CORRELATION BETWEEN GLUTAMATE AND Ca^{2+} UPTAKE IN RAT LIVER MITOCHONDRIA

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1. Introduction

Rat liver mitochondria can accumulate large quantities of Ca^{2+} in the inner matrix compartment by an energy-linked process. It was demonstrated that this Ca^{2+} uptake is associated with a stoichiometric ejection of protons [1–3]. Anions such as phosphate, acetate and bicarbonate which permeate electroneutrally can support and accompany this energy-linked Ca^{2+} transport in respiring rat liver mitochondria [4] and cause acidification of the matrix. In contrast, anions which permeate electrogenically without net transport of protons into matrix (nitrate, thiocyanate) do not accompany Ca^{2+} transport [4]. There is now a wide agreement that the driving force for Ca^{2+} uptake in the presence of permeant anions is the electrical component of the electrochemical proton gradient derived from respiration or ATP hydrolysis [5]. A controversy has been recently developed concerning the ratio H^+ ejected/Ca^{2+} taken up, whether there are two (+) charges [6] or one (+) charge [7] when phosphate movements are inhibited.

We present here data showing that glutamate can accompany Ca^{2+} uptake in respiring rat liver mitochondria. Glutamate can enter rat liver mitochondria by two transport systems: a glutamate/asparate exchange [8–10] and a *N*-ethylmaleimide sensitive glutamate/H^+ co-transport [11–15]. Our data are consistent with a co-transport glutamate/Ca^{2+} mediated by this last transport system.

Abbreviations: P_i, inorganic phosphate; Tris, Tris (Hydroxymethyl) aminomethane; Hepes, *N*-2-hydroxyethylpiperazine-*N'*2-ethanesulfonic acid; Val, valinomycin; Rot, rotenone; Succ, succinate; Glu, glutamate; NEM, *N*-ethylmaleimide

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2. Materials and methods

2.1. *Isolation of mitochondria*

Rat liver mitochondria were isolated according to [16] in 0.25 M sucrose, 2 mM Tris–HCl, pH 7.4. Proteins were determined by the Biuret method [7].

2.2. *[^{3}H]Glutamate incorporation*

The technique used was derived from [12] and recently described in detail [18].

2.3. *Ca^{2+} uptake*

Atomic absorption determinations have been made with a Perkin Elmer spectrophotometer 420.

Changes in the extramitochondrial concentration of free Ca^{2+} were monitored by use of a Ca^{2+} selective electrode (Philips IS561) developed [19]. The electrode potentials were amplified by a Orion 801 A ionometer which drove a Sefram recorder.

Mitochondrial swelling, initiated by addition of 100 μM Ca^{2+}, were monitored at 546 nm in a cell photometer containing 1 ml 100 mM Tris–glutamate, pH 7.2.

3. Results and discussion

3.1. *Stimulation of glutamate uptake by valinomycin and Ca^{2+}*

Figure 1 shows that rat liver mitochondria, incubated in the presence of 5 mM potassium glutamate, absorb approx. 5–6 nmol glutamate/mg protein/min. This absorption is reduced 10–15% by rotenone [18] but is considerably increased by valinomycin which, in the presence of K^+, stimulated the transmembrane ΔpH [20,21]. Addition of 50 natoms Ca^{2+}/mg protein increases glutamate uptake to the same extent as the valinomycin–K^+ system.

In contrast, addition of 5 mM P_i stops glutamate uptake whether Ca^{2+} is present or not. When valinomycin is also present, P_i considerably reduces glutamate uptake (4 nmols [^{3}H]Glu/mg proteins, instead of 22). It has been clearly established that phosphate uptake by mitochondria is controlled by the transmembrane ΔpH [22,23]. In the presence of Ca^{2+} or of valinomycin there appears to be competition between the phosphate/H^+ and the glutamate/H^+ co-transports in relation to the transmembrane ΔpH. The presence of Ca^{2+} favours the phosphate/Ca^{2+} co-transport.

A small part of the reduction in radioactivity measured in the presence of P_i may be due to a P_i/[^{3}H]dicarboxylate exchange resulting from [^{3}H]-glutamate metabolism.

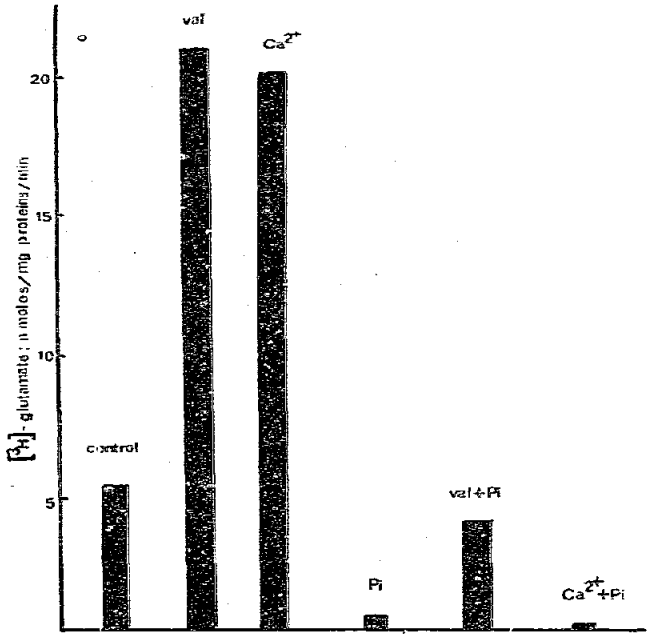

Fig.1. Stimulation of glutamate accumulation by valinomycin and Ca^{2+}. Mitochondria, 2 mg, were preincubated in Eppendorf cups in 1 ml medium (200 mM sucrose, 5 mM $MgCl_2$, 10 mM Hepes–KOH buffer, pH 7.4 and [^{14}C]sucrose). Glutamate transport was initiated by adding [^{3}H]glutamate (K^+ salt), 5 mM final concentration, and stopped by rapid centrifugation with an Eppendorf 3200 microcentrifuge. The supernatant was discarded and the pellet dissolved in 0.2 ml formic acid. The radioactivity (^{3}H and ^{14}C) of the pellet was measured with an Intertechnique SL 40 scintillation counter in the POPOP/PPO system. The [^{14}C]sucrose permeable space was found to range from 1.5 μl to 2 μl/mg proteins. For each experiment, [^{3}H]glutamate in the matrix space (sucrose impermeable) was calculated by substrating [^{3}H]-glutamate present in the sucrose space. Valinomycin 20 ng/mg protein, Ca^{2+} 50 natoms/mg proteins, Pi 5 mM.

3.2. *The effect of glutamate on Ca^{2+} uptake*

3.2.1. Mitochondrial Ca^{2+} determined by atomic absorption

Figure 2 shows that, in the absence of any added substrate, the mitochondria contain about 5 natoms

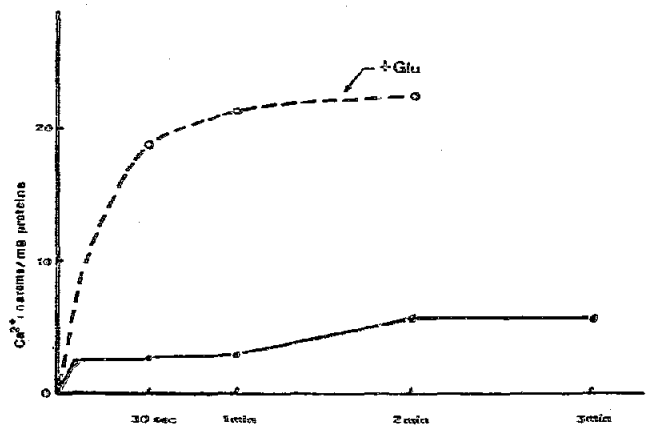

Fig.2. Effect of glutamate on Ca^{2+} fixation. Mitochondria, 2 mg, were incubated in the same conditions as for glutamate uptake essays (fig.1). Ca^{2+}, 50 natoms/mg protein, was initially present in the medium and the reaction was initiated by adding mitochondria and stopped by centrifugation at various times. In each case the pellet was dissolved in 0.2 ml formic acid for Ca^{2+} determination by atomic absorption. (–●–●–) Ca^{2+} incorporation. (- -o- -o- -) Ca^{2+} incorporation with 5 mM Glu.

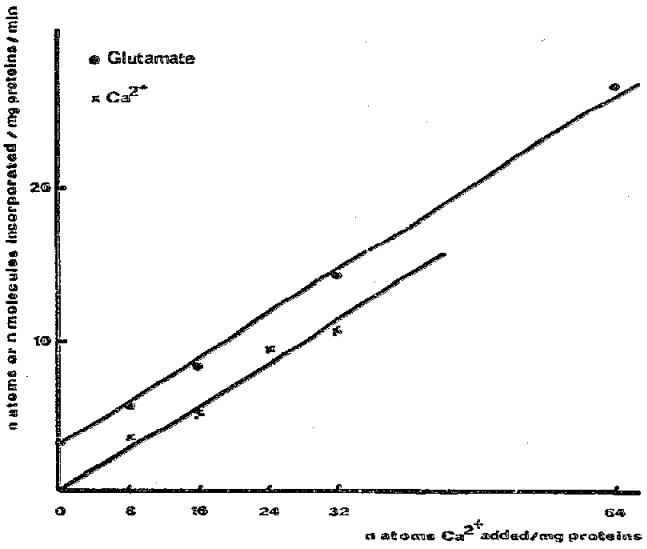

Fig.3. Correlation between glutamate and Ca^{2+} incorporation. [^{3}H]Glutamate and Ca^{2+} incorporation were determined in conditions described in fig.1 ([^{3}H]Glu.) and fig.2 (Ca^{2+}). (—•—•—) Glutamate accumulation. (—×—×—) Ca^{2+} accumulation.

Ca^{2+}/mg protein. In the presence of 5 mM glutamate they accumulate up to 22 natoms Ca^{2+}/mg protein.

Figure 3 shows that there is a linear regression of the quantities of Ca^{2+} and [^{3}H]glutamate absorbed and the concentration of Ca^{2+} in the incubation medium.

3.2.2. Change in extramitochondrial free Ca^{2+} concentration determined by a specific Ca^{2+} electrode

In the absence of any added substrate mitochondria fix relatively little Ca^{2+} (fig.4A). The addition of 5 mM glutamate induces active penetration of mitochondria by Ca^{2+} and after a few minutes all the available Ca^{2+} is fixed. This raises the question of whether, in such an experiment, glutamate is involved only as a substrate generating the energy for Ca^{2+} transport or as an anion, indispensible to Ca^{2+} transport, before it is oxidised.

In fig.4B it can be seen that the addition of succinate in the presence of rotenone (protonmotive force generator) induces a slight Ca^{2+} penetration. In contrast, in the same conditions, glutamate addition gives rise to complete Ca^{2+} fixation. Figure 4C shows

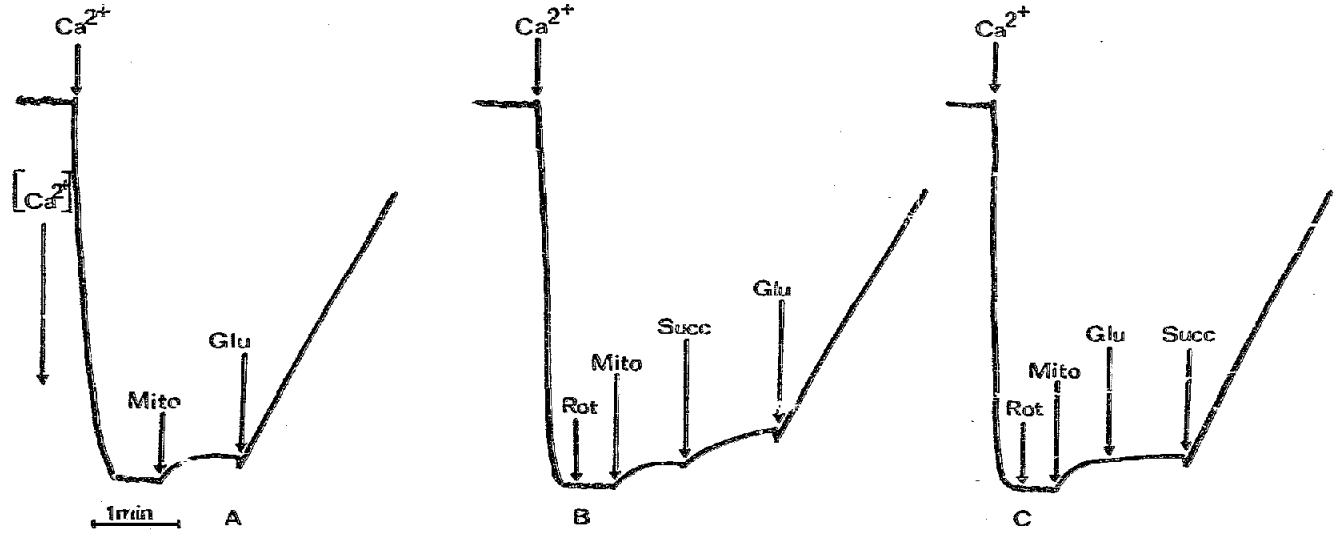

Fig.4. Effect of glutamate on extramitochondrial Ca^{2+} concentration measured with a Ca^{2+} specific electrode. Mitochondria, 1 mg, were incubated in 5 ml medium (20 mM sucrose, 20 mM KCl, 10 mM Hepes–KOH, pH 7.2). Ca^{2+} (100 natoms) was added before mitochondria. Changes in Ca^{2+} concentration were monitored with a Ca^{2+}-specific electrode Philips connected to a Orion 801 A ionometer with a Sefram recorder. Glutamate 5 mM, succinate 5 mM, rotenone 1 μg.

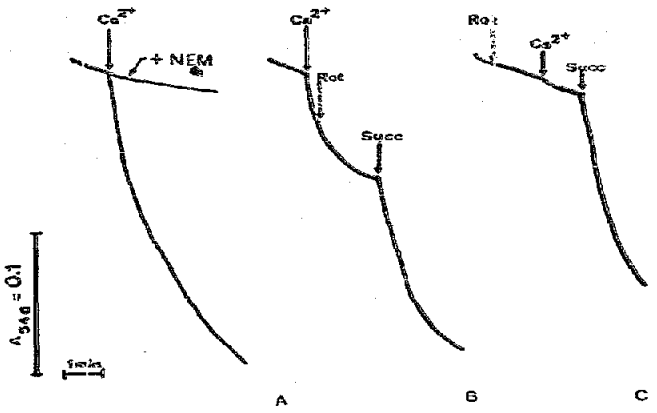

Fig.5. Energy-linked swelling induced by Ca^{2+} of rat liver mitochondria in Tris glutamate. Mitochondria, 1 mg, were added to 1 ml 0.1 μM Tris–glutamate, pH 7.2. Ca^{2+} 100 natoms, NEM 100 nmol, succinate 5 mM, rotenone 1 μg.

that glutamate added in the presence of rotenone, which inhibits its oxidation, does not result in Ca^{2+} fixation. Following succinate addition results immediately in calcium glutamate uptake.

3.2.3. Mitochondria swelling induced by calcium glutamate

When the mitochondria are incubated in the presence of 100 mM Tris–glutamate, no swelling is observed. The addition of 100 μM Ca^{2+} induces swelling which can be inhibited by *N*-ethylmaleimice (fig.5A).

Rotenone addition also causes inhibition whereas succinate re-establishes swelling (fig.5B). Calcium glutamate penetration is thus an energy-requiring process. When glutamate oxidation is inhibited by rotenone, succinate addition after Ca^{2+} results in glutamate–Ca^{2+} transport (fig.5C).

4. Conclusions

Our results suggest that there is cotransport of glutamate and Ca^{2+} in rat liver mitochondria and that the process requires energy. This hypothesis is supported by the following facts:

1. Phosphate, whose active transport is linked to the transmembrane ΔpH, is a powerful inhibitor of glutamate translocation. In our experimental conditions, phosphate utilizes the transmembrane ΔpH to its own advantage.

2. The glutamate/Ca^{2+} co-transport is inhibited if glutamate oxidation is blocked by rotenone. This inhibition is removed if succinate is added to the incubation medium.

3. The inhibition of glutamate/Ca^{2+} co-transport by NEM suggests that the glutamate/H^+ transport system is involved.

Acknowledgements

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