Red Cell Sodium Countertransport and Cotransport in Normotensive and Hypertensive Blacks

MITZY CANESSA, PH.D., ANDA SPALVINS, B.S., NORMA ADRAGNA, PH.D., AND BONITA FALKNER, M.D.

SUMMARY We have previously described elevated Li$\text{-Na}_\text{o}$ countertransport (CT) and Na-K cotransport (CO) in red cells of Caucasian patients from Boston. In this study, we report both transport systems in black patients from Philadelphia. The maximal rate ($V_{\text{max}}$) of CT was assayed by measuring the Na$\text{-stimulated Li efflux from cells containing} \pm 6$ mmol Li/liter. The $V_{\text{max}}$ of outward cotransport was assayed by measuring the furosemide-sensitive component of Na and K efflux into Mg medium from cells containing 50 mmol/liter of both ions. The mean value of CT for 18 normotensive (NT) subjects with no family history of hypertension, (−) FHH, was 0.18 ± 0.05 (mmol/liter cells × hour); and in 14 hypertensive (HT) patients, 0.18 ± 0.07. The mean values of Na and K cotransport were, respectively (mmol/liter cells × hour), in 18 NT subjects with (−) FHH, 0.38 ± 0.24 and 0.50 ± 0.28 in 18 HT subjects, 0.25 ± 0.17 and 0.24 ± 0.14. We conclude that there is no difference in the $V_{\text{max}}$ for CT between the two groups of black subjects, but that the $V_{\text{max}}$ for Na-K CO was significantly reduced in the HT group. Notably, the offspring of HT patients (age 14 years, n = 17) also had a marked reduction in the $V_{\text{max}}$ of Na (0.15 ± 0.17) K cotransport (0.19 ± 0.14) in comparison with the mean value of Na (0.40 ± 0.2) and K (0.60 ± 0.3) cotransport measured in offspring (n = 10) of NT subjects (age 14 years). Two main differences between the patterns of red cell cation transport were found in this sample of black patients in comparison with previous studies in Caucasian subjects. First, hypertensive blacks had no significant elevation of Li-Na countertransport, as previously observed in Caucasians; second, the $V_{\text{max}}$ of Na-K cotransport was significantly lower in hypertensive blacks than a previous sample of hypertensives. These measurements of red cell cation transport have uncovered heterogeneity in the types of disturbances of Na transport that might be present in the hypertensive population in different frequencies. The results indicate that in this subset of black hypertensive patients and their offspring, the capacity to transport Na against its electrochemical gradient is lower than in hypertensive subjects with elevated countertransport and Na-K cotransport. (Hypertension 6: 344–351, 1984)

KEY WORDS • red cell cation transport • essential hypertension • ethnic differences

RECENT studies on the mechanism of ouabain-insensitive Na transport in human red cells have indicated that Na countertransport and Na-K cotransport are more than likely two different transport proteins rather than one single Na transport system with two modes of operation.$^1,2$ The systems differ in their sensitivity to inhibitors such as furosemide and p-chloromercuribenzoate, their $k_{\text{o}}$, for internal Li, and their dependence on chloride.$^1$ The functional role of both transport systems seems to be the extrusion of Na against its electrochemical gradient driven by a Na, K, or proton gradient.

Studies of red cell cation transport in patients with essential hypertension have also shown that at least two types of alterations of Na transport can be found in hypertensive patients: a reduction of the $V_{\text{max}}$ of outward Na-K cotransport,$^3,4$ and a marked elevation in the Li-Na countertransport system$^2$ that is accompanied by normal or elevated Na-K cotransport.$^4,6$ The present study was designed to investigate, in an ethnic group different from the one already studied, such as American blacks, the occurrence of these two types of alterations of Na transport. Although the differences in blood pressure between blacks and Caucasians in the United States are now well documented,$^7$ the causes of these differences have remained unclear.$^8-11$ The higher average diastolic blood pressure of blacks as compared to that of Caucasians in the U.S. is accompanied by the well-documented finding of

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great prevalence of essential hypertension in the black population. The present study is an attempt to investigate the factors controlling membrane transport as causes of these differences between populations. As in previous investigations, in this study we have measured the Na-K cotransport and Li-Na countertransport in cells with a cationic composition that ensures kinetic conditions for maximal rate.

These findings indicate that there are at least two subsets of cation transport alterations (low Na-K cotransport and elevated countertransport) that may affect differently hypertensives of distinct ethnic origin. In the present study in black hypertensives, we found a reduced Na-K cotransport and no elevation of the Li-Na countertransport, in contrast to previous findings in Caucasian hypertensives showing elevated countertransport and Na-K cotransport.

Materials and Methods

We studied four groups of subjects. The control group was composed of 18 normotensive subjects (11 females and 7 males) with diastolic blood pressures under 90 mm Hg and no personal or family history of hypertension. This group took a normal diet, ate salt freely, and was medically normal at the time of the study.

In the second group were 18 established essential hypertensive patients (9 females and 9 males) with no evidence of renal or heart failure or severe retinopathy. One parent of the young was also studied to determine the Na cell transport and blood pressure.

A third group of 10 normotensive adolescents (5 females and 5 males) had negative family history of hypertension. A fourth group of 17 normotensive adolescent subjects (8 females and 9 males) had family history of hypertension (+ FHH). Their age ranged from 10 to 20 years.

In addition to the red cell transport studies, the cardiovascular response to mental stress was examined in 23 offspring of normotensive and hypertensive subjects. The methodology for assessment of mental stress produced by an arithmetic test is identical to that previously described by one of us.

Preparation of Red Cells

Human blood was drawn from donors into heparin vacuum tubes and immediately processed. Plasma anduffy coat were removed by centrifugation for 10 minutes at 3000 g. Cold preservation solution contained (mM): 135 K, 15 Na, 10 Tris-MOPS buffer, pH 7.4. The cell suspensions (50% hematocrit) were shipped by air to Boston in styrofoam buckets containing crushed ice in plastic bags. Upon arrival (approximately 4 hours later), 2 ml of cells were separated for Na loading overnight by the p-chloromercuribenzene sulfonic acid (PCMB) technique. Packed red cells (2 ml) were separated into two tubes for lithium loading. An aliquot of the remaining cells was washed four times with a solution containing (mM): 75 MgCl₂, 95 sucrose, 10 Tris-MOPS, pH 7.4 at 4°C (WS). An aliquot of the pellet was washed twice and suspended at approximately 50% hematocrit in WS. The hemoglobin per liter of cells (optical density at 540 nm) and cation composition of the initial cell suspension were determined in appropriate dilutions in 0.02% Acetoxion detergent (American Scientific Products, McGraw Park, Illinois). The next day, the cell suspension separated for lithium loading was spun, the supernate was removed, and Li-loading solution was added. Blood from members of the laboratory was drawn, preserved, and simultaneously enclosed as a control of cell preservation and internal control of the analytical reproducibility of the flux analysis. Previous results indicated that preservation for up to 3 days does not alter the Na countertransport or the cotransport.

Measurements of the Maximal Activation of Outward \( \text{Na-K} \) Cotransport

The PCMB-loading procedure was performed exactly as previously described. The loading solution contained (mM): 120 NaCl, 30 KCl, 1 MgCl₂, 2.5 Na phosphate buffer pH 7.2, 1 Tris-EGTA, pH 7.2, and 0.02 PCMBs.

Furosemide-sensitive Na and K fluxes were measured into Na-free and K-free Mg medium containing (mM): 75 MgCl₂, 85 sucrose, 10 Tris-MOPS (pH 7.4 at 37°C), 10 glucose, and 0.1 ouabain. Contamination by Na and K of this solution was always less than 6.

<table>
<thead>
<tr>
<th>Population study</th>
<th>Normotensive control</th>
<th>Hypertensive</th>
<th>(-)FHH</th>
<th>(+)FHH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>18</td>
<td>18</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>35 ± 16</td>
<td>38 ± 16</td>
<td>14.6 ± 5</td>
<td>13 ± 6</td>
</tr>
<tr>
<td>Mean SBP</td>
<td>112 ± 1</td>
<td>147 ± 24</td>
<td>110 ± 13</td>
<td>121 ± 18</td>
</tr>
<tr>
<td>Mean DBP</td>
<td>73 ± 13</td>
<td>95 ± 13</td>
<td>64.8 ± 8</td>
<td>75.2 ± 14</td>
</tr>
<tr>
<td>Weight (kg/m²)</td>
<td>20.8 ± 4</td>
<td>29 ± 8.8</td>
<td>20 ± 4</td>
<td>23.3 ± 5</td>
</tr>
</tbody>
</table>

(-)FHH = negative family history of hypertension; (+)FHH = positive family history of hypertension; SBP = systolic blood pressure; DBP = diastolic blood pressure.
μM. Furosemide (20 mM) was freshly prepared by titration to pH 7.4 with Tris base and added to the Mg medium to a final concentration of 1 mM. Washed cells suspended at 40% hematocrit were diluted with the cold media to a final hematocrit of 4%. Triplicate tubes containing 1.5 ml of the flux suspension were incubated for 0 and 60 minutes at 37°C. To stop the reaction, tubes were transferred to 4°C for 1 minute and then centrifuged for 5 minutes at 6000 g. The hematocrit of the incubation media was rigorously maintained since, under these conditions, the rate of the reaction was linear up to 75 minutes.

Na and K concentrations were measured in an atomic absorption spectrophotometer (Model 5000, Perkin-Elmer, Norwalk, Connecticut) with standards of Na and K in water (10–200 μM). The efflux was calculated from the slope of the change of the external cation concentration between 0 and 60 minutes. The regression coefficients were always higher than 0.985. The flux units (mmol/liter cells × hour) were calculated from the measured hematocrit of the cell suspension added to the incubation media. The furosemide-sensitive component was calculated from the difference between the cation fluxes in the presence and absence of the inhibitor. Flux measurements in the PCMBS-loaded cells were computed only when the hemoglobin per liter of cells was 100% ± 2% that of fresh cells. In every group of blood samples analyzed for Na-K cotransport and Li-Na countertransport, the red cells of one control subject were measured in triplicate. The variations of the control samples (in every set of assays, 20%).

Measurements of the Maximal Rate of Li-Na Countertransport

The Li loading and efflux measurements were performed as previously described. The 50% cell suspension was diluted in cold Mg and Na media. At this hematocrit, the Mg concentration in the Na medium is about 7 mM, which gives similar leak fluxes as in 75 mM MgCl₂. Triplicate tubes containing 1.5 ml of flux media were incubated for 0 and 60 minutes at 37°C in a shaker bath. Subsequently, we followed the procedure previously described. Li concentration at zero time in both media was 2 μM.

The Li concentration in Mg medium was determined with standards containing 75 mM MgCl₂. Li standards in water were used for Na medium since no interference was detected by our instrument (Perkin Elmer atomic absorption spectrophotometer model 5000). All Li standards were checked with commercial standard (Alfa Division/Ventron Corporation, Danvers, Massachusetts). All solutions used in these experiments were adjusted to 290 ± 5 mOsm. The concentrations of MgCl₂ and sucrose stock solutions were calculated from their measured osmotic pressure and osmotic coefficient.

Chemicals

KCl, NaCl, LiCl, and MgCl₂, were purchased from Mallinckrodt, Inc. (St. Louis, Missouri). Tris, cysteine, PCMBS, adenine, and ouabain were purchased from Sigma Chemical Company (St. Louis, Missouri). Furosemide was a gift of Hoechst Roussel Pharmaceuticals, Inc. (Somerville, New Jersey). All solutions were made in deionized, double-distilled water.

**Results**

Li-Na Countertransport in Red Cells of Normotensive Subjects and Hypertensive Patients

Table 2 shows the cation composition of the red cells loaded with Li for countertransport measurements and of the cells loaded with Na for measurements of the maximal rate of Na-K cotransport. The cellular lithium concentration used in this assay ensures saturation of internal sites and an adequate Li concentration in the efflux medium (>5 μM). It can also be noticed that, after the lithium-loading procedure, cellular Na is significantly higher in the hypertensive patients and their offspring than in normotensive subjects. The red cells of cases and controls were similarly preserved and incubated for 3 hours in Li-loading solution. It seems, therefore, that the hypertensive group has elevated cellular Na. The cellular concentration of Na achieved by the PCMBS-loading procedure ensures saturation of internal sites with Na and K in order to obtain maximal activation of the furosemide-sensitive Na and K efflux. The Na- and K-

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### Table 2. Cation Composition of Red Cells Loaded for Li-Na Countertransport and Na-K Cotransport

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium (mmol/liter cells)</th>
<th>Potassium (mmol/liter cells)</th>
<th>Lithium (μM)</th>
<th>Hemoglobin (OD/liter cells)</th>
<th>Sodium (mmol/liter cells)</th>
<th>Potassium (mmol/liter cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive (n = 18)</td>
<td>4.02 ± 1.6</td>
<td>94 ± 6.7</td>
<td>5.2 ± 0.6</td>
<td>291 ± 20</td>
<td>39 ± 12</td>
<td>74.7 ± 14</td>
</tr>
<tr>
<td>Normotensive* (+)FHH</td>
<td>7.1 ± 3</td>
<td>97.6 ± 6</td>
<td>6 ± 1</td>
<td>301 ± 17</td>
<td>51 ± 14</td>
<td>60 ± 14</td>
</tr>
<tr>
<td>(−)FHH (n = 10)</td>
<td>4.4 ± 1.5</td>
<td>98 ± 6</td>
<td>5.2 ± 0.6</td>
<td>301 ± 16</td>
<td>43 ± 14</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Hypertensive* (n = 18)</td>
<td>6.2 ± 2.4</td>
<td>93.3 ± 5.2</td>
<td>6.2 ± 0.9</td>
<td>295 ± 18</td>
<td>55 ± 18</td>
<td>59 ± 17</td>
</tr>
</tbody>
</table>

Values are means ± SD. OD = optical density at 540 nm; (−)FHH = negative family history of hypertension, (+)FHH = positive family history of hypertension.

* *p < 0.001.
free medium (zero-trans) provides the maximal gradient and avoids trans-inhibition effects from these cations.

It can be seen in Table 3 that in a normotensive group of subjects without a family history of hypertension and in hypertensive subjects that the Li⁻Na⁺ countertransport is not significantly different in mean value or distribution. Only one hypertensive patient had elevated countertransport (0.4 mmol/liter cells x hour). In the present sample, the rate constant of Li efflux into Mg medium is higher (0.024/hr⁻¹) than in a previous study. Since in these experiments the red cells were preserved for one day, we measured simultaneously the red cells of Caucasian normotensive subjects as internal controls after 1 day of preservation. The mean value for the rate constant of Li efflux into Mg medium was 0.018 ± 0.002 (n = 11), which is not different than the values previously reported (0.016 ± 0.001).

Na-K Cotransport in Red Cells of Normotensive Subjects and Hypertensive Patients

Table 4 shows measurements of the furosemide-sensitive and furosemide-resistant Na and K efflux in red cells of normotensive subjects and hypertensive patients. The mean values of Na-K cotransport do not differ significantly from those previously reported in Caucasian subjects, which also showed a wide variation in the control group. It can be seen that the red cells of the hypertensive patients have significantly lower values of Na-K cotransport than the control group.

A large overlap between the groups was observed. One-third of the normotensive control group had values of red cell Na cotransport below 0.25 mmol/liter cells x hour. Eleven of the 16 hypertensive individuals had cotransport values "lower" than 0.25 mmol/liter cells x hour.

Table 4 also shows the Na/K coupling ratio in normotensive and hypertensive black subjects. It can be seen that the coupling ratio was significantly higher in hypertensive than normotensive subjects. The elevation in Na/K ratio is caused by a larger reduction in the furosemide-sensitive Na efflux as compared to the K efflux.

Na-K Cotransport in Red Cells of Juveniles with Normotensive and Hypertensive Parents

Figure 1 compares the red cell Na-K cotransport and diastolic blood pressure in adolescents of normotensive (n = 10) and hypertensive (n = 13) parents. The distribution of diastolic blood pressure of adolescents of hypertensive parents was shifted to the upper limit of 90 mm Hg. There can also be noticed a marked displacement of the red cell Na-K cotransport to values lower than 0.25 mmol/liter cells in the adolescents of hypertensive parents (Table 5).

In many of these adolescents, a stress test was performed to evaluate their cardiovascular response. An abnormal increase in diastolic blood pressure and cardiac rate in response to the arithmetic test was found in eight of the subjects with reduced values of Na-K cotransport. The Li⁻Na⁺ countertransport of adolescents with normotensive parents was not significantly different from that of adolescents with hypertensive parents (Table 5).

In the adolescent offspring of hypertensive parents (Table 5), the reduced values of Na-K cotransport were not accompanied by significant overweight, as observed in the adults. The results suggest that it might be useful to follow up those individuals with low Vmax Of

### Table 3. Li⁻Na⁺ Countertransport in Red Cells of Black Normotensive and Essentially Hypertensive Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yrs)</th>
<th>Blood pressure (mm Hg)</th>
<th>Rate constant (hr⁻¹)</th>
<th>Li efflux (mmol/liter cells x hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diastolic</td>
<td>Systolic</td>
<td>magnesium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td>18</td>
<td>35 ± 16</td>
<td>73 ± 13</td>
<td>129 ± 22</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>18</td>
<td>38 ± 16</td>
<td>95 ± 13</td>
<td>147 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SD. Rate constant was calculated as Li efflux (mmol/liter cells x hour)/cellular Li (mmol/liter cells).

### Table 4. Na-K Cotransport in Cells of Black Normotensive and Essentially Hypertensive Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Furosemide-sensitive efflux (mmol/liter cells x hr)</th>
<th>Furosemide-resistant rate constants (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
</tr>
<tr>
<td>Normotensives (—)FHH</td>
<td>18</td>
<td>0.38 ± 0.24</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>18</td>
<td>0.25 ± 0.25*</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*p < 0.05.
†p < 0.001.
Na-K cotransport to determine whether they may become hypertensive with weight gain.

Relationship Between Li-Na, Countertransport and K Cotransport in Black Subjects

Figure 2 shows the relationship between Na countertransport and Na-K cotransport found in normotensive and hypertensive black individuals and Caucasian families. No distinction is being made between normotensive and hypertensive individuals. It can be seen that the two Na transport systems are poorly correlated in this sample \((p < 0.02)\). The regression between K cotransport and Li-Na countertransport was not statistically significant \((r = 0.26; p < 0.01;\) Figure 2). In these regression lines, hypertensives are on the left side of the curve.

Discussion

The black population of the United States has a significantly greater incidence of high blood pressure than the Caucasian population at all ages and in both sexes, even when other factors (socioeconomics, salt intake, obesity, rural or urban setting) are evaluated. As an attempt to contribute to the further understanding of human essential hypertension, we have studied three routes of Na transport in the red cells of normotensive and hypertensive subjects and members of their families: the Li-Na countertransport, the diffusional Li leak, and the Na-K cotransport.

The investigation of red cell Na transport in black subjects underlines at least three main differences in their red cell Na transport systems in comparison with

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yrs)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Weight (kg/m²)</th>
<th>Li-Na, countertransport (mmol/liter cells x hr)</th>
<th>Sodium (mmol/liter cells x hr)</th>
<th>Potassium (mmol/liter cells x hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>14.6 ± 5</td>
<td>64.8 ± 8</td>
<td>20.1 ± 4</td>
<td>0.19 ± 0.05</td>
<td>0.40 ± 0.2</td>
</tr>
<tr>
<td>Adolescents with normotensive parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>14.2 ± 6</td>
<td>75.2 ± 14</td>
<td>23.7 ± 0.5</td>
<td>0.13 ± 0.06</td>
<td>0.15 ± 0.17*</td>
</tr>
<tr>
<td>Adolescents with hypertensive parents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.19 ± 0.14†</td>
</tr>
</tbody>
</table>

Values are means ± sd.

\(^*p < 0.05.\)

\(^†p < 0.001.\)
Na-K COTRANSPORT AND Li-Na COUNTERTRANSPORT IN HYPERTENSIVE BLACKS/Canessa et al. 349

Figure 2. Relationship between the maximal rate of Na and K cotransport and Li$_2$Na$_o$ countertransport of black subjects. $Y = 0.11 \pm 0.42X; n = 44; r = 0.37; p < 0.02$.

A previous case-control study in Caucasians. Table 6 summarizes these findings. First, hypertensive blacks do not seem to have higher values of Li-Na countertransport, as previously found in some Caucasian subjects in Boston, Salt Lake City, Ann Arbor, Chicago, Milan, Parma, Copenhagen, Beer-Sheva, Paris, and Leeds. A slight increase in red cell Na countertransport and no racial differences were reported with a different procedure.

Second, the maximal rate of furosemide-sensitive Na and K efflux was found reduced in subjects with hypertension in comparison to normotensive subjects, as previously reported by Garay et al. The mean value of Na cotransport in normal subjects was not significantly different from that found in our study and other case-controlled studies in the Caucasian population. A large crossover was found between the normotensive and hypertensive subjects. In our study, 33% of the normotensive individuals and 68% of hypertensive patients had values of red cell Na-K cotransport lower than 0.25 mmol/liter cells x hour. In the study of Ivory Coast blacks, 13 of 18 (72%) normotensive subjects had Na cotransport values below 0.25 mmol/liter cells x hour, while in seven subjects it was completely absent. In none of the nine hypertensive subjects studied was the Na and K cotransport over 0.25 mmol/liter cells x hour.

The large crossover of Na cotransport between controls and hypertensives has also been reported by Davidson et al. in South African blacks. In both studies,

### Table 6. Comparison of Alterations in Cation Transport in Normotensive (NT) and Hypertensive (HT) Patients

<table>
<thead>
<tr>
<th>Population</th>
<th>Li efflux (mmol/liter cells x hr)</th>
<th>Na-K COTRANSPORT (mmol/liter cells x hr)</th>
<th>Furosemide-sensitive efflux</th>
<th>Regression CT vs Na CO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnesium (hr$^{-1}$)</td>
<td>Countertransport</td>
<td>Sodium</td>
<td>Potassium</td>
</tr>
<tr>
<td>Normotensives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blacks</td>
<td>0.024 ± 0.006</td>
<td>0.18 ± 0.05</td>
<td>0.38 ± 0.24</td>
<td>0.5 ± 0.28</td>
</tr>
<tr>
<td>Caucasians</td>
<td>0.015 ± 0.001</td>
<td>0.28 ± 0.12</td>
<td>0.31 ± 0.12</td>
<td>0.37 ± 0.20</td>
</tr>
<tr>
<td>Hypertensives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blacks</td>
<td>0.021 ± 0.001</td>
<td>0.18 ± 0.017</td>
<td>0.25 ± 0.17</td>
<td>0.24 ± 0.14</td>
</tr>
<tr>
<td>Caucasians</td>
<td>0.021 ± 0.004</td>
<td>0.51 ± 0.14</td>
<td>0.51 ± 0.20</td>
<td>0.60 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± se. See reference 6. CT = countertransport; CO = cotransport.

* $p < 0.02$ (NT + HT).
† $p < 0.005$ (NT + HT).
the cells were loaded to contain 25 to 30 mmol Na and K per liter of cells, while in our study, the cells contained 55 mmol Na and K per liter. This abnormality of Na-K cotransport adds up with other characteristics of hypertension in blacks, such as volume expansion, low renin hypertension, resistance to furosemide, and propranolol, and low urinary K excretion. The low K efflux in red cells of hypertensive blacks is in agreement with the reduced total K efflux into sucrose medium reported in Nigerian hypertensive subjects.

Another finding of our study is the detection of the same abnormality of furosemide-sensitive K efflux in adolescents with hypertensive parents. One of 10 adolescents with normotensive parents had a furosemide-sensitive K efflux lower than 0.25 mmol/liter cells × hour. In contrast, eight of 13 adolescents of hypertensive parents had the furosemide-sensitive K efflux markedly reduced.

In previous studies, one of us demonstrated that adolescents with borderline hypertension have a greater risk for progression to sustained hypertension. A genetic background positive for hypertension in the families of the adolescents was associated with a pronounced increase in blood pressure and heart rate in response to stress. The baseline values of blood pressure and heart rate were greater than in the control group but, probably more important, the absolute increase in systolic blood pressure, diastolic blood pressure, and heart rate were significantly greater in the offspring of hypertensives than in the normotensive control group.

Characteristics of those adolescents progressing to sustained hypertension were, therefore, a uniformly strong family history of hypertension accompanied by high cardiovascular response to mental stress and reduced K cotransport.

Finally, another parameter distinguishing the black from the Caucasian population is the relationship between Li-Na, countertransport and Na and K cotransport. The elevated countertransport described in hypertensive Caucasians correlated significantly with an elevated maximal rate of the Na-K cotransport. In the hypertensive blacks, the Na cotransport correlated poorly with countertransport (ρ < 0.02) and had a significantly lower slope (0.11 ± 0.042). These data indicate that simultaneous measurements of the maximal rate of both Na transport systems can define at least two types of alterations of cation transport in hypertensive subjects. In some hypertensives, the reduction of the V_{max} of Na-K cotransport seems to be accompanied by a normal or elevated V_{max} of the ouabain-sensitive Na pump. In hypertensive blacks, we have also observed a reduction of the V_{max} of the Na-K pump and of the Na-K cotransport (measured in nystatin-loaded cells), which was interpreted to identify a subset of salt-sensitive individuals. The low values of Na transport (and the elevation of cell Na) indicate that the red cell membranes of these individuals have a substantially lower capacity to extrude Na than hypertensive subjects with normal or elevated Na pump, Na-K cotransport, and Li-Na exchange.

It is not yet clear whether the subset of hypertensive patients with reduced Na-K cotransport represents a different clinical entity (moderate hypertension) from those having elevated countertransport. The hypertensive blacks studied here had a "moderate" type of hypertension similar to that of many Caucasian hypertensives with normal values of countertransport. Several researchers who have only measured cotransport have not confirmed the reduction of Na-K cotransport. Unfortunately, some investigators have used flux assays under inadequate kinetic conditions to isolate a single transport pathway at its maximal rate. Li influx or Na influx into fresh cells is dependent on intracellular Na and does not measure the V_{max} of the Na exchange system; it also comprises an inward diffusional leak pathway and a small fraction of the Na-K cotransport. Furosemide-sensitive Na efflux from fresh cells, being sigmoidally dependent on intracellular Na, cannot discriminate whether the change in rate is caused by different cell Na, different affinity, or different maximal rate of the Na-K cotransport. Furosemide-sensitive Rb influx into fresh cells does not measure the maximal activation of the inward Na-K cotransport; it comprises a heteroxchange K/Rb pathway of the Na-K cotransport system, which varies with cell Na in the physiological range.

Moreover, it remains to be investigated whether the Na-K cotransport system is altered in the outward but not in the inward direction. The search for a transport marker of the prehypertensive state might face formidable obstacles in its application to the human population. The data flowing from different parts of the world seem to indicate that the type of alteration of ion transport, as well as the fraction of affected subjects in the hypertensive population, varies from subject to subject and probably from one place to another in small samples of case-controlled studies. In none of these reports has the clinical and physiopathological evaluation of the patients been adequately studied. The alterations of red cell cation transport systems display strong familial aggregation and ethnic differences. These properties are characteristic of the genetic polymorphism traits whose frequency distribution may vary substantially in some human populations.

The presence of populations of genes (demes) of red cell cation transport in the human population might be important for developing a new approach to the epidemiology of human hypertension as well as for defining better clinical subtypes of essential hypertensives or a subset of "salt-sensitive or vasoconstrictive" hypertensives. Case-control designed studies might not reflect the true distribution of hypertensive subtypes in several human populations. Some of these questions might be answered by using adequate kinetic assays of cation transport to investigate the frequency distribution of alterations in ion transport in unselected black and Caucasian populations. It might be important to define whether the higher incidence of hypertension in the black population is related to an overall lower capacity to extrude cellular Na in response to their Na intake.
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