RESPIRATORY INHIBITION OF TCA CYCLE AND CONTROL OF GLUTAMIC ACID SYNTHESIS BY AMMONIA IN RAT LIVER MITOCHONDRIA

Nobuhiko Katunuma and Mitsuko Okada

Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima, Japan.

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Inorganic ammonia is well known to be a strong cytoplasmic poison, although the method in which it inhibits cytoplasmic function is not yet known. This paper reports on poisoning effects of ammonia on cytoplasmic respiration in mitochondria, and on the feed back control of glutamate biosynthesis by ammonia. The effect of ammonia on several components of the TCA cycle was studied. Ammonia was found to be most inhibitory when citrate was used as a substrate for mitochondrial respiration. This respiratory inhibition caused to depress α-ketoglutarate produced, and subsequently glutamate formation by the OTA reaction coupled with the TCA cycle was also inhibited in the presence of ammonia.

In living cells glutamate is synthesized from α-ketoglutarate by several enzymes in the TCA cycle; i.e. GOT_M, GPT_M(Katunuma et al.,1961a,b,1962a,b,Huzino et al.,1963), GDH and OTA which has been purified in our laboratory(Matsuda, Tomino and Katunuma, 1963,Katunuma and Okada, 1962b). The glutamate formed by the above mechanisms is converted to aspartate through one of the GOT_Mcycles (Katunuma and Okada, 1962a,b,c).

The following abbreviations are used in this paper; GOT_M; Mitochondrial glutamic oxalacetic transaminase, GPT_M; Mitochondrial glutamic pyruvic transaminase, GDH; Glutamic dehydrogenase, OTA; Ornithine-δ-transaminase.

When too much glutamate accumulates, inorganic ammonia is liberated from glutamate in the GDH reaction. In fact the addition of glutamate and aspartate or malonate to a mitochondrial system results in the liberation of sufficient ammonia to inhibit the glutamate formation.

The present paper describes the regulation of glutamate formation by the OTA reaction in rat liver mitochondria.

METHODS

α-Ketoglutarate and amino acids were measured with 2,4-dinitrophenyl hydrazine and ninhydrin, respectively, as described in the previous papers(Katunuma and Okada, 1962a,b,c).

The rat liver mitochondria preparation and the incubation medium used were as described previously. Mitochondria were preincubated with ammonium chloride for 10 to 15 minutes at 37°C before the addition of substrates. The reaction period was usually 30 minutes. Preincubation of mitochondria with ammonia prior to the addition of substrates was essential to demonstrate the feed back control mechanism.

RESULTS AND DISCUSSION

Table I Inhibitory Effects of Ammonium salts on Respiration and Glutamate Formation in Mitochondria.

System(acetate;10μM+ succinate;1μM)	Inhibition% Respiration	Glutamate formation
Ammonium sulfate;2.5μM	48	-
Ammonium sulfate;2.5μM +ornithine;5μM	49	34
Ammonium acetate;5μM	49	-
Ammonium chloride;5μM	43	-
Ammonium chloride;7.5μM +ornithine;5μM	60	43
Sodium chloride;5μM	8	-

The effects of various ammonium salts added to mitochondria were examined when acetate and a small amount of succinate were used as the substrates. Respiratory inhibition of 40 to 60% was observed in regard to all kind of ammonium salts employed as shown in Table I. Adding ornithine to the system, glutamate formed by the OTA reaction was determined and a considerable depression of the glutamate formation was found in the presence of ammonium salts. Under this condition it has been previously confirmed that an effective glutamate formation by the GDH reaction is not recognized. In the same system above a decrease in the glutamate formation with increasing amounts of ammonium chloride added was found and at the same time less ornithine was consumed(Fig.1).

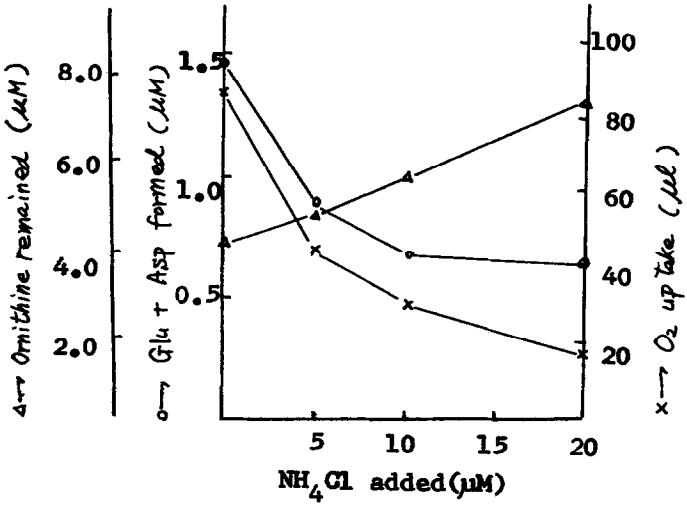

Fig.1 Inhibitory Effect of Ammonium Chloride on Glutamate Formation

The system contained 10μM acetate, 1μM succinate and 10μM ornithine in a final volume of 2ml.

Addition of ammonium to a mitochondrial system(containing citrate and medium) resulted in a decrease in the content of α-ketoglutarate(Fig.2). On the other hand since negligible amounts

of glutamate formation to illustrate the decrease of α-ketoglutarate were only detected, ammonia added to the system is assumed to regulate the production of α-ketoglutarate in the TCA cycle.

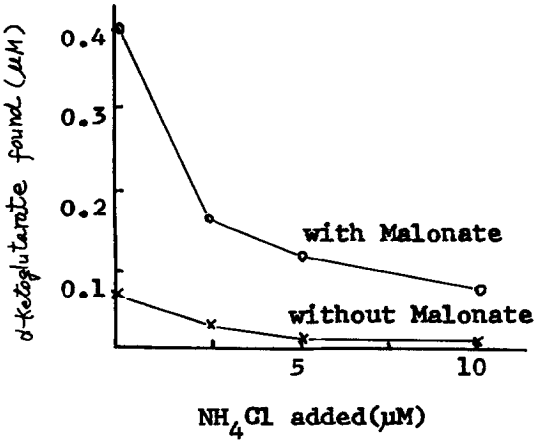

Fig.2 Control of α-Ketoglutarate Formation in the TCA cycle in the presence of the Ammonium Ion.
The system contained 5μM of citrate as substrate and the final volume was 2ml.

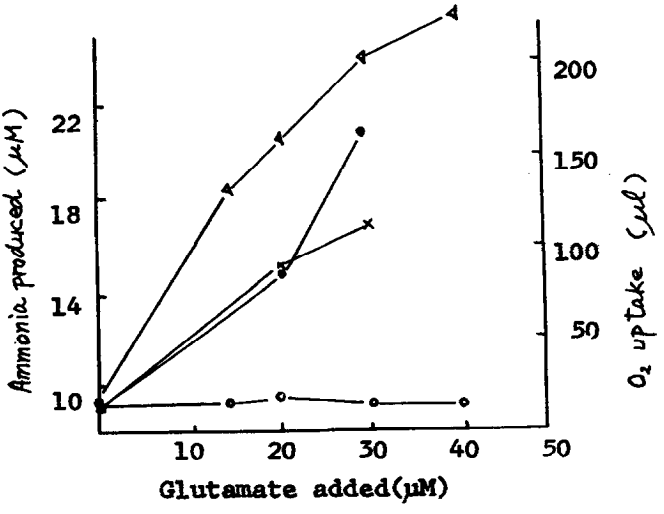

Fig.3 Malonate Inhibition of Converting Glutamate into Aspartate in Mitochondrial system.
•; NH_3 production with 10μM malonate, ×; O_2 up take with 10μM malonate, ○; NH_3 production without adding malonate, ◁;O_2 up take without malonate.

Because glutamate is usually produced from α-ketoglutarate generated in the TCA cycle in living cells, all routs of synthesis of glutamate will be indirectly inhibited by ammonia. The ammonia liberated from glutamate by GDH reaction results in a decrease of α-ketoglutarate formation and as the result since further formation of glutamate is inhibited, there is a apparently a feed back control by ammonia on glutamate formation. The inhibitory mechanism of ammonia on the TCA cycle is remained as an interesting problem to illustrate the feed back control of glutamate formation.

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