SUMMARY

In a previous study, we demonstrated that the red blood cell Na⁺ concentration and Na⁺,K⁺-ATPase activity are sex-dependent and race-dependent: a higher intracellular Na⁺ concentration in blacks and men was associated with a lower Na⁺,K⁺-ATPase activity. To examine whether the low Na⁺,K⁺-ATPase activity is due to a decreased number of enzyme units, altered structure of the enzyme, or the presence of an endogenous digoxinlike substance, ouabain binding studies were performed on the same subject group. The measurements included displacement of [³H]ouabain from its specific binding sites by unlabeled ouabain or potassium. The results demonstrate that groups with lower enzyme activity manifest lower numbers of total specific ouabain binding sites on the surface of the red blood cell (mean ± SD: blacks, 654 ± 24.4; whites, 806 ± 18.3; women, 806 ± 26.9; men, 728 ± 21.2). Other kinetic parameters of [³H]ouabain displacement appear to be the same among the groups. The respective red blood cell Na⁺ and K⁺ concentrations were negatively and positively correlated with the number of ouabain binding sites. Our findings suggest that the lower activity of red blood cell Na⁺,K⁺-ATPase in blacks and men is a function of a lower number of Na⁺-K⁺ pump units. The results also indicate that sex and race should be considered when red blood cell ouabain binding is examined. (Hypertension 8: 1050-1057, 1986)

KEY WORDS • sodium • potassium • Na⁺,K⁺-ATPase • blacks • whites

INTRACELLULAR Na⁺ appears to be a regulatory factor in the contraction of vascular smooth muscle cells. Despite some controversy (for review, see Reference 4), a substantial number of reports have demonstrated higher intracellular Na⁺ levels in human essential hypertension and genetic hypertension in rats (for review, see Reference 11). Norotensive offspring of patients with essential hypertension, a group predisposed to high blood pressure later in life, also manifest a higher Na⁺ concentration in their red and white blood cells. Blacks and men have a higher incidence of essential hypertension than whites and women. In a previous study, we demonstrated that red blood cells (RBCs) of blacks and men have a higher intracellular Na⁺ concentration than that of RBCs of whites and women. A lower Na⁺,K⁺-ATPase activity appeared to be at least partly responsible for the differences. The lower activity of the enzyme may reflect intrinsic, qualitative or quantitative differences in the Na⁺,K⁺-ATPase system or the presence of plasma modulators. Analysis of ouabain binding to its specific cellular receptors can be used to determine the number of Na⁺,K⁺-ATPase units. It also provides information about differences in enzyme structure and the presence of certain humoral modulators. The main purpose of the present investigation was to delineate, by analysis of ouabain binding, the underlying mechanism for the lower Na⁺,K⁺-ATPase activity in RBCs of blacks and men as compared with whites and women.

Subjects and Methods

This study was initiated 6 months after the completion of an investigation devoted to studying racial and sex-related differences in RBC Na⁺,K⁺-ATPase. The subjects recruited were the same as those in our previous study with the exception of one black woman.
and one black man who could not be recalled. The remaining 8 black women, 8 black men, 9 white women, and 10 white men were members of the faculty, student body, and resident and nursing staffs of the University of Medicine and Dentistry of New Jersey. The subjects had no history of hypertension, renal disease, hemolytic or sickle cell anemia, or thyroid or neurological disease. No dietary restrictions were applied. No significant weight difference or changes in the subjects’ dietary habits were noted as compared with the initial study. The subjects were not taking diuretics, antihypertensive or thyroid medications, oral contraceptives, or estrogens. Pregnancy, obesity (Quetelet index [lb/in² × 100] greater than 5), systolic blood pressure (BP) greater than 140 mm Hg, or diastolic BP exceeding 90 mm Hg excluded potential subjects from the study. Subjects’ BP was the average taken in both arms in a sitting position, using the Korotkoff first and fifth components.

Blood was drawn the morning after an overnight fast, while the subjects were in a sitting position after they had ambulated for 1 hour. For plasma renin activity (PRA) assay, the blood was taken into ethylenediaminetetraacetic acid, while heparin (20 U/ml) was the anticoagulant for all the other determinations. Measurements of RBC Na⁺ and K⁺ were performed as previously described. The Na⁺ and K⁺ concentrations were determined by flame photometry. Creatinine was determined by a modified picrate method, and PRA was measured with the Squibb radioimmunoassay kit (Princeton, NJ, USA).

The ouabain binding studies were conducted as follows. First, 8 ml of blood was centrifuged at 270 g for 10 minutes, and the platelet-rich plasma was aspirated. The RBC pellet was suspended in a 10 mM Na⁺ phosphate buffer solution (1 mM MgCl₂, 150 mM NaCl, pH 7.42) and centrifuged at 1100 g for 10 minutes. The supernatant was discarded, and the washing procedure was repeated two more times. The cells were resuspended in the Na⁺ phosphate buffer solution at a 20 to 25% hematocrit. Aliquots of 0.5 ml of this cell suspension were added to tubes that contained 8.34 × 10⁻⁸ M [³H]ouabain (27–33 Ci/mmol; Amersham, Arlington Heights, IL, USA) and various concentrations of unlabeled ouabain. Preliminary studies, it was demonstrated that the steady state for ouabain binding was achieved within 60 minutes of incubation at 37°C. In subsequent experiments, the test tubes were incubated with gentle agitation for 2 hours in a water bath. At the end of this period, 100-μl aliquots of the cell suspension were removed and added to 2 ml of ice-cold phosphate buffer solution. Cells were centrifuged at 3000 g for 2 minutes, and the supernatant was discarded. Two additional washings followed in 2 ml of buffer solution (the third washing solution was free of any radioactivity), and 0.2 ml of 10% perchloric acid was added to the cell pellet to extract the bound, labeled ouabain. Following centrifugation, the supernatant was placed into 10-ml scintillation fluid (Hydroflour). To ensure complete removal of bound ouabain, the extraction procedure was repeated two more times. The efficiency of the radioactive counting was 22%. Cell number was determined by a Coulter counter (Coulter Electronics, Hialeah, FL, USA).

The derivation of the ouabain binding parameters is described in detail in our previous publication. The binding parameters depend on the radiochemical specifications of the labeled ligand (specific activity and radioactive concentration). We, therefore, checked the accuracy of the manufacturer’s specifications. Briefly, the displacement of the labeled ouabain from its specific binding sites by unlabeled ouabain can be expressed as:

\[
\frac{1}{B} = \frac{K_d + L}{B_m + L} \times \frac{L}{B_m \times L} \times c
\]

where \( B = \) specific ouabain, \( B_m = \) maximum specific ouabain binding, \( K_d = \) equilibrium dissociation constant for ouabain binding, \( L = \) concentration of the labeled ouabain (kept constant), and \( c = \) concentration of the unlabeled ouabain (variable). Therefore:

\[
B_m = \frac{1}{b \times L}
\]

\[
K_d = \frac{a}{b} - L
\]

where \( a = \) the y axis intercept of the straight line that is described by Equation 1 and \( b = \) the slope of the straight line. Two straight lines can be obtained by keeping \( L \) constant at two different concentrations and by varying \( c \). The \( x \) coordinate of the intercept of the two straight lines with each other corresponds to \((-)K_d\). This \( K_d \) value is independent of the manufacturer’s specifications, as is the \( a/b \) ratio in Equation 3. Thus, by substituting the real \( K_d \) value in Equation 3, the real \( L \) value can be calculated. We checked two different batches of the Amersham [³H]ouabain preparation (Batches 20 and 21) and found \( L \) values 80 and 51% higher than those calculated on the basis of the manufacturer’s specifications (\( L = \) radioactive concentration/specific activity). The two curves, the intercept of which was used for testing Batch 21 (specific activity = 27 Ci/mmol), are presented in Figure 1. In all further calculations, we used the corrected \( L \) values. Discrepancies between the concentration of labeled ouabain and that claimed by the supplier have also been reported by Akera and Cheng.

Statistical evaluations consisted of weighted linear regression analysis of the pooled, unlabeled ouabain displacement curves. Two-parameter nonlinear regression analysis was used for the \( K^+ \) displacement curves:

\[
B = B_m \times \frac{L}{K_a \left(1 + \left(\frac{L}{K_a}\right)^n\right) + L}
\]

where \( i = K^+ \) concentration, \( K_a = \) apparent equilibrium dissociation constant for \( K^+ \), and \( n = \) Hill coefficient analogue. The values of \( B_m \) and \( K_a \) were obtained from the unlabeled ouabain displacement studies and substituted into Equation 4. One-way analysis of vari-
Figure 1. Dixon transformation of [3H]-ouabain displacement from human red blood cells by unlabeled ouabain. Each point represents the mean of two observations. 1/B = reciprocal of specific [3H]ouabain binding. [3H]-ouabain concentrations (based on specifications by the manufacturer): L1 = 3.6 x 10^{-8} M; L2 = 1.2 x 10^{-7} M. The real Kd derived from the intercept of the two straight lines is 2.97 x 10^{-8} M. The Kd calculated from the straight line of L1 according to Equation 3 in Methods is 4.57 x 10^{-7} M, while the Kd calculated from the straight line of L2 is 1.0 x 10^{-7} M. The L-values calculated from the real Kd using Equation 3 in Methods are 5.3 x 10^{-8} M for L1 and 1.9 x 10^{-7} M for L2.

Results

Age, systolic and diastolic BP values, Quetelet index, and plasma Na+, K+, and creatinine concentrations are presented in Table 1. The white female group manifested a relatively low systolic and diastolic BP. Plasma creatinine concentration, as expected, was lower in women than in men. The RBC Na+ and K+ concentrations and PRA values are presented in Table 2. Duncan’s test revealed that black men had the highest RBC Na+ concentration and Na+/K+ ratio. The interaction test disclosed strong race and sex interrelations with the RBC Na+ concentration and the RBC Na+/K+ ratio. The latter values are not shown in Table 2 (p = 0.016 and p = 0.032 for race and sex, respectively). Blacks and men exhibited higher values for

Table 1. General Clinical and Laboratory Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>Quetelet index (lb/in² x 100)</th>
<th>[Na⁺] (mEq/L)</th>
<th>[K⁺] (mEq/L)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>32.6±7.1</td>
<td>113.0±6.0</td>
<td>74.0±5.8</td>
<td>3.29±0.49</td>
<td>139.8±1.4</td>
<td>4.0±0.2</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td>Men</td>
<td>28.1±1.4</td>
<td>118.6±9.9</td>
<td>78.5±5.8</td>
<td>3.42±0.39</td>
<td>141.1±2.2</td>
<td>4.2±0.4</td>
<td>1.08±0.16</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>31.0±3.4</td>
<td>108.9±7.8</td>
<td>70.1±3.5</td>
<td>3.17±0.25</td>
<td>140.6±1.5</td>
<td>4.2±0.3</td>
<td>0.83±0.15</td>
</tr>
<tr>
<td>Men</td>
<td>29.6±3.6</td>
<td>120.1±8.7</td>
<td>75.7±4.9</td>
<td>3.41±0.30</td>
<td>140.6±2.0</td>
<td>4.2±0.5</td>
<td>1.05±0.12</td>
</tr>
<tr>
<td>All blacks</td>
<td>30.3±5.5</td>
<td>115.8±8.4</td>
<td>76.3±6.0</td>
<td>3.35±0.43</td>
<td>140.4±1.9</td>
<td>4.1±0.3</td>
<td>1.02±0.16</td>
</tr>
<tr>
<td>All whites</td>
<td>30.3±3.5</td>
<td>114.8±9.9</td>
<td>73.4±4.8</td>
<td>3.30±0.30</td>
<td>140.6±1.7</td>
<td>4.2±0.4</td>
<td>0.95±0.17</td>
</tr>
<tr>
<td>All women</td>
<td>31.8±5.4</td>
<td>110.8±7.1</td>
<td>72.4±4.8</td>
<td>3.23±0.38</td>
<td>140.1±1.5</td>
<td>4.1±0.2</td>
<td>0.88±0.14</td>
</tr>
<tr>
<td>All men</td>
<td>28.9±2.9</td>
<td>119.4±9.0</td>
<td>76.9±5.3</td>
<td>3.41±0.33</td>
<td>140.8±2.0</td>
<td>4.2±0.4</td>
<td>1.06±0.14</td>
</tr>
</tbody>
</table>

Values are means ± SD. SBP = systolic blood pressure; DBP = diastolic blood pressure. 
*p < 0.05 (Duncan’s test) vs white men. †vs black men. ‡vs white women. 
§p = 0.005, |p| = 0.01, |p| = 0.001 (interaction test), vs all women.
RACE- AND SEX-DEPENDENT [3H]OUABAIN BINDING/Hopp et al.

TABLE 2. Blood Chemistry Values

<table>
<thead>
<tr>
<th>Group</th>
<th>[Na+] (mEq/L)</th>
<th>[K+] (mEq/L)</th>
<th>PRA (ng ANG I/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>7.45 ±1.78</td>
<td>88.2 ±9.3</td>
<td>1.59 ±1.57</td>
</tr>
<tr>
<td>Men</td>
<td>9.76 ±2.59*</td>
<td>88.2 ±7.0</td>
<td>2.55 ±1.57</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>6.42 ±1.29</td>
<td>91.2 ±17.1</td>
<td>2.79 ±1.74</td>
</tr>
<tr>
<td>Men</td>
<td>7.66 ±1.48</td>
<td>93.4 ±5.6</td>
<td>3.37 ±1.58</td>
</tr>
<tr>
<td>All blacks</td>
<td>8.60 ±2.6†</td>
<td>88.2 ±7.9</td>
<td>2.07 ±1.94</td>
</tr>
<tr>
<td>All whites</td>
<td>7.07 ±1.49</td>
<td>92.3 ±12.1</td>
<td>3.10 ±1.64‡</td>
</tr>
<tr>
<td>All women</td>
<td>6.90 ±1.58</td>
<td>89.8 ±13.6</td>
<td>2.23 ±1.73</td>
</tr>
<tr>
<td>All men</td>
<td>8.59 ±2.25§</td>
<td>91.1 ±6.6</td>
<td>3.00 ±1.89</td>
</tr>
</tbody>
</table>

Values are means ± SD. RBC = red blood cell; PRA = plasma renin activity; ANG I = angiotensin I.

* p < 0.05 (Duncan's test) vs black women, white women, and white men.
† p = 0.016 vs all whites; †p = 0.10 vs all blacks; ‡p = 0.007 vs all women.

Table 3. Parameters of [3H]Ouabain Displacement by Unlabeled Ouabain (Bm, Kd) and K+ (Kt, n) from Red Blood Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Bm (1/cell)</th>
<th>Kd (nM)</th>
<th>Kt (nM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>693 ±30.8*</td>
<td>14.4 ±6.0</td>
<td>2.18 ±0.34</td>
<td>1.51 ±0.17</td>
</tr>
<tr>
<td>Men</td>
<td>582 ±32.1†</td>
<td>17.3 ±5.7</td>
<td>2.75 ±0.43</td>
<td>1.57 ±0.19</td>
</tr>
<tr>
<td>White</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>877 ±34†‡</td>
<td>25.3 ±4.3</td>
<td>2.81 ±0.32</td>
<td>1.49 ±0.14</td>
</tr>
<tr>
<td>Men</td>
<td>761 ±18.9</td>
<td>16.8 ±3.8</td>
<td>2.06 ±0.24</td>
<td>1.40 ±0.11</td>
</tr>
<tr>
<td>All blacks</td>
<td>654 ±24.4§</td>
<td>15.3 ±5.1</td>
<td>2.13 ±0.24</td>
<td>1.46 ±0.11</td>
</tr>
<tr>
<td>All whites</td>
<td>806 ±18.3</td>
<td>20.9 ±3.6</td>
<td>2.39 ±0.20</td>
<td>1.45 ±0.09</td>
</tr>
<tr>
<td>All women</td>
<td>806 ±26.9‖</td>
<td>24.2 ±5.0</td>
<td>2.95 ±0.27</td>
<td>1.54 ±0.12</td>
</tr>
<tr>
<td>All men</td>
<td>728 ±21.2</td>
<td>20.7 ±4.4</td>
<td>2.29 ±0.22</td>
<td>1.39 ±0.10</td>
</tr>
</tbody>
</table>

Values are means ± SD. Mean values for the last four groups (all blacks, all whites, all women, all men) were obtained by computing the pooled data. The p values were obtained using Student's t test. Bm = maximum specific ouabain binding; Kd = apparent dissociation constant for ouabain binding; Kt = apparent equilibrium dissociation constant for K⁺; n = Hill coefficient analogue.

* p < 0.05 vs black men and p < 0.001 vs white women.
† p < 0.001 vs white men; †p < 0.01 vs white men; ‡p < 0.01 vs all whites; †‖ p < 0.005 vs all men.

The displacement of [3H]Ouabain from its specific binding sites by unlabeled ouabain is presented in Figure 2. The inset depicts the Dixon transformation of these parameters than whites and women. The PRA showed a similar pattern to that observed in our previous study, with whites demonstrating a mean value 50% higher than that in blacks. Because of the large scatter of the data, however, only very weak race-related interactions could be demonstrated.

The displacement of [3H]Ouabain by ouabain is presented in Figure 2. The inset depicts the Dixon transformation of the data, the weighted linear regressions of which were used to compute the Bm and Kd values (Table 3). The number of ouabain binding sites on the surface of the RBC was significantly lower in blacks than in whites and in men than in women. The Kd values were essentially the same for all groups. The Bm values were correlated with the results of Na⁺, K⁺-ATPase activity.

The data shown in Figure 2 demonstrate that the specific binding of [3H]Ouabain to human red blood cells is prevented by unlabeled ouabain at concentrations in the range of 10⁻⁶ M. These results support the hypothesis that the specific binding of [3H]Ouabain to red blood cells is mediated by the Na⁺, K⁺-ATPase.
at 6 and 10 mEq/L K⁺ as well as at 20 and 100 mEq/L Na⁺ that were reported in our previous communication.¹⁷ The activity of RBC Na⁺, K⁺-ATPase at the two indicated K⁺ concentrations represents the maximal initial reaction velocity (V_{max}). The Na⁺,K⁺-ATPase activity at the lower Na⁺ concentration was the closest to the V_{max}, whereas the higher Na⁺ concentration incorporated the inhibitory effect of the ion on the enzyme.¹⁷ The Na⁺,K⁺-ATPase activity at the two K⁺ concentrations demonstrated a significant correlation with the Bₘ values (r = 0.451, p < 0.01 and r = 0.430, p < 0.01 at 6 and 10 mEq/L K⁺, respectively). The ATPase activity at 20 mEq/L Na⁺ showed a significant correlation with the Bₘ (r = 0.509, p < 0.01). The correlation was much weaker when the Na⁺,K⁺-ATPase activity at 100 mEq/L Na⁺ was compared with the Bₘ (r = 0.349, p < 0.05). This finding probably relates to the inhibitory effect of higher Na⁺ concentration on the enzyme, which may mask correlations between the Bₘ and the V_{max} values.

The results of the K⁺ displacement studies are presented in Figure 3. The figure inset demonstrates the computer-predicted curves according to Equation 4. The computed values of the apparent dissociation constant (K) and Hill-type coefficient (n) in Table 3 show that the differences in the displacement curves among the groups are primarily derived from the Bₘ values and not from the K or n values.

Several correlation coefficients believed to be relevant to regulation of intracellular cation concentration and BP are listed in Table 4. Most striking was the overall negative correlation between the number of ouabain binding sites (Na⁺–K⁺ pump units) and either the intracellular Na⁺ concentration (see Figure 4) or the RBC Na⁺/K⁺ ratio. Significant correlations between these parameters were also noted for several groups. The slight positive correlations of the Bₘ with RBC K⁺ concentration is consistent with these findings. In the white group, renin was negatively related to the Bₘ and, as a complementary finding, positively related to the RBC Na⁺ level and Na⁺/K⁺ ratio. The Quetelet index had a negative relation to the Bₘ. Supporting this finding was a positive correlation between the Quetelet index and RBC Na⁺/K⁺ ratio in several groups. Age showed a negative effect on the RBC K⁺ concentration, particularly in women.

**Discussion**

That intracellular Na⁺ has a role in the contractile process of vascular smooth muscle cells has been suggested for many years.¹⁻² An elevation of the intracellular Na⁺ level favors the contraction of blood vessels and results in vasoconstriction. Through a membrane Na⁺–Ca²⁺ exchange or by the mobilization of Ca²⁺ from intracellular storage sites,¹⁻³ Na⁺ can regulate the amount of cellular ionized Ca²⁺. Intracellular Na⁺ also may enhance the sensitivity of the contractile apparatus for Ca²⁺.² Our finding of a higher RBC Na⁺ concentration in blacks and men as compared with whites and women is consistent with previous studies.¹⁴,¹⁷⁻²⁵ If the same phenomenon exists in vascular tissues, it may predispose blacks and men to hypertension.

In a previous work, we showed that the higher RBC Na⁺ concentration in blacks and men is, at least partly, related to lower Na⁺,K⁺-ATPase activity.¹⁷ The pres-

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Specific [³H]ouabain binding to human red blood cells as a function of extracellular K⁺ concentration. See Figure 2 for explanation of symbols. Inset presents the computer-predicted specific binding curves according to Equation 4 in Methods for black women (— — —), black men (— — —), white women (——), and white men (— — —).
These relative turnover rates are quite similar. For binding sites is quite stable. 26 A relative turnover rate (cell x mole of inorganic phosphate per hour per mg protein per Na+-K+ pump unit) for adenosine 5'-triphosphate hydrolysis by the enzyme can be calculated using  

\[ V_{\text{max}} \]  

values of the present work. Differences in the enzyme structure among the groups could also account for the unequal activity of Na+,K+-ATPase. From a functional perspective, this explanation is not supported by the comparative equalities of the relative turnover rates. In addition, the similarities in the ouabain and K+ binding parameters (Kd, n) provide further, though not compelling, support to the notion that the various enzymatic activities among the four groups are not the result of different enzymatic structures.

Substantial effort has been made to relate the lower activity of Na+,K+-ATPase to circulating inhibitors. 27-30 Several reports have indicated that at least one of these inhibitors is a digoxinlike substance and that its level is elevated in extracellular volume expansion. 28-30 Blacks appear to have several characteristics that can be associated with expanded extracellular fluid volume and increased levels of a circulating Na+,K+-ATPase inhibitor. 31 Strong binding of such a factor could result in reduced Na+,K+-ATPase activity even when measured in vitro. This concept is not supported by the present study, as no between-group differences were demonstrated. Such differences would be expected if various concentrations of an endogenous digitalislike factor compete with the exogenous ouabain for its binding sites. Our experiments cannot entirely exclude the existence of an endogenous digitalislike factor. However, the binding affinity of such a factor should be of a magnitude that eludes the detection of its dissociation within the 1-hour incubation period. Such binding should be considered irreversible and, under such circumstances, the physiological role of an endogenous digitalislike factor would be highly questionable.

The numbers of ouabain binding sites in this investi-
gation correspond to those in several previous studies, but they are substantially higher than those reported in others. The good correlations among the calculated number of ouabain binding sites, the previously measured Na+, K+-ATPase activity, and the intracellular Na+ concentration or Na+/K+ ratio indicate that the majority of these sites are functional Na+-K+ pump units. The observation of less ouabain binding in some previous studies could be due to an inadequate extraction technique of the bound [3H]ouabain. Our observation of 50 to 80% higher labeled ouabain concentration than that stated by the supplier suggests another possibility for differences among laboratories. These results also emphasize the importance of considering sex and race when comparing the number of RBC ouabain binding sites.

The correlation of the number of RBC Na+-K+ pump units with several other parameters may support mechanistic links between hypertension and cellular Na+ regulation. Potassium has been suggested to have a protective role against elevated BP. In view of the age-dependent rise in BP, it is of interest that the RBC K+ concentration in our subjects decreased with age. This finding is in agreement with a previous report on total body K+ loss with aging. The mechanism of K+ protection against BP elevation is not clear at present: natriuresis, decreased sympathetic activity, and inhibition of the renin-angiotensin system are among the many causes that have been suggested.

The negative correlation between the number of ouabain binding sites and PRA in the white group is a new finding, the significance of which is not clear at this time. Based on recent reports pertaining to the cellular uptake of renin and the presence of angiotensin type II receptors in intracellular organelles, such as nuclei, a question can be raised as to the possible role of the renin-angiotensin system in the regulation of Na+, K+-ATPase synthesis. Low Na+, K+-ATPase activity has been related to the pathogenesis of obesity, although this hypothesis has been challenged. The slightly negative correlation between the maximal ouabain binding and the Quetelet index in the whole sample population and in the white group agrees with findings obtained in normal and overweight subjects.

In conclusion, our main findings demonstrate that a lower number of Na+-K+ pump units on the surface of the RBC is the reason for the lower Na+, K+-ATPase activity in blacks and men as compared with whites and women. These differences may contribute to the higher intracellular Na+ concentration in the former two groups. Such a phenomenon, if it occurs in vascular smooth muscle cells, may predispose to vasoconstriction and elevated BP. Plasma renin activity, age, and Quetelet index appear to be related to RBC Na+-K+ homeostasis. These factors should be evaluated in relation to sex and race. Our results indicate the need for more extensive, wide-scale epidemiological studies of intracellular Na+ homeostasis that would include both normotensive and hypertensive groups.

References
21. Akera T, Cheng TK. A simple method for determination of...
affinity and binding site concentration in receptor binding studies. Biochim Biophys Acta 1977;470:412–423
32. Gardner JD, Conlon T. The effects of sodium and potassium on ouabain binding by human erythrocytes. J Gen Physiol 1972;60:609–629
36. Erdman E, Hasse W. Quantitative aspects of ouabain binding to human erythrocyte and cardiac membranes. J Physiol (Lond) 1975;251:671–682
38. Hoffman JF. The interaction between tritiated ouabain and Na-K pump in red blood cells. J Gen Physiol 1969;54:343a–350s
[3H]ouabain binding of red blood cells in whites and blacks.
L Hopp, N Lasker, S Grossman, R Bamforth and A Aviv

Hypertension. 1986;8:1050-1057
doi: 10.1161/01.HYP.8.11.1050

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/8/11/1050

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/