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

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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/>=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)

The current consensus holds that alterations in the cellular regulation of cytosolic free calcium (Ca$_i$) and cytosolic sodium (Na$_i$) participate in the pathophysiology of essential hypertension. This concept has emerged from numerous studies demonstrating that several ion transport systems are altered in this disease. Recently, the focus of a number of investigations has been on human platelets, inasmuch as these circulating cells are responsive to a variety of agonists that regulate Ca$_i$ and Na$_i$. Moreover, platelets share characteristics with vascular smooth muscle cells, which play an important role in the increased peripheral vascular resistance that occurs in essential hypertension. One of the transport systems that has gained a substantial interest is the Na-H antiport (exchange), a ubiquitous sodium transport system that is activated by a variety of stimuli, including mitogenic and growth factors, vasoconstrictors, phorbol esters, and osmotic and pH changes.1-5 It has been proposed that this transport system may play a pivotal role in essential hypertension.6-8 To examine this concept, several investigators have used an electronic cell sizing technique as an indirect method for measuring the Na-H antiport in platelets of essential hypertensive subjects.9 These studies10,11 have concluded that the activity of the platelet Na-H antiport is increased in essential hypertension. Using the same technique, other investigators have also shown that the activity of the Na-H antiport is increased in cells of the spontaneously hypertensive rat,12,13 an animal model that has been commonly used to study genetic hypertension.

We have recently developed a technique using the fluorescent dye 2'7-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) to evaluate the kinetics of extracellular sodium (Na$_e$) and cytosolic pH (pH$_i$) activa-
tion of the Na-H antiport in platelets and other cells. 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 age, race, gender, and diagnosis of subjects in the various groups are presented in Table 1. It should be noted that the hypertensive group was older than the normotensive group, and normotensive blacks were younger than normotensive whites. In addition, the body mass index (BMI), expressed as body weight (kg)/height (m)$^2$, was greater in the hypertensive than the normotensive group. Correlation analyses revealed that, for the entire group, age was positively correlated with the systolic (r=0.416, p=0.0006) and diastolic (r=0.376, p=0.0020) blood pressures. The BMI was also positively correlated with the systolic (r=0.491, p=0.0001) and diastolic (r=0.479, p=0.0001) blood pressures.

Blood pressure measurements were obtained with a Mercury sphygmomanometer between 9:00 AM and 12:00 noon after a period of 5-10 minutes during which the subject was sitting in a comfortable position. 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.

#### Platelet Preparation, Acidification, and pH 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

## 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>
<td></td>
<td></td>
<td></td>
</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>
</tr>
<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>

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 \( \text{Na}^+ \) and \( \text{pH} \) activation of the Na-H antiport. However, the former method yielded platelets with a more alkaline basal pH (\( \text{pH}_{\text{b}} \)).

Blood was centrifuged at 200g for 10 minutes at room temperature. The platelet-rich plasma was centrifuged at 1,000g for 10 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(\( \beta \)-aminoethyl ether)\( \cdot \)N, N', N'-tetraacetic acid (EGTA) 0.2, and 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) 10 (\( \text{pH} = 7.40 \)). EGTA was omitted from the third washing, which also included 0.1% fatty acid–free bovine serum albumin (BSA). Platelets were loaded with \( 5 \mu \text{M BCECF-AM} \) (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 (\( \text{pH} = 7.40 \)).

Some platelets underwent measurements of \( \text{BpH}_{\text{b}} \) in HEPES (\( \text{pH} 7.40 \)) after washing off the extracellular BCECF-AM. Other platelets were acidified using 0.5 \( \mu \text{g/ml} \) nigericin in sodium-free HBS of different \( \text{pH} \) values (sodium was replaced isomotically by Na-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 \( \text{pH} \) at the initiation of the sodium-dependent alkalinization (\( \text{pH}_{\text{b}} \)). After 7 minutes of treatment with nigericin, 0.1% BSA was added, and the Na-H exchange was activated by adding 150 \( \mu \text{l} \) aliquots (approximately 3 x 10^6 platelets) to 3 ml HBS (0.1% BSA) (\( \text{pH} 7.4 \)) 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 \( \text{pH} \) recovery of platelets acidified by 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 \( \mu \text{g/ml} \) nigericin. After acidification, cells were washed once with sodium-free HBS plus 0.5 \( \mu \text{g/ml} \) nigericin and resuspended in 150 \( \mu \text{l} \) 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 \( \text{pH} \) recovery profiles between platelets acidified in sodium-containing and sodium-free media. In previous studies, \(:\text{Na}^+ \) recovery of acidified human platelets was completely inhibited by 10 \( \mu \text{M} \) of (\( \beta \)-N-methyl-N-isobutyl)amiloride. Calibration of \( \text{pH} \) was performed by subjecting platelets to 5 \( \mu \text{g/ml} \) nigericin in HBS (minus BSA) of different \( \text{pH} \) values with 145 mM potassium (isosmotically substituted for sodium). Standard curves were constructed for each platelet preparation.

\( \text{BpH} \), or changes in \( \text{pH} \), 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 \( \text{pH} \)-dependent recoveries from acidification were obtained through iterative curve fitting of the data to the following model:

\[
\text{pH}_{\text{b}}(t) = \text{pH}_{\text{b0}} - \left[ \text{pH}_{\text{b0}} - \text{pH}_{\text{b1}} \right] \times (1 - k^t) \times t
\]

where \( \text{pH}_{\text{b0}} \) is \( \text{pH} \) at a given time \( t \), \( \text{pH}_{\text{b1}} \) is \( \text{pH} \) at the new steady state, \( \text{pH}_{\text{b0}} \) is the initial \( \text{pH} \) 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 \( \text{pH}_{\text{b0}} \) 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 \( \text{pH} \) 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 \( \text{pH} \) recovery according to the expression:

\[
\text{IR} = k \times \left[ \text{pH}_{\text{b1}} - \text{pH}_{\text{b0}} \right]
\]

Platelet buffering power was measured at \( \text{BpH} \) by treatment with 10 mM NH\(_4\)Cl. The buffering power was determined from the change (\( \Delta \)) in cellular NH\(_4^+\) and \( \text{pH} \) (\( \Delta \text{pH} = \Delta \text{pH} = \Delta \text{pH} / \Delta \text{pH} \)) as previously described. 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 \( \text{pH} \).

In experiments examining the kinetics of sodium activation of the Na-H antiport, the target \( \text{pH}_{\text{b0}} \) was set for 6.7. Initial rates of proton equivalent efflux at different Na\(^+\) concentrations (10, 20, 30, 40, 60, 100, and 140 mM) were used in the model:

\[
v = \text{MR} \times \left[ \text{Na}^+ \right]^N / \text{K}_{\text{Na}}^N + \left[ \text{Na}^+ \right]^N
\]

where \( v \) is the IR of proton equivalent efflux, \( \text{MR} \) is maximal IR of the proton equivalent efflux, \( \left[ \text{Na}^+ \right] \) is extracellular sodium concentration, \( \text{K}_{\text{Na}} \) is half maximal activation of the antiport for a given \( \text{pH} \), and \( N \) is the Hill coefficient. Parameters of kinetics of sodium activation of the Na-H antiport were
FIGURE 1. Panel A: Illustration of fit of exponential model described by equation 1 (solid lines) to observed data (symbols) from platelets from one subject pH_i, cytosolic pH; IP, the first data point; pH_i0, extrapolated, initial pH at the moment of activation of the Na-H antiport. Cells were acidified to different pH_i levels. Panel B: Illustration of fit of model described by equation 2 (solid line) to initial rates of proton equivalent efflux (symbols) from platelets of one subject IR, initial rate. Panel C: Illustration of linear regression describing the relation of the pH_i dependency for activation of the Na-H antiport in platelets from one subject X_i, apparent pH_i set point for activation of the Na-H antiport; Y_0, extrapolated activity of the Na-H antiport at pH_i of 6.0.

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 regression (i.e., models using sigmoid, logarithmic, or exponential 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_i0 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_i 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_0) 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 analysis, 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 various 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 medications manifested no differences in these parameters from the untreated, newly diagnosed hypertensive 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, respectively). 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 parameters for the various cohorts. There were no statistically 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 scattergram 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-


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

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>pH(B) (pH)</th>
<th>pH(pH) × pH</th>
<th>MR (mmol/liter) × sec</th>
<th>K_M (mM)</th>
<th>N</th>
<th>pH(N) [mmol/liter] × sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>High BP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</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>4.60 ± 4.606</td>
<td>2.09 ± 0.163</td>
<td>7.45 ± 0.052</td>
</tr>
<tr>
<td>White women</td>
<td>7.26 ± 0.025</td>
<td>19.6 ± 1.76</td>
<td>6.70 ± 0.051</td>
<td>4.65 ± 4.794</td>
<td>1.80 ± 0.195</td>
<td>7.04 ± 0.089</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>35.75 ± 8.050</td>
<td>1.78 ± 0.326</td>
<td>7.66 ± 0.148</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>40.77 ± 6.691</td>
<td>1.67 ± 0.188</td>
<td>7.20 ± 0.082</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>32.37 ± 5.193</td>
<td>2.08 ± 0.198</td>
<td>7.13 ± 0.034</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>43.71 ± 6.256</td>
<td>1.71 ± 0.222</td>
<td>7.05 ± 0.036</td>
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<tr>
<td>Black men</td>
<td>7.22 ± 0.028</td>
<td>18.1 ± 1.33</td>
<td>6.67 ± 0.035</td>
<td>41.14 ± 7.400</td>
<td>1.81 ± 0.180</td>
<td>7.50 ± 0.110</td>
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<tr>
<td>Black women</td>
<td>7.23 ± 0.049</td>
<td>17.4 ± 1.09</td>
<td>6.60 ± 0.037</td>
<td>44.76 ± 9.144</td>
<td>1.99 ± 0.256</td>
<td>7.20 ± 0.176</td>
</tr>
</tbody>
</table>

*p* values

<table>
<thead>
<tr>
<th>Race</th>
<th>0.7357</th>
<th>0.3909</th>
<th>0.3069</th>
<th>0.9012</th>
<th>0.9665</th>
<th>0.4852</th>
<th>0.0016</th>
<th>0.0900</th>
<th>0.0003</th>
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<tr>
<td>Gender</td>
<td>0.1515</td>
<td>0.3066</td>
<td>0.4134</td>
<td>0.2797</td>
<td>0.1884</td>
<td>0.3484</td>
<td>0.0001</td>
<td>0.9744</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>0.2249</td>
<td>0.8755</td>
<td>0.6546</td>
<td>0.9079</td>
<td>0.9698</td>
<td>0.6772</td>
<td>0.0835</td>
<td>0.6056</td>
<td>0.1011</td>
</tr>
</tbody>
</table>

*pH*, cytosolic pH; *BpH*, basal pH; *pH*, initial pH at the start of sodium activation; *MR*, maximal initial rate (IR) for sodium activation; *K_M*, Michaelis constant; *N*, Hill coefficient; *X*, apparent pH set point for activation of the Na-H antiport; *Y*, apparent IR at *pH* of 6.0; Q, *X* - BpH.

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

**8.2**

**8.0**

**7.8**

**7.6**

**7.4**

**7.2**

**7.0**

**6.8**

**6.6**

**White**

**Black**

**Females**

**Males**

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Potentially wider distribution (mean group values of 7.05-7.66) (Table 2). In fact, five black men and one black woman showed *X* values greater than 7.8, and two white men exhibited *X* values greater than 7.6 (Figure 2). Moreover, there were tendencies for higher *X* values in hypertensive men than in normotensive men. Although, as a group, essential hypertensive subjects did not manifest a significant increase in *X*, diastolic blood pressure for the entire population was weakly but positively correlated with the *X* value lower than BpH,. 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 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 for *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 pH regulation in human platelets are revealed by data summarized in
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 $pH_{in}$ 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 $pH_{in}$ 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 $pH_{in}$ 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 $Y_t$ value (namely, the extrapolated activity of the Na-H antiport at $pH_i$ of 6.0) was derived from a different set of experiments examining the $pH_i$ dependency of the exchanger. The significant correlation between the MR and $Y_t$ ($r=0.500, p=0.0001$) (Table 5) strongly suggests that, in relative terms, the rate of the Na-H antiport at a $pH_i$ used for the sodium-dependent kinetics reflects a similar trend at a wider range of $pH_i$. This conclusion is also expressed in the linear relation between the $pH_{in}$ and the activity of the antiport, derived from experiments examining the $pH_i$-dependent activation of the Na-H antiport.

The relations between the $K_{0.5}$ and MR ($r=0.475, p=0.0001$) or between $K_{0.5}$ and $Y_t$ ($r=0.331, p=0.0104$) (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 $K_{0.5}$ (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 us for human fibroblasts and platelets and by Semplicini et al 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.20-23 These findings relate to the diminished maximal reaction velocity ($V_{max}$)22-25 and lower density ($B_{max}$)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 blacks 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 antiport.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>$r^2$ (%)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.25</td>
<td>0.2500</td>
</tr>
<tr>
<td>SBP</td>
<td>0.07</td>
<td>0.2537</td>
</tr>
<tr>
<td>DBP</td>
<td>0.05</td>
<td>0.2635</td>
</tr>
<tr>
<td>BMI</td>
<td>1.54</td>
<td>0.2696</td>
</tr>
</tbody>
</table>

$r^2$, coefficient of determination; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

### Table 4. Stepwise Multiple Regression Analysis for $X_t$

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r^2$ (%)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.31</td>
<td>0.2596</td>
</tr>
<tr>
<td>SBP</td>
<td>0.79</td>
<td>0.2337</td>
</tr>
<tr>
<td>DBP</td>
<td>0.08</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>

$r^2$, coefficient of determination; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
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. 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 of platelets from a number of subjects. All demonstrated a variable, but a definite, decline in pH 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 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 set point to a more alkaline pH.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 turnover rate or elevated Ca 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 can in itself activate the Na-H antiport through protein kinase C-independent mechanisms1,37,38, it appears that in these cells Ca 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 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
increased sensitivity to agonists that raise Ca, 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 under basal conditions, associated with a more pronounced serum-induced Ca, signal and serum activation of the Na-H antiport. 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, 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. In addition to a higher elevation of Ca, serum-treated skin fibroblasts from blacks manifest a greater Ca, efflux, probably through the calcium pump, than their counterparts from whites. Thus, fibroblasts from blacks appear to have a higher calcium turnover rate than those from whites, and the same phenomenon may also be present in their platelets. Resink et al 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. In fact, our work suggests that, irrespective of racial extraction or gender, platelets of essential hypertensive persons exhibit a lower V,^ for calcium ATPase. Platelets with a lower V,^ 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,.

Elevated Ca, 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. Such a phenomenon would also account for an alkaline shift in the X, without a change in BpH. Whether mediated through protein kinase C-dependent or protein kinase C-independent mechanisms, an alkaline shift in the X, 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,. Such an adaptation would maintain the pH, close to basal level without the necessity of a "trade off" (i.e., the lowering of the pH, to activate the Na-H antiport to accommodate for increased proton entry or production).

Using an electronic cell sizing method, other workers 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 with the present work are difficult because they do not measure the same parameters. It is noteworthy, however, that Schmounder and Weder, 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, 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, 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, cannot be linear at any time. We have demonstrated that, indeed, this is the case for human platelets. 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, recovery, are increasingly inaccurate.

In previous studies, we have shown a negative correlation for the V,^ for erythrocyte Na,K-ATPase and a positive correlation for erythrocyte sodium and the systemic blood pressure. Parameters of erythrocyte Na,K-ATPase are also influenced by gender and racial extraction. 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,^ for the Na,K-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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