Studies finding an increased maximal rate of Na-Li countertransport in red blood cells from persons with essential hypertension and their normotensive offspring have raised the possibility that Na-Li countertransport may serve as a marker for the genetic predisposition to hypertension. We studied Na-Li countertransport in 238 randomly selected blood donors representative of the population of Rochester, Minnesota. The mean value (± SD) for Na-Li countertransport in units of mmoles of lithium efflux per liter of red blood cells per hour was 0.29 ± 0.12. The distribution of Na-Li countertransport values among the donors was continuous. An analysis for multimodality, however, detected significant evidence of bimodality with 72% of the population predicted to belong to the lower mode with a mean of 0.24 mmol/L red blood cells per hour and 28% of the population to belong to the upper mode with a mean of 0.42 mmol/L red blood cells per hour. There was a positive association between Na-Li countertransport and blood pressure; after adjustment for weight and age, Na-Li countertransport predicted approximately 3% of the variation in blood pressure. Persons belonging to the upper mode of the Na-Li countertransport distribution may be at increased risk of acquiring elevated blood pressure as they age. (Hypertension 7: 955-962, 1985)

KEY WORDS • red blood cell • membrane transport • hypertension
We also estimated the contribution of gender, weight, and age to the normal variability of this trait and determined its relation to blood pressure.

Subjects and Methods

The 238 subjects studied were randomly selected from a total of approximately 9000 adult blood donors at the Mayo Clinic in Rochester, Minnesota, between October 1981 and August 1982. More than 90% of the blood donors at the Mayo Clinic live within the city or its surrounding rural areas encompassing a 50-mile radius. The population of this area is 98% white and primarily of northern European descent. All of the subjects in this study were white, and no donor was sampled more than once.

A medical history was taken from all potential donors, and a limited examination was conducted to evaluate each person’s general health status. Persons with histories of acute or chronic illnesses were excluded from blood donation, as were pregnant women and women during their menses. Administration of drugs (e.g., antihypertensive agents) other than oral contraceptive agents was cause for exclusion. Donors were required to be afebrile (i.e., oral temperature < 37.5 °C), have a regular pulse at a rate between 50 and 100 beats/min, and weigh at least 50 kg. All donors had eaten within 4 hours before blood drawing.

Blood pressures were taken and recorded before blood donation by a blood bank nurse who had no knowledge of which donors would be selected for our study nor any information regarding the nature of the study. All blood pressures were taken in the left arm after the donor had been sitting quietly for at least 5 minutes. Depending on arm size, standard or large adult blood pressure cuffs (Baumanometer, W. A. Baum Co., Inc., Copiague, NY, USA) were used to ensure that the cuff covered at least two-thirds of the upper arm and the bladder encompassed most of the circumferences of the arm without overlapping. Pressures were read on an aneroid sphygmomanometer (Littman Wall Model 2224, Medical Products Division/3M, St. Paul, MN, USA) that was calibrated weekly with a mercury manometer to ensure accuracy within plus or minus 2 mm Hg. Korotkoff phase I and V sounds were recorded as the systolic and diastolic blood pressures respectively. Persons with systolic blood pressure less than 90 mm Hg or greater than 190 mm Hg or a diastolic blood pressure less than 50 mm Hg or greater than 100 mm Hg were not accepted as donors.

Sodium-Lithium Countertransport Assay

Blood samples anticoagulated with heparin were drawn from the donors between 0600 and 0900 hours, and all measurements of Na-Li countertransport were made on the day of phlebotomy. The Na-Li countertransport was determined as the rate of sodium-gradient-dependent lithium efflux from lithium-loaded RBCs using methods previously described in detail by Canessa and Tosteson and Smith et al. The RBCs were washed four times in a solution consisting of 75 mM magnesium chloride, 85 mM mannitol, 10 mM glucose, and 10 mM tris(hydroxymethyl)aminomethane (morpholino)propanesulfonic acid (Tris-MOPS), pH = 7.4. For loading with lithium, the washed RBCs were suspended at a hematocrit of approximately 20% in a solution consisting of 150 mM lithium chloride, 10 mM glucose, and 10 mM Tris-MOPS, pH = 7.4. This cell suspension was incubated in a shaking water bath for 3 hours at 37 °C. After loading, the cells were washed four times in the washing solution to remove external lithium. A final suspension of RBCs in washing solution (approximately 50% hematocrit) was prepared and kept at 4 °C for the lithium efflux measurements. The hematocrit of this RBC suspension was determined by the microhematocrit method.

To measure lithium efflux, the lithium-loaded RBCs were incubated in parallel in solutions containing either 150 mM sodium chloride, 85 mM mannitol, 10 mM glucose, 10 mM Tris-MOPS (pH = 7.4), and 0.1 mM ouabain (sodium medium) or 75 mM magnesium chloride, 85 mM mannitol, 10 mM glucose, 10 mM Tris-MOPS (pH = 7.4), and 0.1 mM ouabain (magnesium medium). The cold RBC suspension was pipetted (in 0.15-ml aliquots) into nine microfuge tubes, each containing 1.35 ml of sodium medium, and nine microfuge tubes, each containing 1.35 ml of magnesium medium at 4 °C, and the transport assay was started by placing the microfuge tubes in a shaking water bath at 37 °C. At 10 minutes, 20 minutes, and 30 minutes three tubes containing sodium medium and three tubes containing magnesium medium were removed from the water bath, cooled quickly to 4 °C in an ice water bath, and centrifuged at 10,000 g for 1 minute in a Beckman microfuge (Model 12; Beckman Instruments, Inc., Palo Alto, CA, USA) at 4 °C. The supernatant was quickly pipetted off the cell pellet and saved for triplicate determinations of lithium concentration in the two types of media.

Lithium concentrations in the efflux media were determined using an Instrumentation Laboratory atomic emission spectrophotometer (Model 357; Lexington, MA, USA) at a wavelength of 670.8 nm. Lithium concentrations in sodium media were read against standard solutions of lithium chloride (0.5, 10, 20, 30, and 40 mM) prepared in sodium medium, and lithium concentrations in the magnesium-containing samples were read against standard solutions of lithium chloride prepared in magnesium medium. The lithium content of each efflux sample was expressed per volume (in liters) of RBC in the sample, and the triplicate determinations at each time point were averaged. The lithium efflux rates for RBCs incubated in sodium and magnesium media were taken as the slopes of the linear regression lines calculated with lithium efflux (in units of mmoles of lithium per liter of RBC) as a function of time (hours). The relation between lithium efflux and time was linear in sodium and magnesium media. The data were considered acceptable only when the regression lines explained greater than 95% of the variation in lithium efflux. The difference be-
between the slope of the regression lines for sodium and magnesium samples was taken as the measure of Na-Li countertransport expressed in units of mmoles per liter of RBC per hour.

The reliability of the Na-Li countertransport assay in our laboratory was tested previously for duplicate blood samples drawn from five healthy subjects and analyzed the same day. The technical error in measuring Na-Li countertransport was 0.02 mmol/L RBC/hr, or 7.63% expressed as a percentage of the mean value for Na-Li countertransport. The stability over time of Na-Li countertransport was assessed by three to five measurements during a 3-month period in the same five subjects. The coefficient of variation averaged 13.0 ± 2.8% in these subjects. These levels for technical error and intra-individual variation in Na-Li countertransport are similar to those reported by others using the same methods.

Analysis of Data

Multiple linear regression was used to estimate the contribution of weight, age, and gender to the sample variability in Na-Li countertransport. The method of Day was employed to fit multiple normal distributions to the data after adjustment for weight, age, and gender differences by the best-fitting linear regression model. The association of Na-Li countertransport and blood pressure was estimated by product-moment correlation, rank correlation, and the method of association arrays. The latter is a non-parametric alternative method that estimates the monotonic relationship between two random variables and is not restricted to the linear dependence implicit in product-moment correlation. Statistical tests were considered significant if the values were less than or equal to 0.05.

Materials

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

Results

The descriptive statistics for the sample of blood donors selected for this study are presented in Table 1, and the frequency distribution histogram for Na-Li countertransport is shown in Figure 1. The average value for Na-Li countertransport (± SD) in the pooled group of 238 donors was 0.29 ± 0.12 mmol/L RBC/hr. Male donors had significantly greater Na-Li countertransport than did female donors (Table 1). The standard deviations did not differ significantly between male and female donors for any of the variables measured.

Associations between Na-Li countertransport and weight, age, and blood pressure were evaluated separately for each sex (Table 2). Measurements of all four variables were available in a total of 209 of the 238 blood donors. The associations of Na-Li countertransport with the other variables were not consistent between the sexes. Among female donors, Na-Li countertransport was significantly correlated with weight and systolic and diastolic blood pressure. Among male donors, Na-Li countertransport showed no significant associations with the other measured variables. Considered individually, the correlation coefficients between Na-Li countertransport and systolic blood pressure, weight and systolic pressure, and weight and age were significantly greater in female than in male donors. The anticipated associations between systolic and diastolic pressures, weight, and age were observed.

We next estimated the extent to which variability in Na-Li countertransport was attributable to the gender difference and to weight and age variability. The difference between the male and female donor means accounted for 2.8% of the total Na-Li countertransport...
TABLE 2. Matrix of Pearson Product-Moment Correlation Coefficients Between Variables Measured in Male and Female Blood Donors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 123)</th>
<th>Women (n = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-Li countertransport</td>
<td>—</td>
<td>0.24*</td>
</tr>
<tr>
<td>Weight</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.03†</td>
<td>0.17†</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.03†</td>
<td>0.17†</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.11</td>
<td>0.33*</td>
</tr>
</tbody>
</table>

BP = blood pressure.

*Significant difference between correlation coefficients for male and female donors at p < 0.05.

The frequency distribution histogram for the weight-adjusted, age-adjusted, and gender-adjusted Na-Li countertransport values in the 209 blood donors is shown in Figure 2. The distribution is skewed toward higher values, and an analysis of multimodality rejected the hypothesis of a single gaussian distribution to explain the adjusted Na-Li countertransport measurements (chi square = 13.96, 2 degrees of freedom, p < 0.0001). Analysis of raw or square root transformed data did not alter the conclusion that two distributions fit better than a single distribution. The best-fitting theoretical distributions are shown superimposed on the frequency distribution histogram in Figure 2.

The maximum likelihood estimates of the two means, one proportion, and one standard deviation to describe the dispersion within each mode are given for the pooled data and for each gender separately in Table 3. There was no significant difference between the sexes for the estimates of the parameters of bimodality after correction for the mean difference between genders. A model including separate standard deviations for each mode did not improve the fit of the two-mode model in any case. The analysis predicted that 72% of the population from which this sample was drawn belongs to the first distribution with the lower mean for Na-Li countertransport of 0.24 mmol/L RBC/hr, and 28% of the population belongs to the second distribution with the higher mean of 0.42 mmol/L RBC/hr (Table 3).

We explored the relationship between weight-adjusted and age-adjusted Na-Li countertransport and blood pressure in each gender using three different measures of association. First, using the Pearson product-moment correlation, we noted a low but marginally significant correlation between Na-Li countertransport and systolic (r = 0.23, p < 0.05) and diastolic blood pressure (r = 0.19, p < 0.08) only among female donors. We estimated that 3.5% of the weight-adjusted and age-adjusted systolic blood pressure and 2.4% of the weight-adjusted and age-adjusted diastolic blood pressure in female donors were attributable to variation in Na-Li countertransport. This finding compares with 34% and 36% attributable to age and weight for systolic and diastolic blood pressure respectively. Because of bimodality of Na-Li countertransport, we also considered a nonparametric rank correlation procedure that only assumes that the distributions for Na-Li countertransport and blood pressure are continuous. The general inferences from this strategy were consistent with those derived from the product-moment correlation (i.e., low but statistically signifi-
significant correlations between Na-Li countertransport and both systolic and diastolic blood pressures in female donors).

Since these two correlation methods (product-moment and rank) estimate linear dependence between two random variables, we further considered an association array technique that enables one to explore the nonlinear dependence between blood pressure and Na-Li countertransport and the heterogeneity of association over the range of the Na-Li countertransport distribution. In female donors, the association of Na-Li countertransport with systolic and diastolic blood pressures was consistent over the entire range of values. In male donors, however, the association arrays indicated that a positive association between Na-Li countertransport and systolic and diastolic blood pressure was present only for higher values of Na-Li countertransport.

Discussion

The purpose of this study was to describe the normal interindividual variation of Na-Li countertransport, to estimate the contribution of gender, weight, and age to the observed variability, and to examine the association of Na-Li countertransport with blood pressure in the population at large. Early investigations suggested that Na-Li countertransport was diminished in a subpopulation of patients with manic-depressive illness, and more recent reports indicate that it is continuously distributed in the population. Based on observations in selected groups of normotensive and hypertensive subjects, which suggests that sex-specific factors may affect Na-Li countertransport. The small contribution of weight to normal Na-Li countertransport variation in female donors may also reflect the presence of gender-specific determinants of Na-Li countertransport. Age was not significantly correlated with Na-Li countertransport in either sex (Table 2) and made no significant contribution to variability in Na-Li countertransport once the effects of weight were accounted for. Although longitudinal follow-up data are not available, cross-sectional observations from this and other studies suggest that Na-Li countertransport in adults is not altered with aging.

Additional factors that affect Na-Li countertransport and could contribute to interindividual variation include racial differences, hemodialysis treatment in patients with end-stage renal disease, and pregnancy. However, none of these variables was a consideration in our study. Environmental factors demonstrated to have little or no effect on Na-Li countertransport include fasting versus fed state, low versus high sodium intake, and treatment with antihypertensive drugs. Women taking oral contraceptive pills were not excluded from our study since previous reports suggested that pill ingestion does not affect Na-Li countertransport. We were unable to detect a significant difference in Na-Li countertransport between the subgroup of women taking the pill (0.29 ± 0.09 mmol/L RBC/hr; n = 11) and those not (0.26 ± 0.10 mmol/L RBC/hr; n = 83); nevertheless, a confounding influence cannot be entirely ruled out for those women using the pill in this study.

Analysis of this large, randomly selected sample of healthy persons indicates that Na-Li countertransport is continuously distributed in the population. Based on the analysis of bimodality (Figure 1; Table 3), however, there is statistically significant evidence for two groups within the population. The hypothesis that the two modes for Na-Li countertransport truly represent subpopulations is supported by the fact that the estimated mean Na-Li countertransport values of the two distributions were very close to those reported in a number of studies of normotensive controls and sub-
jects with EH (Table 4). There is remarkable agree-
ment among the estimates of the means of the normo-
tensive groups and of the average of these with the
group of persons hypothesized to belong to the lower
mode of the Na-Li countertransport distribution rep-
resentative of the randomly ascertained Rochester
sample. Although estimates of the mean for selected
hypertensive groups vary, in most studies they are
significantly increased relative to that of normotensive
controls and their average agrees closely with the mean
for the higher mode estimated from the randomly as-
certained Rochester sample. Even though donors with
known hypertension were not included in our sample,
some may have had unrecognized borderline hyperten-
sion and undoubtedly many were relatives of persons
with EH. Since Na-Li countertransport is increased in
some persons with mildly elevated blood pressure24-26
and in some normotensive first-degree relatives of
persons with EH,5,7,9 we might have predicted that our
sample would consist of two subpopulations with sig-
nificantly different Na-Li countertransport. Long-term
follow-up will be required to determine whether the
donors with elevated Na-Li countertransport values
have an increased risk of acquiring overt EH as they
age.

The evidence suggesting bimodality in the popula-
tion is of interest in view of the reports indicating that
much of the interindividual variation in Na-Li counter-
transport is determined by genetic factors.24-36-38 Al-
though bimodality is not proof of a genetic factor influ-
encing a trait,37 it does suggest the possibility that a
single gene having a major effect on the trait is in-
volved. Preliminary analyses of Na-Li countertrans-
port by our group in 52 randomly selected healthy
nuclear families38 have detected evidence for segrega-
tion of an autosomal locus with a major effect on Na-Li
countertransport, which accounts for just over 50% of
its variability. Estimates of the parameters of the distri-
bution for Na-Li countertransport in the population
based on this family study agree closely with our find-
ings in the randomly selected blood donors and further
support the validity of the bimodality analysis.

The multiplicity of reports of significantly higher
Na-Li countertransport in hypertensive persons com-
pared with that in normotensive controls raises the
question of whether there is a continuous incremental
relation between these two variables in the population
at large. Unfortunately, to our knowledge, no study
has related Na-Li countertransport to blood pressure in
a randomly selected sample in which subjects with the
full range of blood pressure are represented. The find-
ing by Trevisan et al.10 of a significant positive correla-
tion between Na-Li countertransport values and dia-
stolic blood pressure levels among a selected group of
middle-aged men (including both normotensive sub-
jects and untreated hypertensive subjects) is consistent
with a continuous linear relation between these two
variables. Our random sample was truncated by the
exclusion of persons with diagnosed hypertension, but
it suggests a continuous relation between Na-Li coun-
tertransport and blood pressure throughout the range of
these variables only in women. In men, a positive
relationship between Na-Li countertransport and blood
pressure was detected only for higher values of Na-Li
countertransport using the method of association ar-
rays.23 This analysis suggests that although men and
women are heterogeneous for the linear relationship
throughout the distributions of the variable, they are
concordant for a positive association in the upper range
of Na-Li countertransport. Accordingly, we observed
that donors in the highest tenth percentile of the Na-Li
countertransport distribution had significantly greater
mean blood pressure than those in the lowest tenth per-
centile (systolic blood pressure ± 14, 120 ± 14

### Table 4. Comparison of Mean Values for Sodium-Lithium Countertransport from Different Samples of Normotensive and Hypertensive Populations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>t*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canessa et al.5</td>
<td>Boston, MA, USA</td>
<td>0.24</td>
<td>0.55</td>
<td>36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adragna et al.6</td>
<td>Boston, MA, USA</td>
<td>0.29</td>
<td>0.51</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Woods et al.7</td>
<td>Chapel Hill, NC, USA</td>
<td>0.17</td>
<td>0.35</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Canali et al.8</td>
<td>Parma, Italy</td>
<td>0.25</td>
<td>0.33</td>
<td>58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cusi et al.9</td>
<td>Milan, Italy</td>
<td>0.23</td>
<td>0.30</td>
<td>45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Trevisan et al.10</td>
<td>Chicago, IL, USA</td>
<td>0.29</td>
<td>0.37</td>
<td>23</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Williams et al.11</td>
<td>Salt Lake City, UT, USA</td>
<td>0.26</td>
<td>0.32</td>
<td>54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clegg and Morgan13</td>
<td>Leeds, England</td>
<td>0.28</td>
<td>0.53</td>
<td>75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weder et al.15</td>
<td>Ann Arbor, MI, USA</td>
<td>0.27</td>
<td>0.37</td>
<td>29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Wiley et al.33</td>
<td>Melbourne, Australia</td>
<td>0.32</td>
<td>0.35</td>
<td>27</td>
<td>NS</td>
</tr>
</tbody>
</table>

Unweighted average

<table>
<thead>
<tr>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.26</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Present study

Rochester, MN, USA

0.24          0.42

NS = not significant.

*Values are in units of millimoles per liter of red blood cells per hour.
†Significant difference between normotensive and hypertensive groups.
SODIUM-LITHIUM COUNTERTRANSポート AND BLOOD PRESSURE/ Turner et al. 961

mm Hg vs 111 ± 13 mm Hg; p < 0.05; and diastolic blood pressure ± SD, 76 ± 10 mm Hg vs 66 ± 9 mm Hg; p < 0.05). Exclusion of diagnosed hypertensive persons with higher Na-Li countertransport values may account for the failure of the linear correlation statistic to measure a significant positive association between the two variables in healthy male donors in our sample. The use of a single casual blood pressure reading, as opposed to multiple casual blood pressure determinations, may have further biased the results toward lower correlations in both genders.

In summary, we believe this random sample of healthy blood donors provides estimates for the frequency distribution of Na-Li countertransport and its association with blood pressure that are more representative of the population at large. Evidence for bimodality in the Na-Li countertransport distribution and a positive association with blood pressure were present even when moderately and severely hypertensive persons were excluded. Although increased Na-Li countertransport appears to have a relatively small effect on blood pressure variability within the range of levels we examined, it may reflect altered physiological mechanisms contributing to development of EH in a subset of the population. Further studies of mechanisms determining the Na-Li countertransport phenotype may help unravel the heterogeneous etiology of EH.

Acknowledgments

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