Erythrocyte Ghost Na⁺,K⁺-ATPase and Blood Pressure

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SUMMARY To examine the relationship between body mass index, blood pressure, and the Na⁺,K⁺-adenosine triphosphatase (ATPase) system, we measured the erythrocyte ghost Na⁺,K⁺-ATPase and the erythrocyte Na⁺ concentration in 120 blacks and 127 whites (136 males and 111 females). Blacks showed a 13.9% higher erythrocyte Na⁺ (7.63 ± 0.19 vs 6.70 ± 0.11 [SEM] mEq/L; p = 0.0001) and a 16.1% lower erythrocyte ghost Na⁺,K⁺-ATPase activity (140.3 ± 4.2 vs 167.3 ± 4.7 nmol inorganic phosphate/mg protein/hr; p = 0.0002) than whites. Male subjects demonstrated a 6.4% higher erythrocyte Na⁺ (7.35 ± 0.17 vs 6.91 ± 0.14 mEq/L; p = 0.043) and an 11.5% lower Na⁺,K⁺-ATPase activity (145.7 ± 3.7 vs 164.7 ± 5.5 nmol inorganic phosphate/mg protein/hr; p = 0.0015) than female subjects. Significant (p < 0.001) negative correlations were identified for the systolic, diastolic, and mean blood pressure levels and the erythrocyte ghost Na⁺,K⁺-ATPase. These findings were complemented by positive correlations for the blood pressure levels and erythrocyte Na⁺ concentrations. The body mass index was negatively correlated with erythrocyte ghost Na⁺,K⁺-ATPase and it accounted for 6.7%, 5.6%, and 6.1% of the variabilities in the systolic, diastolic, and mean blood pressure levels, respectively. Variabilities of 1.4% systolic, 12.3% diastolic, and 11.1% in mean arterial pressure were attributable to the erythrocyte ghost Na⁺,K⁺-ATPase activity. Provided that findings in erythrocytes also reflect the relative status of the vascular smooth muscle cell Na⁺,K⁺-ATPase, the predisposition of black, male, and obese persons to hypertension may relate, among other factors, to a lower activity of this enzyme system, which results in an increased vascular tone. (Hypertension 10: 259-266, 1987)

KEY WORDS • Na⁺,K⁺-adenosine triphosphatase • Na⁺-K⁺ pump • blacks • whites • hypertension

The positive correlation between systemic blood pressure and body mass index (BMI) is well established; however, the relationship between these parameters and the red blood cell (RBC) Na⁺,K⁺-adenosine triphosphatase (ATPase) system has been a matter of a substantial controversy. Most studies that have addressed this issue attempted to demonstrate the existence in essential hypertension (for a review see References 1–3) and obesity4–6 of abnormalities in erythrocyte Na⁺ and the Na⁺,K⁺-ATPase system. Such studies have usually examined a relatively small number of subjects. Furthermore, by comparing hypertensive with normotensive or obese with nonobese subjects, these studies have treated the BMI and blood pressure level as discontinuous entities rather than as variables with continuous distributions. In the present report we describe the relationships among the erythrocyte ghost (EG) Na⁺,K⁺-ATPase, RBC Na⁺, BMI, blood pressure, as well as other related parameters in a large group of men and women who participated in this investigation without being categorized a priori as hypertensive, normotensive, obese, or nonobese.

Subjects and Methods

A total of 247 subjects without known metabolic diseases were studied. Two of these were adolescent (a 15-year-old white boy and a 16-year-old black girl); the rest were adults, 19 to 68 years of age. Subjects
were recruited from the student body, faculty, resident staff, and medical staff of the University of Medicine and Dentistry of New Jersey. The male/female composition of the white group was 73:54, whereas that of the black group was 63:57. Of the 127 whites participating in this study, 26 (18 males and 8 females) had both parents of Jewish extraction. In addition, 3 white males had one Jewish parent. Subjects were excluded from the study if they were taking diuretics, antihypertensive or thyroid medications, oral contraceptives, or estrogen. No pregnant women were studied. Subjects identified as having blood pressure values higher than 140 mm Hg systolic, or 90 mm Hg diastolic (or both) were referred for a further follow-up in the hypertension clinic or by a member of the research team. Blood pressure measurements and blood sampling were performed as described previously. All subjects, including parents of the two adolescents, signed an informed consent approved by the Research Committee of the University of Medicine and Dentistry of New Jersey.

The assay method of EG Na⁺,K⁺-ATPase in this investigation was different from that described in our previous work, and it may account for the differences in the values of the activity of the enzyme between the two studies. However, the results of both investigations are in agreement. The change in methodology was dictated by the need to accommodate the large number of samples. Preparations of EGs for the Na⁺,K⁺-ATPase assay and measurements of RBC Na⁺ and K⁺ concentrations were performed as previously described. The Na⁺,K⁺-ATPase assay in this work was based on the ouabain-sensitive hydrolysis of radiolabeled [γ-³²P]adenosine 5'-triphosphate (ATP) and the chromatographic separation of adenosine 5'-diphosphate (ADP) and inorganic phosphate (P) from ATP. In the previous work the P, in the assay medium was measured using the Fiske and Subbarow method. In addition, in the present work the activity of EG Na⁺,K⁺-ATPase was assayed only at its apparent, maximal initial reaction velocity (Vₐₚ). RBC ghosts were added at a protein concentration of 100 to 200 μg/ml to 12 x 75-mm test tubes containing NaCl, 40 mM; KCl, 10 mM; MgCl₂, 5 mM; EGTA, 1 mM; imidazole, 50 mM; pH, 7.4; with or without 0.1 M ouabain. Tubes were preincubated for 10 minutes at 37°C, and the reaction was started by the addition of ATP containing tracer amounts of radiolabeled ATP (final concentration, 3 mM; final volume, 1 ml). All assays were performed in triplicate, and values were corrected for nonenzymatic hydrolysis. Ouabain-sensitive ATP hydrolysis was linear for at least 60 minutes of incubation. At 60 minutes, poly(ethyleneimine)cellulose strips (EM Reagents, Merck, Darmstadt, Germany) were spotted with 2 to 5 μl of reaction mixture and allowed to air dry (approximately 10 minutes). Separation of ADP and P, from ATP was achieved in 30 minutes in a solvent system containing 0.5 M LiCl/1.5 M formic acid. In this solvent system ATP migrates with a relative mobility (R) of 0.4 whereas P, comigrates with ADP with an R of 0.8. Visualization of the spots is achieved by a short-wave ultraviolet illumination under which the ATP and ADP spots appear dark. Spots were cut out, placed in scintillation vials, and counted by Cerenkov radiation. Verification of the migration pattern for the isotope was made by autoradiography. Fractional hydrolysis was obtained by dividing the counts in the P₂-ADP spot by total counts (ATP counts plus P₂-ADP counts). The fractional hydrolysis multiplied by the amount of ATP in the reaction vessel equals P, liberated. Results are expressed as nanomoles of P, per milligram of membrane protein per hour. The intra-assay coefficient of variation was 9.38%. The relationship between EG Na⁺,K⁺-ATPase activity and RBC Na⁺ is depicted in Figure 1. A highly significant negative correlation exists between the two variables.

Data were analyzed using multiple regressions and a two-way analysis of variance (ANOVA) with further comparisons (unless otherwise indicated) using the Duncan's multiple range test with a two-tailed level of significance.

**Results**

**Effect of Age, Gender, Race, and Body Mass Index on Blood Pressure**

Higher blood pressure levels were observed in blacks and males as compared with whites and females (Table 1). The blood pressure levels were increased with age (p<0.001, r = 0.210 for systolic blood pressure; p<0.005, r = 0.191 for diastolic blood pressure; and p<0.001, r = 0.218 for mean blood pressure). The BMI, defined by the Quetelet index (expressed as kilograms of body weight per height squared in meters) was also increased with age (p<0.025, r = 0.146). In addition, the blood pressure levels were positively cor-
related with the BMI (Figure 2). These findings have been documented by multiple studies that examined the epidemiology of essential hypertension (for a review see References 8–10).

### Effect of Race, Gender, and Ethnic Extraction on Cellular and Extracellular Na⁺ and K⁺, and Erythrocyte Ghost Na⁺,K⁺-ATPase Activity

Blacks demonstrated a 13.9% higher RBC Na⁺ than whites, and males showed a 6.4% higher RBC Na⁺ than females (see Table 1). Differences in RBC Na⁺ were highly significant ($p = 0.0001$) with respect to race and less significant ($p = 0.043$) with respect to gender. Blacks had a 16.1% lower EG Na⁺,K⁺-ATPase activity ($p = 0.0002$) than whites, and males manifested an 11.5% lower activity of the enzyme than females ($p = 0.0015$). Males with two Jewish parents had an 11.9% higher RBC Na⁺ concentration (7.51 ± 0.36 mEq/L) than other white males (6.71 ± 0.15 mEq/L; $p < 0.025$ by a 2-tailed Student’s $t$ test). The RBC K⁺ concentration was lower in the former group (72.26 ± 0.36 mEq/L) than other white males (67.1 ± 0.15 mEq/L; $p < 0.025$ by a 2-tailed Student’s $t$ test). The RBC K⁺ concentration was lower in males than in females. The biological importance of the third observation is uncertain as the difference was quite small.

### Relationships Among Blood Pressure, Body Mass Index, Erythrocyte Ghost Na⁺,K⁺-ATPase Activity, and RBC Na⁺ and Plasma K⁺ Concentrations

There were highly significant ($p < 0.001$) negative correlations between the systolic, diastolic, and mean blood pressure levels, and EG Na⁺,K⁺-ATPase activity (Figure 3). Complementing these findings, the blood pressure parameters were positively correlated with the RBC Na⁺ concentration (Figure 4). The BMI was negatively correlated with the activity of the enzyme (Figure 5) and positively correlated with the RBC Na⁺ ($p < 0.025$, $r = 0.162$). In addition, the plasma K⁺ was negatively correlated with the diastolic and mean blood pressures but not with the systolic blood pressure (Figure 6).

Most (22) subjects with a Quetelet index that was equal to or greater than 30 were black. Thus, data were also analyzed after exclusion of these subjects. The results were not substantially changed by this exclusion. For instance, correlations of the Quetelet index and the systolic, diastolic, and mean blood pressure levels continued to be significant after exclusion of these subjects ($p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively; see Figure 2). The significance persisted after exclusion of these subjects from the correlation of EG Na⁺,K⁺-ATPase and RBC Na⁺ ($p < 0.025$, $r = 0.162$). Furthermore, we also analyzed the data excluding 16 subjects whose RBC Na⁺ was equal to or higher than 10 mEq/L, as this subgroup also consisted primarily of blacks (see Figures 1 and 4). After this exclusion, the correlation of EG Na⁺, K⁺-ATPase and RBC Na⁺ was maintained at a $p$ level less than 0.01. Additionally, the significance of the
correlations between RBC Na\(^+\) and systolic, diastolic, and mean blood pressures remained significant \((p<0.05, p<0.02, \text{ and } p<0.02, \text{ respectively})\). Taken together, these findings indicate that the relationships of the RBC Na\(^+\) with Na\(^+\), K\(^+\)-ATPase and blood pressure exist throughout a broad range of BMI and RBC Na\(^+\) and that they are not mere reflections of racial extraction and extreme variations in the BMI.

Relative Contribution of the Tested Parameters to Variabilities in Blood Pressure Values

To examine the contribution of all the measured parameters on blood pressure, we used a stepwise multiple regression analysis that enters variables in the order of their relative contribution to variation in the dependent variable. This procedure generates a \(r^2\) value referred to as the "coefficient of determination." This value indicates, as a percentage, the contribution of a particular parameter to the variance in the dependent variable. The blood pressure levels were arbitrarily defined as dependent variables. The results of this analysis are presented in Table 2. The BMI apparently contributed more to the variability of the systolic blood pressure than the EG Na\(^+\), K\(^+\)-ATPase did, whereas the activity of the enzyme was a more prominent determinant of the variability in the diastolic and mean arterial blood pressure levels. In addition, age contributed more to the variability in the systolic than in the diastolic blood pressure, a phenomenon that is well established. Finally, the extracellular K\(^+\) also appeared to influence both the diastolic and mean arterial blood pressures. The extracellular K\(^+\) may, indeed, be an important regulator of the Na\(^+\)-K\(^+\) pump. The stepwise linear regression analyses did not demonstrate that RBC Na\(^+\) was one of the major determinants of the blood pressure despite the statistically signifi-
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100
90
80
70
60
50

FIGURE 4. Relationships between RBC Na\(^+\) (mEq/L cells) and blood pressure levels (mm Hg) in black females ( ), black males ( ), white females ( ), and white males ( ). The slopes of the correlations indicate that an increase of 1 mEq/L in RBC Na\(^+\) was associated with elevations of 1.19, 1.17, and 1.18 mm Hg in the systolic, diastolic, and mean arterial pressures, respectively.

cant correlations between the intracellular Na\(^+\) concentrations and the blood pressure values when these analyses were performed separately. The reason for this phenomenon is probably the colinearity of the EG Na\(^+\),K\(^+\)-ATPase activity and the RBC Na\(^+\). 

Discussion

Racial and sex-related differences in the RBC Na\(^+\) concentrations have been demonstrated previously.\(^3,12-17\) Although these differences are relatively small, they reflect substantial alterations in the activity of the Na\(^+-\)K\(^+\) pump, assuming that the affinity of the pump to intracellular Na\(^+\) is not different between blacks, whites, men, and women. Small changes in cellular Na\(^+\) concentration near physiological levels have a profound effect on the activation of Na\(^+\),K\(^+\)-ATPase in RBCs\(^7\) and vascular smooth muscle cells,\(^18\) as the slope of the Na\(^+\) activation curve of the enzyme at these concentrations is quite steep. Thus, a higher RBC Na\(^+\) concentration is likely to represent a substantial reduction in Na\(^+\)-K\(^+\) pump activity if a major determinant for this elevation is a lower activity of this transport system. However, if higher RBC Na\(^+\) concentrations in blacks and men relate to factors other than the Na\(^+\)-K\(^+\)-ATPase system, one would expect the Na\(^+\)-K\(^+\) pump activity to be accelerated. In a previous investigation we showed that the lower activity of EG Na\(^+\),K\(^+\)-ATPase in blacks and men is associated with a relatively lower number of RBC Na\(^+\)-K\(^+\) pump units.\(^12\) This finding suggests that higher RBC Na\(^+\) in blacks and men could be the result of lower Na\(^+\)-K\(^+\) pump activity. Additionally, the observation that RBC K\(^+\) is lower in men than in women fits well with the reduced activity of Na\(^+\),K\(^+\)-ATPase. However, statistically significant differences in cellular K\(^+\) concentrations were not demonstrated between blacks and whites, suggesting that, in addition to racial differences in the Na\(^+-\)K\(^+\) pump, other factors are likely to contribute to the discrepancies in RBC Na\(^+\) and K\(^+\) regulation between the two groups. For instance, recent studies indicate that the RBC Na\(^+\)-K\(^+\) cotransport system behaves differently in hypertensive and normotensive blacks as compared with whites.\(^19,20\)

It has been suggested that K\(^+\) intake correlates negatively with blood pressure levels\(^21-23\); one of these studies showed that higher K\(^+\) intake is associated with a higher plasma K\(^+\) concentration.\(^22\) Our findings of negative correlations between plasma K\(^+\) and diastolic and mean blood pressure support the concept that variations in K\(^+\) intake may exert their effect through alterations in plasma K\(^+\) even within narrow physiological boundaries.
number of Na⁺-K⁺ pump units, could reduce the activity of the Na⁺-K⁺ pump in erythrocytes of blacks.

There is a lack of unanimity regarding the relationship between the BMI and the activity of the RBC Na⁺,K⁺-ATPase. Some studies suggest that such as association exists, while others do not support this concept. Relationships among the BMI, systemic blood pressure, RBC Na⁺, and Na⁺,K⁺-ATPase activity are likely to be established only when large numbers of subjects are investigated, as the activity of the Na⁺-K⁺ pump and cellular Na⁺ concentration depend on multiple factors in addition to racial origin or gender. For instance, Beutler et al. studied the number of ouabain binding sites and ouabain-sensitive ATP hydrolysis in RBCs of 26 obese, 17 formerly obese, and 60 normal subjects; of these, 31 subjects were Jewish and 9 were black. These investigators concluded that differences in Na⁺,K⁺-ATPase activity may relate to ethnic origin rather than to obesity per se, as both Jews and blacks manifested a lower enzyme activity than did non-Jewish whites. Findings of the present study appear to support the lower activity of RBC Na⁺,K⁺-ATPase in Jews (i.e., our observations of higher intracellular Na⁺ concentration, lower intracellular K⁺, and lower EG Na⁺,K⁺-ATPase activity in Jewish males). The last parameter, however, did not reach statistical significance, probably because of the relatively small number of Jewish males. No statistical analyses were attempted with respect to Jewish and white females as the number of subjects in the former group was limited. Most studies attempting to relate blood pressure levels and RBC Na⁺ exhibit deficiencies similar to those addressing the issue of obesity, that is, they have examined relatively small samples divided into discontinuous categories of hypertensive and normotensive subjects. For instance, Munro-Faure et al. and Weller and Walter and Distler did not identify differences in RBC Na⁺ between hypertensive and normotensive subjects, whereas D'Armico and Losse et al. demonstrated higher RBC Na⁺ values in the former group.

We and others have found a lower urinary excretion of K⁺ in blacks. Thus, the additional finding of significantly lower plasma K⁺ concentration in blacks as compared with whites may have some physiological implication. Although K⁺ concentration in the plasma is close to the Vₐₐₑₑ for activation of Na⁺,K⁺-ATPase, a lower extracellular K⁺ level, coupled with a lower plasma K⁺ concentration, lower intracellular K⁺, and lower EG Na⁺,K⁺-ATPase activity in Jewish males. No statistical analyses were attempted with respect to Jewish and white females as the number of subjects in the former group was limited. Most studies attempting to relate blood pressure levels and RBC Na⁺ exhibit deficiencies similar to those addressing the issue of obesity, that is, they have examined relatively small samples divided into discontinuous categories of hypertensive and normotensive subjects. For instance, Munro-Faure et al. and Weller and Walter and Distler did not identify differences in RBC Na⁺ between hypertensive and normotensive subjects, whereas D'Armico and Losse et al. demonstrated higher RBC Na⁺ values in the former group.

**Figure 6.** Relationships between plasma K⁺ (mEq/L) and blood pressure levels (mm Hg) in black females (●), black males (●), white females (○), and white males (○). An increase of 1 mEq/L in plasma K⁺ concentration was associated with a decline of 4.93 and 4.27 mm Hg in the diastolic and mean arterial blood pressures, respectively.

**Table 2.** Results of Stepwise Multiple Regression Analysis

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable (in order of addition)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>QI, Sex, Age, Race, Na⁺,K⁺-ATPase</td>
<td>22.20</td>
</tr>
<tr>
<td>r² (% )</td>
<td>6.66*</td>
<td>5.81†</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>QI, Na⁺,K⁺-ATPase, Age, Sex, Race, Plasma K⁺, Race, Na⁺,K⁺-ATPase</td>
<td>28.26</td>
</tr>
<tr>
<td>r² (%)</td>
<td>12.34†</td>
<td>5.63§</td>
</tr>
<tr>
<td>MAP</td>
<td>QI, Sex, Race, Age, Plasma K⁺</td>
<td>30.96</td>
</tr>
<tr>
<td>r² (%)</td>
<td>11.08†</td>
<td>6.10†</td>
</tr>
</tbody>
</table>

QI = Quetelet index; BP = blood pressure; MAP = mean arterial pressure; r² = coefficient of determination.

*p<0.02, †p<0.001, §p<0.05, ‡p<0.01, ¶p<0.002.
However, studies using large groups of subjects have demonstrated a highly significant increase in RBC Na\(^+\) of hypertensive subjects. Wessels and Zunkley\(^{36}\) reported this finding in a group consisting of 319 normotensive and 295 essential hypertensive subjects whose racial extraction was not indicated. Aderounmo and Salako\(^{37}\) reported the same observation in a Nigerian study consisting of 100 essential hypertensive and 908 normotensive subjects. When pooled data of the majority of investigations in blacks and whites were reviewed and analyzed by Hilton,\(^{2}\) it became quite evident that hypertensive subjects manifest a higher RBC Na\(^+\) than normotensive subjects.

Finally, although correlations of RBC Na\(^+\), Na\(^+\), K\(^+\)-ATPase with the systemic blood pressure do not necessarily imply a cause-and-effect relationship, they strongly suggest that these variables play an important role in blood pressure control. A lower activity of the Na\(^+\)-K\(^+\) pump and a higher intracellular Na\(^+\) concentration, if they are also present in the vascular smooth muscle cell, can produce an increase in the vascular tone by activating voltage-sensitive Ca\(^{2+}\) channels or by reducing the activity of the Na\(^+\)-Ca\(^{2+}\) exchange.\(^{38}\) The latter transport system has recently been shown to exist in the vascular smooth muscle cell,\(^{39}\) although its contribution to the overall Ca\(^{2+}\) metabolism in this cell is uncertain.

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References

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