Depressed (Ca^{++})-Transport ATPase in Cystic Fibrosis Erythrocytes

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(Ca^{++})-ATPase activity was studied in enzyme preparations isolated from erythrocytes of cystic fibrosis and control patients. At 3 x 10^{-4}M Ca^{++}, specific activity of (Ca^{++})-ATPase in cystic fibrosis was 0.60 umoles Pi/mg protein/hour while in controls it was 0.87 μmoles Pi/mg protein/hour. No significant difference in Mg^{++}-ATPase and (Na^{+} + K^{+})-ATPase activities was found. The depression of (Ca^{++})-ATPase activity was closely related to the severity of the disease. This suggests that a major defect in cystic fibrosis may be in the (Ca^{++})-ATPase portion of membrane transport systems rather than in the (Na^{+} + K^{+})-ATPase portion.

An increase in calcium levels of many glycoprotein-rich secretions is one of the most constant findings in cystic fibrosis (1). This indicates a defect in Ca^{++} reabsorption due to a depressed Ca^{++} -transport mechanism. Such a Ca^{++} -transport mechanism which transports Ca^{++} out of the cell was recently found in the cell membrane of human erythrocytes (2). The energy for this outward transport is derived from a (Ca^{++})-ATPase situated in the red cell membrane. This active outward transport of Ca^{++} results in a high ratio of external to internal free Ca^{++} (3). On the basis of these reports we undertook an investigation of the (Ca^{++})-transport ATPase in erythrocytes from cystic fibrosis patients.

METHODS

We collected samples of blood from patients and from controls of both sexes. The male children ranged in age from one to sixteen years and the females ranged from three to seventeen years of age. Various anticoagulants were used: citrate, heparin and EDTA. Membrane suspensions free of hemoglobin were prepared by the method of Post et al. (4) as soon as possible after the blood was collected. The membrane suspensions were subsequently stored at

4°C. in a Tris-imidazole-histidine buffer pH 7.8. ATPase activities were determined by incubation for thirty minutes at 37° in media containing 0.2 to 0.6 mg enzyme protein, 4mM ATP, 6mM Mg^{++}, 20mM Tris-TES (N-tris (hydroxymethyl)-methyl-2-aminoethanesulfonic acid) pH 7.5, 0.3mM ouabain and 0.1 mM EGTA (ethyleneglycol-bis (aminoethyl)-tetraacetic acid). (Ca^{++})-ATPase activity was determined by the addition of increasing concentrations of Ca^{++}. Mg^{++}-ATPase was determined in the absence of Ca^{++}. (Na^{+} + K^{+})-ATPase activity was determined in the above media by addition of 150mM Na^{+} and 10mM K^{+} and by omitting Ca^{++} and ouabain. Protein determinations were done by the method of Lowry et al. (5) and inorganic phosphate determinations were by the method of Post and Sen (6). Specific activities were reported as umoles of inorganic phosphate released from ATP per mg enzyme protein per hour.

RESULTS AND DISCUSSION

Figure 1 illustrates the (Ca^{++})-dependent ATPase activity as a function of Ca^{++} concentration. The Mg^{++}-ATPase and the (Na^{+} + K^{+})-ATPase activities are shown on the ordinate. Calcium activation appears at 10^{-8}M Ca^{++} and increases to 3 x 10^{-7}M Ca^{++} at which point the rate of increase slows and a plateau is

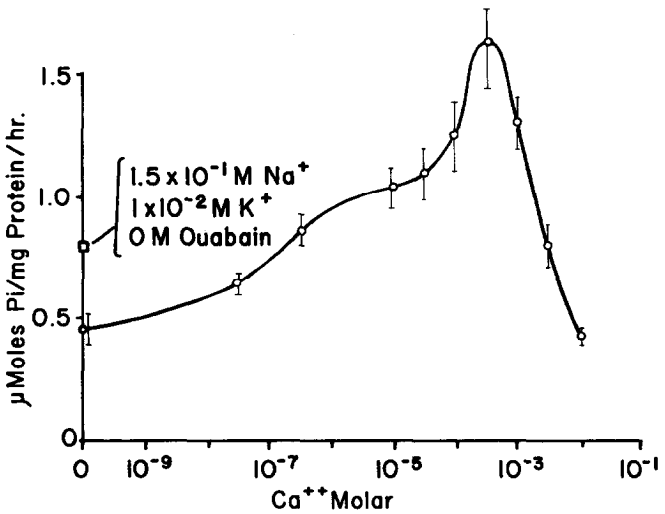

Figure 1. (Ca^{++})-Dependent ATPase Activity in Human Red Cells. Each point represents the mean of four experiments with the standard error of the mean. The free Ca^{++} concentration was calculated from the ratio of EGTA to Ca^{++} (10).

observed up to 3 x 10^{-5}M Ca^{++}. The activity then increases rapidly to an optimum at 3 x 10^{-4}M Ca^{++} in agreement with that reported by other investigators (2,7) and decreases at higher calcium concentration. A comparison of (Ca^{++})-ATPase activity to (Na^{+} + K^{+})-ATPase activity results in a ratio of about 3 in agreement with that reported by Lee and Shin (3). The plateau in the activity curve suggests the presence of two enzyme activities, one at a low calcium concentration and the other at a higher calcium concentration.

Figure 2 shows (Ca^{++})-ATPase activity (Mg^{++}-ATPase activity subtracted) of cystic fibrosis and control enzymes in the range of maximal Ca^{++} activation. The activity in cystic fibrosis enzymes is clearly lower.

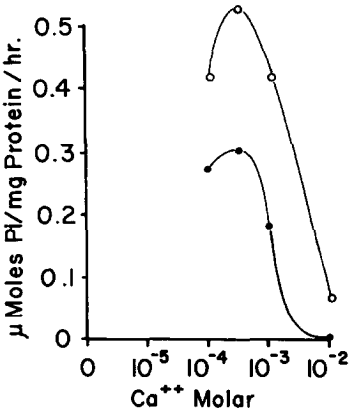

Figure 2. (Ca^{++})-Dependent ATPase Activity in Cystic Fibrosis and Control Red Cells. o-o controls, •-• cystic fibrosis patients. The Mg^{++}-ATPase was subtracted from each value.

The table summarizes the data for various ATPase activities in patients and controls. There is a significant depression of (Ca^{++})-ATPase activity in cystic fibrosis red cells while there is no significant difference in Mg^{++}-ATPase activities and (Na^{+} + K^{+})-ATPase activities between patient and control red cells. Balfe et al. (8) reported a depression of ouabain sensitive (Na^{+} + K^{+})-ATPase in five out of a total of seven cystic fibrosis patients; in our more complete study a comparison of twenty-five controls with twenty-four patients showed no significant difference in (Na^{+} + K^{+})-ATPase activities.

TABLE

Depression of (Ca^{++})-Dependent ATPase Activity

Enzyme	(Ca^{++})-ATPase	(Na^{+} + K^{+})-ATPase	Mg^{++}-ATPase
Specific Activity			
Controls (25)	0.87±0.04	0.34±0.02	0.58±0.03
Patients (24)	0.60±0.03	0.36±0.03	0.52±0.02
Parents of patients (7)	0.83±0.06	0.37±0.01	0.48±0.03

The numbers in parenthesis show numbers of individuals tested. (Ca^{++})-ATPase was tested at 3 x 10^{-4}M Ca^{++}. The specific activity values are reported ± the standard error of the mean. The difference between control and patient (Ca^{++})-ATPase was significant ($p < 0.001$) while differences in other ATPase activities were not ($p > 0.1$).

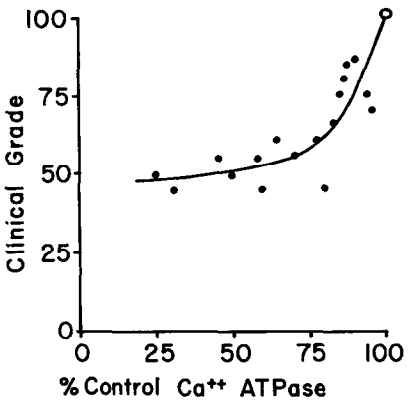

Figure 3. Correlation Between Severity of Cystic Fibrosis and Depression of (Ca^{++})-ATPase. o-control, •-cystic fibrosis. Control at 100% on each axis.

Figure 3 illustrates the correlation between the depression of the (Ca^{++})-ATPase activity and the clinical grade (9) of the patients. This grade is a measure of the severity of the disease. There is clearly a correlation between depression of enzyme activity and clinical grade. This suggests that a major defect in cystic fibrosis may be in the (Ca^{++})-ATPase portion of membrane transport systems rather than in the (Na^{+} + K^{+})-ATPase portion.

ACKNOWLEDGMENTS

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