Red Blood Cell Li\(^+\)-Na\(^+\) Countertransport, Na\(^+\)-K\(^+\) Cotransport, and the Hemodynamics of Hypertension

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SUMMARY Red blood cell Li\(^+\)-Na\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport activities, home blood pressure, invasive systemic hemodynamics, and limb venous compliance were measured in 65 white men (23 normotensive, 22 borderline hypertensive, and 20 mild essential hypertensive subjects). Li\(^+\)-Na\(^+\) countertransport activity was positively and significantly correlated with subject-determined home systolic blood pressure (r = 0.31, p<0.02) and with directly measured systolic (r = 0.29, p<0.02) and diastolic (r = 0.27, p<0.03) blood pressures in the hemodynamic laboratory, independent of potential confounding variables. Analysis of the hemodynamic determinants of blood pressure revealed a significant positive correlation of countertransport with vascular resistance (r = 0.30, p<0.02) but not with cardiac output or cardiac index. High red blood cell Na\(^+\)-K\(^+\) cotransport activity was not independently associated with hypertension or with a characteristic hemodynamic pattern but was related to decreased venous compliance. Red blood cell Li\(^+\)-Na\(^+\) countertransport deserves further study as a marker for the genetic substrate of human essential hypertension. Red cell Na\(^+\)-K\(^+\) cotransport may be altered secondarily by factors related to high blood pressure and seems to be a valid marker for abnormalities of the venous system in hypertension. (Hypertension 9: 459-466, 1987)

KEY WORDS • lithium-sodium countertransport • sodium-potassium cotransport • bumetanide • vascular resistance • venous compliance

INCREASED systemic vascular resistance is the hemodynamic hallmark of established human essential hypertension. Borderline hypertension results from elevated cardiac output in about a third of patients, but even in such "hyperkinetic" hypertensive patients, vascular resistance is considered to be inappropriately elevated for the prevailing cardiac output. Although structural changes may reinforce and amplify the development of elevated vascular resistance, the events initiating hypertension can more plausibly be attributed to a metabolic defect in sodium metabolism in vascular smooth muscle cells. Although cellular sodium metabolism cannot yet be assessed directly in human blood vessels, the intriguing observations of Canessa et al. and Garay et al. relating quantitative disturbances of red blood cell (RBC) Li\(^+\)-Na\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport, respectively, to essential hypertension, have fueled speculations that similar abnormalities could affect vascular smooth muscle cells.

Indirect evidence for an association of RBC transport abnormalities and vascular smooth muscle cell dysfunction in essential hypertension was reported in the 1960s by Losse, Wessels, and their co-workers, who found RBC sodium content\(^6\) and the rate of \(^{22}\)Na\(^+\) influx\(^7\) to be slightly elevated in RBCs from hypertensive subjects. Postnov et al.\(^8\) described a similar increase in \(^{22}\)Na\(^+\) efflux from the RBCs of essential hypertensive subjects and presented evidence that the cause of the increased \(^{22}\)Na\(^+\) permeability is a defect in...
membrane binding of calcium ions, a potentially widespread defect that could alter smooth muscle cell contractility. The possibility that RBC characteristics paralleled more directly pathophysiological cardiovascular functions was tested by Wessels and Zunkley, who demonstrated a positive correlation of RBC-Na⁺ influx rates with pressor reactivity to systemically infused angiotensin II and nonepinephrine. Because of the confounding effects of vascular structural adaptations and of baroreceptor regulation, pressor responsiveness has limitations as a measure of vascular smooth muscle contractility in hypertension. The question raised remains open, however, for following these early efforts, only a few recent studies have attempted to relate RBC countertransport to quantitative hemodynamics. To date, several reports support a positive correlation of countertransport and blood pressure (BP), but only Fujita et al. have investigated the relationship between RBC countertransport and vascular resistance, and they reported a positive correlation of modest strength (r = 0.46, p < 0.02).

During the past 7 years Li⁻-Na⁺ countertransport has emerged as a reliable group marker for hypertension, but skepticism and confusion have surrounded attempts to validate early reports of a link between high BP and Na⁺-K⁺ cotransport. Na⁺-K⁺ cotransport is a mode of ouabain-insensitive cation transport that is functionally and pharmacologically distinct from countertransport. Originally described as having a subnormal capacity in hypertensive persons, maximal cotransport activity has subsequently proved to be inconsistently related to BP. However, as a result of interest stimulated by the original observation, Na⁺-K⁺ cotransport activity (operationally defined by the effect of the loop diuretics furosemide and bumetanide) has been described in vascular smooth muscle in studies undertaken in cell culture and in intact vessels. In addition, Na⁺-K⁺-Cl⁻ cotransport appears to be very active in vascular endothelial cells. Clinical studies of the cardiovascular actions of intravenously administered loop diuretics have revealed a prominent central venodilator activity, an extrarenal pharmacodynamic effect that may represent cotransport inhibition.

We undertook the present analysis in conjunction with a study of the importance of decreased limb venous distensibility, a known characteristic of borderline and mild hypertension. In central hemodynamics and renin release during thigh cuff-induced venous pooling. The results of that study are reported in part elsewhere. In the present report, we describe our findings related to RBC Li⁺-Na⁺ countertransport and Na⁺-K⁺ cotransport. Measured at nominally maximal activities, both countertransport and cotransport correlated positively with BP. Of the two, however, only RBC Li⁺-Na⁺ countertransport was independently correlated with the vascular resistance component of elevated BP. Increased Na⁺-K⁺ cotransport activity seemed to be most closely linked to decreased venous distensibility, which may be a secondary cardiovascular adaptation to hypertension.

Subjects and Methods

Study Protocol

The details of the recruitment of normotensive (BP <140/90 mm Hg), borderline hypertensive (at least one casual BP >140/90 mm Hg and at least one casual BP <140/90 mm Hg within the preceding year), and essential hypertensive (all casual BPs >140/90 mm Hg) subjects and their preparation were outlined more fully in an earlier report. The present analysis was limited to white men, as race has a substantial influence on RBC countertransport and cotransport. At the time of recruitment, all subjects were instructed in the self-determination of BP and were provided with an aneroid sphygmomanometer. Subjects recorded BPs twice daily for 7 days, and averages of the 14 systolic (home SBP) and diastolic (home DBP) readings were used in the analysis. At the time of the hemodynamic assessment, all subjects had been off antihypertensive treatment for a minimum of 4 weeks and had been eating a 100 mEq Na⁺ diet for 4 days. Dietary compliance was assessed by measurement of Na⁺ in a urine specimen collected over the 24 hours prior to the laboratory hemodynamic study. Standard invasive methods were employed for measurement of brachial BPs (laboratory SBP and DBP) and cardiac output (by dye dilution; for further details, see Reference 30). Leg vein distensibility was measured plethysmographically (mercury-in-Silastic, Hokanson EC-4 plethysmograph; Issaquah, WA, USA) as the change in calf circumference after inflation of a 15-cm congesting cuff (Hokanson rapid cuff inflator) applied to the thigh. Duplicate volume/pressure curves, using 5 mm Hg pressure steps ranging from 10 to 50 mm Hg congesting pressures, were obtained on all subjects, and values reported are averages of the two determinations in units of milliliters per 100 g of tissue. The minimum occlusion pressure (MOP) was determined by extrapolation of the mean volume/pressure curve for each subject through zero volume. The venous volume at 30 mm Hg above MOP (VVₐ) was derived by extrapolation from the average volume/pressure curve for each subject.

RBC Studies

Detailed RBC methods, normal values, and reproducibility data for our laboratory have been published. All cell electrolyte determinations were performed on 200-μl hemolysates deproteinized with 10 ml of 5% trichloroacetic acid and measured by atomic absorption (AA) spectrophotometry (Perkin-Elmer 2380 AA spectrophotometer; Norwalk, CT, USA). RBC water content was determined gravimetrically. Li⁺-Na⁺ countertransport was assessed by the method of Canezza et al. as the difference in Li⁺ efflux from Li⁺-loaded RBCs into Na⁺-rich and Na⁺-free media. Na⁺-K⁺ cotransport was determined using cells loaded with Na⁺ by the nystatin technique, as previously described. Unlike our earlier reports, we used bumetanide (Hoffmann-La Roche, Nutley, NJ, USA) instead of furosemide to inhibit cotransport. Although we measured both bumetanide-sensitive Na⁺ and K⁺...
fluxes from the Na⁺-loaded cells, only the Na⁺ fluxes were examined for relationships with cardiovascular functions. As we and others have noted, loop diuretic-sensitive K⁺ efflux is complex.

Statistics

Data were stored on the MTS computer system of the University of Michigan and analyzed with the MIDAS statistical package. Linear relationships between normally distributed variables were examined by product-moment correlation, and the effect of potential confounding variables was controlled by partial correlation. Differences detected following subdivision of the study population by BP levels were subjected to analysis of variance. The significance of differences in rates of high and low flux values between groups was assessed by the chi-square test with the Yates correction for continuity. Group differences between venous distensibility curves were first analyzed by profile analysis. This multivariate technique assumes parallelism of the curves. If the assumption of parallelism was violated, differences between the curves were tested at each pressure level by the Student's t test with Bonferroni corrections. Data are reported as means ± SD. Statistical significance was accepted at the 0.05 level.

Results

Characteristics of the Study Groups

Descriptive data for the 65 subjects (23 normotensive, 22 borderline hypertensive, and 20 mild essential hypertensive subjects) who completed the hemodynamic and RBC transport studies are shown in Tables 1 and 2. As in previous studies, both borderline and mild hypertensive subjects were heavier and bulkier; weight and body mass index were considered potential confounding variables in subsequent analyses. The SBP and DBP levels of borderline hypertensive subjects were in the intermediate range between pressures of normotensive and mild essential hypertensive subjects. Although for the entire study group, indirect home and invasive laboratory BPs were highly intercorrelated (SBP: r = 0.73, p < 0.0001; DBP: r = 0.74, p < 0.0001), laboratory SBP was on average slightly higher (2.7 ± 11.7 mm Hg; p = 0.08) and DBP significantly lower (9.7 ± 7.7 mm Hg; p < 0.0001) than pressures measured at home, observations probably attributable to known systematic differences in the direct and indirect techniques of BP measurement as well as to posture and setting. Consistent with hemodynamic studies from this and other laboratories (see References 36), cardiac output was somewhat higher in borderline hypertensive subjects than in either normotensive or mildly hypertensive subjects, although the observed differences were of only marginal statistical significance (p = 0.09). Cardiac index showed a similar trend, but differences were not significant. Calculated vascular resistance was increased significantly in the mildly hypertensive subjects but was almost identical in the normotensive and borderline hypertensive groups. Thus, as in previous studies, the increased BP in borderline hypertensive subjects reflected a failure of vascular resistance to decrease and accommodate the higher cardiac output. Venous distensibility showed a stepwise decrease with increasing blood pressure, as shown in Table 1 for the standardized VVₓₒ measurement. Figure 1 demonstrates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 23)</th>
<th>Borderline hypertensive (n = 22)</th>
<th>Mildly hypertensive (n = 20)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33.3 ± 9.4</td>
<td>36.2 ± 8.4</td>
<td>38.4 ± 7.9</td>
<td>1.9</td>
<td>0.16</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.5 ± 9.5</td>
<td>93.6 ± 20.0</td>
<td>89.8 ± 12.6</td>
<td>8.4</td>
<td>0.0006</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 2.8</td>
<td>29.1 ± 6.1</td>
<td>28.4 ± 3.4</td>
<td>7.8</td>
<td>0.0009</td>
</tr>
<tr>
<td>Home SBP (mm Hg)*</td>
<td>117.1 ± 6.8</td>
<td>128.5 ± 9.5</td>
<td>144.4 ± 12.3</td>
<td>34.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Home DBP (mm Hg)*</td>
<td>72.8 ± 7.7</td>
<td>82.4 ± 7.3</td>
<td>95.5 ± 5.0</td>
<td>52.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Laboratory SBP (mm Hg)</td>
<td>121.7 ± 8.8</td>
<td>132.6 ± 7.8</td>
<td>146.5 ± 19.6</td>
<td>20.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Laboratory DBP (mm Hg)</td>
<td>65.6 ± 5.2</td>
<td>74.4 ± 6.3</td>
<td>82.9 ± 7.4</td>
<td>40.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>59.6 ± 7.5</td>
<td>65.4 ± 10.9</td>
<td>66.2 ± 12.0</td>
<td>2.8</td>
<td>0.07</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>5.57 ± 1.03</td>
<td>6.48 ± 2.12</td>
<td>5.73 ± 0.83</td>
<td>2.5</td>
<td>0.09</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>2.90 ± 0.60</td>
<td>3.06 ± 0.99</td>
<td>2.77 ± 0.44</td>
<td>0.8</td>
<td>0.44</td>
</tr>
<tr>
<td>SVR (dyn·sec·cm⁻²)</td>
<td>1224 ± 250</td>
<td>1233 ± 296</td>
<td>1500 ± 280</td>
<td>4.0</td>
<td>0.02</td>
</tr>
<tr>
<td>VVₓₒ (ml/100 g)</td>
<td>1.59 ± 0.59</td>
<td>1.33 ± 0.39</td>
<td>1.24 ± 0.35</td>
<td>3.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Uₜₑₗ₀ (mmol/24 hr)</td>
<td>99.5 ± 59.9</td>
<td>87.6 ± 55.1</td>
<td>86.8 ± 29.5</td>
<td>0.4</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure (see Methods for explanation of home and laboratory); HR = heart rate; CO = cardiac output; CI = cardiac index; SVR = systemic vascular resistance; VVₓₒ = increase in venous volume at 30 mm Hg congesting pressure (standardized for minimum occlusion pressure).

* = 16 normotensive subjects.

** = 20 normotensive, 20 borderline hypertensive, and 19 mildly hypertensive subjects.
TABLE 2. RBC Cation Content and Transport

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 23)</th>
<th>Borderline hypertensive (n = 22)</th>
<th>Mildly hypertensive (n = 20)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh RBC contents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol/L cells)</td>
<td>7.2 ± 1.7</td>
<td>7.5 ± 1.3</td>
<td>7.7 ± 1.6</td>
<td>0.5</td>
<td>0.61</td>
</tr>
<tr>
<td>K⁺ (mmol/L cells)</td>
<td>89.5 ± 0.1</td>
<td>93.4 ± 9.1</td>
<td>93.5 ± 10.5</td>
<td>1.3</td>
<td>0.27</td>
</tr>
<tr>
<td>H₂O (%)</td>
<td>64.1 ± 1.5</td>
<td>64.0 ± 1.3</td>
<td>64.4 ± 1.0</td>
<td>0.6</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Li⁺-loaded RBC contents (mmol/L cells)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li⁺</td>
<td>7.9 ± 0.7</td>
<td>8.1 ± 1.6</td>
<td>8.3 ± 0.8</td>
<td>0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Na⁺</td>
<td>2.9 ± 1.0</td>
<td>3.5 ± 1.1</td>
<td>3.5 ± 2.2</td>
<td>0.9</td>
<td>0.41</td>
</tr>
<tr>
<td>K⁺</td>
<td>82.9 ± 10.3</td>
<td>84.9 ± 10.2</td>
<td>85.6 ± 10.5</td>
<td>0.4</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Li effluxes (mmol Li⁺/L cells • hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na medium</td>
<td>0.49 ± 0.12</td>
<td>0.57 ± 0.20</td>
<td>0.66 ± 0.19</td>
<td>5.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Mg medium</td>
<td>0.20 ± 0.05</td>
<td>0.24 ± 0.09</td>
<td>0.30 ± 0.16</td>
<td>4.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Countertransport</td>
<td>0.29 ± 0.12</td>
<td>0.33 ± 0.13</td>
<td>0.36 ± 0.14</td>
<td>1.3</td>
<td>0.29</td>
</tr>
<tr>
<td>High/low</td>
<td>8:15</td>
<td>13:9</td>
<td>13:7</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Na⁺-loaded RBC contents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol/L cells)</td>
<td>39.7 ± 9.9</td>
<td>45.4 ± 11.9</td>
<td>43.8 ± 13.4</td>
<td>1.4</td>
<td>0.26</td>
</tr>
<tr>
<td>K⁺ (mmol/L cells)</td>
<td>50.1 ± 6.4</td>
<td>50.6 ± 9.0</td>
<td>52.7 ± 8.5</td>
<td>0.6</td>
<td>0.55</td>
</tr>
<tr>
<td>H₂O (%)</td>
<td>63.9 ± 2.1</td>
<td>65.3 ± 3.1</td>
<td>63.8 ± 2.7</td>
<td>2.2</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Bumetanide-sensitive effluxes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol/L cells • hr)*</td>
<td>0.55 ± 0.24</td>
<td>0.52 ± 0.29</td>
<td>0.75 ± 0.35</td>
<td>3.9</td>
<td>0.03</td>
</tr>
<tr>
<td>High/low</td>
<td>9:13</td>
<td>10:12</td>
<td>15:5</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>K⁺ (mmol/L cells • hr)*</td>
<td>0.67 ± 0.31</td>
<td>0.61 ± 0.33</td>
<td>0.77 ± 0.24</td>
<td>1.6</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Values are means ± SD. For Li⁺-Na⁺ countertransport, high ≥ 0.32, low < 0.32 mmol Li⁺/L cells • hr; for Na⁺-K⁺ cotransport, high ≥ 0.55, low < 0.55 mmol Na⁺/L cells • hr. Significance testing by chi-square for larger difference.

that compared with normotensive subjects, the venous compliance curves for borderline and mildly hypertensive subjects were progressively displaced toward the pressure axis throughout the entire range of applied venous congesting pressures.

RBC Na⁺, K⁺, and water contents were not significantly different between the groups (see Table 2), although cell electrolyte values were slightly higher in both borderline and mildly hypertensive groups. Mean Li⁺-Na⁺ countertransport activity increased progressively in the three groups, and although the differences were not significant, mean levels for the normotensive (0.29 mmol/L cells • hr) and mildly hypertensive groups (0.36 mmol/L cells • hr) were very similar to the significant differences observed for whites in previous studies. In our own earlier work, as compared with the present study, increased power was conferred by larger numbers, the range of BPs was broader, and sodium balance was not attempted. The linear relationship reported in that study was described in a regression equation of countertransport on BP. We used casual indirect mean BP as the independent variable to calculate expected levels of countertransport for the present borderline (102.8 mm Hg) and mildly (115.1 mm Hg) hypertensive groups, and the predicted values, 0.31 and 0.36 mmol/L cells • hr, respectively, were in generally good agreement with the observed activities. Na⁺-K⁺ cotransport did not differ between normotensive and borderline hypertensive groups but was significantly elevated in the mildly hypertensive group. This differs from our previous findings but probably is explicable by an age effect (see Discussion) or by an influence related to the control of dietary Na⁺ intake.
Relationship of Countertransport and Cotransport to BP

Both countertransport and cotransport were significantly positively correlated with one or more BPs, either subject-recorded indirect BP measurements obtained in the home or directly recorded brachial pressures in the laboratory. Countertransport generally correlated better with SBPs (home: \( r = 0.31, p < 0.02 \); laboratory: \( r = 0.29, p < 0.02 \)) than with DBPs (home: \( r = 0.17, p = 0.20 \); laboratory: \( r = 0.27, p < 0.03 \)), while for cotransport, relationships were similarly positive but of lesser significance (home \( r = 0.18, p = 0.17 \); laboratory \( r = 0.21, p = 0.09 \); home DBP: \( r = 0.25, p = 0.06 \); laboratory DBP: \( r = 0.25, p < 0.05 \)). Although we controlled for race and gender and limited the range of age and BP, the many other variables reportedly influencing either BP (weight, body mass index, age, plasma norepinephrine, plasma renin activity, countertransport [weight, body mass index, age, plasma cholesterol, cholesterol, serum K+, serum K+, body mass index, age, weight, body mass index, and laboratory SBP] were of necessity controlled only by multivariate statistical techniques. Controlling for these factors by partial correlation identified countertransport and weight as independent, strongly correlated with age, which itself could be shown to be an important simple correlate of cotransport (\( r = 0.25, p < 0.05 \)).

Systemic Hemodynamics of Hypertension

Examination of the hemodynamic determinants of BP, cardiac output and calculated systemic vascular resistance, demonstrated significant correlations of vascular resistance with both countertransport (\( r = 0.30, p < 0.02 \)) and cotransport (\( r = 0.28, p < 0.03 \)) for the entire group. Partial correlation revealed that the relationship of vascular resistance and cotransport was largely dependent on age, which was itself the strongest single predictor of vascular resistance (\( r = 0.36, p < 0.003 \)). However, the correlation of countertransport and vascular resistance was still significant (\( p < 0.03 \)) when controlled for age (partial correlation coefficient = 0.30).

The relationship of countertransport and vascular resistance was evident only in the mildly hypertensive subgroup when the subjects were stratified by BP. In mildly hypertensive subjects, after controlling for the effects of age and weight by partial correlation, there was a significant positive correlation of countertransport and vascular resistance (\( r = 0.53, p < 0.03 \)) that was not evident in either the borderline hypertensive or the normotensive subjects.

Examination of plasma volume and total blood volume, cardiac output, stroke index, and heart rate for relationships with RBC transport functions uncovered no significant correlations. Particular attention was paid to the upper quartile of cardiac index in the borderline hypertensive group, but no characteristic RBC transport abnormality was evident in this hyperdynamic subgroup.

Venous Compliance, Hypertension, and RBC Cotransport

Venous distensibility curves of satisfactory quality for analysis were obtained in 59 of the 65 subjects (20 normotensive, 20 borderline hypertensive, and 19 mildly hypertensive subjects). Venous distensibility was significantly decreased in subjects with elevated RBC Na⁺-K⁺ cotransport compared with that of subjects with normal cotransport activity (Figure 2). Of the correlations of the standardized measure of venous distensibility, \( V \), and BP, only the correlation with laboratory SBP was significant (\( r = -0.31, p < 0.02 \)), perhaps because both measurements were obtained under standardized laboratory conditions. Among the other potential confounding variables, weight (\( r = -0.37, p < 0.005 \)) and body mass index (\( r = -0.43, p < 0.001 \)) were significantly related to \( V \), and age was of marginal significance (\( r = -0.24, p < 0.06 \)). RBC cotransport was inversely correlated with \( V \) (\( r = -0.35, p < 0.01 \)), most strongly in the borderline (\( r = -0.53, p < 0.02 \)), and mildly (\( r = -0.46, p < 0.05 \)) hypertensive subgroups. Partial correlation (controlling for cotransport, age, weight, body mass index, and laboratory SBP) identified body mass index and cotransport as the two strongest independent correlates of \( V \) for the entire study group. For the combined subgroup of borderline and mildly hypertensive subjects, RBC cotransport was the only significant independent correlate of \( V \) (\( p < 0.001 \)), and in this group, cotransport alone explained 25% of the variability in \( V \).

Since \( V \) is a derived variable and may be influenced by the correction for MOP, it is important to point out that differences in MOP between the three groups were small and statistically nonsignificant (normotensive group: 6.1 ± 4.2 mm Hg; borderline group: 4.5 ± 5.1 mm Hg; mildly hypertensive group:

**Figure 2.** Venous distensibility curves for 29 subjects (○) with high (>0.55 mmol/L cells·hr) and 30 subjects (●) with normal (≤0.55 mmol/L cells·hr) RBC cotransport activity. All values are means ± SD; p values are given between the curves.
7.9 ± 6.8 mm Hg; \( p > 0.10 \). The three volume/pressure curves were significantly different by profile analysis, diverging over their range, with maximal differences obtained at the highest congesting pressures (see Figure 1).

**Discussion**

The purpose of this study was to examine the relationships between the activities of two RBC transport systems, Li\(^+\)-Na\(^+\) countertransport and Na\(^+\)-K\(^+\) co-transport, and arterial BP, systemic hemodynamics, and venous distensibility in normotensive, borderline, and mildly hypertensive white men. We demonstrated a significant positive correlation of countertransport and BPs obtained both by the subjects themselves at home with cuff and stethoscope and by intra-arterial recordings in the hemodynamics laboratory. These observations confirm and extend our earlier report of a positive correlation of casual clinic BPs and countertransport in whites.\(^{14}\) The correlation with home BP measurements is of particular interest because, as the average of a larger number of readings, home BP, as opposed to laboratory or casual BP, more nearly approximates the true mean BP, which may be the best predictor of cardiovascular complications.\(^{30}\) The significant increase in countertransport in mildly hypertensive subjects demonstrated in the present study was not noted in our previous report,\(^{14}\) but as the correlation in the present group is dependent on age, we continue to assert that countertransport has no independent predictive value for hypertension. The most intriguing finding related to countertransport in the present study was the demonstration of an inverse relationship between countertransport activity and venous compliance.

**RBC Li\(^+\)-Na\(^+\) Countertransport and Vascular Resistance**

A significant positive correlation between systemic vascular resistance and countertransport was described in 1983 by Fujita et al.,\(^{18}\) in a study using noninvasive measurements of BP and cardiac output in 26 borderline hypertensive subjects who were a subgroup of 40 young borderline hypertensive subjects with an overall group mean BP of 108 mm Hg. Compared to the subjects in the study by Fujita et al.,\(^{18}\) mean BPs were considerably lower in our borderline hypertensive subjects (laboratory: 94.8 ± 5.3 mm Hg; home: 97.7 ± 6.4 mm Hg) but quite similar to pressures in our mildly hypertensive subjects (laboratory: 107.5 ± 11.3 mm Hg; home: 111.8 ± 6.7 mm Hg). Therefore, the subjects we have identified as mildly hypertensive, in whom vascular resistance is already clearly increased, are most comparable to the borderline group of Fujita et al.\(^{18}\) and, as in that study, vascular resistance correlated significantly with countertransport in our mildly hypertensive group.

**RBC Li\(^+\)-Na\(^+\) Countertransport as a Marker for Risk of Hypertension**

We found no significant correlation between countertransport and vascular resistance in borderline hypertensive subjects. However, the prevalence of high (Li\(^+\) > 0.32 mmol/L cells·hr) countertransport values in the borderline hypertensive subgroup (59%) was similar to that observed in the mildly hypertensive group (65%). The coexistence of an increased group risk of future hypertension in the borderline hypertensive subjects\(^{21}\) and an increased prevalence of high countertransport activity suggests that countertransport may be a marker for a lesion that predisposes borderline hypertensive persons, characterized as a group by high cardiac output, to the development of high vascular resistance, the hemodynamic basis of established human essential hypertension. Whether a countertransport system analogous to that characterized in RBCs plays any direct role in the development of high vascular resistance (e.g., in vascular smooth muscle cells) cannot be determined from our data.

**RBC Na\(^+\)-K\(^+\) Cotransport and Venous Compliance**

In contradistinction to the demonstrated independence of countertransport as a correlate of BP and vascular resistance, the relationship of cotransport to the same measures, while significant by simple correlation, proved to be due to an effect of age. Neither we\(^{14}\) nor others have previously noted an age effect on cotransport and would regard the correlation observed in the present study as open to question. The correlation of venous distensibility with cotransport was, however, still significant after controlling for age and appears to be a valid association, although, of course, the reason RBC cotransport correlates with venous tone is unknown. Since in the present study, venous stiffness, like cotransport, increased in tandem with BP, it seems likely that abnormal venous compliance is a secondary adaptive consequence of hypertension, perhaps related to the decreased ventricular compliance that seems to be an early effect of hypertension.

We interpret the RBC cotransport findings as a clue that the decrease in venous distensibility may not be due only to structural adaptations. Venodilation is known to follow intravenous administration of furosemide,\(^{26}\) and although experimental evidence supports roles for prostaglandins\(^{52}\) and angiotensin\(^{53}\) in this effect, the possibility that venous smooth muscle tone is chronically dependent on loop diuretic-sensitive cotransport activity has not been excluded. A circulating factor with activity shared with the loop diuretics, earlier termed the chlorothiazide hormone\(^{63}\) and now tentatively linked to atrial natriuretic factor,\(^{55,56}\) is affecting both RBC and venous smooth muscle cotransports, could potentially explain the relationships demonstrated in the current study.

**Sources of Variability in Measurement of RBC Li\(^+\)-Na\(^+\) Countertransport**

The specificity of a marker is weakened if other factors importantly influence its measurement. For RBC Li\(^+\)-Na\(^+\) countertransport activity, at present still a bioassay,\(^{57}\) a number of potential confounding clinical variables have been described, the most consistent of which are weight,\(^{12,17,43,44,48}\) or body mass index,\(^{11}\) plasma cholesterol,\(^{17,44,47,48}\) and race.\(^{14,54}\) The last factor is not an issue in the present study in which only
white men were examined. After hypertension, weight has been the most frequently reported correlate of countertransport in studies of white men, although disagreement certainly exists. In our earlier report, in the present study we noted no significant relationship between RBC countertransport and either weight or body mass index, although the latter two, of course, correlated with each other and with BP. Similarly, we found no relationship of cholesterol to countertransport in the group as a whole or in any of the subgroups based on countertransport level, in agreement with several earlier reports. Since most of our subjects had normal levels of plasma cholesterol (mean, 209 ± 42 mg/dL; range, 110-350 mg/dL; level in 8 subjects, >250 mg/dL), they are not comparable to the predominantly hyperlipidemic normotensive group reported by Corrocher et al. to demonstrate a positive correlation between plasma cholesterol and countertransport nor to the hyperlipidemic patients studied by Behr et al. We did not measure other lipid fractions such as triglycerides or high density lipoproteins, which may be more important than total cholesterol as determinants of countertransport activity.

Other countertransport correlates are less well documented. In this study, we could find no effect of age on countertransport either in the whole group or in subgroups, although age has been reported to be both positively and negatively correlated with countertransport in normotensive subjects. We are therefore in agreement with the several studies that found no interaction between age and countertransport.

Chronic hypokalemia has been reported to increase countertransport activity. None of our subjects was hypokalemic (mean, 4.4 ± 0.4 mEq/L; range, 3.7-5.5 mEq/L), and serum K⁺ within the normal range did not correlate with countertransport activity, in agreement with one earlier report but not with that of Behr et al., who noted an inverse correlation of serum K⁺ and countertransport. Finally, the observation of Brugnara et al. relating levels of stimulated plasma renin activity (1 hour after i.v. administration of 40 mg of furosemide) to countertransport activity cannot readily be compared with the present study, for although we measured both basal and stimulated plasma renin activity (by thigh cuff inflation), we did not administer furosemide. As in the study of Brugnara et al., basal plasma renin activity did not correlate with countertransport activity, and our subjects, unlike theirs, were following a controlled sodium intake, thus eliminating some of the effect of differences in dietary sodium on plasma renin activity. Stimulation of plasma renin activity by lower-extremity venous pooling did not reveal any relationship between Li⁺-Na⁺ countertransport activity and either the magnitude of the change in renin activity or the final absolute plasma renin activity level achieved. Thus, although we cannot directly compare our study with the earlier report, we do not detect any important interaction of RBC countertransport and physiologically stimulated PRA.

In summary, RBC Li⁺-Na⁺ countertransport was positively and independently correlated with BP measurements obtained under home and laboratory conditions. Countertransport was correlated positively with vascular resistance, particularly in the subgroup of mildly hypertensive subjects. RBC cotransport was not an independent correlate of either BP or vascular resistance but was inversely related to venous distensibility.

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