Sodium-Lithium Countertransport in Ambulatory Hypertensive and Normotensive Patients

STEVEN T. TURNER, ERIC BOERWINKLE, MARK JOHNSON, ELLIOTT RICHELSON, AND CHARLES F. SING

SUMMARY Numerous studies have reported the mean value for Na+-Li+ countertransport to be increased in red blood cells from patients with essential hypertension. Although concomitant variables including age, body size, national origin, geographic location, gender, and family history of hypertension may affect Na+-Li+ countertransport values, most case-control studies have failed to assess the contribution of these factors to the differences in Na+-Li+ countertransport between hypertensive and normotensive groups. The present study was undertaken to provide estimates of Na+-Li+ countertransport in hypertensive and normotensive subjects after taking into account these potentially confounding sources of variation. In 187 subjects undergoing medical evaluation at the Mayo Clinic, Rochester, MN, the combined effects of variation in age, height, and weight accounted for 20.6% of the interindividual variability in Na+-Li+ countertransport. After adjustment to remove variability due to these concomitants, differences in national origin, region of birth, and place of current residence made no additional contribution to variability in this trait. There was no significant difference in mean adjusted Na+-Li+ countertransport between men and women (0.41 ± 0.17 vs 0.40 ± 0.12 [SD] mmol Li efflux/L red blood cells/hr; n = 107). The mean value for adjusted Na+-Li+ countertransport was significantly greater (p < 0.001) in subjects with essential hypertension (0.44 ± 0.15 mmol/L red blood cell/hr; n = 104) compared with normotensive subjects (0.31 ± 0.07 mmol/L red blood cells/hr; n = 39) or subjects with borderline blood pressure elevation (0.35 ± 0.11 mmol/L red blood cells/hr; n = 21). Subjects with a family history of hypertension in at least one parent or full sibling had significantly higher (p < 0.02) Na+-Li+ countertransport values (0.42 ± 0.16 mmol/L red blood cells/hr; n = 111) than those with no family history of hypertension (0.37 ± 0.13 mmol/L red blood cells/hr; n = 76). These results suggest that increased mean Na+-Li+ countertransport in hypertensive subjects in this sample cannot be attributed to confounding effects of variation in age, body size, gender, national origin, birthplace, or residence. Forty-eight percent of subjects with essential hypertension had adjusted Na+-Li+ countertransport values above the range observed in normotensive controls. (Hypertension 9: 24–34, 1987)

KEY WORDS sodium transport • blood pressure • hypertension • red blood cell • biological transport • lithium transport

Among reports of altered in vitro cation transport by red blood cells (RBCs) from patients with essential hypertension, those finding an increase in the maximal rate of Na+-Li+ countertransport1-12 have been of particular interest, because interindividual variation in the capacity of this pathway is influenced by genetic factors.13-15 The observation of increased Na+-Li+ countertransport in normotensive first-degree relatives of patients with essential hypertension1,16,17 has led to a hypothesis that Na+-Li+ countertransport may be a genetically determined biochemical alteration that predisposes one to the development of elevated blood pressure.18-20 The failure to observe increased Na+-Li+ countertransport in some persons with secondary forms of hypertension who report no family history of hypertension1 has been interpreted as further support for this hypothesis. Comparisons of Na+-Li+ countertransport in selected hypertensive and normotensive groups have shown...
considerable variability in the magnitude of average difference and degree of overlap between these groups. A few studies have failed to observe a statistically significant difference between the average values for normotensive controls and subjects with essential hypertension. Phenotypic heterogeneity attributable to genetic differences within the normotensive and hypertensive samples may account for this diversity of results; however, additional factors may also explain the differences among studies. Some reports indicate that age, body size, and gender may be important determinants of Na\(^{+}\)-Li\(^{+}\) countertransport. Effects of ancestral origin or geographical location have also been suggested to account for differences between samples. Furthermore, a common practice of selecting hypertensive subjects from a patient population attending diagnostic or treatment facilities and normotensive controls from nonpatient populations may result in samples with a different range of disease-related factors influencing the frequency distribution of the trait. Few studies comparing Na\(^{+}\)-Li\(^{+}\) countertransport in hypertensive and normotensive subjects have assessed or controlled for these potentially confounding sources of variability.

The objective of the present study was to provide estimates of Na\(^{+}\)-Li\(^{+}\) countertransport in a large sample of patients, including hypertensive and normotensive individuals selected under similar medical circumstances. We assessed the contributions to variability in Na\(^{+}\)-Li\(^{+}\) countertransport by making individual differences in age, body size, gender, national origin, birthplace, residence, and family history of hypertension. Our results confirm the previous reports of significantly greater mean Na\(^{+}\)-Li\(^{+}\) countertransport in subjects with essential hypertension compared with normotensive controls and suggest that the difference cannot be attributed to confounding effects of the concomitant variables studied. Although residual variability in Na\(^{+}\)-Li\(^{+}\) countertransport indicates that persons with essential hypertension are heterogeneous with respect to this phenotype, a large fraction of these patients have Na\(^{+}\)-Li\(^{+}\) countertransport elevated above the range observed in normotensive subjects.

Subjects and Methods

Between May 1982 and November 1982, we recruited 187 ambulatory patients for this study from a population of approximately 2079 patients seen for general medical examinations in the Division of Hypertension, Mayo Clinic, Rochester, MN. Approximately 58% of the persons in this population had a diagnosis of hypertension, and 42% were evaluated for reasons other than hypertension. Seventy percent of the subjects studied were consecutive patients seen by one of us (S.T.T.), and the remainder were referred by seven other physicians. No attempt was made to select patients with regard to any medical or nonmedical characteristics, and the assignment of patients to diagnostic categories (see the following sections) was random with respect to the referring physician (chi square = 28.6, p > 0.10).

The protocol for this study was approved by the institutional review committee of the Mayo Clinic, and informed consent was obtained verbally from each participant before phlebotomy. All subjects were white. No participant was a first-degree relative of any other participant in the study. Before examination, each subject was asked to fill out a questionnaire to provide information regarding birthplace, national origin of parents, and history of hypertension in first-degree relatives. The referring physician then reviewed the subject’s personal and family medical history and performed a complete physical examination. None of the women sampled were pregnant or using oral contraceptive agents. Routine laboratory studies of all patients included serum electrolyte and creatinine determinations, urinalysis, chest roentgenogram, and electrocardiogram. Based on clinical information, subjects were placed into one of the following four diagnostic categories.

Normotensive Group

Individuals in the normotensive group (n = 39) had no prior history of elevated blood pressure, diastolic blood pressure less than 90 mm Hg, and systolic blood pressure less than 140 mm Hg at the time of their examination. They were seen for medical problems unrelated to hypertension or for routine health maintenance. All had normal values for routine laboratory studies, and none had evidence of cardiac or renal failure. One individual was receiving a \(\beta\)-blocker because of premature ventricular contractions.

Borderline Hypertensive Group

The borderline hypertensive group (n = 21) was composed of persons undergoing evaluation because of a prior history of diastolic blood pressure elevation greater than 90 mm Hg on at least one occasion. Recheck of the blood pressure on at least one subsequent occasion (i.e., at the time of our initial evaluation or during the next 3–5 days) demonstrated systolic blood pressure less than 140 mm Hg and diastolic blood pressure less than 90 mm Hg. The blood pressures presented in Results are those measured at the time of the initial examination. Two individuals were treated with a diuretic for indications other than hypertension.

Essential Hypertensive Group

Subjects were diagnosed as definitely hypertensive if the diastolic blood pressure was greater than 90 mm Hg or the systolic blood pressure greater than 140 mm Hg on at least two separate and consecutive occasions or if drug treatment had been administered for elevated blood pressure. Essential hypertension was diagnosed in 104 hypertensive subjects for whom the results of medical history, physical examination, and routine laboratory studies did not suggest a cause of secondary hypertension. None of these patients had evidence of cardiac, renal, or hepatic failure, and all had Grade 2 or milder hypertensive changes on funduscopic exami-
nation. The results of additional screening tests for renovascular disease (e.g., excretory urogram or renal arteriogram) in 58 subjects and tests for adenomadel-
dular disease (e.g., urinary metanephrines or catechola-
mines) in 50 subjects were normal. Ninety-four of the
patients with essential hypertension were receiving an-
thypertensive drug therapy at the time of the study. Of
the 10 subjects not receiving antihypertensive drug
therapy, three had never been treated and the others
had discontinued medications for periods varying from
1 week to 3 years before the study.
Secondary Hypertensive Group
Twenty-three hypertensive patients in whom addi-
tional laboratory data suggested possible cause(s) for
the elevated blood pressure were considered to have
secondary hypertension. Six of these subjects had evi-
dence of occlusive renal artery disease on renal arteri-
ograms; 10 had evidence of renal parenchymal disease
based on excretory urography, ultrasound, or renal
clearance determinations; 3 were taking drugs known
to induce hypertension (estrogen or minocorticoid);
and 4 had diabetes mellitus requiring treatment
with insulin or orally administered hypoglycemic
agents. In all subjects, the underlying conditions were
discovered before or at the initial diagnosis of hyper-
tension. The mean serum creatinine for these subjects
(1.23 mg/dl) was slightly greater (p < 0.005) than that
for other subjects (1.02 mg/dl). Eighteen of the 23
subjects in this group were taking antihypertensive
drugs at the time of this study.
Na⁺-Li⁺ Countertransport Assay
Blood samples anticoagulated with heparin were
drawn from subjects between 0730 and 0900 after they
had fasted overnight. The samples were kept at 0 to
4°C on ice until processing for determination of Na⁺-
Li⁺ countertransport began (always within 2 hours).
All assays for Na⁺-Li⁺ countertransport were per-
formed on the day of phlebotomy.
The maximal velocity (V_{max}) for sodium gradient-
dependent lithium efflux was determined using meth-
ods developed by Canessa and colleagues and modi-

dified slightly by Smith et al.6 This technique uses
RBCs loaded with saturating concentrations of internal
lithium to estimate the V_{max} of Na⁺-Li⁺ countertrans-
port as the difference between rate of lithium efflux
into an external medium containing saturating concen-
trations of sodium ion minus the efflux into a medium
in which sodium has been replaced by magnesium ion.
In brief, plasma and buffy coat were removed from
the RBCs after centrifugation (850 g) of the whole
blood for 5 minutes at 0 to 4°C. Packed erythrocytes
were suspended at an approximate hematocrit of 20%
in a washing solution of 75 mM MgCl₂, 85 mM mannit-	ol, 20 mM glucose, and 10 mM Tris MOPS (pH 7.4 at 37°C).
Batches of 150 mL of the packed cells were incubated for
3 hours at 37°C in a solution of 150 mM lithium chloride,
10 mM glucose, and 10 mM Tris MOPS, pH 7.4 at 37°C.
Rates of lithium efflux were determined by parallel
incubations of lithium-loaded RBCs in solutions con-
taining either sodium or magnesium ion. The sodium
medium consisted of 150 mM NaCl, 10 mM glucose,
10 mM Tris MOPS (pH 7.4 at 37°C), and 0.1 mM ouabain;
150 mM NaCl was replaced by 75 mM MgCl₂
and 85 mM mannitol in the magnesium medium.
Rates of lithium efflux were determined by parallel
incubations of lithium-loaded RBCs in solutions con-
taining either sodium or magnesium ion. The sodium
medium consisted of 150 mM NaCl, 10 mM glucose,
10 mM Tris MOPS (pH 7.4 at 37°C), and 0.1 mM ouabain;
150 mM NaCl was replaced by 75 mM MgCl₂
and 85 mM mannitol in the magnesium medium.
External lithium was removed from the loaded cells by four additional washes. A final suspension of the erythrocytes in washing solu-
tion was maintained at 0 to 4°C for use in the lithium
transport incubations. Hematocrit of the cell suspen-
dation (approximately 50%) was determined by the mi-

Microfuge method.

Lithium concentrations in the efflux media were
measured with an atomic emission spectrophotometer
(Model 357; Instrumentation Laboratory, Allied Ana-
lytical Systems, Waltham, MA, USA), at a wave-
length of 670.8 nm. Solutions of lithium chloride (0,
5, 10, 20, 30, and 40 μM) were prepared in sodium and
magnesium media were used as standards. Lithium
content of each efflux sample was expressed per vol-
ume of RBCs in the sample, and the triplicates at each
time point were averaged. The rate of lithium efflux
was determined by the slope of the linear regression
line with lithium efflux (in millimoles of lithium per
liter of RBCs) as a function of time (in hours). Data
were considered acceptable only when the linear re-
gression line explained greater than 95% of the vari-
ation in lithium efflux. The difference between the
trend of the linear regression lines for lithium efflux in sodi-
un and magnesium media was taken as the estimate of
maximal rate of Na⁺-Li⁺ countertransport in units of
millimoles of lithium per liter of RBCs per hour.

We have reported the standard deviation for dupli-
cate measurements of Na⁺-Li⁺ countertransport in
blood samples from five healthy subjects to be 0.02
mmol/L RBCs/hr or 7.63% expressed as a percent of
the mean value for Na⁺-Li⁺ countertransport in our
laboratory.21 The coefficient of variation estimated
from multiple determinations in the same subjects over
a 3-month period averaged 13.0 ± 2.8%. The validity
of a single measurement to represent a subject's Na⁺-
Li⁺ countertransport level was verified further by re-
measurement of Na⁺-Li⁺ countertransport after inter-
vals from 3.7 to 16.6 months (mean, 5.6 months) in 41
healthy blood donors participating in our previous
study.21 The correlation (r) between the two determina-
tions was 0.80 (p ≤ 0.0001).
Data Analysis

A contingency chi square was used to test for homogeneity across the diagnostic classifications of the frequency distribution of subjects among gender, nationality, birthplace, and residence strata. An analysis of covariance was applied to each of five dependent variables — systolic blood pressure, diastolic blood pressure, lithium efflux into magnesium medium, lithium efflux into sodium medium, and Na\(^+\)-Li\(^+\) countertransport — to determine if the regression on age, age\(^2\), height, height\(^2\), weight, and weight\(^2\) was significantly heterogeneous among the eight sex-diagnostic strata. Blood pressure and lithium efflux data were adjusted to remove variability attributable to the combined first-order and second-order effects of age, height, and weight. Contrasts among linear combinations of means for the strata were made using Scheffe's method to control inflation of Type I errors. Test statistics were considered statistically significant when the associated \(p\) value was 0.05 or less.

Materials

Chemicals and biochemicals of the highest grade of purity were purchased from Sigma Chemical Company (St. Louis, MO, USA). All solutions were prepared in deionized, double-distilled water.

Results

Table 1 presents a description of the sample of hypertensive and normotensive patients; 57.2% of the sample were men and 42.8% were women. There was no significant difference in composition of the four diagnostic groups with respect to gender, ancestral origins, birthplace, or residence of the members. Fifteen percent of the subjects were born in Minnesota, and an additional 57.2% were born in other midwestern states. Approximately 20% of the subjects resided in Minnesota at the time of the study, and an additional 60.9% were living in other midwestern states. Subjects born or residing outside the United States comprised only 8.6% and 3.2% of the sample, respectively.

Height and weight were significantly greater in men than in women for all diagnostic groups (Table 2). Among the men, the group with secondary hypertension was significantly older than the other groups, accounting for the statistically significant variation in age between diagnostic categories (\(F = 3.38, p \leq 0.01\)). An analysis of covariance did not detect significant heterogeneity of the regression of blood pressure or any of the lithium transport variables on age, height, and weight among the eight sex-diagnostic categories.

The combined effects of the concomitants accounted for 10.9% of variation in systolic blood pressure.

<table>
<thead>
<tr>
<th>TABLE 1. Gender, Ancestral Origin, and Geographic Origin of Study Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Parents' national origin</td>
</tr>
<tr>
<td>North American</td>
</tr>
<tr>
<td>European</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Not available</td>
</tr>
<tr>
<td>Birthplace</td>
</tr>
<tr>
<td>Minnesota</td>
</tr>
<tr>
<td>Other Midwest</td>
</tr>
<tr>
<td>Other USA</td>
</tr>
<tr>
<td>Not USA</td>
</tr>
<tr>
<td>Not available</td>
</tr>
<tr>
<td>Residence</td>
</tr>
<tr>
<td>Minnesota</td>
</tr>
<tr>
<td>Other Midwest</td>
</tr>
<tr>
<td>Other USA</td>
</tr>
<tr>
<td>Not USA</td>
</tr>
</tbody>
</table>

Values in parentheses are percentage of pooled sample. There was no significant difference in the composition of the diagnostic classes with respect to gender, nationality of parents, birthplace, or residence.

NT = normotension, BH = borderline hypertension, EH = essential hypertension, SH = secondary hypertension; Other Midwest = North Dakota, South Dakota, Nebraska, Kansas, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, Ohio; Other USA = other states in continental United States; Not USA = outside continental United States.
(p<0.001), 2.8% in diastolic blood pressure (p>0.05), 5.2% in lithium efflux into magnesium medium (p>0.05), 17.1% in lithium efflux into sodium medium (p<0.001), and 20.6% in Na+-Li+ countertransport (p<0.001).

After adjustment for age, height, and weight variation, blood pressure and lithium efflux did not differ significantly between categories of national origin, birthplace, or residence (Table 3). No further adjustment of blood pressure or lithium efflux was done to remove variability attributable to differences in national origin, birthplace, or residence, because of the similarity of means within the strata of these variables (see Table 3) and the similar distribution of the strata among diagnostic classes (see Table 1).

Regardless of sex of the subjects, mean systolic and diastolic blood pressures were significantly greater in each of the three hypertensive groups compared with the normotensive group (Table 4). For the sexes pooled, mean Na+-Li+ countertransport was significantly greater in subjects with essential hypertension (0.44 ± 0.15 mmol/L RBCs/hr; n = 104) and secondary hypertension (0.45 ± 0.17 mmol/L RBCs/hr; n = 23) compared with normotensive controls (0.31 ± 0.07 mmol/L RBCs/hr; n = 39). In 21 subjects with borderline hypertension, mean Na+-Li+ countertransport (0.35 ± 0.11 mmol/L RBCs/hr) was significantly lower than in subjects with essential or secondary hypertension (p<0.05) and not significantly greater than the mean for normotensive subjects.

The differences in Na+-Li+ countertransport between hypertensive and normotensive subjects were due to differences in the component of lithium efflux stimulated by external sodium (see Table 4). The sodium-independent efflux of lithium (i.e., when sodium was replaced by magnesium in the external medium) was also significantly greater in subjects with essential (0.22 ± 0.09 mmol/L RBCs/hr) and secondary hypertension (0.24 ± 0.09 mmol/L RBCs/hr) compared with the normotensive subjects (0.18 ± 0.04 mmol/L RBCs/hr); these increases were small compared with corresponding differences in sodium-stimulated efflux.

There was no significant difference in average Na+-Li+ countertransport between men and women after removal of age, height, and weight effects (see Table 4). Therefore adjusted male and female data were pooled to construct frequency distribution histograms for Na+-Li+ countertransport in each diagnostic group (Figure 1). Although there was considerable overlap between distributions, 48.1% of the subjects with essential hypertension had Na+-Li+ countertransport greater than the highest countertransport value observed for normotensive controls (i.e., 0.427 mmol/L RBCs/hr).

To explore the relationship of family history of hy-
persistence to Na\(^+\)-Li\(^+\) countertransport and blood pressure levels, we contrasted these two variables in subjects with and without a history of hypertension in one or more parents or siblings (Table 5). In the pooled sample, a family history of hypertension predicted greater Na\(^+\)-Li\(^+\) countertransport and blood pressure differences within the essential and secondary hypertension groups, while the association with higher systolic and diastolic blood pressures was due primarily to differences within the normotensive and borderline hypertension groups. The finding of significantly greater mean adjusted Na\(^+\)-Li\(^+\) countertransport in the subjects with essential hypertension compared with normotensive controls (see Figure 1 and Table 4) extends previous reports by establishing that the difference cannot be attributed to interindividual differences in age, body size, national origin, birthplace, residence, or gender.

In this sample, Na\(^+\)-Li\(^+\) countertransport correlated significantly with age (r = -0.24, p<0.01), height (r = 0.20, p<0.01), and weight (r = 0.42, p<0.01), and 20.6% of the interindividual variability in Na\(^+\)-Li\(^+\) countertransport could be attributed to the combined effects of these concomitant variables. Contrary to the hypothesis that ancestral background or geographical location might be predictive of Na\(^+\)-Li\(^+\) transport, the present study was undertaken to provide estimates of average Na\(^+\)-Li\(^+\) countertransport and its frequency distribution in hypertensive and normotensive subjects after controlling for extraneous sources of variability that might confound comparisons between these groups. The finding of significantly greater mean adjusted Na\(^+\)-Li\(^+\) countertransport in the subjects with essential hypertension compared with normotensive controls (see Figure 1 and Table 4) extends previous reports by establishing that the difference cannot be attributed to interindividual differences in age, body size, national origin, birthplace, residence, or gender.

In this sample, Na\(^+\)-Li\(^+\) countertransport correlated significantly with age (r = -0.24, p<0.01), height (r = 0.20, p<0.01), and weight (r = 0.42, p<0.01), and 20.6% of the interindividual variability in Na\(^+\)-Li\(^+\) countertransport could be attributed to the combined effects of these concomitant variables. Contrary to the hypothesis that ancestral background or geographical location might be predictive of Na\(^+\)-Li\(^+\) countertransport levels, we conducted an independent and additional contribution to the prediction of mean Na\(^+\)-Li\(^+\) countertransport levels after adjusting for dif-

### Table 3. Effect of National Origin, Birthplace, and Residence on Blood Pressure and Lithium Efflux

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood pressure (mm Hg)</th>
<th>Mg medium</th>
<th>Na medium</th>
<th>Na(^+)-Li(^+) countertransport</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>North American</td>
<td>27</td>
<td>149±22</td>
<td>92±11</td>
<td>0.214±0.083</td>
</tr>
<tr>
<td>European</td>
<td>80</td>
<td>146±23</td>
<td>92±12</td>
<td>0.213±0.078</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>142±24</td>
<td>91±20</td>
<td>0.226±0.041</td>
</tr>
<tr>
<td>Not available</td>
<td>77</td>
<td>146±18</td>
<td>90±11</td>
<td>0.209±0.087</td>
</tr>
<tr>
<td>F</td>
<td>0.17</td>
<td>0.67</td>
<td>0.07</td>
<td>1.24</td>
</tr>
<tr>
<td>P</td>
<td>0.92</td>
<td>0.57</td>
<td>0.97</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Birthplace</th>
<th>Blood pressure (mm Hg)</th>
<th>Mg medium</th>
<th>Na medium</th>
<th>Na(^+)-Li(^+) countertransport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>28</td>
<td>142±19</td>
<td>91±11</td>
<td>0.216±0.076</td>
</tr>
<tr>
<td>Other Midwest</td>
<td>107</td>
<td>147±21</td>
<td>92±12</td>
<td>0.205±0.075</td>
</tr>
<tr>
<td>Other USA</td>
<td>29</td>
<td>148±24</td>
<td>89±12</td>
<td>0.231±0.118</td>
</tr>
<tr>
<td>Not USA</td>
<td>16</td>
<td>148±17</td>
<td>92±12</td>
<td>0.216±0.060</td>
</tr>
<tr>
<td>Not available</td>
<td>7</td>
<td>149±17</td>
<td>93±10</td>
<td>0.207±0.068</td>
</tr>
<tr>
<td>F</td>
<td>0.37</td>
<td>0.45</td>
<td>0.65</td>
<td>0.68</td>
</tr>
<tr>
<td>p</td>
<td>0.828</td>
<td>0.770</td>
<td>0.630</td>
<td>0.606</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residence</th>
<th>Blood pressure (mm Hg)</th>
<th>Mg medium</th>
<th>Na medium</th>
<th>Na(^+)-Li(^+) countertransport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>37</td>
<td>144±18</td>
<td>92±10</td>
<td>0.219±0.084</td>
</tr>
<tr>
<td>Other Midwest</td>
<td>114</td>
<td>147±21</td>
<td>91±12</td>
<td>0.209±0.084</td>
</tr>
<tr>
<td>Other USA</td>
<td>30</td>
<td>147±24</td>
<td>90±13</td>
<td>0.205±0.068</td>
</tr>
<tr>
<td>Not USA</td>
<td>6</td>
<td>145±23</td>
<td>96±15</td>
<td>0.245±0.084</td>
</tr>
<tr>
<td>Not available</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.23</td>
<td>0.34</td>
<td>0.55</td>
<td>1.99</td>
</tr>
<tr>
<td>P</td>
<td>0.872</td>
<td>0.796</td>
<td>0.648</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Values are means ± SD. The effects of interindividual variability in age, height, and weight were removed from the data by adjustment to the grand means for the pooled sample (i.e., age, 55.3 years; height, 166.9 cm; weight, 78.4 kg).

Other Midwest = North Dakota, South Dakota, Nebraska, Kansas, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, Ohio; Other USA = other states in continental United States; Not USA = outside continental United States.
**Table 4: Blood Pressure and Lithium Efflux After Adjustment to Remove Age, Height, and Weight Effects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>NT (n = 39)</th>
<th>BH (n = 21)</th>
<th>EH (n = 104)</th>
<th>SH (n = 23)</th>
<th>Pooled (n = 187)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>132 ± 15</td>
<td>152 ± 11*</td>
<td>150 ± 21*</td>
<td>153 ± 29*</td>
<td>147 ± 21</td>
<td>6.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>124 ± 16</td>
<td>149 ± 15*</td>
<td>151 ± 17*</td>
<td>154 ± 21*</td>
<td>146 ± 20</td>
<td>8.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pooled</td>
<td>128 ± 16</td>
<td>151 ± 12*</td>
<td>151 ± 19*</td>
<td>154 ± 25*</td>
<td>146 ± 16</td>
<td>14.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>1.65</td>
<td>0.19</td>
<td>0.11</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.020</td>
<td>0.667</td>
<td>0.742</td>
<td>0.965</td>
<td>0.230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>83 ± 9</td>
<td>92 ± 6*</td>
<td>95 ± 11*</td>
<td>97 ± 14*</td>
<td>92 ± 12</td>
<td>8.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>76 ± 5</td>
<td>93 ± 13*</td>
<td>93 ± 10*</td>
<td>95 ± 11*</td>
<td>90 ± 12</td>
<td>11.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pooled</td>
<td>80 ± 8</td>
<td>92 ± 10*</td>
<td>94 ± 11*</td>
<td>96 ± 12*</td>
<td>91 ± 12</td>
<td>18.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>3.53</td>
<td>0.06</td>
<td>0.79</td>
<td>0.17</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.062</td>
<td>0.802</td>
<td>0.376</td>
<td>0.684</td>
<td>0.293</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li efflux into Mg medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L RBCs/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.186 ± 0.045</td>
<td>0.200 ± 0.058†</td>
<td>0.222 ± 0.081</td>
<td>0.265 ± 0.112*</td>
<td>0.216 ± 0.079</td>
<td>2.92</td>
<td>0.031</td>
</tr>
<tr>
<td>Women</td>
<td>0.162 ± 0.037</td>
<td>0.203 ± 0.079</td>
<td>0.218 ± 0.101*</td>
<td>0.214 ± 0.048</td>
<td>0.205 ± 0.085</td>
<td>1.90</td>
<td>0.17</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.177 ± 0.043</td>
<td>0.201 ± 0.066</td>
<td>0.220 ± 0.090*</td>
<td>0.241 ± 0.090*</td>
<td>0.212 ± 0.082</td>
<td>4.03</td>
<td>0.003</td>
</tr>
<tr>
<td>F</td>
<td>0.88</td>
<td>0.01</td>
<td>0.08</td>
<td>2.34</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.35</td>
<td>0.928</td>
<td>0.773</td>
<td>0.128</td>
<td>0.330</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li efflux into Na medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L RBCs/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.494 ± 0.088</td>
<td>0.516 ± 0.109‡</td>
<td>0.675 ± 0.221*</td>
<td>0.749 ± 0.274*</td>
<td>0.625 ± 0.214</td>
<td>9.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>0.473 ± 0.074</td>
<td>0.605 ± 0.160</td>
<td>0.635 ± 0.157*</td>
<td>0.637 ± 0.127*</td>
<td>0.602 ± 0.153</td>
<td>3.38</td>
<td>0.008</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.486 ± 0.083</td>
<td>0.554 ± 0.137†</td>
<td>0.657 ± 0.196*</td>
<td>0.695 ± 0.219*</td>
<td>0.615 ± 0.190</td>
<td>11.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>0.13</td>
<td>1.33</td>
<td>1.31</td>
<td>2.37</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.719</td>
<td>0.250</td>
<td>0.253</td>
<td>0.125</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺-Li⁺ countertransport</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L RBCs/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.308 ± 0.072</td>
<td>0.316 ± 0.088‡</td>
<td>0.453 ± 0.178*</td>
<td>0.484 ± 0.198*</td>
<td>0.408 ± 0.168</td>
<td>9.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>0.312 ± 0.081</td>
<td>0.402 ± 0.121</td>
<td>0.417 ± 0.116*</td>
<td>0.422 ± 0.131*</td>
<td>0.396 ± 0.118</td>
<td>2.32</td>
<td>0.073</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.309 ± 0.075</td>
<td>0.353 ± 0.110‡</td>
<td>0.437 ± 0.155*</td>
<td>0.455 ± 0.169*</td>
<td>0.403 ± 0.149</td>
<td>9.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>0.01</td>
<td>1.97</td>
<td>1.71</td>
<td>1.13</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.933</td>
<td>0.162</td>
<td>0.193</td>
<td>0.289</td>
<td>0.600</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. The data were adjusted for age, height, and weight.

*NT = normotension, BH = borderline hypertension, EH = essential hypertension, SH = secondary hypertension.

p < 0.05, significantly greater than NT group.

†p < 0.05, significantly less than SH group.

‡p < 0.05, significantly less than EH and SH groups.

ferences due to age and body size we detected no statistically significant variation in mean Na⁺-Li⁺ countertransport associated with differences in national origin, birthplace, or place of residence (see Table 3). Furthermore, adjusted Na⁺-Li⁺ countertransport values did not differ between men and women in this sample or in the sample studied by Williams et al., which also controlled for body size. Hence, greater weight and body size may account for the findings by others of significantly greater average unadjusted Na⁺-Li⁺ countertransport in normotensive or hypertensive men compared with women. In view of the rela-
tively large contribution that age, height, and weight made to Na+-Li+ countertransport variability in this sample, the diversity in estimates of Na+-Li+ countertransport from other hypertensive and normotensive samples may result in part from a failure to control for heterogeneous composition of the samples with respect to these covariates.

Although variability in Na+-Li+ countertransport was significantly reduced by adjustment for the covariables considered here, considerable unexplained variation remained, and there was broad overlap in the Na+-Li+ countertransport distributions for hypertensive and normotensive subjects (see Figure 1). Fifty-two percent of patients with essential hypertension had Na+-Li+ countertransport within the range of values for normotensive subjects. Failure of the Na+-Li+ countertransport phenotype to distinguish every person with essential hypertension has led some to conclude that it cannot be a useful marker for the disease. Such a view, however, ignores the multifactorial etiology of essential hypertension. Na+-Li+ countertransport may be only one of the genetically determined factors contributing to blood pressure variability. Because there are many other contributing factors, both genetic and environmental, not all persons with essential hypertension are likely to manifest increased Na+-Li+ countertransport. From this perspective, it is indeed remarkable that such a large proportion of persons with essential hypertension have abnormal values of this single biochemical trait.

The source or methods for selection of subjects have not been clearly stated in many previous studies comparing Na+-Li+ countertransport in hypertensive and normotensive groups. We sampled normotensive subjects from the same clinical practice as our hypertensive patients to minimize extraneous variability in Na+-Li+ countertransport that may be attributable to selection of groups from different medical circumstances. Patients with essential hypertension, in particular, were selected without regard to specific characteristics of their disease to reflect the heterogeneity within this diagnostic category. Frequency distributions of age and region of residence in our sample did not differ from those estimated for the overall population of adult patients examined at the Mayo Clinic (unpublished data, 1982). Moreover, average heights were similar to those reported for large sex-matched and age-matched samples from the general population. Thus, we expect the average levels of Na+-Li+ countertransport derived from this sample to be representative of normotensive and hypertensive white adults attending similar medical facilities.

Na+-Li+ countertransport tended to be increased in the subjects with borderline blood pressure in this sample (see Table 5), in agreement with other reports. The mean adjusted levels of both Na+-Li+ countertransport and blood pressure showed an increasing trend among the normotensive, borderline hypertensive, and definitely hypertensive groups (see Table 3). This association of greater mean Na+-Li+ countertransport with higher mean blood pressure is consistent with modest, positive correlations between Na+-Li+ countertransport and systolic or diastolic blood pressure reported in samples of normotensive and untreated hypertensive subjects.

In contrast to reports that Na+-Li+ countertransport is uniformly normal in patients with secondary hypertension, we observed broad distribution of adjusted Na+-Li+ countertransport among patients with secondary causes for hypertension (see Figure 1), and the mean value was no different from that in patients with essential hypertension (see Figure 1). The inability of our diagnostic criteria to rule out coexistent essential hypertension may account for this unexpected finding, although cure of hypertension has not been a criterion for diagnosis of secondary hypertension in other studies. Since elevated Na+-Li+ countertransport has been observed in other patients with secondary hypertension, it seems prudent to delay conclusions regarding Na+-Li+ countertransport in this heterogeneous diagnostic category until larger cohorts can be studied both before and after cure of hypertension.

In the pooled sample, the finding of significantly greater Na+-Li+ countertransport and blood pressure in subjects reporting a history of hypertension in one or more of their first-degree relatives (see Table 5) sup-

---

**Figure 1.** Frequency distributions of sodium-lithium countertransport in the four diagnostic classes studied. The data for males and female subjects were pooled after adjustment for age, height, and weight.
TABLE 5. Family History of Hypertension and Blood Pressure and Lithium Efflux

<table>
<thead>
<tr>
<th>Variable</th>
<th>Family history of hypertension</th>
<th>Diagnostic class</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects*</td>
<td>Positive</td>
<td>12</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>Positive</td>
<td>138 ± 17</td>
<td>153 ± 11</td>
<td>149 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>Positive</td>
<td>85 ± 8</td>
<td>93 ± 11</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>Li efflux into Mg medium (mmol/L RBCs/hr)</td>
<td>Positive</td>
<td>0.185 ± 0.033</td>
<td>0.204 ± 0.075</td>
<td>0.222 ± 0.097</td>
</tr>
<tr>
<td>Li efflux into Na medium (mmol/L RBCs/hr)</td>
<td>Positive</td>
<td>0.488 ± 0.088</td>
<td>0.561 ± 0.162</td>
<td>0.671 ± 0.203</td>
</tr>
<tr>
<td>Na⁺-Li⁺ countertransport (mmol/L RBCs/hr)</td>
<td>Positive</td>
<td>0.303 ± 0.080</td>
<td>0.356 ± 0.123</td>
<td>0.449 ± 0.153</td>
</tr>
</tbody>
</table>

Values for blood pressure and lithium efflux are means ± SD. Data were adjusted for age, height, and weight.

NT = normotension, BH = borderline hypertension, EH = essential hypertension, SH = secondary hypertension.

*Number of subjects with positive or negative family history of hypertension in each diagnostic class. The chi-square statistic (3 degrees of freedom) for heterogeneity between diagnostic classes in the proportion of subjects with positive and negative family history of hypertension was 25.4 (p < 0.001).

ports the hypothesis that variability in Na⁺-Li⁺ countertransport correlates with the familial predisposition to essential hypertension. It is likely that antihypertensive drug therapy in most of the patients with essential and secondary hypertension lowered their blood pressures and may have obscured differences otherwise attributable to family history of hypertension in these subgroups. In studies such as this, relying only on hearsay information to determine family medical history, the increase in mean Na⁺-Li⁺ countertransport associated with a family history of hypertension has been small and, in some instances, not statistically significant. However, studies of the relatives of persons ascertained because of the presence or absence of essential hypertension corroborate the association (i.e., significantly greater Na⁺-Li⁺ countertransport is found in relatives of persons in whom the diagnosis of essential hypertension has been verified by actual examination but not in relatives of persons in whom normal blood pressure readings have been confirmed). Considering the multitude of factors that may contribute to blood pressure variability and the imprecision in our ascertainment of the family history of hypertension, it is not surprising that differences in mean blood pressures and Na⁺-Li⁺ countertransport reached statistical significance only in the large pooled sample. Nevertheless, the consistency of the associations between increased Na⁺-Li⁺ countertransport and family background of hypertension, higher blood pressure levels, and presence of essential hypertension points to Na⁺-Li⁺ countertransport as one of the few known candidates for biochemical phenotypes associated with blood pressure variability.

In summary, this study contributes to the growing body of evidence that sodium ion transport is altered at...
the cellular level in many hypertensive persons. Further investigation is required to elucidate pathophysiological implications of increased Na+/Li+ countertransport in the RBC and to identify additional factors responsible for differences in this trait between hypertensive and normotensive persons. Studies of samples representative of the general population should be designed to determine whether information regarding the Na+/Li+ countertransport phenotype can help predict the occurrence of essential hypertension. Thus, we have undertaken a study of multigeneration families to estimate the contribution that genetic factors determining interindividual differences in Na+/Li+ countertransport make to the prediction of essential hypertension. Preliminary studies suggest that a small but significant fraction of blood pressure variability may be attributable to a hypothesized single genetic factor responsible for bimodality in the Na+/Li+ countertransport distribution.41

Acknowledgments
The authors thank members of the Division of Hypertension, Mayo Clinic, who referred patients for this study. The Typing Service provided secretarial assistance.

References
33. Boerwinkle E, Turner ST, Weinsilboum R, Johnson M,
Richelson E, Sing CF. Genetic analysis of the distribution of sodium lithium countertransport in a sample representative of the general population. Genetic Epidemiology 1986;3:365-368
Sodium-lithium countertransport in ambulatory hypertensive and normotensive patients.
S T Turner, E Boerwinkle, M Johnson, E Richelson and C F Sing

Hypertension. 1987;9:24-34
doi: 10.1161/01.HYP.9.1.24
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 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/9/1/24

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/