Lymphocyte Membrane Sodium-Proton Exchange in Spontaneously Hypertensive Rats

PETER U. FEIG, MICHAEL A. D'OCCIO, AND JOHN W. BOYLAN

SUMMARY The sodium-proton exchange activity was determined in lymphocytes of spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto rats (WKY), and domestic Wistar rats. Uptake of sodium was determined by measuring the osmotic swelling of lymphocytes after activation of the exchanger by suspension of the cells in sodium propionate and consequent intracellular acidification by the permeant weak acid. Fractional swelling (mean ± SEM) in 16 SHR and 16 WKY was 0.44 ± 0.03 and 0.35 ± 0.02, respectively (p<0.01). The swelling was partially inhibitable by amiloride and, at 10^{-4} M concentration, the amiloride-sensitive swelling was 0.21 ± 0.02 in SHR and 0.11 ± 0.01 in WKY (p = 0.001). Progressive extracellular ion substitutions of chloride for propionate or of potassium for sodium showed that the exchange activity was related linearly to cellular acidification; however, the dependence on extracellular sodium displayed saturation characteristics, with the same apparent $K^*_e$ for cells from SHR and WKY and a $V_{max}$ of 0.54 ± 0.03 for SHR and 0.39 ± 0.02 for WKY (p<0.002). External lithium could replace sodium on the exchanger but abolished the differences between strains. Results in the domestic Wistar rats were similar to those of WKY. These results suggest that lymphocytes of the SHR have a greater capacity for sodium uptake through the sodium-proton exchanger, as compared with normotensive strains. If shared by other cells, such an increased capacity could have a pathophysiological role in genetic hypertension. In particular, its presence in proximal renal tubular cells would support the hypothesis of a primary role for the kidney in the pathogenesis of genetic hypertension. (Hypertension 9: 282-288, 1987)

KEY WORDS • genetic hypertension • cation exchange • thymocytes

A variety of cells from humans with essential hypertension, as well as from genetically hypertensive rats, show alterations in membrane transport of ions when compared with cells from their normotensive counterparts.1−3 One of these alterations is an increased Na^{+}-Li^{+} countertransport, which has been shown to be present in erythrocytes of humans with essential hypertension and their relatives.4−18 While, in general, the pathophysiological role of these membrane alterations is in question,1, 2, 19−21 the meaning of an increased Na^{+}-Li^{+} countertransport is particularly puzzling. Even if the alteration is present in cells potentially more relevant than erythrocytes to the pathophysiology of hypertension, this transport system, mediating one-for-one exchange of sodium under physiological conditions, causes no net movement of ions and, thus, has no apparent physiological function. A possible significance for this apparently irrelevant finding has, however, been proposed by Aronson22 and by Funder et al.11 These authors suggested that the Na^{+}-Li^{+} (or Na^{+}-Na^{+}) countertransport serves as a marker for alterations in yet another, but perhaps related, cation exchange system, the sodium-proton (Na^{+}-H^{+}) exchange (countertransport or antiport), a system that has major physiological functions in regulation of intracellular pH, cell volume, and sodium content, in hormone and enzyme activity, and in renal sodium reabsorption.23 Yet, there are no direct studies on the Na^{+}-H^{+} exchange in cells of genetically hypertensive animals, whether humans or rats, probably because of the difficulty in performing the studies in the most easily available cells, the erythrocytes, where the magnitude of Na^{+}-H^{+} exchange is small in humans24 and probably absent in rats (unpublished observations, 1984; S. Dissing et al., personal communications,

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1985). We therefore studied Na⁺-H⁺ exchange in lymphocytes, cells known to be rich in this transport system, using as a model of genetic hypertension the Wistar Okamoto spontaneously hypertensive rats (SHR) and, as controls, the Wistar-Kyoto rats (WKY) and domestic Wistar rats (DWR).

Materials and Methods

Rats (SHR, WKY, and DWR) were obtained from Laboratory Supply Company (Indianapolis, IN, USA) and studied at 4 months of age. Systolic blood pressures were determined by the tail-cuff method (Narco Bio-Systems, Houston, TX, USA). After ether anesthesia, the thymus was removed, placed in a suspending solution (RPMI, Gibco Laboratories, Grand Island, NY, USA; with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) at pH 7.2 at 20°C), cleared of vessels and visible connective tissue, and teased. The thymocytes were then washed through gauze to remove extraneous tissue. The lymphocytes (thymocytes) were washed three times by repeated centrifugation (200 g for 5 minutes at 20°C) and resuspension in the suspending solution. After cell count (Model ZBI; Coulter counter, Hialeah, FL, USA), the cells remained in the suspending solutions for the duration of the studies at a cell concentration of approximately 10⁶/ml. Small aliquots (0.2 ml) of this cell suspension were then resuspended into 20 ml of the appropriate solutions (see the following section) placed in Coulter vials (final cell concentration approximately 10⁶/ml), and cell volumes were measured (Coulter Channelizer C1000 with X-Y Recorder 4) at frequent intervals, from 1 to 120 minutes after the final dilution.

For baseline volume, the cells were suspended in a solution of (mM) 140 NaCl, 1 KCl, 1 CaCl₂, 1 MgCl₂, 10.1 glucose, 20 HEPES (pH ~ 6.8 at 20°C with Tris OH), 295-300 mosm/kg H₂O. In this medium, the cells maintain a constant volume for at least 80 minutes. In order to stimulate the Na⁺-H⁺ exchanger, NaCl was replaced by equimolar sodium propionate. In this medium, the weak acid anion, in its protonated form, permeates the cell membrane and, in the cell, dissociation of the acid leads to intracellular acidification. The consequent activation of the Na⁺-H⁺ exchanger leads to H⁺ extrusion in exchange for Na⁺ uptake and the entering Na⁺, trapped in the cells (presumably as sodium propionate), causes osmotic gain of water and cell swelling that has been shown to be related to isotopic and net sodium influx via the Na⁺-H⁺ exchanger. Preliminary studies in our laboratory have confirmed the findings of Grinstein et al. of stimulatory effect of sodium propionate on Na⁺ influx (data not shown).

The degree of cellular acidification was varied by graded replacement of propionate with chloride as the anion. Dependence of the exchange rate on extracellular sodium was studied by graded replacement of sodium by potassium, a cation that is not transported by the Na⁺-H⁺ exchanger. Lithium propionate replaced sodium propionate to study Li⁺-H⁺ exchange through the Na⁺-H⁺ exchanger. Amiloride was provided by the courtesy of Edward H. Blaine (Merck Institute for Therapeutic Research, West Point, PA, USA).

Cell size was measured by using latex particles as standard and using the factor 1.1 for correction of cell deformability (S. Grinstein, personal communications, 1984). Cell volume gain was expressed as the fractional swelling, as compared with the same cells in isosmotic NaCl medium.

Data were analyzed by unpaired two-tailed Student's t test and analysis of variance (where multiple comparisons were performed) and are presented as means ± SEM.

Results

The systolic blood pressure for 16 SHR was 171.4 ± 6.0 mm Hg, significantly higher than those of 16 WKY (121.4 ± 2.4 mm Hg; p = 1.1 × 10⁻⁸) and DWR (119.0 ± 2.7 mm Hg; p = 1.3 × 10⁻³).

When placed in NaCl medium, the volume of lymphocytes remained constant for 120 minutes of observation and was similar for the cells from the three strains of rats studied, as shown in Table 1. When exposed to 140 mM sodium propionate, cell swelling occurred (see Table 1), reaching a plateau by 20 minutes (results at 80 minutes, not shown, were the same as those at 20 and 40 minutes). As seen, at all times the lymphocytes of SHR had a volume gain significantly (p<0.0001) larger than that of either DWR or WKY.

The effect of small differences in Na⁺-H⁺ exchange activity on the pattern of cell swelling is shown in Figure 1, where lymphocytes from DWR were exposed to sodium propionate without and with a partially inhibitory dose (3 × 10⁻⁶ M) of amiloride. When the results with lymphocytes from all 16 SHR and 16 WKY studied in sodium propionate, without amiloride, were presented in the same manner (Figure 2), a similar difference in Na⁺-H⁺ exchange activity, larger in cells from SHR than in those from WKY, was seen.

Note that we chose 20-minute values (the earliest time at which the reliably measurable plateau in cell swelling was reached) for many of our data (see below); these values were proportional to the smaller and less accurately measurable initial rates of swelling. The reason for the plateau is unclear. It probably is not related to stimulation by increased intracellular sodium of sodium extrusion by the Na⁺-K⁺ pump (presumably, at room temperature and with lowered intracellular pH, the pump is markedly inhibited) or to swelling-induced increase in potassium and chloride permeabilities since neither ouabain nor the potassium channel blocker oligomycin changed the swelling pattern significantly. In studies of lymphocytes of five DWR placed in sodium propionate, the actual values of fractional swelling at 20 minutes were 0.46 ± 0.014 in presence of 10⁻³ M ouabain and 0.53 ± 0.014 in presence of oligomycin, 10 mg/ml, values not statistically different from that of 0.49 ± 0.011, obtained in absence of either ouabain or oligomycin. The reason for the plateau may be a competitive inhibition of the internal transporting sites of the Na⁺-H⁺ exchanger by increased intracellular sodium.
TABLE 1. Volumes of Lymphocytes (μm³) Obtained from Domestic Wistar Rats, WKY, and SHR

<table>
<thead>
<tr>
<th>Strain</th>
<th>NaCl</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWR (n = 7)</td>
<td>119 ± 1</td>
<td>161 ± 1*</td>
<td>164 ± 2*</td>
<td>165 ± 2*</td>
<td>166 ± 2*</td>
</tr>
<tr>
<td>WKY (n = 7)</td>
<td>120 ± 1</td>
<td>164 ± 2*</td>
<td>168 ± 1*</td>
<td>171 ± 1*</td>
<td>173 ± 2*</td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>122 ± 1</td>
<td>181 ± 1</td>
<td>185 ± 1</td>
<td>188 ± 1</td>
<td>187 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Cells were placed in a medium with either 140 mM NaCl (values remain constant over time) or 140 mM sodium propionate (values shown are after 5, 10, 20, and 40 minutes.

DWR = domestic Wistar rats.

*p < 0.0001 (analysis of variance), compared with values in SHR.

Graded substitution of chloride for propionate as the anion accompanying sodium resulted in a proportional decline in cell volume gain (Figure 3A) because intracellular acidification was lower. Similarly, changing extracellular sodium concentration by substitution with potassium, a cation that is not transported by the Na⁺-H⁺ exchanger, also resulted in decreased volume gain (Figure 3B). As shown, there was a proportionality between swelling and extracellular propionate concentration but not between swelling and extracellular sodium concentration (see Figure 3).

The linearity of the dependence of swelling on the extracellular propionate concentration, using the 20-minute values, is shown in Figure 4A (r = 0.997 for both SHR and WKY). When extracellular sodium concentration was varied, however, the relationship between extracellular sodium concentration and the volume gain (20-minute values) showed characteristics of saturation (Figure 4B). Because of the saturation characteristics and because of the apparent proportionality between the initial rates and the 20-minute values for swelling, we performed Lineweaver-Burk plots of the inverse of the volume gain against the inverse of the extracellular sodium concentration in each of the 21 experiments (7 experiments on cells of each of 3 strains) in which the effect of variable extracellular sodium was studied. A representative example of such linear Lineweaver-Burk plots on cells of one of the SHR and one of the WKY is shown in the inset of Figure 4B. The results of these plots showed that the difference between strains was due to the maximum swelling (V_max), which for cells from SHR (0.54 ± 0.03) was higher than for those from WKY (0.40 ± 0.02) or from DWR (0.37 ± 0.01; both at p < 0.001, by analysis of variance). The apparent K_m (mM) was 21 ± 4 for SHR, 18 ± 2 for WKY, and 13 ± 1 for DWR. Since a small fraction of the swelling was not dependent on extracellular sodium, occurring even in absence of sodium (see Figures 3B and 4B) because of the gain of propionic acid, these values are not exactly proportional to the sodium entry through the Na⁺-H⁺ exchanger. Subtraction of the small fractional swelling (about 0.05–0.09) that occurs in the absence of extracellular sodium, predictably increases K_m and decreases V_max values. However, subtraction of these small values from the smallest (and, thus, least accurately measurable) swelling values results in relatively inaccurate estimates of swelling at low extracel-

Figure 1. Fractional volume change of lymphocytes of domestic Wistar rats placed in 140 mM sodium propionate in the absence (□) and presence (▲) of a partially inhibitory dose of amiloride (3 × 10⁻⁶ M).

Figure 2. Fractional volume change of lymphocytes of 16 SHR (●) and 16 WKY (▲) in 140 mM sodium propionate. Values are means ± SEM. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference between groups.
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**Figure 3.** Fractional volume change of lymphocytes of 7 domestic Wistar rats. Values are means ± SEM. A. Cells were placed in 140 mM Na with the anion composed of propionate (at concentrations of 0–140 mM, as indicated) or chloride. B. Cells were placed in 140 mM propionate, with the cation composed of sodium (at concentrations of 0–140 mM, as indicated) or potassium.

luminal sodium concentrations. Under these circumstances, use of the Lineweaver-Burk plot and inversion of volume gain results in excessive "weight" for the least accurate values and thus considerable error in the determination of the slope of the function and the x intercept and $K_m$. Thus, these data are handled best by the use of other methods, in which volume gain is not inverted, such as the Woolf-Augustinsson-Hofstee plot, which circumvents this problem. The latter

**Figure 4.** Fractional volume change of lymphocytes of 7 SHR (■) and 7 WKY (▲) 20 minutes after placement in media with various sodium and propionate concentrations. Values are means ± SEM. A. Data were obtained in 140 mM Na medium and are presented as a function of the propionate concentration (0–140 mM), with chloride used as equimolar replacement for propionate. B. Data were obtained in 140 mM propionate medium and are presented as a function of the sodium concentration (0–140 mM), with potassium used as equimolar replacement for sodium. Inset shows a representative Lineweaver-Burk plot of the inverse of fractional volume change versus the inverse of sodium concentration of the results in a spontaneously hypertensive (■) and a Wistar-Kyoto (▲) rat; $V_m$ represents the maximal volume change, and $K_m$ represents the sodium concentration in which half-maximal swelling was obtained.
method resulted in values for $V_{\text{max}}$ (y intercept) that for cells from SHR were higher than for those from WKY (0.50 ± 0.03 vs 0.34 ± 0.03; p < 0.0001) or from DWR (0.26 ± 0.02; p < 0.005), with no significant difference between WKY and DWR. Values for $K_m$ (mM) using this plot were 27 ± 3 for SHR, 28 ± 3 for WKY, and 15 ± 3 for DWR. Interestingly, by either method, the $K_m$ values for DWR were significantly lower than those for either SHR or WKY; the reason for this increased affinity of the transport system of DWR for external sodium is unclear but may relate to the fact that DWR are from a genetically different strain than the SHR and WKY. The $K_m$ values in this study are consistent with the 59 mM value given by Grinstein et al.\textsuperscript{23} That our values are slightly lower may be because our extracellular pH (6.8) was lower than the 7.2 pH value used in the study of Grinstein et al.\textsuperscript{23} Moreover, the intracellular pH may not be comparable. The results, therefore, show an increase in the capacity to transport sodium through the Na\textsuperscript{+}-H\textsuperscript{+} exchanger in cells from SHR, as compared with those of WKY and DWR.

When sodium in the extracellular medium was replaced by lithium, swelling was present but reduced to about 40% of the magnitude found with sodium (Figure 5), as reported by Grinstein et al.\textsuperscript{23} Of note is that with lithium as substrate on the external site of the exchanger, no differences between SHR and WKY were apparent (see Figure 5).

In lymphocytes, amiloride only partially suppresses the Na\textsuperscript{+}-H\textsuperscript{+} exchanger,\textsuperscript{30,31} with a dose-dependence that, in our studies, did not show complete saturation even at 3 to 10 mM concentrations. Results with amiloride at $10^{-4}$ M and $3 \times 10^{-3}$ M concentrations are shown in Figure 6A. As seen, the component of swelling resistant to either of the two doses of amiloride was the same for the cells of the two strains and the amiloride-sensitive fraction was larger in cells from SHR than in those from WKY. Because of the effects of high doses of amiloride on systems other than the Na\textsuperscript{+}-H\textsuperscript{+} exchanger,\textsuperscript{23} we chose to use a $10^{-4}$ M concentration as representative of more specific amiloride-sensitive Na\textsuperscript{+}-H\textsuperscript{+} exchange. Results on the amiloride-sensitive component of cell volume gain at $10^{-4}$ M concentration are shown in Figure 6B, with significantly higher volume gain in SHR than in WKY ($p < 0.01$ at 10 minutes and after).

**Discussion**

Our findings in lymphocytes represent evidence for an increased sodium transport capacity by the Na\textsuperscript{+}-H\textsuperscript{+} exchanger in cells from SHR, as compared with those of two normotensive control strains, the WKY and the DWR. The data are therefore compatible with the hypothesis proposed by Aronson\textsuperscript{22} and by Funder et al.\textsuperscript{11} mentioned previously, that the increased Na\textsuperscript{+}-Li\textsuperscript{+} countertransport found in red blood cells of patients with essential hypertension may mark the presence of altered Na\textsuperscript{+}-H\textsuperscript{+} exchange activity. While several similarities exist between the two cation exchange systems,\textsuperscript{22,23} evidence for difference also exists, such as the lack of sensitivity of Na\textsuperscript{+}-Li\textsuperscript{+} countertransport to amiloride\textsuperscript{23} and the demonstration that the Na\textsuperscript{+}-Li\textsuperscript{+} countertransport-rich rabbit erythrocyte does not perform significant Na\textsuperscript{+}-H\textsuperscript{+} exchange through the Na\textsuperscript{+}-Li\textsuperscript{+} countertransport system.\textsuperscript{32} However, this does not exclude the possibility that the two transport systems are sufficiently similar that they may be controlled by common genetic or environmental factors (or both). Unfortunately, it is not possible to show any correlation between our findings and erythrocytic Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in these animal models of the disease, because of the absence of Na\textsuperscript{+}-Li\textsuperscript{+} countertransport in rat erythrocytes (personal observations, 1984; M. Canessa et al. and S. Dissing et al., personal communications, 1985).

Whether related or not to the abnormalities in Na\textsuperscript{+}-Li\textsuperscript{+} countertransport, the alterations in Na\textsuperscript{+}-H\textsuperscript{+} exchange demonstrated here may have important implications for the pathophysiology of hypertension. If present in the vascular smooth muscle, such alterations could induce a net gain in sodium and thus, secondarily, increase intracellular calcium concentration and vascular tone\textsuperscript{1,19}; alternatively, it could cause an increase in intracellular pH, with subsequent increase in vascular tone.\textsuperscript{23} Moreover, the effect of Na\textsuperscript{+}-H\textsuperscript{+} exchange on intracellular pH regulation\textsuperscript{23} has a potential effect on many pH-dependent enzymes throughout the organism. It is also of note that this transport system is
involved in many hormonal actions\(^2\) that could play a role in the pathophysiology of hypertension.

Of particular interest is the possibility that this same alteration in Na\(^+\)-H\(^+\) exchange could be present in the cells of the proximal renal tubule. If so, this could cause increased sodium reabsorption and a propensity for hypertension, in particular under conditions of excessive sodium intake.\(^{20, 35-36}\) If this were the case, a generalized cellular membrane sodium transport abnormality, previously often described as secondary to events that follow a primary abnormality in the kidney,\(^{20}\) could be implicated in the known primary role of the kidney in genetic hypertension.\(^{20, 35-41}\) In this regard, recent work has shown an increased lithium reabsorption, presumably by the proximal renal tubules, in both humans\(^{42}\) and rats\(^{43}\) with genetic hypertension. Our finding that the transport of lithium by the Na\(^+\)-H\(^+\) exchanger is no different in SHR than in WKY is not necessarily in conflict with these studies. In the urinary clearance studies in humans\(^{42}\) and rats,\(^{43}\) lithium clearance is a marker for total proximal tubular sodium reabsorption, probably includes cation absorption secondary to Na\(^+\)-H\(^+\) exchange,\(^{23}\) and is not restricted to that transported by the Na\(^+\)-H\(^+\) (or Li\(^+\)-H\(^+\)) exchanger.

The cause of the increased Na\(^+\)-H\(^+\) exchange capacity in cells of SHR is not determined. Since intracellular pH is a regulator of this exchange mechanism,\(^{23, 25, 44}\) the relationship between Na\(^+\)-H\(^+\) exchange activity and intracellular pH should be determined. At this point, we cannot state whether the alterations observed represent intrinsic differences in the exchanger or whether they are due to differences in intracellular acidification or in the allosteric effect of the H\(^+\) ion on an intracellular modifier site.\(^{23, 44}\) In addition, because an increase in intracellular calcium stimulates the Na\(^+\)-H\(^+\) exchanger\(^23\) (albeit contrary evidence\(^23\) also exists) and because intracellular calcium has been shown to be increased in cells from humans\(^{45, 46}\) and rats\(^{46}\) with genetic hypertension, the role of intracellular calcium should also be defined.

In summary, our studies show still another abnormality of ionic transport by membranes in cells of an animal model of genetic hypertension. Because of the numerous physiological roles of cell membrane Na\(^+\)-H\(^+\) exchange, this abnormality has the potential for influencing many important pathophysiological functions. In particular it may constitute, at least in part, a basic abnormality present in the kidney, an abnormality that could account for the primacy of the kidney in genetic hypertension in rats\(^{37, 39, 41}\) and, perhaps, in humans.\(^{38, 40}\)

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