Influence of Race, Sex, and Blood Pressure on Erythrocyte Sodium Transport in Humans

JEAN B. SMITH, MARY B. WADE, NAOMI S. FINEBERG, AND MYRON H. WEINBERGER

SUMMARY Sodium transport of erythrocytes from normotensive and essential hypertensive subjects was evaluated by determining ouabain-sensitive and ouabain-insensitive sodium efflux rates, Na⁺-Li⁺ countertransport rates, Li⁺-K⁺ cotransport rate constants (lithium replacing sodium), intracellular sodium concentrations, and the number of Na⁺,K⁺-adenosine triphosphatase (ATPase) sites per erythrocyte. Subjects included men and women, blacks and whites. Hypertensive subjects had significantly higher sodium transport than did normotensive subjects for ouabain-sensitive sodium efflux (*p < 0.025*) and Na⁺-Li⁺ countertransport (*p < 0.001*). Sexual differences were noted for ouabain-sensitive (*p < 0.001*) and ouabain-insensitive (*p < 0.001*) sodium efflux, for intracellular sodium concentration (*p < 0.025*), and for the Li⁺-K⁺ cotransport rate constant (*p < 0.005*), all with higher values for men than for women. Racial differences were noted for ouabain-insensitive sodium efflux (*p < 0.005*), Na⁺-Li⁺ countertransport (*p < 0.001*), and the Li⁺-K⁺ cotransport rate constant (*p < 0.001*); values were higher in whites than blacks for all three measurements. The number of [³H]ouabain binding sites was lower for blacks (*p < 0.001*) and the intracellular sodium concentration was higher for blacks (*p < 0.001*). Among all subjects, significant (*p < 0.001*) correlations were found between intracellular sodium concentration and the number of Na⁺,K⁺-ATPase sites per erythrocyte (r = —0.78) and between the ouabain-sensitive sodium efflux per site and intracellular sodium concentration (r = 0.85, *p < 0.001*). The values for sodium efflux, intracellular sodium, and the number of adenosine triphosphatase sites per red blood cell were used to calculate a second-order rate constant that was significantly (*p = 0.011*) higher among hypertensive than among normotensive subjects. The results indicate that significant differences in sodium handling are present in erythrocytes of hypertensive subjects and suggest that further elucidation of these abnormalities may provide insights into the pathogenesis of essential hypertension. (Hypertension 12: 254—258, 1988)

KEY WORDS • hypertension • sodium transport • intracellular sodium

The relationship between dietary sodium intake and blood pressure has long been recognized.¹ We now know that heterogeneity of blood pressure response to sodium exists in normotensive and hypertensive subjects.² Sodium-sensitive persons may also have an abnormality in the ability of their kidney to excrete a sodium load.³ Several transport mechanisms, including the Na⁺-K⁺ pump,⁴ Na⁺-Li⁺ countertransport,⁵ and Na⁺-K⁺ cotransport,⁶ have been implicated in the pathogenesis of hypertension. In the erythrocyte, these transport systems have been suggested to be qualitatively similar to those in the kidney,⁷ and their easy accessibility has enabled their study.

The results of previous cation transport studies in erythrocytes frequently have been conflicting, perhaps because of the variety of subjects studied, potentially confounding demographic or genetic factors, and perhaps also because of an incomplete understanding of the relationships among the various parameters measured. Among recent studies comparing hypertensive and normotensive subjects, there have been reports of increased⁸ ⁹ and decreased¹⁰ intracellular sodium concentration ([Na⁺]) among hypertensive subjects as well as reports of no difference between the groups.¹¹ ¹² Several studies¹⁰ ¹¹ ¹² reported a decreased ouabain-sensitive sodium efflux among hypertensive subjects, whereas others reported higher¹⁴ or unchanged values¹⁵ ¹⁶ when compared with normotensive sub-
jects. Similarly, for ouabain-insensitive sodium efflux, there are reports of higher\textsuperscript{15} or unchanged\textsuperscript{16} values for hypertensive subjects. For cotransport as well, some studies have reported higher\textsuperscript{15, 17, 18} lower\textsuperscript{19, 20} or no significant difference\textsuperscript{21, 22} in values for hypertensive patients when compared with values for normotensive subjects. Perhaps the least controversial observations have been in the Na\textsuperscript{+}-Li\textsuperscript{+} countertransport measurements, for which the hypertensive subjects' values are either higher\textsuperscript{23-25} or not significantly different\textsuperscript{26} from those of normal subjects. However, some of the differences in Na\textsuperscript{+}-Li\textsuperscript{+} countertransport have been attributed to effects of factors other than hypertension.\textsuperscript{27} Studies of racial differences in erythrocyte cation transport have been more consistent. Increases in [Na]\textsuperscript{+}\textsuperscript{28-30} and decreases in both cotransport\textsuperscript{29, 31, 33} and countertransport\textsuperscript{28, 30, 34} have repeatedly been reported for black subjects when compared with whites.

To examine the relationships among the various measurements of cation transport in black and white hypertensive and normotensive subjects, we measured the ouabain-sensitive and insensitive sodium efflux rates, the [Na], and the number of [\textsuperscript{3}H]ouabain binding sites of fresh, washed red blood cells (RBCs), as well as the Na\textsuperscript{+}-Li\textsuperscript{+} countertransport rate and the Li\textsuperscript{+}-K\textsuperscript{+} cotransport rate constant of lithium-loaded cells in 80 normotensive subjects and 65 hypertensive subjects, who were not on medication.

**Subjects and Methods**

The racial and gender characteristics of the normotensive and essential hypertensive subjects are shown in Table 1. Subjects ranged in age from 19 to 68 years for the normotensive subjects and from 23 to 74 years for the hypertensive subjects, and they were studied during an unrestricted diet. Informed consent was obtained from each subject after explanation of the studies to be performed. None of the normotensive subjects was taking any medication known to influence sodium transport. Secondary forms of hypertension had been excluded by previously reported techniques.\textsuperscript{35} All hypertensive subjects had mild to moderate uncomplicated hypertension for which withdrawal of anti-hypertensive medication was not deemed to be a major risk. All of the hypertensive subjects had been without medication for at least 18 days.

**TABLE 1. Number of Subjects Studied**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>On washed RBCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>On lithium-loaded cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>34</td>
<td>12</td>
</tr>
</tbody>
</table>

There were four subjects for whom not all of the parameters were measured.

**RBC Preparation**

Venous blood (40 ml) was drawn into heparinized Vacutainer tubes and processed within 1 hour. The RBCs were separated from the plasma anduffy coat after centrifugation for 10 minutes at 1000 g washed three times in a washing solution (150 mM choline chloride), and centrifuged after each wash for 10 minutes at 1000 g. All washing and incubation solutions were adjusted to an osmolality of 295 to 305 mosm. Packed cells (2 ml) were separated and suspended in a buffer solution consisting of 140 mM NaCl, 30 mM HEPES, and 10 mM dextrose for determination of the number of ouabain binding sites. Another aliquot of packed cells (5 ml) was used for the Na\textsuperscript{+}-Li\textsuperscript{+} countertransport and Li\textsuperscript{+}-K\textsuperscript{+} cotransport determinations. The remainder were washed three more times, and a suspension of approximately 50% was prepared in the washing solution to be used for [Na], determination and sodium efflux measurements. The hematocrit of the suspension was determined.

**[\textsuperscript{3}H]Ouabain Binding Assay**

Details of the [\textsuperscript{3}H]ouabain binding assay procedure, a modification of the method of DeLuise et al.,\textsuperscript{36} have been published previously.\textsuperscript{37} Aliquots (0.8 ml) of the cell suspension in HEPES buffer were added to solutions containing 100 \textmu M of [\textsuperscript{3}H]ouabain (0.12 pmol, 20.9 Ci/mmoll, New England Nuclear, Boston, MA, USA) and 100 \textmu M of an unlabeled ouabain solution. The unlabeled ouabain concentration in the final dilution was 0.5, 5.0, or 10.0 nM. Each concentration was assayed in duplicate. Samples were incubated in a shaking water bath at 37 °C for 3 hours. The cells were then washed three times in 150 mM choline chloride (3 ml) to remove unbound ouabain, and the supernatant was removed by aspiration after each wash. A 5% solution of trichloroacetic acid (1.2 ml) was added to release the bound ouabain from the cells, the sample was centrifuged at 800 g for 5 minutes, and 1 ml of the supernatant was added to 10 ml of scintillation fluid (New England Nuclear). The bound [\textsuperscript{3}H]ouabain was measured using a Nuclear Chicago Mark II LSC counter (Tracor, Elk Grove Village, IL, USA). Scatchard plots (bound/free vs total bound ouabain) were constructed to determine the concentration of [\textsuperscript{3}H]ouabain binding sites (x-intercept) in the incubated cell suspension. The mean ± SD of the correlation coefficients for the line was \( r = 0.998 ± 0.004 \). The number of sites per RBC was calculated as follows:

\[
\text{Sites/RBC} = \frac{(x\text{-intercept}) \times (6.02 \times 10^{23})}{\text{number of RBCs in final suspension}}
\]
Intracellular Sodium Concentrations

Further dilution (1:51) of the 50% suspension of cells washed six times was prepared in metal-free nonionic detergent (0.02% cationox A, Scientific Products, McGaw Park, IL, USA), and the sodium concentration was measured by atomic absorption spectroscopy (Instrumentation Laboratories, Andover, MA, USA). The \([\text{Na}]_i\) was calculated from the equation:

\[
[\text{Na}]_i = \frac{\text{Na concentration of 1:51 dilution} \times 51}{\text{hematocrit of suspension}}
\]  

Sodium Efflux Rate Determinations

Further dilutions of 4 ml of the 50% suspension were prepared for sodium efflux rate measurements in 10 ml of a MgCl₂ solution without ouabain (70 mM MgCl₂, 85 mM sucrose, 10 mM KCl, 1 mM glucose, 10 mM Tris–[N-morpholino]propanesulfonic acid [MOPS], pH 7.4 at 37 °C) and 10 ml of a MgCl₂ solution with ouabain (70 mM MgCl₂, 85 mM sucrose, 10 mM KCl, 10 mM glucose, 0.1 mM ouabain, 10 mM TRIS-MOPS, pH 7.4 at 37 °C). The suspensions were incubated at 37 °C in a shaking water bath with duplicate samples removed at 0, 5, and 15 minutes. The suspension was centrifuged, and the supernatant was determined for sodium analysis by atomic absorption spectroscopy. Plots of the sodium concentration in the supernatant versus the incubation period yielded the rate of sodium efflux into solution with and without ouabain. The difference was calculated to be the ouabain-inhibitable sodium efflux rate. This method of determining the efflux rate yields the same value as multiplying the initial \([\text{Na}]_i\) by the slope obtained from plots of \(-\ln[\text{Na}]_i\) versus time for conditions in which less than 5% of the \([\text{Na}]_i\) has effluxed.

A second-order rate constant \(k_2\) for sodium efflux was calculated from the relationship of sodium efflux per RBC with \([\text{Na}]_i\) and the sites/RBC:

\[
\text{Na efflux/RBC} = k_2 \times [\text{Na}]_i \times \text{sites/RBC}
\]

Na⁺-Li⁺ Countertransport and Li⁺-K⁺ Cotransport

Na⁺-Li⁺ countertransport and Li⁺-K⁺ cotransport were determined according to a previously published method. A 5-ml aliquot of packed RBCs was added to 20 ml of 150 mM LiCl and incubated at 37 °C for 3 hours in a shaking water bath. The cells were washed five times in the choline chloride washing solution to remove extracellular lithium, and a suspension (~50%) of the cells in the choline chloride solution was prepared. The hematocrit of the suspension was determined, a dilution was prepared for intracellular lithium determination, and 2.0 ml was added to 10 ml each of the following solutions: A (150 mM NaCl, 0.10 mM ouabain, 10 mM glucose, 10 mM TRIS-MOPS, pH 7.4 at 37 °C), B (150 mM choline chloride, 0.10 mM ouabain, 10 mM glucose, 10 mM TRIS-MOPS, pH 7.4 at 37 °C), C (150 mM choline chloride, 0.10 mM ouabain, 10 mM glucose, 1 mM furosemide, 10 mM TRIS-MOPS, pH 7.4 at 37 °C).

These suspensions were incubated at 37 °C in a shaking water bath, with samples removed at 45 and 90 minutes. The samples were centrifuged for 5 minutes at 1000 g, and the supernatant was removed to be analyzed for lithium by atomic absorption spectroscopy. The lithium efflux into each of the solutions was calculated from graphs of lithium concentration versus time. The Na⁺-Li⁺ countertransport is the difference between the efflux into Solutions A and B; the Li⁺-K⁺ cotransport rate constant is the difference between Solutions B and C divided by the intracellular lithium concentration. The ouabain-insensitive, furosemide-insensitive lithium efflux rate constant is the efflux into Solution C divided by the intracellular lithium concentration.

The coefficient of variation for the assays established by repeated (2–8) measurements for the same normotensive subjects (n = 21) over a 14-month period was 6.3% for \([\text{Na}]_i\), 6.1% for sodium efflux, 5.1% for the number of sites/RBC, 8.6% for \(k_2\), 10.0% for Na⁺-Li⁺ countertransport, 23.2% for Li⁺-K⁺ cotransport, and 14.0% for the ouabain-insensitive, furosemide-insensitive lithium efflux rate constant.

Statistical Methods

Group values are expressed as means ± SD. Preliminary analyses included two-way analysis of variance by race and sex within blood pressure group to determine which groups could be pooled for comparisons of hypertensive and normotensive subjects.

Linear correlation coefficients were used to estimate the strength of the relationship between two variables. A correlation coefficient for curvilinear relationship between \([\text{Na}]_i\) and ouabain sites was analyzed using the reciprocal of the number of ouabain sites and linear regression. Since the intercepts were not significantly different from zero, the regressions were performed forcing the lines through the origin. Regression lines for comparisons of correlations were also computed separately for each of the groups. If regressions did not differ, a single regression was computed for that blood pressure group. Comparisons of regressions between normotensive and hypertensive subjects were made only when no race or sex difference was evident.

Results

As shown in Table 2, both \([\text{Na}]_i\) and the number of sites/RBC showed very significant \((p < 0.001)\) differences due to race. The \([\text{Na}]_i\) was higher and the number of sites/RBC was lower for blacks, except in hypertensive black women. Men had significantly \((p < 0.025)\) higher \([\text{Na}]_i\) than did women. Significant sexual differences were observed for both ouabain-sensitive \((p < 0.001)\) and ouabain-insensitive sodium efflux rates \((p < 0.001)\), with men having higher values than women. Analysis
showed significantly \((p = 0.02)\) higher ouabain-sensitive sodium efflux rate for essential hypertensive subjects even though the group mean for normotensive black women was higher than that for hypertensive black women. The rate constant for ouabain-sensitive sodium efflux \((k_2)\) was significantly \((p = 0.011)\) higher for hypertensive subjects than for normotensive subjects, even though hypertensive black women had lower \(k_2\) values than did normotensive black women. The significance of differences for the sodium transport parameters analyzed by race, sex, and blood pressure status are summarized in Table 3.

For \(Na^+/Li^+\) countertransport significant \((p < 0.001)\) differences were noted between whites and blacks and between normotensive and hypertensive subjects \((p < 0.001;\) Figure 1). For the cotransport constant, blacks had significantly \((p < 0.001)\) lower values and men had significantly \((p = 0.003)\) higher values (Figure 2). No difference due to blood pressure was evident for cotransport. The rate constant for ouabain-insensitive, furosemide-insensitive efflux \((\text{lithium efflux into Solution 3})\) was significantly \((p = 0.02)\) higher for blacks than for whites.

The relationship between the ouabain-sensitive sodium efflux per site and \([Na]\) was similar for blacks and whites, men and women, and normotensive (Figure 3A) and hypertensive subjects (Figure 3B). Significant inverse correlations were found between \([Na]\) and the sites/RBC for normotensive (Figure 4A) and hypertensive subjects (Figure 4B). The transformation \((\text{[Na]} = \text{slope} \times \text{1/sites} + \text{intercept})\) gave correlation coefficients for both hypertensive \((r = 0.74)\) and normotensive \((r = 0.79)\) subjects that were significant \((p < 0.001)\). The equations for the lines were not significantly different between the two groups.

**Discussion**

Sodium efflux from RBCs occurs primarily via four mechanisms: the Na\(^+/\)K\(^+\)-adenosine triphosphatase (ATPase)-mediated Na\(^+\)-K\(^+\) pump, Na\(^+\)-K\(^+\) cotransport, Na\(^+\)-Na\(^+\) countertransport, and sodium leak, which is not inhibited by ouabain or furosemide. Under physiological conditions, the Na\(^+\)-K\(^+\) pump is the principal transporter, accounting for 1.4 to 2.0 mmol Na/RBC/hr. The Na\(^+\)-K\(^+\) cotransport and the sodium leak pathways are each responsible for approximately 0.2 mmol/L RBCs/hr, and the Na\(^+\)-Na\(^+\) exchange (measured as Na\(^+\)-Li\(^+\) countertransport) accomplishes no net transfer.

In our study of these transport systems in RBCs from essential hypertensive subjects not currently medicated, both the ouabain-sensitive sodium transport and the Na\(^+\)-Li\(^+\) countertransport showed significant differences between hypertensive and normotensive subjects while the Na\(^+\)-Li\(^+\) countertransport and Li\(^+\)-K\(^+\) cotransport showed a significant \((p < 0.001)\) racial effect. Na\(^+\)-Li\(^+\) countertransport showed significant differences between the two groups for whites and blacks, men and women, and normotensive and hypertensive subjects. The rate constant for Na\(^+\)-Li\(^+\) countertransport \((k_2)\) was significantly \((p = 0.011)\) higher for hypertensive subjects than for normotensive subjects, even though hypertensive black women had lower \(k_2\) values than did normotensive black women. The significance of differences for the sodium transport parameters analyzed by race, sex, and blood pressure status are summarized in Table 3.

**Table 2. Sodium Efflux Measurements of Washed Erythrocytes**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sodium efflux (mmol/L RBCs/hr)</th>
<th>[Na] (mmol/L RBCs)</th>
<th>Sites</th>
<th>(k_2 \times (10^{-4})) (sites(^{-1})·hr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normotensive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black ((n = 13))</td>
<td>1.50±0.17</td>
<td>0.36±0.15</td>
<td>7.18±2.34</td>
<td>358±143</td>
</tr>
<tr>
<td>White ((n = 23))</td>
<td>1.60±0.18</td>
<td>0.41±0.11</td>
<td>6.80±1.58</td>
<td>380±80</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black ((n = 18))</td>
<td>1.47±0.16</td>
<td>0.27±0.09</td>
<td>7.15±1.72</td>
<td>314±67</td>
</tr>
<tr>
<td>White ((n = 26))</td>
<td>1.48±0.22</td>
<td>0.39±0.11</td>
<td>5.87±1.46</td>
<td>394±91</td>
</tr>
<tr>
<td><strong>Hypertensive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black ((n = 10))</td>
<td>1.72±0.22</td>
<td>0.38±0.16</td>
<td>8.54±1.70</td>
<td>276±52</td>
</tr>
<tr>
<td>White ((n = 34))</td>
<td>1.66±0.16</td>
<td>0.39±0.13</td>
<td>6.25±1.14</td>
<td>395±80</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black ((n = 9))</td>
<td>1.42±0.19</td>
<td>0.24±0.11</td>
<td>6.95±1.22</td>
<td>376±90</td>
</tr>
<tr>
<td>White ((n = 11))</td>
<td>1.57±0.17</td>
<td>0.27±0.07</td>
<td>6.01±1.03</td>
<td>337±56</td>
</tr>
</tbody>
</table>

Values are means ± SD. \([Na]\) = intracellular Na concentration; \(k_2\) = second-order rate constant.

**Table 3. Significance of Differences in Sodium Transport Parameters**

<table>
<thead>
<tr>
<th>Transport Parameter</th>
<th>Diagnosis</th>
<th>Race</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain-sensitive sodium efflux</td>
<td>(p = 0.02)</td>
<td>NS</td>
<td>(p &lt; 0.001)</td>
</tr>
<tr>
<td>Ouabain-insensitive sodium efflux</td>
<td>NS</td>
<td>(p = 0.002)</td>
<td>(p &lt; 0.001)</td>
</tr>
<tr>
<td>[^3H]Ouabain binding sites/RBC</td>
<td>NS</td>
<td>(p &lt; 0.001)</td>
<td>NS</td>
</tr>
<tr>
<td>[Na]</td>
<td>NS</td>
<td>(p &lt; 0.001)</td>
<td>(p &lt; 0.025)</td>
</tr>
<tr>
<td>Ouabain-sensitive rate constant ((k_2))</td>
<td>(p = 0.01)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Na(^+)-Li(^+) countertransport</td>
<td>(p &lt; 0.001)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Li(^+)-K(^+) cotransport rate constant</td>
<td>(p &lt; 0.001)</td>
<td>NS</td>
<td>(p &lt; 0.005)</td>
</tr>
<tr>
<td>Ouabain-insensitive, furosemide-insensitive lithium efflux rate constant</td>
<td>NS</td>
<td>(p = 0.02)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\([Na]\) = intracellular Na concentration; \(k_2\) = second-order rate constant.
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transport separated normotensive and hypertensive groups best (p < 0.001), yet there was considerable overlap in the values. Observation of racial differences for [Na], and the number of sites/RBC (p < 0.001) confirmed previous studies;29-34-43 however, the ouabain-sensitive sodium efflux and the product of [Na] and sites were similar for blacks and whites.

The ouabain-insensitive sodium efflux into a sodium-free medium is composed of Na\(^+\)-Li\(^+\) cotransport as well as other, yet unidentified sodium transports collectively referred to as the "sodium leak." The Li\(^+\)-K\(^+\) cotransport measured in this study may be an estimate of Na\(^+\)-K\(^+\) cotransport, since lithium replaces sodium in many transport systems and Li\(^+\)-K\(^+\) cotransport has been shown to correlate with Na\(^+\)-K\(^+\) cotransport.39

The group mean for neither [Na], nor the number of sites/RBC was significantly different for essential hypertensive subjects in comparison to values for normal subjects. This finding contrasts with previously reported higher8 ? and lower 10 concentrations. The difference between our results and those of Simon and Conklin10 could be due, at least in part, to the units used for reporting. Our values are expressed in mmoles per liter of RBCs, while theirs are in mmoles per kilogram of cell water. They also reported a significantly smaller cell volume for RBCs from hypertensive subjects than for normotensive subjects. Conversion of their data to mmoles per liter of RBCs still showed a lower mean [Na], for hypertensive subjects, but the difference was not significant when expressed in these units. Thus, their data may be confounded by factors influencing cell size and water content.

Ringel et al.44 have reported significantly higher [Na], and lower adenosine triphosphatase (ATPase) sites/RBC among previously treated hypertensive subjects who had been withdrawn from treatment for at least 6 weeks but not among untreated hypertensive subjects. Even though our hypertensive subjects had also been withdrawn from treatment, we did not find significant differences for [Na], and ATPase sites. Perhaps our results differ from those of Ringel et al.44 because our subjects included black and white men and women and the differences noted by Ringel et al.44 are more prevalent among black men. Our group of black men was too small for the differences between hypertensive and normotensive subjects to be significant.

A significant (p < 0.001) correlation between sodium efflux/site and [Na], was evident for both normotensive (see Figure 3A) and hypertensive subjects (see Figure 3B). This interpersonal relationship, showing that subjects with higher sodium efflux have a higher, rather than a lower, intracellular sodium, suggests that [Na], is not determined by but instead may be a determinant of Na\(^+\)-K\(^+\) pump activity. The relationship between the efflux/site and [Na], indicates that 79% of the interpersonal variation in efflux/site can be attributed to differences in [Na]. This conclusion suggests that the
FIGURE 3. Relationship between the ouabain-sensitive sodium efflux per site (mole per liter of RBCs per hour) and the intracellular sodium concentration \([\text{Na}]_i\) in normotensive (A) and hypertensive (B) white men (\(\bullet\)), black men (\(\Delta\)), white women (\(\sigma\)), and black women (\(\circ\)). The equation for the line for the normotensive subjects (A) is \(y = 0.609x + 0.067, \ r = 0.88, \ p < 0.001\). The equation for the line for the hypertensive subjects (B) is \(y = 0.669x - 0.091, \ r = 0.82, \ p < 0.001\). The equations are not significantly different.

relationship between efflux/site and \([\text{Na}]_i\) can be expressed by the equation:

\[
\text{Na efflux/site} = k_2 [\text{Na}]_i
\]  

which is equivalent to Equation 3. (See Subjects and Methods.) Using the independently determined values of sodium efflux/RBC, \([\text{Na}]_i\), and sites/RBC, we have calculated \(k_2\) (a second-order rate constant) for the normotensive and hypertensive subjects. For normotensive subjects, the interpersonal variation in \(k_2\) is 15.1%, comparable to the variation in sodium efflux (16.1%), but smaller than the interpersonal variations in \([\text{Na}]_i\) (27.2%) or the sites/RBC (26.8%).

For RBCs from a single source loaded to varying intracellular sodium concentrations Garay and Garrahan\(^4\) have expressed the relationship between sodium efflux per cell and \([\text{Na}]_i\) by the following equation:

\[
\frac{\text{Na efflux/RBC}}{V_{\text{max}}(1 + K_{\text{Na}}[\text{Na}]_i)}
\]

where \(V_{\text{max}}\) is the maximum efflux, proportional to the number of ATPase sites/RBC, and \(K_{\text{Na}}\) is the apparent dissociation constant. If it is assumed that the proportionality constant relating \(V_{\text{max}}\) and the number of sites is the same for all normotensive subjects, \(K_{\text{Na}}\) can be calculated for each subject. Comparison of \(K_{\text{Na}}\) calculated from Equation 5 with \(k_2\) calculated from Equation 3 for each subject gives a very highly significant correlation (\(r = -0.92, \ n = 142, \ p < 0.001\)), suggesting that \(k_2\) and \(K_{\text{Na}}\) may be indicators of the same membrane property (i.e., \(k_2\) is an estimate of the association constant of the ATPase sites for sodium, while \(K_{\text{Na}}\) is an estimate of the dissociation constant).

Of the Na\(^+\)-K\(^+\) pump parameters, \(k_2\) differentiated best between hypertensive and normotensive subjects (\(p = 0.011\)). We have previously reported significantly elevated \(k_2\) values among women taking oral contraceptives\(^4^6\) and among patients with primary hyperaldosteronism, which remitted after correction of excessive aldosterone production and hypertension.\(^4^7\)

The hypertensive black women were distinctive in their values for several of the Na\(^+\)-K\(^+\) pump parameters. They had lower ouabain-insensitive sodium efflux than the normotensive black women, while other hypertensive subjects had higher values than their normotensive counterparts. The number of sites/RBC for hypertensive black women was lower than that of their normotensive counterparts, while all other hypertensive groups had a higher \(k_2\) value. This observation may have relevance to the increased sodium sensitivity of blood pressure demonstrated by this subgroup.\(^3\)

The inverse relationship between \([\text{Na}]_i\) and the number of sites/RBC (see Figure 4A and B) was similar for all groups of subjects and comparable to previously reported results for normotensive subjects.\(^4^6\) Thus, it is possible that the relationship between \([\text{Na}]_i\), and sites defines a compensatory mechanism that limits excessive sodium transport.

Detection of abnormalities in RBC sodium transport of hypertensive subjects is of relevance if similar abnormalities exist in other cells, such as those responsible for sodium reabsorption in the kidney or for the contraction of vascular smooth muscle cells. Although the hypothesis is still controversial,\(^4^6\) the fact that Na\(^+\)-Li\(^+\) countertransport has been reported to inversely correlate with urinary lithium clearance\(^4^9\) has led to the suggestion...
that it may be a marker of sodium reabsorption in the proximal tubule. Both cotransport and the Na\textsuperscript{+}-K\textsuperscript{+} pump influence sodium reabsorption in the distal tubular system of the kidney.\textsuperscript{50} The results of the present study showing increased activity for several parameters of sodium transport add further support to the notion that sodium transport abnormalities may be involved in the development of sodium-sensitive blood pressure elevation.\textsuperscript{51} Further elucidation of factors accounting for the differences in cation transport observed between normal and hypertensive subjects may provide new insights regarding the pathogenesis and treatment of essential hypertension. They may also provide a marker for the identification of persons at increased risk for the development of hypertension.

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