Na\textsuperscript{+}-H\textsuperscript{+} Antiport Activity in Skin Fibroblasts from Blacks and Whites

Norio Hatori, Jeffrey P. Gardner, Haruo Tomonari, Burton P. Fine and Abraham Aviv

The predisposition of black people to salt (NaCl)-sensitive essential hypertension may relate to racial differences in cellular Na\textsuperscript{+} metabolism. This tenet was investigated by examining the Na\textsuperscript{+}-H\textsuperscript{+} antiport in serially passed skin fibroblasts from blacks and whites. Na\textsuperscript{+}-dependent stimulation of the Na\textsuperscript{+}-H\textsuperscript{+} antiport by cellular acidification resulted in a greater maximal velocity (V\textsubscript{max} (mean±SEM) of this transport system in quiescent fibroblasts from blacks than fibroblasts from whites; the V\textsubscript{max} for recovery from cellular pH (pH\textsubscript{c}) of 6.6 was 5.84±0.50 versus 4.39±0.34 mmol H\textsuperscript{+}/lx20 seconds for blacks and whites, respectively (p<0.05). Although the Na\textsuperscript{+} concentration producing 50% stimulation of the Na\textsuperscript{+}-H\textsuperscript{+} antiport for blacks (35.1±5.7 mM) was greater than for whites (24.1±3.5 mM), this difference was not statistically significant. No racial differences were observed in the Hill coefficient (n, 1.35±0.21 for blacks and 1.46±0.28 for whites). Compared with whites, cells from blacks exhibited a greater response to cytoplasmic acidification over the range of pH\textsubscript{c} values 6.20–6.60, as exhibited by an augmented rate of recovery in the pH\textsubscript{c}. These differences were not due to different basal pH\textsubscript{c} values or cellular buffering capacities, which were similar for blacks and whites. Na\textsuperscript{+}-H\textsuperscript{+} antiport activity was not correlated with family history of hypertension. Increased activity of the Na\textsuperscript{+}-H\textsuperscript{+} antiport in fibroblasts from blacks was confirmed without cellular acidification by stimulating quiescent cells with 10% human serum. This study demonstrates innate racial differences in cellular membrane Na\textsuperscript{+}-H\textsuperscript{+} antiport activity. (Hypertension 1990;15:140–145)

As a group, blacks exhibit a higher frequency of essential hypertension, particularly the salt-sensitive form of the disease. The cellular mechanisms that underlie this racial difference are poorly understood. This investigation focuses on racial (black vs. white) differences in the Na\textsuperscript{+}-H\textsuperscript{+} antiport of skin fibroblasts because 1) nonquiescent fibroblasts from blacks exhibit both an increased rate of Na\textsuperscript{+} turnover and a higher cytosolic Na\textsuperscript{+} concentration, and 2) fibroblasts from blacks show a greater cytosolic free Ca\textsuperscript{2+} response to agonists in serum. Agonist-mediated Ca\textsuperscript{2+} response is frequently coupled with activation of the Na\textsuperscript{+}-H\textsuperscript{+} antiport, a transport system that plays an important role in Na\textsuperscript{+} homeostasis (for review of its characteristics, see References 9 and 10). In this report, we show that the activity of the Na\textsuperscript{+}-H\textsuperscript{+} antiport is higher in cultured fibroblasts from blacks as compared with whites. If this racial difference is also present in epithelium of the renal proximal tubule, it may increase Na\textsuperscript{+} reabsorption and contribute to the propensity for salt-sensitive essential hypertension to develop in blacks.

Methods

Subjects

Fibroblasts were derived from our frozen stock of cultured cells. They originated from 19 normotensive black and 23 normotensive white subjects. Studies with these cells were performed in two phases separated by a 1-year interval. Experimental protocols in phase 1 were performed (by N.H.) in cells from 15 black (11 with a family history of essential hypertension) and 15 white (10 with a family history of essential hypertension) subjects, and experiments in phase 2 were performed (by J.G.) with cells from 11 black (eight with a family history of hypertension) and 11 white (five with a family history of hypertension) subjects. Sex, age, and blood pressure values of individuals contributing cells for the two phases are...
described in Table 1. Informed consent, approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey, was obtained from all subjects.

Methods

Biopsy specimens (approximately 2×3 mm) were taken from the medial aspect of the left arm and processed to obtain cultured skin fibroblasts as previously described. Cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) (95% air and 5% CO₂) with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and antibiotics (50 μg streptomycin and 50 units penicillin/ml). Once sufficient quantities of cells were obtained, cells were harvested and stored in liquid nitrogen for future use. Before each phase of experiments, preparations from the stock of frozen cells were matched according to the donor’s race, age, and gender. Cells were defrosted and grown as above. Four days before experiments, 5×10⁵ cells were inoculated on 13.8×30 mm glass coverslips placed in Nunc-6-well plates and grown without antibiotics.

Confluent monolayers of fibroblasts (passages 6–11) were made quiescent by a 24-hour FBS depletion. This step was undertaken to eliminate the possible effect of growth on any observed differences in Na⁺-H⁺ activity. No effects of passage number, gender, or age were noted on the examined cellular parameters.

Measurements of cytosolic pH (pHₜ) were performed with minor modifications as previously described. All procedures were performed at 37°C. In the first phase, cells were washed twice with 3 ml HEPES solution of the following composition (mM): NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 1, HEPES 10, glucose 10, pH 7.10. They were loaded for 1 hour at 37°C in the same buffer with 5 μM 2',7’-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM). Cells were then washed twice with 3 ml HEPES buffer solution (HBS) containing 0.1% fatty acid–free bovine serum albumin (BSA), pH 7.40. In the second phase, fibroblasts were loaded in DMEM (instead of HBS) with 5 μM BCECF-AM. After 60 minutes, cells were rinsed twice with HBS. During these experiments, it was noted that after BCECF loading, the pH, declined (0.1–0.3 pH units) until stabilization occurred after about 5–7 minutes.

Cytoplasmic acidification was performed by subjecting cells to 0.5 μg nigericin/ml HBS (minus BSA), the pH of which was adjusted to different levels (6.30–6.60). The pH reached a new steady state within less than 4 minutes incubation (see inset to Figure 1); this maneuver was reproducible for a given extracellular pH. In preliminary experiments, we showed that treatment with this concentration of nigericin for 10 minutes caused no apparent changes in cytosolic Na⁺ and K⁺ concentrations. After acidification, cells were rapidly washed twice with Na⁺-free HBS. NaCl was isosmotically replaced with choline chloride. In the second phase, it was replaced by N-methyl-d-glucamine (NMDG). The Na⁺-H⁺ antiport in acidified cells was activated by the addi-

<table>
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<tr>
<th>Phase</th>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Age (yr)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
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<tr>
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<tr>
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<tr>
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<td>6</td>
<td>33.4±3.2</td>
<td>110.1±3.8</td>
<td>70.7±1.8</td>
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<tr>
<td></td>
<td>White</td>
<td>5</td>
<td>6</td>
<td>35.3±3.4</td>
<td>114.5±4.0</td>
<td>74.2±2.6</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Figure 1. Line graph showing Na⁺-dependent activation of Na⁺-H⁺ antiport in acidified (cytosolic pHₜ=6.30) fibroblasts from 15 black (●) and 15 white (○) subjects. Individual points with significant differences are indicated in figure (*p<0.02). Curves represent fit of model to data as described in Methods; parameters of Na⁺-dependent alkalinization are given in text. Basal pHₜ levels in HEPES buffer solution (HBS) (7.33±0.04 and 7.29±0.03 for fibroblasts from blacks and whites, respectively) were not significantly different. Inset, a composite demonstrating methodology for measuring pHₜ of acidified cells exposed to varying extracellular sodium concentrations ([Na⁺]₀). In typical experiment, cells were acidified with nigericin (NIG) for 5 minutes. After two rapid washes in Na⁺-free HBS (pH 7.40), cells were subjected to varying [Na⁺]₀, and the pHₜ was monitored. In first phase of study, cells on same coverslip were subjected to repeated acidifications followed by sequential activation at increasing [Na⁺]₀. In the second phase, activations of Na⁺-H⁺ antiport with different [Na⁺]₀, were performed in different slides.
tion of HBS with Na+ concentrations ranging from 0 to 140 mM. For Na+-dependent pHj recoveries, acidified cells were monitored for the initial 30 seconds (first phase) or 20 seconds (second phase) after exposure to Na+, when changes in pHj (ΔpH) appeared linear.

Fluorescence measurements were performed in two different spectrophotometers equipped with thermostatically controlled (37° C) cell holders. For the first phase, experiments were performed in a Perkin-Elmer (Norwalk, Connecticut) LS-5 spectrophotometer (excitation wavelength 500 nm, emission wavelength 530 nm; slits 5x10 nm). Experiments in the second phase were performed with a SPECT (Edison, New Jersey) Fluorolog II spectrophotometer (model CM-3) equipped with a beam splitter and two excitation monochromators enabling alternate excitation of BACEF at 440 and 503 nm (slits at 3.6 nm). Emission wavelength was set at 530 nm. For these experiments, the integration time was set at 0.2 second and data were collected over 1-second intervals.

Calibration of the fluorescent intensity of intracellular BACEF against pH was performed in parallel with the same number of cells. These cells were subjected to 5 μg nigericin/ml in a solution containing (mM): KCl 150, CaCl2 2, MgCl2 1, HEPES 10, glucose 10, pH 6.20–7.60. The cytoplasmic buffering capacity was measured by subjecting cells to 5 mM NH4Cl or potassium propionate (first phase) or 10 mM NH4Cl (second phase). The buffering capacity was determined from changes in cellular [NH4+] or [propionate−] and the ΔpH (Δ[NH3]/ΔpH) or [propionate−]/ΔpH).14 H+ equivalent efflux rates were determined as the product of the buffer capacity of the cells and Na+-dependent pHj recovery.

In the first phase, measurements of the cellular buffering power were not performed at the same time as measurements of the Na+-dependent pHj recovery from nigericin acidification. Thus, the H+ equivalent efflux rates could not be computed for individual subjects. In the second phase, the cellular buffering power for each subject was measured at the same time as the pHj recovery from cellular acidification, and the H+ equivalent efflux was determined for fibroblasts from each individual.

The data for activation of the Na+-H+ antiport by [Na+] were fitted to a model described by

\[ v = V_{\text{max}} \times \frac{[\text{Na}^+]^n}{[\text{Na}^+]^n + K_{0.5}^*} \]

where \( v \) is the initial reaction velocity, \( V_{\text{max}} \) is the maximal initial reaction velocity, \([\text{Na}^+] \) is the extracellular Na+ concentration, \( K_{0.5}^* \) is the concentration for 50% stimulation of the Na+-H+ antiport, and \( n \) is the Hill coefficient. This equation is a modification of a Michaelis-Menten–like reaction, which incorporates a cooperative mode of interaction between Na+ and the Na+-H+ antiport. The fit of the model to the data is indicated by the curves in Figure 1.

Nonlinear regression analyses of activation of the Na+-H+ antiport by [Na+] were performed using an NLIN procedure of the Statistical Analysis System (SAS) on an IBM 3033U computer (first phase) and an IBM-compatible personal computer (second phase). Statistical analyses of the various kinetic parameters from the nonlinear regressions used weighted least squares according to the method described by Johnson and Miliken.15 All other statistical comparisons were performed with the unpaired Student’s t test. Data are presented as mean±SEM.

Results

The kinetics of Na+-H+ antiport activation in human fibroblasts were measured at two different initial pHj values (6.30 in the first phase and 6.60 for the second phase). There were no correlations between parameters of the Na+-H+ antiport and family history of essential hypertension. In the first phase (Figure 1), the maximal reaction velocity (\( V_{\text{max}} \)) of the recovery from a pHj of 630 for fibroblasts from blacks was significantly higher than that for fibroblasts from whites (0.330±0.025 vs. 0.233±0.011 pH units/30 seconds, respectively; \( p<0.02 \)). There were no statistically significant racial differences in the \( K_{0.5} (47.6±6.8 \text{ mM for blacks vs. } 33.5±2.7 \text{ mM for whites}) \) and Hill coefficient (1.31±0.13 for blacks and 1.51±0.14 for whites). The cellular buffering capacity exhibited substantial variability among the subjects. However, no differences in this parameter were observed between blacks and whites, whether NH4+ (blacks 11.9±1.4 and whites 14.8±1.4 mmol/l/pH) or propionate− (blacks 13.5±1.6 and whites 15.0±1.8 mmol/l/pH) were used in the pHj recovery. In further experiments, we examined the effects of varying the degree of cellular acidification on the rate of pHj recovery in 140 mM Na+ (Figure 2). The dependence of the pHj recovery rate in fibroblasts from blacks was shifted to the left, suggesting that the Na+-H+ antiport in fibroblasts from blacks was more sensitive to intracellular H+ than in whites.

Precise estimation of kinetic parameters of the Na+-H+ antiport requires determination of H+ equivalent efflux rates for fibroblasts from each individual. Because this parameter was not available in experiments from the first phase, further studies were conducted. In this set of experiments, the kinetics of Na+-dependent cytoplasmic pHj recovery from a pHj of 6.60 was monitored. Results of these experiments are presented in Table 2, and they show a significant difference in the \( V_{\text{max}} \) for the H+ equivalent efflux for fibroblasts from blacks compared with fibroblasts from white subjects. Similar to experiments presented in Figures 1 and 2, there were no statistically significant racial differences in basal pHj values (7.27±0.05 for blacks and 7.17±0.05 for whites), \( K_{0.5} \) values, or Hill coefficients. Moreover, there were also no differences between blacks and whites in the cellular buffering capacity as measured by the NH4Cl method (26.9±3.9 mmol/l/pH for blacks and 28.3±2.6 mmol/
The difference in the absolute values of the cellular buffering power between cells studied during the first and second phases is unclear. This discrepancy may relate to different growth conditions. For instance, the FBS stocks for growing the cells during the two phases were different.

The Na+-H+ antiport appears to play a role in the mitogenic response, and it is activated by subjecting serum-deprived, quiescent cells to serum. In cells exposed to HBS, the addition of 10% human serum (derived from a single white individual) resulted in a biphasic response of the pHi profile of fibroblasts (Figure 3). In cells from white subjects, an initial and brief period of acidification was followed by sustained alkalinization; the latter phase lasted for at least 10 minutes with a plateau being reached at approximately 6 minutes. In comparison, fibroblasts from blacks demonstrated a slight acidification but a substantially more rapid rate of alkalinization and a higher plateau level in the pHi. The addition of 10% human serum to fibroblasts in Na+-deficient HBS resulted in a dramatic and rapid acidification in cells from both blacks and whites; subsequently, only a slight increase in the pHi toward basal levels was noted (Figure 3).

### Discussion

We have demonstrated increased mean activity of the Na+-H+ antiport in fibroblasts from blacks by using independent modes of stimulation, namely by varying [Na+] and pHi and by the addition of serum. Fibroblasts from blacks exhibit a greater H+ sensitivity for activation of the Na+-H+ antiport. Moreover, the Vmax for the Na+-H+ antiport in acidified cells is significantly higher in fibroblasts from blacks than whites at a pHi of 6.60 or lower. The higher Vmax value implies that either the density of Na+-H+ carriers in these cells is greater or that the turnover rate of each carrier is higher, or both. Higher Na+-H+ antiport activity could explain the increased Na+ turnover rate in nonquiescent fibroblasts from blacks.

The biphasic response in pHi to serum in our study was similar to previous observations in which agonists exert their effect via mobilization of Ca2+ and stimulation of the Na+-H+ antiport. When the second phase of this response is eliminated by inhibition of the Na+-H+ antiport, pronounced acidification ensues after exposure to the agonist. The reason that fibroblasts from blacks demonstrate only slight acidification at the early phase (initial 30 seconds) after exposure to serum in Na+-containing HBS may relate to their high Na+-H+ antiport activity, which overshadows the acidification.

Racial differences in cellular Na+ metabolism have been extensively investigated in erythrocytes. In these...
cellular differences.

be the only cause for hypertension in blacks. How-

differences in cellular Na⁺ regulation are unlikely to
cellular Na⁺ regulation may not be

demonstrate resistance to

distribute in platelets from blacks and whites. Although these cells are
ready available, they demonstrate resistance to amiloride, which may explain their poor specific
binding to amiloride analogues. Thus, it is question-
able whether these cells are appropriate to examine parameters of the Na⁺-H⁺ antiport. We recently
studied kinetics of Na⁺ activation of the Na⁺-H⁺ antiport in platelets from blacks and whites (Toku-
dome G, Aviv A, unpublished observations) and did not demonstrate racial differences in the V_{max} for Na⁺
activation of this system in these cells. However, platelets from blacks showed a significant alkaline
shift in the pH_i set point for activation of the exchanger. Although the pH_i, set point for activation of the Na⁺-H⁺ antiport in fibroblasts from blacks and whites were not examined in the present work, results (Figure 2) suggest an alkaline shift of this parameter in fibroblasts from blacks. One possible cause for differences between cultured cells and circulating cells is the lasting effect of factors in the blood that can obscure abnormalities that may be part of the makeup of circulating cells. Thus, our findings in cultured fibroblasts suggest the existence of racial differences that are innate to the cell membrane without the confounding influence of circu-
lating factors. Such a tenet is based on the assumption that differences in cellular Na⁺ regulation that are observed in vitro are also expressed in vivo. Furthermore, a recent study²² has shown that growth parameters of cultured human skin fibroblasts may be influenced by degree of sun exposure or their site of origin. The contribution of such a factor and melanin content to the racial differences in the Na⁺-H⁺ antiport is unknown.

We speculate that increased Na⁺-H⁺ antiport activity in fibroblasts from blacks may also be expressed in a variety of other cells including vascular smooth muscle and renal tubular cells. In these cells such a phenomenon may indicate greater sensitivity to vasoactive agents and growth factors.¹³,¹⁸,²⁸–³⁰ Because the Na⁺-H⁺ antiport plays an important function in renal tubular Na⁺ reabsorption,¹¹,¹² the postulated increase in its activity in tubular cells could provide one explanation for the propensity of salt-sensitive essential hypertension to develop in blacks.⁴–⁶ It must be emphasized, however, that increased Na⁺-H⁺ antiport activity may not be expressed in all cells.

Inasmuch as our studies were performed on cells from normotensive individuals, the observed racial differences in cellular Na⁺ regulation are unlikely to be the only cause for hypertension in blacks. How-
ever, we suggest that the threshold to hypertension development may be lower in blacks because of these cellular differences.

References


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