SYNERGIC ACTION OF CALCIUM IONS AND DIAMIDE ON MITOCHONDRIAL SWELLING

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Summary: The characteristics of rat liver mitochondria swelling induced by diamide, an oxidizing agent for thiol groups, and by Ca ions are very similar. In both cases the swelling, which is initiated by addition of 0.5-1 mM phosphate or acetate, is prevented by FCCP, antimycin A, EGTA, Mg^{++} and ruthenium red. Diamide potentiates the swelling action of Ca^{++}, while DTE potentiates that of Mg^{++}. The additive effects of calcium and diamide on rat liver mitochondria have been correlated with their synergic action in promoting the release of mitochondrial Mg^{++}. The results strongly indicate that some of the effects of diamide are mediated by a mobilization of endogenous divalent ions and that the antagonism between Ca^{++} and Mg^{++} is closely correlated with the redox state of membrane bound thiol groups.

Introduction: It is well known that metal cations, especially divalent ions, affect the properties of mitochondrial and chloroplast membranes. Slater and Cleland (1) demonstrated that Ca^{++} acts as a "labilizer" of coupled phosphorylation, and consequently proposed EDTA as a mitochondrial stabilizing agent. Conversely, Mg^{++} protects mitochondria from the deleterious action of Ca^{++} (2-6), probably by competing for the binding sites within the mitochondrial membrane (7). Furthermore, Mg^{++} bound to mitochondria is considered an obligatory component of the mitochondrial energy transducing apparatus (8).

On the other hand, thiol oxidizing agents (tellurite, selenite and diamide) induce alterations in mitochondrial structure and function which are reminiscent, from a phenomenological point of view, of those produced by Ca^{++} (9-12). In particular, diamide brings about an impairment in some energy coupled processes such as oxidative phosphorylation and Ca^{++} and K^{+} uptake, which can be restored by successive addition of DTE to functionally damaged mitochondria (11).

The analogy between the effects of Ca^{++} and thiol oxidizing agents on some mitochondrial properties prompted us to study whether changes in the SH/S-S ratio could influence the action of Ca^{++} and Mg^{++} on mitochondrial membrane permeability. The results reported in the present paper demonstrate

that the oxidation of mitochondrial thiol groups strongly potentiates the effect of Ca^{++} on mitochondrial swelling, while their reduction potentiates the antagonistic effects of Mg^{++}.

Experimental: Rat liver mitochondria were isolated according to Schneider (13). Protein concentration was determined by the biuret method (14). Mg^{++} was estimated by atomic absorption spectroscopy of acid extracts (15). Swelling was monitored by absorption at 520 nm using an Aminco-Chance spectrophotometer.

Results: Fig.1 shows the close similarity between rat liver mitochondria swelling induced by diamide and by Ca^{++}. In both cases swelling was initiated by addition of 0.5-1 mM potassium phosphate and was prevented by FCCP, antimycin A, or by omission of the oxidizable substrate. The dependence of diamide and Ca^{++} induced swelling on added phosphate is confirmed by the blocking effect of mercurials (mersalyl and pCMB). Addition of Mg^{++} or of EGTA prevented the swelling induced by diamide as well as that induced by Ca^{++}. EDTA was much less effective than EGTA. Furthermore, in the presence of higher concentrations of phosphate (above 2 mM) or of Ca^{++} (above 0.3 mM), the antagonistic effect of Mg^{++} resulted much less evident. Similar results were obtained by replacing potassium phosphate with potassium acetate (re-

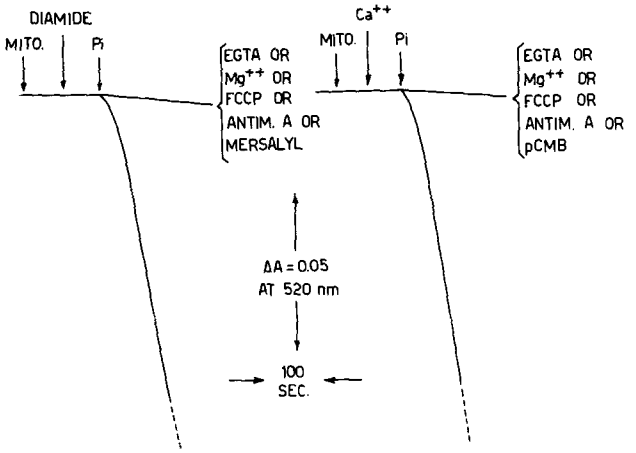

Figure 1 - Similarity of RLM swelling induced by diamide and by Ca^{++}.
Rat liver mitochondria (1.5 mg protein) were suspended in a medium (final volume 2 ml, temperature 25°C) containing: 250 mM sucrose, 10 mM Tris-Cl pH 7.4, 5 mM K-succinate, 2.5 μM rotenone. 0.5 mM Pi was added. When present: 0.1 mM diamide, 0.1 mM Ca^{++}, 0.1 mM EGTA, 0.5 mM Mg^{++}, 1 μM FCCP, 1 μM antimycin A, 10 μM mersalyl, 10 μM pCMB.

sults not shown). Thus, the possibility that the prevention of the swelling by Mg^{++} might be due to an intramitochondrial precipitation of magnesium salts was ruled out.

Fig.2 indicates that the action of diamide and Ca^{++} on one hand, and of DTE and Mg^{++} on the other, are synergic. The results show that in the presence of Ca^{++} plus diamide, the swelling was more pronounced than in the presence of Ca^{++} or diamide added separately, and the antagonistic action of Mg^{++} was much lower. Conversely, when mitochondria were incubated in a medium containing Ca^{++} plus DTE, the swelling was decreased and the antagonistic effect of Mg^{++} was much more pronounced.

The described synergism between Ca^{++} and diamide is in part explained by the following observations.

When diamide and, to a lesser extent, Ca^{++}, were added to the mitochondria suspension separately, Mg^{++} release from mitochondria was induced (Fig. 3). This release was much higher when diamide and Ca^{++} were added together. Phosphate had an additive effect.

Ruthenium red, a known inhibitor of calcium translocation across the mitochondrial membrane (10), entirely blocked Ca^{++} induced swelling and significantly inhibited the swelling induced by diamide (Fig. 4 a and b). The possibility, in the latter case, that ruthenium red prevented the swelling by not interferring with Ca^{++} movements, but simply by inhibiting the respi-

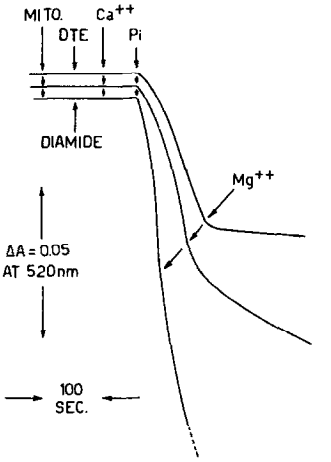

Figure 2 - Synergic action of diamide and Ca^{++} in promoting rat liver mitochondria swelling and synergic antagonistic action of DTE and Mg^{++}.

Experimental conditions and incubation medium as in Figure 1. When present DTE was 1 mM.

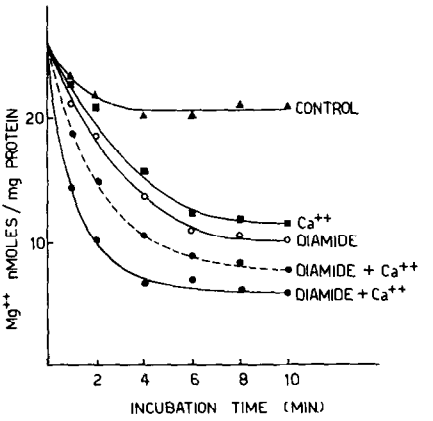

Figure 3 - The effect of Ca^{++} and diamide on the release of mitochondrial Mg^{++}. Rat liver mitochondria (5 mg protein in 2 ml) were incubated under the conditions described in Figure 1. When added, both diamide and Ca^{++} were 0.25 mM. The mitochondria were then isolated by centrifugation. The tubes were carefully blotted dry and the pellets extracted with acids as indicated by Brierley and Knight (15). Mg^{++} was determined by atomic absorption spectroscopy. Dashed line is relative to experiments in the absence of added phosphate.

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ration on which the swelling is dependent, is ruled out by the observation that ruthenium red at this concentration did not affect succinate oxidation (results not shown).

The swelling described was obtained with significantly lower concentrations of diamide than those required for the penetration of the reagent into the matrix space and for the achievement of the maximum degree of oxidation of the mitochondrial thiol groups (16). Thus, it is conceivable that diamide induced swelling is dependent on the oxidation of a few pairs of membrane bound thiol groups.

Discussion: The results show that, under proper conditions, diamide and calcium ions can exert very similar effects on mitochondria. The described swelling of rat liver mitochondria induced by the two agents is dependent on respiration and on the presence of a "permeant" anion, such as phosphate or acetate; it is prevented, or antagonized, by EGTA, Mg^{++}, DTE and ruthenium red. In both cases, the swelling very probably reflects alterations in the permeability properties of the inner membrane as also indicated by the release of endogenous Mg^{++}.

The fact that EGTA, Mg^{++} and ruthenium red are able to prevent not only the swelling induced by Ca^{++}, as expected, but also the swelling promot-

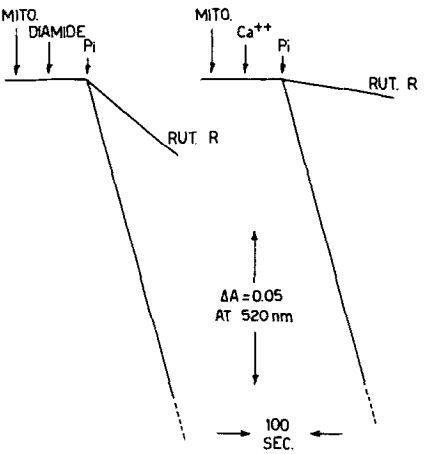

Figure 4 - Inhibition of Ca^{++} and diamide induced swelling by ruthenium red. Experimental conditions and incubation medium as in Figure 1. The concentration of ruthenium red was 5 nmoles/mg protein.

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ed by diamide, would indicate that the observed diamide effect is mediated by a mobilization of endogenous Ca^{++}. In fact when endogenous Ca^{++} are removed by EGTA, or antagonized by an excess of exogenous Mg^{++}, or immobilized by ruthenium red, the diamide effect is abolished and normal mitochondrial permeability is preserved.

Conversely, the antagonistic effect of DTE also on the swelling induced by exogenous Ca^{++}, would suggest that Ca ions are in some way prevented from reaching the sites involved in their deleterious action on mitochondrial permeability when some membrane thiol groups are protected by a proper reducing agent.

The additive effects of diamide and Ca^{++} (see Fig. 2) can be correlated with their synergic action in promoting the release of mitochondrial Mg^{++} (see Fig. 3).

It is conceivable that both added and endogenous Ca^{++}, the latter mobilized by the oxidation of membrane bound thiol groups, induce a displacement of endogenous Mg^{++}, thus weakening the permeability barrier. In other words, the antagonism between Ca^{++} and Mg^{++} seems to be closely correlated with the redox state of membrane thiol groups. When the SH/S-S ratio is high, the stabilizing action of Mg^{++} is preserved; when the SH/S-S ratio is low, endoge - nous Mg ions are lost and the antagonistic labilizing action of Ca^{++} becomes predominant.

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