PROTECTION OF RENAL PHOSPHOENOLPYRUVATE CARBOXYKINASE AGAINST DEGRADATION IN VITRO BY ATP, CYCLIC AMP AND AMINO ACIDS

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Received December 11,1974

SUMMARY:

Acceleration of gluconeogenesis from various substrates by lysine and glutamine is accompanied by elevated activities of PEP-carboxykinase. A study of the kinetics of the activity changes pointed to a protection of the enzyme as the primary cause of the amino acid effect on gluconeogenesis. This interpretation could be substantiated by a study of PEP-carboxykinase inactivation in homogenates of kidney cortex: A rapid inactivation of the enzyme within 4o - 6o minutes is counteracted by glutamine. In addition to amino acids also ATP and cyclic AMP stabilize PEP-carboxykinase. The inactivating system is bound to membranous structures and is activated by sulfhydryl agents. A role of cyclic AMP and amino acids in the control of gluconeogenesis at the level of PEP-carboxykinase degradation is deduced from these results. The effect of glutamine on gluconeogenesis is specific for kidney. This finding points to a special role of this amino acid in control of ammoniagenesis.

In previous studies on the regulatory role of lysine and ornithine in gluconeogenesis from mono- and dicarboxylic acids in kidney cortex slices (1) and tubules (2) the attack of the amino acids could be located below the level of triose phosphate. The ability of ammonium chloride to substitute for lysine in control of gluconeogenesis from lactate has been interpreted with an acceleration of the flow of carbon through the aspartate shuttle, necessary for the transport of carbon from mitochondria to the cytoplasm (3, 4). Since gluconeogenesis from dicarboxylic acids

involves malate as the transport form of carbon through the mitochondrial membrane, the effects of lysine on gluconeogenesis from these substrates (1, 2) hardly can be reconciled with a solely attack at the transport level. Because of a limiting role of phosphoenolpyruvate (PEP)-carboxykinase in gluconeogenesis (5), as an alternative mechanism of lysine action a possible control of this enzyme by amino acids was reinvestigated. The present communication is in support of a protection of PEP-carboxykinase against inactivation by ATP, cyclic AMP and amino acids. This finding gives also an explanation for former negative results in vitro (2) in which addition of an energy providing substrate was neglected.

A preliminary account of this investigation has been published (6).

MATERIAL AND METHODS

Male rats of the Sprague Dawley strain (140-180 g) were used as kidney donors for the preparation of kidney tubules by collagenase treatment as described by Guder et al. (7). For optimal stimulation of gluconeogenesis in vitro the animals had to be kept on a carbohydrate diet almost free of fat and protein (Altromin C-1005, Altrogge, Lage/Lippe, Germany).

Incubations were performed in 80 ml-vessels at 37°C under oxygen. The incubation mixture contained in 5 ml of the saline medium of Krebs and Gasquet (8): Kidney tubules corresponding to 1-2 mg of protein/ml; 10 mM $NaHCO_3$; additional substrates as indicated in the tables and figures. Incucubations were started by the addition of substrates and terminated after 60 minutes by cooling to 0°C, and centri-

fugation at 5oo x g for 1 to 2 minutes. Glucose was determined in the supernatants with the glucose oxidase method. The tubules were broken up by homogenization in 2 ml of o.25 M sucrose supplemented with o.1 % desoxycholate. The activities of PEP-carboxykinase were determined as described by Seubert and Huth (9), supplemented with 1 mM $MnCl_2$.

For a study of the inactivation rates of PEP-carboxykinase in homogenates of kidney cortex (1:2o with Tris-HCl buffer pH 8.1, supplemented with 5 mM cysteine) high basal activities were induced in vivo by a 5o % protein diet (Altromin C-1oo1). This diet causes rises up to 4o-6o units/g wet wt. Additional components of the inactivating system are presented in the tables and figures.

Protein was determined according to Lowry et al. (1o).

RESULTS AND DISCUSSION

Table 1 summarizes the rates of glucose synthesis and the activities of PEP-carboxykinase after incubation of kidney cortex tubules with succinate: Concomitant with an increased glucose synthesis also the activities of PEP-carboxykinase were found to be elevated by lysine and glutamine. Cycloheximide, an inhibitor of protein synthesis neither suppresses glucose synthesis nor the elevation of PEP-carboxykinase. The effect of glutamine on gluconeogenesis and PEP-carboxykinase is specific for kidney, indicating in addition to its role as substrate (table 1 (6)) an important role of this amino acid in control of ammoniagenesis.

Because of similar responses of glucose synthesis and PEP-

Table 1: Effects of lysine and glutamine on tubular gluconeogenesis and PEP-carboxykinase activities (6)

Additions	Glucose Synthesis nmoles/mg·h ± Sem (n)	P	PEP-Carboxykinase munits/mg Prot ± Sem (n)	P
Succinate (5 mM)	152 ± 4 (4)	-	16.9 ± 1.2 (4)	-
Succinate + Lysine (5 mM)	340 ± 7 (4)	< 0.001	27.4 ± 0.9 (4)	< 0.001
Succinate + Lysine + Cyclo-Heximide (2mM)	328 ± 5.8 (3)	< 0.001	29.5 ± 2.1 (3)	< 0.0025
Succinate + Glutamine (5 mM)	353 ± 45 (4)	< 0.0025	29.9 ± 2.2 (5)	< 0.0025

carboxykinase to lysine in tubules from protein fed animals (not shown), showing extreme high basal activities of PEP-carboxykinase, rather a protection of the enzyme against inactivation than its activation was considered as the primary effect of the amino acid on gluconeogenesis. This assumption could be supported by a study of the kinetics of PEP-carboxykinase inactivation in a kidney cortex homogenate, first reported by Ballard et al. (11) to occur in liver. As illustrated in Fig. 1, there is an ATP-dependent protection of PEP-carboxykinase against inactivation. The necessity of unphysiological concentrations of ATP for optimal protection is explained by high activities of an ATPase which causes a rapid decomposition of the energy providing substrate. With a regenerating system (acetokinase and acetyl-P) concentrations as low as o.1 to o.2 mM proved to be sufficient for optimal protection. AMP cannot substitute for ATP in protecting PEP-carboxykinase. In subsequent studies (not shown) the effects of ADP and cycloheximide were investi-

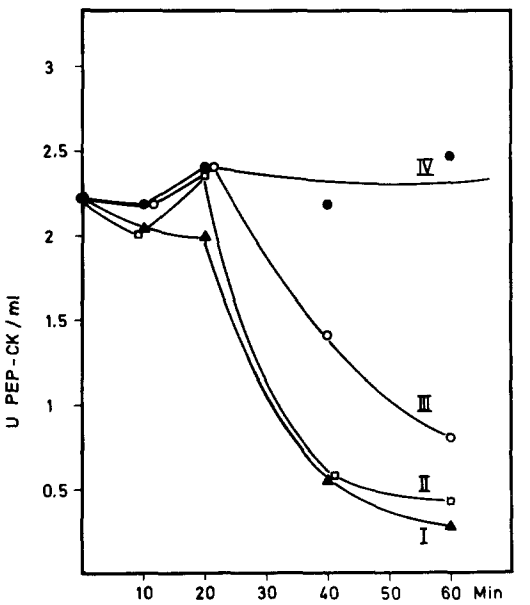

Fig. 1: Kinetics of PEP-carboxykinase inactivation in kidney cortex homogenates

2 ml homogenate (6 units PEP-carboxykinase) were incubated in an end volume of 2.7 ml under shaking at 37°C (o.1 M Trisbuffer pH 8.1; 5 mM cysteine). Aliquots (o.o1-o.o3 ml) were assayed for PEP-carboxykinase activity.

Curve I: No addition; Curve II: 3.7 mM AMP, 7.4 mM Mg^{++}; Curve III: 2.8 mM ATP, 7.4 mM Mg^{++}; Curve IV: 5.6 mM ATP, 7.4 mM Mg^{++}.

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gated: ADP accelerates the inactivation (an interference with the PEP-carboxykinase assay was excluded). Cyclohexiimide does not interfer with ATP.

In table 2 the effects of ATP, glutamine and 3',5'-cyclo-AMP on PEP-carboxykinase inactivation in a kidney cortex homogenate are summarized: As is evident, glutamine stabilizes the enzyme additive to the effect of ATP. The protective effect of the amino acid, however, disappears at optimal ATP-concentrations (not shown), indicating different points of attack of the amino acid and the nucleoside triphosphate. Lysine can substitute for glutamine (not shown). The effects of the former amino acid, however, were

Table 2: Combined action of ATP, glutamine and 3',5'-AMP on PEP-carboxykinase inactivation

Additions	ATP (mM)	Incubation Period (min)	PEP-carboxykinase u/ml ± Sem (n)	P
None	-	0	1.04 ± 0.04 (2)	-
None	-	60	0.32 ± 0.04 (6)	-
None	1.5	60	0.67 ± 0.03 (3)	< 0.0005 *
Glutamine (5 mM)	-	60	0.54 ± 0.02 (3)	< 0.0025 *
Glutamine	1.5	60	0.84 ± 0.03 (3)	< 0.01 **
None	0.75	60	0.49 ± 0.02 (3)	< 0.0025 *
3',5'-C-AMP (10^{-4} M)	-	60	0.51 ± 0.01 (3)	< 0.0025 *
3',5'-C-AMP	0.75	60	0.54 ± 0.04 (3)	N. S. ***

* Significance vs. 60 min. incubation without additions.
** Significance vs. 60 min. incubation with ATP (1.5 mM)
*** Significance vs. 60 min. incubation with ATP (0.75 mM)

not as reproducable than those of glutamine. In addition to ATP and glutamine also cyclic-AMP protects PEP-carboxykinase against inactivation (table 2). In most cases so far investigated, however, this effect was only evident in presence of suboptimal concentrations of ATP. Most likely also in the experiment of table 2 a protective effect of endogenous ATP, continuously regenerated by still intact mitochondria, was potentiated by the cyclic nucleotide. A reactivation of PEP-carboxykinase so far could not be demonstrated after its inactivation.

The data presented support protection of PEP-carboxykinase against inactivation by a membrane bound enzyme system. Whether the nucleotides and the amino acids act at the level of PEP-carboxykinase inactivation or by control of the protecting enzyme system (activation of PEP-carboxykinase or inactivation of the inactivating system) is subject of a current investigation.

This investigation was supported by grants from the Deutsche Forschungsgemeinschaft. The Stiftung Volkswagenwerk provided us with the scientific equipment.

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