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CYCLIC AMP-DEPENDENT INACTIVATION OF HUMAN LIVER PYRUVATE KINASE

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SUMMARY

Human liver pyruvate kinase is rapidly (within 2 min) inactivated by incubation of a human liver supernatant with cyclic AMP, when measured at suboptimal substrate concentrations. Half-maximal inactivation is reached with 0.04 μM cyclic AMP. The apparent $K_{0.5}$ for phosphoenolpyruvate shifts from 0.5 mM to 1.1 mM by incubation with cyclic AMP. It is concluded that cyclic AMP-dependent protein kinase may catalyze the phosphorylation of human liver pyruvate kinase in vivo.

Recently it has been shown that purified L-type pyruvate kinase of rat[1] and pig[2] liver can be phosphorylated by a cyclic AMP-dependent protein kinase. The phosphorylated enzyme is characterized by a lowered affinity to its substrate phosphoenolpyruvate and its allosteric activator fructose-1,6-diphosphate[2,3]. This phenomenon can also be shown with rat hepatocytes incubated with glucagon[4,5] or rat liver homogenate incubated with cyclic AMP[6].

The similarity of the results obtained with cells with those obtained with purified L-type pyruvate kinase indicates that phosphorylation of L-type pyruvate kinase might be a mechanism by which in the liver glucagon stimulates gluconeogenesis. Phosphorylation of pyruvate kinase will lead to a lowered pyruvate kinase activity resulting in a lowered cycling at the level of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and pyruvate kinase[7]. An impairment in the phosphorylation mechanism can lead to an increased cycling through the pyruvate-phosphoenolpyruvate cycle, which results in a reduced net lactate utilization. This might be one of the possible causes for lactate acidosis. No experimental data are known concerning the inactivation by cyclic AMP of human liver pyruvate kinase. Therefore, it is necessary to design experiments in which a possible phosphorylation mechanism in human liver samples can be tested. In this report we present the results obtained for human liver pyruvate kinase.

MATERIALS AND METHODS

Liver samples were obtained post mortem from human infants within

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three hours after death. There were no indications that the liver was involved in the cause of death. Material was frozen in liquid N_2 and stored at -70°C until use.

Homogenates (5% w/v) were prepared in 25 mM Tris-HCl pH 7.5. After addition of 1 mM 2-mercaptoethanol, the homogenate was centrifuged 20 min at 48 000 x g at 4°C.

Pyruvate kinase activity was measured at 30°C in an Aminco DW2 spectrophotometer by coupling with lactate dehydrogenase. In this way the pyruvate kinase activity of 0.2 mg supernatant protein can be accurately measured. Cuvettes contained in a final volume of 3 ml: 25 mM Tris-HCl pH 7.5, 200 mM KCl, 1.0 mM ADP, 0.13 mM NADH, 11 U lactate dehydrogenase and 0.2-0.5 mg protein. Mg^{2+} concentrations are indicated in the legends of the figures. After a 3 min incubation period the reaction was started by addition of the substrate phosphoenolpyruvate.

Incubations with cyclic AMP were started by mixing 0.1 ml supernatant with 0.1 ml medium containing 50 mM Tris-HCl pH 7.5, 125 mM KF, 10 mM theophylline, 10 mM $MgCl_2$, 0.4 mM ATP and 0.4 µM cyclic AMP. Incubations were performed during 2 min at 27°C and the activity of pyruvate kinase was measured. For the experiment described in Fig. 4 the medium contained 1.0 mM ATP and 10 µM cyclic AMP, while the incubation time was 5 min.

Protein was measured according to Lowry et al.[8] with bovine serum albumin as a standard.

RESULTS

Incubation of a human liver supernatant with cyclic AMP leads to a rapid inactivation of pyruvate kinase measured at suboptimal substrate concentrations (Fig. 1). In the absence of Mg-ATP no inactivation is observed. Inactivation is virtually complete within 1 min, while up to 5 min no further change in activity occurs. Controls incubated in the absence of cyclic AMP kept unchanged during the time of incubation.

Fig. 2 gives the dose-response curve of the inactivation. Half-maximal inactivation is reached with 0.04 µM cyclic AMP.

In order to investigate more closely the kinetic change in pyruvate kinase upon incubation, the phosphoenolpyruvate saturation plots are determined after incubation in the absence and presence of cyclic AMP. Supernatant incubated without cyclic AMP (Fig. 3A) as well as supernatant which is not incubated (Fig. 4) shows half-maximal activity ($K_{0.5}$) at 0.5 mM phosphoenolpyruvate. Upon incubation with cyclic AMP this value increases to 1.1 mM (Fig. 3B). The activity in the presence of an excess of the allosteric activator fructose-1,6-diphosphate is not altered upon incubation.

The discontinuity in the Hill plot (insert Fig. 3B) shows that after incubation of the enzyme with 0.2 µM cyclic AMP, two enzyme forms are present. By applying more extreme conditions for inactivation (Fig. 4), the discontinuity in the Hill plot disappears, while the $K_{0.5}$ increases still further.

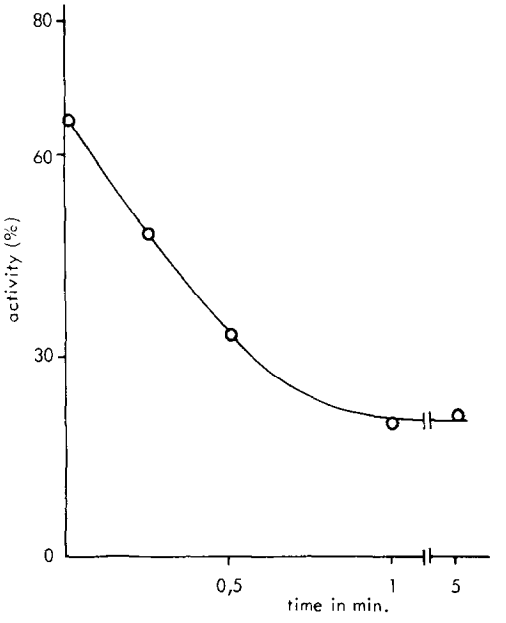

Fig. 1. Time course of the inactivation of human liver pyruvate kinase by incubation with cyclic AMP. Incubation of human liver supernatant was performed as described in Materials and Methods. Activity was measured at 0.5 mM phosphoenolpyruvate and 1.7 mM $MgCl_2$. Activity expressed as percentage of maximal activity, measured in the presence of 0.5 mM fructose-1,6-diphosphate.

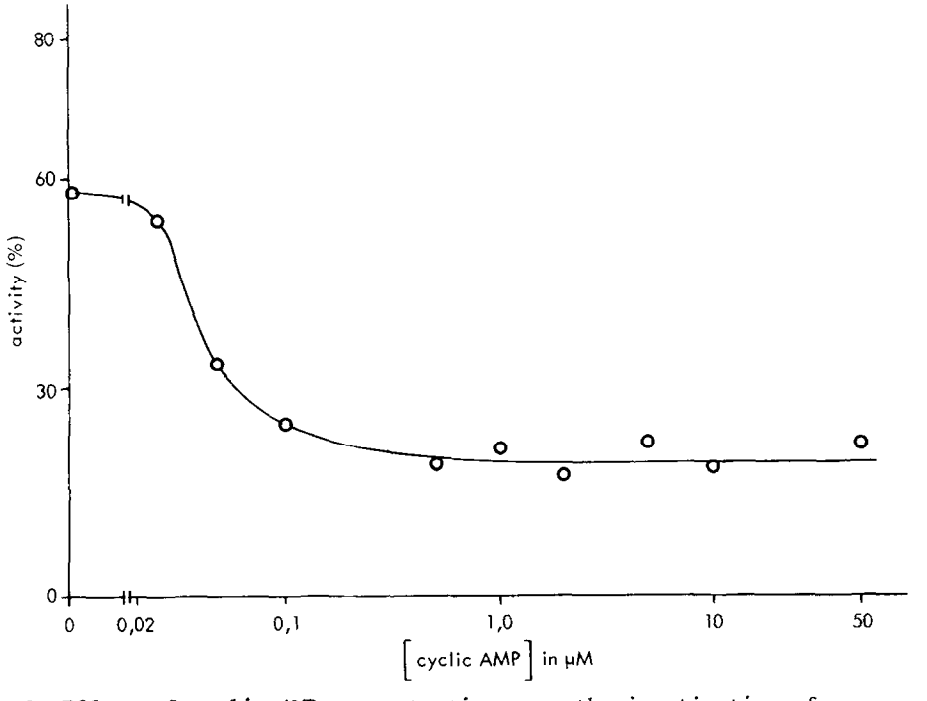

Fig. 2. Effect of cyclic AMP concentration upon the inactivation of human liver pyruvate kinase. Conditions and expression of results as in Fig. 1. Incubation time was 5 min.

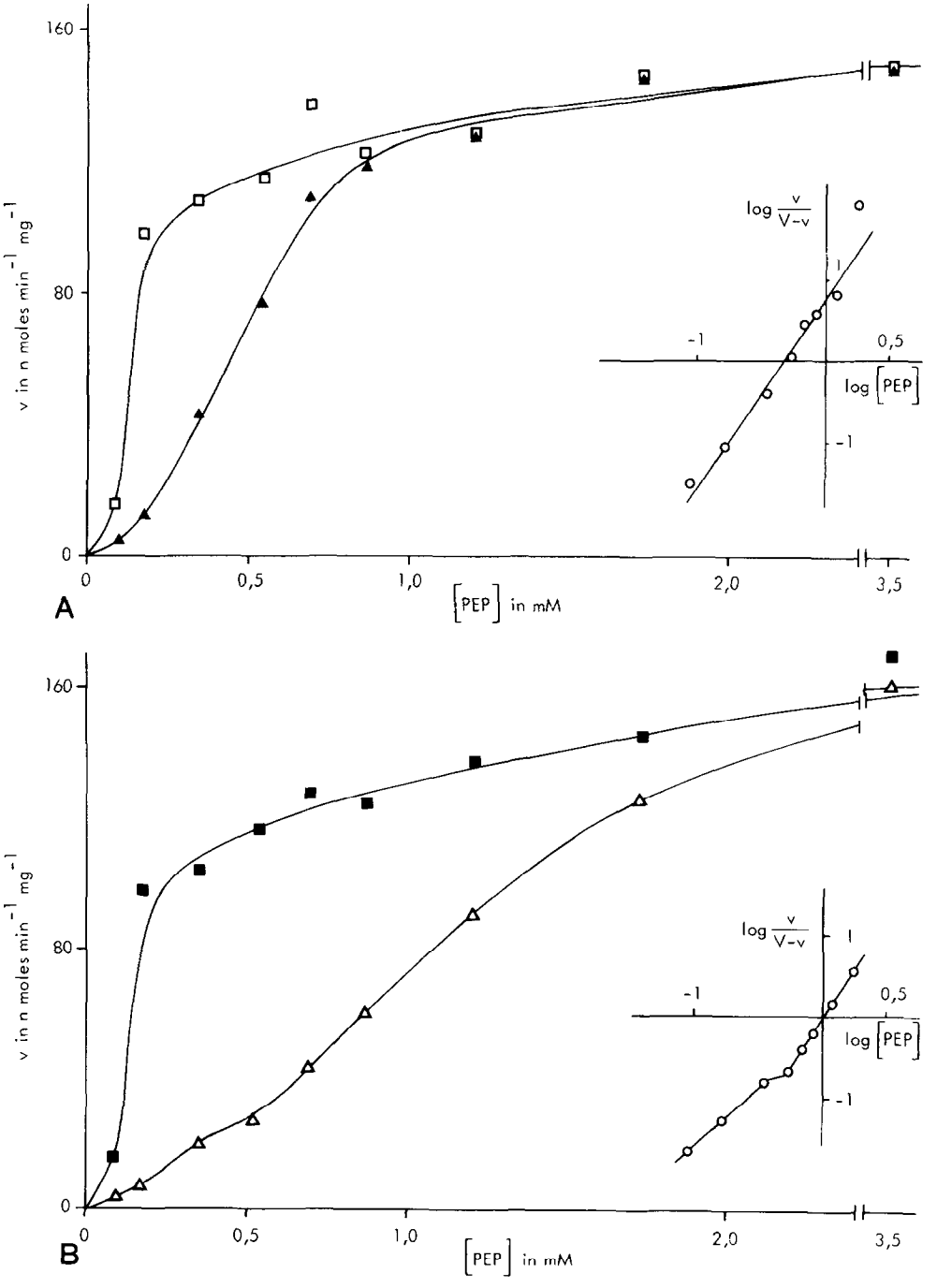

Fig. 3. Phosphoenolpyruvate saturation plot of human liver pyruvate kinase after incubation in the presence and absence of cyclic AMP. Incubation of human liver supernatant was performed as described in Materials and Methods. A: Incubation in the absence of cyclic AMP. B: Incubation in the presence of 0.2 µM cyclic AMP. Activity was measured at 1.7 mM $MgCl_2$ in the absence (triangles) and presence (squares) of fructose-1,6-diphosphate.

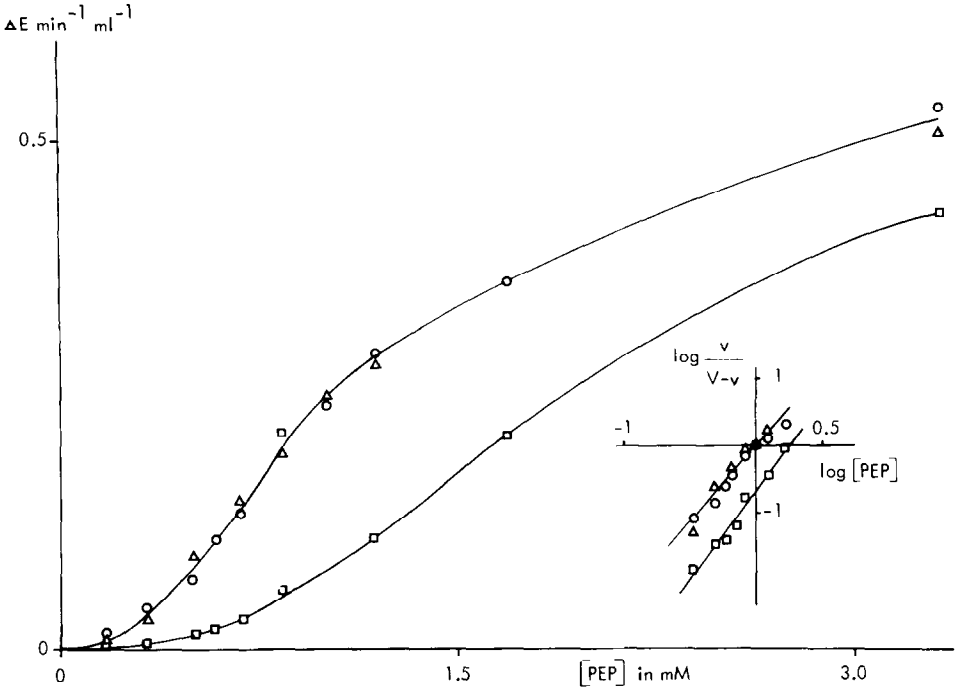

Fig. 4. Influence of incubation on phosphoenolpyruvate saturation of human liver pyruvate kinase. Incubation of human liver supernatant was performed as described in Materials and Methods except that $[Mg^{2+}]_{free}$ was kept constant at 1 mM (ref. 10). o—o, absence of cyclic AMP; □—□, 2 μM cyclic AMP; Δ—Δ, supernatant without incubation.

The described cyclic AMP-dependent inactivation of pyruvate kinase is also found in four other tested human liver samples.

DISCUSSION

The present results show that human liver pyruvate kinase can be inactivated in a human liver supernatant in a cyclic AMP-dependent way. This inactivation requires the presence of Mg-ATP, half-maximal rate of inactivation is obtained in the presence of 0.04 μM cyclic AMP. This apparent K_a is comparable to the values observed for cyclic AMP-dependent protein kinases, which makes it very likely that the cyclic AMP-dependent inactivation of pyruvate kinase is promoted by a human liver protein kinase. This implies that the inactivation is caused by phosphorylation of the enzyme. This conclusion is strengthened by the observed change in $K_{0.5}$ for phosphoenolpyruvate upon incubation while the maximal activity is unaltered.

Although no direct evidence is obtained for in vivo phosphorylation of human liver pyruvate kinase, the fact that pyruvate kinase in the supernatant is inactivated without further addition of protein kinase, and the high sensitivity to physiological amounts of cyclic AMP makes it very likely that this process is of physiological importance.

The importance of the phosphorylation of pyruvate kinase for the hormonal stimulation of gluconeogenesis is under discussion, however, it might be argued that a deficiency in this phosphorylation mechanism will lead to an increased phosphoenolpyruvate-pyruvate cycling. This results in a lowered lactate utilization by the liver i.e. a diminished lactate removal from circulating blood. This might occur in some patients with type B lactic acidosis[9], which makes it important to test the pyruvate kinase inactivation process in these patients.

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