Kinetic Abnormalities of the Red Blood Cell  
Sodium-Proton Exchange in Hypertensive Patients  

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The present study was designed to examine the kinetics of Na⁺-H⁺ exchange in red blood cells of normotensive and hypertensive subjects and its relation to the previously reported abnormalities in Na⁺-Li⁺ exchange. The Na⁺-H⁺ antiporter activation kinetics were studied by varying cell pH and measuring net Na⁺ influx (mmol/l cell x hr=units) driven by an outward H⁺ gradient. The Na⁺-Li⁺ exchange was determined at pH 7.4 as sodium-stimulated Li⁺ efflux. Untreated hypertensive patients (n=30) had a higher maximal rate of Na⁺-Li⁺ exchange (0.43±0.05 versus 0.26±0.02 units, p<0.0003), a higher maximal rate of Na⁺-H⁺ exchange (62.3±6.2 versus 47±4 units; p<0.02), but a similar affinity for cell pH compared with normotensive subjects (n=46). The cell pH activation of the Na⁺-H⁺ antiporter exhibited a lower Hill coefficient than that of normotensive subjects (1.61±0.12 versus 2.56±0.14; p<0.0001). This index of occupancy of internal H⁺ regulatory sites was found reduced in most of the hypertensive patients (73%) whether their hypertension was untreated or treated. Hypertensive patients with Na⁺-Li⁺ exchange above 0.35 units (0.68±0.057 units, n=16) did not exhibit elevated maximal rates of Na⁺-H⁺ exchange (57.3±10 units, NS) in comparison with those with Na⁺-Li⁺ exchange below 0.35 units (66.4±7.6 units, n=26), but both groups exhibited reduced Hill coefficients. Hypertensive patients with enhanced Na⁺-H⁺ exchange activity (more than 90 units) had normal maximal rates of Na⁺-Li⁺ exchange. We propose that the elevated maximal rate of Na⁺-Li⁺ exchange and the low Hill coefficient for Na⁺-H⁺ exchange activation seen in many hypertensive patients might reflect a higher number of antiporter sites or abnormal antiporter regulation by phosphorylation. (Hypertension 1991;17:340–348)

Sodium-proton (Na-H) exchange is present in most eukaryotic cells, and it mediates an electroneutral exchange of external Na⁺ for internal H⁺ with a stoichiometry of 1:1.1-3 The transport system was first proposed to be present in the proximal tubule by Pitts et al4 and subsequently was described in rat kidney brush border membrane vesicles.5 Na-H exchange is important in the regulation of intracellular pH, cell volume, Na⁺ reabsorption in the kidney, and initiation of cell cycle events by growth factors.1-6

The presence of a tightly coupled Na-H exchange in human red blood cells has recently been documented.7-9 The imposition of an outward H⁺ gradient in acid-loaded cells stimulates amiloride-sensitive Na⁺ influx.8 Studies of the kinetics, stoichiometry, and modes of operation of the human red blood cell Na-H exchange have shown a tight coupling between H⁺-gradient-driven Na⁺ influx and the Na⁺-gradient-driven H⁺ efflux. It is only partially (50-70%) inhibited by amiloride and its high affinity analogues8,9 and is stimulated by cytosolic calcium.7 In other cell types (e.g., vascular smooth muscle and adrenal glomerulosa), the Na-H antiporter activity is modulated by second messengers of several receptors such as angiotensin II and growth factors.1

We and others10-13 have noted the striking similarities between Na⁺-Li⁺ countertransport (Na-Li exchange) and the Na-H exchanger. The kinetic interactions of Li⁺ with the Na-H antiporter and of H⁺
with Na-Li exchange are consistent with both exchange processes being driven by the same system, although some differences exist.\textsuperscript{12-14} Red blood cell Na-Li exchange is the best-characterized intermediate phenotype in human essential hypertension. It is known to be elevated in some, but not all, cases of essential hypertension,\textsuperscript{15,16} pregnancy,\textsuperscript{17} hypothyroidism,\textsuperscript{18} hyperlipidemia,\textsuperscript{19} and insulin-dependent diabetes with nephropathy.\textsuperscript{20} Genetic studies have shown that a model of polygenic inheritance or a recessive major gene effect can account for most of the variability of Na-Li countertransport in the human population.\textsuperscript{21,22} Thus, if Na-H exchange indeed mediates elevated Na-Li exchange, then one should be able to demonstrate abnormalities of the pH regulatory system in red blood cells of hypertensive patients. The present study was designed to examine the functional status of the red blood cell Na-H antiporter in hypertensive patients by determining its kinetics of activation by cell pH and by investigating whether alterations in this transporter are associated with alterations in the maximal transport rate ($V_{\text{max}}$) of the Na-Li exchange. We found, in comparison with normotensive subjects, that hypertensive patients exhibit elevated $V_{\text{max}}$ of both Na-Li and Na-H exchanges and a low Hill coefficient ($n_{\text{app}}$) for activation of Na-H antiporter by cell pH. Furthermore, two subgroups of hypertensive patients were identified: one group with normal $V_{\text{max}}$ of Na-Li and enhanced Na-H exchange; the second group exhibited elevated $V_{\text{max}}$ of Na-Li exchange but normal $V_{\text{max}}$ and low $n_{\text{app}}$ for Na-H exchange, which might reflect a higher number of antiporter sites or abnormal regulation by phosphorylation.

**Methods**

A total of 88 subjects were included in this study and were divided into four groups. The control group consisted of 46 normotensive subjects with diastolic blood pressure less than 90 mm Hg; 35 had no family history of hypertension and 11 had a positive family history of hypertension. All were Caucasian, were on antihypertensive therapy. The untreated hypertensive group ($n=30$) was composed of subjects who had never received medication or whose medications were discontinued for at least 2 weeks before the study. Twelve hypertensive subjects were also studied while continuing antihypertensive therapy. Hypertensive subjects were chosen based on their willingness to participate in this study. We excluded subjects whose diastolic blood pressure, when therapy was discontinued, exceeded 110 mm Hg and those who were older than 70 years. Renal function was normal (serum creatinine less than 1.2 mg/dl or creatinine clearance more than 80 ml/min). There were no other selection or exclusion criteria.

The studies were approved by our institution's internal review board and subjects gave informed consent.

**Preparation of Red Blood Cells**

Blood was drawn into heparinized tubes and centrifuged at 2,000g for 4 minutes at 4°C. The plasma and buffy coat were removed by aspiration, and the red blood cells were then washed three times with ice-cold choline wash solution (CWS) that contained (mM): choline chloride 149, MgCl\textsubscript{2} 1, Tris-MOPS 10, pH 7.4 (4°C). The red blood cells were then resuspended to approximately 50% with CWS. Aliquots of this suspension were then used for determination of hemocrit, hemoglobin (optical density at 540 nm), and Na\textsuperscript{+} concentration (atomic absorption spectroscopy) after appropriate dilution with 0.02% Acationox detergent (American Scientific Products, Boston, Mass.) in double-distilled water.

**Modification of Cell Cation Content**

The nystatin procedure was used to prepare both lithium-loaded and sodium-depleted cells as described.\textsuperscript{23,24} All solutions were prepared with deionized, double-distilled water, and the osmolarity was checked using a freezing point osmometer. Washed, packed cells were incubated at 15% hematocrit in cold nystatin loading solution (NLS) containing 40 $\mu$g/ml nystatin for 20 minutes at 4°C and protected from the light. The nystatin was dissolved in dimethyl sulfoxide (5 mg in 1.3 ml). For Na\textsuperscript{+} depletion, the NLS contained (mM): KCl 150, sucrose 50. For Li\textsuperscript{+}-loading, NLS contained (mM): LiCl 10, KCl 140, sucrose 50. The cell suspensions were then warmed to 37°C for 5 minutes, and the red blood cells were washed four times with warm nystatin washing solution (NWS). The NWS had the same composition as the NLS with the pH adjusted to 7.4 with 1 mM KH\textsubscript{2}PO\textsubscript{4} buffer and 10 mM glucose and 0.1% albumin added. The Li\textsuperscript{+}-loaded cells were used for determination of Na-Li countertransport as described below, whereas the Na\textsuperscript{+}-depleted cells were acid-loaded for Na-H exchange measurements.

**Modification of Red Blood Cell pH**

Red blood cells with pH\textsubscript{1} varying from 6.0 to 7.0 were prepared as previously described.\textsuperscript{9,24} In brief, Na\textsuperscript{+}-depleted cells (5% hematocrit) were incubated at 37°C for 10 minutes in six different acid-loading solutions, which contained (mM): KCl 170, MgCl\textsubscript{2} 0.15, ouabain 0.1, bumetanide 0.1 (prepared in dimethyl sulfoxide and added just before the red blood cells), glucose 10, and Tris-MES 20 adjusted to pH 5.8, 6.0, 6.3, 6.5, 6.8, or 7 at 37°C.\textsuperscript{9} The osmolarity of the acid-loading solutions was adjusted to 360 mosm by the addition of 40 mM sucrose. After a 10-minute preincubation with different acid-loading solutions, 4,4-
were resuspended to approximately 50% hematocrit with pH wash solution and stored on ice until used. Aliquots of the cell suspensions were used for determination of hemoglobin, hematocrit, and intracellular Na+ content. The pH was determined with a pH electrode in a cell lysate made with four volumes of 0.02% Acationox detergent. The cellular Na+ concentration was determined by atomic absorption spectroscopy with suitable standards prepared in double-distilled and deionized water. The cation content of the acid-loaded cells was expressed per liter original volume, as determined by relating the absorbance at 540 nm of the cell lysate to that of a known volume of red blood cells. The cell volume was estimated by comparing the hemoglobin per liter of the loaded cells with that of the fresh cells.

Measurement of Na-H Exchange Activity

Na-H exchange was estimated as net Na+ influx (into Na+-depleted, acid-loaded cells), driven by an outward H+ gradient (i.e., Δ extracellular pH [pHe] Na+ influx) as previously reported.9-24 The experiment commenced with the addition of 200 μl of a 50% suspension of the acid-loaded cells to 4 ml Na+ influx media preincubated at 37°C in a shaking water bath. The Na+ influx media contained (mM): NaCl 150, KCl 20, MgCl2 0.15, sucrose 40. After the final wash, the cells were resuspended to approximately 50% hematocrit with pH wash solution and stored on ice until used. Aliquots of the cell suspensions were used for determination of hemoglobin, hematocrit, and intracellular Na+ content. The pH was determined with a pH electrode in a cell lysate made with four volumes of 0.02% Acationox detergent. The cellular Na+ concentration was determined by atomic absorption spectroscopy with suitable standards prepared in double-distilled and deionized water. The cation content of the acid-loaded cells was expressed per liter original volume, as determined by relating the absorbance at 540 nm of the cell lysate to that of a known volume of red blood cells. The cell volume was estimated by comparing the hemoglobin per liter of the loaded cells with that of the fresh cells.

Measurement of Na-Li Exchange

Na-Li exchange was measured as external Na+-stimulated Li+ efflux from nystatin lithium-loaded cells (lithium 8, sodium 0.4 mmol/l cell) as previously described.24 The choline and Na+ media contained (mM): choline chloride or NaCl 148, MgCl2 1, glucose 10, ouabain 0.1, Tris-MOPS 10 (pH 7.4 at 37°C). Duplicate samples were taken at 0, 30, and 60 minutes. The samples were centrifuged immediately (2,000g, 5 minutes, 4°C), and the supernatant was carefully removed and stored for Li+ determination by atomic absorption spectroscopy. The difference in Li+ efflux between the two media represents the Na-Li exchange.

Statistical Analysis

All analyses (Student’s t test, linear regression, covariance analysis) were performed in a CLINFO facility. The data are reported as mean±SEM. The null hypothesis was rejected when p<0.05.

Chemicals

NaCl, MgCl2, dibutyl phthalate, and glucose were obtained from Fisher Scientific Company, Fairlawn, N.J. Ouabain, Tris, MES, MOPS, DIDS, nystatin, and albumin (bovine fraction V) were purchased from Sigma Chemical Co., St. Louis, Mo. and KCl from Mallinckrodt, Inc., St. Louis, Mo. Neptazane was from Lederle Laboratories, Division of the American Cyanamid Co., Pearl River, N.J. Choline chloride (ammonia-free) was obtained from Calbiochem, Behring Diagnostics, San Diego, Calif. Bumetanide was from Leo Laboratories, Vernouillet, France.

Results

Characteristics of Study Population

As observed in Table 1, untreated hypertensive subjects were similar in age but had a significantly higher diastolic blood pressure and weight than the normotensive subjects. Subjects in the hypertensive group under treatment were significantly older and heavier than those in the normotensive group. Men represented 60% of the total number of subjects in every group. Family history of hypertension was ascertained by patient questionnaire. Serum cholesterol levels were not significantly different in normotensive and hypertensive patients (207±10 versus 231±13 mg/dl, respectively).

Kinetics of Na-H Exchange in Normotensive Subjects

In these studies, initial rates of Na+ influx were determined incubating the cells at pHe 6.0 to impose a H+ gradient and at pHe 6.0 to inhibit Na+-H+ exchange. The difference between Na+ influx at pHe 8.0 and at pHe 6.0 (ΔpHe) represents the net Na+ influx driven by an outward H+ gradient (i.e., Na+-H+...
The ΔpH₉Na⁺ influx had values similar to the H⁺ efflux driven by an inward Na⁺ gradient, which is consistent with a stoichiometric ratio of 1:1 Na⁺ for H⁺ exchange. In this assay, inhibition of Na-H exchange by external protons is used to determine the antipporter activity instead of its amiloride sensitivity because in human red blood cells, 1.0 mM amiloride (or 20 μM dimethyl-amiloride) inhibits only 60±10% of the proton gradient-driven Na⁺ influx.

Figure 1A shows that a reduction in cellular pH from 7.0 to 6.0 (increased H⁺) activated red blood cell Na-H exchange (ΔpH₉-driven Na⁺ influx) to give an apparent Vₐₘₙ of 29 FU between pH₉ 5.9 and 6.1 in this representative normotensive subject. Note that when the acid-loaded cells were incubated at pH₉ 6.0, Na⁺ influx was much slower (Vₐₘₙ 10 FU) and linear. This is an apparent Vₐₘₙ because, at 150 mM Na⁺, external Na⁺ sites of the red blood cell Na-H exchanger with a high Kₐ for external Na⁺ (50 mM) might not be fully saturated. The dependence of the Na-H antipporter on cellular H⁺ was analyzed using the Hill equation:

\[ \frac{v}{V_{\text{max}}} = \frac{[H^+]^n}{K'} \]  

where v represents the measured velocity of Na-H exchange, Vₐₘₙ the estimated maximum rate from the pH activation curve, H⁺ the concentration in the cell (pH₉), and n the number of internal H⁺ binding sites. To determine the constant n, a rearranged form of the Hill equation was used:

\[ n = \frac{\log (V_{\text{max}} - v)}{\log v - \log K'} \]  

A plot of log (v) versus log v of Na⁺ influx versus log H⁺ (Figure 1B) yielded a straight line with slope equal to the Hill coefficient (nₐₘₙ); the intercept at the x axis at pH 6.44 gives a Hill coefficient (nₐₘₙ) of 2.7 and the intercept gives a Kₐ of 6.44.

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**Table 1. Clinical Characteristics of Study Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ratio F/M</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives (−FH) (n=35)</td>
<td>15/20</td>
<td>37.7±1.9</td>
<td>66.8±1.8</td>
<td>108.7±1.7</td>
<td>72.2±1.2</td>
</tr>
<tr>
<td>Normotensives (+FH) (n=11)</td>
<td>4/7</td>
<td>35.3±4.2</td>
<td>70.2±3.9</td>
<td>116.5±2.2</td>
<td>76.9±1.8</td>
</tr>
<tr>
<td>Normotensives total (n=46)</td>
<td>19/27</td>
<td>37.1±1.8</td>
<td>67.6±1.7</td>
<td>110.6±1.5</td>
<td>73.3±1.0</td>
</tr>
<tr>
<td>Hypertensives untreated (n=30)</td>
<td>11/19</td>
<td>42.2±1.8</td>
<td>77.3±3.0</td>
<td>140.6±3.0</td>
<td>90.2±1.8</td>
</tr>
<tr>
<td>Hypertensives treated (n=12)</td>
<td>5/7</td>
<td>52.6±1.8</td>
<td>88.5±4.3</td>
<td>128.0±3.1</td>
<td>83.5±0.9</td>
</tr>
<tr>
<td>Hypertensives total (n=42)</td>
<td>16/26</td>
<td>45.3±1.6</td>
<td>80.6±2.6</td>
<td>136.7±2.4</td>
<td>88.9±1.3</td>
</tr>
</tbody>
</table>

*p* values

1 vs. 4 <0.001

3 vs. 6 <0.0001

Mean±SEM. F/M, female/male; SBP, systolic blood pressure; DBP, diastolic blood pressure; FH, family history of hypertension.
TABLE 2. Red Blood Cell Na^+-Li^+ and Na^+-H^+ Exchanges of Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Na^+-Li^+ exchange</th>
<th>Na^+-H^+ exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V_{max}</td>
<td>K_m</td>
</tr>
<tr>
<td>Normotensives (-FH) (n=35)</td>
<td>0.23±0.02</td>
<td>45.1±4.2</td>
</tr>
<tr>
<td>Normotensives (+ FH) (n=11)</td>
<td>0.31±0.04</td>
<td>54.3±10.4</td>
</tr>
<tr>
<td>Normotensives total (n=46)</td>
<td>0.25±0.02</td>
<td>47.0±4.0</td>
</tr>
<tr>
<td>Hypertensives untreated (n=30)</td>
<td>0.43±0.05</td>
<td>62.3±6.2</td>
</tr>
<tr>
<td>Hypertensives treated (n=12)</td>
<td>0.28±0.03</td>
<td>55.6±8.0</td>
</tr>
<tr>
<td>Hypertensives total (n=42)</td>
<td>0.39±0.03</td>
<td>60.3±4.9</td>
</tr>
</tbody>
</table>

p values

1 vs. 4                                     <0.0003           <0.02  NS  <0.0001
3 vs. 6                                     <0.0018           <0.038 NS  <0.0001

Mean±SEM. Values for maximal rate (V_{max}) in mmol/l cell/hr. K_m in pH units. FH, family history of hypertension.

y=0 gives log [H]_{0.5} (i.e., the logarithm of the substrate concentration that yields 50% of the V_{max}, which for the sake of simplicity we call K_m. The constant K'=[(H)_{0.5}]^{n_{app}}.

The Hill coefficient provides an index of the effect of H^+ on Na-H exchange via occupancy of the proton transport and regulatory (allosteric) sites. A Hill coefficient greater than 1.0 indicates positive cooperativity; the greater the Hill coefficient, the greater the degree of cooperativity and the steeper the antipporter response to a fall in pH. In the normotensive subject in Figure 1B, the pK was 6.44 and n_{app}, 2.7, which indicates strong, positive cooperativity between H^+ internal sites.

Table 2 summarizes results obtained from cell pH activation curves for Na-H exchange performed in 46 normotensive subjects. The Na-H exchange had mean values for V_{max} of 47 FU, pK 6.45, and n_{app}, 2.56, but large interindividual differences were observed in the V_{max} of the Na-H antipporter. Because it has previously been shown that the normotensive offspring of hypertensive parents also exhibit elevated Na-Li countertransport,15,21,22 we also examined the kinetics of Na-H exchange in this group (Table 2). The V_{max} of Na-H and Na-Li exchanges were slightly elevated in the normotensive group with a family history of hypertension but were not significantly greater than those with a negative family history.

Kinetics of Na-H Exchange in Hypertensive Patients

In hypertensive patients, the activation of Na-H exchange by cell pH demonstrated less sigmoidicity between pH 7.1 and 5.9 (Figure 2A) than in the normotensive subject (Figure 1A). The abnormal activation of Na-H antiporter activity in the hypertensive subjects was reflected by a reduced value of n_{app} (e.g., n_{app}, 1.4 in the subject displayed in Figure 2B). The K_m for activation was not significantly different from that of the normotensive subject (Figure 1). In some hypertensive patients (but not all of them), Na^+ influx did not reach saturation even at pH 5.8. In these instances, the V_{max} was calculated from a Hanes-Woolf plot. The fact that the actual maximal velocity of the system was not attained does not affect the measurement of the n_{app}, which is independent of the estimated V_{max} value.25

Table 2 summarizes results from cell pH activation curves for Na-H exchange performed in 42 hypertensive patients. In comparison with normotensive subjects, untreated hypertensive patients exhibited elevated V_{max} of Na-H exchange (62.3±6.2 FU, p<0.02), similar K_m, but significantly reduced n_{app} (1.61±0.12;
p<0.0001) (Table 2). This decreased $n_{pp}$ indicates that the cooperative interaction of H at the regulatory sites of Na-H exchange is abnormal in these patients. The 12 hypertensive patients receiving treatment (Table 2) also had decreased $n_{pp}$ ($p<0.019$ for Wilcoxon's test) when compared with the normotensive group.

Figure 3 shows the frequency distribution of the $V_{max}$ of Na-H exchange in normotensive and hypertensive subjects. In the normotensive group, the skewness to the right is produced by a subset of 20% of the subjects having values higher than 60 FU. In the untreated hypertensive group, the frequency distribution was bimodal and shifted to the right (Figure 3). Forty-five percent of the hypertensive subjects have values higher than 60 FU; for this reason, the difference between the two groups was analyzed with a nonparametric test (Wilcoxon's test, Table 2).

In normotensive subjects, the frequency distribution of $n_{pp}$ for Na-H exchange activation was broad, but only 17% of the normotensive subjects displayed $n_{pp}$ values lower than 2.0 (Figure 4). In contrast, the hypertensive group showed a unimodal distribution markedly shifted to lower $n_{pp}$ values; 75% of hypertensive subjects displayed $n_{pp}$ values less than 2.0 (Figure 4).

A linear regression analysis, performed with treated hypertensive subjects excluded, indicated that the $V_{max}$ of Na-H exchange was significantly and positively correlated with diastolic blood pressure ($r=0.43$, $p<0.0001$) and weight ($r=0.33$, $p<0.0001$).

### Na-Li and Na-H Exchanges

Na-Li countertransport was also measured in the red blood cells of all the groups studied. The results are shown in Table 2. In the normotensive group without a family history of hypertension, 88.5% of the subjects had values below 0.35 FU. A "cutoff" point of 0.35 FU was taken as elevated Na-Li exchange based on the distribution in this group. In comparison with normotensive subjects ($V_{max}$ of 0.25±0.02 FU), Na-Li countertransport was found elevated in the untreated (0.43 FU, $p<0.0018$) and treated (0.285 FU, $p>0.05$) hypertensive patients. The hypertensive (treated and untreated) patients were subdivided into two groups according to their normal (less than 0.35 FU) or elevated (more than 0.35 FU) levels of red blood cell Na-Li countertransport. Twenty-six hypertensive patients had normal Na-Li exchange, whereas the remaining 16 had elevated values. This distribution (38%) was similar in untreated (36%) and treated (41%) patients, and it is consistent with previous reports showing that 35–50% of hypertensive individuals have elevated Na-Li exchange.

The Na-H exchange kinetic parameters for hypertensive patients divided into elevated and normal Na-Li exchange are shown in Table 3. In the hypertensive subjects with elevated Na-Li exchange values,

### Table 3. Kinetic Parameters of Red Blood Cell Na-H Exchange in Hypertensive Subjects With Elevated and Normal Na-Li Exchange

<table>
<thead>
<tr>
<th>Variable</th>
<th>$&gt;0.35$ FU</th>
<th>$&lt;0.35$ FU</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>41.1±2.7</td>
<td>43.0±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.8±4.0</td>
<td>77.6±4.6</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>139.0±4.7</td>
<td>142.0±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>87.2±2.9</td>
<td>92.5±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Na-Li exchange</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$ (FU)</td>
<td>0.68±0.05</td>
<td>0.23±0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Na-H exchange</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max}$ (FU)</td>
<td>57.3±10.2</td>
<td>66.4±7.6*</td>
<td>NS</td>
</tr>
<tr>
<td>$K_n$ (cell pH)</td>
<td>6.33±0.08</td>
<td>6.49±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td>1.43±0.20</td>
<td>1.76±0.14</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

| Subjects (n)        | 26         | 16         |          |

Mean±SEM. p values were calculated for the two groups with normal and elevated Na-Li exchange. Analysis included all hypertensive patients. FU, flux units (mmol/l cell x h); SBP, systolic blood pressure; DBP, diastolic blood pressure; $V_{max}$, maximal rate.

*p<0.02 with respect to the normotensive subjects.
the $V_{\text{max}}$ of Na-H exchange was not elevated (57.3±10) but exhibited a significantly reduced $n_{\text{app}}$ of 1.43±0.2 ($p<0.0002$) in comparison with the normotensive group shown in Table 2. In contrast, many hypertensive patients with normal Na-Li countertransport had significantly higher $V_{\text{max}}$ of the antiporter than normotensive subjects ($p<0.02$, Table 3). It appears, therefore, that a reduced $n_{\text{app}}$ for activation of Na-H exchange by $H_+$ (i.e., impaired cell pH regulation) is found in most of the hypertensive subjects regardless of their Na-Li exchange values.

We also examined the Na-Li countertransport activity in the hypertensive subjects, categorizing them according to their $V_{\text{max}}$ of Na-H exchange. Patients were divided in two groups: group 1, Na-H antiporter activity within 2 SDs of the mean for normotensive subjects (less than 90 FU) and group 2, Na-H antiporter activity outside 2 SDs (more than 90 FU). As observed in Figure 5, group 2 with elevated Na-H exchange exhibited normal values of Na-Li exchange and group 1 exhibited elevated Na-Li exchange. Conversely, patients with Na-Li exchange over 0.40 FU had normal $V_{\text{max}}$ of Na-H exchange (Figure 5). A higher cutoff point of 0.4 FU was used for Na-Li exchange in Figure 5 because it included 87% of the normotensive subjects with and without a family history of hypertension; this value reflects that in the normotensive subjects with a family history of hypertension, 13% had values over 0.40 FU compared with only 8% of those without a family history.

**Discussion**

The activation kinetics of Na-H exchange by cellular pH in red blood cells of normotensive and hypertensive patients provides an assessment of functional properties such as the capacity and ability of the antiporter to regulate cytosolic pH in response to an acid load.1-3,7 In red blood cells of normotensive subjects, Na-H exchange has a $V_{\text{max}}$ of 47 FU, pK 6.4 for activation by $H_+$, and a high Hill coefficient ($n_{\text{app}}$ 2.6), which reflects a high degree of cooperativity at the internal $H^+$ regulatory sites. In previous reports,9,27 slightly lower $V_{\text{max}}$ values were obtained in 20 normotensive subjects. In this study, normal subjects exhibited large interindividual differences in the $V_{\text{max}}$ of Na-H antiporter and a skewed distribution; 20% of individuals had activities higher than 60 FU (Table 2 and Figure 3).

In the untreated hypertensive subjects, the $V_{\text{max}}$ of Na-H exchange was significantly elevated, and the Hill coefficient was significantly reduced in comparison with normotensive subjects (Table 2). This kinetic form of the antiporter was also found in red blood cells of some normotensive subjects both with and without a positive family history of hypertension. The data also indicate that Na-H exchange, regardless of the Na-Li exchange, is abnormally regulated by $H_+$ in most of the hypertensive patients. The $H_+$ regulatory site has important physiological implications for the control of cell pH since a small change in pH, can rapidly stimulate the transporter. The occupancy of the $H^+$ regulatory site is believed to induce a conformational change, which activates Na-H exchange without concomitant transport of $H^+$.26-30

Kinetic studies carried out in our laboratory have supported the conclusion that Na-Li exchange is a mode of operation of the Na-H exchanger at a pH greater than 7.0.12,14 However, this does not mean that they have to be linearly correlated as shown in this study. In the hypertensive patients with elevated Na-Li exchange (Table 3, Figure 5), the $V_{\text{max}}$ of Na-H exchange was not elevated in comparison with the normotensive group, but the hypertensive group did exhibit a significantly reduced $n_{\text{app}}$ (Table 3). In contrast, most hypertensive patients with normal Na-Li countertransport had significantly higher $V_{\text{max}}$ of Na-H exchange than normotensive subjects ($p<0.02$, Table 3). Hypertensive patients with elevated Na-H exchange (21%) displayed normal Na-Li exchange. Of those with normal $V_{\text{max}}$ of Na-H exchange, 82% had elevated Na-Li countertransport (Figure 5). Thus, our results do not agree with Aronson's proposal10 that increased Na-Li exchange is caused by increased Na-H exchange activity, but most likely the increase is caused by abnormal regulatory properties.
A similar kinetic study of the red blood cell Na-H exchange performed in insulin-dependent diabetics without nephropathy indicated a much higher \( V_{\text{max}} \) than in hypertensive patients \((81 \pm 4.3, n=26)\) and normal Na-Li exchange activity. In contrast, insulin-dependent diabetic patients with nephropathy exhibited elevated values of Na-Li exchange and elevated \( V_{\text{max}} \) of Na-H exchange and low Hill coefficient for pH activation, as observed in our hypertensive patients. Because these patients are receiving insulin treatment, the results suggest that this hormone may play a role in the regulation of the antiporter in human red blood cells.

Na-Li exchange activity was not a good indicator of the kinetic parameters of the Na-H antiporter; most of the patients (75%) had Hill coefficients lower than 2.0 (Table 3), but only 38% had elevated Li-Na countertransport. The lack of relation between the \( V_{\text{max}} \) of both exchange modes can be understood considering their properties. The exchange of \( Li^+ \) for \( Na^+ \) is measured at \( pH_i=pH_o=7.4 \). At this \( pH_o \), the \( H_+ \) regulatory site is not occupied and its activity is less than 1% of the \( V_{\text{max}} \) of Na-H exchange seen at pH 6.0. Na-Li exchange, therefore, does not provide a reliable estimate of the cation translocation through the cell pH-regulated pathway. Thus, at a pH greater than 7.0, the antiporter is in a conformational state, which transports \( Li^+ \) and \( Na^+ \) at a very low rate; at a pH less than 7.0, when the \( H_+ \) regulatory site is occupied, a conformational change is induced, and \( H^+ \) efflux increases to rates 100 times greater than \( Na^+ \) or \( Li^+ \) efflux. This is the consequence of the pronounced asymmetry in the affinities of the antiporter for transport and regulatory sites. At a pH, less than 7.0, \( H_+ \gg > > > > > > Li_+ \gg > > > Na_+ \) are transported outward and \( Na_+ \gg Li_+ > > > > > > H_+ \) are transported inward. Furthermore, Na-H exchange is partially ATP dependent and amiloride sensitive, whereas Na-Li and Na-Na exchanges are ATP independent and amiloride insensitive.

As a further extension of this model, we hypothesize that Na-Li exchange may provide an indirect estimate of the number of antiporter sites, whereas Na-H exchange estimates the kinetic behavior of the \( H_+ \)-regulated pathway. This may imply that in the patients with elevated Na-Li exchange, normal Na-H exchange \( V_{\text{max}} \) and low \( n_{\text{top}} \) for pH activation, the antiporter is not normally modulated by or is overexpressed as a compensatory mechanism. In the patients with normal rates of Na-Li countertransport, abnormal pH regulation might not be accompanied by an increased number of sites.

Several mechanisms may explain the abnormal pH regulation of the Na-H antiporter observed in hypertensive patients: 1) the Na-H exchanger might be encoded by different structural genes in hypertensive patients; 2) the antiporter undergoes different post-translational modifications (i.e., phosphorylation and glycosylation) in the hypertensive process; or 3) the Na-H exchanger might be overexpressed in the patients with elevated Na-Li exchange. Recent studies by Lifton et al. have excluded the possibility that the Na-H exchange gene present in chromosome 1p is linked to the hypertensive process. However, it is not yet known if there are multiple genes encoding various Na-H antiporter isoforms. Several observations indicate that posttranslational modifications such as phosphorylation and glycosylation modulate the Na-H antiporter activity. It has been shown that cytosolic \( Ca^{2+} \), protein kinase C-dependent phosphorylation, and insulin are important regulators of the antiporter activity in several cell types. The absence of a good marker to estimate the number of antiporter sites makes it difficult to differentiate between an increased number of transporters or increased turnover per transport site.

Several studies have reported measurements of Na-H exchange activity using changes in cell volume as described by Grinstein et al. and have found enhanced Na-H exchange in thymocytes of spontaneously hypertensive rats in comparison with the Wistar-Kyoto normotensive strain as well as in platelets and leukocytes from essential hypertensive patients. Thus, all circulating blood cells display altered antiporter activity in hypertensive patients. At present, it is not known if the abnormal cell pH regulation by the antiporter observed in red blood cells of hypertensive patients may reflect functional properties in other cell types (i.e., the kidney tubule or vascular smooth muscle). However, if the regulation of the antiporter is most likely to be tissue specific, alterations of this antiporter in other cells should be expected. In addition, since Na-H exchange is activated at the initiation of the cell proliferation, induced by growth factors, altered Na-H exchange could play a key role in the development of vascular smooth muscle hyperplasia as reported in the SHR strain.

In summary, our study reveals that red blood cell Na-H exchange exhibits, in most hypertensive patients, abnormal kinetics of activation by cellular pH, as evidenced by low values of the Hill coefficient, regardless of the cells' level of Na-Li exchange. Two subgroups of hypertensive patients could be distinguished: one group with elevated \( V_{\text{max}} \) of Na-H and normal Na-Li countertransport levels; a second group exhibited normal \( V_{\text{max}} \) of Na-H and increased Na-Li exchange, which might reflect an increased number of antiporter sites or abnormal regulation by phosphorylation.

References


KEY WORDS • sodium-hydrogen antipporter • essential hypertension • red blood cell • sodium-lithium countertransport
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