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α-KETOGLUTARATE MODULATION OF GLUTAMINE METABOLISM BY RAT RENAL MITOCHONDRIA*

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SUMMARY: α-Ketoglutarate, (0.2-3.0 mM), had no effect on phosphate-dependent glutaminase activity in rat kidney mitochondria partially disrupted with 0.03% Triton X-100 at near physiological conditions. When the effect on glutamine metabolism of α-ketoglutarate was examined in rotenone-inhibited mitochondria, an increase in glutamate formation was observed. In the presence of glutamine, the addition of 0.5 mM α-ketoglutarate decreased the deamidation of [U-^{14}C]-glutamine, a finding which was accompanied by significant amination of [U-^{14}C]-α-ketoglutarate. α-Ketoglutarate amination was markedly increased in the presence of succinate. The data indicate that the inhibitory effect of α-ketoglutarate on glutamine metabolism in experimental conditions for glutamine transport study (Goldstein 1976) may be due to glutamate formed in the matrix by enhanced reductive amination of α-ketoglutarate.

INTRODUCTION: The metabolism of glutamine, which is the main source for ammonia formation in the kidney, occurs in the mitochondria (1). Two hypotheses have been presented to explain the increase in glutamine metabolism by kidney mitochondria in acidosis. One hypothesis suggests that the activity of the enzyme phosphate-dependent glutaminase is rate-limiting and may be regulated by the level of the reaction product glutamate (2,3). The second hypothesis postulates that glutamine transport across the inner mitochondrial membrane may regulate the supply of substrate to the enzyme (4,5). It was reported that α-ketoglutarate levels, which drop sharply in the kidney in acute acidosis, may affect the influx of glutamine into mitochondria (6). However, α-ketoglutarate may be readily aminated in kidney mitochondria (7,8). Thus the observed

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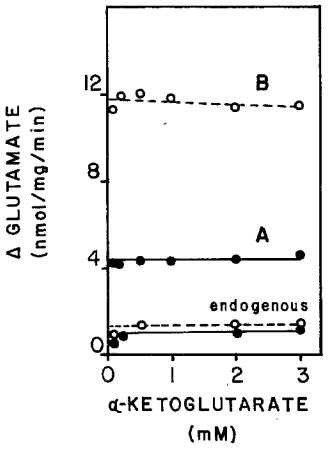

Fig. 1 Effect of α-ketoglutarate on phosphate-dependent glutaminase activity in kidney mitochondria partially disrupted by treatment with 0.03% Triton X-100. The mitochondria (2.3 - 2.7 mg/ml)were incubated at 28° C for 0,4 and 8 min in a medium containing 20 mM MOPS, 10 mM Tris, 130 or 85 mM KCl, 1 μg/ml rotenone, 1 mM aminooxyacetate with 2 mM K_2HPO_4 (open circles) or 20 mM K_2HPO_4 (closed circles) and 1 mM glutamine (A,B) or 0.5 mM NH_4Cl (endog.), at pH 7.6.

inhibition of glutamine deamidation by α-ketoglutarate (6) could be mediated by the resulting glutamate level, rather than by α-ketoglutarate directly. Therefore, the aim of this study was to determine whether significant reductive amination of α-ketoglutarate occurs under experimental conditions for glutamine transport.

MATERIALS AND METHODS: Kidney cortex mitochondria were isolated from Sprague-Dawley rats (200-300 g) by differential centrifugation in a solution of 225 mM mannitol, 75 mM sucrose, 0.1 mM EDTA and 5 mM MOPS[1] at pH 7.2. The mitochondria were purified with a Ficoll density gradient to prevent contamination by phosphate-independent glutaminase (9). The mitochondria (2.5-3.5 mg protein/ml) were incubated at 28°C in a medium containing 138 mM KCl, 20 mM MOPS, 10 mM Tris, 2 mM K_2HPO_4, 5 mM $MgCl_2$, 1 mM EDTA and 1 mM glutamine at pH 7.4. Rotenone (1 μg/ml) and other substrates were introduced into the incubation medium as indicated in the legends of tables. For measurement of phosphate-dependent glutaminase activity the mitochondria were incubated in a medium of 20 mM MOPS, 10 mM Tris, 2 or 20 mM K_2HPO_4 and 130 or 85 mM KCl at pH 7.4 plus 1 mM glutamine, 1 mM aminooxyacetate, 1 μg/ml rotenone and 0.03% Triton X-100. Oxygen uptake was determined by a Clark-type oxygen electrode at 25°C. Samples for metabolite assays were taken at timed intervals from 0 to 16 min and deproteinized by 14% (w/v) perchloric acid. Extracts were then neutralized with a mixture of 3 M KOH-0.5 M MOPS. Glutamate, glutamine and α-ketoglutarate were determined enzymatically (10). In some experiments [U-^{14}C]-glutamine or [U-^{14}C]-α-ketoglutarate was used and glutamine, glutamate and α-ketoglutarate from neutralized samples were separated on Dowex acetate columns (0.7 x 7.5 cm) by elution with 6 ml of water, 7 ml of 0.5 M acetic acid and 2 ml of 4 N HCl, respectively, as previously described (9). Protein was determined by the biuret reaction.

1) MOPS, morpholinopropane sulfonic acid

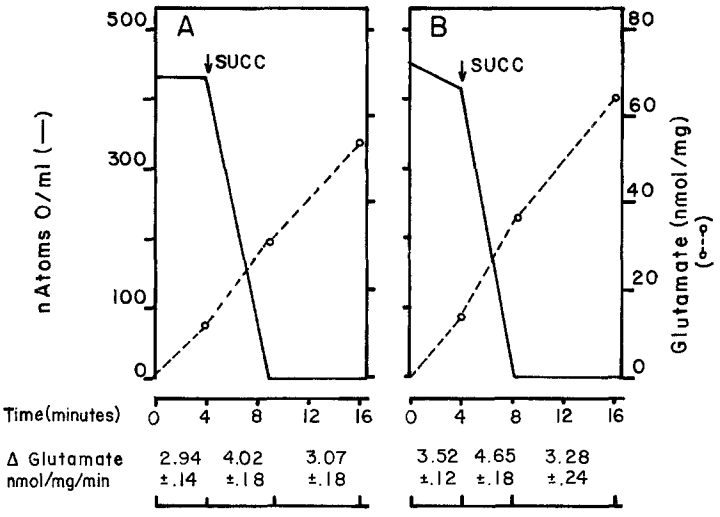

Fig. 2 The stimulation of respiration and glutamate formation in rotenone-inhibited mitochondria by succinate addition in the presence of glutamine (A) or glutamine plus α-ketoglutarate (B). Rat kidney cortex mitochondria (2.7-3.5 mg/ml) were incubated in a medium of 20 mM MOPS, 10 mM Tris, 138 mM KCl, 2 mM K_2HPO_4, 5 mM $MgCl_2$, 1 mM EDTA plus 1 μg/ml rotenone, 1 mM glutamine and 0.5 mM α-ketoglutarate, pH 7.4. 1 mM succinate was added to the medium as indicated (Succ). The values presented are means ± SE from 3-4 separate experiments.

RESULTS AND DISCUSSION: The activity of phosphate-dependent glutaminase in disrupted kidney mitochondria determined at near physiological conditions is shown in Fig. 1. In contrast to previously reported data indicating a weak inhibition by α-ketoglutarate at near optimal enzymatic conditions (11), α-ketoglutarate in concentrations up to 3 mM had no effect on glutaminase activity.

The metabolism of glutamine by rotenone-inhibited mitochondria was stimulated significantly following succinate addition (Fig. 2A). This presumably resulted from energization of the mitochondrial membrane, which promotes glutamine influx (4) or from an increase of phosphate influx (12). The possibility that glutamine metabolism expressed as glutamate formation might be overestimated as a result of α-ketoglutarate amination (7,8) was excluded in this experimental condition. Addition of 50 μM fluorocitrate had no effect on glutamate formation by rotenone-inhibited mitochondria incubated with glutamine plus succinate in spite of almost complete inhibition of pyruvate oxidation by 5 μM fluorocitrate in normal kidney mitochondria (results not shown).

Table I. Glutamine deamidation and α-ketoglutarate amination rates in rotenone-inhibited mitochondria.

Experimental condition		Glutamate formation total	Glutamate formation ^{14}C-labelled
		nmole/mg/min	
[^{14}C]-glutamine	(3)	3.85 ± 0.13	3.73 ± 0.46
[^{14}C]-glutamine, α-KG	(3)	4.05 ± 0.16	2.58 ± 0.10
[^{14}C]-glutamine, Succ.	(4)	4.75 ± 0.22	4.60 ± 0.34
[^{14}C]-glutamine, Succ., α-KG	(4)	5.54 ± 0.14	2.39 ± 0.17
[^{14}C]-α-KG, Succ., NH_4Cl	(3)	0.69 ± 0.20	0.89 ± 0.16
[^{14}C]-α-KG, glutamine	(4)	3.97 ± 0.21	1.92 ± 0.47
[^{14}C]-α-KG, glutamine, Succ.	(4)	5.43 ± 0.30	3.42 ± 0.66

Rat kidney mitochondria were incubated under experimental conditions similar to those described in Fig.1 at 28°C for 4 min with 1 mM [U-^{14}C] glutamine or 0.5 mM [U-^{14}C]α-ketoglutarate ± 1 mM succinate and 1 mM NH_4Cl. Glutamate formation was determined enzymatically and isotopically. Values represent the mean ± SE from 3-4 experiments.

Abbreviations: α-KG, α-ketoglutarate; Succ., succinate

The presence of α-ketoglutarate, which was not oxidized by rotenone-inhibited mitochondria, plus glutamine resulted in slight mitochondrial respiration as well as glutamate formation indicating a leak through the rotenone block (Fig. 2B). (Rotenone was used in the same amount per mg protein in this study as in a previous report (6)). The subsequent addition of succinate potentiated this metabolic response, but only under aerobic conditions. Addition of 0.5 mM α-ketoglutarate depressed ^{14}C-glutamate formation from ^{14}C-glutamine in rotenone-inhibited mitochondria by 31% in the absence and by 48% in the presence of succinate (Table I), a finding which agrees closely with data obtained by Goldstein (6). The use of uniformly labelled ^{14}C-α-ketoglutarate with non-labelled glutamine demonstrated that approximately one-half of the total glutamate formed resulted from the reductive amination of α-ketoglutarate. The required substrates for the reductive amination of exogenous α-ketoglutarate were supplied in this experimental condition by endogenous reducing equivalents (rotenone-inhibited mitochondria) and by ammonia formed from glutamine. Succinate promoted the generation of these precursors. Thus, the rate of α-keto-

glutarate amination was markedly elevated upon addition of succinate. Therefore, it is suggested that the inhibition by α-ketoglutarate of glutamine uptake and metabolism is not caused by α-ketoglutarate directly, as suggested previously (6), but results from glutamate formed intramitochondrially at an enhanced rate upon addition of α-ketoglutarate to the medium. It has been reported that fluorocitrate or fluoroacetate resulted in significant increases in glutamine metabolism in dog kidney (13,14). Both compounds inhibit citrate formation, the primary precursor of α-ketoglutarate, as well as reducing equivalents, which are substrates for glutamate biosynthesis in kidney mitochondria (7,8).

It is interesting that reductive amination of α-ketoglutarate by rotenone-inhibited kidney mitochondria observed in this study was markedly lower when ammonium chloride was introduced into incubation medium without glutamine (Table I). The concentration of ammonium chloride used in this experiment was approximately one order of magnitude higher than the ammonia formed from glutamine, based on the assumption of uniform distribution in the intra- and extramitochondrial space. Thus, the three-fold difference in the rate of α-ketoglutarate amination indicates that significant compartmentation of ammonia occurs during glutamine metabolism in these rotenone-inhibited mitochondria.

Our observations do not support a direct effect of α-ketoglutarate on glutamine metabolism by rat kidney mitochondria. α-Ketoglutarate had no effect on the reaction rate of glutamine deamidation. Moreover, glutamate formation was stimulated by succinate in rotenone-inhibited mitochondria incubated with glutamine plus α-ketoglutarate. On the basis of these experimental data it was concluded that α-ketoglutarate inhibition of glutamine metabolism occurs via formation of intramitochondrial glutamate.

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