Inhibition of phosphoenolpyruvate carboxykinase by 6-phosphogluconate in rat liver

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Summary. Various concentrations of 6-phosphogluconate inhibit rat liver phosphoenolpyruvate carboxykinase activity. 0.04 mM 6-phosphogluconate, which is the concentration found in vivo, caused a 50% inhibition of 6-phosphoenolpyruvate carboxykinase activity. 6-Phosphogluconate lowered the V_{max} of the enzyme and increased the concentration of phosphoenolpyruvate required to achieve one-half of the maximum velocity. The role of 6-phosphogluconate as a regulator of the coordination of fluxes through three metabolic pathways is discussed.
Key words. Rat liver; phosphoenolpyruvate carboxykinase; 6-phosphogluconate.

6-Phosphogluconate (6PG), an intermediate of the oxidative branch of the hexosemonophosphate pathway, activated both hepatic phosphofructokinase [1], the rate limiting enzyme of glycolysis, and pyruvate kinase [2], the second regulated glycolytic enzyme. Under some conditions the activation of phosphofructokinase by 6PG can be enhanced by fructose-2, 6-biphosphate (F-2, 6-P) [1], a potent activator of the enzyme [3]. The role of fructose-2, 6-biphosphate in the regulation of hepatic carbohydrate metabolism has been the subject of much research in recent years [3, 4]. Because of the evidence for their activity on enzymes of the glycolytic pathway, we tested the effect of 6PG as well as of F-2, 6-P on phosphoenolpyruvate carboxykinase (PEPCK), the enzyme catalysing the key step in gluconeogenesis, since the latter is regulated in a coordinated reciprocal manner with its corresponding degradative pathway, glycolysis, in liver homogenates. The results show that 6PG inhibits PEPCK activity.

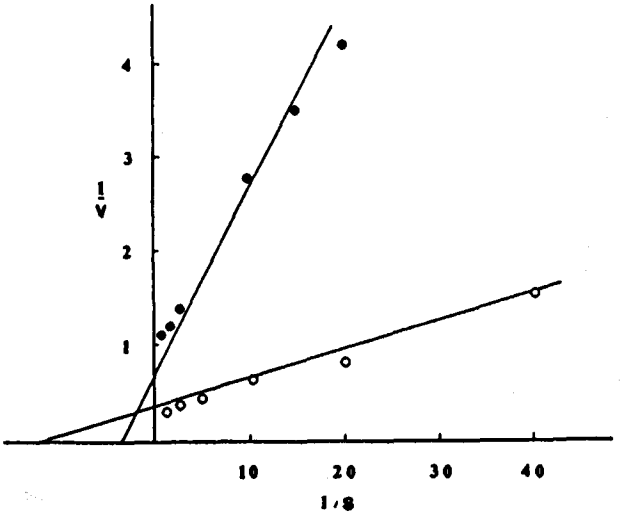

Figure 1. Lineweaver-Burk plots of PEPCK activity in the absence (○) and in the presence (●) of 6PG.

Materials and methods

Male Sprague-Dawley rats which weighed 330–380 g and which had been starved for 18 h were killed by decapitation. Their livers were removed, washed in normal saline and blotted dry on filter paper. The liver homogenate was sedimented at 17,000 rpm for 30 min at 4 °C. The resultant supernatant was saturated with $(NH_4)_2SO_4$. The fraction 25–50% saturated with $(NH_4)_2SO_4$ was used. In this fraction only the PEPCK activity appears, and no pyruvate kinase activity was detected that might interfere with PEPCK activity [5]. PEPCK activity was assayed at 25 °C as described by Opie and Newsholme [6]. Hill coefficients were calculated from the Hill plots. Student's t-test was used to compare the values of the Hill coefficients.

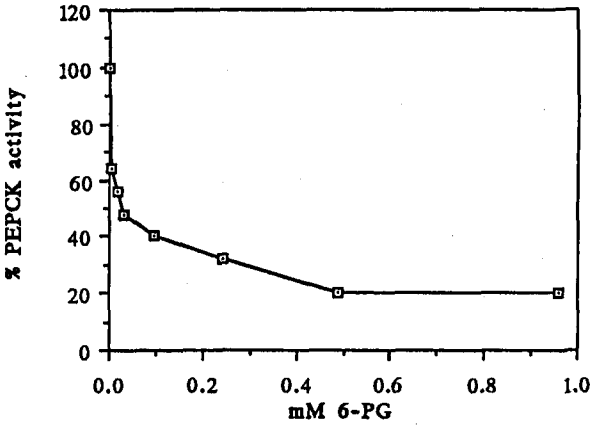

Figure 2. Effect of 6PG on PEPCK activity. Phosphoenolpyruvate concentration was kept in the range of its K_m.

Results and discussion

The data in the table and figures 1 and 2 demonstrate that 6PG inhibits the activity of PEPCK from rat liver. PEPCK activity as a function of PEP concentration shows negative cooperativity (Hill coefficient 0.66). 0.032 mM 6PG significantly decreased the cooperativity (Hill coefficient 0.44), while it increased the apparent K_m for PEP (table). 6PG lowered the V_{max} of the reaction

Effects of 6-phosphogluconate on kinetic parameters of phosphoenolpyruvate carboxykinase

	6-Phosphogluconate (mM)	
	0	0.032
Hill coefficient	0.66	0.44
K_m (mM)	0.08	0.28

from 3.57 to 0.94 μmoles/min/g liver. The K_m for PEP in the presence of 0.032 mM of 6PG is 0.28 mM (fig. 1), which is near the physiological range of PEP concentration in livers from fed rats (0.19 mM)[7]. In the presence of 0.04 mM 6PG, which is the concentration found in vivo[8], 50% of the enzyme activity was inhibited. 80% loss of the enzyme activity was observed in the presence of 0.5–1 mM 6PG. At various 6PG concentrations tested (fig. 2), PEP concentration was kept in the range of its K_m (0.08 mM).

In the range of 0–1 mM of F-2, 6-P no significant alteration of PEPCK activity was observed when PEP concentration was kept at 0.08 mM, 0.12 mM or 0.16 mM. However, this ester is an activator of phosphofructokinase[3,9] only under plethoric energetic conditions[10]. The metabolic function of liver parenchymal cells is zoned according to their location in the acinus[11]. The lack of any effect of F-2, 6-P on the activity of the gluconeogenic enzyme PEPCK, in spite of its positive effect on a key glycolytic enzyme, could be attributed to the fact that the processes of glycolysis and gluconeogenesis take place in different zones of the liver[12–14]. However, it was found that F-2, 6-P was not present in different zones but was evenly distributed between the two regions[15]. Therefore it is suggested that there may be other factors that regulate both glycolysis and gluconeogenesis[16].

6PG, an intermediate of the hexose monophosphate pathway, has been shown to block PEPCK, the key regulatory enzyme in gluconeogenesis, as the present study indicates. On the other hand, 6PG has been shown to activate both the hepatic glycolytic enzymes phosphofructokinase[1,17] and pyruvate kinase[2,18]. As a result glycolysis and therefore acetyl-CoA production is favoured, through the effect of 6PG on the phosphofructokinase and pyruvate kinase reactions. The influence of 6PG on these enzymes may provide one way of coordinating fluxes through the hexose monophosphate and the glycolytic pathways, which contribute NADPH and acetyl-CoA, respectively, for fatty acid synthesis, as Sommercorn and Freedland have also reported for rat liver[1]. In addition, PEPCK may be inhibited by 6PG in order to favour the conversion of cytoplasmic oxaloacetate to malate by malate dehydrogenase. Thus, glycolysis could proceed by using the NAD generated by the previously-mentioned reaction. Alternatively, reducing equivalents yielded by glyceraldehyde phosphate dehydrogenase during glycolysis could be carried through the malate-aspartate shuttle from the cytoplasm into the mitochondrial matrix, and subsequently the transported NADHs might be used either for energy production or for fatty acid synthesis. Therfore 6PG might provide, through its inhibition of PEPCK activity, an extra regulation which is advantageous for the contribution of substrates for fatty acid synthesis. This extra regulation involves one more metabolic pathway, that of gluconeogenesis. The biochemical mechanisms for such control processes warrant further investigation into the role of 6PG in the control of metabolism of genetically obese rats.

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