Red Blood Cell Lithium-Sodium Countertransport in Non-modulating Essential Hypertension

Jamie Redgrave, Mitzy Canessa, Ray Gleason, Norman K. Hollenberg, and Gordon H. Williams

Abnormalities in erythrocyte Li-Na countertransport have been reported in hypertensive subjects, and the available evidence favors familial aggregation and striking heritability of this marker. It is uncertain, however, whether the abnormalities are associated with hypertension per se or whether they may be concentrated in a particular subset of hypertensive subjects. In the present study, maximal rates of Li-Na countertransport were measured in red blood cells of 82 white subjects, including 37 normotensive subjects and 45 normal- or high-renin hypertensive subjects previously classified as non-modulators (n=21) or modulators (n=24). Mean countertransport activity was significantly higher in non-modulators compared with normally modulating hypertensive or normotensive subjects (0.475±0.044 vs. 0.309±0.028 or 0.249±0.012 mmol/l cell×hr, respectively, p<0.001). Modulators did not differ significantly from normotensive subjects with regard to mean countertransport activity. Red blood cell sodium pump and Na-K-Cl cotransport were not significantly different in modulating and non-modulating hypertensive subjects. These relations remained unchanged after adjusting for age, body weight, and plasma cholesterol levels by analysis of covariance. A countertransport value exceeding 0.50 mmol/l cell×hr occurred in 40% of the non-modulators but in only one of the other subjects. In contrast, while one half of the modulators and normotensive subjects had a countertransport value less than 0.235 mmol/l cell×hr, none of the non-modulators did. Thus, elevated countertransport appears to aggregate in the non-modulating subset of essential hypertensive subjects. If these findings are cautiously applied, selecting patients with an elevated countertransport activity (>0.50 mmol/l cell×hr) may serve as a useful method for enriching a sample of the essential hypertensive population with patients who have the non-modulation trait. (Hypertension 1989;13:721–726)

The activity of erythrocyte sodium transport processes may be abnormal in many hypertensive individuals, and, at least for one process, Li-Na countertransport, familial aggregation and high heritability have been documented. These data have caused some to suggest that sodium transport activity may even be a genetic marker for hypertension. High levels of countertransport activity have been particularly common in hypertensive subjects of European extraction with a positive family history for hypertension.7

"Non-modulators" are a subset of the normal- to high-renin essential hypertensive population.8–10 They appear to be a discrete group with a strong family history for hypertension.11,12 They can be called non-modulators and are characterized as such on the basis of the fact that sodium intake does not modify (modulate) renal vascular responsiveness, adrenal responsiveness, or both, to angiotensin II (Ang II).

The present study was designed to investigate whether non-modulators are more likely to have alterations of their red blood cell transport systems than the rest of the hypertensive population. The results indicated that non-modulating hypertensive individuals have a significant elevation of Li-Na countertransport compared with modulating hypertensive persons.
TABLE 1. Demographic and Clinical Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive (n=37)</th>
<th>Modulators (n=24)</th>
<th>Non-modulators (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>32±2</td>
<td>47±3</td>
<td>55±2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73±2</td>
<td>87±4</td>
<td>91±4</td>
</tr>
<tr>
<td>Sex ratio (F/M)</td>
<td>6/31</td>
<td>6/18</td>
<td>2/19</td>
</tr>
<tr>
<td>Admission blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>119±9</td>
<td>153±4</td>
<td>157±4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73±2</td>
<td>95±2</td>
<td>97±3</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>192±5</td>
<td>217±8</td>
<td>250±13</td>
</tr>
</tbody>
</table>

Subjects and Methods

Subjects

Forty-five white patients with normal- or high-renin essential hypertension and 37 normotensive white subjects were studied at the Clinical Research Center (CRC) of the Brigham and Women's Hospital (Table 1). Normal-renin essential hypertension was defined in those who had a plasma renin activity (PRA) of greater than or equal to 2.4 ng/ml/hr in response to upright posture on a 10 meq sodium diet. Each hypertensive subject had outpatient diastolic blood pressure measurements greater than 90 mm Hg on at least three occasions and documented evidence of hypertension for at least 6 months before the study. A clinical laboratory evaluation, designed to rule out secondary causes of hypertension, to establish the renin profile, and to assure normal cardiovascular and renal function as described previously, was performed. Subjects were nongravid and had normal thyroid function tests.

All antihypertensive medications were discontinued for at least 2 weeks before CRC admission. All subjects received constant isocaloric diets throughout the study. Fluid intake was maintained at 2,500 ml/day and dietary potassium at 100 meq/day. Dietary sodium intake was fixed in each subject at either 200 meq/day if the patient was characterized as a non-modulator on the basis of renal blood flow or at 10 meq/day if plasma aldosterone responses were used. Approximately one half of the patients were assigned to the non-modulating group by the adrenal criterion and the other half by the renal criterion. In this study, no subject was studied twice, and the red blood cell sodium transport studies were performed on red blood cells obtained when the patient was on a high salt intake before classification. The non-modulating hypertensive patient was defined as having an increment in plasma aldosterone of less than 15 ng/dl in response to a 3 ng/kg/min infusion of Ang II when in balance on a low-sodium intake or a decrement in renal blood flow of less than 120 ml/min/1.73 m² in response to a 3 ng/kg/min Ang II infusion on a 200 meq sodium intake. The rationale for these classifications has been described previously.

The protocol was approved by the Human Subjects Committee of the Brigham and Women's Hospital, and written consent was obtained from each patient after an explanation of the nature, purpose, and possible risks of the study.

Techniques

Plasma and urine sodium and potassium concentrations were measured by flame photometry, plasma and urine creatinine concentrations by an autoanalyzer method, and PRA, Ang II, and aldosterone levels by radioimmunoassay.

Blood samples for Li-Na countertransport measurement were collected with heparin or EDTA and centrifuged for 10 minutes at 300g to separate the plasma and buffy coat. The red blood cells were used the same day or preserved overnight as previously described. Previous studies have indicated that ion transport measurements were not affected by up to 3 days of preservation. The maximal velocity (V max) of the Li-Na countertransport was assayed from the external sodium-stimulated lithium efflux from lithium preloaded erythrocytes as previously reported. The mean intracellular standard deviation of this assay is 0.03 mmol/l cell x hr. Na-K-Cl cotransport (V max and the Michaelis-Menten dissociation constant [K m]) for sodium was determined by measuring furosemide-sensitive sodium and potassium efflux into choline media (1 mM MgCl₂) from cells loaded with sodium by means of nystatin as previously described.

Statistical Analysis

Data are presented as mean±SEM. The null hypothesis was rejected at a level ≤0.05. Differences among group means were tested for significance by one-way analysis of variance followed by Newman-Keuls test for pairwise comparisons. The influence of age, body weight, and plasma cholesterol levels or mean countertransport levels among study groups was examined by one-way analysis of covariance (ANCOVA) followed by least-squares means procedure for pairwise comparisons. χ² tests with Yates's correction for continuity were used for nonparametric data. The relation between red blood cell Li-Na countertransport level and age, body weight, plasma cholesterol, or blood pressure was tested for significance by Pearson's product-moment correlation.

Results

Patient Characteristics

The hypertensive patients in this study were admitted sequentially to the CRC. The normodulating hypertensive subjects did not differ significantly from the rest of the hypertensive subjects with respect to body weight, sex ratio, or admission blood pressures. Non-modulators, however, were...
significantly older and had higher mean fasting plasma cholesterol levels than did modulators (p<0.05, Table 1). Normotensive subjects were significantly younger and had lower mean body weight, plasma cholesterol, and admission blood pressures than either hypertensive group.

**Erythrocyte Lithium-Sodium Countertransport Activity**

Red blood cell Li-Na countertransport was significantly higher \( (p<0.01) \) for hypertensive patients \( (0.381\pm0.026 \text{ mmol/l cell}\times\text{hr}) \) than for normotensive subjects \( (0.249\pm0.012 \text{ mmol/l cell}\times\text{hr}) \). Non-modulators had a much higher frequency of an elevated level than did the modulating hypertensive patients (Figure 1). Indeed, the mean countertransport level in the non-modulators \( (0.475\pm0.044 \text{ mmol/l cell}\times\text{hr}) \) was highly significantly different \( (p<0.001) \) from that observed in the modulators \( (0.309\pm0.028 \text{ mmol/l cell}\times\text{hr}) \) or the normotensive subjects \( (0.249\pm0.012 \text{ mmol/l cell}\times\text{hr}) \). The impact of the group differences in cholesterol, body weight, and age on this transporter was assessed by ANCOVA (Table 2). Even after correction for these variables, the differences between the normotensive subjects and modulators versus the non-modulators still were highly significant \( (p<0.001) \).

Even though the non-modulators had a significantly higher mean countertransport level than did the modulating hypertensive patients, there was considerable overlap (Figure 1). Thus, the value of the erythrocyte countertransport activity in screening for non-modulation varied, depending on the cut point chosen (Figure 1). Forty percent of the non-modulators had a value greater than 0.5, whereas only one modulating hypertensive and no normotensive subject exceeded that level. A value greater than 0.235 would include not only all the non-modulators but also 52% of the remaining subjects studied. For normal modulation, a value below 0.235 would exclude all non-modulators but would include 50% of the other subjects studied (Figure 1).

**Red Blood Cell Sodium Pump and Sodium-Potassium Cotransport**

The \( V_{\text{max}} \) of the sodium pump was determined by measuring ouabain-sensitive sodium efflux from cells loaded to 50 mmol/l cell sodium and 50 mmol/l cell potassium into media containing 148 mM choline chloride and 10 mM KCl. There were no significant differences between normotensive subjects, modulating hypertensive patients, and non-modulators.

**Table 2. Covariance Analysis of Li-Na Countertransport in Modulating and Non-modulating Hypertensive Subjects**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Unadjusted</th>
<th>Age</th>
<th>Body weight</th>
<th>Serum cholesterol</th>
<th>Age, body weight, and serum cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive (N)</td>
<td>37</td>
<td>0.249±0.012</td>
<td>0.231±0.028</td>
<td>0.248±0.025</td>
<td>0.258±0.022</td>
<td>0.276±0.029</td>
</tr>
<tr>
<td>Modulator (M)</td>
<td>24</td>
<td>0.309±0.028</td>
<td>0.308±0.030</td>
<td>0.297±0.029</td>
<td>0.304±0.027</td>
<td>0.294±0.028</td>
</tr>
<tr>
<td>Non-modulator (NM)</td>
<td>21</td>
<td>0.475±0.044</td>
<td>0.499±0.040</td>
<td>0.482±0.032</td>
<td>0.454±0.031</td>
<td>0.432±0.040</td>
</tr>
</tbody>
</table>

Significance level \( (p) \)

| N vs. M               | NS          | NS          | NS          | NS          | NS          | NS          |
| N vs. NM              | <0.001      | <0.001      | <0.001      | <0.001      | <0.001      | <0.05       |
| M vs. NM              | <0.001      | <0.001      | <0.001      | <0.001      | <0.001      | <0.01       |

Values are mean±SEM.
TABLE 3. Red Blood Cell Sodium Pump and Na-K Cotransport in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=37)</th>
<th>Modulator (n=24)</th>
<th>Non-modulator (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Na</td>
<td>8.6±0.7</td>
<td>8.2±0.9</td>
<td>8.4±0.5</td>
</tr>
<tr>
<td>Na pump*</td>
<td>5.6±0.9</td>
<td>5.1±0.3</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>Na cotransport†</td>
<td>0.88±0.09</td>
<td>1.18±0.11</td>
<td>0.86±0.09</td>
</tr>
<tr>
<td>K cotransport†</td>
<td>1.03±0.12</td>
<td>1.27±0.11</td>
<td>0.93±0.09</td>
</tr>
<tr>
<td>(K_{mNa}) cotransport</td>
<td>10.0±1.0</td>
<td>11.2±0.7</td>
<td>15.9±2.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*Maximal rate of ouabain-sensitive Na⁺ efflux.
†Maximal rate of furosemide-sensitive Na⁺ and K⁺ efflux.
‡Normotensive, non-modulator, \(p<0.05\).

Discussion

As observed in many other reports, the present study confirms that the erythrocyte Li-Na countertransport activity is higher in hypertensive patients than in normotensive subjects.14,7,18 It makes the additional intriguing observation that those individuals with a high maximal rate of countertransport tend to be aggregated in the non-modulating hypertensive subset. Thus, elevated erythrocyte countertransport might be useful as a screening test for non-modulation. However, the sensitivity and specificity do not appear to be sufficiently high for this purpose unless a sample of the hypertensive population enriched with or depleted of non-modulators is desired. For example, a subpopulation of hypertensive individuals with a countertransport value greater than 0.50 mmol/l cell×hr would be comprised of 90% non-modulators while a subpopulation of patients with countertransport levels less than 0.235 mmol/l cell×hr would contain no non-modulators.

During the last several years, we have studied the activity of Li-Na countertransport as a potential marker for abnormalities that might be inherited in families containing hypertensive patients.1,3,5,16 The \(V_{max}\) of this transporter in erythrocytes is familialy aggregated.3,6,7 Studies performed in twins,4,5 families living in Utah,4 and a sample of the general population in Rochester, Minnesota20 have estimated that as much as 80–90% of the individual variance of the countertransporter can be accounted for by heritability.

It has been suggested that non-modulation is also familially aggregated. In a study of more than 50 hypertensive subjects who were classified as non-modulators or normally modulating, 85% of the non-modulators had a positive family history for hypertension when compared with only 30% of the normally modulating hypertensive patients.12 In a large number of normotensive kidney donors in whom family history for hypertension was carefully assessed, those individuals with a positive family history were three to four times more likely to have the characteristics of non-modulation than were those who had a negative family history.21 Finally, in familial studies where the presence or absence of non-modulation was assessed in more than one individual in the family, non-modulation was nine times more likely to be associated with a positive rather than a negative family history for hypertension.22 Thus, it is perhaps not surprising that high countertransport tends to aggregate in those individuals who are non-modulators.

What other factors could explain the differences reported in this study? The association of elevated Li-Na exchange with pregnancy,23 hyperlipidemia,24 hypothyroidism,25 and patients with diabetic nephropathy26 also indicates that abnormalities in this transport system can occur in other pathophysiological situations. In the present study, none of the patients was pregnant, and hypothyroidism was specifically excluded by thyroid function tests in all subjects. There was no evidence of diabetic nephropathy by urinalysis, or by creatinine, renal blood flow, or glomerular filtration rate measurements.

The three study groups showed significant differences in mean age, body weight, and plasma cholesterol levels (Table 2). However, when the mean Li-Na countertransport levels were adjusted for these factors, separately and collectively, the statistical distinction between non-modulators and the other two groups remained significant (Table 2). For the total group of 82 subjects, Li-Na countertransport level was found to be highly correlated with body weight (\(p=0.001\)), age, fasting cholesterol level, and admission blood pressures (all \(p<0.0001\)). Sodium intake was not fixed at the same level in all subjects. There was, however, no consistent difference in the level of sodium intake in the non-modulators versus the modulating hypertensive subjects. Furthermore, we have recently documented that short-term manipulation of sodium intake, as was done in this study, does not effect the level of erythrocyte countertransport either in normotensive or hypertensive subjects.27

Other functional abnormalities have been found associated with elevated Li-Na countertransport in hypertensive patients. Brugnara et al reported a significant correlation (\(r=0.425, p<0.05\)) between countertransport and elevation of PRA after furosemide. Non-modulators also have increased PRA
under similar circumstances.\textsuperscript{29} Weder\textsuperscript{30} found significant correlation ($r=0.48$, $p<0.01$) with the fractional lithium clearance, and Fujita et al\textsuperscript{31} with increased peripheral resistance ($r=0.46$, $p<0.02$). While fractional sodium reabsorption, as assessed by lithium clearance, has not been reported in non-modulators because of their renal vasculature and sodium-handling abnormalities,\textsuperscript{9,12} it is likely that they also may have abnormalities in lithium clearance.

How widely the results of the present study can be extrapolated to other hypertensive populations is uncertain. Several studies have proposed a model with a major gene or a polygenic transmission, or both, for the inheritance of countertransport activity.\textsuperscript{4,20} The available evidence also suggests that non-modulation is transmitted by single gene inheritance.\textsuperscript{22} However, on the basis that hypertension is most likely the result of the interaction of several genes and the environment, there may be circumstances in which abnormalities in erythrocyte countertransport may not track one-to-one with non-modulation. Indeed, the present study does not exclude the possibility of an inheritance of two closely linked but independent entities in the hypertensive population. Particular caution must be used in extrapolating these results to blacks, as they were not included in the present study. It is also well known that blacks tend to have a low level of erythrocyte countertransport activity.\textsuperscript{32} Even with these reservations, it is of interest to speculate how these two abnormalities may be associated. Recently, we have documented that an elevated Li-Na countertransport reflects a kinetic abnormality of the Na$^+/H^+$ exchanger\textsuperscript{13,34} at the cellular level. This exchanger plays an important role in regulating proximal tubular sodium absorption\textsuperscript{35} and vascular\textsuperscript{36,37} and perhaps adrenal responses to Ang II—all areas in which abnormalities have been reported in non-modulators.

In summary, these results confirm an increase in Li-Na countertransport in hypertensive subjects. They further document that the individuals with an elevated erythrocyte countertransport tend to aggregate in the non-modulating subset of the hypertensive population. Non-modulators have the following abnormalities: fixed adrenal and renal vascular responses to Ang II,\textsuperscript{8-11} reduced renin suppression by saline and Ang II,\textsuperscript{38,39} a reduced ability to handle a sodium load,\textsuperscript{9,12} and fixed renal blood flow in response to changes in sodium intake but not to converting enzyme inhibition.\textsuperscript{8,9} To this list can now be added an elevated Li-Na countertransport, thus linking, for the first time, an abnormality involving membrane transport of sodium to a subset of hypertensive individuals who have physiological abnormalities involving sodium handling.

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