Kinetic Parameters of Rubidium Transport Pathways Are Normal in Cystic Fibrosis Red Cells

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ABSTRACT. The abnormalities in ion transport in cystic fibrosis (CF) respiratory and sweat duct epithelia have prompted studies of ion permeability in CF red blood cells (RBC) although previous reports have been contradictory. In this study, the kinetic characteristics of the three major cation transport systems in RBC were evaluated by measuring rubidium (Rb) uptake at various external Rb concentrations. The maximal velocity and affinity for external Rb ($K_{1/2}$) of the NaK pump were normal in CF RBC, as were the maximal velocity and K_m for Rb of the NaK cotransport system. Residual (ouabain and bumetanide insensitive) Rb uptake, and steady state RBC Na and K contents were also normal. These data indicate the NaK pump and cotransport system do not exhibit primary or secondary perturbations in CF RBC, and suggest that the noncarrier-mediated membrane permeability to cations is also normal in these cells. (*Pediatr Res* 24: 495-498, 1988)

Abbreviations

CF, cystic fibrosis
RBC, red blood cell
 V_{max} , maximal velocity
 K_m , R_b , external K or Rb
PBS, phosphate-buffered saline
OS, ouabain-sensitive
Co, cotransport

CF is an autosomal recessive disorder manifesting multiple exocrine abnormalities (1). The diagnostically important elevation of sweat chloride reflects malfunctioning of the salt resorbing mechanisms of sweat duct epithelium. Recent work has indicated that the basis of this dysfunction is low Cl permeability of the apical membrane of the ductal epithelial cells (2). Respiratory epithelia share this defect (3), which appears in these cells to arise from abnormalities of the adrenergic regulatory mechanisms of the Cl channel (4-6). Other recent studies of CF respiratory epithelia have also shown increased permeability of the apical membrane to Na, accompanied by elevated Na pump rates at the basolateral membrane, with increased numbers of [^3H]ouabain binding sites and ouabain-inhibitable oxygen consumption (7). These findings suggest that, in addition to the Cl channel,

certain cation transport systems might also be primarily or secondarily affected in CF.

Numerous studies of cation transport in CF RBC have been prompted by the ready availability and ease of handling of this "biopsy" material and the advantage of interpreting results within the context of the relatively well understood cation transport systems of red cells. The results of early studies, however, were contradictory. Balfe *et al.* (8) found reduced Na pump activity (ouabain-sensitive Na efflux) in CF red cells compared to normal red cells, but this finding was not confirmed by Lapey and Gardner (9) using similar techniques, or by Hadden *et al.* (10), who found normal ouabain-sensitive ^{86}Rb uptake in CF red cells. Studies of NaK ATPase activity also produced conflicting results when comparing CF to normal RBC membranes (10, 11). More recently, however, Reznik *et al.* (12) reported a reduced affinity of the NaK pump for external K(Rb): K_m was 2.2 mM for normal RBC and 4.6 in CF RBC. This finding paralleled their previous report that the affinity for external K of the NaK pump in cultured CF fibroblasts was reduced compared to normal cells (13), but both of these results are inconsistent with the finding of Boucher *et al.* (7) that CF respiratory epithelium had increased Na pump activity (7).

Assessments of the function of the NaK cotransport system in CF RBC have been similarly ambiguous. The portion of Na efflux inhibited by ethacrinic acid (which was later found to block NaK cotransport) was reported by Balfe *et al.* (8) to be reduced in CF RBC, although Lapey and Gardner (9) observed this effect in male and not female CF patients, a phenomenon inconsistent with a single autosomal gene defect. Hadden *et al.* (10) found normal ethacrinic acid inhibitable ^{86}Rb uptake in CF RBC. Interpretation of these studies is hampered by the fact that the NaK cotransport system was not well understood at the time they were published.

These discrepancies might have resulted from the variety of experimental procedures used, including the use of different ions (Rb, K, Li) at different concentrations in the incubation media. Accordingly, physiologically important differences in the kinetic characteristics of the transport pathways studied might have been obscured. For this reason, and in view of the more recent reports of abnormalities in Na pump activity in CF red cells and other tissues (7, 12, 13), kinetic parameters of three major cation transport pathways in red cells were measured: the NaK pump, the NaK Co system, and the ouabain and bumetanide insensitive (residual) pathway. The results indicate that all of these pathways are kinetically normal in CF red cells.

MATERIALS AND METHODS

To assess the kinetic characteristics of the potassium transport systems, net Rb uptake in red cells was measured, which approximates the unidirectional flux as long as internal Rb concentration remains low. The suitability of Rb as a substitute for K has

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been well documented for ion transport studies in the human red cell (14–16). Heparinized blood was obtained with informed consent under a protocol approved by the Institutional Review Board of the University of Alabama at Birmingham, from normal white volunteers (aged 22–50 yr, median 27 yr) or from ambulatory CF patients (aged 11–37 yr, median 16 yr) who were not taking digitalis derivatives or diuretics. Cells were washed three times in PBS, which contained (mM): 110 NaCl, 20 Na_2HPO_4 (pH 7.45), 0.1 EDTA, 10 tetramethylammonium-Cl, 10 glucose. Cells were incubated at 5–10% hematocrit in PBS with various concentrations of external Rb (Rb_o , 0.4–50 mM) with Na varying reciprocally ($\text{Na}_o = 100\text{--}150$ mM). Flasks contained either ouabain (0.1 mM), ouabain plus bumetanide (0.05 mM), or no drug (control). Prewarmed cells were added at zero time, and triplicate samples were taken at 1 h and processed as described previously (17). Cells were lysed and the hemolysates assayed spectrophotometrically for Hb and for Rb by flame emission spectroscopy. Rb uptake was calculated as mmol/liter·cells/h. Supernatant Rb concentrations were measured in each incubation flask. This method has been validated in human (16) and sheep (18) red cells. My preliminary experiments showed that this “one point” method gave results equivalent to procedures involving multiple time points over a 2-h period. Cellular Na and K were measured on fresh, washed cells using similar flame emission techniques.

NaK pump activity was calculated as the OS component of Rb uptake (uptake in control cells minus uptake in ouabain-treated cells) for each measured Rb_o concentration. Results were plotted as OS Rb uptake *versus* Rb_o as shown in Figure 1A. Sachs and Welt (19) showed that simple Michaelis-Menten kinetics did not adequately describe the response of the NaK pump to external K. Indeed, Eadie-Hofstee plots of our data were so curved as to make analysis of $V_{\max}$ and K_m unthinkable. Therefore, the value of OS Rb uptake at 14–16 mM Rb was taken as $V_{\max}$, and $K_{1/2}$ was estimated graphically as the value of Rb_o at which one-half $V_{\max}$ was achieved. The data in Figure 1A demonstrate that OS Rb uptake clearly saturated at these Rb_o concentrations and that $K_{1/2}$ could be unambiguously determined by this method.

NaK cotransport was assayed as the ouabain-insensitive, bumetanide-sensitive Rb uptake (Co Rb uptake), calculated by subtracting Rb uptake of cells treated with ouabain and bumetanide from that of cells exposed to ouabain alone. For some concentrations of Rb_o , bumetanide incubations were omitted due to limitation in the volume of blood samples. However, because Rb uptake in the presence of both ouabain and bumetanide was linear with Rb_o (see below), a value for ouabain- and bumetanide-insensitive uptake could be calculated at any Rb_o for which ouabain-insensitive Rb uptake was measured, allowing the calculation of Co Rb uptake for all ouabain treated samples. The relationship of Co Rb uptake to Rb_o approximated a rectangular hyperbola (Fig. 1B) and linear plots of $(\text{Co Rb uptake})^{-1}$ *versus* $(\text{Rb}_o)^{-1}$ were obtained (inset), allowing calculation of $V_{\max}$ and K_m .

The component of Rb uptake in the presence of both ouabain and bumetanide (residual Rb uptake) has been shown to approximate the permeability of the lipid bilayer (20) in normal RBC. The linear, nonsaturating relationship between residual Rb uptake and Rb_o was confirmed by the data in this study (Fig. 1C) for both normal and CF RBC. Plots of residual Rb uptake *versus* Rb_o were straight lines with an average correlation coefficients (r) of $0.997 (\pm 0.003 \text{ SD}, n = 20)$, and intercepts at zero Rb_o which approximated naturally occurring Rb levels in RBC, providing further validation of the Rb uptake method. The slope of such a plot (by least squares analysis) represents an apparent rate constant k (units: mmol/liter·cell/h/mM), which is related, but not equivalent to a rate coefficient (units: h^{-1}).

All chemicals were reagent grade; water was predeionized and doubly distilled. Ouabain was obtained from Sigma Chemical Co., (St. Louis, MO); bumetanide was a gift from Hoffman-LaRoche, Inc. (Nutley, NJ).

Fig. 1. Concentration dependence of rubidium uptake in normal red cells. Each panel represents data from a single experiment. Points are means of triplicate determinations, the SD of which were smaller than the points as shown. A, OS Rb uptake *versus* Rb_o . $V_{\max}$ from the data of this experiment is 1.23 mmol/liter·cells/h; Rb_o concentration at 1/2 $V_{\max}$ ($K_{1/2}$) was 1.1 mM, as shown by dotted lines. B, Ouabain-insensitive, bumetanide-sensitive (Co) Rb uptake *versus* Rb_o . The curve depicted is the rectangular hyperbola defined by K_m and $V_{\max}$ calculated from the double reciprocal plot shown in the inset. C, Ouabain- and bumetanide-insensitive (residual) Rb uptake *versus* Rb_o . Slope was 0.0183 mmol/liter·cells/h/mM, $r = 0.9996$; intercept was 0.067 mmol/liter·cells/h, compared to a measured naturally occurring Rb level of 0.068 ± 0.002 mmol/liter·cells.

RESULTS

Table 1 below shows cellular Na and K contents in RBC from 10 CF patients and 10 normal controls, along with the kinetic parameters of K(Rb) influx mediated by the NaK pump, NaK Co system, and residual pathway. Steady-state monovalent cation contents were not different in CF RBC from normal RBC, confirming the findings of other investigators (10, 11). It is especially important to note that cellular Na is normal in CF cells because this allows analysis of external NaK pump kinetics with the assurance that the degree of pump activation by internal Na is the same in CF and normal cells. Without this information, comparisons of the kinetic constants of the pump (especially $V_{\max}$) between the two cell types cannot be made. Normal internal cation concentrations also insure that the driving forces for Co-mediated uptake are equivalent for normal and CF cells.

External K(Rb) affinity ($K_{1/2}$), as well as $V_{\max}$ of the NaK pump, were virtually the same in CF and control RBC (Table 1). The scatter plots of these parameters are shown in Figure 2, which

Table 1. Cation transport parameters in normal and CF RBC measured as Rb uptake*

		Normal	CF
Cellular cations†	Na	8.7 (2.8)	8.3 (1.5)
	K	92.4 (9.2)	98.8 (7.7)
		(n = 7)	(n = 10)
NaK pump§	$K_{1/2}$ ‡	1.13 (0.15)	1.21 (0.14)
	$V_{\max}$	1.98 (0.34)	1.93 (0.46)
		(n = 10)	(n = 10)
NaK Co¶	K_m	4.18 (0.85)	5.12 (1.24)
	$V_{\max}$	0.916 (0.400)	0.645 (0.250)
		(n = 10)	(n = 10)
Residual Rb permeability k**		0.0181 (0.0030)	0.0183 (0.0055)
		(n = 10)	(n = 10)

* Cellular cations and kinetic parameters were measured as described in "Materials and methods" and illustrated in Figure 1. Data are presented as means (SD) of single determinations in *n* individuals.

† mmol/liter · cells.

‡ mM.

§ Ouabain-sensitive Rb uptake.

|| mmol/liter · cells/h.

¶ Ouabain-insensitive, bumetanide-sensitive Rb uptake.

** Rate constant for ouabain- and bumetanide-sensitive Rb uptake, mmol/liter · cells/h/mM.

illustrates clearly the tight distributions of these kinetic constants of the NaK pump for both CF and normal populations. These data contrast markedly with those of Reznik *et al.* (12) who found a 2-fold increase in K_m for external K (Rb). For the data reported here, the probability of a type II (β) error for failing to detect a two-fold increase in $K_{1/2}$ is less than 0.001. Indeed, the current data would have a 95% probability of demonstrating a difference in $K_{1/2}$ as small as 20%, based on power analysis for a 2-tailed *t* test with *n* = 10 using a 0.05 significance criterion (21). In agreement with Reznik *et al.* (12), I found no difference in NaK pump $V_{\max}$ between CF and normal RBC, although in their study $V_{\max}$ was 40% lower than reported here.

Interindividual variability in the measurements of K_m and $V_{\max}$ of the NaK cotransport system was much greater than those of NaK pump activity, consistent with the observations in studies of larger populations (22–24). Although the Co $V_{\max}$ for Rb was slightly lower and the K_m was slightly higher in CF RBC (Table 1), these differences were not statistically significant. The probability by unpaired *t* test for a difference between the two groups was 0.10 for $V_{\max}$ and 0.06 for K_m , without consideration of multiple analyses that would tighten significance criteria. In view of the finding of Lapey and Garner (9) that male but not female CF patients showed reduced ethacrinic acid sensitive Na efflux, Co $V_{\max}$ and K_m in CF and normal RBC were examined for gender-associated differences, and none was observed (data not shown). The scatter plots of these data (Fig. 3) demonstrate that for both cotransport K_m and $V_{\max}$, the CF and normal populations cluster together, with approximately 80% overlap of distributions. In the case of $V_{\max}$ in particular, the major contribution to the difference between groups comes from two outlying points. Clearly these parameters are not useful predictors of CF phenotype, and these data are not consistent with a primary or secondary defect in CF reflected in the NaK cotransport system.

The rate constant, *k*, for ouabain- and bumetanide-insensitive Rb uptake was identical in CF and normal RBC (Table 1). This indicates that the basal permeability of the membrane to K(Rb) is normal in CF RBC. Direct measurements of Na permeability are very difficult in RBC because of the high rate of Na:Na exchange, but this finding makes it unlikely that there is a nonspecific increase in cation permeability in CF RBC.

DISCUSSION

This study has demonstrated that the major cation transport pathways capable of net Na and K movements are normal in CF red blood cells. The NaK pump and NaK cotransport are normal in CF cells with respect to both their maximal velocities and affinities for external K(Rb). These results conflict with the

Fig. 2. Kinetic parameters of the NaK pump in normal and CF red cells. $V_{\max}$ and affinity for external Rb ($K_{1/2}$) were determined as described in "Materials and Methods" and illustrated in Figure 1A. Means and SD of these data are found in Table 1.

Fig. 3. Kinetic parameters of the NaK cotransport system in normal and CF red cells. V_{max} and affinity for external Rb (K_m) were determined as described in "Materials and Methods," and illustrated in Figure 1B. Means and SD of these data are found in Table 1.

decreased K(Rb) affinity of the NaK pump in CF RBC reported by Reznik *et al.* (12). The reasons for this discrepancy are not apparent, although we found that the Michaelis-Menten formulation used by Reznik *et al.* (12) did not adequately describe the kinetics of Rb_o interaction with the NaK pump, in agreement with the earlier work of Sachs and Welt (19). The K_m and V_{max} measured in the presence of high external Na for normal RBC in this study (1.1 mM and 1.98 mmol/cells/h/liter·cells) are in close agreement with the values for these parameters under comparable conditions reported originally by Sachs and Welt (1.2 mM and 1.67–1.85 mmol/liter·cell/h) (19). Given the normal cellular Na content of CF RBC, our finding of a normal NaK pump V_{max} provides indirect evidence that the number of pump sites on CF RBC is normal. Thus, the increased Na pump rate and numbers of [³H]ouabain binding sites of CF respiratory epithelial cells (7) are not paralleled in the red cell. Similarly, the kinetic characteristics of the NaK Co system of CF RBC were found to be normal in this study.

The residual permeability of CF RBC to Rb was also normal, suggesting that a nonspecific cation permeability defect does not exist. This conclusion is further supported by normal intracellular cation concentrations and pump and Co rates in CF RBC, because a cell with elevated Na or K permeability would be expected on theoretical grounds to show abnormalities in one or more of these values (25). Moreover, in view of normal internal cation contents and external cation transport kinetics in CF RBC, the hypothesis that perturbations exist in these cells in the internal kinetics of these pathways is extremely unlikely. Accordingly, further exploration of internal pump or cotransport kinetics does not seem warranted and was not undertaken in this study.

Boucher *et al.* (26) have presented evidence that conductive anion (Cl⁻) permeability in CF RBC is also normal, indicating that the defect in Cl permeability or Cl channel regulation manifest in sweat duct and respiratory epithelium (2–6) is not present in RBC. It would appear, therefore, that the RBC is of limited use either as model system for the study of CF membrane transport abnormalities or as the source of phenotypic markers of abnormal ion transport in CF.

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