Abnormal Sodium Efflux in Erythrocytes of Patients with Essential Hypertension

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SUMMARY Erythrocyte sodium efflux as well as sodium, potassium, and water content were studied in 12 untreated men with uncomplicated essential hypertension and in 18 normotensive control subjects. In the patients with essential hypertension, the rate constant for total sodium efflux was significantly lower than in the normotensives (5.96*10^-4 ± 0.45*10^-4 min^-1 vs 6.69*10^-4 ± 0.49*10^-4 min^-1; p < 0.005), which was due to a reduced ouabain-sensitive sodium efflux rate constant. Significant differences in total sodium efflux and ouabain-sensitive sodium efflux, however, could not be demonstrated, since intracellular sodium concentrations, although insignificant, were higher in the patients with essential hypertension (6.11 ± 0.74 mmole/liter vs 5.97 ± 0.66 mmole/liter). The rate constants for ouabain-insensitive sodium efflux, for ouabain-insensitive furosemide-sensitive sodium efflux, and for passive (ouabain-insensitive furosemide-insensitive) sodium efflux were similar in hypertensives and in normotensives.

The cause of the reduced rate constant for ouabain-sensitive sodium efflux is not clear. However, as suggested for other types of altered erythrocyte transport mechanisms described recently, it might be determined genetically. (Hypertension 4: 205-210, 1982)

KEY WORDS • essential hypertension • erythrocyte electrolytes • sodium efflux • ouabain • furosemide

An increased sodium concentration of the arterial wall, of leucocytes, and of erythrocytes has been reported in patients with essential hypertension. Recently, normal sodium concentrations in red blood cells (RBC) of essential hypertensives have been observed as well. Possible disturbances of intracellular sodium concentration might be due to either altered active and/or passive transport mechanisms. Active ouabain-sensitive sodium transport has been found to be reduced in leucocytes of white patients with essential hypertension and in erythrocytes of hypertensive Black Africans. Since active sodium and potassium transport are linked to each other, the report of an increased net potassium influx in sodium-loaded RBCs of patients with essential hypertension appears to contrast with the finding of a decreased ouabain-sensitive sodium efflux rate constant. Recently, also, abnormalities of glycoside-insensitive sodium transport have been described in RBCs of patients with essential hypertension. An increased rate constant for ouabain-insensitive sodium efflux from RBCs has been reported by Postnov et al. Canessa et al. observed an increased RBC lithium-sodium countertransport, and Garay et al. described a decreased maximal rate of furosemide-sensitive Na^+, K^+ -cotransport in patients with essential hypertension.

Data reported on RBC sodium concentration and transport are, at least in part, controversial, and thus far sodium transport mechanisms have only incompletely been investigated in essential hypertension. Therefore, in this paper we report studies on sodium and potassium concentrations and cell water content of RBCs in untreated patients with essential hypertension and in normotensive control subjects. Ouabain-sensitive, and ouabain-insensitive, furosemide-sensitive and ouabain-insensitive furosemide-insensitive rate constants for sodium efflux were determined as well.

Methods

We studied 12 men with uncomplicated essential hypertension (WHO Grades I and II). Secondary causes of hypertension had been excluded by appropriate methods in all cases. No patients had clinical or laboratory evidence of cardiac or renal
failure. Patients with essential hypertension were either untreated or antihypertensive therapy had been withdrawn for at least 4 weeks prior to the study. The mean age of the hypertensives was 33.1 ± 10.1 (± SD) years, and their sitting blood pressure was 171.0 ± 15.2/107.5 ± 11.8 mm Hg (average from two ambulatory visits to the hypertension center). The mean age of the 18 normotensive control subjects was 28.2 ± 5.1 years, and their arterial blood pressure was below 140/90 mm Hg in all cases. All subjects were on a free diet before blood sampling.

Erythrocyte water content was determined using freshly drawn heparinized blood. After centrifugation at 6000 g for 20 minutes, the plasma and buffy coat were discarded and 0.4 ml erythrocytes were dried at atmospheric pressure at room temperature and later on for three additional days in a vacuum over phosphorus pentoxide. The weight loss was determined gravimetrically. Erythrocyte water content was not corrected for trapped plasma which was similar in normotensives and in hypertensives.

Red blood cell (RBC) sodium and potassium were measured using 3.0 ml freshly drawn heparinized blood. The blood samples were centrifuged at 6000 g for 3 minutes at 4°C and washed three times with cold isotonic magnesium chloride solution (rapid washing with isotonic magnesium chloride solution does not affect RBC electrolyte concentrations).1-16 The erythrocytes were hemolyzed with distilled water in a final volume of 10.0 ml. Sodium and potassium were determined by flame photometry (Zeiss Fl 6/7), and the results were corrected for hematocrit, which was determined by a radioisotope dilution procedure in a modification of the method of Burck.14 Following the addition of 1.0 ml freshly drawn heparinized blood to 0.1 ml [22NaCl dissolved in isotonic saline, the suspension was centrifuged immediately. Hematocrit was calculated from the radioactivity of a 0.5 ml sample of the supernatant and the total radioactivity measured previously.

For sodium efflux studies, 6 ml of red blood cells with some residual plasma were preincubated with 0.6 μCl [22Na+ at 37.0 °C for 1 hour. Preliminary experiments had shown that preincubating red blood cells for [22Na+ loading did not affect intracellular electrolyte content. Subsequently, the erythrocytes were washed three times in a nonradioactive incubation medium containing 145 mmoles/liter NaCl, 5.0 mmoles/liter KCl, 1.7 mmoles/liter Na2HPO4, 1.2 mmoles/liter MgCl2, 2.4 mmoles/liter CaCl2, 10.0 mmoles/liter glucose, and 18.0 mmoles/liter tris(hydroxymethyl)aminomethane, adjusted to pH 7.40 at 37.0 °C. The concentration of ouabain was 0.5 mmoles/liter and that of furosemide, 1.0 mmoles/liter. After the last washing procedure, 1.0 ml of erythrocytes was added to 9.0 ml of incubation medium and the resulting suspension was incubated in a shaking bath at 37.0 °C. At the beginning of the efflux studies and after 15, 30, 60, 90, and 120 minutes of incubation, 1.3 ml samples were taken, cooled immediately in an ice-water bath, and centrifuged for 3 minutes at 3000 g at 4 °C; 1.0 ml portions of the supernatant were taken to measure the radioactivity (Nt). The total radioactivity was determined in 1.0 ml of uncentrifuged incubation medium (No). The rate constant for sodium efflux (k) was calculated from the slope of the regression line, which was obtained by plotting the logarithm of residual radioactivity within the erythrocytes ln (1 − Nt/No) against the incubation time.17-19 Linearity was ascertained throughout the incubation period of 120 minutes.

Sodium efflux was determined by multiplying the rate constant for sodium efflux by the concentration of sodium within the erythrocyte, as determined by flame photometry. This way of calculation is consistent with the literature, both in the absence and presence of ouabain and furosemide.14, 19-24 The data were analyzed by the Student's t test. Values are expressed as means ± standard deviation.

### Results

**Erythrocyte Sodium, Potassium, and Red Cell Water Content**

The sodium and potassium concentrations of red blood cells of the normotensives and patients with essential hypertension are shown in table 1. There were no significant differences in red blood cell electrolyte concentrations. After correction for erythrocyte cell water, significant differences of intracellular sodium and potassium could not be demonstrated.

<table>
<thead>
<tr>
<th>Group</th>
<th>Na (mmole/liter RBC)</th>
<th>K (mmole/liter RBC)</th>
<th>Na (mmole/liter cell H2O)</th>
<th>K (mmole/liter cell H2O)</th>
<th>RBC water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>5.97 ±0.66</td>
<td>93.54 ±4.92</td>
<td>9.33 ±0.97</td>
<td>146.36 ±5.79</td>
<td>63.93 ±1.23</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>6.11 ±0.74</td>
<td>90.63 ±4.03</td>
<td>9.58 ±1.22</td>
<td>141.93 ±5.86</td>
<td>63.86 ±0.89</td>
</tr>
</tbody>
</table>

### Table 1. Red Blood Cell Sodium and Potassium Concentrations and Water Content in Normotensives and in Patients with Essential Hypertension (mean values ± SD)
normotensives, ouabain inhibited the rate constant by 74.1%, and in the patients with essential hypertension, by 73.3% (not significantly different) at a ouabain concentration of 0.5 mmole/liter. Higher concentrations of ouabain did not result in a further decrease of the rate constants.

The furosemide-insensitive rate constant, measured in the presence of 1.0 mmole furosemide/liter, was similar in normotensives and hypertensives (table 2, III). In the normotensives, furosemide reduced the rate constant for sodium efflux by 35.5%, and in the hypertensives, by 29.8% ($p < 0.05$). The ouabain-insensitive furosemide-insensitive rate constant, measured in the presence of ouabain and furosemide together, represents the passive permeability of the erythrocyte membrane. Again, there were no significant differences between both groups (table 2, IV). In the normotensives, ouabain + furosemide inhibited total sodium efflux by 87.3% and 85.7% in the hypertensives (difference not significant). The ouabain-insensitive furosemide-sensitive rate constant, represented by the difference between the ouabain-insensitive and ouabain-insensitive furosemide-insensitive rate constant (table 2, II–IV), was also similar in both groups.

The total furosemide-sensitive rate constant for sodium efflux (table 2, I–III) measured in the absence of ouabain was significantly lower in the hypertensives ($p < 0.01$). In both groups total furosemide-sensitive rate constants were higher than the ouabain-insensitive furosemide-sensitive rate constants (II–IV), indicating that furosemide also inhibited a component of ouabain-sensitive sodium efflux. This ouabain-sensitive furosemide-sensitive rate constant [(I–III)–(II–IV)] was significantly lower in the hypertensive patients than the normotensives ($p < 0.05$).

**Table 2. Rate Constants ($10^{-3}$·min$^{-1}$) for Sodium Efflux in Normotensives and in Patients with Essential Hypertension (mean values ± SD)**

<table>
<thead>
<tr>
<th>Patients with essential hypertension</th>
<th>I Normotensives</th>
<th>II Hypertensives</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.69 ± 0.49</td>
<td>5.96 ± 0.45</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>II</td>
<td>1.73 ± 0.21</td>
<td>1.59 ± 0.13</td>
<td>ns</td>
</tr>
<tr>
<td>III</td>
<td>4.41 ± 0.48</td>
<td>4.18 ± 0.50</td>
<td>ns</td>
</tr>
<tr>
<td>IV</td>
<td>0.86 ± 0.11</td>
<td>0.85 ± 0.07</td>
<td>ns</td>
</tr>
<tr>
<td>I-II</td>
<td>4.96 ± 0.49</td>
<td>4.38 ± 0.45</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>II-IV</td>
<td>0.89 ± 0.23</td>
<td>0.74 ± 0.17</td>
<td>ns</td>
</tr>
<tr>
<td>I-III</td>
<td>2.36 ± 0.71</td>
<td>1.78 ± 0.40</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(I-III)-(II-IV)</td>
<td>1.50 ± 0.63</td>
<td>1.03 ± 0.38</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

$I$ = total sodium efflux; $II$ = ouabain-insensitive sodium efflux; $III$ = furosemide-insensitive sodium efflux; $IV$ = ouabain-insensitive furosemide-insensitive efflux; $II-I$ = ouabain-sensitive sodium efflux; $II-IV$ = ouabain-insensitive furosemide-sensitive sodium efflux; $I-III$ = total furosemide-sensitive sodium efflux; $(I-III)-(II-IV)$ = ouabain-sensitive furosemide-sensitive sodium efflux.
Sodium Efflux [mmole/liter RBC-hr]

Total sodium efflux, ouabain-sensitive sodium efflux, ouabain-insensitive sodium efflux, ouabain-insensitive furosemide-sensitive sodium efflux, and ouabain-insensitive furosemide-insensitive sodium efflux in mmole/liter red blood cells per hour are shown in table 3. Since in the hypertensives the intracellular sodium concentration was increased, although insignificantly, no significant differences for sodium efflux could be demonstrated between normotensives and hypertensives.

### Discussion

The results of this study demonstrate that the sodium and potassium concentrations of red blood cells of untreated patients with mild or moderate essential hypertension are not different from those of normotensive controls. Our data agree with the findings of Burck, et al., and Canessa et al., but they conflict with reports of Losse et al., Wessels et al., Gessler, and Fadeke Aderounmu and Salako, who found an increased erythrocyte sodium content in patients with essential hypertension. Also, an increased water content has been reported in erythrocytes of patients with essential hypertension, Garay and Meyer, who found an increased erythrocyte sodium content, 

The discrepancies reported in the literature might be due to various factors: The severity of the hypertensive process might influence the intracellular concentration of electrolytes, since Garay and Meyer observed changes in net sodium and potassium erythrocyte fluxes in patients with essential hypertension, depending on the height of their blood pressure. Burck reported normal sodium erythrocyte concentrations in patients with uncomplicated essential hypertension and elevated concentrations in patients whose hypertension was complicated by heart failure. It is important to note in this context that our patients had moderate hypertension without evidence of heart failure.

Antihypertensive therapy might also cause an elevation of intracellular sodium concentration. We have shown that administration of hydrochlorothiazide to normotensive subjects for 1 week results in an increase in red blood cell sodium by 25%, the intracellular sodium concentration being significantly increased 2 days after starting diuretic treatment and remaining elevated for 4 more days after stopping treatment. Furosemide has been shown to reduce the rate constant for sodium efflux from erythrocytes, which might cause an increase in red blood cell sodium. The beta-blocking agent propranolol has been shown to inhibit active sodium transport. Therefore, in our study the patients were either untreated or drug therapy had been discontinued at least 4 weeks prior to the study.

Data on rate constants for red blood cell sodium efflux have not been published so far for white patients with essential hypertension. In our hypertensives, the rate constant for mean total sodium efflux was increased due to a reduced ouabain-sensitive sodium efflux component. The reduced ouabain-sensitive rate constant might be due to a diminished Na-K-ATPase activity, which has been found in hemolyzed and dialyzed as well as in resealed red blood cells of patients with essential hypertension. Whether an altered permeability for actively transported sodium might also be involved in the reduced rate constant for ouabain-sensitive sodium efflux remains unclear. In potassium-depleted red blood cells of patients with essential hypertension, Garay and Meyer observed a diminished net sodium efflux, which might be due to an increased passive sodium influx. These results, however, cannot be directly compared with our findings, since sodium loading of red blood cells has been shown to lead to a reduction of the rate constant for ouabain-sensitive sodium efflux and to an increased ouabain-sensitive active cation transport, whereas the rate constant for ouabain-sensitive sodium efflux is decreased because the pump is saturable. In our experiments we measured unidirectional sodium efflux in loaded red blood cells at physiological intracellular electrolyte concentrations.

Total sodium efflux and ouabain-sensitive sodium efflux (mmoles per liter red blood cells per hour) were not different in both groups since intracellular sodium concentration was higher in the patients with essential hypertension (although insignificantly). Similar results were reported by Fadeke Aderounmu and Salako, who found a decreased ouabain-sensitive sodium efflux rate constant in erythrocytes of un-

### Table 3. Red Blood Cell Sodium Efflux (mmole/liter erythrocytes · hr) in Normotensives and in Patients with Essential Hypertension (mean values ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Ouabain sensitive</th>
<th>Ouabain insensitive, furosemide sensitive</th>
<th>Ouabain insensitive, furosemide insensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>2.39 ± 0.30</td>
<td>1.77 ± 0.23</td>
<td>0.62 ± 0.11</td>
<td>0.32 ± 0.10</td>
</tr>
<tr>
<td>n = 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensives</td>
<td>2.19 ± 0.32</td>
<td>1.61 ± 0.24</td>
<td>0.58 ± 0.11</td>
<td>0.28 ± 0.09</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
treated African patients with essential hypertension. These authors also reported a significantly decreased ouabain-sensitive sodium efflux in hypertensives. The statistical significance reported, however, apparently is due to an error in their mathematical analysis (active sodium efflux in Control Subject 14, p. 373, table II, is 2.8 instead of 10.5 mmoles/liter). After correction, a statistically significant difference in active sodium efflux is no longer demonstrable). Also, in leucocytes of patients with essential hypertension, a significantly lower total and glycoside-sensitive sodium efflux rate constant has been observed. Similar to our data obtained in erythrocytes, total sodium efflux was not statistically different from that of normotensives since in leucocytes intracellular sodium concentration was increased in the hypertensives.

The ouabain-insensitive sodium efflux rate constant was similar in normotensives and in hypertensives. By contrast, Postnov et al.19 reported an increased ouabain-insensitive sodium permeability of the erythrocyte membrane of patients with essential hypertension. However, they had preincubated the erythrocytes at 2 °C for at least 4 hours. Thus, the discrepancy might be due to different experimental conditions since the erythrocytes in our experiments were constantly kept at 37.0 °C. Our results are consistent with data of Thomas et al.3 who observed a similar ouabain-insensitive permeability of the leucocyte membrane in normotensives and in patients with essential hypertension.

The ouabain-insensitive furosemide-sensitive rate constant for sodium efflux was similar in the normotensive control subjects and in the patients with essential hypertension. This result seems to contrast with the reduction of erythrocyte Na+, K+ — cotransport in essential hypertension, as reported by Garay et al.14 Since cotransport depends on the intra-/extracellular sodium and potassium ratios, the discrepancy of the results might be due to a different methodology. In our experiments the additional inhibition of total sodium efflux by furosemide was of the same order of magnitude as reported by Dunn8 and Rettoni4 in normotensives. The total furosemide-sensitive rate constant for sodium efflux, measured in the absence of ouabain, was significantly decreased in the hypertensive patients. In both groups the total furosemide-sensitive rate constant was significantly higher than the ouabain-insensitive furosemide-sensitive rate constant. These results demonstrate, as has been reported earlier for normotensives,8, 40 that furosemide inhibits an ouabain-insensitive component of sodium efflux. In addition, it inhibits, at least partly, the ouabain-sensitive sodium efflux.

Since the ouabain-insensitive furosemide-sensitive rate constant is similar in normotensives and in patients with essential hypertension, the significantly lower total furosemide-sensitive rate constant observed in the hypertensives has to be attributed to a diminished inhibition of ouabain-sensitive sodium efflux. The ouabain-insensitive furosemide-insensitive rate constant for sodium efflux from erythrocytes was found to be similar in the normotensives and in the hypertensives. In our experiments, ouabain inhibited 74% of the total sodium efflux both in normotensives and in hypertensives. A further 13% of the total sodium efflux could be inhibited by furosemide, and the residual 13% of sodium efflux could neither be inhibited by furosemide nor by ouabain. In the absence of ouabain, glycoside-sensitive sodium efflux was inhibited, at least partly, by furosemide. Similar results have been reported by Dunn8, 19 for normotensives.

The disturbance of erythrocyte sodium transport in the patients with essential hypertension described here was of minor degree and did not result in an increase in red blood cell sodium concentration. Although, as a group, the rate constants for total and for ouabain-sensitive sodium efflux were significantly lower in the hypertensives, markedly reduced values were found only in four of 12 patients with hypertension. It remains uncertain whether these patients represent a distinct subgroup of essential hypertensives since no other criteria are available to prove this hypothesis. The overlap in rate constants between both groups observed in our study is opposed to disturbances in Na+, K+ — cotransport,4 or in sodium-lithium countertransport,8 which were observed in virtually all essential hypertensive patients so studied.

The cause of the reduced rate constant for ouabain-sensitive sodium efflux observed in our patients with essential hypertension is not clear. However, as suggested for other types of altered erythrocyte transport mechanisms,8, 40 it might be determined genetically.

References
10. Walter U, Distler A: Effects of ouabain and furosemide on ATPase activity and sodium transport in erythrocytes of nor-
motivations and of patients with essential hypertension. In Intra-
cellular Electrolytes and Arterial Hypertension, edited by Zumkley H, Losse H. Stuttgart/New York; Georg Thieme
Verlag, 1980, p 170-181


13 Postnov YV, Orlov SN (Shevchenko A, Adler AM): Altered sodium permeability, calcium binding and Na-K-ATPase ac-
tivity in the red blood cell membrane in essential hypertension. Pflügers Arch 371: 263, 1977

14 Garay RP, Dagher G, Pernollet MG, Devynck MA, Meyer P: Inherited defect in a Na+, K+-co-transport system in erythro-


25 Askari A, Rao SN: Studies on the partial reactions catalyzed by the (Na+ + K+)-activated ATPase. III. Relation of K+-
dependent p-nitrophenolphosphatase to Na+-transport in red cell ghosts. Biochim Biophys Acta 241: 75, 1971


30 Burck HCh: Der Elektrolytgehalt der Erythrocyten im Rahmen der Diagnostik der Herzinsuffizienz. Verh dtsch Ges Inn Med 77: 140, 1971

31 Walter U: Red blood cell sodium and potassium after hydro-


33 Müller-Soyano A, Glader BE: Cation specifici of pro-


38 Garrey RP, Garrahan PJ: The interaction of sodium and potas-
sium with the sodium pump in red cells. J Physiol 231: 297, 1973


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U Walter and A Distler

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