Evidence For a Functional Role of Pyruvate Kinase in Decreasing Gluconeogenesis in the Perfused Rat Liver[1] (36682)

J. W. KRAMER[2] AND R. A. FREEDLAND

Department of Physiological Sciences, University of California, Davis, California 95616

Phosphoenolpyruvate (PEP) is a metabolite at the primary junction of gluconeogenesis and glycolysis, two pathways which oppose each other in function, but in part, have a common enzymatic pathway. Pyruvate kinase (PK) (EC 2.7.1.40), phosphoenolpyruvate carboxykinase (PEP-CK) (EC 4.1.1.32) and enolase (EC 4.2.1.11) are three enzymes which have PEP as either a substrate or product. Of these three enzymes only PK and PEP-CK are inducible (1, 2) and essentially physiologically irreversible (3), suggesting that PK and PEP-CK activity may be governed by control mechanisms other than induction. Inhibitors of PK activity have been demonstrated *in vitro* (1), but these inhibitors do not appear to completely inhibit PK activity in perfused livers from starved rats where PEP, synthesized from oxaloacetate (OAA) was reported to be recycled back to pyruvate (4). Another form of control of the conversion of PEP to pyruvate could be the functional compartmentation of PEP, so PEP synthesized from OAA, would not be available as a substrate for PK. This form of compartmentation would require two pools of PEP: one for glycolysis and another for gluconeogenesis.

The study presented here attempted to determine if PEP was functionally compartmented in the cytosol of the hepatocyte on a basis of the following premise. If PEP were functionally compartmented, there would not be a correlation between the amount of PK activity and the rate of gluconeogenesis from lactate, because PEP synthesized from OAA, and destined for gluconeogenesis would not be available as a substrate for PK. However, if the PEP synthesized from OAA were available as a substrate for PK for conversion to pyruvate, then the amount of PK activity would correlate negatively with the rate of gluconeogenesis from lactate. The perfused liver system permitted us to determine the rates of gluconeogenesis from various glucose precursors by livers that had dissimilar enzyme profiles as the result of different dietary and hormonal treatments. Dietary and thyroxine treatments used in this study resulted in different amounts of PK and PEP-CK activities, but no other apparent differences limited gluconeogenesis (5, 6).

Materials and Methods. Livers from 125 to 175 g male Sprague-Dawley rats were perfused by the method of Hems *et al.* (7). Donor rats were divided into 3 groups each being fed one of the following diets: glucose diet (65% glucose, 25% casein, 4% salts, 5% corn oil and 1% vitamins), fructose diet (fructose was substituted for glucose in the high glucose diet), and high protein diet (casein was substituted for glucose in the high glucose diet so the diet was a 90% casein diet) (8). Each rat was injected with 1 mg of thyroxine (T_4) ip daily for 5 days. Treatment with T_4 resulted in lower amounts of liver glycogen that in turn reduced the amount of glucose produced from glycogenolysis during the period of perfusion when gluconeogenesis was being measured (9). In addition T_4 resulted in a greater absolute difference in the amount of PK activity of the fructose fed versus the glucose fed groups, but did not result in an altered amount of PEP-CK activity (7).

The substrates used were L-lactate, glycerol or D-fructose, in amounts sufficient to

[1] Supported in part by U.S. Public Health Service Research Grant No. AM-04732.

[2] Postdoctoral Trainee in Clinical Pathology, supported by U.S. Public Health Service Grant GM 633.

TABLE I. Rates of Gluconeogenesis, the Amounts of Pyruvate Kinase (PK) and Phosphoenolpyruvate Carboxykinase (PEP-CK) Activity in Perfused Livers from Rats Fed Different Diets and Treated with Thyroxine.

Diet	PK[a]	PEP-CK[a]	Gluconeogenesis			PK/PEP-CK
			L-Lactate[b]	Fructose[b]	Glycerol[b]	
65% Fructose	168 ± 9	4 ± 0.5	0.8 ± 0.1	1.9 ± 0.3	0.8 ± 0.1	40
65% Glucose	89 ± 4	4 ± 0.3	1.3 ± 0.1	1.9 ± 0.3	0.9 ± 0.2	23
90% Casein	91 ± 6	10 ± 0.7	1.5 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	9

[a] Enzyme activity is expressed as micromoles of NADH disappearing/min/100 g body weight and are means from 4 or more livers ± SE of mean.

[b] The rates of gluconeogenesis are expressed as micromoles of glucose/min/liver as determined with 4 or more livers ± SE of mean.

make their final concentration in the perfusion fluid 10 m*M*. The substrate was added 37 min after the start of the perfusion, and the perfusion fluid was sampled at 5 min intervals until the end of the 75 min perfusion period. The concentration of glucose in the perfusion fluid was determined by the glucose oxidase method, and the rate of glucose production is reported as micromoles of glucose formed per minute per gram of liver.

The activities of pyruvate kinase (10) and phosphoenolpyruvate carboxykinase (11) were determined after termination of perfusion. Enzyme activities are reported on the basis of units/100 g of body weight.

Results and Discussion. The activities of the enzymes, rates of gluconeogenesis and ratios of PK/PEP-CK activities obtained are summarized in Table I. Rates of gluconeogenesis from fructose and glycerol were the same in all groups, suggesting that the different activities of PEP-CK and PK did not influence that part of the gluconeogenic pathway in which fructose and glycerol were converted to glucose. The rate of gluconeogenesis from lactate increased as either the PK activity decreased or the PEP-CK activity increased, but there was no apparent direct correlation between the rate of gluconeogenesis from lactate and the activities of PK or PEP-CK alone. However, there was an inverse relationship between the rate of gluconeogenesis from lactate and a ratio of PK/PEP-CK activities.

A comparison of the data obtained from the fructose and glucose fed groups suggests that PEP synthesized from OAA for gluconeogenesis was available for conversion to pyruvate by PK. In the fructose and glucose fed groups the activity of PEP-CK and rates of gluconeogenesis from glycerol and fructose were essentailly the same. However, the activity of PK in the fructose fed group was 1.9 times greater than the activity of PK in the glucose group, but the rate of gluconeogenesis from lactate in the fructose fed group was only 0.6 of the value obtained for the glucose fed group. Therefore, the increase in activity of PK, the unchanged activity of PEP-CK and the decrease in the rate of gluconeogenesis from lactate, implies that the activity of PK inversely influenced the rate of gluconeogenesis from lactate in the fructose fed group and may have done so by returning PEP, synthesized from OAA, to pyruvate.

Changes in the activity of PEP-CK did not appear to greatly influence the rate of gluconeogenesis from lactate. This was implied in a comparison of the activities of the enzymes and rates of gluconeogenesis from lactate in the protein fed versus the glucose fed groups. The activity of PEP-CK was 2.5 times greater in the protein fed group than in the glucose fed group, but the rate of gluconeogenesis from lactate and the activity of PK were the same in both groups. If the greater activity of PEP-CK did not cause an increase in the rate of gluconeogenesis from lactate in the protein fed group versus the glucose fed group, then the activity of PEP-CK probably did not limit gluconeogenesis in the fructose fed group where the activity of

PEP-CK was the same as in the glucose fed group. The activity of PEP-CK was the same in the glucose and fructose fed groups, but the rate of gluconeogenesis in the fructose fed group was 0.6 of the rate in the glucose group. If the activity of PEP-CK in the fructose fed group was not limiting, then maybe the rate of gluconeogenesis from lactate may have been determined to a great degree by the activity of PK present.

The activity of PK in liver from rats fed a high protein diet or starved was about 40% of the activity in control rats fed a 65% glucose diet (1, 11)). This lower PK activity would be of significant physiological importance during gluconeogenic conditions when it would be desirable to have a minimum amount of PEP, originally synthesized from amino acids, lactate, and pyruvate for gluconeogenesis, converted to pyruvate by PK. Friedman *et al.* (4) have demonstrated in perfused livers from starved rats, a significant reduction in the conversion of PEP, synthesized from OAA, to pyruvate by PK as compared to fed rats. The decrease in conversion of gluconeogenic PEP to pyruvate in starved rats is associated with a decrease in PK activity (1) and an increase in the rate of gluconeogenesis from pyruvate and lactate (4). The decrease in PK activity observed during gluconeogenic conditions serves to effectively increase the net gluconeogenic capacity of the rate limiting pyruvate carboxylase (EC 6.4.1.1.) (12), by decreasing the amount of PEP being converted back to pyruvate and thus increasing the amount of PEP available for gluconeogenesis.

Summary. The inverse relationship between the rate of gluconeogenesis from lactate and the pyruvate kinase/phosphoenolpyruvate carboxykinase ratio in the perfused livers from rats fed a 65% glucose diet, a 65% fructose diet, and a 90% casein diet suggested that phosphoenolpyruvate synthesized from oxaloacetate was available to pyruvate kinase for conversion to pyruvate. The intermediate or intermediates of gluconeogenesis and glycolysis which may intermix are not clear, but the earliest intermediate in gluconeogenesis at which intermixing could occur is phosphoenolpyruvate. This implied the absence of functional compartmentation between the metabolic intermediates of gluconeogenesis and glycolysis at the level of phosphoenolpyruvate and that control of pyruvate kinase activity serves to regulate the rate of gluconeogenesis. Thus, the observed decrease in pyruvate kinase activity reported for liver during gluconeogenic conditions is probably of considerable physiological significance in permitting a greater rate of glucose production to occur from amino acids, lactate and pyruvate.

The authors acknowledge the technical assistance of Mr. E. H. Avery.

1. Krebs, H. A., and Eggleston, L. V., Biochem. J. **94**, 3c (1965).

2. Foster, D. O., Ray, P. D., and Lardy, H. A., Biochemistry **5**, 555 (1966).

3. Scrutton, M. C., and Utter, M. F., Annu. Rev. Biochem. **37**, 249 (1968).

4. Friedman, B., Goodman, E. H., Jr., Saunders, H. L., Kostos, V., and Weinhouse, S., Metabolism **20**, 2 (1971).

5. Murad, S., and Freedland, R. A., Proc. Soc. Exp. Biol. Med. **124**, 1176 (1967).

6. Murad, S., Doctoral thesis, University of California, Davis, 1968.

7. Hems, R., Ross, B. D., Berry, M. N., and Krebs, H. A., Biochem. J. **101**, 284 (1966).

8. Benevenga, N. J., Stielau, W. J., and Freedland, R. A., J. Nutr. **84**, 345 (1964).

9. Freedland, R. A., Krebs, H. A., Biochem. J. **104**, 45 (1967).

10. Szepesi, B., and Freedland, R. A., J. Nutr. **99**, 449 (1969).

11. Freedland, R. A., Cunliffe, T. L., and Zinkl, J. G., J. Biol. Chem. **241**, 5448 (1966).

12. Exton, J. H., and Park, C. R., J. Biol. Chem. **244**, 1424 (1969).

Received April 6, 1972. P.S.E.B.M., 1972, Vol. 140.