Variations in the Apparent pH Set Point for Activation of Platelet Na-H Antiport

Goro Tokudome, Haruo Tomonari, Jeffrey P. Gardner, Mordechay Aladjem, Burton P. Fine, Norman Lasker, Michael Gutkin, Lawrence H. Byrd, and Abraham Aviv

To explore the role of the Na-H antiport in essential hypertension, we studied the kinetics of cytosolic pH and external sodium activation of this transport system in platelets from 65 normotensive and essential hypertensive subjects on and off antihypertensive medications. Subjects included both blacks and whites, as well as men and women. The fluorescent dye 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein was used to monitor the cytosolic pH in these cells. Platelets from black (hypertensive and normotensive) men and hypertensive white men demonstrated a highly significant alkaline shift in the apparent cytosolic pH set point for activation of the Na-H antiport. For the hypertensive subgroups, the cytosolic pH set point values (mean±SEM) were: white men, 7.45±0.052; white women, 7.04±0.089; black men, 7.66±0.148; and black women, 7.20±0.082. For the normotensive subgroups, the cytosolic pH set point values were: white men, 7.13±0.034; white women, 7.05±0.036; black men, 7.50±0.110; and black women, 7.20±0.176 (p=0.0016 for race and p=0.0001 for gender, using a three-way analysis of variance by race, gender, and hypertension). There were no race-, gender-, or blood pressure-related differences among the various cohorts in the kinetics of sodium activation of the Na-H antiport, the cellular buffering power, and basal pH. These results suggest that at basal pH the Na-H antiport is quiescent in platelets from both black and white women and normotensive white men. However, it can be active at basal pH in platelets from black men (normotensive and hypertensive) and in platelets from hypertensive white men. Our work demonstrates the heterogenous nature of the alterations in the Na-H antiport in essential hypertension and its dependence on gender and racial extraction. (Hypertension 1990;16:180–189)
tion of the Na-H antiport in platelets and other cells.\textsuperscript{14} This method was used to: 1) further understand the mechanism of operation of the Na-H antiport in human platelets, 2) decipher gender- and race-related differences in this transport system, and 3) determine whether characteristics of the Na-H exchange are altered in essential hypertension.

**Methods**

**Characteristics of Subjects and General Procedures**

Hypertensive subjects were recruited from the hypertension clinics of the Veterans Administration (VA) Hospital at East Orange, N.J., the clinic of the Hypertension and Renal Group in Livingston, N.J., and the staff of the University of Medicine and Dentistry of New Jersey (UMDNJ). Normotensive research subjects were also from the staff of the UMDNJ. The hypertensive group comprised 14 treated and 18 untreated patients. The normotensive group included 20 subjects who reported a negative family history of essential hypertension and 13 who reported a positive family history of the disease. The average of three blood pressure measurements, obtained at 2-minute intervals, was used. Diastolic blood pressure was determined as the fifth Korotkoff sound. After blood pressure measurements, 50 ml blood was drawn into acid citrate dextrose buffer (20/1, vol/vol), which included (mM): sodium citrate 14, citric acid 11.8, and dextrose 18. Platelets were studied within 4 hours after drawing the blood.

**Cytosolic Monitoring**

In preliminary studies, we examined the effect of platelet isolation by gel filtration through Sepharose 2B beads (Sigma Chemical Co., St. Louis, Mo.) versus centrifugation on parameters of the Na-H antiport. Both approaches yielded relatively pure platelet preparations (less than 0.5% contamination of other cell types). This method was used to: 1) further understand the mechanism of operation of the Na-H antiport in human platelets, 2) decipher gender- and race-related differences in this transport system, and 3) determine whether characteristics of the Na-H exchange are altered in essential hypertension.

**Table 1. Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>MBP (mm Hg)</th>
<th>BMI (kg/m(^2))</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High BP</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>White men</td>
<td>150 ± 2.5</td>
<td>96 ± 1.6</td>
<td>114 ± 1.4</td>
<td>28.3 ± 0.72</td>
<td>49 ± 2.2</td>
</tr>
<tr>
<td>White women</td>
<td>145 ± 8.4</td>
<td>90 ± 5.0</td>
<td>109 ± 5.7</td>
<td>24.3 ± 1.21</td>
<td>50 ± 3.1</td>
</tr>
<tr>
<td>Black men</td>
<td>137 ± 6.7</td>
<td>92 ± 4.7</td>
<td>107 ± 5.0</td>
<td>26.0 ± 0.85</td>
<td>54 ± 3.6</td>
</tr>
<tr>
<td>Black women</td>
<td>145 ± 3.5</td>
<td>94 ± 2.2</td>
<td>110 ± 2.8</td>
<td>28.3 ± 1.91</td>
<td>48 ± 3.9</td>
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<tr>
<td>Normal BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White men</td>
<td>119 ± 2.5</td>
<td>76 ± 1.2</td>
<td>90 ± 1.3</td>
<td>22.8 ± 0.73</td>
<td>45 ± 3.8</td>
</tr>
<tr>
<td>White women</td>
<td>111 ± 5.3</td>
<td>74 ± 2.5</td>
<td>86 ± 3.4</td>
<td>22.8 ± 1.46</td>
<td>41 ± 4.2</td>
</tr>
<tr>
<td>Black men</td>
<td>120 ± 4.2</td>
<td>78 ± 1.9</td>
<td>92 ± 2.5</td>
<td>25.2 ± 0.97</td>
<td>30 ± 1.0</td>
</tr>
<tr>
<td>Black women</td>
<td>117 ± 1.5</td>
<td>73 ± 1.5</td>
<td>87 ± 1.4</td>
<td>24.5 ± 1.09</td>
<td>34 ± 3.4</td>
</tr>
</tbody>
</table>

*p* values

- Race: 0.6363, 0.9408, 0.7674, 0.0800, 0.0703
- Gender: 0.4974, 0.1183, 0.1817, 0.4479, 0.5780
- Diagnosis: 0.0001, 0.0001, 0.0001, 0.0001, 0.0001

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; BMI, body mass index.
with white cells). Platelets prepared by gel filtration or centrifugation exhibited identical profiles in the kinetics of Na$_3$ and pH$_j$ activation of the Na-H antiport. However, the former method yielded platelets with a more alkaline basal pH$_j$. (BpH$_j$).

Blood was centrifuged at 200g for 10 minutes at room temperature. The platelet-rich plasma was centrifuged at 1,000g for 15 minutes, and cells were washed three times (using centrifugation at 1,000g for 10 minutes) with a buffer consisting of (mM): NaCl 140, KCl 5, glucose 10, ethylene glycol-bis(β-aminoethyl ether)-N, N', N'-tetraacetic acid (EGTA) 0.2, and 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) 10 (pH=7.40). EGTA was omitted from the third washing, which also included 0.1% fatty acid-free bovine serum albumin (BSA). Platelets were loaded with 5 μM BCECF-AM (Molecular Probes, Eugene, Ore.) at 37°C in HEPES-buffered solution (HBS) consisting of (mM): NaCl 140, KCl 5, MgCl$_2$ 1, CaCl$_2$ 1, glucose 10, and HEPES 10 (pH=7.40).

Some platelets underwent measurements of BpH$_j$ in HEPES (pH 7.4) after washing off the extracellular BCECF-AM. Other platelets were acidified using 0.5 μg/ml nigericin in sodium-free HBS of different pH values (sodium was replaced isosmotically by N-methyl-D-glucamine). Most studies by others have used potassium concentrations of 130–145 mM when acidifying cells by nigericin. However, such an approach results in depolarization of the plasma membrane, which may influence parameters of the Na-H antiport. In the present study, we used physiological concentrations of potassium during the acidification process. This approach resulted in a lack of a priori knowledge of the exact pH$_j$ at the initiation of the sodium-dependent alkalinization (pH$_{ic}$). After 7 minutes of treatment with nigericin, 0.1% BSA was added, and the Na-H exchange was activated by adding 150 μl aliquots (approximately 3×10$^6$ platelets) to 3 ml HBS (0.1% BSA) (pH 7.4) containing sodium. The 20-fold dilution of the nigericin plus 0.1% BSA eliminated any further effect of the ionophore. In preliminary experiments, we compared results of pH$_j$ recovery of platelets acidified by the nigericin method in HBS containing sodium versus sodium-free HBS. For the former approach, cells were incubated for 7 minutes in HBS with 0.5 μg/ml nigericin. After acidification, cells were washed once with sodium-free HBS plus 0.5 μg/ml nigericin and resuspended in 150 μl aliquots of sodium-free HBS plus 0.1% BSA. The Na-H antiport was activated as above by the rapid addition of the cells to 3 ml HBS. No apparent differences were observed in the pH$_j$ recovery profiles between platelets acidified in sodium-containing and sodium-free media. In previous studies,$^{14}$ we showed that the sodium-dependent pH$_j$ recovery of acidified human platelets was completely inhibited by 10 μM of 5-(N-methyl-N-isobutyl) amiloride. Calibration of pH$_j$ was performed by subjecting platelets to 5 μg/ml nigericin in HBS (minus BSA) of different pH values with 145 mM potassium (isosmotically substituted for sodium). Standard curves were constructed for each platelet preparation.

BpH$_j$ or changes in pH$_j$ were monitored at 37°C under constant stirring in a SPEX Fluorolog II spectrophotometer (model CM-3, SPEX Industries, Inc., Edison, N.J.). Excitation and emission wavelengths were set at 440/503 nm and 530 nm, respectively. The integration time was set at 0.2 seconds and data points were obtained at 1-second intervals. Autofluorescence of aliquots of platelets not exposed to BCECF-AM was measured in each experiment and fluorescent signals were corrected accordingly.

### Data Analysis

Rate constants of the sodium- and pH$_j$-dependent recoveries from acidification were obtained through iterative curve fitting of the data to the following model:

$$\text{pH}_{ic} = \text{pH}_{ic0} - [\text{pH}_{ic0} - \text{pH}_{ic}] \times e^{-kt}$$  \hspace{1cm} (1)

where pH$_{ic0}$ is pH$_j$ at a given time t, pH$_{ic}$ is pH$_j$ at the new steady state, pH$_{ic0}$ is the initial pH$_j$ at the moment of activation of the Na-H antiport, and k is the rate constant. The first data point (IP in Figure 1A) was obtained at 2 seconds after activation of the Na-H antiport. Thus, the pH$_{ic0}$ was back extrapolated from the fitted data (Figure 1A). Implicit to this extrapolation is the assumption that the pattern described by the model between 2 seconds after activation of the Na-H antiport and the new steady state also holds for the initial 2 seconds after activation. This assumption appears valid in view of the excellent fit of the model to the data during pH$_j$ monitoring after activation of the Na-H antiport. Rate constants, obtained from the curve fitting, were used to calculate the apparent initial rate (IR) of pH$_j$ recovery according to the expression:

$$\text{IR} = k \times [\text{pH}_{ic} - \text{pH}_{ic0}]$$  \hspace{1cm} (2)

Platelet buffering power was measured at BpH$_j$ by treatment with 10 mM NH$_4$Cl. The buffering power was determined from the change (Δ) in cellular NH$_4^+$ and pH$_j$ (ΔpH$_j$/ΔpH$_j$) as previously described.$^{15}$ The proton equivalent efflux rate (representing the activity of the Na-H antiport) was obtained from the product of the cellular buffering power and sodium-dependent rate of change in pH$_j$.

In experiments examining the kinetics of sodium activation of the Na-H antiport, the target pH$_{ic0}$ was set for 6.7. Initial rates of proton equivalent efflux at different Na$_3$ concentrations (10, 20, 30, 40, 60, 100, and 140 mM) were used in the model:

$$v = MR \times [\text{Na}_3]^N / K_{0.5}^N + [\text{Na}_3]^N$$  \hspace{1cm} (3)

where v is the IR of proton equivalent efflux, MR is maximal IR of the proton equivalent efflux, [Na$_3$] is extracellular sodium concentration, $K_{0.5}$ is half maximal activation of the antiport for a given pH$_j$, and N is the Hill coefficient. Parameters of kinetics of sodium activation of the Na-H antiport were...
obtained by a nonlinear curve fitting method. An
illustration of the use of this model for data derived
from a set of experiments in platelets from one subject
is presented in Figure 1B (for further details,
see Reference 14).

For the pH$_i$-dependent kinetics of the Na-H
antiport, cells from each subject were acidified to 6–8
different pH$_i$ levels ranging between 6.0 and 7.0. In
preliminary analyses we compared nonlinear regres-
sion (i.e., models using sigmoid, logarithmic, or ex-
ponential functions) versus linear regressions in
describing pH$_i$-dependent activation of the Na-H
antiport. The best fit for the relation between the
pH$_i$ (x axis starting at pH$_{io}$ of 6.0) and IR of proton
equivalent efflux (y axis) was obtained by a linear
regression (Figure 1C). This is not a unique finding,
inasmuch as others have demonstrated within this pH
range a linear relation between the pH$_i$ and Na-H
antiport activity in different cells. Further analyses,
thus, used linear regressions to evaluate the relations
between pH$_{io}$ and IR. The x intersect (X$_i$) represents
the apparent pH$_i$ set point for activation of the Na-H
antiport. The y intersect (Y$_i$) represents the apparent
activity of the antiport at pH$_i$ of 6.0.

The aforementioned models were fitted to data
derived from platelets of each subject to obtain the
sodium-dependent and pH$_i$-dependent kinetic
parameters of the Na-H antiport. Statistical methods
to evaluate these and other parameters used a three-
way analysis of variance (ANOVA), correlation anal-
ysis, and stepwise multiple regression analyses. All
curve fitting techniques and statistical computations
were performed on an IBM-compatible PC using
SAS REG and GLM programs. Because of technical
reasons, blood samples from six subjects were not
available for analysis of pH$_i$-dependent kinetics
of activation of the Na-H antiport.

Results
To determine possible differences among the var-
ious cohorts of the normotensive group, the initial
step in the analysis of parameters of pH$_i$ and the
Na-H antiport used a three-way ANOVA by race,
gender, and family history of essential hypertension.
Family history of essential hypertension had no effect
on these parameters. However, blacks demonstrated
higher X$_i$ values (p = 0.0126) than whites, and women
a lower X$_i$ value than men (p = 0.0880). The p value
for the influence of a family history of essential
hypertension on X$_i$ was 0.6560. A three-way ANOVA
by race, gender, and the effect of antihypertensive
treatment was then used to analyze the pH$_i$ and
Na-H antiport parameters for the hypertensive
group. Patients treated with antihypertensive medi-
cations manifested no differences in these param-
eters from the untreated, newly diagnosed hyperten-
sive subjects. However, as in the normotensive group,
blacks and men demonstrated higher X$_i$ values than
whites and women (p = 0.0748 and p = 0.0003, respec-
tively). In further analysis, the entire group was
analyzed using a three-way ANOVA by race, gender,
and diagnosis of essential hypertension.

Table 2 presents pH$_i$ and Na-H antiport para-
eters for the various cohorts. There were no statisti-
cally significant differences among the subgroups in
most of the parameters. However, blacks and men
exhibited a highly significant increase in their X$_i$
values as compared with whites and women
(p = 0.0016 and p = 0.0001, respectively). A scatter-
gram depicting the means and distribution of X$_i$
values for hypertensive and normotensive blacks and
whites is presented in Figure 2. Whereas the BpH$_i$
was within a narrow range for all groups (mean group
values of 7.22–7.37), the X$_i$ demonstrated a substan-
values in hypertensive men than in normotensive
to a

\( Q \) indicates a shift of \( X \) to a

higher pH level than \( \text{BpH}_1 \) and a negative \( Q \) indicates an \( X \) value lower than \( \text{BpH}_1 \). Of interest are the following observations: 1) women (black and white, hypertensive and normotensive) exhibited a negative mean \( Q \) value, 2) black men manifested a positive mean \( Q \) value with a higher value associated with hypertension, and 3) white men were positioned in between these two extremes; normotensive white men manifested a negative mean \( Q \), whereas their hypertensive counterparts exhibited a positive mean \( Q \) value. When correlation analyses were applied to the various cohorts, only white men showed significant relations between \( Q \) and the systolic (\( r=0.647, p=0.0015 \)) and diastolic (\( r=0.5253, p=0.0145 \)) blood pressures.

No correlations were observed between \( \text{pH} \) and Na-H antiport parameters and age (Table 3). However, a significant, positive correlation was observed between the BMI and \( X \) (\( r=0.266, p=0.0405 \)). To examine the potential effect of the BMI, we used stepwise multiple regression analysis that ranks variables with respect to their relative contribution to variation in the \( X \) (arbitrarily defined as the dependent variable). Several variables were included in the following order in the analysis: BMI, systolic blood pressure, diastolic blood pressure, race, and sex. Table 4 summarizes the results. Together, these variables accounted for 39.8% of the observed variability in the \( X \). BMI had a minimal (1.31%) and insignificant contribution, whereas the diastolic blood pressure showed a small contribution of 5.08% (\( p=0.0835 \)) to the \( X \). The main contributors to the variability in the \( X \) were gender (19.04%, \( p=0.0001 \)) and racial extraction (13.54%, \( p=0.0035 \)).

Several interesting features concerning characteristics of the Na-H antiport and \( \text{pH} \) regulation in human platelets are revealed by data summarized in

**FIGURE 2. Scattergram for apparent platelet pH, set point for activation of the Na-H antiport (\( X \)) in the various groups.**

### Table 2. Cytosolic pH and Na-H Antiport Parameters

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>BpH (pH)</th>
<th>Buffer power [mmol/liter]</th>
<th>pH in pH</th>
<th>( N )</th>
<th>( X ) (pH)</th>
<th>Y</th>
<th>pHu (pH)</th>
<th>MR (mmol/liter)</th>
<th>( K_d ) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High BP</strong></td>
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</tr>
<tr>
<td>White men</td>
<td>7.26±0.021</td>
<td>18.0±2.03</td>
<td>6.60±0.045</td>
<td>2.29±0.335</td>
<td>40.64±4.606</td>
<td>2.09±0.163</td>
<td>7.45±0.052</td>
<td>3.60±0.458</td>
<td>+0.200±0.055</td>
</tr>
<tr>
<td>White women</td>
<td>7.26±0.025</td>
<td>19.6±1.76</td>
<td>7.70±0.051</td>
<td>2.21±0.352</td>
<td>46.05±7.794</td>
<td>1.80±0.195</td>
<td>7.04±0.089</td>
<td>3.30±0.509</td>
<td>-0.213±0.072</td>
</tr>
<tr>
<td>Black men</td>
<td>7.28±0.025</td>
<td>16.4±1.80</td>
<td>6.67±0.080</td>
<td>1.77±0.372</td>
<td>35.72±6.050</td>
<td>1.78±0.326</td>
<td>7.66±0.148</td>
<td>2.82±0.473</td>
<td>+0.387±0.136</td>
</tr>
<tr>
<td>Black women</td>
<td>7.28±0.028</td>
<td>15.8±0.95</td>
<td>6.66±0.043</td>
<td>1.80±0.300</td>
<td>40.77±6.961</td>
<td>1.67±0.188</td>
<td>7.20±0.082</td>
<td>2.58±0.498</td>
<td>-0.047±0.095</td>
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<td><strong>Normal BP</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White men</td>
<td>7.20±0.028</td>
<td>20.8±2.73</td>
<td>6.77±0.035</td>
<td>1.31±0.159</td>
<td>32.37±5.193</td>
<td>2.08±0.198</td>
<td>7.13±0.034</td>
<td>2.94±0.353</td>
<td>-0.064±0.024</td>
</tr>
<tr>
<td>White women</td>
<td>7.32±0.027</td>
<td>14.4±1.10</td>
<td>6.65±0.039</td>
<td>2.27±0.559</td>
<td>43.71±6.256</td>
<td>1.71±0.222</td>
<td>7.05±0.036</td>
<td>4.19±0.569</td>
<td>-0.257±0.045</td>
</tr>
<tr>
<td>Black men</td>
<td>7.22±0.028</td>
<td>18.1±1.33</td>
<td>6.67±0.035</td>
<td>1.99±0.356</td>
<td>41.14±7.400</td>
<td>1.81±0.180</td>
<td>7.50±0.110</td>
<td>3.36±0.564</td>
<td>+0.272±0.101</td>
</tr>
<tr>
<td>Black women</td>
<td>7.23±0.049</td>
<td>17.4±1.09</td>
<td>6.60±0.037</td>
<td>2.37±0.735</td>
<td>44.76±9.144</td>
<td>1.99±0.256</td>
<td>7.20±0.176</td>
<td>2.61±0.808</td>
<td>-0.017±0.142</td>
</tr>
</tbody>
</table>

**Table 4. Summary of the Analysis**

- **pH**: cytosolic pH; BP, basal pH; pHu, initial pH at the start of sodium activation; MR, maximal initial rate (IR) for sodium activation; \( K_d \), Michaelis constant; N, Hill coefficient; X, apparent pH; set point for activation of the Na-H antiport; Y, apparent IR at pHu of 6.0; Q, X - BpH.
Tables 2 and 5. Platelets of patients of all subgroups manifested a Hill coefficient greater than unity in sodium-dependent activation of the Na-H antiport (Table 2). We have demonstrated a similar phenomenon with respect to other cells.14 There were narrow interindividual variations in pHxo values of platelets that were acidified under identical conditions (Table 2). These variations are explained in Table 5 and Figure 3, showing a significant, positive correlation between the pHxo and the cellular buffering power (r=0.417, p=0.0005). That is, cells with a higher buffering power demonstrate a lesser degree of nigericin-induced acidification under the specific conditions delineated in the Methods section. At the end of the acidification period, the pHxo is at a steady state but not at equilibrium with the extracellular pH because of differences in the transmembrane potassium concentrations. 

The MR was obtained from the analysis of sodium-dependent kinetics of the Na-H antiport in cells acidified to pH of approximately 6.7. The Yt value (namely, the extrapolated activity of the Na-H antiport at pH of 6.0) was derived from a different set of experiments examining the pH dependence of the exchanger. The significant correlation between the MR and Yt (r=0.500, p=0.0001) (Table 5) strongly suggests that, in relative terms, the rate of the Na-H antiport at a pH used for the sodium-dependent kinetics reflects a similar trend at a wider range of pH. This conclusion is also expressed in the linear relation between the pHxo and the activity of the antiport, derived from experiments examining the pH-dependent activation of the Na-H antiport.

The relations between the K0.5 and MR (r=0.475, p=0.0001) or between K0.5 and Yt (r=0.331, p=0.0104) (Table 5) indicate that cells with a lower affinity of the Na-H antiport to Na+, manifest increased maximal initial rate activity of this transport system. Such a phenomenon has been observed in different cells with respect to other sodium transport systems (for example, see Reference 18). It may represent increased density or enhanced turnover of the Na-H carrier to compensate for its lower affinity to Na+. The Hill coefficient greater than 1 in the sodium-dependent activation kinetics (Table 2), coupled with the negative correlation of the Hill coefficient with the K0.5 (Table 5), raise the possibility of more than one external binding site for sodium on the Na-H antiport of human platelets. Another possibility is that platelets manifest a positive cooperativity with respect to sodium activation of the Na-H antiport. This has been demonstrated recently by us14 for human fibroblasts and platelets and by Semplicini et al19 in human erythrocytes.

**Discussion**

Gender- and race (black versus white)-related differences have previously been shown for various sodium transport systems. We and others observed that erythrocytes from blacks and men have a higher sodium concentration than whites and women, respectively.20-22 These findings relate to the diminished maximal reaction velocity (Vmax)23-25 and lower density (Bmax)26 of sodium-pump units in blacks and men. Racial differences have also been observed in erythrocyte Na-K-2Cl cotransport21,27,28 and Li-Na countertransport.27-29 Recently, we identified increased sodium turnover rate under basal conditions in cultured skin fibroblasts from blacks30 and a greater activation of the Na-H antiport in these cells by serum stimulation and nigericin acidification.31 However, in contrast to the present study in human platelets, which did not identify racial differences in the MR of the

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Table 3. Correlation Analyses of Subject Characteristics With Parameters of the Na-H Antiport

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>rip</th>
<th>pHxo</th>
<th>Buffer power</th>
<th>MR</th>
<th>K0.5</th>
<th>N</th>
<th>Xt</th>
<th>Yt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r</td>
<td>0.154</td>
<td>-0.023</td>
<td>-0.168</td>
<td>-0.074</td>
<td>0.156</td>
<td>0.070</td>
<td>-0.058</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.2206</td>
<td>0.8551</td>
<td>0.1810</td>
<td>0.5563</td>
<td>0.2159</td>
<td>0.5966</td>
<td>0.6632</td>
</tr>
<tr>
<td>SBP</td>
<td>r</td>
<td>0.025</td>
<td>-0.037</td>
<td>-0.032</td>
<td>-0.002</td>
<td>-0.052</td>
<td>0.130</td>
<td>-0.180</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.8444</td>
<td>0.7692</td>
<td>0.7995</td>
<td>0.9857</td>
<td>0.6785</td>
<td>0.3272</td>
<td>0.1713</td>
</tr>
<tr>
<td>DBP</td>
<td>r</td>
<td>0.183</td>
<td>-0.060</td>
<td>-0.084</td>
<td>-0.144</td>
<td>0.007</td>
<td>0.249</td>
<td>-0.148</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.1435</td>
<td>0.6362</td>
<td>0.5049</td>
<td>0.2541</td>
<td>0.9549</td>
<td>0.0568</td>
<td>0.2630</td>
</tr>
<tr>
<td>BMI</td>
<td>r</td>
<td>0.005</td>
<td>-0.144</td>
<td>0.070</td>
<td>0.058</td>
<td>-0.001</td>
<td>0.266</td>
<td>-0.062</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.9698</td>
<td>0.2518</td>
<td>0.5773</td>
<td>0.6451</td>
<td>0.9224</td>
<td>0.0415</td>
<td>0.6404</td>
</tr>
</tbody>
</table>

pHxo, cytosolic pH; BpHi, basal pH; MR, maximal initial rate (IR) for sodium activation; K0.5, Michaelis constant; N, Hill coefficient; Xt, apparent pH set point for activation of the Na-H antiport; Yt, apparent IR at pHxo of 6.0.

---

Table 4. Stepwise Multiple Regression Analysis for Xt

<table>
<thead>
<tr>
<th>Variable</th>
<th>r² (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.31</td>
<td>0.2896</td>
</tr>
<tr>
<td>SBP</td>
<td>0.79</td>
<td>0.2837</td>
</tr>
<tr>
<td>DBP</td>
<td>5.08</td>
<td>0.0835</td>
</tr>
<tr>
<td>Race</td>
<td>13.54</td>
<td>0.0035</td>
</tr>
<tr>
<td>Gender</td>
<td>19.04</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

r², coefficient of determination; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
TABLE 5. Correlation Analyses of pH, and Na-H Antiport Parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>r/p</th>
<th>Y</th>
<th>X</th>
<th>N</th>
<th>K_{65}</th>
<th>MR</th>
<th>pH_{lo}</th>
<th>Buffer power</th>
</tr>
</thead>
<tbody>
<tr>
<td>BpH_{i}</td>
<td>0.937</td>
<td>0.305</td>
<td>0.084</td>
<td>-0.111</td>
<td>-0.034</td>
<td>0.122</td>
<td>0.027</td>
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</tr>
<tr>
<td>Buffer power</td>
<td>0.7802</td>
<td>0.0190</td>
<td>0.5060</td>
<td>0.3793</td>
<td>0.7888</td>
<td>0.3337</td>
<td>0.8284</td>
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</tr>
<tr>
<td>pH_{lo}</td>
<td>0.187</td>
<td>-0.032</td>
<td>-0.044</td>
<td>-0.057</td>
<td>0.162</td>
<td>0.417</td>
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</tr>
<tr>
<td>MR</td>
<td>0.1563</td>
<td>0.8083</td>
<td>0.7278</td>
<td>0.6523</td>
<td>0.1959</td>
<td>0.0005</td>
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<tr>
<td>K_{65}</td>
<td>0.044</td>
<td>0.130</td>
<td>-0.094</td>
<td>-0.233</td>
<td>-0.093</td>
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<tr>
<td>N</td>
<td>0.500</td>
<td>-0.107</td>
<td>-0.001</td>
<td>0.475</td>
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<td>...</td>
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</tr>
<tr>
<td>X_{i}</td>
<td>0.0001</td>
<td>0.4202</td>
<td>0.9917</td>
<td>0.0001</td>
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<tr>
<td>p</td>
<td>0.331</td>
<td>-0.242</td>
<td>-0.413</td>
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<td>...</td>
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<td></td>
</tr>
<tr>
<td>p</td>
<td>0.0104</td>
<td>0.0652</td>
<td>0.0006</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>pH_{i}</td>
<td>-0.105</td>
<td>0.062</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>pH_{i}</td>
<td>0.4276</td>
<td>0.6391</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>pH_{i}</td>
<td>-0.252</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>pH_{i}</td>
<td>0.0544</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

pH_{i}, cytosolic pH; BpH_{i}, basal pH; pH_{lo}, initial pH at the start of sodium activation; MR, maximal initial rate (IR) for sodium activation; K_{65}, Michaelis constant; N, Hill coefficient; X_{i}, apparent pH_{i} set point for activation of the Na-H antiport; Y_{i}, apparent IR at pH_{lo} of 6.0.

sodium-dependent activation of the Na-H antiport, our observations indicate that this parameter is higher in skin fibroblasts from blacks than whites. Hypertensive and normotensive women of both races and normotensive white men showed an X_{i} lower than BpH_{i}. However, a shift of the X_{i} to relatively alkaline levels in platelets of black men and hypertensive white men was observed. These findings suggest that, under basal conditions, the Na-H antiport is active in platelets of at least some black men and hypertensive white men. We believe that, indeed, this is the case. After completion of this study, we examined the effect of sodium-deficient medium or treatment with amiloride analogues on BpH_{i} of platelets from a number of subjects. All demonstrated a variable, but a definite, decline in pH_{i} in response to these experimental perturbations. In some the rate of acidification was very slow, but in others it was quite substantial (G. Tokudome and A. Aviv, unpublished data). One explanation for our findings would be that the method of platelet isolation or experimental conditions favored activation of the Na-H antiport. Although this is possible, it is noteworthy that in our hands platelets isolated by gel filtration exhibited a higher BpH_{i} than those obtained by centrifugation. Moreover, if the isolation procedure was the reason for the race-, gender-, and blood pressure-related differences in parameters of the Na-H antiport, the central conclusion of our study still holds. That is, differences in regulatory mechanisms of the Na-H antiport must exist among platelets of the various cohorts to account for their highly significant, differing responses to the experimental handling.

One way to accelerate the activity of the Na-H antiport is by activation of protein kinase C. Such a mode of enhancement of the exchanger is marked by a shift of the pH_{i} set point to a more alkaline pH_{i}.3,32 In this regard, protein kinase C activity was shown to be elevated in platelets of the SHR.33 Thus, it is conceivable that protein kinase C activity is also increased in platelets of black men and hypertensive white men. Such a phenomenon, if it occurs, may be associated with either increased Ca_{i} turnover rate or elevated Ca_{i} levels, which have been shown to be present in platelets of essential hypertensive patients.34-36

Although it is not yet clear whether, in the vascular smooth muscle cell, a rise in Ca_{i} can in itself activate the Na-H antiport through protein kinase C-independent mechanisms1,37,38, it appears that in these cells Ca_{i} is an important modulator and a necessary element for agonist-mediated activation of the enzyme that occurs through phospholipase C.37 Activation of protein kinase C appears, in turn, to attenuate agonist-mediated Ca_{i} response.39-42 It is, therefore, possible that increased activities of both protein kinase C and the Na-H antiport represent an integrated homeostatic process to compensate for

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Plot showing relation between the pH_{lo} (initial pH_{i}) and the cellular buffer power in platelets. Regression line is described by y=18.5x−105.7.
increased sensitivity to agonists that raise $Ca_\text{v}$ through phospholipase C-dependent or phospholipase C-independent mechanisms. We have reached this conclusion after observing that, as compared with those from whites, cultured skin fibroblasts from blacks demonstrate increased sodium turnover rate$^{30}$ under basal conditions, associated with a more pronounced serum-induced $Ca_\text{v}$ signal$^{43}$ and serum activation of the Na-H antiport.$^{31}$ It is possible that the same phenomenon is present in platelets from black men and hypertensive white men.

How then can an alkaline shift in the $X_c$ be present without the concurrent alkaline drift in $BpH$? Such a condition could exist because of a higher metabolic rate generating protons or a higher proton entry through the calcium pump (the physiological analogue of the calcium adenosine triphosphatase [ATPase]), which is a Ca-H exchange mechanism.$^{44}$ In addition to a higher elevation of $Ca_\text{v}$, serum-treated skin fibroblasts from blacks manifest a greater $^{4}Ca$ efflux, probably through the calcium pump, than their counterparts from whites.$^{45}$ Thus, fibroblasts from blacks appear to have an increased calcium turnover rate than those from whites, and the same phenomenon may also be present in their platelets. Resink et al$^{45}$ have suggested a similar process with regard to platelets of essential hypertensive subjects by demonstrating higher activities of both the calmodulin-sensitive and calmodulin-insensitive components of calcium ATPase in these cells. This observation could not be confirmed by our group.$^{46}$ In fact, our work suggests that, irrespective of racial extraction or gender, platelets of essential hypertensive persons exhibit a lower $V_{\max}$ for calcium ATPase. Platelets with a lower $V_{\max}$ for calcium ATPase can still demonstrate increased agonist-mediated calcium turnover rate if calcium entry and mobilization are increased. However, as compared with their counterparts, these platelets will extrude calcium at the same rate but at a higher level of $Ca_\text{v}$.

Elevated $Ca_\text{v}$ in platelets of essential hypertensive patients may increase their metabolic demand and consequently proton generation because of increased ATP hydrolysis and the formation of lactic acid.$^{47}$ Such a phenomenon would also account for an alkaline shift in the $X_c$ without a change in $BpH$. Whether mediated through protein kinase C-dependent or protein kinase C-independent mechanisms, an alkaline shift in the $X_c$ could be an advantageous, compensatory process for increased activity of the Ca-H exchange or the postulated augmentation of the metabolic rate associated with a rise in $Ca_\text{v}$. Such an adaptation would maintain the $pH_i$ close to basal level without the necessity of a "trade off" (i.e., the lowering of the $pH_i$ to activate the Na-H antiport to accommodate for increased proton entry or production).

Using an electronic cell sizing method, other workers$^{10,11}$ demonstrated that platelet swelling associated with sodium propionate acidification is enhanced in essential hypertension. Based on this observation, they concluded that the activity of the Na-H antiport is increased in essential hypertension. The electronic cell sizing method for the measurement of the Na-H antiport is based on the following principles. Treatment of cell suspensions with sodium propionate results in cytosolic acidification because propionic acid rapidly permeates the plasma membrane. The resulting activation of the Na-H antiport increases both sodium influx and the obligatory uptake of water required to maintain cellular osmolality. Thus, a major difference between the nigericin-mediated versus propionate-induced acidification of platelets is that the second approach also entails alteration in osmotic forces across the plasma membrane and substantial changes in platelet volume. In fact, the latter variable serves as the criterion for estimating the activity of the Na-H exchanger.

The aforementioned considerations emphasize the necessity to define the specific conditions for stimulation of the Na-H antiport and methods of assessing its kinetic properties. Hence, strict comparisons of results derived from studies using the electronic cell sizing technique$^{10,11}$ with the present work are difficult because they do not measure the same parameters. It is noteworthy, however, that Schmounder and Weder,$^{11}$ who studied black men and white men, did not show race-related differences in platelet volume alteration resulting from sodium propionate acidification, and Livne and coworkers,$^{10}$ studying whites, did not report gender-related differences in this parameter.

An intrinsic feature of the Na-H antiport is that its stimulation results in a rise in $pH_i$ and consequent deceleration of its activity. Based on this concept, the Na-H antiport-dependent rate of change in cellular volume associated with treatment with sodium propionate and, for that matter, the rate of change in $pH_i$, cannot be linear at any time. We have demonstrated that, indeed, this is the case for human platelets.$^{14}$ Therefore, the IR of proton-equivalent flux is more reliable for measuring the activity of the Na-H antiport. Measurements derived from progressively longer intervals after activation of the exchanger, even if they fall within the apparently linear phase of the $pH_i$ recovery, are increasingly inaccurate.

In previous studies, we have shown a negative correlation for the $V_{\max}$ for erythrocyte Na$_3K$-ATPase and a positive correlation for erythrocyte sodium and the systemic blood pressure.$^{25}$ Parameters of erythrocyte Na$_3K$-ATPase are also influenced by gender and racial extraction.$^{22,23,26}$ A similar conclusion emerges from the present work with respect to the Na-H antiport in platelets. The predisposition to essential hypertension is lower in women and whites than in men and blacks, respectively. Moreover, these groups with a lesser predisposition to the disease manifest a higher $V_{\max}$ for the Na$_3K$-ATPase and a more acidic set point for activation of the Na-H antiport. Both features provide for maintaining a lower cytosolic sodium concentration. These gender-
and race-related variations underscore the heterogeneous nature of the predisposition to and cellular manifestations of essential hypertension.

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KEY WORDS • essential hypertension • sodium transport • race • gender
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