The Li⁺-Na⁺ Exchange and Na⁺-K⁺-Cl⁻ Cotransport Systems in Essential Hypertension

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SUMMARY This review examines the physiological functions of the Li⁺-Na⁺ exchanger and Na⁺-K⁺-Cl⁻ cotransport system in human red blood cells. Both transporters are familial aggregated and determined mainly by genetic factors; they are present in kidney and vascular cells, where they are regulated by vasoactive substances. To assess the physiological function of these two transporters, we investigated their kinetic and equilibrium properties, and their modulation by vasoactive substances. Recent studies in red blood cells indicate that the Li⁺-Na⁺ exchanger may be a mode of operation of the Na⁺-H⁺ exchanger, which plays an important role in the regulation of cell pH, cell volume, and transtubular sodium transport. In vascular cells, Na⁺-H⁺ exchanger is modulated by vasoconstrictors such as growth factors and angiotensin, while Na⁺-K⁺-Cl⁻ cotransport is modulated by vasodilators such as atrial natriuretic factor and bradykinin. Kinetic studies in red blood cells of hypertensive patients and their offspring indicate the presence of subsets with elevated Vmax of Li⁺-Na⁺ exchange or high Km for cell sodium for outward Na⁺-K⁺-Cl⁻ cotransport. The latter alteration is found most frequently in young blacks born of hypertensive parents, and it appears to be dependent on their level of sodium intake. The relationship between the alterations of the red blood cell sodium exchanger and Na⁺-K⁺-Cl⁻ cotransport and risk factors for hypertension indicates that they can provide a tool to examine the interaction of genetic, hormonal, and environmental factors in human hypertension.

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KEY WORDS • Li⁺-Na⁺ exchange • Na⁺-K⁺ cotransport • erythrocyte • hypertension

DURING the past several years, we have studied two transport systems in human red blood cells (RBCs) — Na⁺-Na⁺ exchange and Na⁺-K⁺-Cl⁻ cotransport — with the following goals in mind:

1. To define their physiological function and mechanism of regulation by studying their kinetic, equilibrium properties, and modes of operation.
2. To provide a tool for the study of genetic and environmental interactions in the etiology of human essential hypertension.
3. To assess whether alterations of these transporters in patients with essential hypertension may be due to a) differences in the number of transport sites; b) differences in the Km of the transport protein, which can alter their function; or c) differences in their modulation by vasoactive substances and/or growth factors.

This paper examines the properties of the Li⁺-Na⁺ exchange and the Na⁺-K⁺-Cl⁻ cotransport, their physiological regulation, and their alterations in essential hypertension.

Physiological Role of the Sodium Exchange System: Is the Li⁺-Na⁺ Exchanger a Na⁺-H⁺ Exchanger?

One of the major questions raised concerning the Na⁺-Li⁺ countertransport system is that of its purpose and physiological function. It has been described as a sodium (lithium) for sodium exchange that has defied explanation. Several sodium transport systems can operate in the Na⁺-Li⁺ exchange mode when one of the interacting ions is not present at one side of the membrane. To define the cellular function of the Li⁺-Na⁺ exchange system, it is necessary to determine whether or not it is a mode of operation of another sodium transport system. We have established that it is not a mode of operation of Na⁺-K⁺-Cl⁻ cotransport.¹

Several investigators have suggested that, in cells other than erythrocytes, this transport system may perform sodium-proton exchange.²⁻⁴ Aronson¹ proposed that the elevation of the Li⁺-Na⁺ exchange reflects a...
parallel elevation of the Na\(^{+}\)-H\(^{+}\) exchange in the kidney and higher tubular sodium reabsorption. It has emerged in recent years, however, that the Na\(^{+}\)-H\(^{+}\) exchanger is widely distributed in all cell types as a pH regulatory system; it is certainly an important route for sodium transport in vascular cells, and therefore we have no good reasons to believe that the Li\(^{+}\)-Na\(^{+}\) exchanger is only a probe of tubular sodium reabsorption. Moreover, several aspects of the Na\(^{+}\)-H\(^{+}\) exchanger, such as its modes of operation and equilibrium properties, have not yet been defined. Studies on RBCs under conditions of constant cell volume, membrane potential, and proton and sodium gradients may provide further knowledge of the normal and abnormal functioning of this exchanger in other cell types.

In recent years it has been established that Na\(^{+}\)-H\(^{+}\) exchange is a very important mechanism of pH regulation in a large variety of cell types. Furthermore, the activation of this transporter by shrinkage in some cell types indicates that it plays a role in the regulation of cell volume. Because in human RBCs, cellular pH is regulated by the activity of the anion exchanger and the concentration of nonpermeant solutes (hemoglobin and 2,3-diphosphoglycerate (DPG), the physiological role of the Na\(^{+}\)-H\(^{+}\) exchanger is not yet clear. It is likely that its residual activity in mature RBCs is a vestige of the volume-sensitive Na\(^{+}\)-H\(^{+}\) exchanger present in the erythroblast. Studies carried out in our laboratory indicate that the Na\(^{+}\)-H\(^{+}\) is a “silent” transport system in mature human RBCs at pH 7.4, and that several maneuvers can “turn it on,” such as 1) elevation of cytosolic calcium by means of the ionophore A23187; 2) decrease of cell pH by increasing the nonpermeant anions adenosine 5’-triphosphate (ATP) and 2,3-DPG; 3) acidification (pH, less than 7) of the RBC followed by 4-4’-diisothiacyano-2-2-stilbene disulphonate and acetazolamide treatment (to inhibit anion exchange and carbonic anhydrase); and incubation in alkaline medium to impose an outward proton gradient.

The pH\(_{7}\)-stimulated sodium influx (pH\(_{7}\), 6.5, Na\(^{+}\)\(_{7}\), 75 mM) is of the order of 4 to 6 mmol/L cell/hr, half of that being amiloride sensitive. The Na\(^{+}\)-H\(^{+}\) exchanger reaches V\(_{\text{max}}\) of 20 to 50 mmol/L cell/hr at pH\(_{7}\), 6, pH\(_{7}\), 8, Na\(^{+}\)\(_{7}\), 0, and Na\(^{+}\)\(_{7}\), 140 mM. An important property of the human RBC Na\(^{+}\)-H\(^{+}\) is its poor sensitivity to amiloride (IC\(_{50}\) 400 μM) and its analogues, which can only inhibit 30 to 50% of the sodium influx stimulated by an outward hydrogen gradient. Notably, when the system was activated by a rise in cytosolic calcium, the IC\(_{50}\) for amiloride was 20 μM. The Li\(^{+}\)-Na\(^{+}\) exchange is markedly inhibited by external protons (pK 6.8), but stimulated by cytosolic protons (pH\(_{7}\), <7.2). These properties are similar to those of Na\(^{+}\)-H\(^{+}\) exchange. The Na\(^{+}\)-Na\(^{+}\) exchange, however, is completely inhibited when Na\(^{+}\)-H\(^{+}\) exchange reaches maximal velocity (pH\(_{7}\), 6, pH\(_{7}\), 8, Na\(^{+}\)\(_{7}\), 15 mM, Na\(^{+}\)\(_{7}\), 140 mM) in agreement with the lower affinity for sodium than for lithium. These data support the hypothesis that, under conditions of pH equilibri-um higher than 7, the Na\(^{+}\)-H\(^{+}\) exchanger is in a different conformational state that promotes amiloride-insensitive Li\(^{+}\)-Na\(^{+}\) exchange. Studies in my laboratory on the kinetic effects of hydrogen on the Na\(^{+}\)-H\(^{+}\), Na\(^{+}\)-Na\(^{+}\), and Li\(^{+}\)-Na\(^{+}\) exchanges are being designed to establish that they are indeed a mode of operation of Na\(^{+}\)-H\(^{+}\) exchange, with normal or defective properties. The Li\(^{+}\)-Na\(^{+}\) exchange at pH greater than 7 has low capacity and high affinity for lithium, and it is insensitive to amiloride and to metabolic starvation. The Na\(^{+}\)-H\(^{+}\) exchange has a high capacity and can also transport lithium, but at a lower rate, it is insensitive to phloretin, stimulated by an increase in cytosolic calcium, and inhibited by amiloride and metabolic starvation. The Na\(^{+}\)-H\(^{+}\) exchange, Na\(^{+}\)-Li\(^{+}\) exchange, and Li\(^{+}\)-Na\(^{+}\) exchanges also operate highly asymmetrically with respect to hydrogen, sodium, and lithium ions. Cell hydrogen and lithium can activate Na\(^{+}\)-Li\(^{+}\) and Li\(^{+}\)-Na\(^{+}\) exchanges but inhibit them from the outside. External sodium and lithium ions can also activate Na\(^{+}\)-H\(^{+}\), Li\(^{+}\)-H\(^{+}\), and Na\(^{+}\)+Li\(^{+}\) exchanges.

Alterations of sodium exchange in RBCs of hypertensive subjects also raise the question of the physiological role of the Na\(^{+}\)-H\(^{+}\) exchange in vascular smooth muscle cells. To maintain cellular pH at about 7.3 in a cell with a membrane potential of ~45 mV, the vascular smooth muscle cells need a mechanism for proton efflux against an electrochemical gradient. The regulation of cell pH by an electroneutral exchange of Na\(^{+}\)-H\(^{+}\) can accomplish this task while markedly contributing to the dissipation of the sodium gradient. We have found that Na\(^{+}\)-H\(^{+}\) exchange is the main regulator of cell pH in vascular smooth muscle. Changes in cytosolic protons have profound effect on contractility because acid pH leads to vasodilation and alkaline pH to vasoconstriction. The activation of this pathway leads to an increase in sodium permeability, net sodium entry, and cellular sodium, and dissipation of the sodium gradient, which in turn may decrease calcium efflux through a Na\(^{+}\)-Ca\(^{2+}\) exchanger and influence vascular smooth muscle contractility. Further insight into the relationship between cell pH and vasoconstriction is gained by the activation of the Na\(^{+}\)-H\(^{+}\) exchange of the vascular smooth muscle by the powerful vasoconstrictor angiotensin II. The sustained activation of the Na\(^{+}\)-H\(^{+}\) exchange follows the rapid mobilization of cytosolic calcium and inositol 1,4,5-trisphosphate generation triggered by this peptide. The activation of the Na\(^{+}\)-H\(^{+}\) exchange by angiotensin in renal vasculature might play a pivotal role in the hormonal response to salt intake.

**Factors Determining RBC Li\(^{+}\)-Na\(^{+}\) Exchange**

We examine here the relationship between the alterations of this transporter and the risk factor for essential hypertension.

**Assays of Li\(^{+}\)-Na\(^{+}\) Exchange in Human RBCs**

To study transport systems in RBCs of patients with essential hypertension, the appropriate assay should 1) determine only one transport system, 2) assess a de-
fined mode of operation, 3) measure kinetic variables such as $V_{\text{max}}$ and $K_m$, and 4) demonstrate good inter-assay and intra-assay reproducibility. To fulfill all these requirements, the cation content of the cells must be varied without altering cell volume or metabolism.

The sodium exchange system can be studied measuring the heteroexchange of intracellular lithium for extracellular sodium ($\text{Li}^+\text{-Na}^+_e$ exchange) or intracellular sodium for extracellular lithium ($\text{Na}^+\text{-Li}^+_e$ exchange). We developed an assay of the maximal rate of the $\text{Li}^+\text{-Na}^+_e$ exchange system in human RBCs that takes into account its kinetic properties. The assay is very reproducible and has been useful in investigating the genetic, pathophysiological, and epidemiological aspects of essential hypertension (see Reference 13 for review). Other investigators have measured $\text{Na}^+\text{-Li}^+_o$ exchange. Duhm et al. assayed phloretin-sensitive lithium influx into fresh cells incubated in 2 mM lithium. The assay does not measure the maximal rate, and relies on the nonspecific action of phloretin and not on the stimulation of lithium influx by cell sodium to determine the exchange. Recent kinetic studies by Hannaert and Garay, however, provided evidence of the marked variations in the dissociation constant of internal sodium sites, promoting $\text{Na}^+\text{-Li}^+_e$ exchange. In this elegant kinetic study, Hannaert and Garay measured $\text{Li}^+\text{-Li}^+_o$-stimulated sodium efflux and determined the interindividual variations of the dissociation constant of internal sodium and external lithium sites. The authors concluded that the countertransport mechanism is consistent (“ping-pong”). They also demonstrated that the rate of $\text{Li}^+\text{-Na}^+_e$ exchange was three to four times higher than that of $\text{Na}^+\text{-Li}^+_e$ exchange. These results indicate that the sodium exchanger has different velocities when lithium or sodium occupies internal or external sites. The exchanger has higher affinity for lithium than for sodium at internal and external sites. A practical implication of these studies is that measurements of the $V_{\text{max}}$ of the $\text{Li}^+\text{-Na}^+_e$ exchange are therefore more precise than of $\text{Na}^+\text{-Li}^+_o$ exchange. The $V_{\text{max}}$ of the $\text{Li}^+\text{-Na}^+_e$ exchange is elevated in a fraction (35–70%) of hypertensive individuals (see References 12 and 13 for review). We examine here the relationship between the alterations in $\text{Li}^+\text{-Na}^+_e$ exchange and $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport in human RBCs and the risk factors for essential hypertension.

Genetic Factors and RBC $\text{Li}^+\text{-Na}^+$ Exchange

From family and twin studies, it appears that genetic factors are the main determinants of the $V_{\text{max}}$ of this transport system. Family aggregation of $\text{Li}^+\text{-Na}^+$ exchange was early reported by Cusi et al. and Canessa et al. Later, extensive genetic studies were carried out in Mormon families of Salt Lake City by Williams and associates and by Turner et al. at the Mayo Clinic. A detailed report of the inheritance of the $\text{Li}^+\text{-Na}^+$ exchange by Williams et al. is included in this supplement (pages I-000–I-000).

From a twin study conducted by Lewitter and Canessa, we have established that 98% of the $V_{\text{max}}$ of the $\text{Li}^+\text{-Na}^+$ exchange system and 85% of the $V_{\text{max}}$ of the $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport are determined by heritability. The contribution of heritability to the $V_{\text{max}}$ of the $\text{Na}^+\text{-K}^+$ pump was not statistically significant. A critique of this estimation of the heritability of the $\text{Li}^+\text{-Na}^+$ exchange and $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport is that twin studies may underestimate the effect of shared environment. The racial differences reported by several studies in the $V_{\text{max}}$ of the two systems may be due to genetic differences.

Environmental Factors and RBC $\text{Li}^+\text{-Na}^+$ Exchange

To study the role of environmental factors, we examined the effect of dietary manipulations of the $V_{\text{max}}$ of the $\text{Li}^+\text{-Na}^+$ exchange. We found that reducing salt intake did not modify the maximal rate of exchange in normotensive or hypertensive individuals. We also investigated, in collaboration with Goldzer, Solomon, and Kopin, the effects of changes in caloric intake and weight. The data revealed that neither overweight nor weight reduction modified $\text{Li}^+\text{-Na}^+$ exchange. The $V_{\text{max}}$ of $\text{Li}^+\text{-Na}^+$ exchange was normal in overweight women with normal blood pressure; only hypertensive and overweight women displayed an elevation of the exchange. Moreover, caloric restriction did not significantly change the $\text{Li}^+\text{-Na}^+$ exchange. Similar findings were reported by Weder and associates in young adults. In addition, we have observed that diet supplementation with oleic and linoleic acid did not reveal changes in this transport system. On the basis of these findings, we conclude that dietary manipulations do not affect RBC $\text{Li}^+\text{-Na}^+$ countertransport.

The $\text{Li}^+\text{-Na}^+$ exchange was found to be elevated in normotensive subjects with hyperlipidemia (defined as values of plasma cholesterol and triglycerides higher than 250 mg/dl and 200 mg/dl, respectively). Hypertensive subjects with hyperlipidemia also had a significantly higher $\text{Li}^+\text{-Na}^+$ countertransport than hypertensive subjects with normal plasma lipids. A weak association between hyperlipidemia and elevated countertransport also was reported by Hunt et al. and Duhm and Behr. The finding that exercise can produce a small but significant reduction in the $V_{\text{max}}$ of the $\text{Li}^+\text{-Na}^+$ exchange may also be related to changes in the plasma and cell membrane lipids. Even though these are only small changes in comparison to the influence of heritability factors, further studies in this area should be developed considering the role of genetic factors in hyperlipidemia. It is also possible that the effect of diuretic treatment on the exchange pathway observed in some studies is mediated by changes in lipidemia. The different temperature dependence of the $\text{Li}^+\text{-Na}^+$ exchange of normotensive and hypertensive subjects also suggests that they may have a different membrane lipid environment.

Hormonal Factors and RBC $\text{Li}^+\text{-Na}^+$ Exchange

Several studies agree that the $\text{Li}^+\text{-Na}^+$ exchange is significantly lower in women than in men. In some of the studies, analysis of the partial variance with age and weight was not carried out. Evidence for the effect of hormones on the $\text{Li}^+\text{-Na}^+$ exchange has
been provided by the finding that it almost doubles in the last trimester of pregnancy in Caucasian women.\textsuperscript{34} This finding has been interpreted as evidence that the Li\textsuperscript{+}-Na\textsuperscript{+} exchange is not determined by heritability factors. Many investigators believe that a genetically determined transport system never changes; however, it is possible that either the number of sites or the mechanism of modulation is under genetic control.

For instance, we examined the V\textsubscript{max} of the Li\textsuperscript{+}-Na\textsuperscript{+} exchange in 10 black women in the last trimester of pregnancy. In agreement with either one of these hypotheses, we observed that the changes of the Li\textsuperscript{+}-Na\textsuperscript{+} exchange during pregnancy also show racial differences. The V\textsubscript{max} of Li\textsuperscript{+}-Na\textsuperscript{+} exchange in black non-pregnant women was 0.22 ± 0.059, (n = 11), while in the third trimester of pregnancy the V\textsubscript{max} was 0.29 ± 0.08 (n = 9). This elevation of the countertransport in black women was significantly lower (V\textsubscript{max} 0.07 mmol/L cell/hr) than that observed in Caucasian women (V\textsubscript{max} 0.18 mmol/L cell/hr).\textsuperscript{35}

### Age Dependence

We examined the role of this factor in a previous review.\textsuperscript{13}

### Modulation of the Na\textsuperscript{+}-H\textsuperscript{+} Exchanger

The activity of this pH-regulation system has been studied in recent years in several cell types. The most interesting findings emerged from studies showing the stimulatory effect of serum and growth factors. Several aspects of this problem are presented in this supplement by Glaser and Whiteley (pages 1-000-1-000). Cytosolic calcium as second messenger appears to be involved in several cell types in the regulation of Na\textsuperscript{+}-H\textsuperscript{+} by some growth factors, angiotensin, or bradykinin. Several reviews of the Na\textsuperscript{+}-H\textsuperscript{+} exchange have been published recently.

### Physiological Functions of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} Cotransport

Several transport systems can couple the movement of cation(s) to that of chloride ions. The Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-}, Na\textsuperscript{+}-Cl\textsuperscript{-}, and K\textsuperscript{+}-Cl\textsuperscript{-} cotransport systems have been shown to be present in different cell types. These three systems share the dependence on chloride ions and the inhibition by the loop diuretics bumetanide and furosemide. The K\textsuperscript{+}-Cl\textsuperscript{-} cotransport is chloride-dependent, partially furosemide-sensitive, and bumetanide-insensitive, stimulated by cell swelling. The Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport, on the other hand, is furosemide- and bumetanide-sensitive, chloride-dependent, and, in some cells, stimulated by cell shrinkage.

An important physiological function of the Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport can be to maintain chloride ion concentration far from equilibrium, as happens in vascular smooth muscle. Under those conditions, chloride channels are maintained in a close state. Opening of the channels will dissipate the chloride gradient and depolarize the membrane potential. The activation of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransporter by a second messenger can maintain the cell chloride concentration away from equilibrium. Changes in the activity of this transporter in vascular cells may have profound effect on cell volume and intercellular permeability of the vascular tissue.

### Modes of Operation of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} Cotransport

To gain further insight into the interactions among sodium, potassium, and chloride that can be ascribed to transport through a cotransport system, we have shown\textsuperscript{35} that different modes of sodium and potassium transport are sensitive to furosemide: outward and inward Na\textsuperscript{+}-K\textsuperscript{+} cotransport, K\textsuperscript{+}-K\textsuperscript{+} exchange, Na\textsuperscript{+}, Na\textsuperscript{+}, exchange, and uncoupled sodium and potassium efflux. The stoichiometry for unidirectional fluxes of sodium and potassium is not fixed one-to-one but is variable according to the concentration of sodium and potassium on both sides of the membrane.\textsuperscript{35} The stoichiometry of net sodium, potassium, and chloride movement is close to 1:1:2.

In the physiological range of sodium, potassium, and chloride concentrations of human RBCs, this transport system performs a net outward sodium and potassium movement that is furosemide-sensitive.\textsuperscript{36} However, the net efflux is only a small fraction (10%) of the V\textsubscript{max} of the outward mode.\textsuperscript{36} A decrease in cellular ATP content below 100 \(\mu\)mol/L cell markedly reduces net sodium extraction, outward and inward coupled transport of sodium and potassium, and K\textsuperscript{+}-K\textsuperscript{+} exchange pathway of the Na\textsuperscript{+}-K\textsuperscript{+} cotransport.\textsuperscript{37} Thus, the system requires not only the chemical gradients of sodium, potassium, and chloride, but also ATP. However, the mechanism of modulation has not yet been defined. It would be important to define whether ATP needs to be hydrolyzed by a protein kinase of high affinity for ATP or, alternatively, whether ATP modulation does not require its breakdown.

### Function of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} Cotransport in Vascular Cells

An important finding is that Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport represents an important pathway for cation transport not only of kidney tubular cells but also of vascular endothelial and smooth muscle cells.\textsuperscript{38,39} In aortic endothelial and smooth muscle cells, the fraction of potassium influx transported by Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport is as important as that mediated by the Na\textsuperscript{+}-K\textsuperscript{+} pump. The cell chloride content plays an important role in determining the direction of the net cation and chloride movement through the Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport system. In endothelial cells (low chloride content), the system performs net inward sodium (and possibly potassium and chloride) movement.\textsuperscript{39} The chloride distribution of vascular smooth muscle away from equilibrium can be accounted for by the operation of an electroneutral Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport in the inward direction.

Another important property of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} cotransport is that its activity can be modulated by several vasoactive substances. In contrast to the effect of ouabain-like factors on the sodium pump, the regulation of cotransport seems to be tissue-specific. In fact,
cyclic adenosine 3',5'-monophosphate (cAMP) inhibits the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport in vascular smooth muscle and endothelial cells, while bradykinin and vasopressin markedly stimulate it.\(^{38,39}\) O'Donnell and Owen\(^{40}\) recently showed that vasorelaxant atrial natriuretic factor (ANF) stimulates inward cotransport of potassium in vascular smooth muscle. The activation of the transporter in this case is mediated by the second messenger action of cyclic guanosine 3',5'-monophosphate (cGMP). This response appears to be tissue-specific (see Reference 41 for review).

**Factors Determining RBC Na\(^+\)-K\(^+\)-Cl\(^-\) Cotransport**

**Assays of RBC Na\(^+\)-K\(^+\)-Cl\(^-\) Cotransport**

The Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport has several modes of transport with different kinetic properties.\(^{35}\) Many of the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport assays used to investigate the properties of cotransporter in RBCs of hypertensive patients have yielded conflicting results. Most of the studies in essential hypertension have examined the outward cotransport mode measuring furosemide- or bumetanide-sensitive sodium and potassium efflux into sodium-free medium (see Reference 41 for review).

Several experimental factors influence the values of these measurements, such as variations of the cation loading procedure, variations of cell sodium content, and variations in the composition of the external medium.

We recently examined the role of these factors on the assays of Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport.\(^{41}\) Most of the studies of outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport were performed by incubating the cells in a medium without sodium and potassium ("zero-trans"). Under these conditions the transporter has a higher rate of translocation, and precise kinetic measurements can be performed because, in the presence of sodium or potassium in the external medium, the outward mode is inhibited.\(^{39}\) However, the use of high magnesium concentration (75 mM) to replace sodium and potassium markedly inhibits (50–70%) the cotransporter (\(V_{\text{max}}\)). Using choline medium and 1 mM MgCl\(_2\), the \(V_{\text{max}}\) values are 1000 and 600 \(\mu\text{mol/L cell/hr}\) in white and black subjects, respectively.\(^{43}\) It is also likely that magnesium inhibition overestimates the fraction of hypertensive patients with high \(K_m\) for cell sodium when the \(V_{\text{max}}\) is low.

**Factors Determining RBC Na\(^+\)-K\(^+\)-Cl\(^-\) Cotransport**

The outward cotransport was studied in cells with increasing sodium content to determine the \(K_m\) and the \(V_{\text{max}}\) of the system.\(^{42,43}\) Some researchers have attempted to study the system in fresh cells at ionic concentrations close to the physiological. Other investigators have studied inward potassium (or rubidium) transport into fresh cells (see Reference 41 for review). The main findings of these studies are as follows:

1. The \(V_{\text{max}}\) is family aggregated\(^{16,17,44,45}\) and is largely determined by heritability factor.\(^{23}\) From a twin study\(^\text{2}\) we estimated that heritability can account for 84% of interindividual variability.
2. The \(V_{\text{max}}\) of outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport has very large interindividual variance in normotensive and hypertensive subjects. It can be reduced, normal, or elevated in RBCs of patients with essential hypertension (see Reference 43 for review). These results, therefore, appear as confusing as studies on circulating catecholamines in hypertension. In none of these studies was sodium balance determined.
3. A subgroup of hypertensive patients has markedly higher \(K_m\) for internal sodium to stimulate outward cotransport.\(^{42,44,46}\) This alteration is more frequent in young blacks born of hypertensive parents\(^{43}\) than in whites.\(^{42}\)
4. Several studies agree that the \(V_{\text{max}}\) of the outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport is lower in blacks than in whites (see References 22, 23, 47, and 41 for review).
5. Sodium intake modulates RBC Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport activity. The \(K_m\) for cell sodium of outward sodium cotransport is decreased in hypertensive subjects when sodium intake is reduced.\(^{46}\) On the other hand, young blacks with a family history of hypertension and abnormal \(K_m\) are more likely to increase their blood pressure with 2 weeks of salt loading (10 g/15 days).\(^{43}\) Weder et al.\(^{26}\) showed that caloric restriction on the diet of obese adolescents produced a marked increase in the \(V_{\text{max}}\) of the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport. However, a control of the reduction in sodium intake with such diet was not provided in this study.

In studies carried out in collaboration with Williams,\(^{46}\) a fraction of the hypertensive subjects (30–40%) showed a significant increase in the affinity for internal sodium to activate outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport. Reduction of sodium intake from 200 to 10 mEq/day for 4 to 5 days decreases the \(K_m\) for sodium of hypertensive subjects. Since several hormonal changes take place upon sodium restriction, it remains to be established which one may be involved in this change. These findings may also provide a partial explanation for the conflicting results of low and high values of outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport at only one sodium content.

Studies carried out in young blacks (age 20) indicate that one can also find normotensive individuals born of hypertensive parents with low affinity for internal sodium to activate outward Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport.\(^{43}\) However, the blood pressure of the individuals with abnormal cotransport rose more than 5 mm Hg upon 15 days of salt loading.\(^{43}\) These findings suggest a possible link between red cell Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport and salt-sensitive blood pressure in black subjects. The results also suggest that the regulation by salt intake of the genetically determined Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport may be involved in the vasodilator response.

**Hypothesis for the Pathophysiology of RBC Sodium Transport in Essential Hypertension**

A few years ago many researchers believed that the study of RBC transport could not provide an assessment of vascular and kidney function. For many others, the alterations of a 1:1 Li\(^+\)-Na\(^+\) exchange could not have any meaning for the status of the sodium gradient in vascular cells. It is now clear that the study of ion transporters such as Li\(^+\)-Na\(^+\), Na\(^+\)-H\(^+\) and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport in human RBCs can provide
an assessment of some of the kinetic properties of systems represented in vascular and renal cells.

Further studies should clarify whether or not these sodium gradient-driven transporters are themselves abnormal or whether the biochemical mechanisms involved in their modulation are operating abnormally. The expression of these two transporters in human RBCs permits us to examine some of the functional parameters of ion carriers involved in the hormonal regulation of blood pressure by vasoactive substances that are responsible to salt intake. The interactions between genetic factors (determining kinetic properties of gradient-driven transporters) and the hormonal regulatory system of sodium balance by vasoactive peptides provide powerful mechanisms to regulate blood pressure and renal sodium excretion. A popular hypothesis of hypertension claims that an undefined genetic defect on the kidney is responsible for abnormal production of ouabain-like factors with natriuretic properties (unproved as yet) and vasoconstrictive properties because they reduce sodium gradient for calcium extrusion by Na+-Ca2+ exchange. We think that this is not the only mechanism. Figure 1 summarizes recent findings on ion transport across the vascular cells. These cells possess not only sodium pump and Na+-Ca2+ exchange, but have an array of channels that dissipate chloride, sodium, calcium, and potassium gradients. The sodium and calcium pumps cannot regulate the chloride or pH gradient, the potassium gradients, or the cell volume as the Na+-H+ and Na+-K+-Cl- cotransport systems do.

An important role in the regulation of blood pressure can be played by the regulation of these two sodium transporters across kidney and vascular cells by the action of vasoactive hormones (angiotensin II, bradykinin, and ANF), growth factors (platelet-derived growth factor, epidermal growth factor), and the second messengers (calcium, cAMP, or cGMP). The regulation of these transporters by second messengers assures constant cell pH, cell volume, and sodium, potassium, and chloride gradients dissipated by ionic channels. The regulation of cell pH during vasoconstriction by the Na+-H+ exchanger may play a more critical role than the Na+-Ca2+ exchanger. The occupancy of vasoconstrictor (angiotensin II) or vasodilator (ANF) receptors also determines that their respective second messengers (cytosolic calcium or cGMP) activate Na+-H+ or Na+-K+-Cl- cotransport in vascular cells to regulate cytosolic pH, cell volume, and sodium, potassium, and chloride gradients.

These findings indicate that the link between calcium and sodium ions is not solely determined by the Na+-Ca2+ exchanger. Calcium is a powerful modulator of sodium gradient-driven transporters, present in vascular and kidney cells, such as Na+-H+ exchange and Na+-K+-Cl- cotransport, two transporters involved in the response to vasoconstrictor and vasodilator hormones. It is therefore possible that genetic factors are involved in determining normal and abnormal kinetic variables (Kg and Vmax) of ion transporters whose activity is modulated by vasoactive substances.

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