Erythrocyte Transport Function in Cystic Fibrosis

STEPHEN A. FEIG,^[17] GEORGE B. SEGEL, KENNETH A. KERN, ALAN B. OSHER,
AND ROBERT H. SCHWARTZ

Gwynne Hazen Cherry Memorial Laboratory, the Department of Pediatrics, UCLA School of Medicine, Los Angeles, California,
and Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA

Extract

ATPase activity and monovalent cation transport were examined in erythrocytes (RBC) of subjects with cystic fibrosis (CF). The RBC membrane (Ca^{2+})ATPase activity of homozygous patients and obligate heterozygotes was indistinguishable from that of normal control subjects. This was true similarly for total, ouabain-sensitive, and ouabain-insensitive (Na^{+} - K^{+})ATPase.

Total, ouabain-sensitive, and ouabain-insensitive $^{24}\text{Na}^{+}$ transport were normal in CF cells. Ouabain-induced Na^{+} accumulation was similar in CF and normal RBC. Measurements of K^{+} transport in CF RBC were, likewise, normal.

We conclude that intrinsic ATPase and monovalent cation transport functions are normal in CF RBC. We were unable to confirm several abnormalities in these cells reported previously. The CF RBC is not helpful in defining a transport abnormality in that entity.

Speculation

These studies document normal intrinsic transport function in RBC of patients with CF. There have been recent reports of an abnormal serum factor in patients with CF. This factor might be involved in the production of a transport defect in CF. Further studies are indicated to determine whether CF plasma contains a factor capable of altering membrane transport functions of normal RBC.

Introduction

An increase in the Ca^{2+} or Na^{+} content of various body secretions has reported consistently in patients with CF. This has suggested the presence of a membrane transport defect to many investigators. Because of their ease of availability, RBC have been studied by several workers in an attempt to document an abnormal transport mechanism. The results of these investigations have been remarkably inconsistent.

The content of Na^{+} in CF RBC has been uniformly normal [2, 3, 9]. Balfe *et al.* [2] have described a small decrease in ouabain-sensitive Na^{+} efflux, and a marked reduction in the ouabain-insensitive Na^{+} efflux. This

latter was caused by deficiencies in both the ethacrynic acid-sensitive and -insensitive portions. Paradoxically, they noted decreased ouabain-sensitive (Na^{+} - K^{+})ATPase in CF erythrocyte membranes, whereas the ouabain-insensitive portion was normal.

Lapey and Gardner [9] have also observed a decrease in the ethacrynic acid-sensitive portion of Na^{+} efflux in RBC of homozygous CF males and older females, but the RBC transport function of younger female subjects and heterozygotes was normal. They did not report any studies of RBC ATPase activity.

Cole and Dirks [3] have examined ATPase activity in CF RBC, but have not studied Na^{+} transport. They observed normal ouabain-sensitive ATPase and dimin-

ished ouabain-insensitive ATPase in CF RBC membranes. Cole and Dirks also studied the effect of preincubation of normal RBC in CF and normal plasma. Their data suggests that CF plasma may contain a factor which reduces the ouabain-sensitive but not the ouabain-insensitive ATPase of normal cells [3].

Horton *et al.* [8] have studied ATPase activity in RBC of CF heterozygotes and homozygotes. They reported normal (Na⁺-K⁺)- and (Mg²⁺)ATPase activities, but decreased (Ca²⁺)ATPase activity in CF homozygotes. The degree of abnormality was directly proportional to the clinical severity of disease. They found normal ATPase activities in the RBC of heterozygotes, however.

Hadden *et al.* [6] have recently reported totally normal ⁸⁶Rb and ⁸⁵Sr transport in CF erythrocytes. Because Rb⁺ and Sr²⁺ are transported by the same mechanisms as Na⁺ and Ca²⁺, respectively, they inferred that Na⁺ and Ca²⁺ transport is normal in CF cells. These authors observed no deficiency of ethacrynic acid-sensitive Na⁺ efflux.

We have studied CF RBC membranes in an attempt to clarify the rather confusing picture created by this contradictory series of previous reports. We were totally unable to confirm any abnormality of ATPase function in CF heterozygotes or homozygotes, and also failed to confirm any abnormality of Na⁺ or K⁺ transport in CF RBC.

Methods

Patients with CF were selected from the clinics of the UCLA Center for the Health Sciences and the Strong Memorial Hospital on the basis of their willingness to undergo venipuncture. Obligate heterozygotes were similarly chosen from among the mothers of CF patients. Normal subjects were chosen at random from the clinical, laboratory, and secretarial staffs of the clinics.

For the ATPase assays, cells were washed and lysed by the method of Nakao *et al.* [11]. Protein content was

determined by the method of Lowry *et al.* [10]. Incubations for (Na⁺-K⁺)ATPase were performed by the method of Post *et al.* [13]. Filtrates were prepared with cold 10% trichloroacetic acid.

Incubations for (Ca²⁺)- and (Mg²⁺)ATPase were performed in 50 mM Tris-maleate buffer, pH 6.8, at which pH maximum activity was obtained [4]. The (Mg²⁺)ATPase was defined as the inorganic phosphate (P_i) liberated from ATP in the presence of Mg²⁺ less that liberated in the absence of Mg²⁺. (Ca²⁺)ATPase was defined as the P_i liberated in the presence of Mg²⁺ and Ca²⁺, less that liberated in the presence of Mg²⁺ alone. The concentrations of Mg²⁺ (0.01 M) and Ca²⁺ (0.001 M) were chosen to maximize the reaction rate [4]. The concentration of free Ca²⁺ was calculated from the binding constant of ethylene glycol tetraacetic acid [12] which was present in a final concentration of 0.001 M. ATP was present in a concentration of 0.0025 M, which provided maximum reaction velocity.

The reaction was performed in a volume of 0.5 ml at 37° in an oscillating water bath. The reaction was started by the addition of Tris-ATP, neutralized with KOH. It was stopped by the addition of 0.5 ml iced 10% trichloroacetic acid.

All ATPase incubation filtrates were analyzed for the concentration of P_i by the method of Ames and Dubin [1]. To avoid thermal hydrolysis of ATP, the color was allowed to develop at room temperature and a standard curve of OD *versus* [P_i] was performed with every experiment.

Measurements of ²⁴Na⁺ and ⁴²K⁺ transport were performed [5]. Ouabain-induced Na⁺ accumulation and K⁺ loss were also measured without isotopes. All transport incubations were performed in Krebs-Henseleit buffer as described previously [5].

Results and Discussion

Initial studies of the Ca²⁺- and Mg²⁺-ATPase activities of CF erythrocyte membranes were entirely within

Table I. ATPase activity of cystic fibrosis (CF) erythrocyte membranes¹

	(Mg ²⁺)ATPase	(Ca ²⁺)ATPase	(Na ⁺ -K ⁺)ATPase		
			Total	Ouabain sensitive	Ouabain insensitive
Normal	205 ± 82 (64)	594 ± 276 (64)	488 ± 151 (35)	275 ± 113 (35)	202 ± 76 (35)
CF homozygotes	244 ± 71 (24)	671 ± 245 (24)	467 ± 114 (20)	239 ± 64 (20)	228 ± 123 (20)
CF heterozygotes	237 ± 68 (15)	692 ± 300 (15)	494 ± 85 (15)	293 ± 58 (15)	200 ± 61 (15)

¹ Activity expressed as nanomoles of P_i liberated per milligram of protein per hour ± SD. Numbers in parentheses indicate number of observations.

Table II. Monovalent cation transport in cystic fibrosis (CF) erythrocytes¹

	K ⁺		Na ⁺	
	CF	Normal	CF	Normal
Isotope flux				
Total	1.6 ± 0.1	1.7 ± 0.3	3.1 ± 0.3	2.9 ± 0.3
Ouabain sensitive	1.2 ± 0.1 (4)	1.3 ± 0.3 (6)	2.2 ± 0.2 (4)	2.0 ± 0.2 (6)
Ouabain insensitive	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.9 ± 0.2
Nonisotope flux				
Ouabain induced (K ⁺ leak or Na ⁺ accumulation)	1.2 ± 0.3 (7)	1.2 ± 0.1 (7)	1.5 ± 0.2 (7)	1.4 ± 0.1 (7)

¹ Values expressed as milliequivalents per liter of cells per hour ± SD. Numbers in parentheses indicate number of observations.

normal limits (Table I). We could not confirm the deficiency of Ca²⁺-ATPase reported by Horton *et al.* [8]. No abnormalities were observed in CF heterozygotes.

Furthermore, examination of (Na⁺-K⁺)ATPase activity did not suggest any abnormality in homo- or heterozygote CF erythrocyte membranes (Table I). Our data do not support the previous observations of decreased ouabain-sensitive [2] or ouabain-insensitive [3] (Na⁺-K⁺)ATPase.

Active Na⁺ and K⁺ transport were examined in normal and homozygote CF erythrocytes (Table II). Potassium flux, measured by isotope influx or ouabain-induced loss, was entirely normal in CF RBC. Studies of Na⁺ flux in CF cells were entirely within normal limits also.

We calculate from the data of Balfe *et al.* [2] a 44% decrease in ouabain-insensitive Na⁺ efflux. The calculation is based upon the observation of Hoffman [7] that the ethacrynic acid-sensitive portion is at least 65% of the ouabain-insensitive efflux. Our data show no significant decrease in ouabain insensitive Na⁺ efflux in CF erythrocytes. Therefore, no further studies to dissect the ouabain-insensitive efflux seemed to be indicated.

Summary

These studies demonstrate normal transport function in CF RBC. They were performed on membranes and intact cells in a buffer system. Although the intrinsic transport capability of these cells is normal, these experiments do not examine any effect of CF plasma on normal RBC function. In view of the observations of a factor in CF serum capable of affecting cellular functions [14], further studies must be performed to deter-

mine whether this factor is capable of altering RBC function, as suggested by Cole and Dirks [3].

References and Notes

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15. The authors appreciate the excellent technical assistance of Mr. Paul Dutcher and Mrs. Sara Bassilian.
  16. This research was supported in part by General Research Support Grant no. RR 5354 from the United States Public Health Service and by grants from the National Cystic Fibrosis Research Foundation.
  17. Requests for reprints should be addressed to: S. A. FEIG, M.D., Department of Pediatrics, UCLA School of Medicine, Los Angeles, California 90024 (USA).
  18. Accepted for publication January 14, 1974.