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Glutamine affects glutamate metabolism in isolated rat kidney cortex mitochondria

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The active state respiration of isolated rat kidney cortex mitochondria with 10 mM glutamate as single substrate is substantially increased by the addition of 10 mM glutamine. This increase in respiration was accompanied by a higher transamination rate and was found to be insensitive to the selective inhibition of either the transamination or the desamination pathway of glutamate oxidation. These data can be explained by an approximately 2-fold elevated intramitochondrial glutamate concentration observed in the additional presence of glutamine.

Introduction

It is well known that glutamine can serve, in the additional presence of glutamate, as an effective hydrogen donor of oxidative phosphorylation in rat kidney cortex mitochondria [1,2], playing a substantial role in kidney energy metabolism during acidosis. The reason for this effect, however, remains obscure due to the fact that glutamine is metabolized in kidney cortex mostly via phosphate-dependent glutaminase to form intramitochondrial glutamate [2]. It is well documented that extramitochondrial glutamate is metabolized nearly completely via the transamination pathway [3,5]. This phenomenon is related to the low capacity of the glutamate/OH^- carrier in kidney cortex being rate limiting for the desamination pathway [5]. On the other hand glutamine, the major source of ammonium ions in kidney, is postulated to be metabolized mainly via the desamination route [3,4].

In the present paper we have investigated the reason why glutamine stimulates the oxygen consumption of rat kidney cortex mitochondria oxidizing glutamate. It was found that this stimulation is related to an increase in transamination velocity caused by an increase of intramitochondrial glutamate concentration. Preliminary results have been presented elsewhere [6].

Materials and Methods

Rat kidney cortex mitochondria were isolated as described in [8]. The incubation medium contained 110 mM mannitol, 60 mM Tris-HCl, 60 mM potassium phosphate, 0.5 mM EDTA, 5 mM $MgCl_2$ (pH 7.4). The oxygen consumption was determined at 25°C in a stirred oxygraph vessel using a teflon-coated Clark electrode connected to a custom-built rate meter which allowed the simultaneous determination of oxygen content and rate of respiration. The respiratory control index when using 10 mM glutamate (+10 mM malate) was routinely better than 5. The incubations for the determination of metabolic fluxes were carried out in the presence of 5 mM ADP and such activities of F_1-ATPase (isolated according to Ref. 7, approx. 1 EU/mg prot.) sufficient for active state respiration as described in Ref. 9. The assays of aspartate, glutamate and ammonia were performed by standard spectrophotometric means [10]. The intramitochondrial glutamate concentration was determined by injection of a 3 ml sample into 8 ml NaCl-saturated buffer (pre-cooled to −10°C) and rapid filtration through three Millipore glass prefilters [11]. To remove extramitochondrial metabolites the adsorbed mitochondria were washed with another 8 ml of NaCl-saturated buffer (pre-cooled to −10°C) and extracted from the filter by an organic quench procedure according to [12]. The amount of adsorbed

Abbreviations: PDCA, pyridine-2,6-dicarboxylic acid; AOA, aminooxyacetate.

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TABLE I

Effect of inhibitors on the active state respiration of rat kidney cortex mitochondria oxidizing glutamine or glutamine + glutamate

The rates of respiration are expressed in nmol O_2/min/mg protein. 0.3–0.6 mg/ml rat kidney cortex mitochondria were incubated in the medium (cf. Methods). The rates were determined in the presence of 5 mM ADP and 1 EU/mg F1-ATPase. Additions: glutamine (GLN) 10 mM; glutamate (GLU) 10 mM; aminooxyacetate (AOA) 1 mM; pyridine-2,6-dicarboxylic acid (PDCA) 0.5 mM.

	Control *	+ AOA *	+ PDCS * *	+ AOA + PDCS *
GLU	50.8 ± 8.3	19.6 ± 5.1	38.2 ± 5.3	13.4 ± 4.4
GLU + GLN	76.2 ± 8.4	37.6 ± 4.4	56.4 ± 6.1	16.6 ± 5.4

* Six independent experiments.

* * Four independent experiments.

protein (approx. 50% of total mitochondrial protein) was determined in parallel using a modified Biuret method [13]. To prove the correctness of the applied method the contents of adenine and pyridine nucleotides of rat liver mitochondria adsorbed to the filters were compared with the total nucleotide content of the incubations. For adenine nucleotides a content of 10.3 ± 0.9 nmol/mg prot. was found in the adsorbed mitochondria and 8.8 ± 0.6 nmol/mg prot. in the total extract, respectively; for NAD 3.0 ± 0.5 nmol/mg prot. in the adsorbed mitochondria and 2.6 ± 0.4 nmol/mg in the total extract, respectively; for NADP 3.7 ± 0.3 nmol/mg in the adsorbed mitochondria and 3.2 ± 0.2 nmol/mg in the total extract, respectively (5 experiments). These data show that there is no damage or drastic modification of the content of adsorbed mitochondria during the filtration procedure. Similar results for the content of intramitochondrial nucleotides were obtained in the presence of 5 mM ATP (cf. also [11]), indicating a complete removal of extramitochondrial compounds.

Results and Discussion

In Table I the effect of glutamine addition on the active state respiration of rat kidney cortex mitochondria oxidizing glutamate is shown. In the absence of inhibitors glutamine increases the respiration to 150%. In the presence of aminooxyacetate, an inhibitor of aspartate aminotransferase, the initial glutamate oxidation rate is inhibited to 40% of the control. Under these circumstances glutamine still causes an increase in mitochondrial respiration. To test the possibility of glutamine being metabolized solely via the desamination pathway the glutamate dehydrogenase inhibitor, pyridine-2,6-dicarboxylic acid (PDCA), was applied. As can be seen in Table I, 0.5 mM PDCA causes an inhibition of glutamate oxidation by 25% but does not influence the stimulating effect of glutamine. To prove the effectiveness of the applied concentrations of inhibitors both were added to the incubation. As expected under these circumstances the oxidation of glutamate was largely inhibited and the stimulating effect of glutamine disappeared nearly completely. From these data it seems to be clear that glutamine can stimulate both of the mentioned pathways of glutamate metabolization.

To prove if the stimulatory effect of glutamine on oxygen consumption with glutamate as a substrate is caused by an increase in transamination or desamination velocity the aspartate and ammonium production rates were also determined. In Table II the results of

TABLE II

Effect of aminooxyacetate on the oxygen consumption and the aspartate and ammonium production rates of rat kidney cortex mitochondria oxidizing glutamine, glutamate, glutamine + glutamate or glutamate + malate

Rat kidney cortex mitochondria (0.3–0.8 mg/ml) were incubated as described in the legend to Table I. The rates of oxygen consumption (O_2), aspartate production (ASP) and ammonium production (NH_3) are given in nmol/min/mg protein. Number of independent experiments, 6.

	GLN	GLU	GLN + GLU	GLU + MAL
O_2 control	30.3 ± 4.3	50.8 ± 8.3	76.2 ± 8.4	106.1 ± 15.3
+ AOA	32.8 ± 3.4	19.6 ± 5.1	37.6 ± 4.4	32.6 ± 5.2
ASP control	2.5 ± 0.9	22.7 ± 6.4 *	30.0 ± 5.9 *	39.1 ± 3.2
+ AOA	1.4 ± 0.2	2.7 ± 0.7	3.2 ± 0.5	2.3 ± 0.9
NH_3 control	31.4 ± 5.0	6.1 ± 3.2	15.0 ± 4.3	4.3 ± 2.1
+ AOA	26.1 ± 5.7	5.4 ± 4.6	21.8 ± 4.2	8.2 ± 3.2

* The difference between these values was proven to be significant by paired t-test ($P < 0.002$).

these determinations are summarized. The active state conditions for these experiments were adjusted with soluble F_1-ATPase (according to [9]) to have steady-state metabolic fluxes during the total incubation time (15 min). It can be assumed that under our experimental conditions the aspartate production rate is identical to the transamination velocity (cf. [3]). In agreement with others [3,5] we found very little aspartate production with glutamine alone. With glutamate a significant aspartate production was detected which could be stimulated by the addition of glutamine. In the combined presence of glutamate and malate the highest aspartate production rates were observed.

It can be seen that in agreement with data from the literature [3,5] the rate of ammonium production is the highest with glutamine alone. With the substrate combination, glutamate and glutamine, an approximately two-times lower ammonium production was observed, which increased after the addition of aminooxyacetate nearly to the value observed with glutamine alone. These data clearly support the above mentioned view that the increase in respiration observed by the addition of glutamine is caused solely by glutamate transamination. The ammonium production observed under these circumstances can be attributed to the action of glutaminase.

Significant changes in intramitochondrial glutamate concentration postulated already by Kovacevic et al. [2] could account for the flux changes after glutamine and malate additions. To test this we measured the intramitochondrial glutamate concentrations under our incubation conditions using a specially developed filtration procedure (cf. Materials and Methods) which allowed its determination even in the presence of rather large extramitochondrial glutamate concentrations. In the presence of glutamate or glutamine alone, under our experimental conditions, the intramitochondrial glutamate concentrations were detected to be 11.8 ± 4.7 mM and 16.2 ± 3.1 mM, respectively (assuming a matrix volume of 1 μl, four experiments). In the combined presence of both substrates the intramitochondrial glutamate concentration increased to 26.8 ± 8.4 mM. Similarly, malate addition increased the intramitochondrial glutamate concentration to 31.0 ± 9.1 mM.

The rather high K_m value of aspartate aminotransferase for glutamate ($K_m = 8.9$ mM [14]) can be accounted for by the large effect of a variation in the intramitochondrial glutamate concentration on the transamination velocity. This explanation is supported by the fact that the glutamine stimulation of glutamate oxidation is PDCA-insensitive. Therefore, the separate import pathway of glutamine [15], in combination with the action of glutaminase [2], causes the increased intramitochondrial glutamate concentration which is responsible for the observed stimulating effects of glutamine.

The effect of malate on the glutamate transamination rate seems to be only in part related to its function as precursor of oxaloacetate. It can be assumed that the observed increase in intramitochondrial glutamate concentration in the presence of malate removes the main limitation for the maximal transamination flux. This increase is possibly caused by the dicarboxylate carrier which can import glutamate in exchange for malate with 2.2% of the activity of the phosphate/malate exchange reaction [16].

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