ACTION OF THE NEUROTOXIN β-*N*-OXALYL-L-α,β-DIAMINOPROPIONIC ACID ON GLUTAMATE METABOLISM OF BRAIN MITOCHONDRIA*

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1. Introduction

Mammalian neurolathyrism is a progressive and degenerative disease of central nervous system originating from excessive consumption of *Lathyrus* seeds, particularly those of *L. sativus*. Rao et al. [1] have isolated β-*N*-oxalyl-L-α,β-diaminopropionic acid (ODAP) from the seeds of *L. sativus* and shown this substance to be the neurotoxin [1, 2]. Characterization of the biochemical effect and cellular site of action of ODAP is required for clarifying the relation between chronic ingestion of this substance, its neurotoxicity, and degenerative effects in the brain.

ODAP is structurally analogous to glutamate. Since glutamate and ammonia metabolism are associated with mitochondria, and because acute doses of ODAP led to ammonia toxicity in rats [3], an investigation of the action of ODAP on glutamate metabolism in isolated brain mitochondria was undertaken.

The results presented in this communication show that ODAP affects the metabolism of isolated brain mitochondria through:

a) an apparent inhibition of respiration with glutamate, glutamine or α-ketoglutarate;
b) inhibition of glutamate transport across the inner mitochondrial membrane; and by
c) apparent activation of mitochondrial glutaminase.

* This paper is dedicated to the memory of Professor P.S. Sarma, Department of Biochemistry, Indian Institute of Science, Bangalore, India.

2. Materials and methods

Mitochondria were isolated from bovine brain following the centrifugation schedule of Stahl et al. [4], and using the isolation medium of Moore and Jobsis [5]. Ficoll gradients were not utilized in our procedure; instead, mitochondria were washed once with 8% Ficoll solution in isolation medium.

Protein was determined by a modification of the biuret method [6] using bovine serum albumin as a standard.

Uptake of oxygen by brain mitochondria was determined polarographically at 26° in a 3 ml reaction mixture with the Clark oxygen electrode. The assay medium contained 225 mM mannitol, 75 mM sucrose, 0.5 mM $MgCl_2$, 10 mM phosphate, 10 mM KCl, 5 mM Tris, 0.33 mM EDTA, 0.5 μM FCCP, 1 mg/ml bovine serum albumin, 4 mM substrate and 2–3 mg/ml mitochondrial protein, at pH 7.4.

The uptake of glutamate by brain mitochondria was followed by measuring volume changes by photometric means. The assay medium contained 225 mM mannitol, 75 mM sucrose, 1 mM phosphate, 10 mM KCl, 5 mM Tris, 0.33 mM EDTA at pH 7.4. Usually the final mitochondria concentration was 1 mg/ml.

Assay of glutaminase I in mitochondria was carried out according to Meister [7]. Glutamic dehydrogenase and glutamic-oxaloacetic aminotransferase were estimated by the procedures of Strecker [8] and Karmen [9], respectively.

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Table 1
Effect of ODAP on the rates of respiration of brain mitochondria.

	Oxygen consumption (natoms/min/mg protein)		
	Control	+ 3 mM ODAP	Inhibition (%)
Succinate	57.0	60.0	–
α-Glycero-phosphate	48.0	48.0	–
Glutamate	21.0	9.0	57.1
Glutamine	18.0	6.0	66.7
α-Ketoglutarate	36.0	24.0	33.4

Brain mitochondria (2 mg/ml) in a basic respiration medium of 225 mM mannitol, 75 mM sucrose, 0.5 mM $MgCl_2$, 10 mM phosphate, 10 mM KCl, 5 mM Tris, 0.33 mM EDTA, 0.5 μM FCCP and 1 mg/ml bovine serum albumin, pH 7.4. Respiratory substrates were 4 mM. The results represent the average of 3 independent experiments.

3. Results

The effects of ODAP on the respiration of mitochondria using succinate, α-glycerophosphate, glutamate, glutamine and α-ketoglutarate are summarized in table 1. It is apparent that the respiratory rates decrease only when glutamate, glutamine or α-ketoglutarate are used as oxidizable substrates. The extent of inhibition depends on the relative concentration of glutamate and ODAP and the maximal effect is obtained when the ratio of the substrate to inhibitor is approx. 1:4. This behavior could arise either due to inhibition of the respiratory chain redox reactions or reflects decreased uptake of glutamate across the mitochondrial membrane.

Accordingly, to distinguish between these alternatives, the effect of ODAP on glutamate uptake was studied as a function of mitochondrial volume changes. The experiments were carried out in the presence of rotenone which blocks the oxidation of glutamate. The energy required for glutamate transport was derived through rotenone insensitive oxidation of succinate. The results presented in fig. 1 clearly demonstrate that ODAP inhibits glutamate dependent swelling. Fig. 2 shows that ODAP exerts its effects through fully competitive binding–presumably to the glutamate carrier present in the mitochondrial membrane.

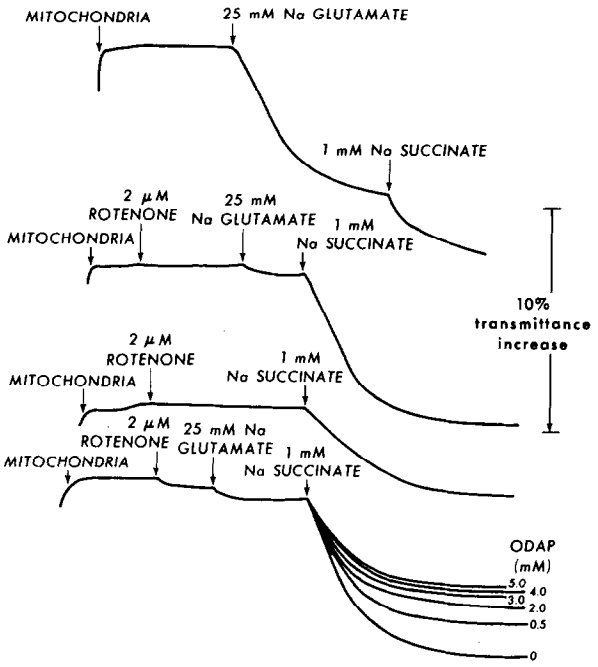

Fig. 1. Effect of ODAP on glutamate-dependent swelling. Assay medium: 225 mM mannitol, 75 mM sucrose, 1 mM phosphate, 10 mM KCl, 5 mM Tris, 0.33 mM EDTA. pH 7.4. Bovine brain mitochondria 1 mg/ml.

Also, approximate calculations suggest that neurolathyrism would occur only when ingestion of *L. sativus* seeds leads to 5–10 mM circulating ODAP. Hence, it is possible that apart from its effect on glutamate transport, ODAP might also influence other reactions in glutamate metabolism, e.g., those catalyzed by glutaminase I, glutamic dehydrogenase or aminotransferases. Indeed, this has been confirmed qualitatively, but the most striking effect is the apparent stimulation of glutaminase activity (table 2).

4. Discussion

In isolated brain mitochondria, ODAP at high concentration inhibits glutamate transport and several metabolic pathways; but stimulates glutamate and ammonia formation by an apparent activation of glutaminase. Cheema et al. [10] have reported that ODAP administration to young rats increases the activity of a soluble glutaminase enzyme and that ODAP has no effect on glutamine synthetase and slightly inhibits glutamic dehydrogenase.

Our findings suggest that ammonia toxicity in the

Table 2
Stimulation of glutaminase activity.

Mitochondria (ml)	Substrate (2×10^{-3} M)	ODAP (M)	nmoles NH_3/ min/mg protein
0.05	Glutamine	–	196.0
–	Glutamine	–	8.0
–	Glutamine	5×10^{-4}	9.2
–	–	2×10^{-3}	0.0
0.05	–	–	0.0
0.05	Glutamine	1×10^{-4}	265.0
0.05	Glutamine	5×10^{-4}	341.0
0.05	Glutamine	2×10^{-3}	471.0

The glutaminase incubation system contained in a total volume of 1 ml: 20 μmoles glutamine; 20 μmoles phosphate buffer pH 7.8; 0.15 mg protein. Incubation at 37° for 10 min. The results are averaged over 6 independent assays.

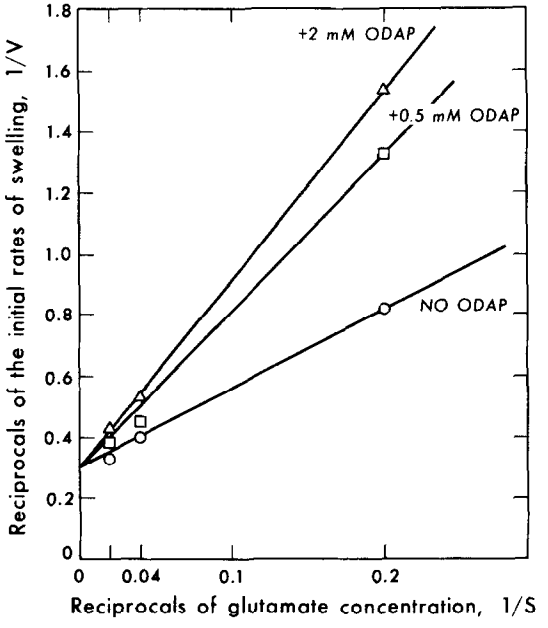

Fig. 2. Competitive inhibition by ODAP of the glutamate-dependent swelling. Experimental conditions as given in fig. 1.

brain could arise after prolonged treatment of an animal with ODAP by combined activation of glutaminase and inhibition of glutamate transport and oxidation. A resulting ammonia accumulation could activate lysosomal hydrolases, consequently causing brain damage. Activation of brain lysosomes has recently been observed by Lakskamanan et al. [11] following acute administration of ODAP.

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