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Erythrocyte Sodium-Potassium Transport in Cystic Fibrosis

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ABSTRACT. Erythrocytes from 15 patients with cystic fibrosis (CF) aged 8 mo to 22 y (mean age 12.8 y) were analyzed for Na^+,K^+-ATPase activity and sodium, potassium, and ATP concentrations. Sodium concentrations and Na^+-K^+ ratio of erythrocytes were statistically significantly lower in the CF patients [6.6 (SD 1.9) *versus* 9.2 (SD 1.1) mmol/L ($p < 0.01$) and 0.070 (SD 0.023) *versus* 0.104 (SD 0.016) mmol/L ($p < 0.01$), respectively]. The Na^+,K^+-ATPase activity was similar compared with that of reference individuals [536 (SD 100) *versus* 488 (SD 92) nmol inorganic phosphate/mg protein/h]. Intraerythrocyte sodium concentration and Na^+-K^+ ratio were thus lower in relation to the recorded Na^+,K^+-ATPase activities in controls, indicating a change of the passive transmembrane movements of sodium ions in CF. There was a rise of erythrocyte sodium and Na^+-K^+ ratio despite unchanged Na^+,K^+-ATPase activity after regular infusion of a fat emulsion rich in essential fatty acids, inferring that an altered membrane composition by essential fatty acid deficiency could explain the low intracellular sodium concentration in CF. (*Pediatr Res* 31: 425–427, 1992)

Abbreviations

CF, cystic fibrosis
Na^+,K^+-ATPase, sodium- and potassium-activated adenosine triphosphatase
Na^+-K^+ ratio, erythrocyte sodium-potassium ratio
AA, arachidonic acid

The altered handling of sodium and chloride ions in sweat glands of patients with CF has proved to be related to a generalized phenomenon of impaired chloride conductance in epithelial cells (1–3). Furthermore, patients with CF have been shown to have an increased renal tubular reabsorption of sodium ions (4) not due to a depletion of sodium, inasmuch as the total exchangeable sodium was normal (5). Active sodium-potassium transport is the main activity exerting proximal tubular sodium reabsorption and is also working in erythrocytes (6). It was therefore indicated to study the enzymatic activity responsible for this transport and the results thereof, namely the Na^+,K^+-ATPase and the intracellular concentrations of sodium and potassium (7, 8). By measuring these parameters in erythrocytes, evidence of a generalized different handling of sodium ions could be obtained.

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MATERIALS AND METHODS

Patients. Fifteen patients (eight males and seven females) were studied. The mean age was 12.8 y and the ages varied between 8 mo and 22 y. All patients had sweat chloride concentrations above 60 mmol/L. All patients had pancreatic insufficiency and pulmonary symptoms. The clinical condition was considered good in seven cases [Schwachman score >70 (9)], moderate in five cases (Schwachman score 50–70), and severe in three cases (Schwachman score <50).

All patients had a standard diet without restrictions. Three of the patients had an extra supply of glucose polymer formulas. All patients had supplementations of pancreatic enzymes and fat soluble vitamins. Four of the patients received regular infusions of a 10% fat emulsion (Intralipid, Kabi Pharmacia, Sweden) during 3 consecutive d every 4 to 6 wk (2 g/kg body wt/8 h). All patients except two had inhalations of N-acetylcystein and 10 had β_2-stimulating agents and bromhexine as inhalations and/or oral medication.

Sixteen age-matched healthy controls were selected from patients admitted for minor surgery and from school children who were healthy according to school health records; their ages ranged from 10 mo to 25 y, mean age 12.2 y. The reference values of some of the healthy controls have been reported previously (10). The sampling of blood was approved by the Ethical Committee of the Medical Faculty, University of Göteborg.

Methods. Na^+,K^+-ATPase in erythrocytes was analyzed according to a method modified by Sigström and Waldenström (11). About 2 mL of EDTA blood was centrifuged. Plasma, white cells, and the top layer of erythrocytes were removed. The remaining erythrocytes were washed twice in a solution containing 5 mmol sodium phosphate and 135 mmol sodium chloride with pH 7.40, with removal of the supernatant and redilution to the hematocrit of about 0.4 in the phosphate-saline buffer. The washed erythrocytes were hemolyzed in 30 volumes of a solution containing 5 mmol Tris buffer and 2 mmol EDTA, pH 7.5 at 2°C. After 15 min, the erythrocytes were centrifuged at 16 000 × *g* for 30 min at 2°C. The Hb-containing supernatant was removed, and the hemolysis procedure and centrifugation were repeated twice. The erythrocyte membranes free from Hb were stored at −20°C for about 18 h. Before incubation, the membranes were thawed and frozen three times and diluted to about 300% of the original packed cell volume with 5 mmol Tris buffer solution, pH 7.5. Aliquots of 0.1 or 0.2 mL of this membrane suspension were added to 0.8 mL of an incubation solution with a final concentration per L of Tris, pH 7.40, 15 mmol; magnesium chloride, 3.9 mmol; potassium chloride, 5.8 mmol; sodium chloride, 42 mmol; EDTA, 0.25 mmol; and ATP, 3 mmol with and without ouabain, 0.5 mmol. Liberated inorganic phosphate was determined according to Fiske and Subarrow (12) after incubation for 120 min at 37°C interrupted by addition of 0.6 mmol trichloroacetic acid. The protein concentration of the membrane suspension was analyzed according to Lowry *et al.*

(13). The activity was expressed as the ouabain-sensitive liberation of inorganic phosphate in nmol/mg protein/h (U).

Erythrocyte sodium and potassium concentrations were measured with a modification of the method of Bengtsson *et al.* (14). About 2 mL of EDTA blood was centrifuged at 600 × *g*, and plasma, white blood cells, and the top layer of erythrocytes were removed. The remaining erythrocytes were washed twice in an isotonic solution containing choline chloride, sodium and potassium chloride, phosphate, and Tris to give a pH of 7.40, each time followed by centrifugation at 600 × *g* and removal of the supernatant. The washed erythrocyte suspension was analyzed in an IL flame photometer (Instrumentation Laboratory, Italy) after dilution of the packed erythrocytes with equal volumes of distilled water in one tube and with 5 mmol of sodium chloride and 10 mmol of potassium chloride in another tube.

Erythrocyte ATP was analyzed in nine of the 15 patients using an analysis kit from Boehringer-Mannheim (Mannheim, Germany) with modification for small volumes. Statistical analyses were performed using the Wilcoxon-Mann-Whitney test. Variation is indicated by SD.

RESULTS

The characteristics of active Na^+-K^+-transport are presented in Table 1. The mean Na^+,K^+-ATPase activity seemed higher in the CF patients, 536 ± 100 U *versus* 455 ± 92 U, but the difference was not statistically significant. The CF patients had statistically significantly lower means of erythrocyte sodium concentration (6.6 ± 1.9 *versus* 9.2 ± 1.1 mmol/L, $p < 0.01$ and Na^+-K^+ ratio (0.070 ± 0.020 *versus* 0.104 ± 0.016, $p < 0.01$). The means for erythrocyte potassium concentration and ATP were similar in both groups, being 93.2 ± 4.7 *versus* 89.4 ± 8.4 mmol/L and 1.02 ± 0.10 *versus* 1.01 ± 0.10 mmol/L, respectively. Figure 1 shows the correlation between the Na^+,K^+-ATPase activity and Na^+-K^+ ratio for each individual among the CF patients compared with the reference values. The confidence interval was originally calculated in 131 healthy individuals and has been published previously (10). The CF patients were all in the lower half of or outside the 95% confidence interval for this correlation.

One CF patient was analyzed after 3 d of treatment with infusions of essential fatty acids, and one after 5 d of treatment. The Na^+,K^+-ATPase values were similar before and after, being 564 U before and 577 U after in one of the patients and 515 U before and 520 U after in the other patient. The corresponding values for erythrocyte sodium and Na^+-K^+ ratio were increased from 5.7 mmol/L and 0.063, respectively, before to 9.2 mmol/L and 0.122 after infusion in one patient and from 7.8 mmol/L and 0.081 before to 10.2 mmol/L and 0.107 after infusion in the second patient. When comparing the four patients who had received regular infusions of essential fatty acids during the analysis with those who had received few or no infusions, the mean numerical product of Na^+,K^+-ATPase and Na^+-K^+-ratio was 45.9 ± 2.8 for the four patients and 34.0 ± 7.8 for the other CF patients ($p = 0.02$).

Table 1. *Erythrocyte Na^+,K^+-ATPase, sodium (Na^+), potassium (K^+), Na^+-K^+ ratio, and ATP in patients with CF and reference individuals of corresponding ages [mean (SD)]*

	CF ($n = 15$)	Reference ($n = 16$)
Na^+,K^+-ATPase (U)*	536 (100)	488 (92)
Na^+ (mmol/L)	6.6 (1.9)†	9.2 (1.1)
K^+ (mmol/L)	93.2 (4.7)	89.4 (8.4)
Na^+-K^+ ratio	0.070 (0.020)†	0.104 (0.016)
ATP (mmol/L erythrocytes)	1.02 (0.10)	1.01 (0.10)

* U = nmol inorganic phosphate/mg protein/h.
† $p < 0.01$.

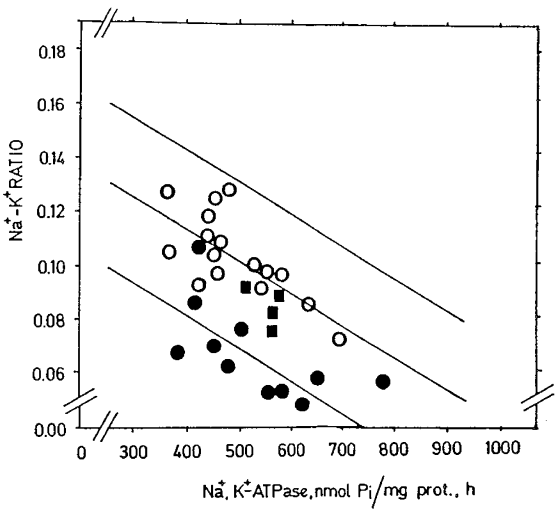

Fig. 1. Relationship between Na^+,K^+-ATPase and Na^+-K^+ ratio in erythrocytes of CF patients without infusions of Intralipid (*filled circles*), CF patients with repeated infusions of Intralipid (*filled squares*), and age-matched controls (*open circles*). *Lines* indicate 95% confidence interval and mean in 131 healthy individuals (10).

DISCUSSION

The findings of a decreased sodium concentration in the urine of patients with CF (4, 15) could be the result of an increased saving of sodium ions to cope with losses of sodium in sweat and other sources, *e.g.* feces. However, the sodium loss through sweat and feces is usually limited in the CF patients (5), and there seems to be little need for an increased renal saving of sodium for this reason. Furthermore, an extra supply of sodium did not change the renal excretion pattern (4). The Na^+,K^+-ATPase activities of erythrocytes are expected to reflect those of renal tubular tissue (6), and the recorded normal or moderately elevated erythrocyte Na^+,K^+-ATPase levels, which indicate a well-maintained or moderately increased active Na^+-K^+ transport, in the CF patients of this study seem to fit into this pattern. In an earlier study of Na^+,K^+-ATPase, sodium and potassium concentrations of erythrocytes of CF patients, Cole and Dirks (16) demonstrated similar values in controls and CF patients. Accordingly, the decreased urinary sodium concentration seems to be another expression of the disease itself. An interesting finding in the CF patients of this study is the low intraerythrocyte sodium concentration and the low Na^+-K^+ ratios in relation to the recorded Na^+,K^+-ATPase activities (Fig. 1). Low intracellular sodium concentrations in erythrocytes (17) and fibroblasts (18) have been reported previously. This suggests that low intracellular sodium concentrations are not only dependent on an intensified active sodium pumping but may also be related to decreased non-ATP-dependent transmembrane movements of sodium ions in some kinds of tissues. The membrane properties of the different walls of tubular cells are, in contrast to erythrocytes, not uniform regarding sodium permeability. An increased renal sodium reabsorption, as seen in CF, can also be explained by an increased sodium permeability of one part of the tubular cell. This may explain why amiloride, which blocks the entrance of sodium into the cell, could normalize the bioelectrical potential difference across respiratory epithelia (19). A similar mechanism may explain a higher efficacy of furosemide in urinary electrolyte excretion in CF patients (20).

One cause of changed sodium permeability might be a decrease of the membrane concentration of some of the essential fatty acids (21, 22). Indirect evidence for this speculation is the increasing intraerythrocyte sodium concentration and increased Na^+-K^+ ratios together with unchanged Na^+,K^+-ATPase activities in two of the CF patients after infusion of an emulsion rich in essential fatty acids. Another interesting observation in the 15

patients of this study was that those four CF patients who for therapeutic reasons got regular infusions of fat emulsion rich in essential fatty acids had comparatively higher Na^+-K^+ ratios in relation to their Na^+,K^+-ATPase activities than those who did not receive this treatment (Fig. 1). Such an interpretation of the results of this study is supported by the finding that the urinary sodium excretion was normalized after a sodium load in CF patients, who normalized their essential fatty acid pattern in serum phospholipids after long-term infusions of fat emulsions (23). Assuming that the findings in erythrocytes reflect a generalized membrane aberration related to a change of the composition of essential fatty acids, this might be secondary to an increased turnover (24, 25). Evidence for this hypothesis has been demonstrated in *in vitro* studies of lymphocytes from CF patients, which showed an impaired inhibition of the AA release induced by dexamethasone (26). One possible explanation for the importance of AA and its metabolites in the renal tubular handling of sodium in CF patients is given by Escalante *et al.* (27). They reported a decreased ^{86}Rb uptake into cells isolated from the thick ascending loop of Henle during incubation with AA and said that this effect was mediated by AA metabolites such as 20-hydroxyeicosatetraenoic acid and 20-carboxy AA. This inhibition of ^{86}Rb uptake could be blocked by eicosatetraynoic acid, indicating an acting and counteracting influence on ^{86}Rb (and sodium) uptake by different metabolites of fatty acids. Accordingly, one of the causes of the different renal sodium handling in CF could be a disturbed balance of fatty acid metabolites. The data from this and previous studies (22) suggest that the disturbed transport in CF might be secondary and might be influenced by essential fatty acid supplementation in contrast to the impaired chloride transport, which is more closely linked to the basic defect of the disease (1–3).

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