Sodium and Potassium Ion Transport Accelerations in Erythrocytes of DOC, DOC-Salt, Two-Kidney, One Clip, and Spontaneously Hypertensive Rats
Role of Hypokalemia and Cell Volume

JOCHEN DUHM, DR.MED., BERND OTTO GÖBEL, DR.MED., AND FRANZ-XAVER BECK, DR.MED.

SUMMARY Sodium (Na⁺) and potassium (K⁺) transport by the furosemide-sensitive Na⁺-K⁺ transport system, the Na⁺-K⁺ pump, and the cation leak(s) were studied in erythrocytes from DOC-water, DOC-salt, two-kidney, one clip (Sprague-Dawley), and spontaneously hypertensive rats (Wistar-Kyoto). Rubidium (Rb⁺) was used as a tracer for K⁺. After 4 weeks of DOC-salt hypertension, inward K⁺ (Rb⁺) transport by the furosemide-sensitive system was increased threefold, and the inward Na⁺ leak and the red cell Na⁺ content were elevated by about 50%. The rise in cell Na⁺ accelerated K⁺ inward and Na⁺ outward transport by the Na⁺-K⁺ pump. DOC-water hypertension caused similar but less pronounced changes. In two-kidney, one clip hypertension, the Na⁺ leak and the Na⁺-K⁺ pump rates were slightly elevated, and furosemide-sensitive Rb⁺ uptake tended to be increased. In spontaneously hypertensive rats, furosemide-sensitive Rb⁺ uptake was accelerated by 50%. The marked hypokalemia in DOC-water and DOC-salt hypertension was associated with a slight loss of red cell K⁺ and an increase in mean cellular hemoglobin content (MCHC), indicative of cell shrinkage. Hypokalemia induced by dietary K⁺ deficiency caused alterations in red cell cation transport, content, and cell volume which were qualitatively similar but more pronounced than those seen in DOC-salt hypertension. Osmotic shrinkage in vitro induced a severalfold acceleration of furosemide-sensitive Rb⁺ uptake, similar to that observed in rat erythrocytes shrunken in vivo in K⁺-deficient states. It is concluded that the acceleration of furosemide-sensitive K⁺ (Rb⁺) transport in erythrocytes of mineralocorticoid hypertensive rats is largely caused by the hypokalemia and consecutive red cell K⁺ loss and shrinkage, respectively. Mean cellular hemoglobin content (MCHC) is thus a parameter that must be considered in studies on Na⁺ and K⁺ transport across the membrane of rat erythrocytes. (Hypertension 5: 642-652, 1983)

KEY WORDS • furosemide • sodium-potassium cotransport • sodium-potassium pump • sodium-potassium leak • potassium deficiency

ALTERED transport and distribution of electrolytes may play an important role in the pathogenesis of several forms of clinical and experimental hypertension.1-4 Evidence has accumulated during the last two decades indicating that the Na⁺ content4-8 and passive Na⁺ permeability9-10 of human erythrocytes are increased in essential hypertension. Similar alterations in Na⁺ content and transport have been reported to occur in red cells of spontaneously11-17 and DOC-salt hypertensive rats.18 In essential hypertension, a reduced activity of the chloride-dependent,
furosemide-sensitive Na\(^+\)-K\(^+\) cotransport system has been discussed as a cause of the elevated red cell Na\(^+\) content.\(^{19}\) In addition, the ouabain-resistant Na\(^+\)-Na\(^+\) (Na\(^+\)-Li\(^+\)) exchange\(^{20}\) as well as Na\(^+\)-K\(^+\) pump activities\(^{21}\) were found to be increased in the erythrocytes of patients with essential hypertension.

Although the alterations of Na\(^+\) transport characteristics of human erythrocytes in essential hypertension described above are of no diagnostic value,\(^{21,22}\) the trend toward higher red cell Na\(^+\) levels in human essential and rat spontaneous hypertension seems to be well documented. It has been suggested that Na\(^+\) movements in vascular smooth muscle of hypertensive rats are similarly altered as in erythrocytes,\(^{5,24-25}\) with the elevated smooth muscle Na\(^+\) content possibly increasing the resting tone of the resistance vessels.\(^{26}\)

We designed our present study to identify those mechanisms responsible for the alterations of red cell Na\(^+\) and K\(^+\) transport in some models of rat hypertension, directing special attention toward the furosemide-sensitive transport system for Na\(^+\) and K\(^+\), the Na\(^+\)-K\(^+\) pump, and the Na\(^+\) and K\(^+\) leaks. We did not study ouabain-resistant Na\(^+\)-Li\(^+\) (Na\(^+\)-Na\(^+\)) exchanger, since rat erythrocytes do not exhibit a typical Na\(^+\)-Li\(^+\) countertransport system.\(^{27}\) We included the models of DOC and DOC-salt hypertension in the study because of their obvious association with the Na\(^+\) and K\(^+\) balance. In addition to these models of low renin hypertension, we studied two-kidney, one clip hypertension, which is considered to be of the high renin type, and also studied red cells of a spontaneously hypertensive Wistar-Kyoto (WKY) strain. We further investigated the in vitro action of DOC and the effects of a nutritional K\(^+\) deficiency mimicking DOC-induced hypokalemia.

Materials and Methods

Rats

Male rats were used throughout the study; they were maintained on a control diet (Altromin C 1320, Na\(^+\) 0.2%, K\(^+\) 1%) or a K\(^+\)-deficient diet (Altromin C 1037, Na\(^+\) 0.35%, K\(^+\) < 0.01%), and tap water (or 1% NaCl) ad libitum. Spontaneously hypertensive WKY rats (stroke-prone strain) and an age-matched WKY control strain were obtained by the courtesy of Dr. D. Ganten, Heidelberg. Sprague-Dawley rats were from Mus Rattus, Brunnthal.

Mineralocorticoid hypertension was induced in Sprague-Dawley rats (150–170 g initial body weight) by subcutaneous injections of 25 mg·kg\(^{-1}\) deoxycorticosterone enanthate (DOC, Cortiron Depot, Schering) 6 times per week over 4 to 5 weeks. Control animals received corresponding volumes of the solvent oil (6/4 mixture of castor oil and benzyl benzoate, vol/vol).

Two-kidney, one clip hypertension was produced in Sprague-Dawley rats (initial weight, 150–290 g). The left kidney was approached through a flank incision, and a steel wire (0.2 or 0.3 mm diameter) was firmly tied to the left renal artery with four loops of cotton thread. After 90 seconds of renal ischemia, the steel wire was removed to allow reperfusion of the kidney through the constricted artery. In the sham-operated control group, the left renal artery was similarly constricted, but both the steel wire and the cotton thread were removed after 90 seconds. The operations were performed 10 to 20 days before blood withdrawal.

Changes in blood pressure were recorded by a tail-cuff plethysmographic method. On the day of blood withdrawal, blood pressure in the left femoral artery was monitored by a Statham pressure transducer with the rats under light ether anesthesia.

Measurement of Red Cell Cation Contents and Net Sodium and Rubidium Transport

We used a standard assay similar to that previously described for human erythrocytes,\(^{28}\) except that external rubidium (Rb\(^+\)) was lowered to 2 mM and ouabain was increased to 5 mM.

The rats were exsanguinated through the abdominal aorta under light ether anesthesia, Na\(^+\) heparin being used as anticoagulant (40 to 50 units per ml of blood). Hematocrit (Clay Adams Autocrit, 10 minutes) and hemoglobin (Merckotest Hemoglobin, Merck, millimolar extinction coefficient of cyaanmethemoglobin (by tetramer) = 44) were determined on whole blood. The red cells were sedimented (5-minute centrifugation at 4600 × g, room temperature), plasma,uffy coat, and the uppermost cell layer were removed, and the sediment was carefully mixed for red cell cation content determination (quadruplicates). The remaining cells were then washed thrice with a fivefold excess of incubation medium (mM): NaCl (145), glucose (5), phosphoric acid (1), morpholinopropane sulfonic acid (10), titrated to pH 7.4 at 37°C with tris-(hydroxymethyl)-aminomethane. The final osmolality (Knauer Halbmikro-Osmometer) was 320 to 325 mOsm·kg\(^{-1}\). All media were nominally free of HCO\(_3\)-.

Washed erythrocytes (0.5 to 0.75 ml) were added to each of three incubation vessels containing 75 ml of influx medium (37°C, final hematocrit 0.6% to 1%) and 1) 75 μl dimethyl-sulfoxide (DMSO); 2) 5 mM ouabain and 75 μl DMSO; and 3) 5 mM ouabain plus 150 μl of a 125.5 mM Na\(^+\)-furosemide stock solution freshly prepared using a 1:1 mixture of distilled water and DMSO (vol/vol) as solvent, the final furosemide concentration being 0.5 mM. After 15 minutes of preincubation to allow for ouabain binding to the relatively ouabain-resistant rat erythrocytes,\(^{28}\) isotonic RbCl was added to initiate the influx period at a final concentration of 2 mM external Rb\(^+\). After each 15, 30, and 60 minutes of continued incubation under gentle shaking (37°C), two 12.5 ml aliquots of the cell suspensions were decanted into polyethylene tubes, which were placed in an ice bath and centrifuged at 0°C for 2 minutes (4600 × g). The red cells were then washed thrice with 2 ml ice-cold choline chloride solution (150 mM) and hemolyzed with 1.6 ml 6% n-butanol in water (vol/vol). The hemoglobin concen-
TABLE 1. Blood Pressure, Body and Kidney Weights, and Plasma and Red Cell Parameters in the Eight Groups of Rats Studied (Mean Values ± 1 so)

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Mean blood pressure (mm Hg)</th>
<th>Body weight (g)</th>
<th>Kidney weight (mg/100 g bw-1)</th>
<th>Plasma</th>
<th>Na+ (mM)</th>
<th>K+ (mM)</th>
<th>Cl- (mM)</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Right</td>
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<td></td>
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<tr>
<td>Sprague-Dawley</td>
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<tr>
<td>Control (n = 23)</td>
<td>105 ± 7</td>
<td>317 ± 47</td>
<td>354 ± 14</td>
<td>138 ± 5</td>
<td>3.84 ± 0.30</td>
<td>104 ± 3</td>
<td></td>
</tr>
<tr>
<td>NaCl (n = 6)</td>
<td>101 ± 6</td>
<td>273 ± 14*</td>
<td>389 ± 28</td>
<td>132 ± 2</td>
<td>3.88 ± 0.06</td>
<td>101 ± 2</td>
<td></td>
</tr>
<tr>
<td>DOC-H2O (n = 6)</td>
<td>129 ± 12‡</td>
<td>252 ± 18‡</td>
<td>456 ± 26‡</td>
<td>136 ± 3</td>
<td>2.66 ± 0.242</td>
<td>99 ± 4*</td>
<td></td>
</tr>
<tr>
<td>DOC-NaCl (n = 11)</td>
<td>160 ± 16‡</td>
<td>226 ± 16</td>
<td>742 ± 110‡</td>
<td>139 ± 6</td>
<td>2.43 ± 0.344</td>
<td>93 ± 6‡</td>
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<tr>
<td>Sham-clipped (n = 4)</td>
<td>109 ± 8</td>
<td>335 ± 33</td>
<td>314 ± 20</td>
<td>137 ± 1</td>
<td>3.80 ± 0.39</td>
<td>103 ± 2</td>
<td></td>
</tr>
<tr>
<td>2-kidney, 1 clip (n = 4)</td>
<td>151 ± 39*</td>
<td>256 ± 64*</td>
<td>535 ± 86‡</td>
<td>137 ± 2</td>
<td>4.00 ± 0.25</td>
<td>102 ± 4</td>
<td></td>
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<tr>
<td>Wistar-Kyoto</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>105 ± 5</td>
<td>366 ± 14</td>
<td>321 ± 17</td>
<td>135 ± 2</td>
<td>3.31 ± 0.16</td>
<td>104 ± 1</td>
<td></td>
</tr>
<tr>
<td>SH (n = 5)</td>
<td>185 ± 23‡</td>
<td>261 ± 25‡</td>
<td>401 ± 22‡</td>
<td>137 ± 3</td>
<td>3.11 ± 0.27</td>
<td>101 ± 3</td>
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</tr>
</tbody>
</table>

The red cell Na+ and K+ contents refer to a MCHC of 5.2. The actual Na+ and K+ contents can be obtained by multiplying the given values with the ratio MCHC 5.2⁻¹. *p < 0.05. Significant differences vs the respective control group, i.e., NaCl, DOC-H2O, and DOC-NaCl vs Sprague-Dawley control, two-kidney, one clip vs sham-clipped, and spontaneously hypertensive vs control Wistar-Kyoto rat. †p < 0.01. ‡p < 0.005.

Results

Blood Pressure, Red Cell Cation Contents, Mean Cellular Hemoglobin Content, and Plasma Potassium

Mean blood pressures were significantly elevated in the DOC, DOC-salt, two-kidney, one clip, and the spontaneously hypertensive rats (table 1). Ratios of kidney to body weight were increased in the two DOC groups, in the spontaneously hypertensive rats, and in the intact kidney of the Goldblatt hypertensive animals. Plasma Na+ and osmolalities were essentially the same among the groups.

Plasma K+ was reduced to 2.7 mM in the DOC group, and lowered to 2.4 mM in the DOC-salt hypertensive animals (table 1). In the two DOC groups, the hypokalemia was associated with a fall in plasma chloride concentration, probably reflecting a metabolic alkalosis. In the DOC-salt animals, red cell Na+ and the mean cellular hemoglobin content (MCHC) were significantly increased, and red cell K+ tended to be reduced (table 1).

Sodium and Potassium Transport

In rat erythrocytes, there are ouabain-resistant, Na+-dependent K+ (Rb+) and K+-dependent Na+ movements that are inhibited by loop diuretics such as bumetanide and furosemide. The furosemide-sensitive K+ and Na+ transport is reduced by about 90% upon replacement of Cl⁻ by NO₃⁻. The kinetic properties of the furosemide-sensitive transport system and the alterations of Na+ and K+ net movements resulting from alterations of the driving forces closely resemble those of the so-called Na+-K+ cotransport system of human erythrocytes (Duhm J, Gobel BO, unpublished results, 1983). Accordingly, all cation movements that are inhibitable by furosemide can be operationally ascribed to the action of a Na+-K+ cotransport system. However, it is not known whether the main function of the furosemide-sensitive, chloride-dependent system(s) of rat erythrocytes is a Na+-dependent K+-K+ exchange and a K+-dependent Na+-Na+ exchange. Therefore, the term "cotransport" is
TABLE 1. (Continued)

| Osmolarity (mOsm) | Red cells | MCHC  
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Na⁺ (μmoles·ml⁻¹)</td>
<td>K⁺ (μmoles·ml⁻¹)</td>
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<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>293 ± 5</td>
<td>3.71 ± 0.57</td>
<td>106 ± 2</td>
</tr>
<tr>
<td>293 ± 3</td>
<td>3.48 ± 0.12</td>
<td>105 ± 2</td>
</tr>
<tr>
<td>293 ± 5</td>
<td>3.92 ± 0.23**</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>291 ± 6</td>
<td>5.26 ± 0.74‡</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>292 ± 6</td>
<td>4.21 ± 0.35</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>291 ± 5</td>
<td>4.17 ± 0.20</td>
<td>111 ± 5</td>
</tr>
<tr>
<td>294 ± 2</td>
<td>3.95 ± 0.13</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>293 ± 7</td>
<td>3.79 ± 0.18</td>
<td>103 ± 4</td>
</tr>
</tbody>
</table>

Avoided and replaced by "furosemide-sensitive transport" in our discussion.

The experimental procedure applied to measure Rb⁺ uptake by the Na⁺-K⁺ pump, the furosemide-sensitive system, and the K⁺ (Rb⁺) leak is demonstrated in figure 1 for the examples of erythrocytes from one control rat and one DOC-salt hypertensive animal (mean blood pressure, 109 and 195 mm Hg, respectively). Rb⁺ is accepted in the place of K⁺ by both the Na⁺-K⁺ pump and the furosemide-sensitive system, the two transport systems being saturated to about 50% and 30% with Rb⁺ at 2 mM external Rb⁺, respectively. In the erythrocytes of the DOC-salt rats, Rb⁺ uptake by the Na⁺-K⁺ pump (difference between curves a and b) and the furosemide-sensitive system (difference between curves b and c) proceeded faster, and the Rb⁺ uptake in the presence of ouabain plus furosemide (curve c), which is attributed to the Rb⁺ (K⁺) leak, was slightly accelerated.

Furosemide-Sensitive Sodium and Potassium Transport

Figure 2 summarizes the individual and mean values for the furosemide-sensitive Rb⁺ uptake determined on a total of 69 rats (only data obtained after 1 hour of incubation are considered). Within the control Sprague-Dawley group, a marked interindividual variability in Rb⁺ uptake is seen, the extreme values differing by a factor of about four. Addition of 1% NaCl to the drinking water for 5 weeks did not alter the transport rate. A 5-week treatment with DOC increased the furosemide-sensitive Rb⁺ uptake by 70% (fig. 2) and the mean blood pressure by 24 mm Hg (table 1). In the DOC-salt animals, mean blood pressure was elevated to 160 mm Hg (table 1), and the mean Rb⁺ uptake was tripled. There is a considerable scattering in the data of the DOC-salt animals, the fastest specimen showing a sevenfold increase above the mean control value (fig. 2).

In two-kidney, one clip hypertensive rats, the mean blood pressure was elevated by 42 mm Hg (table 1), and the furosemide-sensitive Rb⁺ uptake was not significantly different from that found in the sham-operated control group (fig. 2). The small number of animals studied, however, allows no definite conclusion as to whether there is a trend for Rb⁺ uptake to be elevated in two-kidney, one clip hypertension (fig. 2). In addition, the situation seen 10 to 20 days after clipping may well differ from that observed after prolonged duration of renal hypertension.3

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The WKY rats showed a slightly slower furosemide-sensitive Rb⁺ uptake than Sprague-Dawley rats. In the spontaneously hypertensive strain, the Rb⁺ uptake was elevated by about 50% (p < 0.001). Whether the lower scattering in the furosemide-sensitive Rb⁺ uptake of the WKY rats as compared to Sprague-Dawley rats is related to the high inbreeding of the WKY strain, remains to be investigated.

There was no relation between blood pressure and furosemide-sensitive Rb⁺ uptake within each of the four hypertensive groups depicted in figure 2.

Furosemide also affected the transport of Na⁺. After 75 minutes of incubation with ouabain in Na⁺ media (15 minutes without plus 60 min with 2 mM Rb⁺), the Na⁺ content of erythrocytes from control rats was lower by 0.82 μmoles·ml cells⁻¹ in the presence of the drug. The reduction in cell Na⁺ due to furosemide was approximately 60% as great in DOC-salt hypertension (table 2).

It is to be noted that furosemide-sensitive Rb⁺ uptake reflects unidirectional inward transport, whereas the changes in Na⁺ content due to furosemide depicted in table 2 are the net result of bidirectional inward and outward movements of Na⁺. It is an open question whether the unidirectional Na⁺ movements are accelerated to a similar extent as furosemide-sensitive Rb⁺ uptake in the erythrocytes from DOC-salt hypertensive rats. Accordingly, the observation of a reduction of furosemide-sensitive Na⁺ net uptake in DOC-salt hypertension with a concomitant acceleration of furosemide-sensitive Rb⁺ uptake does not necessarily damage the assumption that the furosemide-sensitive movements of Na⁺ and K⁺ (Rb⁺) are mediated by the same system. The reduced furosemide-sensitive Na⁺ net uptake in the erythrocytes from DOC-salt hypertensive rats can easily be interpreted as arising from a lowering of inward driving force resulting from the elevated Na⁺ content in these cells.

### Na⁺-K⁺ Pump

Rb⁺ uptake by the Na⁺-K⁺ pump was significantly elevated in all groups of hypertensive rats (table 2). The accelerations of Rb⁺ transport were associated with an elevated red cell Na⁺ content in the DOC and DOC-salt rats, but not in the two-kidney, one clip and the spontaneously hypertensive animals (table 1).

Ouabain-sensitive Na⁺ net extrusion was enhanced in the two groups treated with DOC and in the two-kidney, one clip group, but not in the spontaneously hypertensive WKY rats (table 2).

### Sodium and Potassium Leak

The Na⁺ and K⁺ leaks were assessed by the inward leakage of Na⁺ and Rb⁺ in the presence of ouabain plus furosemide. The Na⁺ leak was increased in the groups treated with DOC or with DOC plus salt and in the two-kidney, one clip rats (table 2). The K⁺ leak was significantly enhanced in the DOC-salt and spontaneously hypertensive rats (table 2). The dissimilar accelerations in Na⁺ and Rb⁺ leakage in the various models of hypertension may indicate that the leak pathways for the two ions are different.

### Action of DOC In Vitro

Deoxycorticosterone, when added to the incubation media as the water soluble glycoside derivative (concentration range between 10⁻¹⁰ and 10⁻⁵ M, 15 minutes of preincubation) did not affect Rb⁺ or Na⁺ net movements as determined over 1 hour in the standard assay with erythrocytes of control Sprague-Dawley rats.
Changes Due to Dietary Potassium Deficiency

To examine whether the increases in transport rates seen in the animals treated with DOC or with DOC plus salt are somehow causally related to the lowering of plasma K⁺, red cells were studied from rats depleted of K⁺ by a K⁺-deficient diet of 4 weeks’ duration. The dietary K⁺ deficiency reduced plasma K⁺ from the control value of 3.79 ± 0.27 (n = 6) to 1.71 ± 0.20 mM. The dietary K⁺ deficiency reduced plasma K⁺ from the control value of 3.79 ± 0.27 (n = 6) to 1.71 ± 0.20 mM. The K⁺ deficiency reduced plasma K⁺ from the control value of 3.79 ± 0.27 (n = 6) to 1.71 ± 0.20 mM.

Rb⁺ uptake and Na⁺ extrusion were increased by 50% and 80%, respectively. Cell Na⁺ was elevated by 50% while cell K⁺ was reduced from 105 to 78 μmoles/ml cells⁻¹ (fig. 3). The values for the MCHC were 5.20 ± 0.06 and 5.88 ± 0.19 (± SD) in control and K⁺-deficient rats, respectively, as though the cells were shrunken by about 12% in K⁺ deficiency. The Rb⁺ leak amounted to 0.24 and 0.26 μmoles/ml cells⁻¹ hr⁻¹ in the control and K⁺-deficient cells, the difference being not statistically significant. The changes in cellular ion content and transport are thus similar, but generally much more pronounced in severe K⁺ deficiency than those seen in the more moderate hypokalemias of the DOC and DOC-salt hypertensive rats (compare figure 3 with tables 1 and 2).

Furosemide-sensitive Rb⁺ uptake was related to the MCHC of the K⁺-deficient and control rats as depicted in figure 4. The uptake rate rose the more, the higher the MCHC was elevated in vivo. A greater than 10-fold acceleration in furosemide-sensitive Rb⁺ uptake accompanies an in vivo increase of the MCHC from 5.1 to 6.1 μmoles Hb₂/ml cells⁻¹. The data obtained with the DOC and DOC-salt hypertensive rats fit well into the relation in figure 4.

Effect of Cell Shrinkage In Vitro

Figure 5 shows ouabain-resistant Rb⁺ uptake as affected by furosemide and cell volume in erythrocytes from normal rats. The cell volume was osmotically altered in vitro by adding increasing amounts of the impermeant solute sucrose to a 100 mM NaCl medium. Total ouabain-resistant Rb⁺ uptake rose several-fold upon cell shrinkage in hypertonic media, the increase being entirely caused by an acceleration of furosemide-sensitive Rb⁺ uptake. A minimum of furosemide-sensitive Rb⁺ uptake is seen at the normal cell volume. Osmotic swelling also increased the furosemide-sensitive Rb⁺ uptake, but to a lesser extent than cell shrinkage.

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By comparing the curves for furosemide-sensitive Rb⁺ uptake in figures 4 and 5 it becomes apparent that the response of the transport system to cell shrinkage is qualitatively similar, regardless of whether the water loss is caused in vivo or in vitro by either a loss of osmotically active particles from the cells or an increase of the medium osmolality.

Discussion

In the present study, an attempt was made to identify those transport systems responsible for the changes of red cell Na⁺ and K⁺ content and transport occurring in some models of experimental hypertension of the rat. Except for the K⁺ channel activated by internal Ca²⁺, and the anionic Na⁺ transport induced by bicarbonate, all of the transport systems known to be involved in the total Na⁺ and K⁺ movements across the rat erythrocyte membrane were investigated. These include the Na⁺-K⁺ pump, a furosemide-sensitive Na⁺-K⁺ transport system and the residual Na⁺ and K⁺ leak(s). The Na⁺ and K⁺ movements occurring in red cells with both the Na⁺-K⁺ pump and the furosemide-sensitive system blocked are operationally defined here to proceed through the residual leak(s).
Na⁺-K⁺ Pump and Residual Leak(s)

In accordance with previous results of other authors, 13, 14, 17, 18 we observed an elevated red cell Na⁺ content, an accelerated Na⁺ extrusion, and K⁺ (Rb⁺) uptake by the Na⁺-K⁺ pump, and an increased Na⁺ downhill leakage in erythrocytes of DOC, DOC-salt, and two-kidney, one clip hypertensive rats (tables 1 and 2). The primary alteration is probably the enhanced Na⁺ leakage, causing an increase in red cell Na⁺ and a consecutive stimulation of the Na⁺-K⁺ pump. In addition, a Na⁺-K⁺ pump inhibiting "natriuretic" factor 2₄ may possibly be a contributing cause to the increase in red cell Na⁺ in vivo. In the present study, however, the cells were washed free of plasma and the influence of a reversibly binding "endogenous Na⁺-K⁺ pump inhibitor" has thus been removed.

In mineralocorticoid hypertension, the increase in plasma HCO₃⁻ concentration leads to an additional acceleration of Na⁺ inward transport in vivo due to the passage of [NaCO₃]⁻ ion pairs through the anion exchange pathway. 3₁, 3₂ The K⁺ leak was increased in DOC-salt and spontaneously hypertensive rats (table 2). Similar alterations in Na⁺ and K⁺ transport have been observed in vascular smooth muscle. 2₃, 2₅

Plasma renin or another factor changing in parallel with renin is probably not involved in the increase of residual cation leakage, since increases in Na⁺ and/or K⁺ (Rb⁺) leaks were seen both in low renin (DOC-water and DOC-salt) and high renin Goldblatt hypertension. Rather, a passive red cell permeability for Na⁺ and/or K⁺ seems to be a phenomenon generally occurring in the experimental models of rat hypertension examined. The changes in furosemide-sensitive Rb⁺ (K⁺) uptake discussed below, in contrast, seem to develop independently of the hypertensive process in the rat.

It is unlikely that deoxycorticosterone itself caused the transport changes in DOC-salt hypertension by an acute and direct interaction with the red cell membrane, since DOC did not influence Na⁺ and K⁺ (Rb⁺) transport by the Na⁺-K⁺ pump and by the leak of rat erythrocytes in vitro. On the other hand, reports are available indicating that mineralocorticoids can alter the Na⁺-K⁺ pump and Na⁺ permeability of human, rabbit, and dog erythrocytes, both in vivo and in vitro. 3₅-3₇ However, d-aldosterone (10⁻₁² -10⁻₇ M) did not affect ouabain-sensitive Li⁺ uptake by human erythrocytes (Li⁺ replacing external K⁺) in vitro (Duhm J, unpublished results), indicating that the fourfold stimulation of Na⁺-K⁺ ATPase by physiological concentrations of aldosterone reported for human erythrocyte ghosts 3₉ is not associated with a corresponding alteration of the transport function of the Na⁺-K⁺ pump in intact human erythrocytes.

Furosemide-Sensitive Potassium Transport

The basic new finding presented in this paper is a several-fold acceleration of furosemide-sensitive, chloride-dependent K⁺ transport in DOC-salt hypertension in the rat.

The furosemide-sensitive transport system of both rat (fig. 2) and human erythrocytes 1₉, 2₃ exhibits large interindividual differences in activity. In men, the activity of the furosemide-sensitive transport system is thought to be genetically determined. 1₇, 1₉ The rate of furosemide-sensitive Rb⁺ uptake in rat erythrocytes, however, can increase up to sevenfold in vivo within 4 weeks of DOC-salt hypertension (fig. 2) (or up to 11-fold in dietary K⁺ depletion, fig. 3), demonstrating that, in the rat, parameters other than genetic contribute in determining the actual activity of the transport system.

Deoxycorticosterone probably did not activate the furosemide-sensitive transport system by a direct action on the red cell membrane, since in vitro DOC was ineffective. Furthermore, the action of DOC in vivo was quite different when it was given alone or in combination with NaCl (fig. 2). Thus, a factor other than the hormone itself is likely to be responsible for the acceleration of furosemide-sensitive K⁺ transport.

One of the possible factors could be the plasma K⁺ concentration, since the furosemide-sensitive Rb⁺ up-
take was the higher the lower the plasma K⁺ fell due to treatment with DOC or DOC plus NaCl (tables 1 and 2). Accordingly, the effect of a dietary K⁺ depletion was examined. A reduction of plasma K⁺ to 1.7 mM by dietary means was found to result in an up to 11-fold acceleration in furosemide-sensitive Rb⁺ uptake within 4 weeks (fig. 3). The question thus arises as to whether a cause-and-effect relationship or merely an accidental parallelism exists between the increase of furosemide-sensitive Rb⁺ transport in the erythrocytes and the fall in plasma K⁺.

Potential links between plasma K⁺ and the furosemide-sensitive transport system could be the red cell K⁺ content, the red cell volume and the MCHC. As the plasma K⁺ was reduced from the control value of 3.8 to 2.4 mM in DOC-salt hypertension (table 1), and to 1.7 mM in dietary K⁺ deficiency, red cell K⁺ fell from the control value of 106 to 101 in DOC-salt hypertension and to 73 μmole-ml⁻¹ in K⁺ deficiency. These values refer to a normalized red cell hemoglobin content of 5.2 μmole-ml⁻¹ cells⁻¹; the actual K⁺ contents (μmole per ml of fresh packed cells) were 103 in the normal cells, 102 in the DOC-salt animals and 88 in K⁺ deficiency. The K⁺ concentrations in red cell water were 160 mM in control erythrocytes, and 162 and 146 mM in the two experimental groups.

Reduction of red cell K⁺ in severe hypokalemia can be ascribed to an inability of the rat erythrocyte Na⁺-K⁺ pump to maintain normal cellular K⁺ levels at plasma K⁺ concentrations below 2.7 mM. Apparently, acceleration of the Na⁺-K⁺ pump rate resulting from the increase in cell Na⁺ in DOC-salt hypertension and K⁺ deficiency (tables 1 and 2, fig. 3) is insufficient to compensate for the reduced binding of K⁺ at the external aspect of the pump. Also the rise in the density of Na⁺-K⁺ pump molecules possibly occurring in chronic hypokalemia does not prevent the changes in cellular K⁺ and Na⁺ content in erythrocytes, skeletal muscle, and kidney of the rat. In addition, stimulation of the Na⁺-K⁺ pump by elevated intracellular Na⁺ has been shown to cause a loss of cellular cations and water, respectively, even in dehydrated cells. Concomitant with the fall in red cell K⁺, the cells shrink in vivo and the MCHC rose from 5.1 in the control group to 5.32 and 5.88 μmole hemoglobin tetramer per ml cells in the DOC-salt and K⁺-deficient rats.

The furosemide-sensitive Rb⁺ uptake rose sharply, regardless of whether the shrinking was due to an increase in medium osmolality induced by sucrose in vitro (fig. 5) or due to a loss of osmotically active particles in vivo (fig. 4). It is tempting to conclude, therefore, that the cell shrinkage of rat erythrocytes occurring in hypokalemic states is in fact responsible for the increase in furosemide-sensitive Rb⁺ uptake observed both in DOC-salt hypertension and nutritional K⁺ deficiency, at least to a great extent.

Acceleration of furosemide-sensitive Rb⁺ uptake in K⁺-deficient and in vitro shrunk erythrocytes was due to an increase in maximum transport rates. The basic properties of the furosemide-sensitive transport system with respect to chloride-dependence and affinity for external Rb⁺ and Na⁺ (apparent K₅₅ for external Rb⁺ and Na⁺ = 3.5 and 65 mM) remained unaltered in cells shrunk in vitro or in K⁺ deficiency (data not shown). It appears reasonable to conclude, therefore, that enhancement of furosemide-sensitive Rb⁺ uptake in DOC-salt hypertension also resulted from an increase in the maximum transport rate. The observed accelerations of Na⁺ and K⁺ transport by the Na⁺-K⁺ pump, in contrast, can easily be explained by the 50% increase in red cell Na⁺ concentration seen in DOC-salt hypertension.

However, other factors than the cell volume may contribute to the changes of the furosemide-sensitive transport system occurring in vivo. Such factors could be the hypokalemic-hypochloremic alkalosis (table 1), a production of new erythrocytes with different membrane properties, changes in red cell Ca²⁺, or alterations of the membrane of circulating erythrocytes by an unknown humoral factor.

Conclusions

One primary alteration in red cell Na⁺ and K⁺ transport occurring in erythrocytes from DOC, DOC-salt, and two-kidney, one clip hypertensive rats is an enhanced Na⁺ leakage, caused by an unknown mechanism. The accelerated Na⁺ leakage results in an increase in red cell Na⁺ with a consecutive stimulation of the Na⁺-K⁺ pump rate.

The hypokalemia associated with DOC and DOC-salt hypertension or induced by dietary K⁺ deficiency is associated with a reduction of red cell K⁺ content in the rat. The resultant loss of osmotically active particles induces an obliged water flow and a cell shrinkage, respectively. Cell shrinkage, in turn, induces a severalfold acceleration of the furosemide-sensitive Na⁺-K⁺ transport system. The mechanism of this phenomenon remains to be elucidated.

The plasma K⁺ concentration and the red cell volume (MCHC) are thus parameters that require careful consideration in all studies on pathophysiological changes of furosemide-sensitive Na⁺-K⁺ transport across the rat erythrocyte membrane. This holds also for measurements of uninhibited Na⁺ and K⁺ movements, since the furosemide-sensitive transport system of rat erythrocytes can contribute with up to 50% of the total cation movements.

A shrinking of human erythrocytes does not markedly increase furosemide-sensitive Rb⁺ uptake. Moreover, human erythrocytes do not lose K⁺ nor do they shrink during K⁺ depletion to an extent comparable to rat erythrocytes. Thus, it is not to be expected that K⁺ deficiency induces accelerations of furosemide-sensitive transport in human erythrocytes similar to those seen in rat red blood cells.

Nucleated bird erythrocytes show an increase in furosemide-sensitive K⁺ uptake upon cell shrinkage, just as reported here for rat erythrocytes. It remains an open question, however, whether the rat
and bird red cell "cotransport" systems have similar functions (e.g., volume regulation 46).

It is not unlikely that a furosemide-sensitive Na⁺-K⁺ transport system contributes to the Na⁺, K⁺, and Cl⁻ movements in vascular smooth muscle. If this were so and if an electroneutral, bidirectional transport with a strict coupling of 1 Na⁺:2 Cl⁻ is assumed, then the normal ion distribution would favor an inward Na⁺ transport in vascular smooth muscle, as calculated according to Equation (4) of Haas et al. Consequently, a defect of the furosemide-sensitive transport system would result in a fall in cellular Na⁺, and not in an elevated Na⁺ concentration as postulated by Garay et al. Rather, an acceleration of furosemide-sensitive transport such as seen here in erythrocytes would cause a rise in cell Na⁺ of vascular smooth muscle cells.

Likewise, the present observation of an increased furosemide-sensitive Rb⁺ uptake in spontaneously hypertensive Wistar Kyoto rats is difficult to reconcile with the findings of an unaltered furosemide-sensitive Na⁺ uptake 15 and a reduced Na⁺ extrusion 16 observed in spontaneously hypertensive Wistar Kyoto rats as well as with a low cotransport activity in essential hypertension.19

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