Essential Hypertension: Improved Differentiation by the Temperature Dependence of Li Efflux in Erythrocytes

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SUMMARY  Kinetic and thermodynamic properties of red cell lithium (Li) efflux were examined in patients with essential hypertension; the maximal rate of Li efflux as affected by temperature was measured at the range of 12° to 42°C. Fifty-two patients with essential hypertension and 22 normotensives were studied. The mean Li efflux, both into sodium (Na) medium and Li-Na countertransport, was higher in hypertensive than in normotensive persons, but the distinction between the two groups was limited by extended scatter and overlap. The distinction could be markedly improved by determining the effect of temperature on Li efflux. While all the normotensives exhibited Arrhenius plots of Li efflux with a change in slope ("break") around 30°C, the corresponding "break" for most (75%) of the hypertensives was about 20°C. Consideration of both the rate and the temperature dependence of Li efflux further improved the differentiation of hypertensive patients. Analysis of normotensive offspring of hypertensives and of patients with secondary hypertension indicates that the temperature dependence of Li efflux may serve as a genetic marker for essential hypertension.

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KEY WORDS  • essential hypertension  • secondary hypertension  • normotension  • offspring  • erythrocytes  • Arrhenius plots

E SSENTIAL hypertension apparently results from a combination of genetic and environmental factors.1, 2 Ideally, genetic markers for essential hypertension could permit recognition of different forms of the disease, allow early detection of hypertension, and assist in the study of pathogenesis of the disorder.3 Alterations in cellular properties (mainly of blood cells) have been described in humans and in animal models in association with essential hypertension.4-10 Furthermore, two erythrocyte transport systems, Li-Na countertransport11 and Na-K cotransport,12 have been reported as possible genetic markers of essential hypertension. These transport assays appear useful in distinguishing between essential and secondary hypertension and for detection of predisposition for hypertension among offspring of affected parents.3, 11-22 Yet, certain difficulties may limit the application of the transport assays in the present form. Li-Na countertransport and Na-K cotransport exhibit marked interindividual differences even within the normotensive group.1 It has been shown in case-controlled studies that the fraction of affected patients as well as the extent of overlap between the tested groups may vary.18, 19, 21, 23, 24 Furthermore, physiological processes can temporarily alter Li-Na countertransport.25 In addition, no abnormality in erythrocyte cation transport could be demonstrated in other studies of essential hypertension.26, 27

In the present study we examined a concept that a combination of a transport measurement (which is potentially a useful genetic marker) with an analysis of another erythrocyte membrane alteration (also associated with the incidence of essential hypertension) might aid in overcoming some of these limitations. It was demonstrated that the profile of temperature dependence of Li efflux in erythrocytes allows an improved differentiation of patients with essential hypertension.

Methods

We studied 52 patients with essential hypertension (36 men, 16 women) in whom secondary causes of hypertension were excluded by investigation in the Hypertensive Clinic of Soroka Medical Center. Only three patients had additional illnesses; one had mild diabetes mellitus, one had chronic stable angina, and another had chronic obstructive lung disease. All pa-
tients had normal renal function with normal creatinine clearance. Two subgroups were distinguished on the basis of family history: 1) 28 patients with an established family history of hypertension; and 2) 24 patients with an uncertain family history of hypertension. Duration of hypertension in the entire group ranged from 2 months to 11 years. Twenty-nine of the patients had not received medication at all or at least for 1 year before the study. The 23 patients on medication were taking diuretics and beta-blocking agents. Five of the patients had severe hypertension, 19 moderate, 20 mild, and eight other patients were diagnosed as borderline hypertensive. Eleven patients of the hypertensive group underwent a renin axis study.

Ten patients with secondary hypertension (five men, five women) were included in the study. Their diagnosis was: chronic glomerulonephritis (3 cases), renovascular hypertension (3), polycystic kidney (1), chronic renal failure (1), thyrotoxicosis (1), and lupus erythematosus (1). With the exception of two of these patients, the parents of patients with secondary hypertension were normotensive.

Twenty-two normal subjects were studied (12 men, 10 women) after careful selection; both parents were normotensive, as were all living relatives; there was no history of hypertension-related mortality in deceased relatives. All normal subjects had no illness of any kind. Eight other normotensive persons, who were first-degree relatives of the hypertensives (namely, one of the parents with essential hypertension), were included in the study as a separate group.

**Determination of Plasma Renin**

Patients analyzed for plasma renin activity were on free diet, without medication, and with normal urinary sodium excretion in the urine. Blood samples were taken twice; following a baseline supine sample, furosemide (40 mg) was injected intravenously and the patient kept walking for an hour prior to the second blood sample. Plasma renin activity was determined by radioimmunoassay, as described.

**Determination of Lithium Efflux**

Blood was drawn into heparin solution (25 units per ml) and processed within 30 minutes. Separation of red blood cells, loading the cells with Li, and measurements of Li efflux were as described by Canessa et al. Efflux was performed simultaneously into Na-rich medium (150 mM NaCl, 10 mM glucose, 0.1 mM ouabain, and 10 mM Tris·Mops) and into Na-free medium (same medium but NaCl replaced by 75 mM MgCl2, and 85 mM sucrose), at various temperatures. A graded temperature device allowed the simultaneous assays of Li efflux at the desired temperatures (commonly 13 temperature points) in the range of 12° to 40°C. The pH of the medium varied from 7.44 (at 12°C) to 7.24 (at 40°C). The effect of this difference in pH on Li efflux rate when tested at a constant temperature was found to be negligible, within the experimental error. Lithium was determined by means of an atomic absorption spectrophotometer (Varian Techtron Model AA6, Melbourne, Australia), and calibrated by standards corresponding to the medium used. The flux was computed from the linear regression of Li loss within 30 minutes. The differences between the rate of lithium efflux into sodium-rich medium and sodium-free medium was taken as countertransport.

**Statistical Analysis**

The differences in means were analyzed by Student’s t test. The plots were drawn as least-squares regression lines and tested by analysis of variance.

**Results**

Table 1 compares the blood pressure of the normotensive and hypertensive persons studied and their respective rate of Li efflux. Patients with essential hypertension are presented as one group and also separated into two subgroups according to the available information about their family history of hypertension. In confirmation of earlier reports, patients with essential hypertension exhibited a higher rate of Li-Na countertransport. The elevated rate of Li efflux result-

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**Table 1: Lithium Efflux in Erythrocytes from Normotensive and Hypertensive Persons**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs)</th>
<th>Blood pressure</th>
<th>Li efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diastolic (mm Hg)</td>
<td>Systolic (mm Hg)</td>
</tr>
<tr>
<td>Normotensives (n = 22)</td>
<td>38 ± 3</td>
<td>78 ± 3</td>
<td>119 ± 2</td>
</tr>
<tr>
<td>Essential hypertensives (n = 52)</td>
<td>39 ± 2</td>
<td>100 ± 2†</td>
<td>153 ± 6†</td>
</tr>
<tr>
<td>1. With definite family history of HT (n = 28)</td>
<td>36 ± 3</td>
<td>100 ± 4†</td>
<td>151 ± 7†</td>
</tr>
<tr>
<td>2. Without definite family history of HT (n = 24)</td>
<td>42 ± 2</td>
<td>101 ± 3†</td>
<td>155 ± 7†</td>
</tr>
<tr>
<td>Secondary hypertensives (n = 10)</td>
<td>48 ± 5*</td>
<td>103 ± 6†</td>
<td>177 ± 8†</td>
</tr>
</tbody>
</table>

Values expressed as means ± SEM. (n) is the number of persons examined except that Li efflux into Na-free medium (and countertransport) was determined for 14 of the normotensives and 36 of the hypertensives, of which 21 and 17 were of Subgroups 1 and 2, respectively; HT = hypertension.

Probabilities (compared to the normotensives): *p < 0.01; †p < 0.001; no sign indicates no significant difference (p > 0.3).
ed primarily from an increase in the Li efflux in the presence of external Na, since Li efflux into Na-free medium was the same in the various groups. No correlation could be discerned between the efflux rates of the hypertensives and either the severity of the disease, sex, age, or drug treatment (r values were at the range of 0 to 0.15). Renin plasma activity among 11 patients was classified as follows: low renin, three patients; normal, six; high renin, six. There was no correlation between renin activity and the efflux rates. The patients with secondary hypertension exhibited mean rates of Li efflux and countertransport similar to those of the normotensives.

Figure 1 is a scatter diagram of Li efflux rates, which exhibits a marked overlap between the normotensives and the hypertensives. To improve the potential usefulness of the flux measurements as a diagnostic tool, we examined an additional membrane property associated with essential hypertension; the erythrocyte membrane shows modified temperature-dependence of Li efflux in essential hypertension. To assure a clear distinction of the temperature-dependence curves, we examined Li efflux at a wider temperature range (12°C to 42°C) and at shorter increments (about 2°C) than analyzed earlier.

Typical temperature-dependence plots of Li efflux for a normotensive person and for a patient with essential hypertension are shown in figure 2. The rates are plotted as a function of temperature in °C (Form A) and as logarithm (Arrhenius plots) as a function of the reciprocal of the absolute temperature, in °K⁻¹ (Form B). Temperature plots (Form A) are concave, but may be fitted with straight lines for the temperature range of 18°C to 35°C. For this range, the normotensive persons exhibited a uniform pattern of a discontinuous temperature-dependence of Li efflux both in sodium-rich medium and countertransport, with a change in slope at about 25°C, in agreement with an earlier report. Patients with essential hypertension were heterogeneous with respect to the temperature dependence of the Li efflux. Most of them (75%) exhibited a single straight line, as exemplified in figure 2, while the other patients showed a discontinuous temperature dependence, similar to the normotensives.

The Arrhenius plot (Form B) of Li efflux is composed of two straight lines, intersecting at a "break." The plots shown in figure 2 indicate a major difference in the "break" temperature: around 30°C for the normotensive person but about 20°C for the patient with essential hypertension. Figure 3 presents a scatter diagram of the "break" temperatures, deduced from Arrhenius plots of Li efflux in Na medium. Countertransport values (not shown) were distributed in a similar fashion. The normotensives exhibited relatively uni-

![Figure 1](http://hyper.ahajournals.org/Downloaded from http://hyper.ahajournals.org/)

**Figure 1.** Lithium efflux, either into sodium-rich medium or countertransport in erythrocytes of normotensives and hypertensive patients, determined at 37°C. PHT = patients with a family history of hypertension, P(T) = patients whose family history of hypertension is unclear; SEC = patients with secondary hypertension. Horizontal lines indicate the mean values ± SEM. Open circles among the essential hypertensives represent borderline cases. Black triangles denote secondary hypertensives with a family history of essential hypertension.
form Arrhenius plots, with a mean "break" temperature of 28.5 ± 0.3°C. The patients with essential hypertension appeared divided into two groups. The major one, which included 39 patients of the 52 studied, exhibited an Arrhenius plot "break" at 21.0° ± 0.2°C; these same patients also showed the modified temperature dependence according to Form A. The minor group of patients exhibited values within the normotensive range. The "break" values for patients with secondary hypertension were at the normotensive range, except for the two patients with a family history of essential hypertension.

As indicated, essential hypertension was equally detected by the two forms of temperature dependence. However, Arrhenius plot is a preferable form, since its slopes are definitely linear, while the Form A plot is curvilinear. For practical purposes it is useful to combine the rate of Li efflux and the temperature dependence into a single numerical expression. To this end we computed the slopes of the Arrhenius plots of Li efflux at two ranges: i) 40° to 30°C; and ii) 30° to 20°C. The ratio of the slopes (i/ii) multiplied by the efflux rate at 37°C is termed the "efflux index." A scatter diagram of efflux index values, based on Li efflux in Na medium, is shown in figure 4. The relative distribution of efflux index of countertransport values was very similar.

Lithium efflux was analyzed in a group of eight first-degree relatives of hypertensives. Their blood pressure was normal, while one of the parents of each examined person had essential hypertension. Efflux rate and index were used as a basis to evaluate whether the rela-
Li EFFLUX AND TEMPERATURE DEPENDENCE IN HYPERTENSION/Levy et al.

Figure 3. “Break” temperature of Arrhenius plots of Li efflux in erythrocytes of normotensive and hypertensive subjects. Li efflux was assayed in Na-rich medium. The symbols are as in figure 1.

Figure 4. Index of lithium efflux in erythrocytes of normotensive and hypertensive subjects. The index was determined as described in the text. The symbols are as in figure 1.

tives correspond to a “normotensive” or “hypertensive” type. Efflux values lower or higher than the mean ± SD of the normotensive group indicated a correspondence to a normotensive or hypertensive type, respectively. Table 2 shows that the classification on the basis of efflux rate and efflux index was similar for four of the offspring but dissimilar for four others. Six of the offspring (listed first) were characterized by the unique plots of the temperature dependence of the Li efflux, typical for most of the hypertensives.

Discussion

Essential hypertension is a multifactorial disease. Thus, a single genetic marker, such as Li-Na countertransport, may not be entirely effective for distinction between healthy and affected individuals. Limitations for distinction are overlapped between normotensive and hypertensive populations, natural variability within each group, and some experimental factor typical of the measurement of a flux rate. Furthermore, rates of Li-Na countertransport are changing during biological processes, such as pregnancy.25 In an attempt to im-

| Table 2. Lithium Efflux in Erythrocytes of Normotensive Offspring of a Hypertensive Parent: Comparison of Rate and Index |

<table>
<thead>
<tr>
<th>Family</th>
<th>Person</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Rate (mmol [liter RBC hr]⁻¹)</th>
<th>Correspondence to NT or HT based on Rate</th>
<th>Correspondence to NT or HT based on Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Z.A.</td>
<td>26</td>
<td>F</td>
<td>0.48</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>S.A.</td>
<td>24</td>
<td>F</td>
<td>0.36</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>A.V.</td>
<td>24</td>
<td>M</td>
<td>0.46</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>G.A.</td>
<td>34</td>
<td>F</td>
<td>0.44</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>N.H.</td>
<td>28</td>
<td>F</td>
<td>0.80</td>
<td>HT</td>
<td>HT</td>
</tr>
<tr>
<td>6</td>
<td>E.H.</td>
<td>20</td>
<td>F</td>
<td>0.66</td>
<td>HT</td>
<td>HT</td>
</tr>
<tr>
<td>7</td>
<td>Y.S.</td>
<td>17</td>
<td>M</td>
<td>0.26</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>M.A.</td>
<td>26</td>
<td>M</td>
<td>0.52</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT = normotensive; HT = hypertensive.

*Li efflux in Na medium; countertransport values showed an identical pattern.
prove the diagnostic capability of Li efflux in erythrocytes, we have extended our earlier study\textsuperscript{1} and examined the temperature dependence of Li efflux. The previous study, which originally demonstrated a modified temperature dependence of Li efflux in essential hypertension, was limited to 10 patients and to measurements at just eight temperatures. The present study deals with a larger number and a greater variety of cases, with reference to family history of hypertension, offspring of hypertensives, and cases of secondary hypertension. Furthermore, a mechanical device developed for the project has allowed the simultaneous flux measurements at a wider temperature range and at more finely spaced temperature intervals than earlier.

Our present study demonstrates that the analysis of Li efflux in erythrocytes by temperature "break" on the Arrhenius plots allows a clearer distinction of hypertensive cases than possible on the basis of efflux rate alone. The hypertensive patients who remained undifferentiated from the normotensive range on the basis of the "break" temperature included 10 cases of established essential hypertension and three borderline cases. The number of cases left in the normotensive range was changed to five established and five borderline cases by the introduction of the efflux index (fig. 4). A prospective study may show whether such borderline hypertensives will develop permanent hypertension in the future, as only about 20–40\% of the borderline cases do.\textsuperscript{2} Secondary hypertensive patients included in the study demonstrated the normotensive pattern of temperature dependence, except for two who had a clear family history of essential hypertension. This finding supports the specificity and the reliability of the method presented here. Furthermore, the association of the unique temperature dependence of the erythrocyte Li efflux with essential hypertension and with first degree relatives suggests that this property might be family aggregated.

The determination of the efflux index requires multiple analyses for each blood sample and thus poses difficulty for routine clinical assays. The practical difficulty in applying our approach can be partly overcome by assaying just Li efflux into Na medium, not countertransport, in order to determine the efflux index. This recommendation is based on the following considerations: 1) Li efflux into Na medium and countertransport show, in our study, essentially identical trends of temperature dependence; 2) figure 5 shows that the two parameters are correlated, as the regression coefficient ($r = 0.82$) and the slope (1.06) of the plot are close to one. Furthermore, the two parameters are also tightly correlated at the various temperatures studied (altogether about 900 determinations); 3) Li efflux into Na medium is a direct measurement, while countertransport is a derived parameter, namely, the difference between the flux into Na and into Na-free medium.

Despite the need for added assays, the application of the efflux index could be highly valuable for cases of particular importance, such as hypertension during pregnancy. We have recently found\textsuperscript{3} that, although Li efflux rates in pregnant women are clearly higher than in nonpregnant women, the individual's pattern of temperature dependence of Li efflux remained unchanged during pregnancy. This finding is of great significance, since no difference in countertransport rates could be detected between hypertensive and normotensive pregnant women, due to the marked elevation of the flux rate during pregnancy, irrespective of hypertension.\textsuperscript{25}

References
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