Essential Hypertension: Sodium-Lithium Countertransport in Erythrocytes from Patients and from Children Having One Hypertensive Parent

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SUMMARY This report deals with the possibility that there is a specific change of the lithium transport across the membrane of erythrocytes from patients with essential hypertension. Sodium-lithium countertransport was significantly increased (p < 0.005) in erythrocytes from 17 males with essential hypertension (mean 0.7 mmole (liter cells × hr)^{-1}, range 0.4-1.6) compared to a group of 16 normotensive males (mean, 0.4 mmole (liter cells × hr)^{-1}, range 0.3-0.6). A considerable overlap between the values from patients and controls was found. No significant increase of the transport function was found in a group of 14 female patients (mean 0.4 mmole (liter cells × hr)^{-1}, range 0.2-0.6) compared with 10 normotensive female controls (mean 0.3 mmole (liter cells × hr)^{-1}, range 0.1-0.6). Determination of sodium-lithium countertransport in red blood cells from nine children with and 14 without known familial disposition for essential hypertension did not demonstrate a close coupling between genetic disposition and the membrane transport function. In spite of the very small intraindividual variability of the transport function, studies of changes in sodium-lithium countertransport are hampered by considerable interindividual variability of the transport in red cells from apparently normal individuals. (Hypertension 4: 703-709, 1982)

KEY WORDS - sodium-lithium countertransport • essential hypertension • offspring of hypertensive patients • red cell membrane • erythrocytes

This study was prompted by the findings of Canessa et al.1 who examined lithium transport in red cells from 36 patients with essential hypertension and concluded that the disease is accompanied by a distinct functional increase of a specific transport mechanism: the so-called sodium-lithium countertransport system.

Lithium can be transported across the human red cell membrane by several transport mechanisms:

1. The Active Sodium-Potassium Transport System. Active lithium influx can be observed at low extracellular potassium concentrations, and active lithium efflux can be seen in the absence of intracellular sodium.2 Both types of active lithium transport are completely abolished by ouabain.

2. The Anion Exchange Mechanism. Lithium transport across the human red cell membrane is increased in bicarbonate containing media.3 This increased passive lithium flux is ascribed to transport of negatively charged carbonate ion pairs (LiCO₃) via the physiologically important chloride-bicarbonate exchange system of the red cell membrane. The transport by this pathway is completely blocked by specific inhibitors of anion transport, and it does not occur in bicarbonate-free KCl or NaCl media.4

3. The Sodium-Potassium Cotransport System. This transport mechanism mediates a coupled transport of potassium and sodium which is inhibited by the diuretic furosemide.5 Recently it has been shown that this system is able to mediate transfer of potassium plus lithium but at relatively low intracellular lithium concentrations this transport mode is insignificant in cells containing sodium.6

4. The Sodium-Sodium Exchange System. Transport via this ouabain insensitive transport mechanism was discovered independently by two research groups.7,4 The transport process is a tightly coupled exchange mechanism, coupling the efflux of one cation (lithium in our experiments) to the influx of another (sodium in the present work). Operating in this mode the system has been called the sodium-lithium countertransport system. Transport is blocked with
phloretin, and is efficiently inhibited by removing exchangeable cations from the trans-side of the membrane, as when lithium efflux takes place into sodium-free potassium-, choline-, or magnesium chloride media.

5. The Lithium Leak. After blocking the four preceding transport systems, lithium is still transported across the red cell membrane at a low rate. This residual flux is proportional to the lithium concentration gradient across the membrane, and is believed to represent a diffusion component, e.g., electrodiffusion of lithium ions through the lipid phase of the membrane. Lithium transport via this pathway cannot be inhibited by any known inhibitor.

The findings of Canessa et al.\textsuperscript{1} that essential hypertension is accompanied by increased sodium-lithium countertransport via the sodium-sodium exchange system (Mechanism 4) may clearly be of great value for the differential diagnosis of hypertension in its various forms. Therefore, we have compared the functioning of the sodium-lithium countertransport in normal erythrocytes with the transport in red cells from patients with essential hypertension. In addition we have compared the transport in red cells from children without known genetic disposition with the sodium-lithium exchange in red cells from a genetic risk group: the offspring of parents with essential hypertension. The findings of Canessa et al.\textsuperscript{1} that essential hypertension is accompanied by increased sodium-lithium countertransport in normal erythrocytes with the transport in red cells from patients with essential hypertension. In addition we have compared the transport in red cells from children without known genetic disposition with the sodium-lithium exchange in red cells from a genetic risk group: the offspring of parents with essential hypertension. The studies were carried out under experimental conditions ensuring that only lithium transport by the countertransport system (Mechanism 4) and by the lithium leak pathway (Mechanism 5) was included in the flux measurements.

Materials and Methods

The sodium-stimulated efflux of lithium was examined after preloading erythrocytes with lithium. Red blood cells from the four groups of individuals were examined.

Group 1

Adults (14 women and 17 men) with mild-to-moderate essential hypertension, with a diastolic blood pressure > 100 mm Hg before treatment, comprised Group 1. Secondary hypertension was excluded by laboratory investigations that included excretory urograms and/or isotope renography. Eight of the patients were untreated; 23 were treated, seven with a thiazide only, 10 with thiazide and betablocker, and six with thiazide, betablocker, and a vasodilator.

Group 2

The control group consisted of 26 adults (10 women and 16 men) with normal blood pressure who were either healthy normal persons or patients admitted to the medical department (blood pressure: systolic < 150 mm Hg, diastolic < 90 mm Hg). Diabetics and patients treated with hormones or cardiovascular drugs were excluded. There was no family history of disposition for hypertension in 24 persons; no family history was available for two men, but the countertransport flux values were within 1 SD of the normal mean value.

Group 3

A group of nine children (four girls and five boys) was studied, in which one of the parents presented essential hypertension, thus potentially disposing the offspring to essential hypertension. At the time of the investigation all of these children had normal blood pressure corresponding to normal values for age and sex. None received any kind of medication.

Group 4

A group of 14 children with no family history of hypertension (five girls and nine boys) was studied, selected from the Children's Hospital. None of the children received any kind of medication.

Statistical Evaluation

For statistical evaluation, the unpaired Student t test was used; differences were accepted as significant if a p value of < 0.05 was found.

Experimental Protocol

Erythrocytes were loaded with lithium in a lithium bicarbonate medium, taking advantage of the observation that lithium influx is 10 to 15 times higher from an isotonic lithium bicarbonate medium than from a lithium chloride medium.\textsuperscript{3, 4} To remove all bicarbonate, the cells were carefully washed with the potassium chloride medium after loading. The efflux of lithium

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No.</th>
<th>Lithium (mmole/liter cells)</th>
<th>Sodium (mmole/liter cells)</th>
<th>MCHC (mmole/liter cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean</td>
<td>sd</td>
<td>mean</td>
</tr>
<tr>
<td>Essential hypertensive adults</td>
<td>M</td>
<td>17</td>
<td>6.9</td>
<td>0.8</td>
<td>6.5</td>
</tr>
<tr>
<td>F</td>
<td>14</td>
<td></td>
<td>7.0</td>
<td>1.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Normotensive adults</td>
<td>M</td>
<td>16</td>
<td>7.3</td>
<td>1.6</td>
<td>5.3</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td></td>
<td>6.9</td>
<td>1.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Normotensive children with one hypertensive parent</td>
<td>M</td>
<td>5</td>
<td>7.2</td>
<td>1.3</td>
<td>5.2</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive children with normotensive parents</td>
<td>M</td>
<td>9</td>
<td>7.6</td>
<td>0.7</td>
<td>6.1</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td></td>
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</tbody>
</table>
SODIUM-LITHIUM COUNTERTRANSPORT IN ERYTHROCYTES

Lithium efflux into potassium and sodium chloride media was determined in subsequent efflux experiments. Lithium efflux into the sodium chloride medium is comprised of at least two components. One is the sodium-lithium countertransport (Mechanism 4) and the other is an efflux component, which increases linearly with the intracellular lithium concentration, here called the lithium leak component (Mechanism 5). The efflux of lithium into a sodium-free medium exclusively occurs through the leak pathway under the experimental conditions used. The sodium-sensitive lithium countertransport could therefore be determined by difference, subtracting the leak flux (Mechanism 5) found in the absence of sodium from the total lithium efflux in the sodium chloride medium (Mechanisms 4 and 5) (fig. 1).

Electrolyte Media

The loading medium included LiHCO₃ 150 mM, KCl 5 mM, and glucose 10 mM. The medium was prepared from lithium carbonate and titrated with CO₂ to pH 7.4 at 38°C, pCO₂ = 250 Torr.

The sodium medium included NaCl 150 mM, glucose 10 mM, tris (hydroxymethyl)-aminomethan 10 mM, and ouabain 0.1 mM. The potassium medium included KCl 150 mM, glucose 10 mM, tris (hydroxymethyl)-aminomethan 10 mM, and ouabain 0.1 mM. The media were titrated to pH 7.4 at 38°C with 7.5 mmole HCl/liter. In a few experiments, potassium was substituted with 150 mM choline chloride or with 75 mM magnesium chloride (table 2). The isotonic magnesium chloride medium in addition contained 85 mM sucrose. All chemicals employed were reagent grade.

Preparation of Cells

Media samples of 12 ml of heparinized blood were washed thrice in the loading medium and resuspended at a hematocrit of 20%. The cells were loaded with lithium by incubating the cell suspension for 15 minutes at 38°C. The bicarbonate-stimulated lithium influx is about 25 mmoles (liter cells X hr)-¹ so that the intracellular lithium concentration after loading was 7 mmoles/liter cells (range 5 to 10) corresponding to about 10 mmoles/liter cell water. Variation in the lithium concentration between 2 and 21 mmoles/liter cells (fig. 1 and table 3) was obtained by varying the incubation time in the loading medium between 3 and 50 minutes. After loading, the cells were washed 5 times with large volumes of the potassium chloride medium with the double purpose of removing extracellular lithium and all bicarbonate from the cell suspension. After the last wash, the cells were resuspended in the potassium medium to a hematocrit of 50% and used for the subsequent efflux experiments. A sample was removed for the following analyses: hemoglobin, hematocrit, mean cellular hemoglobin concentration.

### Table 2. Leak Efflux of Lithium from Erythrocytes into Four Media Not Permitting Lithium-Sodium Countertransport

<table>
<thead>
<tr>
<th>Electrolyte medium</th>
<th>Lithium efflux (mmole/liter cells X hr)-¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>0.29</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.30</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.30</td>
</tr>
<tr>
<td>NaCl + phloretin</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The cells contained 8.1 mmoles lithium/liter. Lithium efflux into a sodium chloride medium (total efflux) was 0.8 mmoles/liter cells X hr. The total efflux is composed of countertransport (0.8 - 0.3) = 0.5 mmoles (liter cell X hr)-¹ and a leak of 0.3 mmoles (liter cells X hr)-¹.

### Table 3. Intraindividual Variation of the Countertransport System

<table>
<thead>
<tr>
<th>Donor</th>
<th>Countertransport (mmole/liter cells X hr)-¹</th>
<th>Li⁺ (mmole/liter cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.40-0.51</td>
<td>6.1-16.4</td>
</tr>
<tr>
<td>B</td>
<td>0.92-1.10</td>
<td>5.9-9.2</td>
</tr>
<tr>
<td>C</td>
<td>0.29-0.44</td>
<td>6.3-17.4</td>
</tr>
</tbody>
</table>

The number of flux determinations carried out on red cells from three donors over a 5-month period, the range of countertransport, and the range of intracellular lithium concentrations in the erythrocytes at the start of the experiment. Intracellular concentration of lithium was varied within the range where the countertransport is saturated (see Fig. 1).

There was no correlation between the intracellular lithium concentration and the magnitude of the countertransport.
(MCHC), and erythrocyte content of sodium and lithium.

Lithium Efflux Experiments

The cell suspension and the incubation media were preheated to 38°C and the flux experiments were run at this temperature in a thermostated water bath. The efflux experiments were started by adding 0.5 ml samples of the cell suspension to lithium-free test tubes containing 4.5 ml of the potassium medium or of the sodium medium respectively. Duplicate samples from each incubation medium were centrifuged at the start of the experiments and after 30, 60, and 90 minutes of incubation, and the supernates were isolated for lithium analysis.

Analytical Methods

Lithium and sodium concentrations were determined by emission flame photometry (Eppendorf, GMBH, Hamburg). Lithium concentration in the supernatants of the efflux samples was determined without any dilution. The standard solutions contained 0, 0.05, 0.1, and 0.15 mM lithium dissolved in the medium used in the particular experiment. The calibration graphs were perfectly linear in both media. Unstable readings were obtained in a magnesium medium like that used by Canessa et al. Therefore, we preferred a potassium medium for the experiments carried out in the absence of sodium. Lithium and sodium in erythrocytes were determined after lysing 0.5 ml of the cell suspension in 9.5 ml distilled water. The standards for these determinations all contained 2 mM KCl. The coefficient of variation on duplicate determinations was 3.7%, n = 100 (0.01 - 0.11 mmole/liter lithium in supernates); 1.6%, n = 50 (erythrocyte lithium); and 3.0%, n = 50 (erythrocyte sodium). Sodium and lithium content of the red cells was calculated per liter of cells by relating the amount of alkali metals found in supernates; 1.6%, n = 50 (erythrocyte lithium); was 3.7%, n = 100 (0.01 - 0.11 mmole/liter lithium in supernates). Lithium concentration in the medium (A Li mM X liter cells/hr) was determined in sodium and potassium media. Figure 1 shows that the leak component (Mechanism 5) of lithium efflux into the sodium-free medium is a linear function of intracellular lithium concentration:

\[ J^{m} = k \times (Li^+) \]

where \((Li^+)\) is the intracellular lithium concentration (mmole (liter cells)\(^{-1}\)) and \(k (hr^{-1})\) is the rate constant of leak efflux, \(J^{m} (mmole (liter cells \times hr)^{-1})\). When the efflux took place in a sodium medium, a saturable flux component (the counterexchange) was superposed on the linear leak component. The sodium-lithium countertransport has been reported to display saturation kinetics, attaining a half maximum flux at an intracellular lithium concentration of about 1 mmole (liter cells)\(^{-1}\). Our results confirm that the saturation of the countertransport system has taken place, when intracellular lithium is above 5 mmoles (liter cells)\(^{-1}\).

The leak component of the lithium efflux was subjected to closer examination to justify that it is meaningful to subtract the leak flux found in the potassium medium from the total flux found in the sodium medium in order to obtain the counterexchange flux. We demonstrated that the leak flux is independent of the chemical nature of the cation used to replace sodium and that phloretin, which inhibits the counterexchange, reduced the lithium efflux from cells suspended in a sodium medium to a value, which was identical with the leak flux found in the sodium-free media (table 2).

The reproducibility of the flux determination was controlled by determining the lithium transport repeatedly on fresh blood samples from the same donor over a 5-month period. Duhm and Becker have demonstrated that the transport capacity of the countertransport system may vary considerably from one donor to another, but that it remains constant over periods of months in cells from the same donor. This result was confirmed by repeated flux determination in red cells from three donors (table 3).

Since it may be inconvenient to perform the flux experiments immediately after blood sampling, we have examined whether the lithium transport remains constant in erythrocytes that are washed and stored at 4°C in the potassium medium, before the cells are loaded with lithium. The results shown in table 4 indicated that neither the leak component nor the sodium-lithium counter transport are affected by storing the cells up to three days before the flux experiments are carried out.

Control Experiments

A number of experiments were performed to ensure the reliability of the technique. The saturability of the countertransport was checked as shown in figure 1. Cells from a single donor were loaded with lithium with concentrations varying from 2 to 21 mmoles (liter cells)\(^{-1}\), and the efflux of lithium was subsequently determined in sodium and potassium media. Figure 1 shows that the leak component (Mechanism 5) of lithium efflux into the sodium-free medium is a linear function of intracellular lithium concentration:

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**Results**

Mean values and ranges of the lithium efflux measurements in erythrocytes from the four groups are shown in table 1. The individual values of lithium-sodium countertransport are shown as scatter diagrams in figure 2.

The lithium leak efflux into a potassium medium was of the same magnitude in all groups (0.2-0.3 mmole (liter cells · hr)^{-1}). Consequently the variations found were due to differences between the capacities of the sodium-lithium counterexchange mechanism, which is saturated under the experimental conditions employed. Lithium efflux into the sodium medium measures the sum of leak flux and countertransport. It was of the order of 0.6 mmole (liter cells · hr)^{-1} except in the group of adult hypertensive males, in which it was 50% higher with a mean of 0.9 mmole (liter cells · hr)^{-1}. This group showed the highest mean value of countertransport 0.7 mmole (liter cells · hr)^{-1}. The mean values of countertransport in the rest of the material were 0.3-0.4 mmole (liter cells · hr)^{-1}, the lowest value being found in erythrocytes of children of normotensive parents. By statistical evaluation it was found that the mean values of the normotensive and hypertensive males differed significantly (p < 0.005) whereas no significant difference was demonstrated between the mean values found for normotensive and hypertensive females (p > 0.2). Further subdivision of the relatively small material according to severity of hypertension, graded by the doses and numbers of drugs needed to normalize blood pressure, did not reveal any significant differences between subgroups.

The mean value of countertransport in red cells from children with normotensive parents was not significantly different from that of children with possible genetic disposition from a hypertensive father or mother (0.05 < p < 0.1) nor was there any difference in the countertransport in red cells from boys and girls (fig. 2).

**Discussion**

The present study was prompted by the findings of Canessa et al.\(^1\) demonstrating a distinct difference in the magnitudes of lithium-sodium countertransport in red cells from normal persons and from patients with essential hypertension, with almost no overlapping between the individual flux values found in the two groups. Our findings are only partially concordant with these results. The increase of lithium countertransport in red cells from hypertensive males (0.3 mmole (liter cells · hr)^{-1}) was highly significant, and of the same magnitude as in the Boston study.\(^1\) However, we did not find a significant difference between the mean values from normotensive and hypertensive females (0.07 mmole (liter cells · hr)^{-1}) in contrast to the sig-

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**Table 4. Stability of Transport Functions in Stored Erythrocytes**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.16</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>B</td>
<td>0.22</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td>C</td>
<td>0.22</td>
<td>0.27</td>
<td>0.22</td>
</tr>
<tr>
<td>D</td>
<td>0.16</td>
<td>0.24</td>
<td>0.23</td>
</tr>
</tbody>
</table>

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significant difference of 0.2 mmole (liter cells • hr)⁻¹ found by Canessa et al.¹.

A detailed comparison of the results of the two investigations (table 5 of the present material, tables 1 through 3 of Canessa et al.¹) reveals that the difference is solely due to the values found for countertransport in cells from normal individuals, which in our material were 1.5 to 1.8 times higher than the mean values found by Canessa et al.¹. In contrast, the values for countertransport in erythrocytes from patients were almost identical in the two investigations. The small difference between the leak fluxes in the two materials vanishes, taking into consideration that the leak is a linear function of intracellular lithium concentration, which was about 30% higher in the material of Canessa et al.¹ (13 vs 10 mmol/liter cell water). The rate coefficients of the leak effluxes are accordingly identical in the two materials, about 0.03 hr⁻¹ (see Methods section).

It is difficult to decide what is the reason for the difference between the countertransport values in the two reports. We have previously shown that the bicarbonate-induced increase of lithium permeability used for the loading procedure is completely reversed when bicarbonate is replaced with chloride.⁴ We were not able to detect any difference between the leak fluxes into the magnesium medium used by Canessa et al.¹ and the potassium medium used by us (table 2). Latent hypertension was excluded by repeated blood pressure recordings, but it is clear that the absence of knowledge of a family history of hypertension does not exclude that an apparently normal person is the bearer of genetic disposition for the disease. Also, it of course should be recognized that the diagnosis of essential hypertension is unlikely to represent a well-defined entity of disease with a single etiology. It is likely that the overlapping between the countertransport values found in normal persons and patients (fig. 2) is due to the considerable interindividual variability of the transport capacity in red cells from normal persons that has previously been demonstrated by Duhm and Becker.¹⁰

Duhm and Becker¹⁰ found a fivefold variation of lithium countertransport in influx experiments on red cells from 39 healthy members of the staff of a physiology department. A detailed kinetic analysis of the sodium-stimulated efflux from cells of four normal individuals showed a maximum countertransport varying from 0.5 to 1.4 mmole (liter cells • hr)⁻¹, and three of these donors had a countertransport above 0.8 mmole (liter cells • hr)⁻¹. None of these persons had hypertension or a known disposition for the disease (Duhm, personal communication). Even though it is possible that the technique employed by Duhm and Becker¹⁰ may include a small lithium leak in the alleged countertransport values, it seems clear that their normal values are at least as high as those reported by us.

Canali et al.¹² studied a group of hypertensive patients with a family history of hypertension using the same methods as Canessa et al.¹. In their study there is also a considerable overlap between countertransport values from normal persons and patients, although they found a statistically significant increased lithium-sodium countertransport in the patient group (mean 0.32 mmole (liter cells • hr)⁻¹ vs a mean value of 0.21 mmole (liter cells • hr)⁻¹ in the normotensive control group. The countertransport in erythrocytes from hypertensive persons in the Canali material is considerably lower than the values reported by Canessa et al.¹ and by us.

Okpaku et al.¹³ in a recent pilot study found evidence that racial factors influence the activity of the lithium-sodium countertransport system, countertransport being low in the red cells obtained from Negroes. Accumulation of individuals lacking the sodium-lithium countertransport system in a Negro family has been reported by Pandey et al.¹⁴ These findings, if confirmed, are of evident importance for the comparison of flux values found in various parts of the world.

A statistically significant elevation of lithium-sodium countertransport in red blood cells from male hypertensive patients was found both in our material and in that of Canessa et al.¹, whereas the elevation was much less pronounced in hypertensive females in both investigations. It is an open question whether this difference is of genetic origin or whether it is caused by endocrinological or external environmental factors. Evidence that genetic factors are of importance for the capacity of the sodium-lithium exchange system has been found by several authors. Dorus et al.¹⁵ measured

### Table 5. Distribution of the Material: Lithium Efflux in Potassium and Sodium Media and the Countertransport in the Four Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No.</th>
<th>Age (yrs)</th>
<th>Lithium efflux in potassium medium (mmole (liter cells x hr)⁻¹)</th>
<th>Lithium efflux in sodium medium (mmole (liter cells x hr)⁻¹)</th>
<th>Countertransport (mmole (liter cells x hr)⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential hypertensive adults</td>
<td>M</td>
<td>17</td>
<td>47.5 (30-67)</td>
<td>0.20 (0.12-0.46)</td>
<td>0.88 (0.52-2.01)</td>
<td>0.68 (0.35-1.55)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>14</td>
<td>51.5 (27-74)</td>
<td>0.17 (0.09-0.34)</td>
<td>0.58 (0.31-0.95)</td>
<td>0.41 (0.20-0.61)</td>
</tr>
<tr>
<td>Normotensive adults</td>
<td>M</td>
<td>16</td>
<td>49.5 (18-88)</td>
<td>0.22 (0.12-0.39)</td>
<td>0.64 (0.51-0.88)</td>
<td>0.42 (0.30-0.64)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10</td>
<td>43.5 (19-69)</td>
<td>0.21 (0.14-0.34)</td>
<td>0.55 (0.44-0.79)</td>
<td>0.34 (0.14-0.56)</td>
</tr>
<tr>
<td>Normotensive children with one hypertensive parent</td>
<td>M</td>
<td>5</td>
<td>13.0 (10-18)</td>
<td>0.22 (0.11-0.30)</td>
<td>0.57 (0.43-0.68)</td>
<td>0.35 (0.18-0.46)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive children with normotensive parents</td>
<td>M</td>
<td>9</td>
<td>13.0 (7-17)</td>
<td>0.27 (0.19-0.37)</td>
<td>0.57 (0.36-0.80)</td>
<td>0.30 (0.16-0.52)</td>
</tr>
</tbody>
</table>

Values are means, with ranges in parentheses.
the in vitro lithium distribution in red cells from 291 members of 120 families without finding evidence for a dominant hereditary effect, but the covariations found could be ascribed to the existence of genes with additive effects. An interesting sex difference in a sodium exchange system has recently been reported in a study of sodium transport in red cells from spontaneous hypertensive Wistar-Kyoto rats by Wiley et al. Sodium influx was significantly increased in red cells from the genetically hypertensive male rats, whereas no increase was found in cells from hypertensive female siblings. This study of hereditary hypertension in rats suggests that there may be a genetic sex linkage or that endocrine factors modulate the genetically determined activity of a sodium exchange mechanism which is similar to the transport system studied by us. The last mentioned explanation could be the reason why we do not find any elevation of the sodium-lithium countertransport in red cells from children with a possible genetic disposition.

Several years ago it was suggested that red cells from patients with manic depressive illness have a genetic lithium transport defect. It has later become clear that the alleged deficiency is a low capacity of the lithium-sodium countertransport system, which appears to be found only in a subgroup of patients with bipolar mental disease. It would be of obvious interest to learn whether such patients have a reduced risk for developing essential hypertension, and whether disposition for essential hypertension is protective against the mental disease.

As pointed out by Parker, it is not possible to suggest the role that an increased sodium-lithium countertransport system can play in the pathogenesis of essential hypertension. The sodium-sodium exchange cannot mediate any net transport of sodium across cell membranes. Blaustein has presented an attractive hypothesis according to which the tone of the vascular smooth muscle cell is regulated by a subtle balance between the transport of sodium and calcium ions. However, attempts to demonstrate that the sodium-sodium transport system can mediate sodium-calcium exchange have been unsuccessful so far. Recent investigations suggest that the transport system may mediate sodium/proton exchange across red cell membranes.

It is clear from the present study that determination of sodium-lithium countertransport is not a unique test for the identification of the about 9% of the population that has or is destined to develop essential hypertension. Further research is needed to clarify the possible inheritability of an altered transport function in the red cells of a subgroup of predominantly male patients that displays a clearcut increased capacity for sodium-lithium exchange across the red cell membrane.

References


10. DHah J, Becker BF: Studies on the lithium transport across the red cell membrane IV. Interindividual variations in the Na + -dependent Li + countertransport system of human erythrocytes. Pfluegers Arch 370: 211, 1977


Essential hypertension: sodium-lithium countertransport in erythrocytes from patients and from children having one hypertensive parent.
K K Ibsen, H A Jensen, J O Wieth and J Funder

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