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THE AUTOSOMAL FORM OF PHOSPHORYLASE KINASE DEFICIENCY IN MAN : REDUCED ACTIVITY OF THE MUSCLE ENZYME

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SUMMARY : A muscle biopsy from a boy with the autosomal form of phosphorylase kinase deficiency has been analysed. The glycogen content was higher than normal; phosphorylase was mostly in the *b* form, and the activity of phosphorylase kinase was undetectable at pH 6.8 and reached about 15 % of the mean control value at pH 8.3. The residual activity could be enhanced by trypsin and inhibited by EGTA. Cyclic AMP-dependent and independent protein kinases were normally active.

Phosphorylase kinase deficiency is known to occur as a sex-linked character in both mice (1,2) and man (3). In mice the deficiency exists in muscle but not in the liver (1,2,4) whereas in man phosphorylase kinase is inactive in liver, leucocytes and erythrocytes but is active in the muscle (3,4,5). Another type of phosphorylase kinase deficiency affects both girls and boys (6) and is therefore transmitted as an autosomal character. The deficiency appears to be nearly complete in liver and partial in erythrocytes and leucocytes (4). Only recently a muscle biopsy from one of these patients has been made available, and this paper reports its chemical and enzymatic analysis.

METHODS

Phosphorylase kinase was measured in the presence of 50 U/ml of phosphorylase *b*, 50 mM-Tris-β-glycerophosphate, 6 mM-ATP and 10 mM-magnesium acetate adjusted at the indicated pH (4). Protein kinases were measured by the phosphorylation of histones in the presence and absence of cyclic AMP and of its heat-stable protein inhibitor; the activity measured in the presence of the inhibitor is attributed to cyclic AMP-independent protein kinases (7). Amylo-1,6-glucosidase was measured by the incorporation of [^{14}C]glucose into glycogen and acid α-glucosidase by its ability to form glucose from maltose (8). Proteins were determined according to Lowry *et al.* (9). Trypsin digestion was performed by incubating a 1.3 % muscle homogenate at 30°C in the presence of 40 μg trypsin per ml. Samples of this solution were removed at various times and mixed with an excess (40 times the weight of trypsin) of Soybean

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trypsin inhibitor. Other methods used in the analysis of the biopsies are those described by Hers (10) with one exception : muscle phosphorylase a was measured in the presence of 0.5 mM caffeine (11) instead of AMP.

CASE REPORT

The siblings H.L. (male) and H.H. (female) are cases 12 and 13 in the series reported previously by Lederer *et al.* (4). In this publication, both patients were reported to have a partial deficiency of phosphorylase kinase in their haemolysate whereas a more complete deficiency of this enzyme was observed in the liver of the girl (see also table I). A muscle biopsy of the boy has now been made available for biochemical analysis. Muscle from 3 to 5 subjects was used as the control for phosphorylase, phosphorylase kinase and protein kinase activity. No biochemical abnormality was detected in the control muscles except in one of them which was from a patient with type III glycogenosis. The values found in the latter sample were in the normal range except for the high glycogen content and the deficiency in amylo-1,6-glucosidase.

RESULTS

Glycogen content and enzyme activities in biopsies

In the muscle of patient H.L. (table I), the concentration of glycogen was greater than 2.4 standard deviations above the mean control value. Phosphorylase a activity was barely detectable, although total phosphorylase activity was normal, and no phosphorylase kinase activity could be detected at pH 6.8. Cyclic AMP-dependent as well as cyclic AMP-independent protein kinases, amylo-1,6-glucosidase and acid α-glucosidase activities were within the normal range.

For the purpose of comparison, table I shows data previously published (4) concerning the liver of the sister H.H. In this tissue, the glycogen concentration was markedly elevated and phosphorylase kinase activity was deeply depressed.

Characterization of the residual activity of phosphorylase kinase in the deficient muscle

Although no phosphorylase kinase activity was detectable at pH 6.8 (table I), a definite activity could be measured at higher pH values. At pH 8.3, the activity in the deficient muscle was about 10 % of the mean value of the controls (fig. 1).

Table I : Biochemical analysis of tissue samples

	MUSCLE		LIVER	
	H.L. (boy)	control	H.H. (girl)	control
Glycogen (%)	2.04	0.93 ± 0.07 (40)	12	3.2 ± 0.3 (20)
Phosphorylase (U/g)				
a	7	64 ± 11 (16)	-	-
total	100	108 ± 15 (5)	13	14.3 ± 3 (16)
Phosphorylase kinase (U/g)	0	110 ± 36 (5)	2.2	41.9 ± 4.7 (10)
Protein kinase (mU/g)				
- cyclic AMP	57	46 ± 3 (5)	-	-
+ cyclic AMP	149	135 ± 22 (5)	-	-
+ heat-stable inhibitor	10	11 ± 3 (5)	-	-
Proteins (mg/g)	127	152 ± 24 (40)	103	146 ± 11 (20)

Control values are means ± S.E.M. with the number of observations between parentheses. Amylo-1,6-glucosidase and acid α-glucosidase were in the normal range for the muscle and the liver. Phosphorylase kinase was measured at pH 6.8.

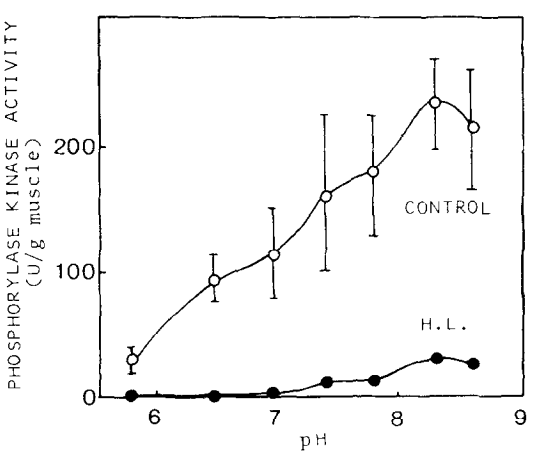
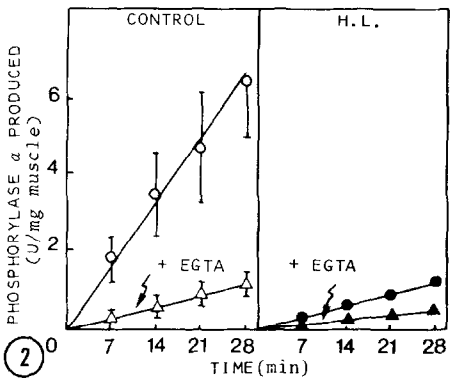


Figure 1. Effect of pH on the activity of muscle phosphorylase kinase. Control values shown are means ± S.E.M. (n = 3).



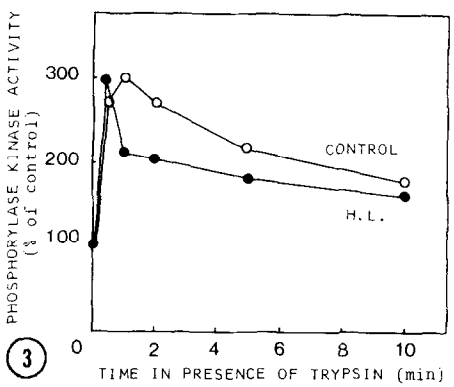


Figure 2. Effect of EGTA on the activity of muscle phosphorylase kinase. Phosphorylase kinase was measured at pH 8.3 in the presence or absence of 3 mM-EGTA. Control values shown are means ± S.E.M. (n = 3).

Figure 3. Effect of trypsin on the activity of muscle phosphorylase kinase. Phosphorylase kinase was measured at pH 8.3.

A well-known property of muscle phosphorylase kinase is to be inhibited by the calcium-chelating agent EGTA (12). It is shown in fig. 2 that this property of phosphorylase kinase could be demonstrated in both the control and the enzymatically deficient muscle. Another property of phosphorylase kinase is to be activated by trypsin (12). Figure 3 shows that trypsin digestion brought about a 3-fold activation of the residual phosphorylase kinase observed at pH 8.3 in the diseased muscle and also of the enzyme from the controls. In both cases, the maximal effect was reached after 30 seconds and was followed by a subsequent inactivation.

DISCUSSION

The decreased activity of phosphorylase kinase in the muscle of the patient H.L. offers a satisfactory explanation for the markedly reduced proportion of phosphorylase a and for the increased content of glycogen in the same tissue. Since the activity of both cyclic AMP-dependent and cyclic AMP-independent protein kinases were normal, the low activity of phosphorylase kinase cannot be attributed to a defect in the activation by protein kinase but rather to a primary defect in phosphorylase kinase itself. A deficiency of protein kinase has indeed been suggested as the primary defect in a

somewhat similar case (13). Rabbit muscle phosphorylase kinase has a high molecular weight and is known to be made of 4 different polypeptide chains with apparently specific functions (14,15,16). The fact that the residual activity of phosphorylase kinase measured in the muscle of our patient, at pH 8.3 was increased by trypsin digestion, indicates that the subunits α and β which are trypsin-sensitive were present. The sensitivity of the residual enzyme to calcium suggests that subunit δ was also present.

A deficiency of phosphorylase kinase in the muscle has been until now described only in mice where it is transmitted as a sex-linked character and does not affect the liver (1,2,4). In contrast, the sex-linked deficiency of phosphorylase kinase in humans affects liver, erythrocytes and leucocytes but not muscle (3,4,5). As indicated in the present paper, the autosomal form of the deficiency in human affects a larger number of tissues. It may be of interest to mention that very recently an autosomal deficiency of phosphorylase kinase has been found in rats where it was apparently restricted to the liver (17). It appears therefore that the complex structure of phosphorylase kinase and the equally complex genetic pattern of its deficiencies does not permit a simple conclusion concerning the primary molecular defect responsible for the disorder.

When this work was in progress we have been informed that a deficiency of muscle phosphorylase kinase in an autosomal type of glycogen storage disease had also been observed by Dr. S.W. Moses (Beer-Sheva, Israël) and his coworkers (personal communication).

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