Human Lymphocyte Sodium-Hydrogen Exchange
The Influences of Lipids, Membrane Fluidity, and Insulin

Patrick Carr, Nicholas A. Taub, Gerald F. Watts, and Lucilla Poston

The relation between serum lipids, membrane fluidity, insulin, and the activity of the sodium-hydrogen exchanger was investigated in human lymphocytes from 83 subjects. Subjects had a wide range of serum lipids and no concurrent disease. Lymphocyte membrane anisotropy (inversely related to membrane fluidity) was measured with a fluorescence polarization method. Sodium-hydrogen exchange maximal proton efflux rate, affinity for external sodium, and resting pH were determined with the intracellular pH-sensitive fluorochrome 2',5'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein. Sodium-hydrogen exchange maximal proton efflux rate was negatively correlated with the age of the subject (p=0.03). Membrane anisotropy correlated with serum triglyceride (p=0.04). Multiple regression analysis demonstrated that the maximal proton efflux rate in human lymphocytes was significantly related to age (p=0.005), systolic blood pressure (p=0.04), membrane anisotropy (p=0.03), and serum cholesterol (p=0.03). Incubation of lymphocytes with insulin failed to affect sodium-hydrogen exchange kinetics, intracellular buffering power, or resting intracellular pH. These results suggest that membrane-bound transport proteins may be influenced by serum lipids and the fluidity of the lipid membrane in which they are bound, but they are unlikely to be affected by insulin. (Hypertension 1993;21:344-352)

Key Words: lymphocytes • ion exchange • hyperlipidemia • insulin • membrane fluidity • fluorescent dyes

Abnormalities of a number of transport processes have been described in essential hypertension. These include increased Na+-H+ exchange,1-3 increased Na+-Li+ countertransport,4-5 and reduced Na+,K+-ATPase.6 The most frequently described is abnormal erythrocyte Na+-Li+ countertransport, which has been suggested as a genetic marker of essential hypertension.4 Recently, however, the suggestion has been made that Na+-Li+ countertransport in essential hypertension may be influenced by serum lipids (for review, see Reference 7) and insulin levels,8,9 particularly in relation to insulin resistance. This may be relevant to elevated Na+-Li+ countertransport in hypertension, because this condition commonly coexists with abnormal lipids and insulin resistance. It also questions the idea of a purely genetically determined level of countertransport activity. Canessa et al10 have presented good evidence that Na+-Li+ countertransport is a mode of operation of Na+-H+ exchange, although this remains controversial.7 Na+-H+ exchange is involved in the control of cell pH and volume, response to humoral vasoactive agents, renal reabsorption of sodium, and cell proliferation and growth.11 All of these have been implicated in the pathogenesis of hypertension. Na+-H+ exchange is also elevated in essential hypertension, so it is important to ascertain whether Na+-H+ exchange is influenced by serum lipids and insulin in parallel with Na+-Li+ countertransport.

Relations between serum lipids, which are in dynamic equilibrium with membrane lipids, and various transport processes other than Na+-H+ exchange have been described extensively.5,12,13 In vitro perturbations of membrane lipids and fluidity also affect transport activity.14,15 Because membrane fluidity is determined in part by lipid composition, it would seem reasonable to anticipate that hyperlipidemia may affect Na+-H+ exchange through changes in fluidity. Hyperinsulinemia and insulin resistance commonly coexist with hypertension and hyperlipidemia. Together with the in vivo evidence of a relation between insulin levels and Na+- Li+ countertransport,8,9 in vitro studies have demonstrated that Na+-Li+ countertransport and Na+-H+ exchange may be affected by incubation with insulin.16,17 However, the reports in vitro have been inconsistent.

In this study, therefore, we examined whether serum lipids or membrane fluidity (measured with a fluorescent probe) are related to Na+-H+ exchange activity. This was performed in peripheral blood lymphocytes (a common cell type used for studying Na+-H+ exchange18,19) from subjects with a wide range of serum lipids. In view of the proposed effect of insulin on Na+-Li+ countertransport and Na+-H+ exchange, we...
also determined whether insulin modifies Na\(^{+}\)-H\(^{+}\) exchange activity by incubating lymphocytes from normal donors with human insulin.

**Methods**

**Subjects**

Eighty-three subjects were recruited from the lipid clinic and from the hospital and medical school staff. The subjects were normotensive and medication-free for the month preceding the study, on an unrestricted diet, and had no concurrent illnesses. Specifically, hypertension, diabetes, and symptomatic vascular disease were excluded. Otherwise, subjects were unselected. Sixty percent of the subjects were lipoprotein phenotype IIa, 12% type IIb, 1% type III, 5% type V, and 22% had normal values. They were not selected on diagnostic grounds, because a heterogeneous group of dyslipidemias would allow study of the effect of a wide range of cholesterol and triglyceride profiles.

Blood pressure was recorded on at least three occasions after 5 minutes rest with subjects in the semirecumbent position, and the mean value was recorded. All studies took place in the morning after a 12-hour fast. Venous blood, taken with minimal stress, was used for routine hematological and biochemical profiles, fasting glucose, and fasting lipid and lipoprotein estimation. Fifty milliliters of the same blood sample was used for the lymphocyte Na\(^{+}\)-H\(^{+}\) exchange kinetics studies.

The insulin incubation experiments were performed on lymphocytes from 14 fasted, healthy, normotensive volunteers (age range, 24–33 years).

This study was approved by the West Lambeth Health Authority Ethical Committee. All subjects gave informed consent.

**Materials**

Physiological saline solution (PSS) contained (mM) NaCl 116, KCl 5.9, CaCl\(_2\) 1.8, Na\(_2\)HPO\(_4\) 1.1, MgSO\(_4\) 0.8, glucose 5.6, and NaHCO\(_3\) 26; osmolality was 285–295 mosm/kg (gassed with 5% CO\(_2\), 37°C, pH 7.4). Double-distilled, deionized, sterile water was used in preparation of all solutions. The acetoxymethyl ester of the pH-sensitive fluorescent dye 2',5'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) was obtained from Calbiochem Novabiochem, Nottingham, UK. Nicergin, nonesterified essentially fatty acid-free bovine serum albumin, and HEPES were obtained from Sigma Chemical Co., St. Louis, Mo. Trimethylammonium-diphenylhexatriene (TMA-DPH) was from Molecular Probes, Eugene, Ore. Lymphoprep was purchased from Nycomed UK Ltd., Sheldon, Birmingham, UK. Insulin (Human Actrapid) was from Novo Laboratories Ltd., Basingstoke, Hampshire, UK. All other chemicals were from Sigma or BDH Chemicals (Poole, Dorset, UK).

**Lymphocyte Isolation**

Lymphocyte isolation was achieved by a modified version of the method first described by Boyum.\(^{20}\) Fifty milliliters of heparinized venous whole blood was diluted with an equal quantity of PSS buffer. This solution was layered onto a sterile sodium metrizoate (9.6%)/Ficoll (5.6%) mixture (Lymphoprep) and centrifuged (700g, 20 minutes, 20°C). The thin lymphocyte layer resting on the density interface was collected and resuspended in a large volume of PSS buffer gassed with 5% CO\(_2\) (pH 7.4, 37°C). Two further washes were carried out; the final suspension contained 94–96% lymphocytes, the major contaminant cell type being monocytes.

One aliquot of cells was removed for anisotropy estimation and the rest incubated for 30 minutes with BCECF-AM (6 \(\mu\)M, 37°C, pH 7.4). The cell concentration used in the fluorometer at this and subsequent stages was 2–4 \(\times\) 10\(^{9}\)/L.

**Anisotropy**

Membrane fluidity is a biophysical property of membranes that quantitatively expresses the mobility and rate of rotational motion of membrane lipid molecules. The fluorescent probe TMA-DPH is anchored at the external cell surface by its cationic TMA group, while the DPH moiety is intercalated between the outermost portions of the fatty acyl chains. Fluorescence anisotropy (inversely related to membrane fluidity) was measured by placing the cells in 3 ml PSS buffer in a quartz cuvette mounted in a fluorometer with computer-controlled excitation and emission polarizers (model LS-50, Perkin-Elmer, Beacnsfield, Bucks, UK). TMA-DPH (5 \(\mu\)M) was added; fluorescence intensity \(I\) was measured at excitation and emission wavelengths of 350 and 430 nm, respectively (slits, 10 nm), and anisotropy \(A\) was calculated according to the equations\(^{31}\):

\[
A = \frac{I_{vv} - 2G I_{vh}}{I_{vv} + 2G I_{vh}} \quad (1)
\]

\[
G = \frac{I_{hv}}{I_{hh}} \quad (2)
\]

where the first element of the subscript pair is the excitation orientation and the second of the pair is the emission orientation (vertical, \(v\); horizontal, \(h\)), and \(G\) is the correction factor for the optical system (evaluated before each anisotropy measurement). Preliminary experiments showed that the measured anisotropy was independent of cell number but sensitive to temperature and length of incubation, so these were controlled to 37°C and 5 minutes, respectively. The fluorescence of the unlabeled cells and the buffer was negligible. Coefficient of variation for the anisotropy measurement was 0.8%.

**Measurement of Resting Intracellular pH and Calibration**

After incubation with BCECF-AM, cells were centrifuged and then resuspended in PSS buffer (20 minutes, 37°C) to allow any esterified dye to diffuse out of the cells. One aliquot was placed in the fluorometer for resting pH measurement, another was resuspended in a K\(^{+}\)/HEPES/nicergin (a K\(^{+}\)-H\(^{+}\) ionophore) medium (K\(^{+}\), 140 mM; HEPES, 10 mM; nicergin, 6 \(\mu\)M; pH 6.2), and the remainder was used for the Na\(^{+}\)-H\(^{+}\) exchange kinetics (see below). Resting fluorescence was recorded over 40 seconds as the ratio of emissions at 530 and 430 nm (slit widths, 5 and 2.5 nm, respectively). Calibration of pH performed with every sample was according to the method of Thomas et al\(^{22}\) using nicergin to abolish the transmembrane pH gradient and small aliquots of po-
tassium hydroxide (0.1 M) to accomplish intracellular pH (pH) changes in the suspension. External pH was measured using a pH meter with microelectrode (model PHA 270, Whatman Labsales Ltd., Maidstone, Kent, UK). The relation established between pH, and fluorescence ratios was sigmoidal but did not differ significantly from linearity between pH 6.2 and 7.6. The correlation coefficient for least-squares linear regression across this range was always >0.995.

**Na+-H+ Exchange Kinetics**

Recovery from intracellular acidosis induced by pH clamping using nigericin is almost entirely due, in lymphocytes, to Na+-H+ exchange. This was confirmed in this study using sodium-free medium and 5-(N,N-hexamethylene) amidorile, a specific inhibitor of Na+-H+ exchange. The rate of pH recovery was found to be close to maximal at pH 6.2.

Intracellular acidosis (pH, 6.2) was induced using a K+/HEPES/nigericin buffer (K+, 140 mM; HEPES, 10 mM; nigericin, 4 μM; pH 6.2). The cells were centrifuged and the pellet resuspended in an identical buffer without nigericin but with the addition of nonesterified essentially fatty acid–free bovine serum albumin (5 g/L) to scavenge the residual ionophore. Aliquots (100 μL) were placed in a series of cuvettes to each of which was added 2.9 mL HEPES buffer (pH 7.4) of different sodium concentrations (140, 70, 30, 20, 10, and 0 mM, with equimolar substitution of choline for sodium). Fluorescence ratios were recorded continually from the moment of addition for 40 seconds (wavelengths and slit widths were as for the calibration above). The first 20 seconds of recovery was essentially linear, fitted by first-order kinetics (r>0.95):

\[
\frac{[\text{Na}]}{\text{Rate of change of pH}} = V_{\text{max}} \times \frac{1}{V_{\text{max}}} \times [\text{Na}] \tag{3}
\]

This transformation was chosen because it has been shown to give markedly superior estimates of \(V_{\text{max}}\) and \(K_m\) in comparison to the commonly used Lineweaver-Burk method. This was confirmed in our own analysis.

**Buffering Capacity**

At any given pH, H+ efflux rate = buffering capacity \times rate of change of pH. Buffering capacity was measured in parallel acid-loaded cells (pH, 6.2) using the partitioning of ammonia across the cell membrane according to the method of Roos and Boron. Ammonium chloride (final concentration, 1 mM) produced a pH change of approximately 0.2 units. This minimal change in pH consistent with accurate measurement is important, because buffering capacity changes dramatically with pH, and this method minimizes the systematic error.

Assuming that the \(pK_a\) for dissociation of ammonia at 37°C is 8.89 and that NH₃ (not NH₂⁺) is freely permeable across the cell membrane, then buffering capacity can be calculated from the following equations:

\[
[\text{NH}_4^+]_0 = [\text{NH}_3]_0 \times 10^{pK_a - pH_0} \tag{4}
\]

\[
[\text{NH}_4^+]_0 = [\text{NH}_3]_0 \times 10^{pK_a - pH} \tag{5}
\]

Buffering capacity = \(\delta[\text{NH}_4^+] / \delta(pH)\) \tag{6}

The coefficient of variation for the buffering capacity measurement was 4.8%.

**Lipid and Lipoprotein Analyses**

The serum concentration of cholesterol was measured by the cholesterol oxidase–4-aminophenozone (CHOD-PAP) enzymatic colorimetric method (Boehringer, Lewes, UK), with an interassay coefficient of variation of <3.5%. Serum triglyceride was also measured by an enzymatic method (Wako Chemicals, Neuss, Germany), with an interassay coefficient of variation of <3%. High density lipoprotein (HDL) cholesterol was assayed after the precipitation of apolipoprotein B with MnCl₂ and heparin. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation (if triglyceride <4.5 mmol/L) or was measured after isolation of LDL at solution density \(d=1.063\) g/mL using preparative ultracentrifugation.

<table>
<thead>
<tr>
<th>Table 1. Descriptive Statistics for the 83 Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Anisotropy (TMA-DPH)</td>
</tr>
<tr>
<td>Resting intracellular pH</td>
</tr>
<tr>
<td>Maximal proton efflux rate (Kₘ) (mmol/L per minute)</td>
</tr>
<tr>
<td>Buffering capacity at pH 6.2 (mmol/L per pH unit)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
</tr>
<tr>
<td>Log₁₀ triglyceride</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
</tr>
</tbody>
</table>

TMA-DPH, trimethylammoniumdiphenylhexatriene; HDL, high density lipoprotein; LDL, low density lipoprotein. Distribution of triglyceride was normalized by log₁₀ transformation; all other distributions were approximately normal.
TABLE 2. Single Linear Correlations of $K_{\text{max}}$, $K_m$, and Anisotropy With Relevant Variables

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variable</th>
<th>n</th>
<th>Correlation coefficient ($r$)</th>
<th>Regression coefficient ($b$)</th>
<th>95% Confidence interval for $b$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal proton efflux rate ($V_{\text{max}}$)</td>
<td>Age</td>
<td>78</td>
<td>-0.25</td>
<td>-0.20</td>
<td>-0.37 to -0.02</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>Anisotropy</td>
<td>77</td>
<td>-0.20</td>
<td>-1.14</td>
<td>-2.41 to 1.14</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>77</td>
<td>0.11</td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}$triglyceride</td>
<td>77</td>
<td>0.09</td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure</td>
<td>78</td>
<td>0.01</td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure</td>
<td>78</td>
<td>-0.16</td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>$K_{\text{m}}$ for sodium</td>
<td>Age</td>
<td>78</td>
<td>-0.17</td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Anisotropy</td>
<td>77</td>
<td>-0.26</td>
<td>-465</td>
<td>-861 to -68</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>77</td>
<td>0.09</td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}$triglyceride</td>
<td>77</td>
<td>0.18</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure</td>
<td>78</td>
<td>0.07</td>
<td></td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure</td>
<td>78</td>
<td>-0.05</td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>Anisotropy</td>
<td>Total cholesterol</td>
<td>81</td>
<td>-0.19</td>
<td>-0.0016</td>
<td>-0.0034 to 0.0024</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>$\log_{10}$triglyceride</td>
<td>81</td>
<td>-0.23</td>
<td>-0.0107</td>
<td>-0.0210 to -0.0004</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure</td>
<td>82</td>
<td>0.01</td>
<td></td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure</td>
<td>82</td>
<td>-0.03</td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Fasting glucose</td>
<td>74</td>
<td>-0.21</td>
<td>-0.0069</td>
<td>-0.0147 to 0.0009</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Pearson's correlation coefficients are given with regression coefficients ($b$) and 95% confidence intervals for $b$ if $p<0.1$. *$p<0.05$.

Effect of Physiological Concentrations of Insulin

All subjects were fasted overnight. Blood (50 mL) was taken by venesection and insulin (50 milliunits/L) added to one half throughout the lymphocyte preparative procedure and during the estimation of Na⁺-H⁺ exchange kinetics. The second half was treated identically but in the absence of insulin to act as a control. The procedures for isolation of lymphocytes and measurement of kinetics and buffering capacity were the same as those outlined above. The insulin concentration used approximates the level found in insulin-resistant states in vivo.

Statistical Analysis

All variables measured had approximately normal distributions, with the exception of triglyceride, which was normalized by $\log_{10}$ transformation. In view of the continuous nature of the variables, linear relations between variables were examined using single and multiple linear regression. Values of Pearson's correlation coefficient ($r$) were also calculated. Variables included in the multiple linear regression model were chosen as those most likely to influence Na⁺-H⁺ exchange on the basis of published work. Arithmetic means and 95% confidence intervals of the variables measured are given, together with ranges where appropriate. Table 2 gives the results of least-squares linear regression on important variables.

Na⁺-H⁺ exchange $V_{\text{max}}$ in lymphocytes was significantly and positively correlated with age of the subject ($p=0.03$) (Figure 1). However, $K_{\text{max}}$ was not associated with any of the measured lipid variables nor with the membrane anisotropy. The affinity of the Na⁺-H⁺ exchanger for external sodium ($K_m$) was significantly negatively correlated with the membrane anisotropy ($p=0.02$) (Figure 2) but paradoxically not with any of the lipid variables.

TMA-DPH anisotropy exhibited a significant negative correlation with $\log_{10}$triglyceride ($p=0.04$) (Figure 3) but was not related to total cholesterol ($p=0.09$) (Figure 4).

Both total cholesterol and LDL cholesterol were also associated with age ($p<0.001$, $p=0.001$, respectively), systolic blood pressure ($p=0.009$, $p=0.01$, respectively), diastolic blood pressure ($p=0.04$, $p=0.03$, respectively), and fasting glucose ($p=0.03$, $p=0.02$, respectively).

A multiple linear regression model for maximal proton efflux rate ($V_{\text{max}}$) was constructed using the predictor variables in Table 3. These were selected as being most likely to influence the observed $V_{\text{max}}$. The effect of age observed in single linear regression was still evident even after adjusting for the effect of other variables. Systolic blood pressure was significantly associated with $V_{\text{max}}$ ($p=0.04$) in this normotensive group of subjects. Membrane anisotropy and total cholesterol also corre-
FIGURE 1. Scatterplot shows lymphocyte maximal proton efflux rate (V_max) of Na^+-H^+ exchange against age of subject with linear regression.

lated with this estimate of maximum Na^+-H^+ exchange activity. These predictor variables together only account for 32.4% of the observed variance in V_max. Similar multiple regression for K_m and resting pH, revealed no significant correlations.

The effects of incubation of the lymphocytes with insulin (50 milliunits/L) on Na^+-H^+ exchange V_max, K_m for external sodium, and resting pH are shown in Figure 5. In summary, no change in Na^+-H^+ exchange activity or resting pH was demonstrated. Buffering capacity of the cells (not shown) was similarly unaffected.

Discussion

In this study, we have attempted to evaluate the association between human lymphocyte Na^+-H^+ exchange, membrane fluidity, and serum lipids. We also sought to determine whether the activity of the Na^+-H^+ antiport may be affected by insulin levels that approximate the highest found in vivo. When attempting to characterize membrane transport processes or changes induced in them by disease, it is important that both the maximal velocity (V_max) and the affinity (K_m) of the transporter are estimated. Recent studies on Na^+-H^+ exchange have described antiporter kinetics solely in terms of an apparent "maximal" H^+ efflux rate under experimental conditions such that it approaches V_max. It has been demonstrated for Na^+-Li^+ countertransport using similar kinetic analysis that changes in the apparent "maximal" rate of this transporter are greatly influenced by the K_m value for external sodium. In the absence of a measurement of K_m, observed changes in the apparent maximal rate may be due to either a change in the true V_max or a change in the K_m of the antiporter. We have estimated both V_max and K_m in this study, but their validity in vivo depends on two further assumptions related to the pH, at which they were obtained. First, a pH of 6.2 must stimulate maximal Na^+-H^+ exchange. Second, a high concentration of hydrogen ions may induce conformational changes in the transport protein, which modify its activity indepen-
Table 4. Correlation of Erythrocyte Sodium-Lithium Countertransport with Plasma Lipids

<table>
<thead>
<tr>
<th>Study</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r  p</td>
<td>r  p</td>
<td>r   p</td>
</tr>
<tr>
<td>Carr et al* (n=100)</td>
<td>0.47 &lt;0.001</td>
<td>-0.29 0.01</td>
<td>-0.29 0.01</td>
</tr>
<tr>
<td>Hunt et al† (n=809)</td>
<td>0.34 &lt;0.0001</td>
<td>0.05 NS</td>
<td>-0.13 0.001</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 2½ years</td>
<td>0.32 &lt;0.0001</td>
<td>0.09 &lt;0.01</td>
<td>-0.11 &lt;0.01</td>
</tr>
<tr>
<td>Corrocher et al‡ (n=52)</td>
<td>0.299 0.05</td>
<td>0.376 0.01</td>
<td>0.071 NS</td>
</tr>
<tr>
<td>Turner and Michel§ (n=543)</td>
<td>0.512 &lt;0.001</td>
<td>-0.424 &lt;0.01</td>
<td>... ...</td>
</tr>
<tr>
<td>Men (n=543)</td>
<td>0.559 &lt;0.001</td>
<td>-0.256 &lt;0.05</td>
<td>... ...</td>
</tr>
<tr>
<td>Women (n=589)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein.
* r = Spearman's rank correlation coefficients.
† r = Pearson's correlation coefficients adjusted for age and sex.
‡ r = Partial regression coefficients (predictor variables: triglyceride, body mass index, cholesterol).

A previous study from our laboratory in mixed leukocytes from essential hypertensive patients showed a positive correlation between H+ efflux and triglyceride and a negative correlation between resting pH and cholesterol. These findings have not been confirmed in this study, although the relation between $V_{\text{max}}$ and blood pressure was similar.

Table 5. Table of Published Studies of Anisotropy of Cell Membranes in Hyperlipidemia Measured by Fluorescence Polarization (TMA-DPH)

<table>
<thead>
<tr>
<th>Study</th>
<th>Tissue</th>
<th>Group</th>
<th>TMA-DPH anisotropy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Quan Sang et al§</td>
<td>Human platelets (hypertensive subjects)</td>
<td>Control</td>
<td>0.278±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>† Cholesterol</td>
<td>0.270±0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.271±0.004</td>
</tr>
<tr>
<td>Malle et al§</td>
<td>Human platelets</td>
<td>Control</td>
<td>0.197±0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIA</td>
<td>0.195±0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIB</td>
<td>0.193±0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV</td>
<td>0.180±0.018</td>
</tr>
<tr>
<td>Muller et al§</td>
<td>Human erythrocytes (unsealed membranes)</td>
<td>Control</td>
<td>0.260±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIA</td>
<td>0.258±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIB</td>
<td>0.255±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV</td>
<td>0.254±0.002</td>
</tr>
<tr>
<td></td>
<td>Human erythrocytes (resealed membranes)</td>
<td>Control</td>
<td>0.270±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIA</td>
<td>0.266±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIB</td>
<td>0.266±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV</td>
<td>0.261±0.002</td>
</tr>
<tr>
<td></td>
<td>Human platelets</td>
<td>Control</td>
<td>0.287±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIA</td>
<td>0.287±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IIB</td>
<td>0.285±0.002</td>
</tr>
</tbody>
</table>
| TMA-DPH, trimethylammoniumdiphenylhexatriene. Hyperlipidemias are stratified according to lipoprotein phenotype. Values are mean±SEM for Le Quan Sang et al and mean±SD for other studies. *p<0.05 compared with controls.
been shown to inhibit and activate the Na\(^+\)-H\(^+\) antiporter, respectively.\(^{1,2}\) However, this work was carried out acutely in cultured lymphoblasts and may not be easily comparable with our results in lymphocytes in vivo. In addition, the changes in membrane composition induced by these methods were very large and may not have been representative of conditions encountered in vivo. Ng and Davies\(^{3}\) found a strong negative correlation between Na\(^+\)-H\(^+\) antiport activity and both total cholesterol and LDL cholesterol in mixed leukocytes of normotensive, normolipidemic subjects. Our results may differ because of the cell type used and the details of the method for estimating Na\(^+\)-H\(^+\) exchange activity. No kinetic data have previously been published on the Na\(^+\)-H\(^+\) exchange in normotensive hyperlipidemic individuals.

Multiple regression analysis demonstrated a positive correlation between Na\(^+\)-H\(^+\) exchange \(V_{\text{max}}\) and systolic blood pressure. Although increased activity of this exchanger in the blood cells of hypertensive subjects has been previously well documented,\(^{4,5}\) to our knowledge this is the only demonstration that Na\(^+\)-H\(^+\) exchange is correlated with systolic blood pressure in normotensive individuals.

TMA-DPH anisotropy was negatively correlated with the \(V_{\text{max}}\) of Na\(^+\)-H\(^+\) exchange by multiple regression. This finding suggests that there may be a link between the fluidity of the membrane and the activity of the Na\(^+\)-H\(^+\) exchange protein embedded in it. We are not aware of any previous reports concerning Na\(^+\)-H\(^+\) exchange activity and anisotropy. The negative correlation between the \(K_m\) for external sodium and membrane anisotropy was not seen on multiple linear regression, suggesting that the association was due to the effect of the other variables in the model.

The importance of insulin and insulin resistance (both associated with essential hypertension) in relation to Na\(^+\)-Li\(^+\) countertransport has only recently been recognized, and many earlier studies have not attempted to estimate their influence. Two recent investigations have demonstrated a relation between insulin resistance (measured by the euglycemic hyperinsulinemic clamp technique) and Na\(^+\)-Li\(^+\) countertransport \(V_{\text{max}}\).\(^{6,7}\) In one of these, Na\(^+\)-Li\(^+\) countertransport was also correlated with fasting insulin levels, but no information about the relation with serum lipids was given.\(^{6}\) In addition, and in contrast to the work in vivo, Foyle and Drury\(^{8}\) have demonstrated that in vitro incubation of erythrocytes in physiological levels of insulin causes a dose-dependent reduction in the \(V_{\text{max}}\) of Na\(^+\)-Li\(^+\) countertransport, although \(K_m\) was not measured. However, Canessa et al\(^{17}\) using similar levels of insulin, could not demonstrate any change in the \(V_{\text{max}}\) of Na\(^+\)-Li\(^+\) countertransport but a marked increase in \(K_m\) for external sodium. These findings, although directly contradictory, suggest that circulating insulin levels may explain some of the variation in Na\(^+\)-Li\(^+\) countertransport seen in the previous studies. As insulin resistance and hyperinsulinemia are features of essential hypertension\(^{9,10}\) and insulin appears to modulate Na\(^+\)-Li\(^+\) countertransport, it is clearly important to establish whether increased activity of the Na\(^+\)-H\(^+\) antiporter is due to a direct action of circulating insulin. Incubation of the lymphocytes in high physiological concentrations of insulin failed to show any change in the kinetic parameters of the Na\(^+\)-H\(^+\) exchanger. In vitro, Pontremoli et al\(^{17}\) demonstrated increased erythrocyte Na\(^+\)-H\(^+\) exchange \(V_{\text{max}}\), but no change in the \(K_m\), in response to insulin levels between 30 and 100 milliunits/L. In vivo, Delva et al\(^{14}\) could find no correlation between insulin levels and erythrocyte Na\(^+\)-H\(^+\) exchange \(V_{\text{max}}\) in essential hypertensive patients. The disparity between these findings and our own may be due to a more complex interaction in vivo or to variation in the expression of insulin receptors on the cell surface in different cell types, disease states, and races.

In summary, this study has demonstrated a strong relation between the \(V_{\text{max}}\) of Na\(^+\)-H\(^+\) exchange and age of the subject. An association between the activity of the antiport and blood pressure was also evident in normotensive subjects. Multiple linear regression analysis demonstrated that the maximal activity of the exchanger is related to both the fluidity of the membrane and serum cholesterol. Despite previous assertions to the contrary, we were unable to demonstrate any effect of insulin on this transporter in vitro. We conclude that alterations in lipids and fluidity may be important determinants of Na\(^+\)-H\(^+\) exchange activity. This may be of relevance to diseases associated with abnormal lipids and membrane fluidity, such as hypertension and diabetes.

References

15. Poli de Figueiredo CE, Ng LL, Davis JE, Lucio-Cazana FJ, Ellory 
   JC, Hendry BM: Modulation of Na-H antiporter in human lym-
   phoblasts by altered membrane cholesterol. Am J Physiol 1991;261:
   C138-C142
16. Foyle WJ, Drury PL: Reduction of Li+\textsuperscript{-}\textsuperscript{-}Na\textsuperscript{+} countertransport by 
17. Canessa M, Zerbini G, Laffel LMB: Sodium activation kinetics of 
   red blood cell Na\textsuperscript{+}/Li\textsuperscript{+} countertransport in diabetes: Methodology 
   Pagano E, Tammaro P, Canessa M: Sodium-hydrogen exchange 
   and cardiac hypertrophy in patients with primary hypertension. 
   J Hypertens 1991;9:S306-S307
19. Grinstein S, Clarke CA, Rothstein A: Activation of Na\textsuperscript{+}/H\textsuperscript{+} 
   exchange in lymphocytes by osmotically induced volume changes 
   and by cytoplasmic acidification. J Gen Physiol 1983;82:619-638
20. Böyum A: A one-stage procedure for isolation of granulocytes and 
   lymphocytes from human blood. Scand J Clin Lab Invest Suppl 
   1968;21:51-76
21. Le Quan Sang KH, Montenay-Garestier T, Devynck MA: Alter-
   tions of platelet membrane microviscosity in essential hyperten-
22. Thomas JA, Buchsbaum RN, Zimniak A, Racker E: Intracellular 
   pH measurements in Ehrlich ascites tumor cells utilizing spectro-
  oscopic probes generated in situ. Biochemistry 1979;18:2210-2218
   Na\textsuperscript{+}/H\textsuperscript{+} exchanger. Clin Sci 1990;79:531-536
   sity Press, 1977, p 33
25. Dowd JE, Riggs DS: A comparison of estimates of Michaelis-
   Menten kinetic constants from various linear transformations. 
   J Biol Chem 1965;240:863-869
27. Ng LL, Dudley C: Intracellular pH clamping of human leucocytes: 
   A technique for determination of cell buffering power and Na\textsuperscript{+}/H\textsuperscript{+} 
28. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the con-
   centration of low-density lipoprotein cholesterol in plasma, with-
   out use of the preparative ultracentrifuge. Clin Chem 1972;18:
   499-502
29. Aronson JK: Methods for expressing the characteristics of trans-
30. Frighi V, Ng LL, Lewis A, Dhar H: Na\textsuperscript{+}/H\textsuperscript{+} antiport and buffering 
   capacity in human polymorphonuclear and mononuclear leuco-
31. Rutherford PA, Thomas TH, Wilkinson R: Increased erythrocyte 
   sodium-lithium countertransport activity in essential hypertension 
   is due to an increased affinity for extracellular sodium. Clin Sci 
   1990;79:365-369
32. Hunt SC, Williams RR, Ash KO: Changes in sodium-lithium coun-
   tertransport correlate with changes in triglyceride levels and body 
   mass index over 2½ years of follow-up in Utah. Cardiovasc Drugs 
   Ther 1990;6:357-362
33. Corrocher R, Steinmayr M, Ruzzenente O, Brugnara C, Bertinato 
   L, Mazzi M, Furri C, Bonfanti F, De Santre G: Elevation of red cell 
   sodium-lithium countertransport in hyperlipidemias. Life Sci 1985; 
   36:649-655
34. Turner ST, Michels VV: Sodium-lithium countertransport and 
   hypertension in Rochester, Minnesota. Hypertension 1991;18: 
   183-190
35. Carr SJ, Thomas TH, Laker MF, Wilkinson R: Lipid lowering 
   therapy leads to a reduction in sodium-lithium countertransport 
   activity. Atherosclerosis 1991;87:103-108
36. Engelmann B, Duhm J: Effect of cholesterol and dipalmitoyl phos-
   phatidylcholine enrichment on the kinetics of Na-Li exchange of 
37. Le Quan Sang K-H, Mazeaud M, Levenson J, Del Pino M, Pithois-
   Merli I, Simon A, Devynck MA: Hypercholesterolaemia alters 
   platelet reactivity and the antihypertensive effect of nitrendipine. 
   J Hypertens 1991;9:S410-S411
38. Malle E, Sattler W, Prenner E, Leis HJ, Karädi I, Knipping G, 
   Romics L, Kostner GM: Platelet membrane fluidity in type II A, 
   type II B and type IV hyperlipoproteinaemia. Atherosclerosis 
   1991;87:159-167
   properties and membrane fluidity of red blood cells and platelets 
   in primary hyperlipoproteinaemia. Atherosclerosis 1990;83:231-237
40. Shattil SJ, Cooper RA: Membrane microviscosity and human 
41. Gleason MM, Medow MS, Tulenko TN: Excess membrane chole-
   terol alters calcium movements, cytosolic calcium levels, and mem-
   brane fluidity in arterial smooth muscle cells. Circ Res 1991;69:
   216-227
42. Ng LL, Davies JE: Lipids and cellular Na\textsuperscript{+}/H\textsuperscript{+} antiport activity in 
43. Epstein M, Sowers JR: Diabetes mellitus and hypertension. Hy-
   pertension 1992;19:403-418
44. Delva P, Pastori C, Degan M, Zamboni M, Aronso E, Lechi C, 
   Guzzo P, Armellini F, Lechi A: Erythrocyte Na\textsuperscript{+}/H\textsuperscript{+} exchange activity in a group of 
Human lymphocyte sodium-hydrogen exchange. The influences of lipids, membrane fluidity, and insulin.
P Carr, N A Taub, G F Watts and L Poston

Hypertension. 1993;21:344-352
doi: 10.1161/01.HYP.21.3.344
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 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/21/3/344