Abnormal net Na\(^+\) and K\(^+\) fluxes in erythrocytes of three varieties of genetically hypertensive rats

cell membrane ionic permeability/genetics/hypertension

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ABSTRACT Net Na\(^+\) and K\(^+\) fluxes were measured in Na\(^+\)-loaded and K\(^+\)-depleted erythrocytes of three varieties of genetically hypertensive rats. In Okamoto spontaneously hypertensive rats (4 and 10–12 weeks of age), Na\(^+\) extrusion was reduced as compared to normotensive controls (Wistar/Kyoto). Na\(^+\) extrusion was also reduced in the hypertension-prone substrain of the Hebrew University Sabra rats compared to the Na\(^+\)-resistant substrain. K\(^+\) fluxes were similar in both groups. In both Okamoto spontaneously hypertensive rats and the hypertension-prone substrain, hypertension was severe and developed rapidly. In the Lyon spontaneously hypertensive rats, in which the blood pressure elevation is less severe than in other genetically hypertensive rats, erythrocyte net Na\(^+\) extrusion was the same as in normotensive controls, but net K\(^+\) gain was slightly increased. These erythrocyte abnormalities, observed in three varieties of genetically transmitted hypertension of the rat, are in several aspects similar to those previously described in accelerated and benign human essential hypertension. Erythrocyte Na\(^+\) and K\(^+\) net flux alterations may thus represent biochemical markers of primary hypertension.

Two experimental procedures have been developed for inducing genetic hypertension in the rat. The first one consists of brother–sister inbreeding among offspring of parents with blood pressure at the upper limit of the normal range. Several strains such as the Okamoto (1), the New Zealand (2), the Milan (3), and the Lyon (4) strains were obtained accordingly. In the Okamoto strain hypertension is severe and becomes established within a few weeks. In the other strains hypertension is milder and develops more slowly. The second procedure consists of loading animals with Na\(^+\) and inbreeding individuals exhibiting the greatest elevation in blood pressure. The Dahl/Na\(^+\)-sensitive Sprague–Dawley strain (5) and the Hebrew University Wistar Sabra hypertension-prone substrain (6) were selected on this basis. In these animals, hypertension can evidently be considered as the result of both Na\(^+\) excess and genetic susceptibility.

An abnormally low ratio of Na\(^+\)/K\(^+\) net fluxes in Na\(^+\)-loaded/K\(^+\)-depleted erythrocytes of human essential hypertensives was recently demonstrated (7). The absence of this abnormality in normotensive families, as in true secondary hypertension, and its presence in some young normotensives born of hypertensive parents led us to suggest that it could be an inherited character related to hypertension.

In order to test this hypothesis, an investigation of net Na\(^+\) and K\(^+\) fluxes has been undertaken in three representative strains of genetically hypertensive rats, the Okamoto strain, the Lyon variety, and the hypertension-prone substrain derived from the Hebrew University Sabra rats.

MATERIALS AND METHODS

Rats. Male rats were used throughout this study. Okamoto spontaneously hypertensive rats (SHR) (1) and the Wistar/Kyoto normotensive controls (WK) derived from the NIH stock were supplied by Ifaa-Credo (Les Oncins, France). They were studied at 4 and 10–12 weeks of age. Systolic arterial blood pressure was recorded by tail plethysmography. Values (mm Hg; 1 mm Hg = 133 Pa) (mean ± SEM) were as follows: at 4 weeks, SHR = 135 ± 4 (n = 8), controls = 123 ± 3 (n = 9); at 10–12 weeks, SHR = 192 ± 4 (n = 9), controls = 137 ± 4 (n = 10).

Rats belonging to the 18th generation of the Lyon hypertensive (LHS) and normotensive (LNS) strains were isolated by selective inbreeding of a CFE albino strain derived from Sprague–Dawley (Ifaa-Credo) (4). They were studied at 10 weeks of age, their systolic blood pressure being: LHS = 147 ± 6 (n = 7) and LNS = 120 ± 4 (n = 8); the difference between the two values was statistically significant (P < 0.001).

Hypertension-prone (H) and sodium-resistant (N) rats were derived from the Hebrew University Sabra rats. The two substrains were obtained by brother–sister inbreeding and selected according to their respective sensitivity or resistance to deoxycorticosterone acetate/Na\(^+\)-induced hypertension (6). They were studied at 15 weeks of age. Systolic blood pressures were H = 182 ± 10 (n = 5) and N = 115 ± 4 (n = 4).

Measurement of Erythrocyte Net Na\(^+\) and K\(^+\) Fluxes. Na\(^+\) and K\(^+\) net fluxes were measured in erythrocytes loaded with Na\(^+\) and depleted from internal K\(^+\) by incubation at 4°C in a K\(^+\)-free solution containing p-chloromercuribenzenesulfonate (PCMBS) according to the procedure described by Garrahan and Rega (8).

Composition of Solutions. The following solutions were prepared. Medium 1 contained (mM): 150 NaCl, 1 MgCl\(_2\), 2.5 sodium phosphate buffer (pH 7.2 at 25°C); medium 2 contained (mM): 145 NaCl, 5 KCl, 1 MgCl\(_2\), 10 glucose, 3 phosphate buffer, 4 cysteine, 2 adenine-HCl, 5 inosine, 3 Tris (pH 7.35 at 25°C); medium 3 contained (mM): 145 NaCl, 5 KCl, 1 MgCl\(_2\), 10 glucose, 3 phosphate buffer (pH 7 at 25°C). Solution A was medium 1 containing 0.020 mM PCMBS, and solution B was medium 1 containing 0.015 mM PCMBS.

Abbreviations: PCMBS, p-chloromercuribenzenesulfonate. Rat strains are: SHR, Okamoto spontaneously hypertensive; WK, Wistar/Kyoto normotensive; LHS, Lyon hypertensive; LNS, Lyon normotensive; H, hypertension-prone Hebrew University Sabra; N, sodium-resistant Hebrew University Sabra.
Determinations of Net Fluxes. Arterial blood was sampled while the animal was under pentobarbital anaesthesia (25 mg/kg), from a catheter implanted in a carotid artery. One hundred microliters of 155 mM NaCl containing 400 international units of heparin per ml was injected intra-arterially per 100 g of animal weight. Two milliliters of blood was collected in a chilled tube containing 1 ml of the same heparin-supplemented saline. In addition, 0.5 ml of blood was withdrawn for hematocrit and hemoglobin determinations. After plasma and buffy coat had been discarded, the erythrocytes were washed three times in 150 mM NaCl. Hematocrit was measured in the final pellet. Adult rat cells were suspended in solution A at a 2.3% (vol/vol) ratio, and young rat cells in solution B at a 1.6% (vol/vol) ratio.

The erythrocytes were then agitated on a reciprocal shaker at a speed of 30 cycles per min. The loading solution was renewed after 3 hr. At the end of a 20-hr loading period, the PCMBs solutions were removed and the cells were incubated for 1 hr at 37°C in medium 2. The medium was then removed and replaced by 20 ml of medium 3. Aliquots (2 ml) were incubated 0, 1.5, 3, and 5 hr for adult rat erythrocytes, and 0, 20, 45, and 60 min for young rat erythrocytes. During incubation, cells were thoroughly mixed every 10 min. Incubation was stopped by sudden cooling at 4°C. Cells were washed three times with a cold 100 mM MgCl2 solution containing 0.1 mM ouabain and hemolyzed with 1 ml of distilled water. Ghosts were removed by centrifugation at 6000 x g for 10 min. Media 1 and 2 were kept for hemolysis estimation. Na+ and K+ were measured by flame photometry. Hemolysate and whole blood hemoglobin were determined by the cyanmethemoglobin method of Van Kampen and Zilijstra (9), and hematocrit by the capillary microhematocrit technique.

The method described in this study is similar to that used in our previous clinical studies (7). However, some modifications were found indispensable for rat erythrocytes. The cell-to-PCMBs ratio described here must be carefully respected in order to avoid unsatisfactory Na+ loading, hemolysis, or reduction of Na+ and K+ fluxes. Agitation during loading and incubation was necessary because of the fast rate of erythrocyte sedimentation. Internal ion concentrations were measured after washing the cells with MgCl2 and not with choline chloride because rat erythrocytes, unlike human erythrocytes, lose Na+ during washing with choline chloride (10).

At the end of the loading procedure, intracellular Na+ was 120–150 mmol/liter of cells, and internal K+ was 15–30 mmol/liter of cells; mean water gain was 12%, and 10% of erythrocytes were hemolyzed.

Net Na+ and K+ fluxes in erythrocytes are the resultant of various ion movements due to the different transport systems. Each of these systems shows a complex kinetic dependence on the external and internal cation concentration. In human and adult rat erythrocytes the net Na+ and K+ fluxes have a slow rate and can be approximated by linear functions. On the contrary, in young rat erythrocytes rates of net Na+ and K+ fluxes are fast and nonlinear and can be approximated by exponential functions (see Appendix).

### RESULTS

Japanese rats. In 12-week rats, net Na+ extrusion from erythrocytes is significantly lower in SHR than in WK controls (Fig. 1). No difference in net K+ influx was observed between SHR and WK rats (Table 1). Both Na+ extrusion and K+ influx were considerably faster in 4-week than in 10- to 12-week SHR and WK rats, but net Na+ extrusion and net K+ influx were significantly slower in SHR than in WK rats, as shown in Fig. 2 and Table 1.

Lyon Spontaneously Hypertensive Rats. Values of both Na+ and K+ net fluxes were lower in the Sprague-Dawley strain than in the Wistar strain. As shown in Fig. 1, no difference between net Na+ fluxes from LHS and LNS rats could be detected. However, LHS rats were characterized by a slightly but significantly faster net K+ influx than LNS rats (Table 1).

H and N Substrains of the Hebrew University Sabra Rats. Net K+ influx was similar in the two substrains (Table 1), but the rate of net Na+ extrusion from erythrocytes of the hypertension-prone H strain was slower than from erythrocytes of the sodium-resistant N rats.

Ratio of Net Na+ and K+ Fluxes. As indicated in Table 2, the ratio of net Na+ flux to net K+ flux calculated in adult hypertensive rats is significantly lower than that of their respective controls. It appears to be close to the ratio observed previously in human essential hypertension.

### DISCUSSION

The abnormalities in net Na+ and K+ fluxes observed in erythrocytes from different varieties of genetically hypertensive rats are in several respects similar to those previously described in human essential hypertension (7).

The lower rate of Na+ extrusion observed in adult Okamoto spontaneously hypertensive rats and in the hypertension-prone

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**Table 1. Net erythrocyte K+ influx (mmol/liter of cells per hr) in hypertensive rats**

<table>
<thead>
<tr>
<th></th>
<th>Japanese strain</th>
<th>Lyon strain, 10 weeks</th>
<th>Sabra strain, 15 weeks</th>
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<tbody>
<tr>
<td></td>
<td>10–12 weeks</td>
<td>4 weeks</td>
<td>10 weeks</td>
</tr>
<tr>
<td>WK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRs</td>
<td>7.19 ± 0.55</td>
<td>53.7 ± 5.7</td>
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<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>7.31 ± 0.51</td>
<td>25.9 ± 1.78**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>LNS</td>
<td>2.61 ± 0.16</td>
<td>3.09 ± 0.43*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>LHS</td>
<td>5.26 ± 0.33</td>
<td>5.52 ± 0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>5.52 ± 0.37</td>
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<td></td>
<td>(n = 4)</td>
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</tbody>
</table>

Data are shown as mean ± SEM. Numbers of animals are given in parentheses.

* P < 0.05 (LHS compared to LNS), Mann–Whitney test.

** 0.001 < P < 0.01 (SHR compared to WK), Student's t test.
substrain of the Hebrew University rats, which develop severe hypertension, can be compared to a similar abnormality previously observed in erythrocytes of sustained human essential hypertension. Furthermore, the increased K⁺ influx in the erythrocytes of the Lyon spontaneously hypertensive rats, which develop mild hypertension, is comparable to that described in benign human essential hypertension.

In young rats of the Okamoto strain, erythrocytes fluxes are considerably faster than in adults. In humans, net Na⁺ and K⁺ fluxes are not affected by age (7). In rats, other differences have been reported between young and adult erythrocytes such as different electrophoretic mobilities (12) and a higher binding capacity for β-adrenergic ligands (13) in the young rats.

No attempt was made in the present investigation to characterize the transport system responsible for the abnormal erythrocyte fluxes in various strains of genetically hypertensive rats. However, it is interesting to note that the ratio of net Na⁺ to net K⁺ flux in human and adult rat genetic hypertension is close to the stoichiometry of the Na⁺,K⁺-pump—i.e., 1.5—thus suggesting that Na⁺ and K⁺ fluxes stem predominantly from the action of the pump. In normotensive subjects born of normotensive parents, it has been recently demonstrated that internal sodium is expelled not only by the Na⁺,K⁺-pump but also by a Na⁺,K⁺-cotransport system and that it is this cotransport system that is defective in erythrocytes of essential hypertensives (11).

Several investigators have reported increased Na⁺ and Li⁺ unidirectional fluxes from erythrocytes of SHR and of H rats (14–16). Thus, all studies performed so far coincide to demonstrate a membrane abnormality in the erythrocytes of genetically hypertensive rats. However, the technique used in the present study has the advantage of amplifying a membrane defect that can ultimately result in intracellular Na⁺ retention.

If this transport defect were also present in other tissues, particularly in excitable cells, the increased intracellular Na⁺ concen could conceivably determine the changes leading to the development of high blood pressure (17).

Several findings from the present study suggest that abnormalities of Na⁺ or K⁺ fluxes are genetically associated with hypertension, because they are present in three distinct strains of genetically hypertensive rats. In addition, the presence of a reduced erythrocyte Na⁺ extrusion in young rats at a prehypertensive stage indicates that the erythrocyte abnormality is not secondary to an increase in blood pressure. Erythrocyte Na⁺ and K⁺ net flux alterations may thus represent biochemical markers of primary hypertension, particularly useful in genetic studies.

APPENDIX

Method of Net Na⁺ and K⁺ Flux Calculation in 4-Week Rats. The net Na⁺ loss can be estimated by the exponential function:

$$\frac{dN_{ai}}{dt} = k_{Na}Na_i,$$  \[1\]

in which $k_{Na}$ is the rate constant for Na⁺ extrusion and $Na_i$ is intracellular Na⁺ concentration.

The rate of K⁺ gain can be approximated by:

$$\frac{dK_i}{dt} = k_KK_0 + k'_KK_0,$$  \[2\]

where $K_i$ and $K_0$ representing intracellular and extracellular K⁺ concentrations, respectively. Then the internal concentration at the steady state $K_i(\infty)$ is:

$$K_i(\infty) = \frac{k'_K}{k_K}K_0.$$  \[3\]

The time dependence of erythrocyte Na⁺ and K⁺ contents may be approximated by the functions:

$$Na_i(t) = Na_i(0)e^{-k_{Na}t},$$  \[4\]

and

$$K_i(t) = [K_i(0) - K_i(\infty)]e^{-k_Kt} - K_i(\infty),$$  \[5\]

in which $Na_i(t)$ or $K_i(t)$ and $Na_0(0)$ or $K_0(0)$ are the Na⁺ and K⁺ concentrations at times $t$ and 0.

Eqs. 4 and 5 were transformed into:

$$\ln Na_i(t) - \ln Na_0 = -k_{Na}t$$  \[6\]

and

$$\ln K_i(t) - K_i(\infty) - K_i(0) = -k_K(t).$$  \[7\]

Using $K_i(\infty) = 115$ mmol/liter of erythrocytes, the left side of Eqs. 6 and 7 were plotted as a function of time and $k_{Na}$ and $k_K$ were obtained from the slopes.
The initial rates of net Na\(^+\) flux (\(\phi_{\text{Na}}\)) and K\(^+\) flux (\(\phi_{\text{K}}\)) were obtained using:

\[
\phi_{\text{Na}} = k_{\text{Na}}\text{Na}(0)
\]

and

\[
\phi_{\text{K}} = k'\text{K}_0,
\]
in which \(\text{K}_0\) is 4 mM.

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