not assessed. Several mechanisms have been described whereby changes in Mg\(^{2+}\), could regulate Ca\(^{2+}\), in vascular smooth muscle cells. Most promising candidates are the Na\(^+\)/Mg\(^{2+}\) and Ca\(^{2+}\)/Mg\(^{2+}\) exchangers, which have been described in muscle cells as well as in RBCs.\(^39\) During the high-salt challenge, we observed a higher Ca\(^{2+}\),/Mg\(^{2+}\), ratio in SS AA compared with SS white subjects, suggesting that these alterations might contribute to the pathophysiology of SS hypertension in AA but not in whites.

Our data suggest that the higher Na\(^+\), in AA in general and in SS AA more specifically might have been secondary to decreased activity of Na,K-ATPase. However, we could not definitively prove this relation. RBCs have low sodium pump activity when compared with other cell types. Thus, the inability to demonstrate a racial difference in pump activity might have been due to the limitation of this cell type rather than a rejection of our primary hypothesis. It is also of interest that the number of sodium pumps was inversely related to change in BP despite the fact that we did not observe group differences (parameter estimate, \(-0.005\pm 0.002\) [SEM], \(P=0.006\)). The importance of K\(^-\) and BP regulation has long been appreciated, wherein low dietary potassium is associated with higher BPs, and supplemental dietary K\(^-\), lowers BP, especially in AA.\(^40\)\(^,\,\)42 Our study subjects demonstrate that there are racial differences in the relation between these ions and the presence of SS. This study also documents that SS AA display a significantly higher ratio of Na\(^+\), to K\(^-\), than do white subjects.

**Perspectives**

In summary, in similarly matched AA and white postmenopausal women, the prevalence of SS is similarly high in both races. Although the prevalence of SS is similar, the magnitude of BP increase is greater in AA, especially in AA hypertensives. SS in AA was associated with higher Na\(^+\), and Ca\(^{2+}\), than in corresponding white subjects. The Ca\(^{2+}\),/Mg\(^{2+}\), ratio was also correlated with SS in AA but not in whites.

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**References**

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