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CLINICAL INVESTIGATION

A kinetic study of cation transport in erythrocytes from uremic patients

DALILA B. CORRY, DAVID B.N. LEE, and MICHAEL L. TUCK

Departments of Medicine, Olive View Medical Center, Sylmar and Sepulveda VA Medical Center, Sepulveda; UCLA School of Medicine, Los Angeles, California, USA

A kinetic study of cation transport from uremic patients. We previously described in red blood cells (RBCs) from uremic patients on dialysis a reduction in sodium (Na) efflux through the Na, potassium (K) cotransport system (Na,K CoT) while Na efflux through the Na,K pump was normal. We then examined Na efflux in fresh cells and in cells loaded to obtain one level of intracellular sodium (Na_i) concentration at about 25 mmol/liter cell. In the present study we used similar cation flux methodology to examine the kinetics of cation efflux through the Na,K pump and Na,K CoT in uremic patients on dialysis. RBCs were Na-loaded to attain five different levels of Na_i concentration over a range of 5 to 50 mmol/liter cells using the ionophore nystatin. At each level of Na-loading, the Na_i achieved was similar in RBCs from controls and patients. Ouabain–sensitive Na efflux through the Na,K pump showed no difference in rate between normals and dialysis patients. When the kinetic parameters of this transport pathway were considered, the apparent affinity ($K_{0.5}$) for sodium was not significantly different between controls and patients (18.4 ± 2.3 vs. 20.0 ± 2.6 mmol/liter cell) and the maximal velocity of efflux (V_{max}) was also not different between controls and patients (9.6 ± 0.7 vs. 8.5 ± 1.2 mmol/liter cell/hr). Comparison of Na_i-activated Na versus K efflux rates through the Na,K CoT in normal subjects demonstrated similar saturation kinetics, ($K_{0.5}$ 15.8 ± 3.3 vs. 12.2 ± 2.8 mmol/liter cell, V_{max} 0.81 ± 0.1 vs. 0.78 ± 0.1 mmol/liter cell/hr) consistent with the known stoichiometric ratio of 1 Na:1 K:2 Cl described for this mechanism. In dialysis patients Na_i-activated, Na,K CoT-mediated Na efflux was markedly reduced. Analysis of the kinetic parameters of Na_i-activated Na efflux showed that the reduced RBC Na,K CoT is due to reduction in V_{max} and not to a change in $K_{0.5}$. Maximum furosemide–sensitive K efflux rate was also reduced in dialysis patients. However, instead of exhibiting the anticipated saturation kinetics observed for Na, the K efflux rates were high at low levels of Na_i and remained unchanged with increasing Na_i concentrations. Ouabain– and furosemide–resistant Na and K effluxes were not significantly different between normals and dialysis patients. We conclude that Na efflux through RBC Na,K pump is intact over a wide range of Na_i concentrations in dialysis patients. On the other hand, the furosemide–sensitive co-efflux of Na and K, which in normal RBCs displayed a typical 1 Na to 1 K transport characteristic, was quantitatively and qualitatively altered in dialysis patients. The maximum efflux rate of both Na and K was reduced and in addition, the usual stoichiometric ratio for Na and K exit through this furosemide–sensitive pathway was no longer observed.

Cation content and sodium (Na) transport in uremic RBCs have been studied to delineate the effect of chronic renal failure on ionic cellular transport mechanisms [1–3]. Most recent studies have focused on the ouabain–sensitive Na, potassium (K) pump in RBCs from chronically hemodialyzed patients [4–6]. However, in the human RBC, Na transport is mediated through other pathways, including the ouabain–insensitive, furosemide–sensitive Na,K cotransport system (Na,K CoT) and the Na,Li counter transport system (Na,Li CTT). We have previously found in RBCs from patients undergoing chronic intermittent hemodialysis and chronic ambulatory peritoneal dialysis (CAPD), normal intracellular Na (Na_i) and K content, normal Na and K passive permeability, and normal Na efflux through the Na,Li CTT and the Na,K pump. We did observe, however, a markedly reduced Na efflux through the Na,K CoT [7]. This observation has also been noted by others [8, 9]. In order to further characterize the ouabain–sensitive and –insensitive cation transport pathways in patients undergoing dialysis, we examined the kinetics of Na efflux through the Na,K pump and Na and K effluxes through the Na,K CoT in RBCs, by measuring cation effluxes at five Na_i concentrations

Methods

Subjects

Fourteen Caucasian males with end–stage renal disease (ESRD) and receiving chronic intermittent hemodialysis were studied. Clinical and biochemical findings are depicted in Table 1. The age range was 29 to 68 years (mean 58.0 ± 3.6). The etiologies of ESRD and the number of patients, in parenthesis, were as follows: chronic glomerulonephritis (4) polycystic kidney disease (4), diabetic nephropathy (2), essential hypertension (2) chronic interstitial nephritis (1), and Alport's syndrome (1). The control population consisted of six females and eight males; eleven subjects were Caucasian and three subjects were Oriental. The age of the controls ranged from 23 to 48 years (mean 40.8 ± 6.7) and all were normotensive with no significant medical problems.

Methods

RCBs preparation. Fresh venous blood was drawn between 0800 and 1000 hours in control subjects and in patients just before starting hemodialysis. Blood was collected into heparinized tubes, centrifuged at 3,000 g and the plasma and buffy coat were removed by aspiration. RBCs were washed four times with a washing solution containing 152 mM choline chloride, 1 mM $MgCl_2$, 10 mM Tris-Mops, pH 7.4 at 4°C.

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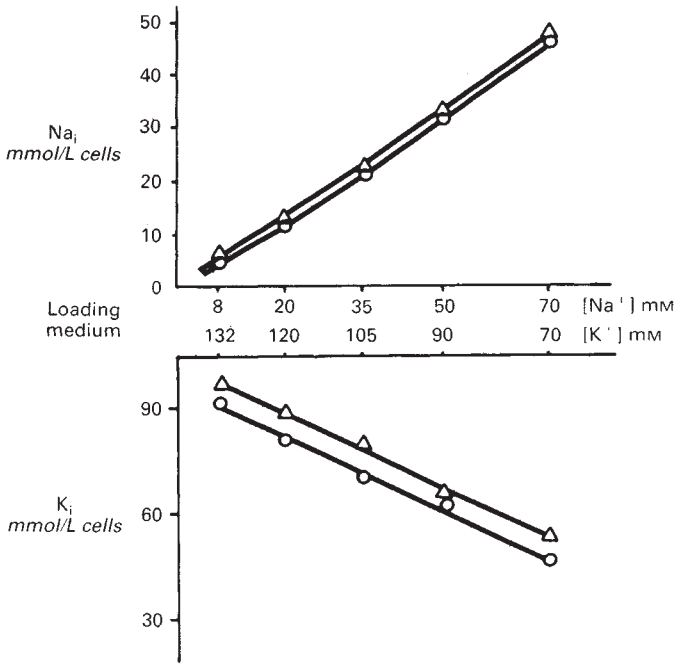

Fig. 1. *Intracellular sodium (Na_i) and potassium (K_i) concentrations simultaneously achieved at each level of Na-loading in 14 normal controls (O) and 14 dialysis patients (Δ).*

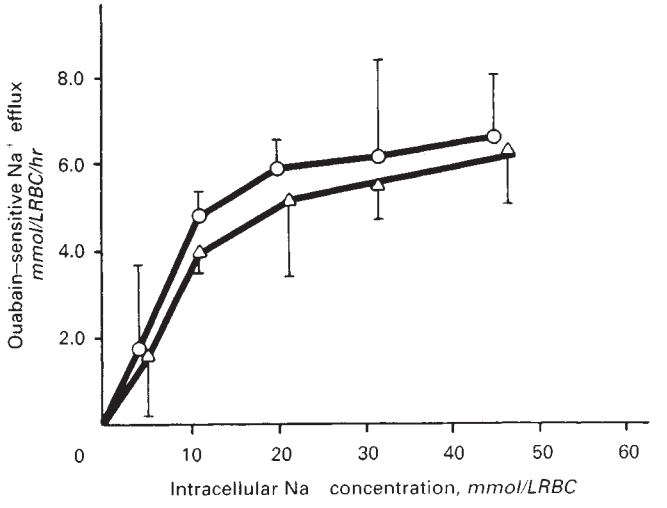

Fig. 2. *Ouabain-sensitive Na efflux rates as a function of five levels of intracellular Na^+ concentrations in normal controls (O) and dialysis patients (Δ).*

Nystatin-loading procedure. The procedure is that of Canessa et al [10] which is modified from Cass and Dalmark [11] using lower amounts of the ionophore to facilitate complete removal by washings. The nystatin-loading solution contained 55 mM sucrose and 140 mM total cations containing a mixture of NaCl and KCl. The Na and K concentrations in the loading solution were reciprocally changed according to the final Na_i desired. Na-loading was carried out at five different levels of external Na_i from 8 to 70 mM (Fig. 1). One ml of packed cells from the cell suspension was added to 5 ml of cold loading solution containing 40 μg/ml of nystation dissolved in DMSO, and the cell suspension incubated for 20 minutes at 4°C. After centrifugation and removal of the supernatant, the cells were incubated for another 20 minutes in 5 ml of the same loading solution but without nystatin. Then nystatin removal was done by washing the cells four times at room temperature again using the same loading solution but now with the addition of 1 mM of K phosphate buffer, 10 mM glucose and 0.1% albumin at pH 7.4. In order to remove the external cations, five additional washes were done using the choline chloride wash solution (described above) at 4°C. Changes in cell volume during nystatin-loading was determined by measuring the hemoglobin per liter cell in fresh and loaded cells.

Table 1. Clinical and biochemical parameters in dialysis patients (N = 14)

Age *yr*	58.0 ± 3.6
Weight *kg*	78.6 ± 3.6
Systolic blood pressure *mm Hg*	150.0 ± 5.6
Diastolic blood pressure *mm Hg*	80.0 ± 3.2
Hemoglobin *g/dl*	10.2 ± 0.8
MCV mm^3	84.6 ± 2.6
MCHC %	34.3 ± 1.6
Blood urea nitrogen *mg/dl*	72.8 ± 3.4
Serum creatinine *mg/dl*	13.6 ± 0.7
Albumin *g/dl*	3.0 ± 0.1
Serum Na *mEq/liter*	137.0 ± 1.0
Serum K *mEq/liter*	4.9 ± 1.5
Serum HCO_3 *mEq/liter*	18.2 ± 1.0
Serum Ca *mg/dl*	9.5 ± 0.2
Serum P *mg/dl*	4.9 ± 0.3

Intracellular cations. Aliquots of a 50% cell suspension were taken for hematocrit and intracellular Na and K determination in fresh and Na-loaded RBCs. Washed RBCs were completely lysed in 0.02% Acationex detergent (American Scientific Products, McGraw Park, Illinois, USA). After centrifugation, the Na and K concentrations in the supernatant were measured using an atomic absorption spectrophotometer (Model 5000, Perkin Elmer, Norwalk, Connecticut, USA). Results are expressed in mmol/liter cell.

Cation efflux measurements. The ouabain-sensitive fraction of Na efflux was measured in Na-loaded RBCs, suspended at 1% Hct in an efflux medium containing 130 mM choline chloride and 10 mM KCl with and without 0.1 mM ouabain. The efflux times were 5 and 25 minutes at 37°C, with triplicate samples. The furosemide-sensitive Na and K effluxes were measured in a 2% Hct suspension of RBCs in an medium containing 140 mM choline and 0.1 mM ouabain with or without 1.0 mM furosemide. The incubation times were 5 and 65 minutes, and all measurements were done in triplicate samples. All the media used for measuring cation effluxes through Na,K CoT also contained 1 mM of $MgCl_2$, 10 mM glucose and 10 mM Tris-Mops, pH 7.4 at 37°C and were Na and K free. The length of incubation was selected in order to measure cation fluxes during their linear rates. The Na,K pump activity was estimated as the difference in Na accumulation in the efflux media with and without ouabain. The difference in Na and K accumulation between the efflux media with and without furosemide was estimated as Na,K CoT activity.

Data analysis. Cation efflux rates are expressed in mmol/liter

Table 2. Kinetic parameters for cation effluxes through the Na,K pump and Na,K CoT

	Na,K pump Na efflux		Na,K CoT			
			Na efflux		K efflux	
	$K_{0.5}$[a]	V_{max}[b]	$K_{0.5}$[a]	V_{max}[b]	$K_{0.5}$[a]	V_{max}[b]
Controls (N = 14)	18.4 ± 2.3	9.6 ± 0.7	15.8 ± 3.0	0.81 ± 0.1	12.2 ± 2.8	0.78 ± 0.1
Patients (N = 14)	20.0 ± 2.6	8.5 ± 1.2	14.9 ± 2.8	0.30 ± 0.1	—	—

[a] mmol/liter cell
[b] mmol/liter cell/hr

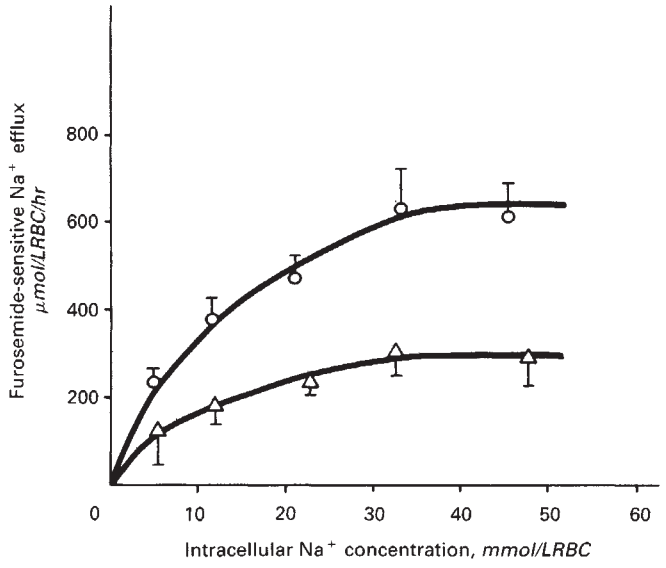

Fig. 3. *Furosemide-sensitive Na efflux rates as a function of five levels of intracellular Na+ concentrations in normal controls (O) and dialysis patients (Δ).*

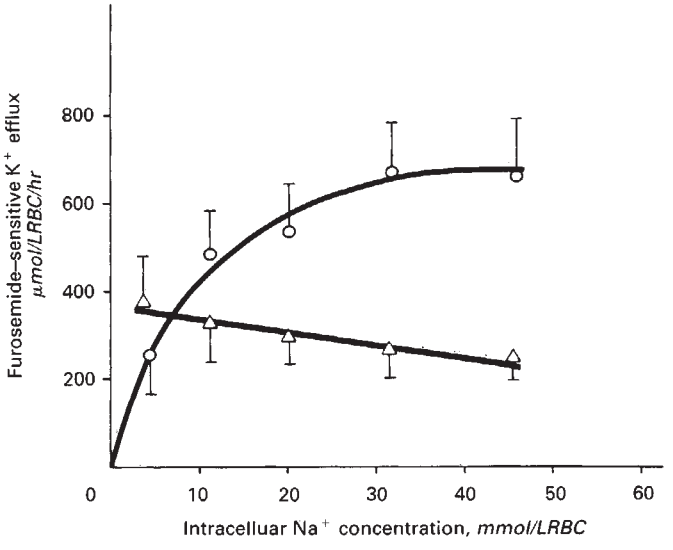

Fig. 4. *Furosemide-sensitive K efflux rates as a function of five levels of intracellular Na^+ concentrations in normal controls (O) and dialysis patients (Δ).*

cell/hr. Data are reported as mean ± SEM. Statistical significance was determined using Student's unpaired t-test. Kinetic parameters ($K_{0.5}$ and V_{max}) of Na_i-activated Na efflux through the Na,K pump were calculated according to the Hanes–Woolf Plot [12]. The same equation was used to calculate the kinetic parameters of Na and K effluxes through the Na,K CoT.

Results

Intracellular cation and cell volume.

In fresh RBCs there was no significant difference between controls and patients in mean intracellular Na (7.2 ± 0.4 vs. 7.1 ± 0.5 mmol/liter cell) or K (96.2 ± 3.6 vs. 95.9 ± 3.3 mmol/liter cell). With nystatin Na-loading there was no difference in the level of Na_i achieved between controls and patients (Fig. 1). Determination of hemoglobin concentration in fresh cells and at the five final Na_i showed mild cell shrinkage with recovery of 98 ± 3% of the initial volume in both study groups.

Na,K pump.

Figure 2 depicts mean values for ouabain-sensitive Na efflux rates as a function of increasing concentrations of Na_i. There was no significant difference in ouabain-sensitive Na efflux rate at all five levels of Na_i between controls and dialysis patients. Calculation of the $K_{0.5}$ and V_{max} using the Hanes–Woolf plot for Na,K pump mediated Na efflux showed no difference between normals and dialysis patients in either of the two parameters (Table 2).

Na,K Cotransport

Na,K CoT, estimated as furosemide-sensitive Na efflux were markedly reduced in dialysis patients at each level of Na_i as depicted in Figure 3. The calculated $K_{0.5}$ for Na efflux through the Na,K CoT was not different between the two study groups, while the V_{max} was markedly reduced in dialysis patients (Table 2). We also examined the furosemide-sensitive K efflux as a function of increasing Na_i (Fig. 4). In normal subjects, K efflux increased with increasing Na_i in a fashion similar to the Na efflux with a calculated $K_{0.5}$ of 12.2 ± 2.8 mmol/liter cell and V_{max} of 0.78 ± 0.1 mmol/liter cell/hr. In contrast, Na,K CoT-mediated K efflux in dialysis patients was independent of the changes in Na_i

Ouabain- and furosemide-insensitive fluxes

Ouabain and furosemide-resistant Na efflux increased progressively with increasing Na_i and were similar at every level of Na-loading for both study groups (Fig. 5). Ouabain- and furosemide-resistant K efflux was slightly higher in dialysis patients but the difference was not significant.

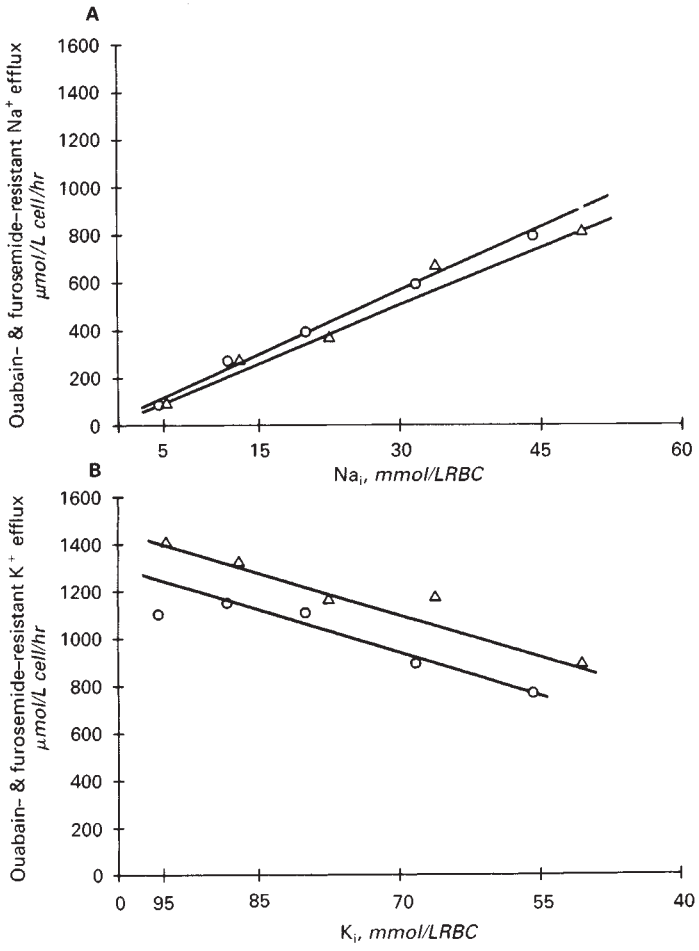

Fig. 5. *Ouabain- and furosemide-resistant Na and K efflux rates as a function of five levels of (A) intracellular sodium (Na_i) and (B) potassium (K_i) concentrations in normal controls (O) and dialysis patients (Δ).*

Discussion

The present study extends our previous results to examine Na,K CoT-mediated outward Na and K fluxes in RBCs from dialysis patients. We used nystatin to Na-load RBCs to five different levels of Na_i in a range from below physiologic levels to levels fivefold greater. The results indicate that in RBCs from dialysis patients there is a normal $K_{0.5}$ but a major reduction in V_{max} for Na efflux through the Na,K CoT. As previously noted, this markedly reduced Na,K CoT is found in almost all uremic patients and occurs independently of blood pressure level or level of RBC Na,K CoT activity observed in first degree relatives [7]. Na and K effluxes through the Na,K CoT have been found to be reduced in RBCs from some patients with essential hypertension [13]. Analysis of the kinetic parameters of Na,K CoT in essential hypertension show an abnormality in the $K_{0.5}$ with only a minority of subjects displaying a reduction in the V_{max} [13]. These findings contrast with Na,K CoT-mediated Na efflux in dialysis patients where $K_{0.5}$ is normal but there is a marked reduction in V_{max} occurring independent of the presence or absence of hypertension.

An important consideration in interpreting Na,K CoT function in dialysis patients is the change in intracellular volume in RBCs during Na-loading. Furosemide-sensitive Na,K CoT in nucleated RBCs is sensitive to acute volume changes [14, 15], the pathway being suppressed by cell swelling and stimulated by cell shrinkage. In human RBCs cation fluxes can also be altered by cell volume changes [16–18] but the effect on Na,K CoT is variable. Adragna et al [17, 18] demonstrated that decreasing RBC water content by both osmotic and non-osmotic methods did not change Na,K CoT activity whereas increasing RBC intracellular water greater than 6% decreased furosemide-sensitive Na efflux, and increased furosemide-resistant K efflux. The methodology of the nystatin Na-loading procedure is designed to maintain cell volume relatively constant. Nonetheless, there was a 2 to 3% reduction in cell volume, as reflected by hemoglobin concentration, during the loading procedure in normal RBCs and in those from dialysis patients. Based on the findings of the above studies [16, 18], alterations in RBC volume in our study would still not be expected to influence Na,K CoT activity. Thus, in dialysis patients the marked decrease in furosemide-sensitive Na efflux cannot be accounted for by cell volume alterations. Duhm and Gobel [19] also described in human RBCs a positive correlation between furosemide-sensitive Na,K CoT and mean cellular hemoglobin-concentration (MCHC). As MCHC was not decreased in dialysis patients, this factor could not account for the change in Na,K CoT in this population.

A second alteration in the RBC Na,K CoT pathway in dialysis patients is the complete uncoupling of 1:1 relationship between Na_i-activated Na and K effluxes. Thus, there was an absence of correlation between Na_i concentrations and K efflux rates in RBCs from patients compared to the typical saturation kinetics in controls. This dissociation of the coupling of cations through the Na,K CoT in dialysis patients has no readily apparent explanation but could represent an alternative furosemide-sensitive K pathway, such as the Ca^{++}-dependent K^+ efflux pathway first described by Gardos [20]. This pathway, however, seems to require the presence of external K in the millimolar range in order to be activated [21], whereas in this study Na,K CoT assays were performed in media free of external K. An alternative explanation is that the recently described K,Cl CoT [22] could predominate in uremia. Both pathways, however, display only weak inhibition by furosemide [22].

Many of the original studies of ouabain-sensitive Na,K pump in renal failure patients reported reduced values in frankly uremic subjects [1–3]. With the advent of dialysis and sampling of a less severely ill population both normal [4, 6] and abnormal [5] Na,K pump function has been found. We previously reported [7, 8] normal RBC ouabain-sensitive Na efflux in RBCs from dialysis patients studied both in cells Na-loaded to one level of Na_i and in fresh cells. The results of the present study confirm the presence of almost identical pump-mediated Na efflux rates in dialysis patients and controls over a wide range of RBC Na_i. Analysis of kinetic parameters shows similar $K_{0.5}$ and V_{max} in cells from both study groups. Hence, as measured by RBC Na-efflux methodology, the Na,K pump can perform at maximal capacity in optimally dialyzed patients.

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Reprint requests to Dalila B. Corry, M.D., Olive View Medical Center, 14445 Olive View Drive, Sylmar, California 91342, USA.

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