CYSTIC FIBROSIS: EFFECT OF MEDIA FROM CULTURED CYSTIC FIBROSIS FIBROBLASTS ON ATPase ACTIVITY

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SUMMARY

Membrane associated ATPase activities of human red blood cells were examined following the addition of spent fibroblast culture media from normal and cystic fibrosis cell lines. In comparison to normal and unspent media, the cystic fibrosis media consistently depressed the ATPase activities.

A factor or factors derived from cystic fibrosis fibroblast media and sera have been reported to cause cessation or asynchrony of ciliary activity on oyster gills (1,2). Investigations to elucidate the nature of this inhibitory activity are continuing (3). The various ion dependent ATPases are likely candidates. Earlier studies have indicated a defect in Ca^{++} reabsorption, due to a depressed Ca^{++} transport mechanism in C/F individuals (4). Depressed transport activity, both Ca^{++} (transport) ATPase (5) and ouabain sensitive ATPase (6), has been found in C/F erythrocytes. It has been postulated by Cole (7) that the toxic factor found in the plasma of patients with C/F might interfere with the metabolism of normal erythrocytes. Using normal erythrocyte membranes as a source of membrane ATPases, we

Abbreviation: C/F, cystic fibrosis

have examined the effect of spent media from normal and C/F fibroblast cultures on a number of ion dependent ATPases.

Materials and Methods

Human fibroblast cells of normal and of C/F individuals were purchased from the American Type Culture Collection Cell Repository, Rockville, Maryland. The C/F cell lines were designated CRL 1154 and CRL 1143. The normal fibroblast cell line was CRL 1147. The fibroblast cells were grown in a 5 to 10% CO_2 atmosphere with a Hsu's Modified McCoy's 5A medium (Gibco) containing 10% fetal calf serum. Culture methods of Bowman and coworkers (2) were followed. Medium was collected and replenished at 2 to 3 day intervals from cultures of confluent-nondividing cells. Culture media were pooled and stored frozen until concentrated. Media within a dialysis membrane with a retention of 3,500 daltons (Spectrum Industries) were concentrated by applying Ficoll (Pharmacia Fine Chemicals) externally to the membrane at 5°C. Thirty-fold concentration was completed in 2 days. The concentrated medium was dialyzed exhaustively against 0.02M Tris-Tes (N-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid) at pH 7.5 in the above dialysis membrane.

Erythrocyte membranes were prepared free of hemoglobin by the method of Post (8). Outdated human blood was used as the source of erythrocytes. Membrane suspensions were stored at 5°C in 0.008M Tris-HCl at pH 7.5 for no more than one week prior to use.

Red blood cell membrane ATPase activities were examined by incubation of 1 mg of membrane protein, 0.7 to 3.5 mg of fibro-

Table 1. Effect of C/F and Normal Media on Red Blood Cell Membrane ATPase Activity.

Preparation 1

ATPase	C/F	Normal	Tris-TES
Na^+/K^+	3.00	6.50	1.00
Mg^{++}	2.66	5.57	0.75
$Ca^{++}10^{-6}$	3.30	7.00	1.00
$Ca^{++}10^{-5}$	2.16	7.00	0.90
$Ca^{++}10^{-4}$	2.50	5.00	2.25

Preparation 2

ATPase	C/F	Normal	Unspent Media
Na^+/K^+	0.94	2.50	5.1
Mg^{++}	1.21	4.97	5.0
$Ca^{++}10^{-6}$	1.20	3.25	2.9
$Ca^{++}10^{-5}$	1.21	2.75	2.5
$Ca^{++}10^{-4}$	1.20	3.50	4.8

Preparation 3

ATPase	C/F	Normal
Na^+/K^+	0.18	0.43
Mg^{++}	0.34	0.61
$Ca^{++}10^{-6}$	0.52	0.98
$Ca^{++}10^{-5}$	0.75	0.88
$Ca^{++}10^{-4}$	0.38	0.88

ATPase activity is reported in ugPi/mg membrane protein/hour. Each membrane preparation was tested for Na^+/K^+ ATPase, Mg^{++} ATPase and Ca^{++} ATPase at 10^{-6} M, 10^{-5} M and 10^{-4} M $CaCl_2$ concentrations. Membrane preparation 1 represents the effect of 0.7 to 1.5mg of media proteins from indicated sources. Preparation 2 represents the effect of 2.0 to 2.7mg of media proteins on ATPase activity. Preparation 3 represents the effect of 3.0 to 3.5mg of media proteins on ATPase activity.

blast medium proteins, 5 x 10^{-3} M ATP, 5 x 10^{-3} M $MgCl_2$, 2 x 10^{-2} M Tris-Tes at pH 7.5 and 3 x 10^{-4} M ouabain at 37°C for 40 minutes. Calcium ATPase was determined by the addition

of increasing concentrations of $CaCl_2$ to the above mixture (10^{-6} M, 10^{-5} M, and 10^{-4}). Ouabain was used to inhibit sodium-potassium ATPase activity. Magnesium ATPase activity was determined in the absence of $CaCl_2$ but in the presence of ouabain. Sodium-potassium ATPase activity was determined in the mixture by the addition of 0.15 M NaCl and 0.01 M KCl and by omitting $CaCl_2$ and ouabain. The quantity of inorganic phosphorus was determined by the method of Post and Sen (9). Protein estimations followed the method of Lowry and coworkers (10).

Results and Discussion

ATPase activity varied between membrane preparations depending on the source and length of storage of the red blood cells. Relative ATPase activity was consistently depressed in samples containing C/F fibroblast media in comparison to the samples containing normal fibroblast media (Table 1). Na^+/K^+ ATPase, Mg^{++} ATPase as well as Ca^{++} ATPase activities averaged 50% lower with the C/F medium.

Significantly lower ATPase activities were observed (Table 1, preparation 1) in assays containing only 0.02 M Tris-TES buffer when compared to the activities of the media preparations. Bader (11) found that immunogammaglobulins had a stimulatory effect on certain ATPase activities. This finding may explain the differences between buffer and media preparations since the media contained 10% fetal calf serum. Unused media when applied to the assay system paralleled the results of the normal media additions more closely (Table 1, preparation 2).

These data suggest that C/F fibroblasts in culture produce a substance or group of substances, which have an inhibitory effect on membrane associated ATPases.

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