Erythrocyte Sodium Transport and Blood Pressure in White Subjects

Jean B. Smith, Mary B. Wade, Naomi S. Fineberg, and Myron H. Weinberger

The mechanisms that define the relation between blood pressure and sodium handling are not yet well understood. Although several abnormalities in sodium transport have been associated with hypertension, a link between the blood pressure of normotensive subjects and the erythrocyte sodium-potassium adenosine triphosphatase pump, the principal sodium transporter of sodium, has not been previously demonstrated. Data from independent measurements of erythrocyte intracellular sodium, ouabain-sensitive sodium efflux, and the number of sodium pump sites per red blood cell were used to calculate a second-order rate constant for ouabain-sensitive sodium efflux. Among 20 normotensive white subjects, this rate constant correlated significantly ($p<0.005$) with mean arterial blood pressure. A significant correlation was not observed between the rate constant and the blood pressure of 22 hypertensive subjects. A hypothesis is proposed, which suggests that the sodium efflux rate constant of erythrocytes is related to the control of sodium reabsorption via the sodium pump of the renal tubules and that an elevated erythrocyte rate constant may be associated with chronic increased sodium reabsorption, which leads to volume expansion and the development of hypertension. (Hypertension 1989;13:716–720)

Even though a relation between dietary sodium intake and blood pressure has long been recognized, an understanding of the mechanism by which sodium affects blood pressure is not yet clear. Abnormal sodium handling by renal tubular or vascular smooth muscle cells is most likely to influence blood pressure. However, because red blood cells are more accessible, many investigators have sought a link between sodium handling of erythrocytes and blood pressure. Numerous studies have reported abnormalities in erythrocyte sodium transport systems among hypertensive patients, but there has been considerable disagreement among investigators regarding abnormalities of intracellular sodium concentration and sodium pump activity. Racial factors have also been reported to influence several sodium transport parameters in normotensive subjects. The transport abnormality that has been most consistently observed in hypertensive subjects is an elevation in the sodium-lithium exchange system, measured in lithium-loaded red blood cells; however, the physiological significance of this transport is not yet clear. Because the major sodium transport mechanism responsible for intracellular sodium content is the sodium-potassium adenosine triphosphatase (ATPase)-mediated sodium pump, we have chosen to examine the relation between several parameters that affect sodium pump activity in red blood cells and blood pressure of hypertensive and normotensive white subjects.

We previously reported a significantly increased sodium efflux rate per erythrocyte among hypertensive subjects when compared with those with normal blood pressure. This increase was not associated with significant changes in either intracellular sodium concentration or the number of Na,K-ATPase sites per erythrocyte but was apparently due to an alteration in the rate constant for the reaction of Na,K-ATPase with Na+. A subsequent report focused on the effect of an acute saline load on the functioning of the sodium pump among white subjects. The hypertensive subjects in that study also demonstrated a higher sodium efflux per erythrocyte and an elevated rate constant; however, after the saline load, both sodium efflux and the rate constant were significantly decreased and no longer were significantly different from the values of normotensive subjects.

In the present report, we describe a relation between blood pressure and several parameters of
the sodium pump of normotensive white subjects and propose a hypothesis to explain these observations. Because there are significant differences between blacks and whites for many of the sodium transport parameters, we have included only white subjects in this report.

Subjects and Methods

Subjects

Twenty-two hypertensive subjects (13 men, nine women) and 20 normotensive subjects (seven men, 13 women) were in the study; all were white. Normotensive subjects were 38.0±13.0 years old, weighed 79.8±23.8 kg, and had a height of 168.3±11.3 cm; hypertensive subjects were 52.7±10.8 years old, weighed 79.5±14.4 kg, and had a height of 168.6±8.6 cm. Hypertension was defined as a diastolic blood pressure greater than 90 mm Hg and a systolic blood pressure greater than 140 mm Hg on repeated determinations or prescription of antihypertensive medication. All of the hypertensive subjects had a mild-to-moderate uncomplicated essential hypertension for whom withdrawal of antihypertensive medication was not deemed to be a significant risk and had been without medication for at least 18 days. Secondary forms of hypertension had been excluded by a comprehensive protocol. Among the 22 hypertensive subjects, six were classified as low-renin hypertensives.

Protocol

Subjects were admitted to the Clinical Research Center on the evening before the study. The patient was awakened at 6:00 AM and required to assume an upright posture (standing or walking) until 8:00 AM; then blood pressure was measured and blood was drawn for the red blood cell sodium pump evaluation and plasma renin activity (PRA). Mean arterial blood pressure for the normotensive subjects was 81.3±6.1 and for the hypertensive subjects was 101.4±11.8 mm Hg (p<0.001).

Analytical Methods

The red blood cells were separated from the plasma and buffy coat after centrifugation for 10 minutes at 1,000g, washed three times in a washing solution (150 mM choline chloride), and centrifuged after each wash for 10 minutes at 1,000g. Packed cells (2 ml) were separated and suspended in a buffer solution (140 mM NaCl, 30 mM HEPES [N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid], and 10 mM dextrose) for determination of the number of ouabain-binding sites; the remainder were washed three more times, and an approximately 50% suspension was prepared in the washing solution to be used for Na\textsubscript{i} determination and sodium efflux measurements. The hematocrit of the suspension was determined.

Details of the methods for determining the number of sites per red blood cell from \[\text{^3H}\]ouabain binding, the ouabain-sensitive sodium efflux, and Na\textsubscript{i} have been previously published.

Sodium efflux per erythrocyte into a solution with an established potassium concentration is a function of the Na\textsubscript{i} concentration, the number of ATPase sites per erythrocyte, and the affinity of the ATPase for sodium. For washed, unloaded erythrocytes the Na\textsubscript{i} concentration is 5-10 mM. At these concentrations, Na\textsubscript{i} is not sufficient to saturate the ATPase, and sodium efflux per red blood cell can be expressed by the general equation for second-order reactions; thus, sodium efflux per cell is dependent on both Na\textsubscript{i} and the number of ATPase sites per red blood cell.

\[\text{Na efflux} = K_2 \times \text{sites} \times \text{Na}_i \] (1)

The apparent second-order rate constant of this equation (K\textsubscript{2}) is related to the affinity constant of the Na-K-ATPase for Na\textsubscript{i} and can be determined from independent measurements of sodium efflux, the number of ATPase sites per erythrocyte, and Na\textsubscript{i} with washed unloaded red blood cells:

\[K_2 = \text{Na efflux/site}/\text{Na}_i \] (2)

PRA was measured with a GammaCoat \textsuperscript{125}I Radioimmunoassay Kit (Clinical Assays, Cambridge, Massachusetts).

Statistics

Data are given as mean±SD. Normotensive and hypertensive subjects were compared with a t test. Because the PRA data were skewed, a square root transformation was performed to normalize the distribution of the PRA values. Linear regression was used to describe the relations of blood pressure and PRA with sodium pump parameters.

Results

Mean values for the sodium pump parameters and the correlation of these parameters with blood pressure are given in Table 1. A highly significant (p<0.005) correlation between K\textsubscript{2} and blood pressure was evident for the normotensive subjects (r=0.64, Figure 1). The correlation (r=-0.01) for the hypertensive subjects alone was not significant.

In an analysis including all subjects, the PRA measured on blood drawn at 8:00 AM showed a significant negative correlation (r=-0.40) with K\textsubscript{2} and a significant positive correlation (r=0.30) with the number of \[^3H\]ouabain-binding sites per erythrocyte. The correlation between the sites and PRA was similar for hypertensive (r=0.39) and normotensive (r=0.37) subjects, but the correlation between the sites per red blood cell and PRA was due to the strength of the relation (r=0.51, p<0.02) among the normotensive subjects (Figure 2); the hypertensive subjects alone did not show a significant correlation (r=0.21).

Discussion

This study is the first to show a significant relation between blood pressure and a parameter of erythrocyte sodium efflux of normotensive white
TABLE 1. Sodium Pump Parameters and Correlation With Blood Pressure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensive subjects (n=20)</th>
<th>Hypertensive subjects (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na efflux (mmol/l RBC/hr)</td>
<td>1.46±0.26 (r=0.43)</td>
<td>1.65±0.21* (r=0.11)</td>
</tr>
<tr>
<td>Naj (mM)</td>
<td>5.75±1.41 (r=0.27)</td>
<td>6.32±1.12 (r=0.11)</td>
</tr>
<tr>
<td>Sites/RBC</td>
<td>369±98 (r=0.43)</td>
<td>331±55 (r=0.14)</td>
</tr>
<tr>
<td>$K_2$ (×10^4 sites/hr)</td>
<td>7.28±1.27 (r=0.64)†</td>
<td>7.99±0.90* (r=0.01)</td>
</tr>
</tbody>
</table>

Na efflux, ouabain-sensitive sodium efflux; Naj, intracellular sodium; site/RBC, sodium pump sites per red blood cell; $K_2$, second-order rate constant for sodium efflux.

*p<0.05 compared with normotensive subjects. †p<0.005.

subjects, strengthening the link between sodium transport and blood pressure at the cellular level. The parameter showing the significant correlation with blood pressure is $K_2$ for sodium efflux, and it is not accompanied by significant correlations between blood pressure and either Na$_i$ concentration or the number of Na,K-ATPase sites per erythrocyte. The correlation is much stronger in normotensive than in hypertensive subjects.

Previously, we have reported significant differences between blacks and whites for Na$_i$ and the number of Na,K-ATPase sites per erythrocyte as well as sodium-lithium countertransport and lithium-potassium cotransport. Although we did not find differences between blacks and whites for ouabain-sensitive sodium efflux or the rate constant for the sodium pump, the fact that so many sodium transport parameters differed in normotensive white and black subjects suggests that the blood pressure response of blacks and whites to sodium may also differ. We have previously reported that normotensive blacks have an impaired excretion of a saline load compared with normotensive whites similar to that found among hypertensive white subjects. Inclusion of normotensive subjects of both black and white races in the analyses may mask relations that are only observed in normotensive white subjects. Thus, in this study we have examined sodium transport parameters of white subjects, separated by blood pressure status.

A recent review by Hilton describes numerous studies in which differences have been noted in erythrocyte sodium handling between normotensive and hypertensive subjects when compared as separate groups; however, significant correlations between blood pressure and parameters of the sodium pump have been noted only in studies that include large numbers of subjects. Stokes et al found significant correlations between diastolic blood pressure and both $^{22}$Na efflux and $^{36}$Rb influx (a measure of sodium pump activity) for 137 hypertensive and normotensive white males. Rygielski et al reported significant correlations between erythrocyte Na$_i$ content and both systolic and diastolic blood pressures as well as between Na,K-ATPase activity and blood pressure for a population of 247 black and white hypertensive and normotensive subjects.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Plot of relation between mean arterial blood pressure and sodium pump rate constant ($K_2$) of white subjects. †, normotensive subjects; ○, hypertensive subjects. Equation for line, $y=3.12\times 10^4 x + 58.8$ ($r=0.64$, $p<0.005$), fits normotensive data only.
Previous studies of sodium efflux have used the equation derived by Garay and Garrahan\(^{20}\) to describe the relation between sodium efflux and Na,

\[
\text{Na efflux} = \frac{V_{\text{max}}}{1 + K_{\text{Na}}/\text{Na}_0} \tag{2}
\]

where \(V_{\text{max}}\) is the maximum sodium efflux measured at saturating concentrations of Na\(_0\), and \(K_{\text{Na}}\) is the apparent dissociation constant. This equation is valid for red blood cells from a single source loaded to varying Na\(_0\) and can be used to determine \(V_{\text{max}}\) and \(K_{\text{Na}}\) with blood pressure. Results from these studies have not shown correlations of \(V_{\text{max}}\) or \(K_{\text{Na}}\).

In a previous publication,\(^{11}\) the relation between Equation 2, which is valid for a wide range of Na\(_0\), and Equation 1, which is valid for Na\(_0\) up to 20 mM, was described; thus, \(K_2\) is inversely related to \(K_{\text{Na}}\).

In our study of 42 white subjects, significant correlations of blood pressure with sodium efflux and \(K_2\) were evident, which suggests that sodium efflux and \(K_2\) may be better indicators of the relation between sodium handling and blood pressure than the parameters previously studied. A possible reason that the data reported here have revealed a significant correlation between a parameter of sodium efflux and blood pressure whereas other studies have not may be that the experiments were performed on recently drawn, washed red blood cells that had experienced less manipulation, less alteration in intracellular ion concentrations, and a shorter time since blood drawing than occurs when loaded cells are used. Another factor that may have permitted significant correlations to be evident is that the blood pressure measurements were made in a standardized fashion after the subject had been hospitalized overnight.

The fact that there was a correlation between blood pressure and \(K_2\) among normotensive subjects but not hypertensive subjects suggests that this parameter might be a clue to a controlling mechanism that does not function properly in the hypertensive state. We, therefore, propose the following speculative hypothesis to explain the existence of this relation among normotensive subjects and its absence among hypertensive subjects. The distal tubule of the kidney is the area in which sodium reabsorption is primarily due to the sodium pump. We have assumed that the erythrocyte sodium pump reflects the function of the sodium pumps of the distal tubules. If this is true, increased sodium efflux would represent increased renal sodium reabsorption. Perhaps those subjects with an elevated \(K_2\) indicative of an increased sodium reabsorption are those who experience long-term increased sodium retention. Short-term variations appear to be handled by the inverse relation between Na\(_0\) and the number of sites per red blood cell. For example, our studies\(^{21}\) of the effect of digoxin administration showed that the number of ATPase sites was decreased and Na\(_0\) was increased but that the sodium efflux per red blood cell remained relatively constant. Others\(^{22}\) have shown that when digoxin is administered for a longer period, the number of sites and the Na\(_0\) concentration gradually return to pretreatment values.

We propose that elevated \(K_2\) is indicative of chronic increased sodium reabsorption and consequent volume expansion for which the Na\(_0\)\times sites variability does not adequately compensate. Under these conditions, elevated blood pressure develops. The resultant volume expansion may stimulate production of an as-yet-unidentified inhibitor of Na,K-ATPase activity. Thus, our observations and hypothesis are consistent with the theory of the pathogenesis of hypertension proposed by Blaustein\(^{23}\) and Haddy and Overbeck.\(^{24}\)

Our previous report,\(^{12}\) which shows that an acute saline load caused a decrease in sodium efflux and \(K_2\) among hypertensive but not among normotensive subjects, also supports this explanation since the saline load might be expected to cause the
production of a Na,K-ATPase inhibitor in the hypertensive subjects but not in normotensive subjects because normotensive subjects have appropriate compensatory responses to the saline load. Our hypothesis that an elevated \( K_e \) is associated with increased sodium reabsorption, volume expansion, and elevated blood pressure is further supported by studies of subjects with hyperaldosteronism.\(^2\) These subjects, who are considered to be a model for sodium-sensitive, volume-expanded hypertension, had significantly elevated \( K_e \) and ouabain-sensitive sodium efflux, both of which returned toward normal after removal of hyperaldosteronism.

In addition, we observed an inverse correlation between PRA and \( K_e \) \((r=-0.40, p<0.01)\) among all the subjects in the present study, again with the greatest strength of the correlation observed among the 20 normotensive subjects \((r=-0.53, p<0.025)\). If the increased \( K_e \) observed in the erythrocyte is paralleled by increased renal sodium reabsorption, the elevated sodium and volume could be expected to decrease PRA. This would be consistent with the present findings. Taken as a whole, these observations provide further support for the validity of studies of red blood cell sodium transport as an index of sodium handling by the kidney. Further studies of the interactions of sodium transport in red blood cells, renal tubules, and vascular smooth muscle in relation to blood pressure may provide new insights into the etiology of hypertension.

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References

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