HEPATIC PHOSPHORYLASE DEFICIENCY: A BIOCHEMICAL STUDY

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SUMMARY: Two boys with hepatomegaly had increased glycogen content in the liver and no activity of liver phosphorylase, even in the presence of 5'AMP. The biochemical differences between phosphorylase- and phosphorylase b kinase deficiency are discussed, and a differential diagnostic procedure proposed.

In 1959, Hers (1) reported cases of glycogen accumulation in the liver with a low phosphorylase activity in this organ. Hülsmann et al. (2) and Williams and Field (3) showed that in those patients the low hepatic phosphorylase activity is reflected by a low phosphorylase activity in the leucocytes. Huijing (4) subsequently showed that in the majority of those cases the leucocytes showed a deficiency of the phosphorylase activating system rather than a deficiency of phosphorylase itself. This deficiency is expressed by an increased K_m value of phosphorylase b kinase for the substrate phosphorylase b (4). It will be shown that the low leucocyte phosphorylase activity of these phosphorylase kinase defective patients is strongly stimulated by 5'AMP, whereas in controls no or little influence has been found. In a few patients, however, the low residual phosphorylase activity is not stimulated by 5'AMP, suggesting deficiency of phosphorylase itself. Only few cases of true liver phosphorylase deficiency have been reported (5,6,7). It is the purpose of the present report to present the biochemical results obtained with various tissues from two Turkish brothers, who were suffering from hepatic phosphorylase deficiency. Furthermore, it will be reported that with the leucocyte phosphorylase acti-

vity assay in combination with glucagon administration, evidence can be obtained for hepatic phosphorylase deficiency and to differentiate this deficiency from phosphorylase b kinase deficiency. The clinical part of this study will be presented elsewhere.

METHODS

Liver tissue was obtained by needle biopsy. The leucocytes were isolated by a method published elsewhere (8). The various tissues were homogenized in 0.05 M NaF. In these homogenates the following enzymes were tested: glucose-6-phosphatase (9), debranching enzyme (10, with modifications), phosphorylase (2,11) and phosphorylase b kinase (4). The phosphorylase activity was measured in two ways; the P_i liberation from glucose-1-phosphate during synthesis of glycogen (10) and the reverse reaction from glycogen to glucose-1-phosphate (3). Both assays were performed in the absence and presence of 5'AMP. Crystalline phosphorylase b was isolated from rabbit muscle according to the method of Fischer et al. (12). The obtained rabbit muscle phosphorylase exhibited activity only in the presence of 5'AMP. Glycogen was determined with the method of Huijing (13).

RESULTS

With the screening method for liver glycogen diseases (14) it was found that administration of glucagon to these two patients (who are brothers) gave a flat blood sugar response. The two patients were, therefore, suspected to have debranching enzyme deficiency (glycogenosis Type III). However, Table I shows that the activity of debranching enzyme is normal in leucocytes. The activity of the leucocyte debranching system is representative for the liver enzyme (15). Accordingly, the liver exhibits a normal debranching enzyme activity (Table II). This was only measured in

Table I

ENZYME ASSAYS AND GLYCOGEN DETERMINATION IN THE LEUCOCYTES OF TWO PATIENTS

		Patient M.T.	Patient O.T.	Controls
phosphorylase[a]	-AMP	5.6	7.9	15.8-47.9(nmoles/min/mg prot)
	+AMP	6.1	9.8	18.0-50.1
debranching enzyme		2.18	2.63	1.43-5.04 (nmoles gluc/min/mg prot)
phosphorylase b kinase[b]		0.29	0.65	0.37-0.65(U phosph.activated/min/mg prot)
glycogen		120	134	102 - 163(µg/mg prot)

[a]Measured with the spectrophotometric method with the coupled enzyme reaction(cf. ref.4)

[b]This activity is measured at a phosphorylase b concentration of 20 U/ml.

Table II

ENZYME ASSAYS AND GLYCOGEN DETERMINATION IN THE LIVER OF TWO PATIENTS

	Patient M.T.	Patient O.T.	Controls
phosphorylase -AMP[a]	0	0	see Table III(nmoles/min/mg prot)
+AMP	0	0	
debranching enzyme	6.96	-	9.09 (nmoles gluc/min/mg prot)
phosphorylase b kinase	0.61	0.45	0.27-0.98(U phosph.b act./min/mg prot)
glucose-6-phosphatase	26.2	32.9	5.9-93 (nmoles P_i/min/mg prot)
glycogen	1156	1358	480-780 (µg/mg prot)

[a]Phosphorylase activity has been measured in two ways (cf. refs. 4,12)

the liver of one of the brothers. Table I shows that the glycogen content of the leucocytes is normal, but that the glycogen content of the liver is markedly increased (Table II). The phosphorylase b kinase activity in the leucocytes is normal, but the phosphorylase activity is quite low in comparison to the control values. The phosphorylase activities of the patients are not stimulated by 5'AMP, and this is in contrast to the results obtained with phosphorylase b kinase deficient patients (Table III). In the liver of both patients the other enzyme activities measured are normal (glucose-6-phosphatase, phosphorylase b kinase), but in this tissue the phosphorylase

Table III

PHOSPHORYLASE ACTIVITIES IN THE LEUCOCYTES OF PHOSPHORYLASE KINASE DEFICIENT PATIENTS

Case	-AMP	+AMP
1	3.9	16.5
2	3.6	17.0
3	0.0	13.6
4	3.8	23.0
5	3.8	75.0
6	2.8	12.9
7	3.4	12.8
8	8.3	17.9
9	0.4	34.8
Controls	15.8-47.9	18.0-50.1

The activities are expressed as nmoles/min/mg protein.

activities are zero, even in the presence of 5'AMP (Table II). This has been measured with the two available assays for phosphorylase (see METHODS). That no activation by 5'AMP on the phosphorylase activities occurs, indicates that the phosphorylase protein is absent or structurally damaged and excludes the possibility that the phosphorylase is in the inactive b form (16). Table IV shows the control

Table IV

PHOSPHORYLASE ACTIVITIES IN THE LIVER OF CONTROLS

nr.	-AMP	+AMP
1	4.4	4.3
2	8.3	9.5
3*	2.5	10.0
4	13.0	10.0
5	13.0	11.0
6	25.1	29.1

The activities are expressed as nmoles/min/mg protein.

*This child had an insulinoma.

values for liver phosphorylase activity. In general, the addition of 5'AMP does not alter the phosphorylase activity, except in one case (nr. 3). However, this child had an insulinoma. It is known that under conditions of carbohydrate feeding (meaning a high insulin level) the phosphorylase is shut off, while the glycogen synthetase is activated. Therefore case 3 could have had considerable amounts of phosphorylase b, which would explain the in vitro stimu-

lation of 5'AMP.

Investigation of the phosphorylase activity in the cultured fibroblasts from these patients, showed that these cells exhibit normal activity (J.F. Koster, R.G. Slee and M.F. Niermeyer, unpublished results).

DISCUSSION

For practical purposes Hers and Van Hoof (17) classified all patients with hepatomegaly, growth retardation, increased hepatic glycogen content and decreased hepatic liver phosphorylase activity as glycogenosis type VI. Also Illingworth and Brown (18) consider all patients with an increased hepatic glycogen concentration, low phosphorylase activity and a not well-defined glycogenosis as glycogenosis Type VI. As has been mentioned earlier most of these cases will probably be phosphorylase kinase deficient patients (Type VIII) rather than true phosphorylase deficiency (Type VI). The two brothers described in the present paper, however, did suffer from true phosphorylase deficiency. The absence of 5'AMP stimulation of phosphorylase excludes the possibility that the enzyme is present in the inactive form. Also the low activity of the leucocyte phosphorylase is not stimulated by 5'AMP, and this is in contrast to the results obtained with phosphorylase b kinase deficient children.

Hepatic phosphorylase deficiencies were earlier reported by Hug and Schubert (5), Drummond *et al.* (6) and Guibaud and Mathieu (7). In the case described by Drummond *et al.* glucagon administration gave a small and delayed blood sugar response. Furthermore, the deficiency of phosphorylase was detected on frozen liver. It is claimed by Hug *et al.* (19) that phosphorylase is a rather labile enzyme. In our hands, the stability of phosphorylase is rather variable. In contrast to our case, the patient of Hug and Schubert showed a hyperglycemic response to glucagon administration. In case of a true phospho-

rylase deficiency this is rather difficult to understand. Glucagon administration to phosphorylase kinase defective patients gives a rise of blood sugar. However, the hyperglycemic maximum is somewhat delayed compared to control values (14). These data give evidence that the delayed hyperglycemic response is due to synthesis of 5'AMP through the intermediate formation of 3',5'-(cyclic) AMP. The possibility of increased gluconeogenesis after glucagon administration is excluded by the fact that debranching enzyme deficient patients do not respond to glucagon administration. If this hypothesis of 5'AMP stimulation of phosphorylase b after glucagon treatment is true it also explains why with phosphorylase deficiency proper no hyperglycemic response after glucagon administration occurs. Hug and Schubert have not measured the phosphorylase b kinase activity of their patient, although they stated that the activating system should have been intact. This was based on the observation that after 30 min preincubation of the patients liver homogenate, the addition of rabbit muscle phosphorylase b resulted in the demonstration of active phosphorylase. However, this activation could be due to the formation of 5'AMP during the preincubation of the liver homogenate. Moreover, kinetic data of Huijing (4) show that addition of a large excess of muscle phosphorylase b to phosphorylase b kinase deficient homogenates may result in phosphorylation (activation) of phosphorylase b.

The patients described by Guibaud and Mathieu (7) in our opinion have a modified phosphorylase protein, which still responds to 5'AMP, and therefore to glucagon administration, but is an inadequate substrate for the phosphorylase b kinase.

Fibroblasts from one of our patients exhibited a normal phosphorylase activity, meaning that the enzyme of these cells is different from the hepatic enzyme. It was reported earlier (20,21) that also the muscle enzyme is different from the fibroblast enzyme.

In conclusion we think that it is possible to make the diagnosis phosphorylase deficiency with the aid of the investigation of the leucocyte enzyme activity as well as the investigation of the pattern of blood glucose changes after glucagon administration. A low leucocyte phosphorylase activity, which cannot be stimulated by 5'AMP, together with the absence of a hyperglycemic response of the patient to glucagon administration is suggestive of phosphorylase deficiency. If the low leucocyte phosphorylase activity is stimulated by 5'AMP and the patients blood sugar increases on glucagon injection, true phosphorylase deficiency can be excluded, but a modified phosphorylase or a phosphorylase b kinase deficiency are likely to be present.

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