Mg^{2+} RESTORES MEMBRANE POTENTIAL IN RAT LIVER MITOCHONDRIA DEENERGIZED BY Ca^{2+} AND PHOSPHATE MOVEMENTS

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1. Introduction

Inorganic phosphate and Ca^{2+} in the incubation medium induces a closely parallel efflux of Mg^{2+} and adenine nucleotides from rat liver mitochondria [1]. These effluxes are respiration-dependent and prevented by external Mg^{2+} [1,2] and by agents inhibiting the transport of Ca^{2+} and phosphate across the inner membrane, i.e., EGTA, ruthenium red and *N*-ethylmaleimide [3,4]. At first no release of accumulated Ca^{2+} occurs during Mg^{2+} efflux; however, as soon as ~50% endogenous Mg^{2+} has been lost, Ca^{2+} begins to escape as well.

Here, we report that the membrane potential of liver mitochondria incubated in the presence of Ca^{2+} and phosphate is preserved for a time period dependent on $[Ca^{2+}]$; then the potential drops and accumulated Ca^{2+} are released. At this point, addition of Mg^{2+} restores the original membrane potential and confers to mitochondria the full capability to reaccumulate the Ca^{2+} lost.

2. Experimental

Rat liver mitochondria were isolated in 0.25 M sucrose and 5 mM Hepes (pH 7.4) by a conventional centrifugation method. Mitochondrial protein concentration was assayed by a biuret method with bovine serum albumin as standard.

Mitochondrial incubations were carried out at 20°C with 1 mg mitochondrial protein/ml in the following standard medium: 200 mM sucrose, 10 mM Hepes (pH 6.8), 2 mM sodium phosphate (pH 6.8), 5 mM sodium succinate, 1.25 μM rotenone. Final $[Ca^{2+}]$ was brought to 30 μM after having determined Ca^{2+} content of media by atomic spectroscopy.

Membrane potential ($\Delta\psi$) was measured by monitoring the movements of tetraphenylphosphonium across the mitochondrial membrane with a tetraphenylphosphonium-selective electrode [5].

Ca^{2+} transport was measured with a Ca^{2+}-selective electrode (Radiometer F 2112) with a calomel electrode (Radiometer K 401) as the response electrode. The logarithmic response of the Ca^{2+} electrode was linearized by connecting the electrodes to a Beckman Selection TM 5000 Ion Analyzer.

Ca^{2+} movements in the presence of added Mg^{2+} were estimated by atomic absorption spectroscopy of the supernatant [3].

Mg^{2+} movements were determined in the mitochondrial pellet as following: at defined times, 1.0 ml portions of the incubation mixture were withdrawn and layered in a centrifuge tube containing 0.15 ml 13% sucrose and 0.4 ml silicone oil (AR 100/AR 150 (2:1); Wacker-Chemie GmbH, Munich) and centrifuged for 60 s in an Eppendorf 3200 centrifuge. After decanting supernatant and silicon oil, 0.85 ml 1 mM sodium EDTA, 0.1% NaCl, 0.9% deoxycholate (pH 7.4) were added and Mg^{2+} in the dissolved pellet was measured by atomic spectroscopy.

Oxygen uptake was measured with a Clark oxygen electrode.

3. Results

Rat liver mitochondria incubated in the presence of 30 μM Ca^{2+} and 2 mM phosphate (concentrations not far from those existing in the cytosol) and 5 mM succinate quickly acquired a membrane potential of ~200 mV and concomitantly accumulated external Ca^{2+} (fig.1). In the typical experiment reported this

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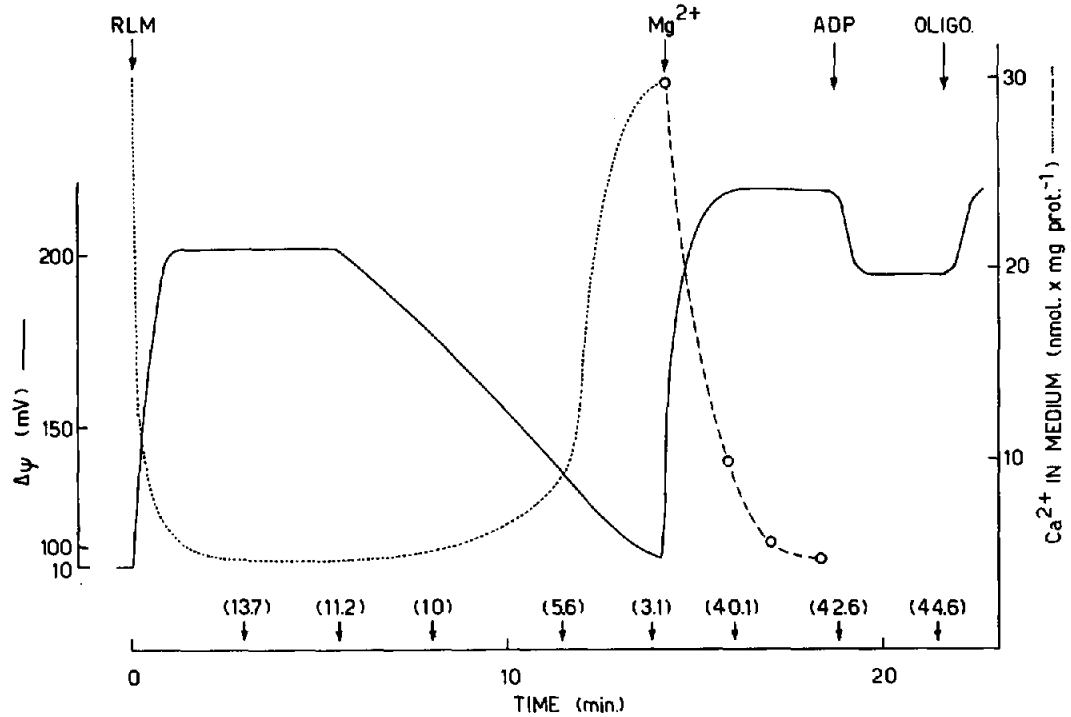

Fig.1. Effect of Mg^{2+} on restoration of $\Delta\psi$ collapsed by Ca^{2+} cycling. Rat liver mitochondria (1 mg/ml) were suspended in the standard medium. At the arrows, the following additions were made: 2 mM Mg^{2+}, 0.2 mM ADP. $\Delta\psi$ measurement (——), Ca^{2+} and Mg^{2+} fluxes were determined in the same vessel. Ca^{2+} fluxes were measured by Ca^{2+} selective electrode (. . .) and atomic absorption spectroscopy of the supernatant (– – –). Numbers in parentheses refer to nmol/mg protein Mg^{2+} determined in the pellet at times indicated; Mg^{2+} content at zero time was 23.5 nmol/mg protein.

potential was preserved for 6 min and began gradually to drop when mitochondrial [Mg^{2+}] was decreased by 51%. As soon as the potential reached the value of 175 mV and the mitochondrial [Mg^{2+}] was concomitantly decreased by 58%, Ca^{2+} was no longer retained. It has to be noted that the time length of membrane potential preservation was inversely related to the amount of [Ca^{2+}] in the incubation medium (not shown). Addition of 2 mM Mg^{2+} to deenergized mitochondria resulted in a quick restoration of the original potential and a complete reaccumulation of Ca^{2+} released (fig.1). The lower steady state of membrane potential resulting from ADP addition and the subsequent restoration to previous values upon addition of oligomycin can be ascribed to the energy utilization for the phosphorylation process [6].

The ability of added Mg^{2+} to restore the coupled state of mitochondria was also confirmed by the inhibition of released respiration (fig.2). Under the experimental conditions adopted the release of resting respiration may reflect an acceleration of Ca^{2+}

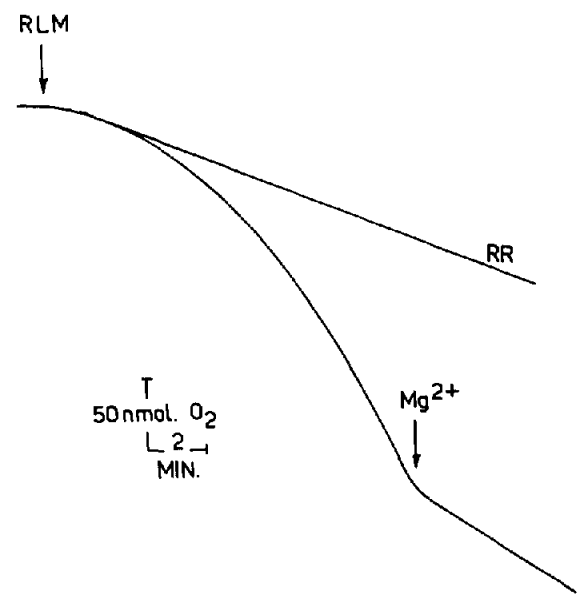

Fig.2. Inhibition by Mg^{2+} on the release of resting respiration. Experimental conditions as in fig.1. Where indicated 2 mM Mg^{2+} was added. When present 0.2 μM ruthenium red (RR).

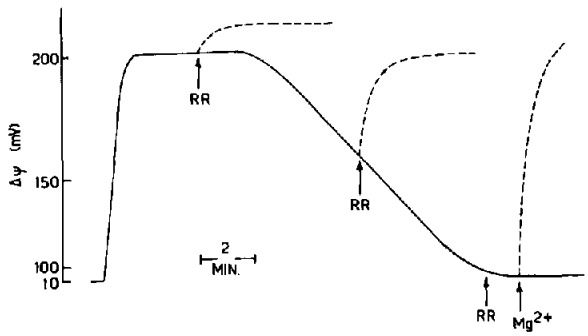

Fig.3. Effect of ruthenium red on mitochondrial $\Delta\psi$ in conditions of Ca^{2+} cycling. Experimental conditions as in fig.1. Where indicated 0.2 μM ruthenium red (RR) and 2 mM Mg^{2+} were added.

cycling, since the release was fully prevented by ruthenium red (fig.2, see also [3,7]). Hence, added Mg^{2+} appears to be able both to inhibit the increase of resting respiration induced by Ca^{2+} and phosphate [1] and to restore the original state of mitochondria, by repairing the damage induced by these two ions.

This view is supported by the results in fig.3 which show that ruthenium red prevented the drop of membrane potential and restored the original potential when added before it dropped below 150 mV. Unlike added Mg^{2+}, ruthenium red resulted completely unable to restore the potential when completely collapsed (fig.3). Similar results have been obtained with mersalyl (not shown, see [8]) which, inhibiting P_i transport, blocks Ca^{2+} movement.

4. Discussion

These results show that the functional changes, at least the most significant, induced to mitochondria by Ca^{2+} and phosphate are reversible. External Mg^{2+} not only prevents [1,4,5,9] these alterations, but also restores the original membrane potential and the capability to accumulate Ca^{2+}, when these linked properties have been lost.

The mechanism by which external Mg^{2+} exerts its restitutive effect is certainly related to its capability to bind to mitochondrial membranes thus replacing endogenous Mg^{2+} released (note numbers in parentheses in fig.1) as a consequence of Ca^{2+} and phosphate fluxes across the inner mitochondrial membrane. Also the findings [10] that Mg^{2+} and Ca^{2+} compete for the binding sites within mitochondrial membrane are relevant to this point. That Ca^{2+} and phosphate movements may induce the observed decay of mitochondrial potential is clearly demonstrated by the preventing and partially restoring action of ruthenium red or mersalyl. However, as shown in [1], the energy-dependent Mg^{2+} efflux induced by Ca^{2+} and phosphate is prevented either by ruthenium red or mersalyl, thus indicating a close dependence on Ca^{2+} and phosphate transport. Evidently the block of Ca^{2+} and phosphate transport allows energized mitochondria to retain endogenous Mg^{2+} and consequently to preserve and possibly to regain the original potential provided that its drop is only partial and the loss of endogenous Mg^{2+} below a critical value.

The ability of external Mg^{2+} to fully reconstitute the permeability barrier required to reset the original coupling state, provides further evidence to the view that Mg^{2+} may control ion flux across inner mitochondrial membrane [11,12] and rationalizes the old concept that Mg^{2+} 'stabilizes' mitochondrial structure and function [13]. The physiological meaning of these results is not clear owing to the lack of information on the free intracellular Mg^{2+} at rest and its changes following metabolic perturbations [14].

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References

[1] Zoccarato, F., Rugolo, M., Siliprandi, D. and Siliprandi, N. (1981) Eur. J. Biochem. 114, 195–199.
[2] Harris, E. J. and Cooper, M. B. (1981) Biochem. Biophys. Res. Commun. 103, 788–796.
[3] Siliprandi, D., Toninello, A., Zoccarato, F. and Siliprandi, N. (1977) Biochem. Biophys. Res. Comm. 78, 23–27.
[4] Siliprandi, N., Siliprandi, D., Toninello, A., Rugolo, M. and Zoccarato, F. (1978) in: The Proton and Calcium Pumps (Azzone, G. F. et al. eds) pp. 263–271, Elsevier Biomedical, Amsterdam, New York.
[5] Lotscher, H. R., Winterhalter, K. H., Carafoli, E. and Richter, C. (1980) Eur. J. Biochem. 110, 211–216.
[6] Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, Y. (1979) J. Membr. Biol. 49, 105–121.

[7] Stucki, J. W. and Ineichen, E. A. (1974) Eur. J. Biochem. 48, 365–375.
[8] Rugulo, M., Siliprandi, D., Siliprandi, N. and Toninello, A. (1981) Biochem. J. 200, 481–486.
[9] Siliprandi, N., Rugolo, M., Siliprandi, D. and Toninello, A. (1981) in: Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F. et al. eds) pp. 265–275, Elsevier Biomedical, Amsterdam, New York.
[10] Scarpa, A. and Azzi, A. (1968) Biochim. Biophys. Acta 150, 473–481.
[11] Brierley, G. P. (1976) Mol. Cell. Biochem. 10, 41–62.
[12] Barnard, P. A. and Cockrell, R. S. (1982) Biochim. Biophys. Acta 679, 68–74.
[13] Lehninger, A. L. (1962) Physiol. Rev. 42, 467–517.
[14] Scarpa, A. and Brinley, J. (1981) Fed. Proc. FASEB 40, 2646–2652.