Recovery of Erythrocyte Na⁺-K⁺-Cl⁻ Cotransport Activity by Enalapril

Ramiro A. Sanchez, Maria I. Gimenez, Bernardo H. Gilbert, Carlos Giannone, Enrique J. Marco, and Agustin J. Ramirez

We studied total exchangeable sodium, ion transport activity at maximal rate, and erythrocyte Na⁺ content in response to angiotensin converting enzyme inhibition in mild-to-moderate essential hypertensive patients with normal renal function. Twenty-five patients (mean age 56 years, range 40–62 years) who had abnormal red blood cell Na⁺-K⁺-Cl⁻ cotransport or red blood cell Li⁺-Na⁺ countertransport were treated with either enalapril (20 mg daily) or hydrochlorothiazide (50 mg daily) during a 30-day period. During the period of enalapril treatment, Na⁺-K⁺ pump and Na⁺-K⁺-Cl⁻ cotransport increased significantly from 4,282±255 to 5,236±325 μmol/l red blood cell/hr (p<0.01) and 166±21 to 220±24 μmol/l red blood cell/hr (p<0.05), respectively. Mean intracellular Na⁺ content in erythrocytes decreased from 11.4±0.40 to 10.0±0.33 mmol/l (p<0.01) and exchangeable Na⁺ from 39.8±0.6 mmol/kg to 35.6±0.6 mmol/kg (p<0.001). Sodium reduction correlated with the recovery of Na⁺-K⁺-Cl⁻ cotransport activity (r=—0.65, p<0.01). During treatment, systolic and diastolic blood pressures were reduced significantly (p<0.01). In 12 patients treated with hydrochlorothiazide, Na⁺-K⁺-Cl⁻ cotransport, Na⁺-K⁺ pump, Na⁺-Li⁺ countertransport, and Na⁺ permeability did not change significantly while Na⁺ content decreased from 11.7±0.3 to 10.3±0.2 mmol/l (p<0.01). Thus, in essential hypertension the reduction of erythrocyte Na⁺ content during enalapril treatment could be related to an increase in Na⁺-K⁺-Cl⁻ cotransport and the Na⁺-K⁺ pump either by preventing the effects of an endogenous inhibitor or by altering the number of transport proteins in the cell membrane. (Hypertension 1991;17:334-339)

In addition to the Na⁺-K⁺ pump, the human erythrocyte contains other minor Na⁺ transport systems such as the chloride-dependent Na⁺-K⁺-Cl⁻ cotransport and the Na⁺-Li⁺ countertransport. In recent years, there has been convincing evidence that abnormalities in red blood cell (RBC) Na⁺ transport may play a role in the pathogenesis of essential hypertension. These abnormalities include a reduced RBC Na⁺-K⁺ pump activity, a decreased apparent affinity of the Na⁺-K⁺-Cl⁻ cotransport for internal Na⁺, and an enhancement of the Na⁺-Li⁺ countertransport. Furthermore, it has been found that some of these Na⁺ transport abnormalities are associated with an increased renal Na⁺ reabsorption.

Saitta and coworkers have shown that the Na⁺-K⁺-Cl⁻ cotransport mode of operation may provide an extra energy necessary to behave as a second pump, compensating by this way other transport alterations. Thus, the Na⁺-K⁺-Cl⁻ cotransport system seems to play two different roles in essential hypertension: 1) a defective second pump in patients with a decreased cotransport affinity for internal Na⁺ and 2) a compensatory pump when there is a defective extrusion of excess cell Na⁺ content.

The above observations raised our interest in analyzing whether converting enzyme inhibitors may correct abnormalities in RBC Na⁺ transport, mainly in Na⁺-K⁺-Cl⁻ cotransport, since it is known that these compounds are able to induce, along with the antihypertensive effect, a reduction in intracellular Na⁺ content.

Thus, the aim of this study was to evaluate the effect of enalapril on RBC Na⁺ transport and exchangeable Na⁺ in essential hypertensive patients with elevated intracellular Na⁺ content and low outward cotransport.

**Methods**

We selected 25 patients with abnormalities in Na⁺-K⁺-Cl⁻ cotransport (n=21) and Na⁺-Li⁺ countertransport (n=6); two patients had both abnormalities. Fifteen patients were men (45–62 years old).
and 10 were women (40–59 years old). Antihypertensive treatment was discontinued 4 weeks before the study. After a 2-week placebo run-in phase, 13 patients (12 with lower Na⁺-K⁺-Cl⁻ cointransport and one with higher Na⁺-Li⁺ cointransport, seven men and six women) received enalapril maleate (20 mg once a day) for up to 30 days; these patients were placed on a low Na⁺ diet (80 mmol Na⁺ daily) both during the placebo period and the active treatment period. The remaining 12 patients (seven with lower Na⁺-K⁺-Cl⁻ cointransport, three with higher Na⁺-Li⁺ cointransport, and two with both abnormalities) received, after 2 weeks of placebo, 50 mg hydrochlorothiazide daily during a similar period of 30 days. Systolic and diastolic blood pressures were registered with a Dinamap apparatus (Critikon, Tampa, Fla.) during placebo and in each week of the active treatment.

Blood samples were taken at the end of the placebo period and after 30 days of treatment with enalapril or hydrochlorothiazide. In women, blood samples were drawn 10 days after menstruation.

**Laboratory Determinations**

Exchangeable sodium was measured with a previously published method in 11 patients receiving enalapril treatment. In these patients 5–8 μCi sodium chloride (²²Na⁺, The Radiochemical Center, Amersham, England) was administered orally. Values were expressed in millimoles per kilogram.

Plasma renin activity was determined in the same 11 enalapril-treated patients by a previously described radioimmunoassay method. The enzyme activity was expressed as nanograms per milliliter per hour.

Na⁺-Li⁺ countertransport measurements were performed in all the patients according to the method described by Canessa et al. and Adragna et al. For loading, 2 ml washed RBCs (hematocrit 20%) were incubated for 3 hours at 37°C in loading solution (mM): LiCl 150, Tris-MOPS 10, pH 7.4 at 37°C, and glucose 10. Li⁺ was removed by washing six times with a solution of (mM) MgCl₂ 75, sucrose 85, and Tris-MOPS 10, pH 7.4 at 4°C. A 50% cell suspension in washing solution was used for measurement of hemoglobin, intracellular sodium content, and fluxes.

For flux measurements, Li⁺ efflux was measured in Mg⁺ and Na⁺ medium (4–5% hematocrit). Na⁺ medium contained (mM): Na⁺ 150, glucose 10, Tris-MOPS 10, pH 7.4 at 37°C, and ouabain 0.1. Mg medium contained (mM): MgCl₂ 75, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10, sucrose 85, and ouabain 0.1. The 50% suspension was diluted to a 4–5% hematocrit with the cooled (4°C) Mg⁺ and Na⁺ media, and the resultant cell suspension was divided into three portions and incubated for 60 minutes at 37°C. Li⁺ concentration was determined in the Mg⁺ and Na⁺ media using an atomic absorption spectrophotometer.

**Na⁺-K⁺ Pump and Na⁺-K⁺ Cointransport Maximum Na⁺ Efflux Rate Measurements**

Measurements were carried out with sodium-loaded RBCs by the nystatin method as described by Canessa et al.

For Na⁺ loading, 2 ml washed RBCs was added to 10 ml loading solution (mM: NaCl 70, KCl 70, Tris-MOPS 10, sucrose 55, pH 7.4 at 4°C) that contained 150 μl nystatin solution (10 mg in 2.5 ml dimethyl sulfoxide) and that was incubated for 20 minutes at 4°C. Nystatin was removed by centrifuging the solution at room temperature and washing four times at 35°C with (mM): NaCl 70, KCl 70, Tris-MOPS 10, pH 7.4 at 4°C, sucrose 55, K₂HPO₄ buffer 1, pH 7.4, glucose 10, and albumin 0.1%. In the first wash, the cells were equilibrated for 10 minutes in the water bath and for 4 minutes in the last three washes. The cells were subsequently washed with cold (4°C) choline washing solution (mM): choline chloride 149, MgCl₂ 1, Tris-MOPS 10, pH 7.4 at 4°C. A suspension at 50% hematocrit was made for measurement for hemoglobin, hematocrit, intracellular sodium content, and fluxes.

**Flux measurements:** To measure Na⁺-K⁺ pump function, 0.2 ml of the 50% suspension was added to 7 ml medium (mM): choline chloride 140, KCl 10, MgCl₂ 1, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10, without and with ouabain (0.1 mM). To measure cointransport, 0.8 ml of the 50% suspension was added to 7 ml medium (mM): choline chloride 148, MgCl₂ 1, Tris-MOPS 10, pH 7.4 at 37°C, glucose 10, ouabain 0.1, without and with 1 mM furosemide (33 mg in 100 μl 1 M Tris base and 1 M furosemide, and then made up to 1 mM with medium). After the addition of the cell suspension to each flask, 1.5 ml flux medium was put into each of five tubes. Duplicate tubes were incubated at 37°C for 5 minutes, and triplicate tubes were incubated for 25 minutes for pump medium and 65 minutes for cointransport medium.

**Cellular Na⁺ content** was measured by means of suitable standards. All solutions used for flux measurements required adjustment to 298±5 mOsm/l. Two normal controls for Na⁺-K⁺ pump and cointransport were repeated five times, obtaining a variation coefficient lower than 10%. Hemoglobin concentration was measured throughout the experiment, discarding flux measurements when variations were greater than 3% when compared with the original cells.

Sodium passive permeability was estimated as the relation between the concentration of Na⁺ in the medium with ouabain and nystatin related the intracellular Na⁺ content.

Sodium transport and Na⁺ content values were compared with those from 13 sex-, age-, and weight-matched normotensive subjects. The normal values obtained were: Na⁺-K⁺ cointransport, 190–560 μmol/l RBC/hr; Na⁺-Li⁺ cointransport, 220–360 μmol/l RBC/hr; Na⁺-K⁺ pump, 3,600–6,000 μmol/l RBC/hr;
TABLE 1. Enalapril Effects on Blood Pressure, Red Blood Cell Na⁺ Transport and Content, Plasma Renin Activity, and Exchangeable Na⁺

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>162.1±4.0</td>
<td>145.3±3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>106.7±2.0</td>
<td>92.1±2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Na⁺-K⁺ pump (μmol/l red blood cell/hr)</td>
<td>4,282±255</td>
<td>5,236±325</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Na⁺ permeability (μmol/l red blood cell/hr)</td>
<td>0.026±0.010</td>
<td>0.029±0.010</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺ cotransport (μmol/l red blood cell/hr)</td>
<td>166±21</td>
<td>220±24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Na⁺-Li⁺ countertransport (μmol/l red blood cell/hr)</td>
<td>302±42</td>
<td>302.4±22</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺ content (mmol/l)</td>
<td>11.4±0.4</td>
<td>10.06±0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>2.2±0.3</td>
<td>11.3±2.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E Na⁺ (mmol/l/kg)</td>
<td>39.8±0.6</td>
<td>35.6±0.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Before treatment and after treatment values of systolic (SBP) and diastolic (DBP) blood pressure, Na⁺-K⁺ pump activity (Na⁺-K⁺ pump), Na⁺ permeability, Na⁺ content (intracellular sodium content), Na⁺-K⁺-Cl⁻ cotransport, Na⁺-Li⁺ countertransport; exchangeable Na⁺ (E Na⁺), and plasma renin activity (PRA).

In vitro incubation with enalapril. In seven subjects enalapril effect on RBC sodium transport was tested in vitro. Enalapril (100 μg/ml) was added to 10 ml blood and incubated for 30 minutes at 37°C. Sodium transport determinations after incubation were then compared with basal values in the same subjects.

The protocol was approved by the Research Committee of the Hospital Instituto de Cardiologia, Academia Nacional de Medicina. Written, informed consent was required for all patients before entering in the study.

Statistical analysis was performed using a nonparametric test (Wilcoxon). An unpaired Student's t test was used for comparison of data between patients receiving enalapril or hydrochlorothiazide. Values were expressed as mean±SEM.

Results

Sodium-potassium pump, Na⁺-K⁺-Cl⁻ cotransport, Na⁺-Li⁺ countertransport, intracellular Na⁺ content, and passive Na⁺ permeability results for both groups are shown in Tables 1 and 2.

All 25 patients evaluated completed the 30-day treatment with enalapril and hydrochlorothiazide. Systolic blood pressure and diastolic blood pressure decreased significantly after treatment with both drugs. Mean value of Na⁺-K⁺-Cl⁻ cotransport (166±21 μmol/l RBC/hr) in essential hypertensive patients increased significantly after enalapril (220±24 μmol/l RBC/hr; p<0.05; Table 1, Figure 1). Of the 12 patients with initial abnormal Na⁺-K⁺-Cl⁻ cotransport, eight were within the range of normotensive values after treatment (Table 1, Figure 1). No significant differences were found in Na⁺-Li⁺ countertransport (302±42 versus 302.4±22 μmol/l RBC/hr) after enalapril (Table 1). Although the mean value of the Na⁺-K⁺ pump was within the normal range before treatment (4,282±255 μmol/l RBC/hr), the activity of the pump was increased after treatment by enalapril (5,236±325 μmol/l RBC/hr; p<0.01; Table 1, Figure 2). No significant difference was found in Na⁺ passive permeability during treatment with enalapril.

Conversely, intracellular sodium content (11.4±0.4 mmol/l) decreased significantly after enalapril (10.06±0.3 mmol/l; p<0.01).

Seven of the 13 patients who were studied had a higher pretreatment Na⁺ content. After enalapril treatment, five of the seven reached normal range values, whereas the other two achieved borderline values (Figure 3).

In 12 hypertensive patients treated with hydrochlorothiazide, Na⁺-K⁺-Cl⁻ cotransport, Na⁺-K⁺ pump, Na⁺-Li⁺ countertransport, and Na⁺ permeability did not change significantly; whereas Na⁺ content decreased from 11.7±0.3 to 10.3±0.2 mmol/l; p<0.01 (Table 2).

There were no differences in Na⁺ transport parameters between the enalapril or hydrochlorothiazide groups before treatment. In 11 essential hypertensive
patients, plasma renin activity (2.2±0.3 ng/ml/hr) increased significantly (11.3±2.0 ng/ml/hr; p<0.05; Table 1) and exchangeable Na⁺ (39.8±0.6 mmol/kg) decreased significantly (35.6±0.6 mmol/kg; p<0.001; Table 1) after treatment with enalapril.

A significant negative correlation was found between Na⁺-K⁺-Cl⁻ cotransport and intracellular Na⁺ content (r=-0.67, p<0.01) after enalapril treatment (Figure 4), whereas these parameters failed to show any correlation before active treatment (r=0.43). Similarly, using linear regression, no significant correlation was found between blood pressure decrease and either Na⁺-K⁺-Cl⁻ cotransport or intracellular Na⁺ content changes, nor was there a significant correlation between Na⁺-K⁺ pump and Na⁺ content.

In in vitro determinations, there were no differences in either the RBC Na⁺ transport parameters or the Na⁺ content after incubation with enalaprilat for 30 minutes (Table 3).

Discussion

Converting enzyme inhibitors have provided a new approach to therapy in hypertension along with a better understanding of the underlying mechanisms in essential hypertension. In our study, enalapril given in a single dose of 20 mg daily reduced both systolic and diastolic blood pressure, similar to the effect of hydrochlorothiazide 50 mg daily. In addition to blood pressure reduction, enalapril induced a significant decrease in both exchangeable Na⁺ and RBC Na⁺ content and an increase in the activity of the Na⁺-K⁺ pump and outward Na⁺-K⁺-Cl⁻ cotransport. Na⁺-K⁺-Cl⁻ cotransport is a bidirectional symport system that plays an important role in the vectorial transport of Na⁺ and water and in the regulation of cellular volume. In the present study, we also found after enalapril treatment a significant negative correlation between RBC Na⁺ content and Na⁺-K⁺-Cl⁻ cotransport, which suggests that enalapril could enhance the activity of Na⁺-K⁺-Cl⁻ cotransport to extrude intracellular Na⁺ content. However, with this data it cannot be assumed that the decrease in RBC Na⁺ content after treatment was exclusively due to an improvement in Na⁺-K⁺-Cl⁻ cotransport rather than in the pump activity.

Natriuresis has been reported in essential hypertensive patients treated with either captopril or enalapril. Although we did not report 24-hour urinary sodium excretion before and after enalapril treatment, the reduction in exchangeable Na⁺ observed in our study, in accordance with previous results, confirms the natriuretic effect of enalapril. The possibility of changes in Na⁺-K⁺-Cl⁻ cotransport and
Na⁺-K⁺ pump as a consequence of the natriuresis induced by enalapril was also explored. The observation that enalapril and not hydrochlorothiazide induced changes in Na⁺-K⁺ pump activity and Na⁺-K⁺-Cl⁻ cotransport would indicate that other mechanisms not associated with natriuresis may be involved in the improvement of erythrocyte Na⁺ transport reported above. A direct action of converting enzyme inhibitors on protein cell membrane (alterations of the protein’s number or changes of its affinity for Na⁺-K⁺) or an extrinsic effect not related to protein synthesis could be possible alternative mechanisms. In this regard, a direct effect of enalapril on erythrocyte membrane is unlikely since we were unable to demonstrate in vitro direct effect of enalapril on erythrocyte membrane isosmotic in vitro. Nevertheless, to fully investigate this hypothesis it would be necessary to prolong the administration of enalapril for more than 30 minutes. The recent characterization in human plasma of endogenous inhibitors of the sodium pump (ouabainlike²⁰) and cotransport (bumetanide³¹) may provide an alternative explanation of our findings. Thus, enalapril could enhance the activity of the Na⁺-K⁺ pump and Na⁺-K⁺-Cl⁻ cotransport by decreasing the activity of the inhibitors due to a specific interaction. The absence of in vitro changes in the Na⁺-K⁺ pump and Na⁺-K⁺-Cl⁻ cotransport activity, in contrast to the in vivo effects, and the lack of a clear effect on Na⁺ transport with hydrochlorothiazide might give further support to this hypothesis.

Occasional reports on any potential specific interaction of enalapril or similar converting enzyme inhibitors with cell membranes and transport phenomena have been published elsewhere. Tuck et al.²² reported an increase in Na⁺-K⁺-Cl⁻ cotransport efflux occurring in three essential hypertensive patients receiving converting enzyme inhibitors, and Santucci et al.²³ showed a reduction in the concentration of lymphocyte Na⁺ after acute or chronic treatment with captopril. In accordance with our results, these studies support the enhanced Na⁺-K⁺-Cl⁻ cotransport and the reduction in Na⁺ content associated with the administration of converting enzyme inhibitors in hypertension.

In the present study, we were unable to demonstrate any relation between lowering blood pressure and changes in Na⁺ content or Na⁺ transport parameters. Although these findings appeared contrary to expectation, it must be considered that the data were analyzed from a preselected group of patients and therefore, particular caution must be taken in extrapolating these results to the entire hypertensive population. In conclusion, in addition to hypotensive and natriuretic effects, enalapril seems to increase Na⁺ pump and cotransport activities by preventing the effects of endogenous inhibitors or by altering the number of transport proteins in the cell membrane.

### References


7. Saitta MN, Hanaaert PA, Rosati C, Diaz AS, Senn N, Garay RP: [Na⁺,K⁺,Cl⁻]-cotransport function and dysfunction in dif-

### Table 3. In Vitro Effects of Enalaprilat on Red Blood Cell Na⁺ Transport and Content

<table>
<thead>
<tr>
<th>Subject</th>
<th>Enal</th>
<th>Na⁺ content</th>
<th>Na⁺-K⁺ pump</th>
<th>Na⁺ cot</th>
<th>Na⁺-Li⁺ CTT</th>
<th>Na⁺ permeab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>12.2</td>
<td>3,970</td>
<td>170</td>
<td>306</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>12.2</td>
<td>3,570</td>
<td>151</td>
<td>286</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>10.1</td>
<td>4,580</td>
<td>209</td>
<td>290</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10.5</td>
<td>4,420</td>
<td>221</td>
<td>301</td>
<td>0.011</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>11.2</td>
<td>5,208</td>
<td>190</td>
<td>301</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>11.0</td>
<td>5,008</td>
<td>170</td>
<td>290</td>
<td>0.029</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>7.8</td>
<td>6,204</td>
<td>301</td>
<td>260</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7.6</td>
<td>5,508</td>
<td>290</td>
<td>240</td>
<td>0.019</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>9.2</td>
<td>4,802</td>
<td>261</td>
<td>308</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>8.9</td>
<td>4,504</td>
<td>252</td>
<td>300</td>
<td>0.015</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>7.2</td>
<td>7,804</td>
<td>209</td>
<td>408</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7.2</td>
<td>7,924</td>
<td>215</td>
<td>398</td>
<td>0.018</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>11.4</td>
<td>6,005</td>
<td>224</td>
<td>308</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>11.0</td>
<td>5,803</td>
<td>204</td>
<td>321</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Enal, enalaprilat; in presence (+) or in absence (−); Na⁺ cot, Na⁺ cotransport; Na⁺-Li⁺ CTT, Na⁺-Li⁺ countertransport; Na⁺ permeab, Na⁺ permeability.
different forms of primary hypertension. *Am J Hypertens* 1988;1:60S-63S.


**KEY WORDS** • erythrocytes • sodium-potassium-chloride cotransport • enalapril • essential hypertension.
Recovery of erythrocyte Na(+)–K(+)–Cl- cotransport activity by enalapril.
R A Sanchez, M I Gimenez, B H Gilbert, C Giannone, E J Marco and A J Ramirez

Hypertension. 1991;17:334-339
doi: 10.1161/01.HYP.17.3.334

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/3/334