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A NEW TYPE OF GLYCOGEN STORAGE DISEASE CAUSED BY DEFICIENCY OF CARDIAC PHOSPHORYLASE KINASE*

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SUMMARY. A five-month-old Japanese boy was found to have marked glycogen accumulation only in the heart. A survey of enzymes revealed normal activities of phosphorylase, cyclic AMP-dependent protein kinase, acid maltase and amylo-1,6-glucosidase. However, the heart had capacity of activating neither rabbit muscle phosphorylase b nor endogenous phosphorylase b, which was converted to active form only when supplemented rabbit muscle phosphorylase kinase. In contrast to the heart, activities of phosphorylase kinase were found within normal levels in other organ tissues so far tested. These findings indicate that the present case of the cardiac glycogenosis is caused by deficiency of cardiac phosphorylase kinase.

Glycogen phosphorylase kinase (EC 2.7.1.38) is one of the key enzymes of glycogen metabolism. This enzyme has been purified from skeletal muscle[1], liver[2,3] and heart[4] and its properties have been well investigated. It appears that these enzyme molecules differ from one another[5]. Phosphorylase kinase from white skeletal muscle is a multisubunit enzyme with a composition of α_4, β_4, γ_4 and δ_4, where the δ-subunit is identical to the calcium-binding

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protein termed calmodulin[6]. Furthermore, this enzyme can be activated through the interaction of a second molecule of calmodulin (termed δ'-subunit)[7]. Cardiac phosphorylase kinase differs from the white muscle isozyme in that a slightly smaller α'-subunit replaces the α-subunit[8] and exogenously added calmodulin (δ'-subunit) cannot be an activator[9]. In addition, it is not presently known that the cardiac enzyme contains the δ-subunit. Although liver phosphorylase kinase contains an α-subunit identical in molecular weight to the white skeletal muscle isozyme[3], these enzymes are immunologically distinguishable[10,11]. The presence of phosphorylase kinase isozymes was first suggested during the study of glycogen storage disease. Several reports show the phosphorylase kinase deficiency in liver but not in skeletal muscle [12-14]. In muscle phosphorylase kinase-deficient mice, various organs such as heart, aorta and brain showed low phosphorylase kinase activity[15,16]. However, to our knowledge, deficiency of only cardiac phosphorylase kinase has not been reported. In the present study, we characterized a new type of glycogen storage disease in which phosphorylase kinase is deficient only in the heart.

MATERIALS AND METHODS

The patient was a Japanese boy who died at 5-months of sudden aggravation of heart failure. Autopsy was performed 13 hours after death. The organ specimens were quickly frozen at -80°C. As control, the specimens of a 2-year-old girl who died of idiopathic respiratory distress syndrome were used. Before the assay of enzymes, samples were thawed, weighed and homogenized at 0-4°C with 5 volumes of 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose and 0.1 mM phenylmethylsulfonyl fluoride using an Ultra-turrax. The homogenates were centrifuged at 0-4°C for 20 min at 16,500 x g. The supernatant was dialyzed against 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 10 mM 2-mercaptoethanol and 0.1 mM phenylmethylsulfonyl fluoride and used for the assay of phosphorylase kinase, phosphorylase and cyclic AMP-dependent protein kinase. For the assay of acid maltase and amylo-1,6-glucosidase, samples were homogenized with 9 volumes of water, centrifuged for 10 min at 1,000 x g and the supernatant was used.

Phosphorylase kinase was assayed by measuring the conversion of rabbit skeletal muscle phosphorylase b to phosphorylase a as described previously (11). The activity of total (active plus inactive) phosphorylase was measured in the presence of 1 mM 5'-AMP for heart and skeletal muscle, or with 1 mM 5'-AMP and 10 %

ethylene glycol dimethyl ether for liver, kidney and spleen. Cyclic AMP-dependent protein kinase was assayed as described previously (17). Acid maltase was assayed by measuring the formation of glucose from maltose (18). The activity of amylo-1,6-glucosidase was measured by the incorporation of ^{14}C-glucose into glycogen (18). Glycogen was determined as glucose with anthrone reagent (19).

Rabbit skeletal muscle phosphorylase b and phosphorylase kinase were purified by the methods of Fischer and Krebs (20) and Cohen (1), respectively. Cyclic AMP-dependent protein kinase was purified from rabbit skeletal muscle as described previously (17) and further purified by Sephadex G-100 column chromatography. Other chemicals were obtained from commercial sources.

RESULTS AND DISCUSSION

Autopsy of the patient showed apparent cardiac enlargement (weighing 160 g) with a high glycogen content in the heart, 300-fold higher in the patient than in the control heart (Table I). Laboratory examinations including measurement of glycogen content, PAS and Best's carmine stainings revealed no abnormal deposition of glycogen in the liver, skeletal muscle or the other systemic organs[1]. As shown in Table I, the activities of acid maltase, amylo-1,6-glucosidase, phosphorylase and cyclic AMP-dependent

TABLE I

Glycogen content and enzyme activities in the heart

	Control	Patient
Glycogen content (% wet weight)	0.020	6.203
Acid maltase (nmol/min/mg protein)	0.246	0.306
Amylo-1,6-glucosidase (nmol/min/mg protein)	1.95	4.18
Phosphorylase kinase (μmol/min^2/mg protein)	0.32	Not detectable
Total phosphorylase (μmol/min/mg protein)	0.432	0.272
Cyclic AMP-dependent protein kinase (nmol/min/mg protein)		
without cyclic AMP	0.30	0.33
with cyclic AMP	0.53	0.43

Experimental conditions for each assay are described under Materials and Methods.

[1]Histopathological details will be submitted elsewhere.

TABLE II

Phosphorylase kinase activity in various organs

	Control	Patient
	(μmol/min^2/mg protein)	(μmol/min^2/mg protein)
Heart	0.32	Not detectable
Liver	0.042	0.104
Muscle	5.8	13.9
Kidney	0.094	0.082
Spleen	0.054	0.083

The control heart, liver and muscle samples were obtained as described under Materials and Methods. The kidney and spleen samples were obtained from a 26-day-old girl who died of congenital heart disease. The mean values of the enzyme activity were measured in duplicates.

protein kinase were within normal range, however, phosphorylase kinase activity is not detectable in the patient heart. Table II shows phosphorylase kinase activity in various organs. Except for the heart, the values of other organ tissues of the patient, such as liver, muscle, kidney and spleen did not differ significantly from the values of control. The data shown are for the left ventricle but the results were the same for the right auricle (data not shown). These results indicate that the lack of phosphorylase kinase is the cause of the abnormal accumulation of glycogen in the heart. Additional evidence to support this finding is shown in Fig. 1. The patient heart preparations were unable to activate endogenous phosphorylase, which was converted to active form only when supplemented with rabbit muscle phosphorylase kinase. Muscle phosphorylase kinase alone had no phosphorylase activity (data not shown). Cyclic AMP-dependent protein kinase had no effect on the activation.

Among many types of glycogen storage disease, Pompe's disease is known to involve abnormal deposition of glycogen in the heart, with death from heart failure early in life. However, in Pompe's disease the abnormal deposition of glycogen is found not only in the heart but also in liver, muscle and other organs. The present

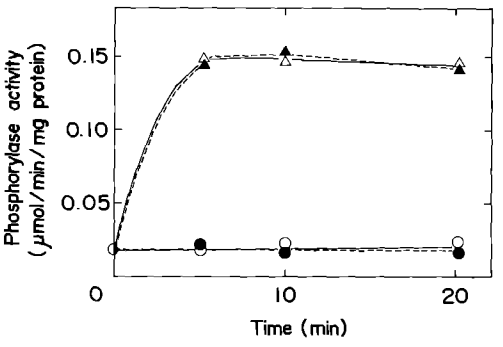

Fig. 1. *Activation of endogenous phosphorylase. Effect of rabbit muscle phosphorylase kinase and cyclic AMP-dependent protein kinase.* The preincubation mixture (50 μl) contained 2.5 μmoles of Tris-HCl at pH 7.4, 10 nmoles of $CaCl_2$, 1 μmole of 2-mercaptoethanol, 0.1 nmole of cyclic AMP, 50 nmoles of ATP, 0.5 μmole of $MgCl_2$, 0.85 μmole of creatinephosphate, 2 units of creatine phosphokinase and 192 μg protein of the supernatant fraction from the patient heart. Where indicated, 9 μg of rabbit skeletal muscle phosphorylase kinase and 2 μg of cyclic AMP-dependent protein kinase were added to the mixture. The mixture was incubated at 30°C for the time indicated in the figure. The reaction was stopped by the addition of 0.8 μmole of EDTA, and measured the activity of produced phosphorylase a as described in Materials and Methods. The incubation time was 10 min. (○——○), without rabbit muscle phosphorylase kinase and cyclic AMP-dependent protein kinase; (△——△), with phosphorylase kinase; (●----●), with cyclic AMP-dependent protein kinase; (▲----▲), with phosphorylase kinase and cyclic AMP-dependent protein kinase.

case differs from Pompe's disease in that the accumulation was specific to the heart and acid maltase activity was within normal range, and it is postulated that deficiency in cardiac phosphorylase kinase is responsible for the cardiac glycogenosis. If one or more of the subunits of cardiac phosphorylase kinase are coded by different genes from those of other isozymes, it would be reasonable to expect that a deficiency of the enzyme might be limited to the heart. It appears unlikely for the following reasons, although not completely excluded, that the enzyme was inactivated in the deep freeze: (a) The phosphorylase kinase activity was normal in the patient's liver, muscle, kidney and spleen. (b) The patient heart showed normal activity of other enzymes such as phosphorylase, cyclic AMP-dependent protein kinase, acid maltase and amylo-1,6-glucosidase. (c) The control hearts, which were

preserved in the same manner as the patient heart, showed normal phosphorylase kinase activities.

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