A POSSIBLE MECHANISM FOR RESPIRATION-DEPENDENT EFFLUX OF Mg IONS FROM LIVER MITOCHONDRIA

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Summary: Addition of Pi or diamide to a suspension of rat liver mitochondria induced a net efflux of Mg^{++} which is dependent on coupled respiration. This Mg^{++} efflux is prevented by EGTA and by Ruthenium red, both of which also prevent the increased rate of state 4 respiration induced by Pi or by diamide. It is assumed that an accelerated recycling of endogenous Ca^{++} induced by Pi or by diamide through an altered permeability of inner membrane to Ca^{++} is responsible for Mg^{++} efflux, and accounts for its apparent dependence on coupled respiration.

A respiration-dependent efflux of Mg^{++} from heart (1) and liver mitochondria (2-7) has been recently described. Respiration linked Mg^{++} efflux from heart mitochondria occurs spontaneously, provided that the extramitochondrial concentration of Mg^{++} is less than about 2.5 mM (1). Efflux of Mg^{++} from liver mitochondria, in addition to coupled respiration, requires the addition of an agent [Ca^{++} (2,3,4), diamide (2), Pi (2,4,5) or low concentrations of ionophore A 23187 (6,7)] which may conveniently modify inner membrane permeability properties.

The results reported in the present paper provide evidence that Mg^{++} efflux from liver mitochondria, induced by diamide or by Pi, is a consequence of a recycling of endogenous Ca^{++} which, in turn, is dependent on coupled respiration.

Experimental: Rat liver mitochondria were isolated according to Schneider (8). Protein concentration was determined by the biuret method (9). Oxygen uptake was measured with a Clark oxygen electrode. Mg^{++} and Ca^{++} movements were estimated by atomic absorption spectroscopy on the supernatant (1), and total cation amount by acid extraction of the pellet (10).

Results: The time course of Ca^{++} and Mg^{++} movements into and out of rat liver mitochondria, incubated in the presence and absence of 2 mM Pi, is reported in Fig. 1a. Practically all calcium ions, initially present in the incubation medium as contaminants, were taken up rapidly, both in the presence and in the absence of Pi, and apparently retained by the mitochondria until an anoxic sta-

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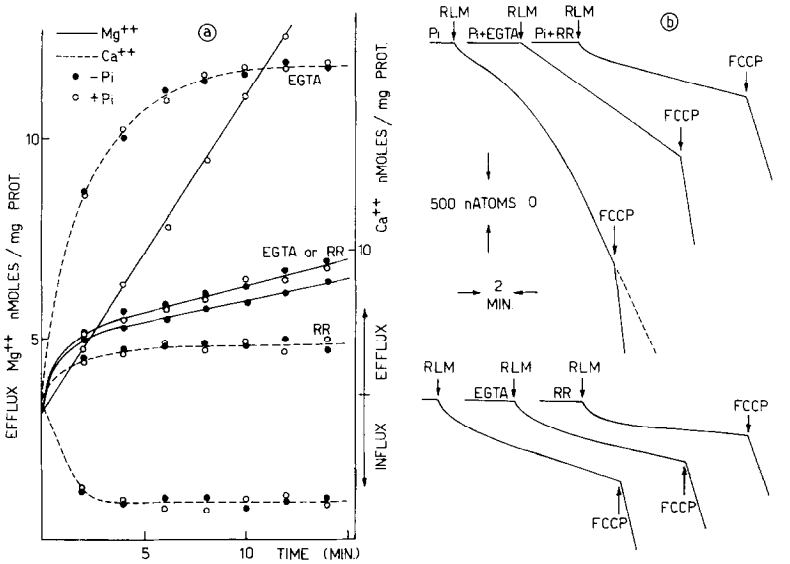

Figure 1 - Effect of phosphate on the movement of endogenous Ca^{++} and Mg^{++} (a) and on state 4 respiration (b).

Rat liver mitochondria (10 mg protein) were suspended in a medium (final volume 10 ml, temperature 25°C) containing: 170 mM sucrose, 10 mM Tris-Cl pH 6.5, 5 mM Na-succinate, 1.25 µM rotenone. When present: 2 mM Pi (pH 6.5), 5 µM Ruthenium red (RR)(5 nmoles/mg prot), 1 mM EGTA, 0.8 µM FCCP, 1 µM Antimycin A. Cation contents at zero time were in nmoles per mg protein: calcium, 18; magnesium, 22.

te was reached.

As expected, FCCP, Antimycin A (results not reported) and EGTA prevented initial Ca^{++} uptake, and induced a rapid release of endogenous Ca^{++}. Ruthenium red prevented both Ca^{++} uptake and release.

A linear efflux of Mg^{++} was constantly observed from liver mitochondria incubated in the presence of Pi, but not from mitochondria incubated in the absence of Pi. Mg^{++} release was completely prevented by FCCP, Antimycin A (results not reported) and also by Ruthenium red and EGTA (EDTA was much less efficient).

The inhibition of Mg^{++} efflux by uncouplers and by respiratory chain inhibitors indicates its dependence on coupled respiration, while inhibition of Mg^{++} efflux by Ruthenium red and EGTA indicates a dependence on Ca^{++} movements across the mitochondrial membrane. As known Ruthenium red blocks the Ca^{++} pump, and EGTA chelates Ca^{++} present outside the mitochondria and, very probably, within the intermembrane space (11).

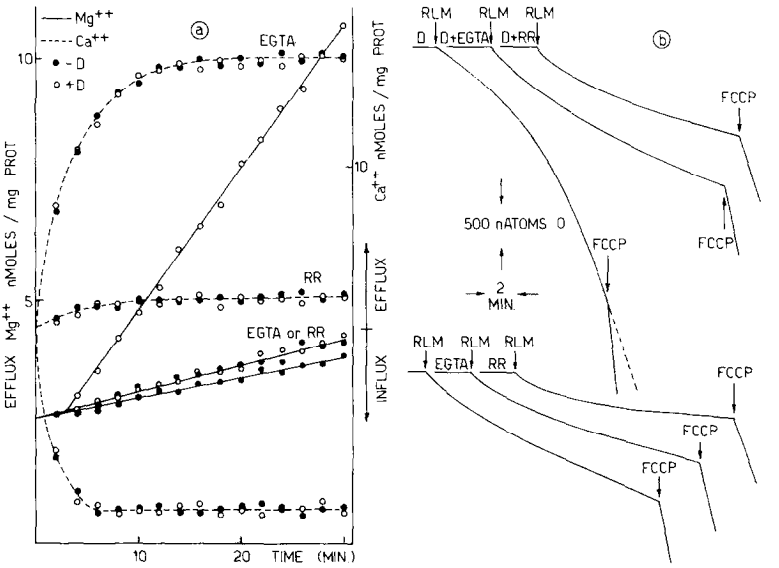

Figure 2 - Effect of diamide on the movement of endogenous Ca^{++} and Mg^{++} (a) and on state 4 respiration (b).

Experimental conditions and incubation medium as in Figure 1, except for 10 mM Tris-Cl, pH 7.4. When present, 0.15 mM diamide (D) corresponding to 150 nmoles/mg protein. Cation contents at zero time were in nmoles per mg protein: calcium, 13,6; magnesium, 21.

Fig.1b also shows that Pi increased significantly the rate of state 4 respiration, and that this effect was abolished by both Ruthenium red and EGTA.

It should be noted that these results were obtained by suspending rat liver mitochondria in a sucrose-Tris medium at pH 6.5. At pH 7.4, added Pi induced the same effect but with a more reduced rate of Mg^{++} efflux.

Approximately the same results were obtained by exposing rat liver mitochondria to 0.15 mM diamide. This reagent induced the same movements of Ca^{++} and Mg^{++} and an analogous increase in state 4 respiration (see Fig.2). Other thiol reagents, such as NEM and Mersalyl, failed to induce any release of Mg^{++} (results not reported).

The amount of Mg^{++} released upon incubation for 15 and 30 minutes in the presence of Pi or diamide was about 66 and 50 per cent respectively of the total mitochondrial Mg^{++}; nevertheless no release of Ca^{++} occurred. On the other hand, no Mg^{++} efflux accompanied the consistent release of Ca^{++} from uncoupled or non respiring mitochondria similarly treated with Pi or with diamide. It appears therefore that Mg^{++} and Ca^{++} release from mitochondria are not necessarily correlated events. This is in some disagreement with previ-

ous observations (obtained under different conditions) that Mg^{++} accompanies the loss of Ca^{++} (4).

Discussion: The results reported in the present paper show that a progressive (linear) efflux of Mg^{++} from liver mitochondria can be induced by diamide as well as by Pi. It is conceivable that, although with different mechanisms, these two unrelated compounds induce inner membrane alterations which have the same, or very similar effects on the movements of divalent ions. Diamide and Pi also share the property of increasing the rate of state 4 respiration, without reaching a complete uncoupled state. The coupled state of liver mitochondria, treated with either 0.15 mM diamide or 2 mM Pi, is demonstrated by their capacity to retain Ca^{++} (see Fig.1a and 2a). On the other hand, the necessity of a coupled state of mitochondria appears to be an indispensable requirement for Mg^{++} efflux, since it is blocked by FCCP or antimycin A. The coupled respiration-dependent efflux of Mg^{++} also appears dependent on Ca^{++} movements, since it is very sensitive to both Ruthenium red and EGTA.

The fact that Ruthenium red and EGTA also slow down state 4 respiration of diamide - or Pi - treated mitochondria, makes it reasonable to postulate a correlation between Mg^{++} efflux and Ca^{++} recycling across inner membrane.

Drahota et al. (12) reported that the retention of low amounts of Ca^{++} is not a result of an irreversible sequestration of this ion, but instead reflects a steady state in which the efflux of Ca^{++} down its chemical gra - dient is compensated by an energy-linked re-uptake during state 4 respiration. The energy-dissipating recycling of endogenous calcium has been more thoroughly substantiated by Pfeiffer et al. (6) using the ionophore A23187.

Under our conditions, a recycling of endogenous Ca^{++} is recognizable in the acceleration of state 4 respiration by Pi or by diamide, and in its sensitivity to Ruthenium red and EGTA. By oxidizing some pairs of thiol groups diamide induces perturbations of the inner membrane (13) which could be compatible with an increase in passive Ca^{++} efflux, thus engaging more energy for its re-uptake. An analogous mechanism may be assumed for Pi. According to Rossi and Lehninger (14), Pi added to liver mitochondria at 2 mM concentration "either prevents the binding of Ca^{++}, or causes the discharge of bound Ca^{++}, so that a large part of the added Ca^{++} remains free in the medium and thus continues to activate the respiratory chain".

Our observation that 2 mM Pi induced a more rapid release of Mg^{++} at pH 6.5 than at pH 7.5 is compatible with an electroneutral exchange of extramitochondrial protons for intramitochondrial Mg^{++} (6).

How the recycling of calcium ions, stimulated by diamide or by Pi, might in turn induce a release of Mg^{++}, is a matter for speculation. However, con-

sidering that Mg^{++} and Ca^{++} could compete for their binding sites within the mitochondrial membrane (15) it is conceivable that continuous recycling of Ca^{++} across the inner membrane could induce a progressive displacement of endogenous Mg^{++}.

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