

A Kinetic Analysis of Na-Li Countertransport in Human Red Blood Cells

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ABSTRACT We examined the kinetic properties of the interactions between inner and outer cation sites of the Na-Li countertransport system in human red blood cells. Li-stimulated Na efflux [$V(\text{Na})$] was measured as a function of external Li [$(\text{Li})_o$] and internal Na [$(\text{Na})_i$] contents. At each $(\text{Li})_o$, a Hanes plot of $(\text{Na})_i/V(\text{Na})$ vs. $(\text{Na})_i$ allowed us to calculate the apparent dissociation constant for internal Na (K_{Na}^i) and the maximal rate of Na efflux [$V_{\text{max}}(\text{Na})$]. In erythrocytes from 10 different subjects, the $V_{\text{max}}(\text{Na})/K_{\text{Na}}^i$ ratios were independent of the external Li concentrations. In other experiments, Na-stimulated Li efflux [$V(\text{Li})$] was measured as a function of external Na and internal Li contents. In three subjects studied, the $V_{\text{max}}(\text{Li})/K_{\text{Li}}^i$ ratios were independent of the external Na concentrations. The data strongly suggest that the countertransport mechanism is consecutive ("ping-pong").

INTRODUCTION

The rate of transport reactions (V) has classically been studied as a function of each substrate concentration (x, y) in a one-variable analysis [$V = f(x)$; $V = g(y)$]. However, this approach has never been sufficient to provide a simple kinetic model that would quantitatively fit the experimental data. This led several investigators to study transport kinetics by means of a two-variable analysis [$V = f(x, y)$].

Cation fluxes catalyzed by the Na-K pump in human red cells were extensively investigated as a function of two variables: internal and external cation contents (Hoffman and Tosteson, 1971; Garay and Garrahan, 1973; Chipperfield and Whittam, 1976; Sachs, 1977, 1983). This analysis provided an understanding of the transinteractions between inner and outer pump sites. However, the two-variable analysis still did not result in a quantitative kinetic model, because the rate of pump fluxes is usually the sum of several modes of operation: Na-K, Na-Na, and K-K exchanges, "uncoupled Na-efflux," and "reversal." An additional complication arises from the fact that three substrates (internal Na, external K, and ATP) are converted into four products (external Na, internal K, ADP, and inorganic phosphate). For these reasons, we have focused on elucidating the Na transport mechanism in a system catalyzing only one simple transport operation, i.e., one-to-one Na-Na exchange.

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A new Na-Na countertransport system was recently described in human red cell membranes by Haas et al. (1975) and independently confirmed by Duhm et al. (1977). Li ions can also be transported as Na-Li countertransport. Sarkadi et al. (1978) extensively studied Na-Li countertransport fluxes by a one-variable analysis, i.e., Li efflux = $f(\text{internal Li})$, Li efflux = $g(\text{external Na})$, Li influx = $h(\text{external Li})$, and Li influx = $i(\text{internal Na})$. This elegant kinetic study, however, was unable to discriminate among different molecular mechanisms.

In the present study, we have used a two-variable analysis to provide a simple kinetic model of Na-Li countertransport in human red cells. On the basis of our findings, we propose a quantitative consecutive ("ping-pong") model of Na-Li countertransport.

GLOSSARY

$(\text{Na})_i$	internal Na content
$(\text{Li})_i$	internal Li content
$(\text{Na})_o$	external Na concentration
$(\text{Li})_o$	external Li concentration
ΔC	increase in external cation concentration
Na-Li countertransport	Na-Li exchange transport system
(Na:Li) countertransport	exchange of internal Na for external Li
(Li:Na) countertransport	exchange of internal Li for external Na
V	cation efflux
$V(\text{Na})$	Li-stimulated Na efflux
$V(\text{Li})$	Na-stimulated Li efflux
$V_{\text{max}}(\text{Na})$	Li-stimulated Na efflux at saturating $(\text{Na})_i$
$V_{\text{max}}(\text{Li})$	Na-stimulated Li efflux at saturating $(\text{Li})_i$
$V_{\text{maxmax}}(\text{Na})$	$V(\text{Na})$ at saturating $(\text{Na})_i$ and $(\text{Li})_o$
$V_{\text{maxmax}}(\text{Li})$	$V(\text{Li})$ at saturating $(\text{Li})_i$ and $(\text{Na})_o$
K_{Na}^i	apparent dissociation constant for internal Na
K_{Li}^i	apparent dissociation constant for internal Li
K_{Na}^o	apparent dissociation constant for external Na
K_{Li}^o	apparent dissociation constant for external Li
Kr_{Na}^i	real dissociation constant for internal Na
Kr_{Li}^i	real dissociation constant for internal Li
Kr_{Na}^o	real dissociation constant for external Na
Kr_{Li}^o	real dissociation constant for external Li

METHODS

14 Caucasian males were included in this study. 13 of these subjects were healthy blood donors or members of the professional staff of the Hôpital Necker, aged 20–40 yr. One subject was an essential hypertensive patient aged 60 yr. In repeated determinations, systolic blood pressure varied between 180 and 190 mmHg and diastolic blood pressure varied between 110 and 120 mmHg. No associated disease was detected after complete clinical, radiological, and laboratory examination. Antihypertensive treatment was suppressed 1 mo before the study.

Preparation of Red Cells

30–40 ml of venous blood was collected in heparinized tubes and centrifuged at 1,750 g for 10 min at 4°C. The plasma and buffy coat were aspirated and the red cell pellet was used immediately.

Variation in Erythrocyte Cation Concentration

Na-loading procedure. Red cells were washed twice with cold 150 mM NaCl (or 150 mM KCl) and resuspended to a hematocrit of ~10% in the different loading media. Three to six different loading media were prepared by mixing various amounts of Na-loading medium and K-loading medium. The loading media contained: 150 mM XCl, 1 mM MgCl₂, 10 mM X-phosphate buffer (pH 7.4 at 37°C), 2 mM adenine, 10 mM inosine, and 10 mM glucose (where X is Na or K; cells incubated in K-loading medium were previously washed with 150 mM KCl).

Red cell suspensions were incubated at 37°C for 4 h. At the end of the incubation period, the loading media were renewed and the red cell suspensions were further incubated at 4°C for 20 h (it is important to note that the pH of the loading media was adjusted to 7.4 at 4°C). At the end of this treatment, the cells were centrifuged at 1,750 g for 4 min and the supernatants were discarded.

Na- or K-loaded erythrocytes were washed five times with 110 mM of cold MgCl₂. After the last wash, the cells were suspended in Mg-sucrose medium at a hematocrit of 10–12%. The Mg-sucrose medium contained: 75 mM MgCl₂, 85 mM sucrose, 10 mM 3-*N*-(morpholino)propanesulfonic acid (MOPS)-Tris buffer (pH 7.4 at 37°C), 0.1 mM ouabain, 0.02 mM bumetanide, and 10 mM glucose. A portion of each cell suspension was set aside to measure hematocrit, intracellular Na and K by flame photometry, and hemoglobin concentration by spectrophotometry.

Internal Na contents ranged from 2 to 20 mmol/liter cells. The hemoglobin contents per liter of loaded cells were much the same as those of untreated cells, which suggests that there was no change in cell volume secondary to the loading procedure.

Li-loading procedure. Red cells were loaded with Li according to the nystatin method of Cass and Dalmark (1973), as modified slightly by Canessa et al. (1982). Briefly, cells were washed at room temperature with K-sucrose medium containing 135 mM KCl and 40 mM sucrose. The cells were resuspended in K-sucrose medium at a hematocrit of ~50% and titrated with CO₂ to a final pH of 6.85 at room temperature (22°C).

The cells were then washed three times with K-sucrose medium at room temperature. The suspension was incubated for 20 min at 4°C in different Li-loading media at 10% hematocrit. The Li-loading media contained: 0–15 mM LiCl, 10 mM NaCl, 125–110 mM KCl, 40 mM sucrose, and 70 µg/ml nystatin.

After the loading period, the cell suspensions were centrifuged and the red cell pellet was resuspended in the same Li-loading media without nystatin and with 2 g/liter bovine serum albumin. The cell suspensions were incubated for 5 min at 37°C with the same media. The cells were then washed four times with cold 110 mM MgCl₂ and resuspended in Mg-sucrose medium at a hematocrit of 10–12%. A portion of each cell suspension was set aside to measure hematocrit, intracellular Li by atomic absorption, internal Na and K by flame photometry, and hemoglobin content by spectrophotometry.

The Li content of the cells varied from 0.4 to 10 mmol/liter cells. The Na content was kept constant at 7.0 (subjects 11 and 12) or 18.0 (subject 13) mmol/liter cells (see Results and Table II). The cell volume remained unchanged after the loading procedure (see Canessa et al., 1982, for further details).

Measurement of Cation Fluxes

Li-stimulated Na efflux. Li-stimulated Na efflux was measured in aliquots of cells with different Na contents (see *Na-loading procedure* above).

0.5 ml of cell suspensions in Mg-sucrose medium was added to four tubes containing 1 ml of different efflux media. Three to six efflux media were prepared by mixing appropriate amounts of Mg-sucrose medium and Li medium. The Li medium contained

150 mM LiCl, 10 mM MOPS-Tris buffer (pH 7.4 at 37°C), 0.1 mM ouabain, 0.02 mM bumetanide, and 10 mM glucose. The osmolality of all solutions was adjusted to 295 ± 5 mosmol. For each different efflux medium, external Na concentrations were measured at time 0 and after 1 h incubation at 37°C (in control experiments, we observed that fluxes were linear for >1–2 h, depending on the external Li and internal Na contents). At the end of the incubation period, the tubes were chilled at 4°C for 1 min and centrifuged at 1,750 *g* for 4 min at 4°C. The supernatants were transferred to tubes for Na analysis in an Eppendorf (Hamburg, Federal Republic of Germany) flame photometer. Na standards (checked with commercial standards, E. Merck, Darmstadt, FRG) were prepared in water and compared with those prepared in the different efflux media.

In control experiments, no evidence of red cell lysis during the incubation in the efflux media could be detected (a small contamination with Na caused by slight initial hemolysis, at time 0, was subtracted from all the tubes).

For each sample of cells (containing a given internal Na content and incubated in an efflux medium of given Li concentration), Na efflux (*V*) was calculated from the difference in external Na concentration between the initial and final incubation times (ΔC) according to the following formula:

$$V = \frac{\Delta C \times (100 - Ht)}{f \times Ht \times t}, \quad (1)$$

where *Ht* is the final hematocrit, *t* is the incubation time (1 h), and *f*(0.85) is the correction factor for the interference of the Mg-sucrose or the Li medium on the Na reading on the flame photometer.

At each internal Na content, Li-stimulated Na efflux [*V*(Na)] was obtained by subtracting Na efflux in the absence of external Li from that in the presence of the different external Li concentrations.

Na-stimulated Li efflux. Na-stimulated Li efflux in Li-loaded cells was measured by a protocol similar to the one used for Li-stimulated Na efflux. Briefly, 0.5 ml of washed Li-loaded cells, suspended in Mg-sucrose medium at a hematocrit of 10–12%, was added to four tubes containing 1 ml of different efflux media. The efflux media were prepared by mixing appropriate amounts of Mg-sucrose and Na media. The Na medium contained 150 mM NaCl, 10 mM MOPS-Tris buffer (pH 7.4 at 37°C), 0.1 mM ouabain, 0.02 mM bumetanide, and 10 mM glucose. For each different efflux medium, external Li concentrations were measured at time 0 and after 1 h incubation at 37°C (in control experiments, we verified that internal cation contents remained almost constant and fluxes were linear for at least 1 h of incubation; indeed, similar results were obtained in experiments where the incubation time was reduced to 30 min). To stop the reaction, tubes were chilled at 4°C for 1 min and then centrifuged for 4 min at 1,750 *g* at 4°C. The supernatants were transferred into tubes for Li analysis in an atomic absorption spectrophotometer (model 457, Instrumentation Laboratory, Inc., Lexington, MA). Li standards were prepared in each efflux medium and checked against commercial standards (E. Merck).

For each sample of cells (containing a given internal Li content and incubated in an efflux medium of given Na concentration), Li efflux (*V*) was calculated from the difference in external Li concentration between the initial and final incubation times (ΔC) in accordance with Eq. 1 (where *f* = 1).

At each internal Li content, Na-stimulated Li efflux [*V*(Li)] was obtained by subtracting Li efflux in the absence of external Na from that in the presence of different external Na concentrations.

Measurement of Fluxes Catalyzed by the Na-K Pump and Na-K Cotransport System

Ouabain-sensitive Na efflux in fresh erythrocytes was taken as a measure of Na-K pump activity (for details, see Garay et al., 1984). Furosemide-sensitive Na efflux in fresh erythrocytes was taken as a measure of outward Na-K cotransport fluxes (for details, see Garay et al., 1984).

RESULTS

Experimental Conditions for Measurement of Outward Na Movements Catalyzed by the Na-Li Countertransport System

The Na-Li countertransport system exhibits a higher affinity for Li than for Na (Sarkadi et al., 1978). On the other hand, the main transport pathways for Li in human red blood cells are the Na-K pump (where Li acts as a K analogue) and the anion carrier (LiCO₃ ion pair transport) (Becker and Duhm, 1978; Pandey et al., 1978). Thus, in order to determine accurately the small Na-Li countertransport fluxes, other investigators have preferred to measure Li rather than Na fluxes and to do so in bicarbonate-free media containing ouabain.

Canessa et al. (1982) have recently reported that Li can also move across the red cell membrane via the furosemide- (or bumetanide-) sensitive Na-K cotransport system. We therefore suspected that the use of bumetanide would allow us to measure precisely not only Li but also Na fluxes via the Na-Li countertransport system. We thus compared in dose-response curves the effects of bumetanide on the Na-K cotransport and Na-Li countertransport systems.

We studied the effect of variable concentrations of bumetanide on: (a) furosemide-sensitive Na efflux, (b) Na-stimulated Li efflux, and (c) ouabain-sensitive Na efflux. Fluxes were measured according to previously published methods (Canessa et al., 1980; Garay et al., 1984). We observed that the Na-K cotransport system was inhibited by very low doses of bumetanide ($IC_{50} = 5 \times 10^{-7}$ M), whereas inhibition of Na-Li countertransport (and of the Na-K pump) occurred only at concentrations higher than 10^{-4} M (data not shown). Therefore, experiments were conducted in the presence of 2×10^{-5} M bumetanide.

Stimulation of (Na:Li) Countertransport by Internal Na

The use of bumetanide allowed us to accurately measure outward Na fluxes, stimulated by external Li [(Na:Li) countertransport]. Fig. 1 shows this flux [$V(\text{Na})$] as a function of internal Na content. It can be seen that $V(\text{Na})$ is stimulated by $(\text{Na})_i$ after a saturable function. Fig. 1 (inset) shows a Hanes plot of the data. The straight line obtained indicates that $V(\text{Na})$ is a Michaelis-like function of $(\text{Na})_i$ (Hill coefficient = 1). The apparent dissociation constant for internal Na (K_{Na}^i) was obtained from the intercept with the x axis and the maximal rate of Na efflux [$V_{\text{max}}(\text{Na})$] was calculated from the intercept with the vertical axis (Fig. 1, inset).

Effect of External Li on the Stimulation of (Na:Li) Countertransport by Internal Na

(Na:Li) countertransport fluxes tend to be very small at low external Li concentrations. In order to overcome this limitation, we used red cells from an essential hypertensive patient whose Na-Li countertransport fluxes were three- to fourfold increased with respect to normal.

Fig. 2A shows Li-stimulated Na efflux as a function of external Li and internal Na contents. These data were analyzed by means of Hanes plots to determine the K_{Na}^i and $V_{max}(Na)$ corresponding to each external Li concentration (Fig. 2B).

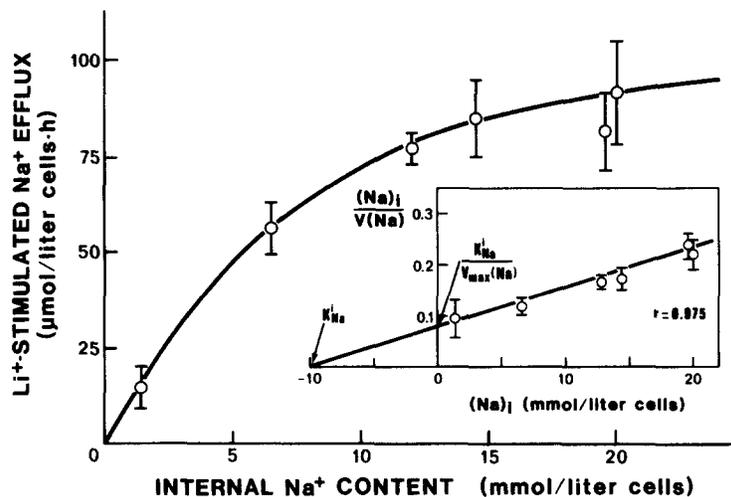


FIGURE 1. Li-stimulated Na efflux [(Na:Li) countertransport] as a function of internal Na content. Fluxes were performed in a medium containing Mg, sucrose, ouabain, bumetanide, and 10 mM of LiCl (cells from a normotensive subject not represented in Tables I and II). Each point is the mean of duplicate determinations and the range is given as a measure of variability. Inset: Hanes plot. The apparent dissociation constant for internal Na (K_{Na}^i) was obtained from the intercept with the horizontal axis and the maximal rate of Na efflux [$V_{max}(Na)$] was calculated from the intercept with the vertical axis (Hill coefficient = 1).

Fig. 3A shows $V_{max}(Na)$ as a function of the external Li concentration. It can be seen that external Li markedly stimulates the maximal rate of Na efflux by (Na:Li) countertransport. The linearity of this function in a Hanes plot indicated a Michaelis-like stimulation (data not shown).

Fig. 3B (open circles) shows the measured K_{Na}^i as a function of the external Li concentration. It can be seen that external Li markedly increased the apparent dissociation constant for internal Na. However, we observed that some of these values were overestimated because Li enters into the cells during the flux period (1 h) and competitively decreases the apparent affinity for internal Na (Sarkadi et al., 1978). To control this artifact, we measured the Li content of the cells at the end of the experiment and we calculated the inhibition of Na efflux by using

the kinetic constants of Sarkadi et al. (1978). We estimated a significant flux inhibition only in cells that contained low Na contents and had been incubated in high-Li media. We thus recalculated the values of K_{Na}^i taking into account this artifact (solid circles in Fig. 3B). A comparison between the measured and corrected K_{Na}^i values in Fig. 3B shows that Li gain introduces an important

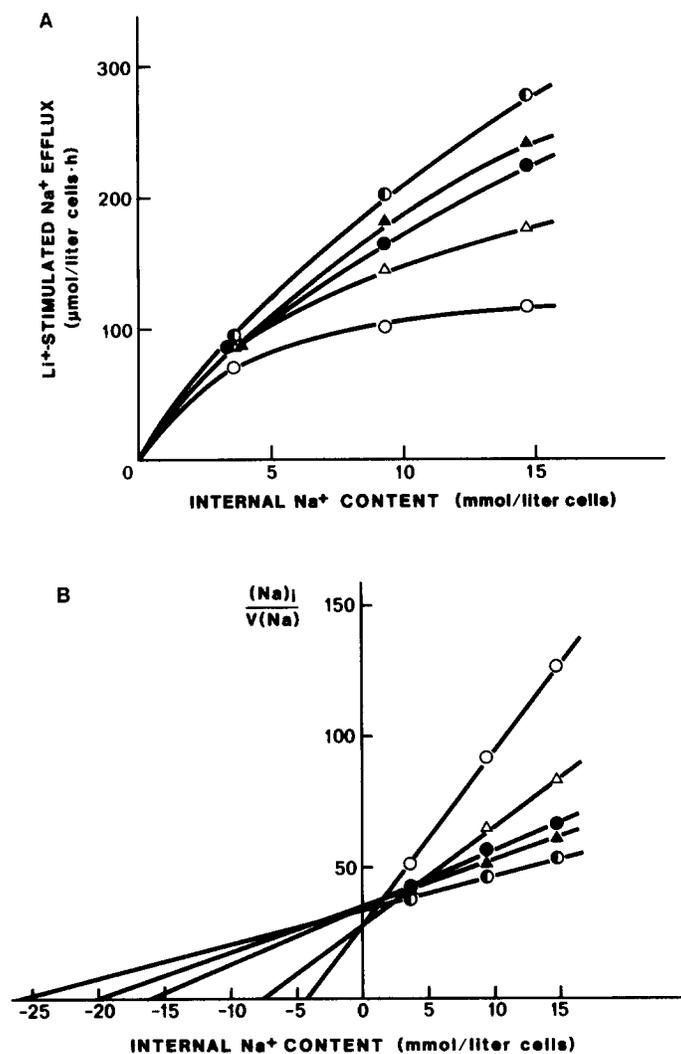
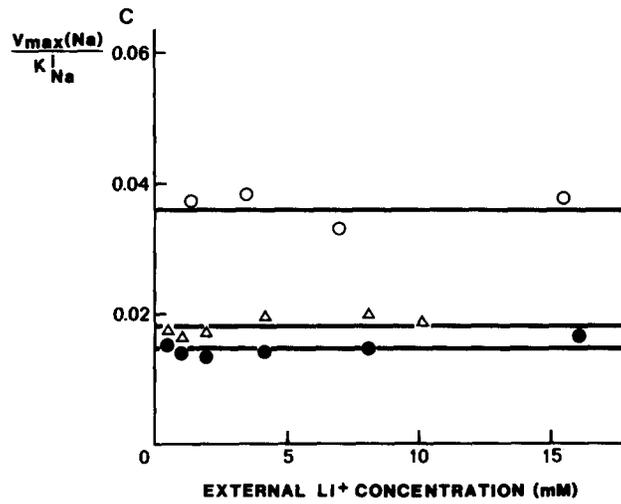
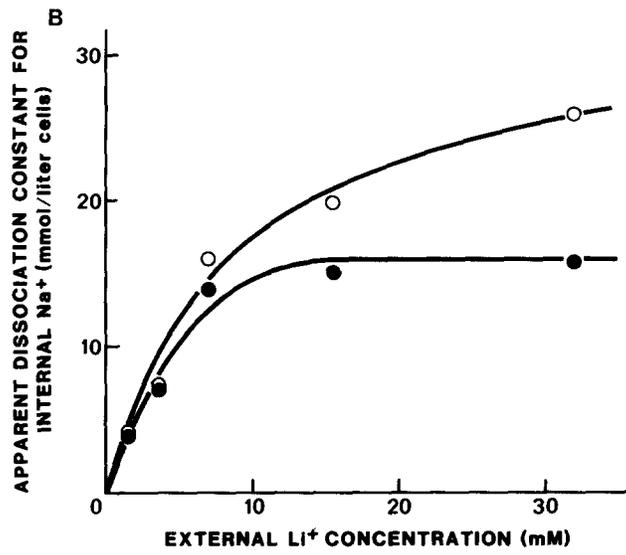
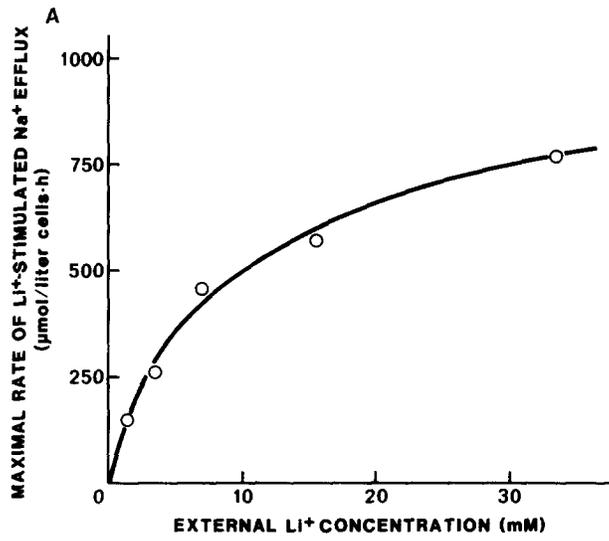


FIGURE 2. (A) The effect of external Li on the stimulation of (Na:Li) countertransport fluxes by increases in internal Na content. For the sake of simplicity, the experimental errors (which are similar to those in Fig. 1) are not indicated. Red cells were drawn from an essential hypertensive patient (subject 1 in Table I) with countertransport fluxes three to four times higher than normal. (B) Hanes plot of A. External Li (mM): ○, 1.4; △, 3.5; ●, 7.0; ▲, 15.5; ●, 31.8.



difference ($\geq 10\%$) only for external Li concentrations higher than 16 mM. Therefore, we worked in a range of 0.5–16 mM external Li concentration in order to reduce this artifact to a minimum. Corrected values of K_{Na}^i as a function of external Li concentration were adequately represented by Michaelis-like functions (data not shown). It is important to note that the $V_{max}(Na)$ values of Fig. 3A correspond to saturating $(Na)_i$ and are thus independent of $(Li)_i$.

The $V_{max}(Na)/K_{Na}^i$ ratios corresponding to each external Li concentration were calculated from Fig. 3, A and B. Fig. 3C shows that this ratio is a constant function of external Li. This result was confirmed in two further experiments carried out in the same hypertensive patient and in nine other experiments carried out in different normotensive subjects (see kinetic constants in Table I).

Effect of External Na on the Stimulation of (Li:Na) Countertransport by Internal Li

The Na-Li countertransport system exhibits a high apparent affinity for internal Li (Sarkadi et al., 1978). The measurement of this parameter requires the preparation of cells with extremely low Li contents, which cannot be maintained in stationary state during the flux period. Therefore, we decreased this affinity by loading the cells with a constant amount of Na (7 or 18 mmol/liter cells, Table II).

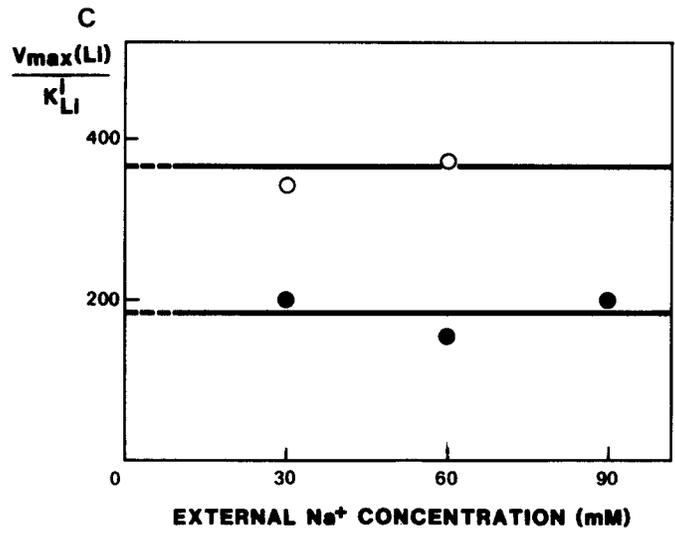
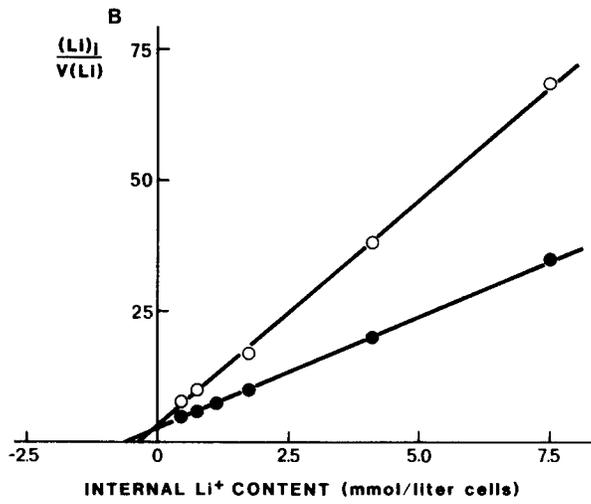
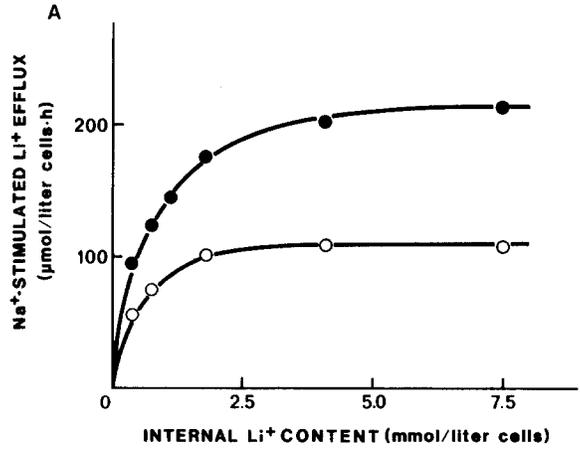
Fig. 4A shows the stimulation of (Li:Na) countertransport by the increase in internal Li content at two external Na concentrations $[(Na)_o]$. A Hanes plot of each curve allowed us to obtain the maximal rate of Li efflux $[V_{max}(Li)]$ and the apparent dissociation constant for internal Li (K_{Li}^i) (Fig. 4B). Fig. 4C shows a plot of the $V_{max}(Li)/K_{Li}^i$ ratio as a function of $(Na)_o$. It can be seen that this ratio is a constant function of $(Na)_o$. A similar result was obtained in two other subjects (Fig. 4C and Table II).

Kinetic Model for Na-Li Countertransport

The independence of the $V_{max}(Na)/K_{Na}^i$ ratio from $(Li)_o$ (Fig. 3C) and of $V_{max}(Li)/K_{Li}^i$ from $(Na)_o$ (Fig. 4C) provides evidence for a consecutive ("ping-pong") model of Na-Li countertransport (see, for instance, Sachs, 1977).

In Fig. 5, we show a consecutive reaction scheme for Na-Li countertransport. The rate-limiting step of the overall reaction is cation translocation. Using this assumption, K 's are equilibrium ("intrinsic" or "real") dissociation constants. Their values are independent of the *trans* cation concentrations and competitively modified by a second *cis* cation. The model predicts that (Na:Li) countertransport fluxes $[V(Na)]$ can be described by the following equation:

FIGURE 3. (*opposite*) (A) Maximal rate of (Na:Li) countertransport $[V_{max}(Na)]$ as a function of the external Li concentration. Values were obtained from the Hanes plot of Fig. 2A. (B) Apparent dissociation constant for internal Na (K_{Na}^i) as a function of the external Li concentration. Open circles indicate measured values. Solid circles indicate values corrected for Li gain into the cells. This artifact becomes important ($>10\%$) for external Li concentrations higher than 16 mM. (C) $V_{max}(Na)/K_{Na}^i$ ratio as a constant function of the external Li concentration. The values of K_{Na}^i represented in this figure correspond to the corrected ones of B. (○, cells from subject 1; ●, cells from subject 3; △, cells from subject 6; Table I). Similar results were obtained in seven other (normotensive) subjects.



$$V(\text{Na}) = \frac{V_{\text{maxmax}}(\text{Na})}{1 + [Kr_{\text{Na}}^i/(\text{Na})_i] + [Kr_{\text{Li}}^o/(\text{Li})_o]}, \quad (2)$$

where $V_{\text{maxmax}}(\text{Na})$ represents Li-stimulated Na efflux at saturating external Li and internal Na contents.

Eq. 2 can be rearranged into:

$$V(\text{Na}) = \frac{V_{\text{max}}(\text{Na})}{1 + [K_{\text{Na}}^i/(\text{Na})_i]}, \quad (3)$$

where

$$V_{\text{max}}(\text{Na}) = \frac{V_{\text{maxmax}}(\text{Na})}{1 + [Kr_{\text{Li}}^o/(\text{Li})_o]} \quad (4)$$

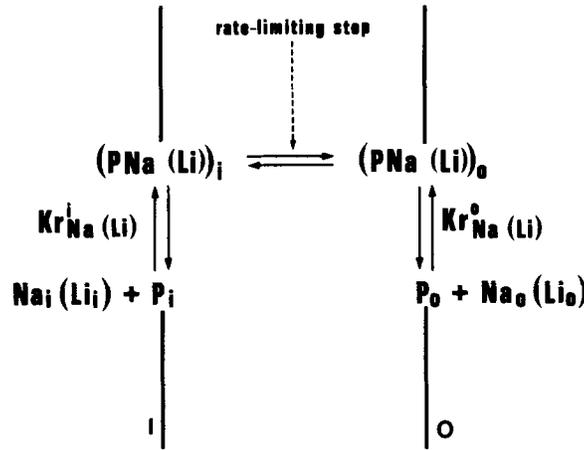


FIGURE 5. A consecutive (“ping-pong”) model for Na-Li countertransport. This is basically similar to the model of Sarkadi et al. (1978). *P* represents a carrier or an active site. “I” and “O” are inside and outside, respectively. Ion translocation is the rate-limiting step.

and

$$K_{\text{Na}}^i = \frac{Kr_{\text{Na}}^i}{1 + [Kr_{\text{Li}}^o/(\text{Li})_o]}. \quad (5)$$

Fig. 6 shows a plot of $(\text{Li})_o/K_{\text{Na}}^i$ as a function of $(\text{Li})_o$. The straight line obtained is in agreement with Eq. 5. The real dissociation constants for internal Na (Kr_{Na}^i) and external Li (Kr_{Li}^o) were calculated from the intercepts with the y and x axes, respectively.

Table I lists the kinetic constants of the model for nine normotensive and one hypertensive subject. It is interesting to note that the hypertensive patient exhibited not only an increased V_{max} , as previously reported by Canessa et al. (1980), but also an increased Kr_{Na}^i .

FIGURE 4. (opposite) (A) The effect of external Na on the stimulation of (Li:Na) countertransport by internal Li (cells from subject 12, Table II). (B) Hanes plot of A. (A and B) $(\text{Na})_o = 30 \text{ mM}$ (○) and 60 mM (●). (C) $V_{\text{max}}(\text{Li})/K_{\text{Li}}^i$ ratio as a constant function of the external Na concentration (○, cells from subject 12; ●, cells from subject 13; Table II).

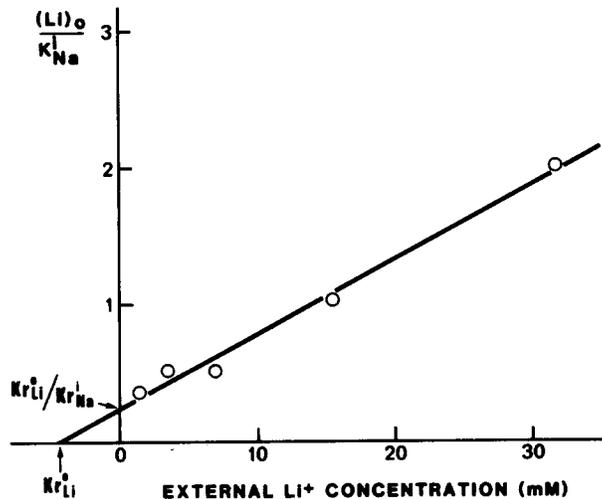


FIGURE 6. A method for the calculation of the kinetic constants of the consecutive model of Fig. 5. This is applied to (Na:Li) countertransport fluxes as a function of external Li and internal Na contents (cells from subject 1, the essential hypertensive patient in Table I). This method allowed us to calculate the kinetic constants given in Tables I and II.

Regarding (Li:Na) countertransport fluxes, the model of Fig. 5 predicts the following equation:

$$V(\text{Li}) = \frac{V_{\text{maxmax}}(\text{Li})}{1 + [Kr_{\text{Li}}^i/(\text{Li})_i] + [Kr_{\text{Na}}^o/(\text{Na})_o]}, \quad (6)$$

where $V_{\text{maxmax}}(\text{Li})$ represents Na-stimulated Li efflux at saturating external Na and internal Li contents.

Using a kinetic analysis similar to that in Eqs. 3–5, we can easily obtain Kr_{Li}^i and Kr_{Na}^o . Table II lists these kinetic constants for the three subjects studied. It

TABLE I
Kinetic Constants of (Na:Li) Countertransport

Subject	Kr_{Na}^o <i>mmol/liter cells</i>	Kr_{Li}^i <i>mM</i>	$V_{\text{maxmax}}(\text{Na})$ <i>μmol/liter cells · h</i>
1*	19.6±3.0	4.5±3.0	870±60
2	4.5±1.4	3.2±1.2	225±40
3	9.2±3.5	2.5±1.3	145±20
4	3.8±1.6	1.7±0.6	80±10
5	4.2±1.8	1.9±0.6	120±15
6	10.7±3.0	1.9±0.5	200±20
7	5.6±1.5	4.8±1.0	205±15
8	9.4±1.7	3.4±0.8	255±25
9	13.0±3.0	2.6±0.4	320±20
10	13.5±2.0	0.8±0.1	380±30

Values in this table are given with their ranges as a measure of variability.

* Essential hypertensive patient.

is important to note that the apparent dissociation constants for internal Li were measured in the presence of a constant amount of internal Na [$Kr_{Li}^i(\text{Na})_i$]. In order to obtain Kr_{Li}^i , we assumed a competitive interaction of internal Na and Li (see Sarkadi et al., 1978). Using this simple assumption, Kr_{Li}^i obeys the following kinetic equation:

$$Kr_{Li}^i = \frac{Kr_{Li}^i(\text{Na})}{1 + [(\text{Na})_i/K_{Na}^i]}, \quad (7)$$

where $K_{Na}^i = 9$ mmol/liter cells (Sarkadi et al., 1978).

The $V_{\text{maxmax}}(\text{Na})$ values of the nine normotensive subjects of Table I were two- or threefold lower than the $V_{\text{maxmax}}(\text{Li})$ values of the three subjects of Table II.

TABLE II
Kinetic Constants of (Li:Na) Countertransport

Subject	Kr_{Li}^i *	Kr_{Na}^i	$V_{\text{maxmax}}(\text{Li})$
	<i>mmol/liter cells</i>	<i>mM</i>	<i>μmol/liter cells · h</i>
11	0.5±0.2	130±60	690±90
12	1.9±0.7	280±100	1,300±200
13	1.0±0.3	120±40	650±50

Values in this table are given with their ranges as a measure of variability. Erythrocyte Na content was 7.0 ± 0.5 mmol/liter cells for subjects 11 and 12 and 18.0 ± 0.5 mmol/liter cells for subject 13.

* Calculated for an Na-free cell using Eq. 7 (see Results).

DISCUSSION

In spite of the extensive literature on ion transport in human red cells, a kinetic model has never been provided to quantitatively fit all the fluxes catalyzed by any of the Na transport systems.

In the present paper, we have investigated fluxes catalyzed by the Na-Li countertransport system by means of a two-variable kinetic analysis, i.e., as a function of internal and external cation concentrations. Our main finding is that the V_{max}/K ratios are independent of *trans* cation concentrations, as predicted by consecutive ("ping-pong") mechanisms of the transport reaction (Fig. 5). This allowed us to calculate all the kinetic constants of the simple consecutive model represented in Fig. 5 (Tables I and II). The introduction of the calculated constants into the kinetic equations of the model (Eqs. 2 and 6) generates functions that quantitatively fit all fluxes catalyzed by the Na-Li countertransport system (as a function of external and internal cation contents). We believe that this complete kinetic description resulted from the fact that, in contrast to other erythrocyte Na transport systems, the Na-Li countertransport system catalyzes only one simple transport operation—one-to-one Na-Na (or Na-Li) exchange.

A major difficulty in assessing Na-Li countertransport fluxes in human erythrocytes is technical, i.e., a high degree of experimental precision is required to measure small fluxes. Other investigators have increased the relative fraction of countertransport fluxes by blocking the pump with ouabain and by working in bicarbonate-free media in order to prevent LiCO_3 ion pair transport (see, for

instance, Pandey et al., 1978; Becker and Duhm, 1978). Moreover, the authors preferred to measure Li rather than Na fluxes.

We were able to determine precisely the Na efflux (Na:Li countertransport) as a function of internal Na and external Li concentrations by means of the following experimental protocol. (a) Internal Na content was varied by simple incubation in media with different salt compositions (Na-loading with *p*-chloromercuribenzenesulfonate [PCMBBS] results in countertransport inhibition; Canessa et al., 1982), (b) Na transport by the Na-K cotransport system, which accounts for one-third of ouabain-resistant Na efflux (Garay et al., 1984), was selectively inhibited with bumetanide. (c) Inhibition of Na efflux by Li gain was reduced to a minimum by performing the flux experiment in media containing low Li concentrations. To further increase the experimental precision, we used erythrocytes from an essential hypertensive patient whose countertransport fluxes were three to four times higher than normal. It is important to note that the resulting consecutive model was not a consequence of the hypertensive disease, because similar results were obtained in erythrocytes from nine other normotensive subjects.

(Li:Na) countertransport appears to be two- or threefold faster, and thus more precisely measurable, than (Na:Li) countertransport (a clear demonstration of this asymmetry requires further determination of both parameters on the same subjects). We therefore tested the above kinetic model by measuring (Li:Na) countertransport fluxes. In these experiments, cells were loaded with Li by using the nystatin method (Canessa et al., 1982), and internal Na content was kept constant to allow a better estimate of the apparent affinity for internal Li (which in Na-free cells is very high; Sarkadi et al., 1978). A kinetic analysis of Li efflux [(Li:Na) countertransport] as a function of external Na and internal Li contents further confirmed a consecutive mechanism of transport reaction (Fig. 5).

Tables I and II show the kinetic constants (real) of the model for all the subjects studied. All the calculated kinetic constants displayed a high degree of interindividual variation. Interestingly, the hypertensive patient was characterized by an increased $V_{\max\max}$ and Kr_{Na}° and a normal Kr_{Li}° (which is in agreement with a previous observation by Canessa et al. that K_{Na}° is normal in hypertensives with increased $V_{\max\max}$). This is very similar to the asymmetry of abnormalities in inner and outer sites of the Na-K cotransport system (Price et al., 1984). It is important to stress that for clinical purposes it is more precise to determine (Li:Na) than (Na:Li) countertransport fluxes. The only advantage of the latter is that it can be measured in parallel with the Na efflux through the pump and cotransport system (Garay et al., 1984). All these aspects deserve further investigation in the hypertensive population.

The consecutive model of Fig. 5 (with the real kinetic constants of Tables I and II) predicts numerical values for any kinetic constant obtained in "single-variable" experiments. We thus calculated "single-variable" constants (data not shown) that were in agreement with those obtained by Sarkadi et al. (1978), with the exception of K_{Na}° , which was almost one order of magnitude higher. It is important to note that we measured K_{Na}° (an external constant) by replacing external Na with Mg (and not with K as Sarkadi et al. did). However, it is hard

to believe that Mg (a divalent cation) could interact with the external sites of the countertransport system. Indeed, we have no explanation for this discrepancy.

Becker and Duhm (1979) have shown that external Na and Li induce a conformational change in Na-Li countertransport (see also Levy and Livne, 1984). This may reflect the translocating conformational transition of Fig. 5, where the inner cation sites of the protein are transformed into outer sites.

In conclusion, we have found that a consecutive ("ping-pong") model can fully account for Na-Li countertransport kinetics. This is the first time that an erythrocyte Na transport system has been described entirely with a simple kinetic model.

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