Inhibition of glutamate dehydrogenase activity in rabbit renal mitochondria by phosphoenolpyruvate

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The effect of phosphoenolpyruvate on glutamate dehydrogenase activity was studied in both intact and Triton X-100-treated rabbit renal mitochondria. The intramitochondrial phosphoenolpyruvate content was modulated by application of both 3-MPA, an inhibitor of phosphoenolpyruvate carboxykinase, and BTCA, which inhibits the tricarboxylate-transporting system. The data indicate that: (i) phosphoenolpyruvate is a potent inhibitor of glutamate dehydrogenase activity; and (ii) its inhibitory effect on the enzyme may be abolished by leucine and ADP, activators of glutamate dehydrogenase.

Glutamate dehydrogenase *Mitochondria* *Phosphoenolpyruvate*

1. INTRODUCTION

Glutamate dehydrogenase (EC 1.4.1.3) is known to be regulated by reversible polymerizations and metabolite effectors of diverse nature; e.g., nucleotides (GTP, ADP, ATP), amino acids (leucine, isoleucine, methionine, norvaline), substrates and coenzymes and certain steroid hormones and lipids (see [1,2] for review). Importance of the regulatory effects of both energy level and leucine on glutamate dehydrogenase activity in isolated rabbit kidney-cortex mitochondria has been reported in [3]. Here, an inhibition of glutamate dehydrogenase activity in rabbit renal mitochondria by phosphoenolpyruvate as well as reversal of this effect by leucine and ADP are described.

Abbreviations: 3-MPA, 3-mercaptopicolinate; 1,2,3-benzenetricarboxylate; FCCP, carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone; PEP, phosphoenolpyruvate

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2. MATERIALS AND METHODS

Mitochondria were prepared from kidney-cortex of white male rabbits (about 2–3 kg body wt) by a minor modification [3] of the method in [4].

The rate of glutamate deamination was determined in the standard incubation mixture containing 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 75 mM Tris–HCl buffer, 10 mM potassium phosphate buffer, 10 mM potassium glutamate, 0.2 mM aminooxyacetate, 0.1–0.2 μM FCCP, about 3 mg mitochondrial protein/ml and 15 mM mannitol (derived from the mitochondrial preparation); the final pH was 7.4. When 1,2,3-benzenetricarboxylate (BTCA) was added to the reaction medium, corresponding amounts of Tris–HCl were omitted. Reactions were started by the addition of the substrate followed by incubation of the mitochondrial suspension for 3 min in the reaction mixture. Other conditions of incubation are described in [5]. For measurement of the distribution of phosphoenolpyruvate between mitochondria and the medium, the silicone layer technique was applied [6].

Glutamate dehydrogenase activity in Triton X-100-treated mitochondria was assayed in the

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direction of glutamate synthesis [7] in the presence of 0.02% Triton X-100.

Determination of phosphoenolpyruvate in both extramitochondrial and intramitochondrial spaces was done spectrophotometrically and fluorimetrically, respectively, as in [8]. Ammonia was estimated as [9] following the distillation of ammonia from the sample to sulphuric acid as described in [10]. Mitochondrial protein was determined by the biuret method [11] as described in [12].

BTCA and leucine were obtained from Sigma (St. Louis, MO), FCCP and 3-MPA were gifts of Dr P. Heytler and Dr T. Tondys, respectively. Lactate dehydrogenase and pyruvate kinase used for the assay of phosphoenolpyruvate were purchased from Polskie Odczynniki Chemiczne, Gliwice and Reanal (Hungary), respectively, while phosphoenolpyruvate was provided by Koch and Light Labs (Colnbrook). All other chemicals were of analytical grade.

3. RESULTS AND DISCUSSION

Kidney-cortex mitochondria of rabbit contain high phosphoenolpyruvate carboxykinase activity [13,14] and synthesize efficiently phosphoenolpyruvate from glutamate, providing both GTP and oxaloacetate, substrates of phosphoenolpyruvate carboxykinase [3,15]. The highest rates of glutamate deamination and thus of phosphoenolpyruvate formation were observed in uncoupled mitochondria. Therefore we have applied uncoupled mitochondria to study the effect of phosphoenolpyruvate on glutamate deamination. As shown in table 1, on the addition of 3-mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase [16,17], a stimulation of ammonia formation was observed, probably due to a decrease of intramitochondrial phosphoenolpyruvate level resulting from an inhibition of phosphoenolpyruvate generation. In order to increase the intramitochondrial phosphoenolpyruvate content we have applied BTCA, an inhibitor of the tricarboxylate-transporting system [18–20], which is known to elevate significantly the phosphoenolpyruvate level in rabbit renal mitochondria [5,21]. The addition of BTCA to uncoupled mitochondria resulted in the 10-fold increase of intramitochondrial phosphoenolpyruvate concentration, accompanied by a marked decline of both glutamate deamination and phosphoenolpyruvate formation. In contrast, when both 3-MPA and BTCA were included into the reaction medium, an increased rate of glutamate deamination was maintained since under these conditions phosphoenolpyruvate formation as well as its accumulation were very low.

Table 1

Effect of 3-MPA, BTCA and leucine on ammonia formation and phosphoenolpyruvate production and accumulation in rabbit renal mitochondria

Additions	Ammonia formation	PEP production	PEP accumulation
	(nmol.min^{-1}.mg protein^{-1})		(nmol.mg protein^{-1})
None	8.5 ± 0.6	6.1 ± 0.3	1.1 ± 0.2
3-MPA	10.5 ± 0.6[a]	2.8 ± 0.2[b]	0.6 ± 0.1[c]
BTCA	1.6 ± 0.2[a]	2.1 ± 0.2[b]	8.1 ± 0.8[b]
BTCA + 3-MPA	11.7 ± 0.7[c]	0.9 ± 0.3[b]	0.6 ± 0.1[c]
Leucine	12.2 ± 0.8[c]	9.5 ± 0.6[b]	0.9 ± 0.2
BTCA + leucine	9.5 ± 0.3	2.6 ± 0.6[b]	7.8 ± 0.5[b]

Significantly different from the corresponding controls: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.02$

The mitochondrial suspensions were incubated for 20 min in the reaction medium described in section 2. 3-MPA, BTCA and leucine were added at 2.5 50, and 5 mM, respectively, where indicated. For measurements of rates of ammonia and phosphoenolpyruvate production samples were removed from the incubation mixture at 5-min intervals, while the accumulation of PEP was determined after 10 min incubation. Values are means ± SEM of 3–4 expt.

In agreement with our previous observations [3] leucine, an activator of glutamate dehydrogenase [22,23], increased both ammonia production and phosphoenolpyruvate formation, while it did not alter the intramitochondrial phosphoenolpyruvate content. On the addition of leucine to mitochondrial suspension containing BTCA the rate of glutamate deamination was stimulated despite a high intramitochondrial phosphoenolpyruvate level. This indicates that leucine abolishes the inhibitory effect of phosphoenolpyruvate on glutamate dehydrogenase activity. In contrast, this amino acid did not increase the rate of phosphoenolpyruvate production. Presumably, an elevation of mitochondrial phosphoenolpyruvate concentration slows down its synthesis by product inhibition of phosphoenolpyruvate carboxykinase, so the total phosphoenolpyruvate synthesized is low when the exit of this compound is prevented.

As shown in fig.1, phosphoenolpyruvate also inhibits glutamate dehydrogenase activity, when studied in the direction of glutamate synthesis in Triton X-100-treated mitochondria incubated with low 2-oxoglutarate concentrations. As in intact mitochondria, leucine abolished the inhibitory effect of phosphoenolpyruvate on activity of this enzyme in Triton X-100-solubilized mitochondria.

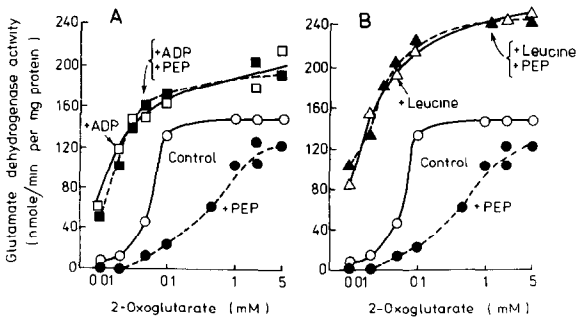

Fig. 1. Reversal of the inhibitory effect of phosphoenolpyruvate on glutamate dehydrogenase activity in Triton X-100-solubilized renal mitochondria by ADP (A) and leucine (B). Glutamate dehydrogenase activity was assayed in the direction of glutamate synthesis in rabbit kidney-cortex mitochondria (0.32 mg protein) incubated in the presence of 0.02% Triton X-100 in the reaction mixture described in [5] containing various concentrations of 2-oxoglutarate. Phosphoenolpyruvate, ADP and leucine were added at 5, 0.1 and 1 mM concentrations, respectively, where indicated.

A reversal of the inhibition of glutamate dehydrogenase activity by phosphoenolpyruvate was also observed in the presence of ADP, another activator of the enzyme (see [1,2] for review).

Since in kidney the glutamate dehydrogenase reaction is of central importance in ammonia production and in disposal of glutamine or glutamate carbon [24], the reversal of the inhibitory effect of phosphoenolpyruvate on glutamate deamination by ADP and leucine could be of particular significance in metabolic acidosis preserving: (i) an increased ammoniagenesis to maintain the acid-base homeostasis; and (ii) a high phosphoenolpyruvate production for gluconeogenesis.

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