Red-Cell Sodium-Lithium Countertransport and Fractional Excretion of Lithium in Normal and Hypertensive Humans

Myron H. Weinberger, Jean B. Smith, Naomi S. Fineberg, and Friedrich C. Luft

To examine the relations between erythrocyte sodium-lithium countertransport and renal proximal tubular sodium handling, we measured countertransport, and then subjected 30 normal and 32 hypertensive subjects, both white and black, to provocative maneuvers of volume expansion and contraction. The fractional excretions of sodium and lithium were measured simultaneously. In agreement with previous studies, we found that countertransport in erythrocytes was elevated in hypertensive patients compared with normal subjects. We also observed that whites have a higher level of countertransport than blacks. In the basal state, we found that fractional sodium excretion of hypertensive patients was no different than in normal subjects, whereas the fractional lithium excretion of hypertensive persons was increased compared with normotensive values. Volume expansion with 2 L 0.9% saline administered intravenously during a 4-hour period provoked an exaggerated natriuresis and a greater increase in fractional lithium clearance in hypertensive patients compared with the control group. With volume expansion and contraction, fractional lithium clearance and countertransport were directly correlated. Our data suggest that hypertensive persons do not have increased proximal tubular sodium reabsorption compared with normal subjects. Further, the exaggerated natriuresis of hypertension is, in part, the result of increased distal solute delivery. The fact that our hypertensive patients were older may partially explain the discrepancies between this report and previous observations. (Hypertension 1989;13:206–212)

An association between elevated in vitro sodium-lithium (Na⁺-Li⁺) countertransport in erythrocytes and human hypertension was first reported by Canessa et al. Aronson, as well as Mahnensmith and Aronson, raised the possibility that this countertransport mechanism may be involved in the pathogenesis of hypertension. They noted similarities between red blood cell Na⁺-Li⁺ countertransport and cation countertransport mediated by the sodium-hydrogen ion exchanger of the renal proximal tubular brush border. They speculated that if increased red blood cell countertransport were paralleled by increased proximal tubular sodium-hydrogen exchange, net proximal tubular sodium reabsorption would be increased. Such a stimulus to increase renal sodium reabsorption could be pivotal to several hypotheses proposed for the development of salt-sensitive human hypertension. Weder was the first to report results testing this linkage in human hypertension. He used lithium as a marker for proximal tubular sodium reabsorption. Lithium is freely filtered by the glomerulus and is reabsorbed in the proximal tubule in a fashion parallel to sodium. Distal tubular lithium handling is minimal, allowing the fractional excretion of lithium to serve as a marker of proximal tubular sodium reabsorption. Weder found that Na⁺-Li⁺ countertransport was increased in hypertensive subjects, corroborating the results of others (see Reference 14 for review). He also observed that his hypertensive subjects had a lower fractional lithium excretion than did normal subjects. He extended these observations by identifying a reduced fractional lithium excretion in the normotensive first-degree relatives of essential hypertensive patients compared with those without a family history of hypertension. Finally, Weder described a significant inverse correlation between Na⁺-Li⁺ countertransport in erythrocytes and fractional lithium excretion within his entire subject population. Weder concluded that hypertensive patients and their first-degree relatives may have increased proximal tubular sodium reabsorption as
subjects were free to move about. Urine was collected after the furosemide day. Mean arterial blood pressure to the diastolic pressure.

The subjects again emptied their bladders completely. At 8:00 AM, another urine specimen was obtained. At that time, each subject was asked to remain recumbent over a 4-hour period. A 4-hour urine specimen was collected. Thereafter, the subjects were free to move about. Urine was collected from 12 noon until 10:00 PM and from 10:00 PM until 8:00 AM the next morning.

Day 3, furosemide. The subjects were then given a diet containing 150 meq sodium and 70 meq potassium. Furosemide (40 mg) was given orally at 10:00 AM, 2:00 PM, and 6:00 PM. Lithium carbonate (600 mg) was again given at bedtime. At 6:00 AM on the following day, the subjects again emptied their bladders completely. At 8:00 AM, another urine specimen was obtained. Blood pressure was measured in both recumbent and standing positions with a mercury manometer by a specially trained nurse before saline administration, after the saline infusion, and after the furosemide day. Mean arterial blood pressure was calculated by adding one third of the pulse pressure to the diastolic pressure.

Sodium-Lithium Countertransport

Na\textsuperscript{+}-Li\textsuperscript{+} countertransport was determined according to a previously published method,\textsuperscript{1} except that a choline chloride solution was used for determination of nonsodium-stimulated lithium efflux. We have observed that magnesium chloride produces an artificially high Na\textsuperscript{+}-Li\textsuperscript{+} countertransport.\textsuperscript{16} Blood was collected into heparinized evacuated tubes, centrifuged at 100g for 10 minutes, and the plasma along with the buffy coat removed. The red blood cells were washed three times in a 150 mM choline chloride solution. All solutions were adjusted to an osmolality of 295–305 mosm/kg H\textsubscript{2}O. An aliquot of packed red blood cells (5 ml) was added to 20 ml of 150 mM lithium chloride and incubated at 37\degree C for 3 hours in a shaking water bath. The cells were washed five times in the choline chloride washing solution to remove extracellular lithium. A suspension (50%) of cells in the choline chloride solution was prepared. The hematocrit of the suspension was determined, and a 1:51 dilution was prepared in a 0.02% cationox solution for subsequent determination of intracellular lithium (Scientific Products, McGaw Park, Illinois). Two milliliters erythrocytes were added to 10 ml each of the following solutions (mM): (A) NaCl 150, ouabain 0.10, glucose 10, Tris-MOPS 10, pH 7.4 at 37\degree C; (B) choline chloride 150, ouabain 0.10, glucose 10, Tris-MOPS 10, pH 7.4 at 37\degree C. These suspensions were incubated at 37\degree C in a shaking water bath with samples removed at 45 and 90 minutes. The samples were centrifuged for 5 minutes at 1,000g, and the supernatant was removed to be analyzed for lithium by atomic absorption spectroscopy (Instrument Laboratories, Andover, Massachusetts). The lithium efflux into each of the solutions was calculated from graphs of lithium concentration versus time. The Na\textsuperscript{+}-Li\textsuperscript{+} countertransport is the difference between the efflux into solutions A and B. The coefficient of variation for the assay established by repeated (2–8) measurements for the same normotensive subjects (n=21) over a 14-month period was 10.0%.

Fractional Excretion

Sodium, creatinine, and lithium were measured in specimens of serum and urine. Sodium was determined by flame photometry (Instrumentation Laboratories, Lexington, Massachusetts), creatinine was measured by an automated method (Beckman Instruments, Fullerton, California), and lithium was measured by atomic absorption. Fractional excretion (FE) was calculated from the formula: $\text{FEX} = \frac{(UX)(SCr)}{(SX)(UCr)}$ expressed as percent, where UX is the urinary concentration of X, SCr the serum creatinine concentration, SX the serum concentration of X, and UCr the urinary creatinine concentration. This expression represents the excretion of X per the amount of X filtered expressed as percent. Plasma renin activity was determined by radiimmunoassay.\textsuperscript{15}

Statistical Analysis

Comparisons between normotensive and hypertensive subjects or between blacks and whites were
TABLE 1. Demographic Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal group</th>
<th>Hypertensive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>37±12</td>
<td>51±11*</td>
</tr>
<tr>
<td>Race (W/B)</td>
<td>21/9</td>
<td>23/9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81±22</td>
<td>85±20</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168±10</td>
<td>169±8</td>
</tr>
<tr>
<td>UNaV (meq/24 hr)</td>
<td>176±66</td>
<td>181±41</td>
</tr>
<tr>
<td>ClCr (ml/min)</td>
<td>106±44</td>
<td>101±41</td>
</tr>
<tr>
<td>PRA (ng Ang I/ml/90 min)</td>
<td>7.2±7.0</td>
<td>5.4±12.9</td>
</tr>
</tbody>
</table>

Values are mean±SD. Values of hypertensive subjects exceed those of normal subjects (p<0.001). *p<0.05.

calculated with t tests. Two-way analysis of variance (ANOVA) was used to examine the simultaneous effects of race and hypertension. A significant interaction term would indicate differential effects of hypertension on blacks and whites. Repeated-measures ANOVA was used to verify fractional excretion of sodium and fractional excretion of lithium changes with saline infusion. The presence of hypertension was a fixed effect, but basal and saline measurement comparisons were performed in each subject. A significant interaction term indicates a differential effect of saline infusion in normotensive and hypertensive subjects. Pearson correlation coefficients were used to assess relations between variables. Stepwise linear regression analysis was done as appropriate. Since plasma renin activity was not normally distributed, statistical analysis was performed on the square root of the value. A p value <0.05 was accepted as significant. Data are expressed as mean±SD.

Results

Table 1 provides the demographic data. The hypertensive subjects were significantly older than the normotensive subjects. The distributions of white to black and men to women were not significantly different between normotensive and hypertensive subjects. Neither height nor weight were different between normotensive and hypertensive subjects. Twenty-four-hour urinary sodium excretion on day of admission; ClCr, creatinine clearance; PRA, plasma renin activity before saline infusion; Ang I, angiotensin I.

Table 2 outlines the blood pressures of normal and hypertensive subjects before and subsequent to the provocative maneuvers. The hypertensive subjects had higher mean blood pressures than the normotensive subjects at every point. With volume expansion the population mean blood pressure increased, and with volume contraction blood pressure decreased. The mean value for intracellular lithium of lithium-loaded cells was 6.08±0.59 mmol; it was not significantly different among the groups. The incremental changes in blood pressure in response to these maneuvers were not different between white and black subjects in either population.

Table 2 presents the Na+-Li+ countertransport values in normotensive subjects and hypertensive patients by race. The values for the hypertensive patients were significantly greater than for the normotensive subjects (p<0.001). Furthermore, the values for the white subjects were significantly greater than for the black subjects (p<0.02). There was no interaction between hypertension and racial effects (p=0.67).

Table 4 presents the fractional excretions of sodium (FENa) and lithium (FELi) under basal and volume expanded conditions. Under basal conditions, FENa was not different between normotensive and hypertensive subjects, which reflects similar glomerular filtration rates and similar states of sodium balance in the two groups. On the other hand, FELi was increased in hypertensive compared with normotensive subjects. With the administration of saline, FENa and FELi increased significantly in both hypertensive and normotensive subjects. However, the increase of both FENa and FELi was greater in hypertensive compared with normotensive subjects (interaction term; p<0.001). Table 5 displays the correlation coefficients between Na+-Li+ countertransport, FELi, FENa, and mean arterial blood pressure. No significant correlation between Na+-Li+ countertransport and
basal FELi was observed; however, with acute perturbations of extracellular fluid volume, significant correlations were observed in all subjects, in whites, and in blacks subjected to volume contraction. These correlations were direct, rather than inverse. Na\(^+-\)Li\(^+\) countertransport was not correlated with FENa under any of the conditions studied. Na\(^+-\)Li\(^+\) countertransport was directly correlated with mean arterial blood pressure under basal and volume expanded conditions. With volume contraction, these significant correlations were no longer identified.

A stepwise linear regression analysis was performed. Age, weight, race, sex, hypertension or normotension, creatinine clearance, basal mean blood pressure, and plasma renin activity (square root of the value) were the independent variables. With FELi as the dependent variable only mean blood pressure entered the equation \((r=0.43, p<0.001)\). The higher the mean blood pressure, the greater the FELi. With FENa as the dependent variable, normotension, female gender, and mean blood pressure entered the relation. The relation was direct \((r=0.53, p<0.001)\). With Na\(^+-\)Li\(^+\) countertransport as the dependent variable, only white race and the presence of hypertension entered the relation \((r=0.53, p<0.001)\), which was again direct. With basal mean blood pressure as the dependent variable, only the presence of hypertension and black race entered the relation \((r=0.75, p<0.001)\). Plasma renin activity was not a factor in any of the relations examined.

### Discussion

The ability to exchange intracellular lithium ions for extracellular sodium ions is a well-established feature of red-cell membranes. The normal function of this countertransport system in erythrocytes is incompletely understood because in physiological media (those with no lithium ions) it appears to promote a 1:1 sodium-sodium exchange, although there is no net effect on the internal ionic concentrations. It has been suggested that the system may result in the exchange of external sodium for internal hydrogen ions \((H^+)^{10}\). Na\(^+-\)H\(^+\) exchange is an important mechanism of pH regulation in a large variety of cell types. In addition, this transport system may play a role in the regulation of cell volume. Canessa and associates\(^{19-21}\) report that the Na\(^+-\)H\(^+\) exchange in erythrocytes can be activated and influenced by elevation of cytosolic calcium, by decreasing the intracellular pH, and by incubating the cells in an alkaline medium. Aronson,\(^2\) Mahnensmith and Aronson,\(^3\) and Funder et al\(^{22}\) raised the possibility that the Na\(^+-\)H\(^+\) countertransport in the renal proximal tubule may be related to erythrocyte Na\(^+-\)Li\(^+\) countertransport. Currently, there is no direct evidence for this hypothesis. Indirect evidence has been presented from studies of spontaneously hypertensive rats that have increased erythrocyte Na\(^+-\)Li\(^+\) countertransport\(^{23}\) and increased Na\(^+-\)H\(^+\) antiporter activity in renal proximal tubules\(^{24}\) compared with Wistar-Kyoto control rats. Recently, acid-base alterations have been described in spontaneously hypertensive rats that may be in part responsible for a compensatory increase in renal tubular Na\(^+-\)H\(^+\) antiporter activity.\(^{25-26}\)

Evidence has also been presented that suggests Na\(^+-\)Li\(^+\) countertransport and renal Na\(^+-\)H\(^+\) exchange are quite different. Kahn\(^{27}\) showed that the Na\(^+-\)H\(^+\) exchange in human and rabbit proximal renal brush border vesicles is amiloride sensitive. However, Na\(^+-\)Li\(^+\) countertransport in erythrocytes of both species could not be inhibited by amiloride under a variety of conditions. On the other hand, Kahn\(^{27}\) demonstrated that extracellular, proton-stimulated sodium efflux in rabbit erythrocytes was inhibited by amiloride. Kahn suggested that the Na\(^+-\)H\(^+\) exchange and Na\(^+-\)Li\(^+\) countertransport are possibly mediated by the same system, but that the affinity for amiloride may be absent or reduced when the system operates in a sodium-for-lithium exchange mode. Alternatively, he suggested that the two exchange mechanisms may be distinct, but that both may be influenced by a physicochemical disturbance that exists in cell membranes of hypertensive patients or in cell mem-

### Tables

#### Table 4. Fractional Excretion of Sodium and Lithium in Basal State and During Volume Expansion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal group</th>
<th>Hypertensive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal FENa (%)</td>
<td>0.75±0.52</td>
<td>0.62±0.32</td>
</tr>
<tr>
<td>Saline FENa (%)</td>
<td>1.31±0.59</td>
<td>1.90±1.29*</td>
</tr>
<tr>
<td>Basal FELi (%)</td>
<td>16.7±4.3</td>
<td>18.9±5.9*</td>
</tr>
<tr>
<td>Saline FELi (%)</td>
<td>20.0±5.3</td>
<td>26.5±7.8*</td>
</tr>
</tbody>
</table>

Values are mean±SD. FENa, fractional excretion of sodium; FELi, fractional excretion of lithium.

#### Table 5. Correlation Coefficients of Sodium-Lithium Countertransport With Fractional Lithium and Sodium Excretion and Mean Arterial Blood Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n=62)</th>
<th>Whites (n=44)</th>
<th>Blacks (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FELi (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.24</td>
<td>0.27</td>
<td>0.08</td>
</tr>
<tr>
<td>Saline</td>
<td>0.28*</td>
<td>0.32*</td>
<td>0.02</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0.54*</td>
<td>0.41*</td>
<td>0.64*</td>
</tr>
<tr>
<td>FENa (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>-0.05</td>
<td>0.01</td>
<td>-0.13</td>
</tr>
<tr>
<td>Saline</td>
<td>0.17</td>
<td>0.20</td>
<td>-0.13</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0.06</td>
<td>-0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.37*</td>
<td>0.51*</td>
<td>0.39</td>
</tr>
<tr>
<td>Saline</td>
<td>0.28*</td>
<td>0.45*</td>
<td>0.23</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0.30*</td>
<td>0.32*</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Values are mean±SD. FELi, fractional excretion of lithium; FENa, fractional excretion of sodium; MABP, mean arterial blood pressure.

*\(p<0.05\); all significant correlations were direct.
branes of those who are prone to the development of hypertension.

Indirect evidence for increased proximal renal tubular sodium reabsorption in persons genetically susceptible to hypertension was presented by Skrabal and coworkers. We provided compelling data for a relation between erythrocyte Na\(^+\)-Li\(^+\) countertransport and increased renal proximal tubular sodium reabsorption in patients with hypertension and in first-degree relatives of hypertensive individuals. FELi, a marker for proximal tubular sodium reabsorption, was decreased in untreated white hypertensive men. Furthermore, FELi was inversely correlated with red blood cell Na\(^+\)-Li\(^+\) countertransport in Weder's subject population.

The data presented here do not confirm the results reported by Weder. We found that hypertensive patients had increased erythrocyte Na\(^+\)-Li\(^+\) countertransport, which is consistent with Weder's observations and those of others. We also identified a decreased Na\(^+\)-Li\(^+\) countertransport in blacks compared with whites, consistent with previous reports. Our normotensive and hypertensive subjects were in similar states of basal sodium balance, yet we were unable to identify a decrease in FELi in our hypertensive subjects. Their FELi value was increased compared with normotensive individuals, which suggests they had less proximal tubular sodium reabsorption rather than more. Correlations between FELi and erythrocyte Na\(^+\)-Li\(^+\) countertransport were identified both after volume expansion and volume contraction; however, these correlations were direct rather than inverse.

Numerous factors exist that have an important bearing on the measurement of Na\(^+\)-Li\(^+\) countertransport in erythrocytes. Important among these are body weight, body mass index, plasma cholesterol, plasma renin activity, and race. Our subjects differed from Weder's in several respects that include body weight, possibly cholesterol values, and race, which may explain the discrepancies. More importantly, Weder studied young men. His hypertensive subjects had a mean age of 25 years, as opposed to our considerably older subjects. His population did not include blacks or women. The pathogenesis of hypertension in blacks and whites may differ; we have identified a much higher incidence of salt-sensitive high blood pressure in blacks.

The operative mechanism of hypertension may be quite different in young compared with older individuals. We emphasized that hypertension may be initiated by one pathogenetic mechanism and may then be supported by quite another, thereby making the initiating mechanism more difficult to identify. He described differences in young and older hypertensive patients in terms of total body and exchangeable sodium and potassium contents to support this view. It is possible that Weder studied young hypertensive subjects who were in the developmental phase of their disease, whereas in the present study, we studied older, established hypertensive patients whose blood pressure may be elevated by quite different underlying mechanisms. Julius and Schork previously observed that young men with borderline hypertension had an increase in sympathetic nervous system activity. Differences in sympathetic tone could also account for the differences in renal tubular sodium reabsorption observed in our subjects in the present study and those of Weder.

FELi and FENa increased in response to volume loading in our hypertensive subjects to a greater degree than in our normal subjects. The renin values of normal and hypertensive subjects were not significantly different in the present investigation. Eight of our hypertensive subjects had "low renin" hypertension, defined according to criteria we have previously described. Twenty-three subjects had normal renin values. We cannot explain the differences in natriuretic response on the basis of differences in renin values. Recently, Holstein-Rathlou et al used lithium clearance methods to examine mechanisms involved in the exaggerated natriuresis of hypertension. They also found that FELi increased with saline administration in their hypertensive subjects compared with normal individuals. They concluded that the exaggerated natriuresis involved an increased delivery of sodium and water from the proximal to the distal tubule. Our present findings are consistent with this point of view.

Stepwise regression analysis showed that blood pressure was the variable most closely and
directly associated with FELi as well as with FENA and Na"-Li" countertransport. Renin did not enter the relations. It is possible that had we had larger numbers of younger hypertensive patients the relations would have been more revealing.

In conclusion, the present study indicates that patients with established hypertension have higher erythrocyte Na"-Li" countertransport values than normal subjects. White persons have higher Na"-Li" countertransport values than black persons. Hypertensive individuals do not have increased proximal renal tubular reabsorption of sodium as reflected by lithium clearance methodology. The exaggerated natriuresis of hypertension is, at least in part, a function of decreased proximal renal tubular sodium reabsorption. Further studies will be necessary to compare the Na"-Li" countertransport and proximal sodium reabsorption in younger normotensive and hypertensive persons with that in older normotensive and established hypertensive individuals.

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