Pediat. Res. *1:* 24–26 (1967)

Adenosine triphosphate
cystic fibrosis of the pancreas
enzymes
ouabain
sodium chloride
sweat gland
sodium transport

Quantitative Microdetermination of Enzymes in Sweat Gland

V. Ouabain-Sensitive Sodium-Potassium Activated Adenosinetriphosphatase

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Extract

The excessive concentration of sodium chloride in sweat in cystic fibrosis may be due to failure of a 'sodium pump' mechanism. In view of a possible relationship between electrolyte transport and ouabain sensitive ATPase, determination of this enzyme activity was undertaken in isolated sweat gland tissue from cystic fibrosis and control subjects. In each case, determinations were made with optimal Na^+, K^+ and Mg^{++} in the substrate mixture, with or without ouabain. With activities expressed as moles phosphate liberated per kg dry sweat gland tissue per hour, seven cystic fibrosis patients gave a mean ouabain sensitive ATPase activity of 2.0 ± 1.1 (SD). Seven controls gave 2.9 ± 1.6. This difference was not significant statistically ($t = 1.1$).

Speculation

Contrary to what might have been suspected from reported results on a relationship between ouabain sensitive ATPase and sodium transport in various sites, namely, crab nerve tissue, avian salt gland, human and sheep erythrocytes and various other tissues, the present study involving skin biopsies from cystic fibrosis patients and controls fails to show this enzyme to be deficient in relation to the defect in sodium transport in the sweat gland in cystic fibrosis.

Introduction

One of the distinguishing characteristics of the disease, cystic fibrosis, is an excessive concentration of sodium chloride in the sweat. That this excessive concentration may be due to some failure in the 'sodium pump' mechanism, which normally transports sodium ions out of the sweat gland and into the extracellular fluid, is a plausible theory. Many investigators have demonstrated a correlation between the active transport of sodium and the activity of certain enzymes, in particular, the enzyme ouabain-sensitive sodium-potassium activated adenosinetriphosphatase. A correlation has been noted between the activity of this enzyme and the active transport of cations by Skou [7] in crab nerve tissue, by Hokin [4] in the avian salt gland, by Post *et al.* [6] in the human erythrocyte, by Bonting *et al.* [1] in various tissues, and by Tosteson *et al.* [8] in sheep erythrocytes. In view of these results, which seem to indicate a parallelism between the activity of this particular ATPase and the active transport of Na^+ and K^+, we undertook to see if there was any abnormality in the activity of the enzyme in sweat glands of fibrocystic patients.

Methods

For isolation of sweat gland tissue [2], skin biopsies were taken with a high speed rotary drill of 3 mm dia-

meter [9], using 1 % procaine as a local anesthetic. The plugs (6 to 10 from each patient) were imbedded in partially frozen tragacanth gel, frozen at —20° C, and sliced at 32 μ thickness with a cryostat microtome. The slices were placed in holes (7 mm diameter) bored in aluminium plates and were dessicated under vacuum in the cryostat until the pressure was reduced to below 30 microns. When ready for use, the slices were brought to room temperature under vacuum and sweat gland fragments were dissected from the surrounding tissue. These fragments were weighed on a quartz fiber fish-pole balance [5], the weight of the dry samples being between 0.6 micrograms and 5.0 micrograms. These fragments were used as the basis for enzyme assay, a minimum of six determinations being done for each patient, the enzyme assay being done in triplicate with and without ouabain.

Enzyme Assay

The enzyme was assayed by determining the amount of inorganic phosphate formed by the action of the enzyme on adenosinetriphosphate. The inorganic phosphate formed was measured colorimetrically using the method of GOMORI [3]. The concentrations of the substrate, buffer, activating cations, and ouabain used were those reported by SKOU [7] as producing optimum activity of the ouabain-sensitive, Na^+–K^+ activated ATPase. These final concentrations were ATP–6 mM/L; Mg^{++}–6 mM/L; Na^+–100 mM/L; K^+–20 mM/L; histidine–30 mM/L; and ouabain–0.5 mM/L. In the determination of the ATPase activity without ouabain, distilled water was used in place of an aqueous solution of ouabain in the preparation of the reaction mixture. The mixtures were incubated in a Dubnoff shaking incubator for one hour at 37° C, after which they were immediately immersed in an ice bath and 0.04 ml of 2N H_2SO_4 were added. After this, the color producing reagents were added, and the optical density of the solutions was read 10 minutes later in microcells in a Beckman DU Spectrophotometer at 670 μ. From the optical densities recorded, the activity of the enzyme in moles of phosphate liberated per kilogram dry weight of sweat gland per hour of incubation was calculated. The calculation involved subtraction of incubated reagent blanks.

Results and Comment

As indicated in table I, the activity of ouabain-sensitive Na^+–K^+ activated ATPase was determined as the difference between two activities. The determina-

Table I

Cystic fibrosis			Controls		
Without ouabain	With ouabain	Difference (without ouabain—with ouabain)	Without oaubain	With ouabain	Difference (without ouabain—with ouabain)
4.66*	2.03	2.63	5.99	1.59	4.40
6.43	2.52	4.11	10.49	5.17	5.32
3.08	1.88	1.20	3.08	2.64	0.44
2.69	1.19	1.50	4.52	1.07	3.45
2.98	1.83	1.15	4.47	2.70	1.77
2.67	1.81	0.86	4.21	0.66	3.55
3.18	0.39	2.79	2.58	1.39	1.19
Mean 3.67 ± 1.39	Mean 1.66 ± 0.68	Mean $\bar{x}_1 = 2.03$	Mean 5.05 ± 2.64	Mean 2.17 ± 1.52	Mean $\bar{x}_2 = 2.87$
		S.D.$_1$ = 1.09			S.D.$_2$ = 1.64

Standard error of the difference = $\sqrt{\frac{(S.D._1)^2}{n_1} + \frac{(S.D._2)^2}{n_2}} = 0.75$ Significance = $\frac{x_2 - x_1}{S.E.D.} = 1.12$

* Each figure is the average of 3 determinations. It represents moles of phosphate liberated per kilogram of dry sweat gland, per hour.

Cystic fibrosis patients were of the following age and sex: 2 years M, 3 years M, 20 years M, 16 years F, 9 years M, 2 years M, 6 years F. Controls: 8 years M – skin infection, 6 years M—battered child, 5 years M—normal, 17 years M—pneumonia, 18 months M—anemia, 23 years M—normal, 5 years M—postencephalitis.

tions conducted without ouabain indicate the total activity of all ATPases which may be found in the sweat gland, while those determinations with ouabain indicate the activity of those ATPases not sensitive to ouabain. Thus the difference between the activities without and with ouabain indicates the activity of the ouabain-sensitive ATPase. No significant difference was found in the activity of this ouabain-sensitive, Na^+–K^+ activated ATPase between normal and fibrocystic sweat gland. The effect of ouabain has been considered to be to offset the Na^+–K^+ activation [4].

In order to determine whether any inorganic phosphate was being produced by reactions other than the activity of the ATPases, dry sweat gland fragments were kept at atmospheric pressure and room temperature for one or more days, and then run through the same procedure of determining enzyme activity. In almost all cases, no inorganic phosphate was detected, and in those instances where it was found, the amount was negligible compared to the inorganic phosphate formed by the activity of the ATPases. The mean of the total ATPase activity (without ouabain) was 3.67 for fibrocystic sweat gland, which agrees quite well with results obtained in this laboratory before [2]. The mean of the total ATPase activity in the normal sweat gland, 5.05 was somewhat higher than that reported previously.

Summary

Because of the reported correlation between activity of ouabain-sensitive Na^+–K^+ activated ATPase and active cation transport in certain tissues, this particular ATPase was studied in the normal and fibrocystic sweat gland. No significant difference was found.

References and Notes

1. BONTING, S.L.; CARAVAGGIO, L.L. and HAWKINS, N.M.: Studies on Sodium-Potassium—activated adenosinetriphosphatase. IV. Correlation with cation transport sensitive to cardiac glycosides. Arch. Biochem. *98:* 413 (1962).
2. GIBBS, G.E.; REIMER, K.; KOLLMORGEN, R.L. and YOUNG, P.G.: Quantitative microdetermination of enzymes in sweat gland. Amer. J. Dis. Child. *105:* 249 (1963).
3. GOMORI, G.: A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. J. Lab. clin. Med. *27:* 955 (1942).
4. HOKIN, M.R.: Studies on a $Na^+ + K^+$-dependent, ouabain-sensitive adenosine triphosphatase in the avaian salt gland. Biochim. biophys. Acta *77:* 108 (1963).
5. LOWRY, O.H.: A quartz fiber balance. J. biol. Chem. *140:* 183 (1941).
6. POST, R.L.; MERRITT, C.R.; KINSOLVING, C.R. and ALBRIGHT, C.D.: Membrane adenosinetriphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. biol. Chem. *235:* 1796 (1960).
7. SKOU, J.C.: Further investigations on a $Mg^{++} + Na^+$-activated adenosinetriphosphatase, possibly related to the active, linked transport of Na^+ and K^+ across the nerve membrane. Biochim. biophys. Acta *42:* 6 (1960).
8. TOSTESON, D.C.; MOULTON, R.H. and BLAUSTEIN, M.: An enzymatic basis for the difference in active cation transport in two genetic types of sheep red cells. Fed. Proc. *19:* 128 (1960).
9. URBACH, F. and SHELLEY, W.B.: A rapid and simple method for obtaining punch biopsies without anesthesia. J. invest. Derm. *17:* 131 (1951).
10. This investigation was supported by USPHS Research Grant AM-03154 and by a Care, Teaching and Research Center Grant of the National Cystic Fibrosis Research Foundation.