Kinetic Aspects of Red Blood Cell Sodium Transport Systems in Essential Hypertension

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SUMMARY A kinetic study of the interaction of internal sodium with four different erythrocyte sodium transport pathways (ouabain-sensitive Na⁺-K⁺ pump, bumetanide-sensitive Na⁺-K⁺ cotransport system, Na⁺-Li⁺ countertransport, and Na⁺ leak) has facilitated the distinction of the following subgroups of patients with essential hypertension: 1) Leak (+), exhibiting increased passive sodium permeability; 2) Co (-), showing low apparent affinity of the Na⁺-K⁺ cotransport system for internal sodium; 3) Counter (+), characterized by increased maximal rates of Na⁺-Li⁺ countertransport; and 4) Pump (-), characterized by an abnormally low apparent affinity of the Na⁺-K⁺ pump for internal sodium. We present here a new and simple sodium-loading method that allows a simultaneous kinetic study of the above abnormalities. The use of this kinetic assay may improve estimation of the frequencies, clinical features, and other properties of each subgroup of hypertensive patients in different populations. (Hypertension 10 [Suppl I]: I-11–I-14, 1987)

KEY WORDS • hypertension • erythrocytes • biological transport • sodium transport • sodium permeability

EPIDEMILOGICAL, clinical, and experimental evidence led Dahl and others to postulate that one of the factors involved in the pathogenesis of essential hypertension was an "inborn error of sodium metabolism." This likely implies the presence of inherited defects in sodium transport proteins, some of which exist in human red blood cells (Figure 1).

As early as 1960 Losse et al. reported that sodium content tended to be slightly increased in erythrocytes of patients with essential hypertension. Later, the presence of such abnormality was clearly established by Wessels and Zumkley, who studied almost 300 hypertensive patients and found a statistically significant 12% increase in mean sodium content with a large overlap between normotensive and hypertensive persons.

The increased erythrocyte sodium content was suspected to derive from abnormal transport of sodium across the cell membrane. In 1979 we proposed that the abnormal transport could be a useful marker for detecting subjects with a genetic predisposition to develop hypertension and for distinguishing between primary and secondary hypertension.

The discovery of new sodium transport systems in erythrocytes and the development of simple assays useful for large-scale studies opened up the search for the "inborn error of sodium metabolism" at a molecular level (see Figure 1). Generally speaking, these studies were based on the belief that (1) transport proteins in disease can be explored like enzymes, that is, by measuring the activity of one or more sodium transport systems at some fixed experimental condition, and (2) the hypertensive population as a whole was characterized by some unique abnormality in transport activity.

In 1980 we reported that erythrocytes from patients with essential hypertension and some of their normotensive offspring exhibited abnormally low outward Na⁺-K⁺ cotransport fluxes. At the same time, Canessa et al. reported increased maximal rates of Na⁺-Li⁺ countertransport. The potential clinical, genetic, and epidemiological implications of these findings strongly encouraged several investigators to measure cotransport and countertransport fluxes (or any other erythrocyte transport pathway). The obtained results varied from laboratory to laboratory and can be summarized as follows.

First, depending on the experimental conditions fixed for the assay and on the hypertensive patients in consideration, fluxes catalyzed by the Na⁺-K⁺ cotransport system were reported to be low, normal, or high, and those catalyzed by the Na⁺-Li⁺ counter-
transport were normal or high (see, for instance, References 9–11). On the other hand, Na⁺-K⁺ pump fluxes were reported to be decreased or increased. Second, some abnormalities were simultaneously found in some hypertensive patients (increased Na⁺-K⁺ pump and decreased Na⁺-K⁺ cotransport, increased Na⁺-K⁺ cotransport and Na⁺-Li⁺ countertransport, and others). Finally, conditions such as pregnancy, hypokalemia or hyperkalemia, and others, and taking oral contraceptives appeared to induce changes in transport activity similar to or even more marked than in red blood cell membranes.

Regarding the heterogeneity of sodium transport abnormalities, we may note that this hypothesis agreed with the classic observation that essential hypertension is a heterogeneous clinical and therapeutic entity.

Unfortunately, an experimental protocol based on the above approach required about 1000 flux determinations, which can be compared with the 40 to 100 flux determinations of previous studies of transport activity (see References 7–14). Nevertheless, it was used for investigating 1) the interaction of internal sodium with the Na⁺-K⁺ cotransport system, the Na⁺-Li⁺ pump, and Na⁺-Li⁺ countertransport and 2) the sodium leak in large numbers of hypertensive patients. These kinetic studies revealed that erythrocytes from persons with essential hypertension could present one or more of four stable sodium transport abnormalities:

1. Increased passive entry of sodium (Leak (+)). This first sodium transport abnormality was suspected by Wessels et al. in 1967 after measuring unidirectional Na⁺ influx, and recently confirmed by more specific studies in about 15 to 30% of hypertensive humans. A Leak (+) abnormality may be compensated by an increase in the maximal rate of the Na⁺-K⁺ pump and the Na⁺-K⁺ cotransport system. Indeed, internal sodium contents (and blood pressure levels) in Leak (+) hypertensive patients are inversely correlated to these compensatory phenomena.

2. Decreased apparent affinity of the Na⁺-K⁺ cotransport system for internal sodium (Co [−]). About 30 to 40% of hypertensive humans exhibit a decreased apparent affinity of the Na⁺-K⁺ cotransport system for internal sodium. This results in decreased ability of the Na⁺-K⁺ cotransport system to extrude a cell sodium load. The maximal rate of the Na⁺-K⁺ cotransport system may be decreased, normal, or increased as a function of the severity of high blood pressure and several other factors. In addition, most Co (+) hypertensive patients exhibit a normal interaction with external potassium.

3. Increased maximal rate of Na⁺-Li⁺ countertransport (Counter [+]). First described by Canessa et al. in 1980, this abnormality is present in about 20 to 40% of hypertensive humans and is frequently associated with an increased maximal rate of outward Na⁺-K⁺ cotransport.

FIGURE 1. Sodium transport systems in human red blood cells and kidney. Three erythrocyte sodium transport systems (the ouabain-sensitive Na⁺-K⁺ pump, the furosemide- or bumetanide-sensitive Na⁺-K⁺ cotransport system, and the Na⁺-Na⁺ countertransport system) appear to be representative of those present in different segments of the nephron (the Na⁺-K⁺ pump of basolateral membranes, the Na⁺-K⁺ cotransport system of the luminal side of Henle’s loop, and the Na⁺-H⁺ exchange of the luminal side of the proximal tubule, which appears to be the same as the erythrocyte Na⁺-Na⁺ countertransport). Conversely, the amiloride-sensitive sodium channel of distal and collecting tubules does not appear to be represented in human red blood cell membranes. It is important to note that some other sodium transport systems, such as Na⁺-Ca²⁺ exchange, that are present in other cells appear to be lacking in human red blood cell membranes.

Transport kinetics is much more complicated than enzyme kinetics because of the numerous substrates and effectors acting in the different compartments. One can therefore conceive a profound effect on a transport system leading to low, normal, or high transport activity depending on the experimental condition. For instance, cholesterol depletion (which increases maximal pump rate and decreases affinity for internal sodium) is translated in high, normal, or low pump activity depending upon the use of sodium-loaded, fresh, or sodium-depleted erythrocytes, respectively. Sodium transport activity is therefore a very complicated function of several variables and, in particular, of cell sodium content. A minimum kinetic description of a given erythrocyte sodium transport system thus requires the measurement of apparent affinities and maximal translocation rates for at least internal sodium.
4. Decreased apparent affinity of the Na\(^{+}\)-K\(^{+}\) pump for internal sodium (Pump [—]). We recently observed a fourth stable sodium transport abnormality, decreased apparent affinity of the Na\(^{+}\)-K\(^{+}\) pump for internal sodium in about 5 to 10% of hypertensive humans.\(^{19}\) This abnormality appears to be compensated by an increase in the maximal rates of the Na\(^{+}\)-K\(^{+}\) pump and Na\(^{+}\)-K\(^{+}\) cotransport system.

Kinetic studies of red blood cell sodium transport may have future genetic, clinical, and epidemiological applications. We thus tried to facilitate these investigations by developing a simple assay that allows the simultaneous measurement of the apparent affinities for internal sodium and maximal rates of the Na\(^{+}\)-K\(^{+}\) pump, the Na\(^{+}\)-K\(^{+}\) cotransport system, and Na\(^{+}\)-Li\(^{+}\) countertransport; and the sodium leak in human erythrocytes.

Methods
Preparation of Red Blood Cells
Venous blood, collected in heparinized tubes, was centrifuged at 1750 \(g\) for 10 minutes at 4°C, and the plasma and buffy coat were removed. The cell pellet was divided into two aliquots, one washed twice with 110 mM MgCl\(_2\), and the other washed twice with 300 mM sucrose. These aliquots were resuspended in the same washing solution at a hematocrit of about 50%.

Variation in Erythrocyte Sodium Content
We and other investigators previously changed erythrocyte cation composition by using mercurials, ionophores, and other nonphysiological chemicals. We thus decided to develop here a less harmful sodium-loading method, based on the stimulation of the physiological NaHPO\(_4^{-}\} influx through the anion carrier.

For each studied subject, five aliquots of cells containing different sodium contents were prepared. The red blood cell pellet washed with MgCl\(_2\) was kept in the cold and used as a reference sample (fresh cells). The red blood cell pellet washed with 300 mM sucrose was divided into four aliquots, which were added to different loading media at a final hematocrit of 4%. Depending on the desired internal cation composition, the different loading media were prepared by mixing suitable proportions of sodium medium and potassium medium. These media contained (in mM): 100 X\(_2\)HPO\(_4^{-}\) and 75 sucrose (where X = Na\(^{+}\) or K\(^{+}\)). The solutions were hypertonc to prevent cell swelling during the loading period. The cell suspensions were incubated for 90 minutes in a water bath at 37°C. The suspension media were renewed every 30 minutes. In control experiments we verified that the cells were loaded with an inorganic phosphate content of 18 to 22 mmol/L cells.

At the end of the loading period, the cells were spun down at 1750 \(g\) for 4 minutes at 4°C and the supernatants were discarded. The red blood cell pellets were resuspended at a hematocrit of 10% in a recovering medium containing (in mM): 150 XCl, 10 4-morpholinopropanesulfonic acid (MOPS)-Tris buffer (pH 7.4 at 37°C), 10 glucose, 10 inosine and 5 adenine (where X = Na\(^{+}\) or K\(^{+}\)). The final pH was adjusted to 7.4 at 37°C with Tris base. The cell suspensions were incubated at 37°C for 80 minutes (the recovering media were renewed once at 40 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.

Measurement of Sodium Efflux
Sodium efflux catalyzed by the different erythrocyte sodium transport systems and the sodium leak were measured by a previously published method.\(^{25}\) Briefly, each aliquot of cells (including reference fresh cells) was washed five times with cold 110 mM MgCl\(_2\), and resuspended in Mg-sucrose medium at a hematocrit of 20 to 25%. The Mg-sucrose medium contained (in mM): 75 MgCl\(_2\), 85 sucrose, 10 MOPS-Tris buffer (pH 7.4 at 37°C), and 10 glucose. A portion of each cell suspension was set aside to measure hematocrit, intracellular sodium and potassium by flame photometry, and hemoglobin absorbance at 540.5 nm by spectrophotometry.

The red blood cell suspensions (0.5 ml) were added in the cold (final hematocrit 4–5%) to tubes containing 2 ml of Mg-sucrose medium as a basic constituent plus the following additions (in mM): 2 KCl, 0.1 ouabain, 0.1 ouabain + 0.02 bumetanide, and 10 LiCl + 0.1 ouabain + 0.02 bumetanide. The osmolalities were maintained at 295 ± 5 mosm.

At \(t = 0\), the tubes were transferred to a 37°C water bath for further incubation. At times (in minutes): 0 (media 3 and 4), 30 (medium 1), 60 (medium 4), and 120 (media 2 and 3) the tubes were transferred to the cold and spun down for 4 minutes at 1750 \(g\) at 4°C (in control experiments we observed that fluxes were linear during such incubation time periods). External sodium concentrations were measured in the supernatants by using an Eppendorf flame photometer (Eppendorf Geratebau Netheler & Hinz GmbH, Hamburg, FRG). Sodium standards (checked with commercial standards, Merck, Darmstadt, FRG) were prepared in water and compared with those prepared in the different efflux media. In control experiments no evidence of red blood cell lysis could be detected during incubation in the efflux media.

The Na\(^{+}\)-K\(^{+}\) pump activity was taken as the ouabain-sensitive sodium efflux, calculated by subtracting sodium efflux in medium 2 from that in medium 1. Outward Na\(^{+}\)-K\(^{+}\) cotransport was equated to the bumetanide-sensitive sodium efflux, calculated by subtracting sodium efflux in medium 3 from that in medium 2. The Na\(^{+}\)-Li\(^{+}\) countertransport was taken as the lithium-stimulated sodium efflux (calculated by subtracting sodium efflux in medium 4 from that in medium 3). Sodium efflux in medium 3 was equated to passive sodium permeability (for further details see Reference 25).

The apparent affinities for internal sodium and the maximal translocation rates of each sodium transport
system and the sodium leak were calculated by using previously published methods.15, 19, 21

Perspectives

Investigation of red blood cell sodium transport kinetics15, 18, 19, 21 has confirmed the prediction of Canessa that the population of patients with essential hypertension is heterogeneous regarding sodium transport abnormalities.16 It is important to note, however, that these studies only represent a preliminary approach to such heterogeneity because (1) other important sodium transport systems such as $\text{Na}^+\cdot\text{H}^+$ and $\text{Na}^+\cdot\text{Ca}^{2+}$ exchanges have not yet been explored, (2) sodium transport systems may show abnormalities in the interaction with other substrates and effectors besides internal sodium, and (3) some hypertensive persons may have primary abnormalities in calcium transport or other proteins involved in the regulation of ion transport systems.

Investigation of red blood cell transport kinetics in genetically hypertensive rats may help to define to which subgroup of hypertension each strain of rats is correlated. For instance, Sabra rats of the hypertensive-prone substrain had a stable Leak (+) abnormality (which is compensated by increased maximal rates of the $\text{Na}^+\cdot\text{K}^+$ pump).26 The physiopathological aspects of hypertension in each strain of genetically hypertensive rats may be then extrapolated to each subgroup of hypertensive humans.

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