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CALCIUM AND MAGNESIUM ION LOSSES IN RESPONSE TO STIMULANTS OF EFFLUX APPLIED TO HEART, LIVER AND KIDNEY MITOCHONDRIA

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SUMMARY

The efflux of Ca^{2+} from previously Ca^{2+} -loaded heart, liver or kidney mitochondria is accompanied by an approximately equal loss of endogenous Mg^{2+} irrespective of the agent applied to stimulate efflux which may be Na^{+}, a mercurial, a long chain fatty derivative or thyroxin. The proportion of Mg^{2+} (and of the accompanying adenine nucleotide) in relation to the Ca^{2+} is diminished if Mg^{2+} is added to the medium. The similarity between data from different mitochondria and with different agents accords with the ion losses involving a common factor such as generation within the membrane of lysophospholipid by Ca^{2+}in transit. It is shown that lysophospholecithin stimulates Ca^{2+} efflux with a hyperbolic concentration dependence.

INTRODUCTION

Studies of the loss of mitochondrial Mg^{2+} which accompanies loss of Ca^{2+} have been made with liver mitochondria to which a load of Ca^{2+} has been given(1,2). The loss of both components set in after more or less delay depending on the conditions; increased (P_i) shortens or abolishes the delay while ADP can prolong it indefinitely. The release of the Ca^{2+} can however be stimulated in a controlled way by diamide and by thiol reagents (such as NEM) and with these, as well as with raised (P_i) there is analytical evidence for an enhanced activity of the endogenous phospholipase A_2(3,4,5). The data presented here indicate that there is a quantitative relation between the losses of Mg^{2+} and of Ca^{2+} holding irrespective of the source of the mitochondria (heart, liver or kidney) and of the way in which Ca^{2+} efflux is stimulated, for which addition of Na^{+} ions is an effective and physiologically interesting means additional to those already cited. A reasonable inference is that

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any way of increasing the local concentration of free Ca^{2+} within the locus of the membrane phospholipase A_2 leads to a common mechanism of structural damage evidenced by losses of Mg^{2+}(3,6) and other components such as adenine nucleotides (1,7). We find that indeed a product, lysophospholecithin, of the lipase activity is a potent stimulator of Ca^{2+} and Mg^{2+} efflux from all three types of mitochondria.

MATERIALS AND METHODS

Rat heart and liver mitochondria were prepared as described before (7,8). Kidney cortex mitochondria were prepared in a way similar to that cited for liver with the difference that mannitol was substituted for sucrose as osmotic support. In all preparations the final wash was given by resuspension in a medium with 300 mM mannitol, 20 mM Tris Hepes, pH 7.4 and 0.1% purified bovine serum albumin. Final suspension was in 300 mM mannitol with 20 mM Tris Hepes, pH 7.4; after taking a sample for protein measurement by a biuret reaction the mitochondrial suspension was supplemented with serum albumin at 2 mg per ml. The movements of Ca^{2+} between the mitochondria and a buffered mannitol medium supplemented with 0.5 mg purified albumin per ml, Tris malate and Tris pyruvate 1.2 mM each, Tris phosphate 0.6 mM and purified Arsenazo III 100 µM. The absorbance of the Arsenazo III was monitored between 685 and 665 nm using a Chance-Aminco D.W.2 spectrophotometer as described before (7,8). The concentrations of total Mg^{2+} in the supernatants from quick separation of portions of suspension withdrawn from the spectrophotometer cuvette were measured by flame atomic absorbtion (Techtron AA 100). A Coleman Microfuge was used for the sample separations. Mitochondria carrying [^{14}C] ADP were prepared by adding 0.5µC [U-^{14}C] ADP (Radiochemical Centre, Amersham) to the intermediate of the three washes given to the mitochondria during their preparation (2,7). The ion movements were always measured after the addition of Ruthenium Red (0.8µM) inhibit reuptake of the Ca^{2+}.

RESULTS

Following loading with 30-50 nmol Ca^{2+} per mg protein Ruthenium Red was added to the suspension; a sample of suspension was withdrawn for centrifugal separation. In the first series of experiments providing the data for Fig.1 the stimulant of Ca^{2+} efflux was then added and final samples were taken after 10 to 15 min. The increase of Ca^{2+} efflux was then added and final samples were taken after 10 to 15 min. The increase of Ca^{2+} seen on the spectrophotometer trace, which was calibrated by making small additions of aliquots of 1 mM Ca Cl_2, was estimated and the total Mg^{2+} contents of aliquots of separated supernatant and of a perchloric acid (1.5M) extract of the pellet

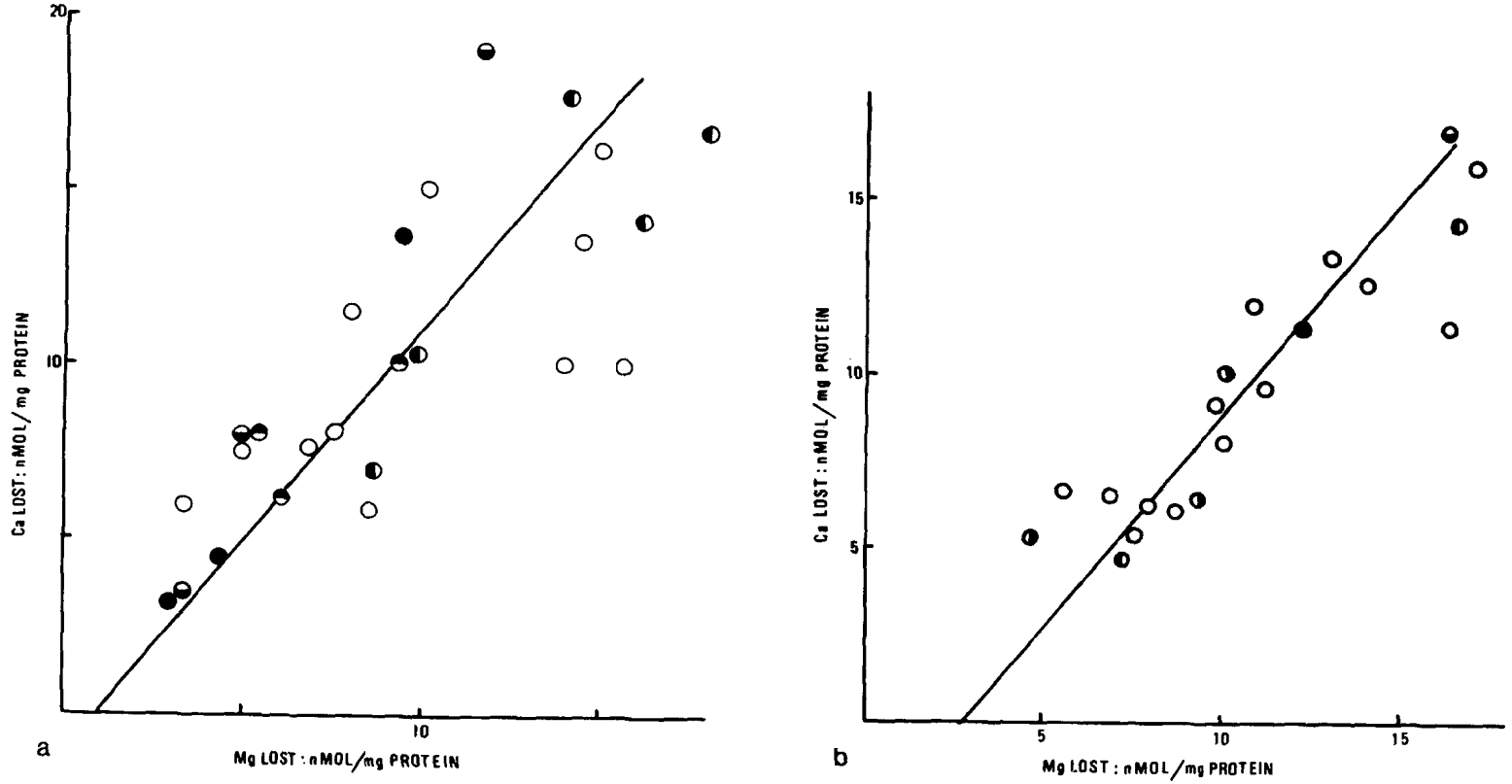
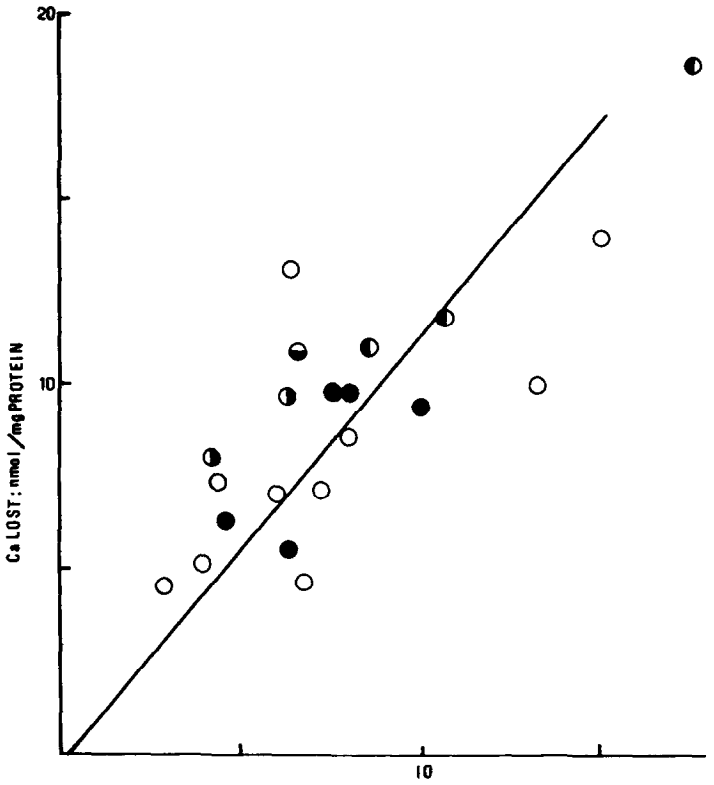


Fig. 1. RELATIONS BETWEEN LOSSES OF Ca^{2+} AND Mg^{2+} FROM MITOCHONDRIA

A) Losses of Ca^{2+} and Mg^{2+} from rat heart mitochondria which had previously been loaded with 30 to 50 nmol Ca^{2+} per mg protein and then exposed to the various agents noted below.

B) Similar data but obtained using rat liver mitochondria.



C

Fig. 1 continued.

C) Similar data but obtained from rat kidney cortex mitochondria. The experiments were made in a medium having 300 mM Mannitol, 20 mM Tries Hepes at pH 7.4 or 7.6 with 0.4 to 0.6 mg mitochondrial protein per ml. The agents used to induce the efflux of Ca^{2+} were: ○ 10 mM NaCl; ◑ Thyroxin, 12-40 µM; ◐ Lysolecithin, 25-50 µM; ● Mersalyl, 40-120 µM; ◓ Oleate, 10-30 µM; ◒ Palmitoyl CoA 10-30 µM.

were found. The change of Mg^{2+} was related to the change of external Ca^{2+} observed between the samples. An increased number of samples, drawn before and during the efflux, were taken in the later experiments (as shown in Fig 2) by commencing with more material in the cuvette. The results for Mg^{2+} lost as a function of Ca^{2+} lost are shown for the

three different preparations in Figs 1a, b and c. Regression lines were estimated by the method of least squares. They are:

For liver mitochondria $\Delta Mg^{2+} = 2.81 + 0.82\Delta Ca^{2+}$ from 17 pairs of values with 95% confidence limits for the constant 4.85 to 0.77 and for the factor applied to ΔCa^{2+} of 1.00 to 0.63 and the correlation coefficient is 0.92.

For kidney mitochondria $\Delta Mg^{2+} = 0.24 + 0.85\Delta Ca^{2+}$ from 24 pairs of values with 95% confidence limits for the constant 2.48 to -1.99 and for the factor applied to ΔCa^{2+} of 1.06 to 0.63 and the correlation coefficient is 0.87.

For heart mitochondria $\Delta Mg^{2+} = 0.96 + 1.07\ \Delta Ca^{2+}$ from 26 pairs of values with 95% confidence limits for the constant 1.75 to -3.67 and for the factor applied to ΔCa^{2+} of 1.32 to 0.81 and the correlation coefficient is 0.87.

Since added Mg^{2+}, in common with ADP, protects against or reverses the induction of a high permeability by Ca^{2+} movement (2,7,8,9,10) it is likely that much of the variability of the above results is attributable to differences of Mg^{2+} content of different batches of the respective preparations from heart, liver and kidney. If a low concentration of Mg^{2+} was added after the loading with Ca^{2+} the induction of Ca^{2+} efflux with Na^{+} applied to liver mitochondria led to relatively less loss of Mg^{2+} and adenine nucleotide (Fig.2). Mg^{2+} is known to diminish Ca^{2+} efflux from heart mitochondria (10) so a similar effect on Mg^{2+} loss would be anticipated which might well leave the Mg^{2+}/Ca^{2+} ratio unaltered.

The similarity between the behaviour of the different mitochondria in response to a number of different stimulants of Ca^{2+} efflux points to there being a common feature in the mechanism. This could be the activation by even small Ca^{2+} movement of the membrane bound phospholipase A_2 as argued by Siliprandi et al (3) and others (11,5,6). Although the products of phospholipase activity, fatty acid and lysphospholipid are

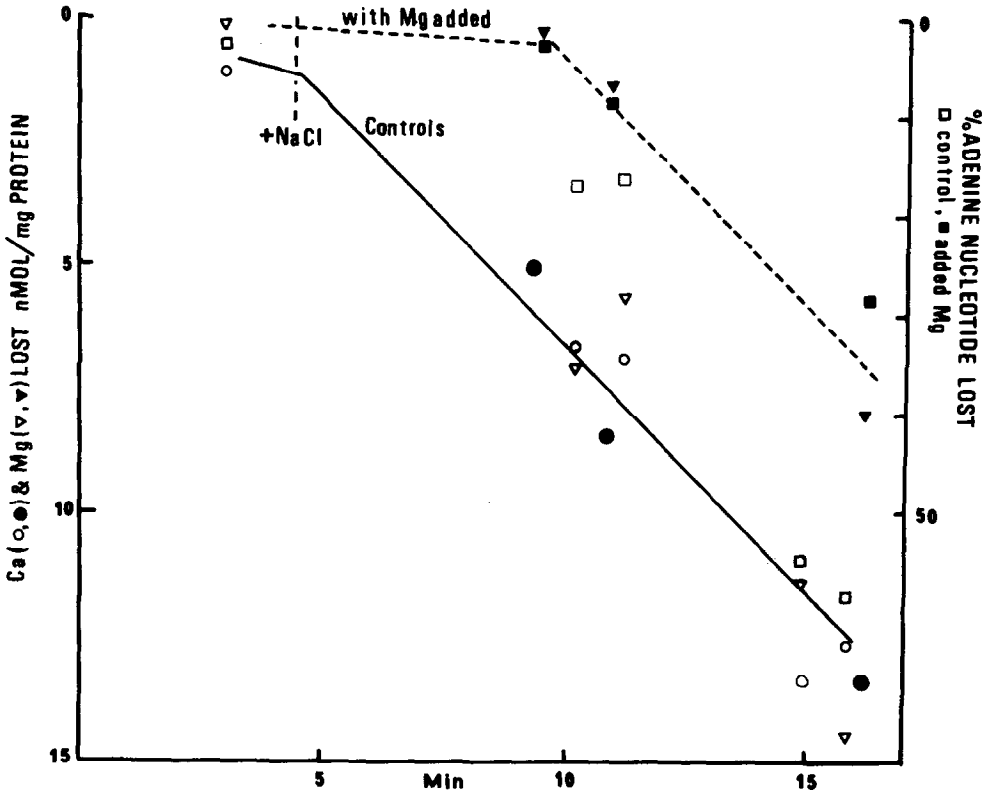


Fig.2. THE EFFECT OF ADDED Mg^{2+} ON LOSSES OF Mg^{2+} AND ADENINE NUCLEOTIDE ACCOMPANYING THE Na^{+} - STIMULATED LOSS OF Ca^{2+} FROM LIVER MITOCHONDRIA.

Results from the control experiment are shown with open points, ○ Ca, ▽ Mg and □ nucleotide. The full line indicates the time course of Ca^{2+} and Mg^{2+} losses. In the companion experiment 20 M Mg^{2+} was added with the Ruthenium Rad after loading with Ca^{2+} at 50 n mol per mg protein. The losses of Ca^{2+}, shown by filled-in points (●), are close to the controls but losses of Mg^{2+}(▼) and of nucleotide (■) are diminished. The time course of these is indicated by the dashed line.

The NaCl addition was 10 mM.

both potential stimulants of Ca^{2+} release, their effects are likely to be less when added externally than when generated in the membrane. The release obtained with externally applied lysolecithin is depicted in Fig.3; this agent promotes release of Ca^{2+} with a hyperbolic rather than

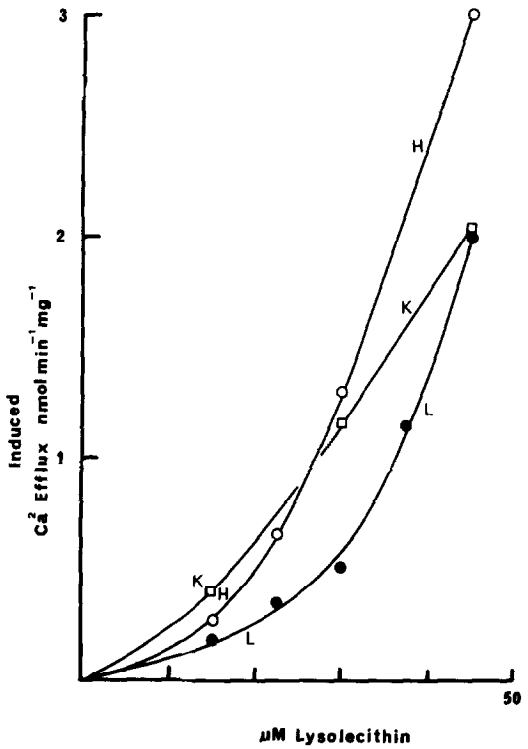


Fig. 3. LYSOLECITHIN STIMULATION OF Ca^{2+} EFFLUX FROM MITOCHONDRIA.

The rates of efflux of Ca^{2+} from heart (H), liver (L) and kidney cortex (K) mitochondria which had first received a loding of 50 nmol Ca^{2+} per mg protein were measured as a series of aliquots of lysolecithin were titrated into the suspensions.

a linear concentration dependence which is likely to be due to the removal of the first additions by adsorption on the proteins. Its stimulant effect can be removed by adding albumin to the suspension. Oleate behaves similarly and is known to induce a change of structure similar to that induced by Ca^{2+}(12).

DISCUSSION

Our results show that in absence of added Mg^{2+} the efflux of Ca^{2+} from mitochondria is accompanied by loss of approximately equivalent Mg^{2+} even when the movements are relatively small. This fact will need to

be taken into account, together with the accompanying movements of adenine nucleotide and P_i when seeking to balance the movement of electrical charge accompanying the Ca^{2+} efflux. The near equivalence we see makes it unlikely that the transition of the membrane to a highly permeable state on account of the Ca^{2+} efflux merely sets up an uncontrolled loss of Mg^{2+}. It seems necessary to think of each emergent Ca^{2+} as activating a phospholipase whose effect is potentially to free a bound Mg^{2+} whose loss depends on the external Mg^{2+}. When a bound Mg^{2+} is lost there appears also to be a loss of bound adenine nucleotide; it is the loss of these substances which leads to high unspecific membrane permeability since adding them can restore specific permeability (13,14).

Support for the proposition (4,5,11) that all passive Ca^{2+} movements lead to activation of the endogenous phospholipase with accompanying losses of Mg^{2+} and ADP leading to an increased permeability is provided by: (1) Sr^{2+} inhibits the Ca^{2+} activated phospholipase (15) and Sr^{2+} efflux is typically at 1/10 that of Ca^{2+}(16); (2) dibucaine (nupercaine) can inhibit phospholipase (17) and it also inhibits Ca^{2+} efflux (18); (3) the non-energised swelling of mitochondria in response to thyroxin and phenyl mercuric acetate has an absolute requirement for Ca^{2+}(19). There is also evidence that Ca^{2+} activates phospholipase A_2 by a conformational change (15,20,21) which might well explain how Mg^{2+} held on an adjacent site could be liberated.

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