Sodium Transport Kinetics in Erythrocytes from Spontaneously Hypertensive Rats

CLELIA ROSATI, PHILIPPE MEYER, AND RICARDO GARAY

SUMMARY Rat erythrocytes with five different amounts of \textit{Na}$^+\text{ content have been prepared by using a new, nondetrimental Na}$^+\text{-loading method (net NaHPO}_4^\text{- influx through the anion carrier). This method allowed the determination of 1) maximal translocation rates and apparent dissociation constants for internal Na}$^+\text{ of the Na}$^+\text{-K}^+\text{ pump, outward Na}$^+\text{-K}^+\text{ cotransport, and Na}$^+\text{-Li}^+\text{ countertransport and 2) rate constants of Na}$^+\text{ leak in erythrocytes from spontaneously hypertensive rats of the Okamoto strain and Wistar-Kyoto normotensive controls aged 2 to 26 weeks. Two major abnormalities were found in erythrocytes from spontaneously hypertensive rats: 1) a decreased cotransport affinity for internal Na}$^+\text{, which was constantly observed from 2 to 26 weeks of age (mean intracellular Na}$^+\text{ content for half-maximal stimulation of outward Na}$^+\text{-K}^+\text{ cotransport = 33.1 ± 7.0 [SD] mmol/L cells in spontaneously hypertensive rats vs 16.7 ± 4.7 mmol/L cells in Wistar-Kyoto rats; p<0.001), and 2) a decreased maximal pump rate in adult (15- to 26-week-old) spontaneously hypertensive as compared with that for age-matched Wistar-Kyoto rats (9-37 vs 34-70 mmol/L cells/hr). Therefore, the low cotransport affinity for internal Na}$^+\text{ appears to be a stable, possibly genetic defect of spontaneously hypertensive rats. Conversely, the decreased maximal pump rate may be a secondary event, possibly reflecting the appearance of endogenous pump inhibitors in the plasma of adult spontaneously hypertensive rats. (Hypertension 11: 41-48, 1988)

KEY WORDS  • ion transport • sodium transport • hypertension • membranes • erythrocyte • rats • spontaneously hypertensive rats

Epidemiological, clinical, and experimental studies have suggested that an inborn error of Na$^+$ metabolism is involved in the pathogenesis of primary hypertension.\textsuperscript{1} This hypothesis has been extensively investigated by measuring Na$^+$ content and Na$^+$ flux in circulating cells from rats and humans with primary hypertension (for a review, see Reference 2). The results were very difficult to interpret in both animals and humans. In the former, abnormal cell Na$^+$ handling was frequently observed, but the nature of the transport alteration differed markedly among different strains (see, for instance, Reference 3). In the latter, marked discrepancies characterized the results of various clinical investigations.\textsuperscript{2} Much of this variability in the data can be accounted for by methodological problems. The flux studies were performed by measuring transport activity under a fixed experimental condition, although Na$^+$ transport is a complex function of the concentrations of substrates and effectors, particularly of cell Na$^+$ content.\textsuperscript{4-6} For instance, cholesterol depletion, which increases the maximal pump rate and decreases the affinity for internal Na$^+$, leads to high, normal, or low pump activity depending on the use of Na$^+$-loaded, fresh, or Na$^+$-depleted erythrocytes, respectively.\textsuperscript{7}

The minimum kinetic description of a given erythrocyte Na$^+$ transport system thus requires the measurement of apparent affinities and maximal translocation rates for at least internal Na$^+$. The reversible increase in membrane cation permeability induced by \textit{p}-chloromercuribenzenesulfonate (PCMB)\textsuperscript{4,5,7} and nystatin\textsuperscript{8,9} in human red blood cells allowed the modification of internal Na$^+$ content [Na$^+$], in order to investigate Na$^+$ transport kinetics in erythrocytes from essential hypertensive patients. These studies revealed that at least four patterns of Na$^+$ transport abnormalities may be found in erythrocytes from different subgroups of essential hypertensive patients.\textsuperscript{10-19} For instance, the "Leak(+)" pattern (observed in about 10-30\% of hypertensive humans consists of a stable increase in
erythrocyte Na⁺ leak, which can be compensated for by increased maximal rates of the Na⁺-K⁺ pump and outward Na⁺-K⁺ cotransport. Erythrocyte Na⁺ content in Leak(+) hypertensive patients is inversely correlated to these compensatory phenomena. A kinetic description of the three other patterns of Na⁺ transport abnormalities is beyond the scope of this article (see, for instance, Reference 16). Nevertheless, the heterogeneity of the hypertensive population and the kinetic complexity of erythrocyte Na⁺ transport abnormalities suggest that the variability of the results hitherto published was due at least in part to the absence of a kinetic approach to the phenomena. In addition, Na⁺ transport in essential hypertensive patients may be affected by several other, not always well-controlled factors besides hypertension.

The complexity of these results pushed us to continue our kinetic study in a more homogeneous and better-controlled system: rats with spontaneous hypertension. Unfortunately, we were unable to change the internal cation composition of rat erythrocytes by using PCMBs and nystatin techniques without inducing an important and irreversible increase in membrane cation leak (R. Garay, unpublished data, 1986).

We recently developed a nondetrimental procedure for loading human red blood cells with Na⁺, in which Na⁺ rapidly enters the red blood cell in a fashion similar to that for an anion (NaHPO₄⁻) through the very active anion carrier. We adapted this mild Na⁺-loading method to rat erythrocytes and in the present study we measured 1) maximal translocation rates and apparent dissociation constants for internal Na⁺ of the Na⁺-K⁺ pump, outward Na⁺-K⁺ cotransport, and Na⁺-Li⁺ countertransport and 2) rate constants of Na⁺ leak in erythrocytes from spontaneously hypertensive rats of the Okamoto strain (SHR) at different ages. This study revealed that SHR have at least two abnormalities in Na⁺ transport: a consistently low cotransport affinity for internal Na⁺ and, in adults only, a decreased maximal pump rate.

Materials and Methods

Rats
Male Okamoto SHR and their normotensive controls, Wistar-Kyoto rats (WKY; derived from the original Okamoto stock and supplied by Centre d'Élevage Roger Janvier, Le Genest, France), were studied at 2 to 26 weeks of age. Values for systolic arterial blood pressure (recorded by tail plethysmography) and weights are indicated in Table 1. All rats were fed a standard diet containing 0.2% NaCl (UAR, Villemaison, France) and were given tap water to drink.

Blood Sampling and Preparation of Red Blood Cells
Arterial blood was sampled with the rats under pentobarbital anesthesia (intraperitoneal dose, 60 mg/kg) from a catheter implanted in the aorta. Whole blood was collected in heparinized tubes and centrifuged at 1750 g for 10 minutes at 4°C. The plasma and buffy coat were aspirated, and the red blood cell pellet was washed twice with 300 mM sucrose and used immediately. As about 10 to 15 ml of blood was required to run all assays on the same pooled sample, red blood cell pellets from two to 10 animals were pooled in experiments with young rats.

Na⁺-Loading Procedure
In each experiment, five aliquots of cells containing different [Na⁺], were prepared by using a slightly modified previously described method. This method is based on the stimulation of physiological NaHPO₄⁻ influx through the anion carrier. Briefly, five different aliquots of washed red blood cells were resuspended to a hematocrit of about 4% in Na⁺-loading medium and incubated for 0 (control), 20, 45, 90, and 180 minutes in a water bath at 37°C. The Na⁺-loading medium contained 100 mM NaHPO₄ and 75 mM sucrose. The suspension medium of the last aliquot of cells was renewed once after 90 minutes of incubation. The loading medium was 1) alkaline to increase the fraction of Na⁺ under the undissociated NaHPO₄⁻ form (pH was buffered by the cells, reaching a value of about 9.5–10 after 180 minutes) and 2) slightly hypertonic to prevent cell swelling.

At the end of the loading period, the cells were spun down at 1750 g for 5 minutes at 4°C and the supernatants were discarded. The red blood cells were resuspended at a hematocrit of 10% in a recovery medium (in mM) 150 NaCl, 2.5 Na⁺ phosphate buffer (pH 7.4 at 37°C), 1 MgCl₂, 10 glucose, 3 inosine, and 2 adenine. The control aliquot was incubated with a recovery medium containing (in mM) 145 NaCl, 5KCl, 2.5 Na⁺ phosphate buffer (pH 7.4 at 37°C), 1 MgCl₂, 10 glucose, 3 inosine, and 2 adenine. The final pH was adjusted to 7.4 at 37°C with Tris base. The cell suspensions were incubated at 37°C for 40 minutes (the recovery medium was renewed once after 20 minutes of incubation). At the end of this recovery step, the cells were spun down at 1750 g for 5 minutes at 4°C and the supernatants were discarded. In control experiments we verified that cell pH, inorganic phosphate content, and hemoglobin absorbance per
liter of cells (an indirect indicator of changes in cell volume) returned to normal levels. This indicated that 1) Na⁺ uptake in the alkaline phosphate media was associated with an equivalent K⁺ loss and 2) a negligible fraction of the final [Na⁺], was therefore in the NaHPO₄⁻ form.

**Measurement of Na⁺ Efflux**

Erythrocytes were washed five times with cold 110 mM MgCl₂ and resuspended in cold Mg-sucrose medium at a hematocrit of 20 to 25%. The Mg-sucrose medium contained 75 mM MgCl₂, 85 mM sucrose, 10 mM 4-morpholinopropanesulfonic acid–Tris buffer (pH 7.4 at 37°C), and 10 mM glucose (calcium is usually omitted from media used for measuring Na⁺ transport in red blood cells, because, under the effect of drugs or other experimental conditions, it can enter the cell and dramatically induce adenosine 5'-triphosphate depletion, opening of K⁺ channels, and cell shrinkage). A portion of each cell suspension was set aside to measure hematocrit, intracellular Na⁺ and K⁺ by flame photometry, and hemoglobin absorbance at 540.5 nm by spectrophotometry. The [Na⁺]₀ of unloaded cells was taken as the basal erythrocyte Na⁺ content.

Next, 0.5 ml of the red blood cell suspension was added (final hematocrit, 4–5%) to tubes containing 2 ml of cold Mg-sucrose medium as a basic constituent plus the following additions: 1) 2 mM KCl, 2) 1 mM ouabain, 3) 1 mM ouabain + 0.1 mM bumetanide, and 4) 10 mM LiCl + 1 mM ouabain + 0.1 mM bumetanide. The osmolalities were maintained at 295 ± 5 mosm. At time zero, the tubes were transferred to a 37°C water bath for further incubation. At 0 (Media 3 and 4), 12 (Medium 1), and 60 (Media 2–4) minutes, the tubes were chilled at 4°C for 1 minute and spun down for 4 minutes at 1750 g at 4°C (in control experiments we observed that fluxes were linear during such periods). External Na⁺ concentrations were measured in the supernatants in an Eppendorf flame photometer (Hamburg, FRG). Na⁺ standards (checked with commercial standards, Merck, Darmstadt, West Germany) were prepared in water and compared with those prepared in the different efflux media. In control experiments, we studied the effect of increasing doses of ouabain on Li⁺-stimulated Na⁺ efflux in fresh erythrocytes. We found that 1) in the absence of ouabain, Li⁺ stimulated a Na⁺ efflux of 150 to 200 μmol/L cells/hr and 2) this Li⁺-stimulated Na⁺ efflux was resistant to ouabain. Thus, Li⁺-stimulated Na⁺ efflux in rat erythrocytes does not appear to result from a Na⁺-Li⁺ exchange catalyzed by the Na⁺-K⁺ pump.

**Calculation of Apparent Affinity for Internal Na⁺ and Maximal Rate of Na⁺ Efflux**

Na⁺ efflux catalyzed by each studied transport system was plotted as a function of [Na⁺]₀ (see Figure 1A). Regarding the Na⁺-K⁺ pump in human red blood cells, this function can be fitted by a modified Hanes equation that is not Hill's equation (for details, see Reference 4):

\[
V_p = \frac{V_{p_{max}}}{1 + K_{p_{max}}/\left[\text{Na}^+\right]}^3
\]

where \(V_p\) represents the ouabain-sensitive Na⁺ efflux, \(V_{p_{max}}\) is the maximal rate of \(V_p\), \(K_{p_{max}}\) represents the apparent dissociation constant for \([\text{Na}^+]_o\), and \(3\) is the number of inner pump sites for \([\text{Na}^+]_o\).

Equation 2 can be rearranged as follows:

\[
\left[\text{Na}^+\right]/(V_p)^{1/3} = \frac{K_{p_{max}}/(V_{p_{max}})^{1/3} + \left[\text{Na}^+\right]/(V_{p_{max}})^{1/3}}
\]

The left side of Equation 3 was plotted against \([\text{Na}^+]_o\) (Hanes plot; see Figure 1B). \(K_{p_{max}}\) and \(V_{p_{max}}\) were obtained from the intercept with the abscissa and slope, respectively (see Reference 4 for further details).

Outward Na⁺-K⁺ cotransport (\(V_c\)) as a function of \([\text{Na}^+]_o\) also follows an equation similar to Equation 2 in human red blood cells.²¹

\[
V_c = V_{c_{max}}(1 + K_{c_{Na}}/\left[\text{Na}^+\right])^3
\]

where \(V_{c_{max}}\) is the maximal rate of bumetanide-sensitive Na⁺ efflux and \(K_{c_{Na}}\) represents the apparent dissociation constant for \([\text{Na}^+]_o\). \(V_{c_{max}}\) and \(K_{c_{Na}}\) were obtained by a similar method to the one used for the Na⁺-K⁺ pump (Hanes plot in Equation 3). The exponent 3 does not necessarily mean that the Na⁺-K⁺ cotransport system has three inner sites for Na⁺. Equation 4 only describes a phenomenological function that fits the sigmoidal dependence of outward cotransport fluxes as a function of cell Na⁺ content.

\[
K_{c_{Na}} = 3.85 K_{c_{Na}}
\]

Na⁺-Li⁺ countertransport is a Michaelis-like function of \([\text{Na}^+]_o\) in human red blood cells.⁴ It follows the equation:

\[
V_{c_{Na}} = V_{c_{max}}(1 + K_{c_{Na}}/\left[\text{Na}^+\right])
\]
where \( V_{\text{cn}} \) is the maximal rate of Li\(^+\)-stimulated Na\(^+\) efflux and \( K_{\text{cn}} \) represents the apparent dissociation constant for internal Na\(^+\). \( V_{\text{cn}} \) and \( K_{\text{cn}} \) were obtained from a Hanes plot of the data (for details, see Reference 6).

Passive Na\(^+\) permeability was obtained by dividing passive Na\(^+\) efflux from unloaded cells by \([\text{Na}^+]_i\) (similar values were obtained by calculating the slope of passive Na\(^+\) efflux as a function of \([\text{Na}^+]_i\), for the five erythrocyte Na\(^+\) concentrations).

**Statistical Analysis**

Data plotted according to Equation 3 (for the Na\(^+\)-K\(^+\) pump) and to related equations (for outward Na\(^+\)-K\(^+\) cotransport and Na\(^+\)-Li\(^+\) countertransport) were fitted by linear regression analysis (Hanes plots). Differences in means were tested by using the two-tailed Student's \( t \) test. Values are given as means ± SD.

**Results**

**Kinetic Properties of the Interaction of Internal Na\(^+\) with Na\(^+\) Transport Systems in Rat Erythrocytes**

Figure 1A shows the stimulation of Na\(^+\) transport systems by the increase in \([\text{Na}^+]_i\) in erythrocytes from normotensive Wistar rats. It can be seen that the Na\(^+\)-K\(^+\) pump and the Na\(^+\)-K\(^+\) cotransport system exhibited a sigmoidal (S-shaped) dependence on \([\text{Na}^+]_i\). Conversely, Na\(^+\)-Li\(^+\) countertransport fluxes exhibited a Michaelis-like dependence on \([\text{Na}^+]_i\). In addition, cotransport and countertransport fluxes were much lower than fluxes catalyzed by the pump (see Figure 1A).

Figure 1B shows a Hanes plot of the data. As predicted by Equations 2, 4, and 6, respectively, linear functions were obtained for fluxes catalyzed by the Na\(^+\)-K\(^+\) pump, Na\(^+\)-K\(^+\) cotransport, and Na\(^+\)-Li\(^+\) countertransport systems. The apparent dissociation constants for internal Na\(^+\) and the maximal rates of Na\(^+\) translocation for each Na\(^+\) transport system were calculated as described in Methods. On the other hand, passive Na\(^+\) efflux was a linear function of \([\text{Na}^+]_i\), further suggesting that it represents a Na\(^+\) leak (data not shown).

The influence of age on the kinetic parameters of WKY was investigated first. Figure 2 shows that the maximal pump rate (\( V_{\text{p}} \)) decreased slightly with age, a change that was at the limit of statistical significance (\( p<0.07 \)). Na\(^+\) leak also tended to decrease with age, but this change was not significant (data not shown). All other kinetic parameters were independent of age. The kinetic constants of animals of different ages were therefore pooled (with the exception of \( V_{\text{p}} \)). Table 2 shows the obtained values.

**Kinetic Properties of the Interaction of Internal Na\(^+\) with Erythrocyte Na\(^+\) Transport Systems in SHR**

The interaction of internal Na\(^+\) with the Na\(^+\)-K\(^+\) pump, Na\(^+\)-K\(^+\) cotransport, and Na\(^+\)-Li\(^+\) countertransport systems and Na\(^+\) leak were studied in erythrocytes from SHR. Maximal translocation rates and apparent dissociation constants for internal Na\(^+\) of these three Na\(^+\) transport systems and the rate constants of Na\(^+\) leak were obtained as described in Methods.

The only kinetic parameter that changed significantly with age was the maximal pump rate (\( V_{\text{p}} \)). Figure 2 shows a significant decrease of \( V_{\text{p}} \) with age in SHR erythrocytes (\( p<0.01 \)). It can be seen that \( V_{\text{p}} \) was normal (or slightly increased) in young SHR and that it tended to decrease in SHR aged 16 to 26 weeks: \( V_{\text{p}} = 28.2 ± 10.7 \) mmol/L of cells/hr in SHR vs \( 55.0 ± 14.5 \) mmol/L of cells/hr in WKY (\( n = 5 \), \( p<0.03 \)).

The kinetic constants of SHR of different ages were pooled (with the exception of \( V_{\text{p}} \)). Table 2 shows that \( K_{\text{c50}} \) was significantly increased in SHR erythrocytes. All other pooled kinetic parameters were not
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1. SODIUM TRANSPORT IN HYPERTENSION

1.1. Sodium Transport in Hypertension

1.1.1. Maximal Rate of the Na⁺-K⁺ Pump (V̇p Na⁺) in SHR and WKY at Different Ages

**Figure 2.** Maximal rate of the Na⁺-K⁺ pump (V̇p Na⁺) in SHR and WKY at different ages. The straight lines were obtained by linear regression analysis. V̇p Na⁺ decreased significantly with age in SHR (p < 0.01, r = 0.77). A less important decrease was found in WKY (p < 0.07, r = 0.47).

1.1.2. Apparent Dissociation Constant of the Na⁺-K⁺ Cotransport System for Internal Na⁺ in Erythrocytes (Kc Na⁺) from SHR and WKY at Different Ages

**Figure 3.** Apparent dissociation constant of the Na⁺-K⁺ cotransport system for internal Na⁺ in erythrocytes (Kc Na⁺) from SHR and WKY at different ages.

1.1.3. Stimulation of Outward Na⁺-K⁺ Cotransport Fluxes by Increases in Na⁺ Content of Erythrocytes from SHR Aged 15 Days

**Figure 4.** Stimulation of outward Na⁺-K⁺ cotransport fluxes by increases in Na⁺ content of erythrocytes from SHR aged 15 days. A decreased apparent affinity of the Na⁺-K⁺ cotransport system for internal Na⁺ was present before the development of high blood pressure.

1.2. Basal Erythrocyte Na⁺ and K⁺ Contents

Basal erythrocyte Na⁺ content was slightly but significantly increased in SHR erythrocytes (4.64 ± 1.38 mmol/L of cells [n = 17] vs 3.78 ± 0.75 mmol/L of cells [n = 17] in WKY; p < 0.05). Conversely, basal erythrocyte K⁺ content was within normal limits (114.7 ± 11.4 mmol/L of cells [n = 17] in SHR vs 113.4 ± 12.2 mmol/L of cells [n = 17] in WKY [NS]).

**Table 2.** Kinetic Constants of Na⁺ Transport Systems in Erythrocytes from SHR

<table>
<thead>
<tr>
<th>Rat</th>
<th>Kp Na⁺ (mmol/L cells)</th>
<th>Kc Na⁺ (mmol/L cells)</th>
<th>V̇p max (mmol/L cells/hr)</th>
<th>Kc Na⁺ (mmol/L cells)</th>
<th>V̇c max (mmol/L cells/hr)</th>
<th>pP Na⁺ (hr⁻¹×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>8.2 ± 1.9 (16)</td>
<td>16.7 ± 4.7 (17)</td>
<td>1.86 ± 0.99 (17)</td>
<td>13.8 ± 3.6 (11)</td>
<td>1.16 ± 0.42 (11)</td>
<td>115 ± 59 (18)</td>
</tr>
<tr>
<td>SHR</td>
<td>7.4 ± 2.2 (14)</td>
<td>33.1 ± 7.0 (14)*</td>
<td>2.04 ± 0.95 (14)</td>
<td>15.9 ± 5.0 (10)</td>
<td>1.39 ± 0.43 (10)</td>
<td>142 ± 56 (18)</td>
</tr>
</tbody>
</table>

Values are means ± SD. Number of rats is shown in parentheses.

Kp Na⁺ = apparent dissociation constant of the pump for internal Na⁺ content; Kc Na⁺ = internal Na⁺ content required for half-maximal stimulation of outward Na⁺-K⁺ cotransport; V̇p max = maximal rate of bumetanide-sensitive Na⁺ efflux; Kc Na⁺ = apparent dissociation constant of countertransport for internal Na⁺; V̇c max = maximal rate of Li⁺-stimulated Na⁺ efflux; pP Na⁺ = passive Na⁺ permeability.

* p < 0.001, compared with values for WKY (by two-tailed Student’s t test).

Discussion

Rat erythrocytes with five different amounts of Na⁺ content were prepared by using a new Na⁺-loading method (net NaHPO₄⁻ influx through the anion carrier). Na⁺ efflux catalyzed by the Na⁺-K⁺ pump, Na⁺-K⁺ cotransport system, and Na⁺-Li⁺ countertransport as a function of [Na⁺], in rat erythrocytes had qualitative kinetic aspects very similar to those previously
observed in human red blood cells. In addition, the numerical values of the apparent affinities for internal Na⁺ of these three Na⁺ transport systems were also similar to those values of human erythrocytes. Conversely, the maximal translocation rates and Na⁺ leak were almost one order of magnitude higher than those found in human red blood cells. These differences may be related to the fact that rat erythrocytes are smaller and thus the surface area per liter of cells is higher.

The study of Na⁺ transport kinetics in SHR erythrocytes required about 705 flux determinations (a number that can be compared with the 40–100 flux determinations of studies conducted previously on transport activity, most of which are reviewed in Reference 2). This kinetic study revealed a significant abnormality in only two of the seven parameters studied: 1) the apparent affinity of the Na⁺-K⁺ cotransport system for internal Na⁺, which was constantly decreased in young and adult SHR erythrocytes, and 2) the maximal pump rate, which was only decreased in adult SHR.

The apparent affinity of the Na⁺-K⁺ cotransport system for internal Na⁺ depends, among other factors, on internal K⁺. However, internal K⁺ does not appear to be responsible for the cotransport abnormality of SHR because 1) erythrocyte K⁺ content in unloaded SHR erythrocytes was within normal limits and 2) in human red blood cells the inner K⁺ sites are saturated with very small amounts of internal K⁺ (Kc,0.25 for internal K⁺ of about 4 mmol/L of cells). Conversely, the cotransport abnormality of SHR may reflect a conformational change in the translocating inner Na⁺ sites. This conformational change could result from 1) a genetic change in the primary structure of the cotransport protein (amino acid composition of the active sites or of the regions involved in conformational changes), 2) a change in the proteolipid environment, or 3) a change in some regulatory mechanism, such as a cyclic adenosine 3’,5’-monophosphate–dependent protein kinase.

The maximal rate of outward Na⁺-K⁺ cotransport in SHR was within normal limits. However, this result was obtained under the following kinetic conditions: 1) initial rate of bumetanide-sensitive Na⁺ efflux, 2) stationary state for internal Na⁺ and K⁺ contents, and 3) absence of the products of the reaction (external Na⁺ and K⁺). Under physiological conditions about half of the cotransport units have the external sites occupied because 1) erythrocyte K⁺ content in unloaded SHR erythrocytes was within normal limits and 2) in human red blood cells the inner K⁺ sites are saturated with very small amounts of internal K⁺ (Kc,0.25 for internal K⁺ of about 4 mmol/L of cells). Conversely, the cotransport abnormality of SHR may reflect a conformational change in the translocating inner Na⁺ sites. This conformational change could result from 1) a genetic change in the primary structure of the cotransport protein (amino acid composition of the active sites or of the regions involved in conformational changes), 2) a change in the proteolipid environment, or 3) a change in some regulatory mechanism, such as a cyclic adenosine 3’,5’-monophosphate–dependent protein kinase.

The physiological role of the Na⁺-K⁺ cotransport system in nonepithelial cells is illustrated by recent results obtained in mouse macrophages. The Na⁺-K⁺ cotransport system in these cells exhibits the following characteristics: 1) The K⁺ to Na⁺ stoichiometry is about 2 or 3 to 1 at physiological cell Na⁺ content and tends to be 1 to 1 in Na⁺-loaded macrophages; 2) outward and inward cotransport fluxes are almost equal at physiological cell Na⁺ content; and 3) increases in cell Na⁺ content are counterbalanced by a strong stimulation of net outward cotransport fluxes. These results suggest that the Na⁺-K⁺ cotransport system should not perform any relevant function under physiological conditions because an outward Cl⁻ efflux threefold to fourfold higher than the Na⁺ efflux may provide the energy required to remain at equilibrium. Conversely, any increase in cell Na⁺ content above physiological levels may be rapidly extruded by the Na⁺-K⁺ cotransport system (and by the Na⁺-K⁺ pump) if a normal affinity for internal Na⁺ ensures a strong stimulation of Na⁺ efflux by slight increases in physiological Na⁺ content (see Figure 4). A Co(−) defect in nonepithelial cells therefore may be translated into an abnormal homeostasis of any increase in cell Na⁺ content, thus resulting in temporary Na⁺ retention by the cell. Interestingly, temporary Na⁺ retention has been found in SHR erythrocytes under an in vivo Na⁺ load. A Co(−) defect in noradrenergic neurons may seriously disturb cell function. Indeed, temporary Na⁺ retention in noradrenergic endings after an action potential may provoke increased noradrenergic activity through inhibition of norepinephrine reuptake. This action may explain the increased noradrenergic activity of Co(−) hypertensive patients and SHR.

Preliminary results from our laboratory indicate that the Na⁺-K⁺ cotransport system in hypertensive rats of the Milan strain does not have a decreased apparent affinity for internal Na⁺ (P. Ferrari, P. Hannaeert, R. Garay, unpublished data, 1987). This finding suggests that our results in SHR cannot be directly extrapolated to other experimental models of spontaneous hypertension.

We have previously found that a subgroup of essential hypertensive patients had the same abnormality as SHR: a decreased apparent affinity of the Na⁺-K⁺ cotransport system for internal Na⁺ (Co(−)). This Co(−) defect may not affect red blood cell function because the Na⁺-K⁺ cotransport system catalyzes small fluxes in these cells and because basal erythrocyte Na⁺ content depends on the equilibrium between Na⁺ extrusion by the pump and Na⁺ entry by membrane leak. The slight increase in basal erythrocyte Na⁺ content of SHR erythrocytes therefore appears to reflect the decreased maximal pump rate of the adult SHR. However, the situation could be different in other nonepithelial cells with higher cotransport fluxes or in Na⁺-loaded cells.

The study of Na⁺ transport kinetics in SHR erythrocytes was obtained under the following kinetic conditions: 1) The K⁺ to Na⁺ stoichiometry is about 2 or 3 to 1 at physiological cell Na⁺ content and tends to be 1 to 1 in Na⁺-loaded macrophages; 2) outward and inward cotransport fluxes are almost equal at physiological cell Na⁺ content; and 3) increases in cell Na⁺ content are counterbalanced by a strong stimulation of net outward cotransport fluxes. These results suggest that the Na⁺-K⁺ cotransport system should not perform any relevant function under physiological conditions because an outward Cl⁻ efflux threefold to fourfold higher than the Na⁺ efflux may provide the energy required to remain at equilibrium. Conversely, any increase in cell Na⁺ content above physiological levels may be rapidly extruded by the Na⁺-K⁺ cotransport system (and by the Na⁺-K⁺ pump) if a normal affinity for internal Na⁺ ensures a strong stimulation of Na⁺ efflux by slight increases in physiological Na⁺ content (see Figure 4). A Co(−) defect in nonepithelial cells therefore may be translated into an abnormal homeostasis of any increase in cell Na⁺ content, thus resulting in temporary Na⁺ retention by the cell. Interestingly, temporary Na⁺ retention has been found in SHR erythrocytes under an in vivo Na⁺ load. A Co(−) defect in noradrenergic neurons may seriously disturb cell function. Indeed, temporary Na⁺ retention in noradrenergic endings after an action potential may provoke increased noradrenergic activity through inhibition of norepinephrine reuptake. This action may explain the increased noradrenergic activity of Co(−) hypertensive patients and SHR.

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A second abnormality of SHR erythrocytes was a decreased maximal pump rate only in the adult SHR. Unfortunately, rat erythrocytes have a low apparent affinity for ouabain, and this hampered a systematic study of ouabain binding to distinguish if the decreased maximal pump rate results from a decrease in the number of transport units or in the turnover rate of cation translocation, or in both. The decreased maximal pump rate may reflect the appearance of endogenous pump inhibitors in plasma of adult SHR. These endogenous factors have been found to be increased in some hypertensive patients (for a review, see References 28, 29), in some forms of experimental hypertension, and recently, in SHR. Interestingly, the development of high blood pressure in SHR was correlated with a progressive decrease in maximal pump rate in erythrocytes. This finding agrees with the hypothesis of others suggesting that the onset of hypertension may arise from pump inhibition in vascular smooth muscle cells through an increase in basal cell Na⁺ content or partial membrane depolarization, or both.

DeWardener and MacGregor postulated that the causal abnormality of primary hypertension was increased renal Na⁺ reabsorption, leading to a compensatory secretion of endogenous (natriuretic) pump inhibitors. On the other hand, catecholamines decrease renal Na⁺ excretion. Thus, increased noradrenergic activity due to a Co(2+) defect in renal noradrenergic endings may be the primary defect in SHR, and increased vascular contractility caused by pump inhibition in the vascular wall may be the final consequence.

In conclusion, SHR erythrocytes are characterized by the presence of 1) a stable decrease in the apparent affinity of the Na⁺,K⁺ cotransport system for internal Na⁺ and 2) a progressive decrease of maximal pump rate in adult animals. Similar membrane transport defects in kidney and vascular wall of SHR may be in- volved in the pathogenesis of high blood pressure.

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Appendix: Calculation of $K_c^{50%}$
Outward $\text{Na}^+-\text{K}^+$ cotransport reaches half-maximal rate at an internal $\text{Na}^+$ content equal to $K_c^{50%}$.

\[ \frac{V_c}{V_{c_{\text{max}}}} = 0.5 = 1/(1 + \frac{K_c^{50%}}{K_c^{50%}}) \]

and

\[ K_c^{50%} = 3.85 \cdot K_{C_N}^{\text{Na}} \]

where $K_{C_N}^{\text{Na}}$ is the internal $\text{Na}^+$ content required for half-maximal stimulation of outward $\text{Na}^+-\text{K}^+$ cotransport, $V_c$ is the bumetanide-sensitive $\text{Na}^+$ efflux, $V_{c_{\text{max}}}$ is the maximal rate of bumetanide-sensitive $\text{Na}^+$ efflux, and $K_{C_N}^{\text{Na}}$ is the apparent dissociation constant for internal $\text{Na}^+$. 

\[ (K_c^{50%}/K_c^{50%}) = (2)^{0.5} - 1 \]
Sodium transport kinetics in erythrocytes from spontaneously hypertensive rats.
C Rosati, P Meyer and R Garay

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