Journal of Bioenergetics and Biomembranes, Vol. 13, Nos. 3/4, 1981

Inhibition and Stimulation of Respiration-Linked Mg^{2+} Efflux in Rat Heart Mitochondria

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Received November 12, 1980

Abstract

Respiration-driven Mg^{2+} efflux from rat heart mitochondria has been studied in different conditions. Almost total release of Mg^{2+} from the mitochondria occurs upon addition of a proton/bivalent cation exchanger, A23187. The content of Mg^{2+} remaining in mitochondria after A23187 treatment is the same if part of the mitochondrial Mg^{2+} has already been extruded through the energy-linked mechanism. Some inhibition of Mg^{2+} efflux is observed in the presence of high concentrations of La^{3+} (100 μM). A proton/monovalent cation exchanger, nigericin, completely prevents Mg^{2+} efflux, whereas a cation conductor, valinomycin, considerably stimulates it. The results indicate that the main part of mitochondrial Mg^{2+} is present in a membrane-bounded compartment, probably in the matrix space. The driving force of the Mg^{2+} efflux appears to be the proton gradient (ΔpH) created by mitochondrial respiration.

Key Words: Mg^{2+} efflux; heart mitochondria; respiration.

Introduction

The role of Mg^{2+} in cellular functions is well known (Bygrave, 1967; Aikawa, 1978) even if little information exists concerning the regulation of this cation at the cellular level. Only small changes in total cellular or tissue Mg^{2+} contents occur in different conditions (Somlyo *et al.*, 1966; Van Rossum, 1970; Palaty, 1971; Page and Polimeni, 1972) even if several lines of evidence indicate that specific transport systems for this cation exist in the cell (Van Rossum, 1970; Palaty, 1971; Page and Polimeni, 1972; Wallach *et al;* 1966; Beauchamp *et al;* 1971). Mg^{2+} has several effects on mitochondrial functions. This cation is required for oxidative phosphorylation (reviewed in Brierley, 1976), it decreases the permeability of the mitochondrial membrane toward

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0145-479X/81/0800-0133$03.00/0

monovalent cations (Brierley, 1976; Packer *et al;* 1966) and inhibits mitochondrial Ca^{2+} uptake (Vainio *et al.,* 1970; Sordahl, 1974; Crompton *et al.,* 1976a; Hutson *et al.,* 1976; Åkerman *et al.,* 1977; Åkerman, 1977). At high extramitochondrial Mg^{2+} concentrations an energy-linked uptake of this cation occurs in heart mitochondria (Brierley, 1976; Brierley *et al.,* 1962; Schuster and Olson, 1974), in contrast to liver mitochondria, which only very slowly translocate this cation (Lehninger *et al.,* 1967). Recently energy-linked Mg^{2+} efflux from mitochondria has also been described. Mg^{2+} extrusion from liver mitochondria requires endogenous Ca^{2+} in combination with added phosphate (Höser *et al.,* 1976; Siliprandi *et al.,* 1977) or an uncoupler of oxidative phosphorylation in combination with ADP (Kun *et al.,* 1969). Mg^{2+} efflux from heart mitochondria, on the other hand, is inhibited by Ca^{2+} (Crompton *et al.,* 1976b) except at very high Ca^{2+} concentrations, in which case a stimulation of Mg^{2+} efflux occurs (Vial *et al.,* 1978). The release of Mg^{2+} from heart mitochondria shows other features that are different from liver mitochondria (Crompton *et al.,* 1976b), indicating that a specific transport system for Mg^{2+} might exist in the membrane of heart mitochondria. Also smooth muscle mitochondria appear to extrude Mg^{2+} in a similar manner as heart mitochondria (Sloane *et al.,* 1978).

The aim of the present study was to characterize further the mechanism of energy-linked Mg^{2+} extrusion from heart mitochondria.

Methods and Materials

Rat heart mitochondria were prepared from young male Wistar rats according to Scarpa and Grazziotti (1973). Mg^{2+} fluxes were measured in a medium containing 120 mM KCl, 20 mM Tris, and 10 mM Hepes, pH adjusted to 7.2 with HCl, and the various reagents as indicated in the figure legends. The mitochondria were incubated at 25 °C with constant mixing, and at certain time intervals aliquots (0.5 ml) were withdrawn and centrifuged at 20,000 *g* for 2 min in a Janetzki bench centrifuge. The supernatant was withdrawn and the pellets subsequently extracted with 0.1 N HCl and 1% $LaCl_3$ for 48 h, and the Mg^{2+} content was determined using a Perkin-Elmer atomic absorption spectrometer. Protein was determined according to Lowry with bovine serum albumin as standard.

Nigericin was a gift from Dr. M. Gorman (Eli Lilly Co.) and A23187 from Dr. Hamill (Eli Lilly Co.). Valinomycin and oligomycin were obtained from Sigma Chemicals Co., St. Louis, Missouri, and ruthenium red from BDH Chemicals Ltd., Poole England. The ruthenium red was recrystallized according to Luft (1971) before use. All the other reagents used were commercial products of highest grade.

Results

The Mg^{2+} content in different preparations of heart mitochondria incubated in the experimental medium varies somewhat and is usually between 15–20 nmol/mg protein. An addition of succinate (in the presence of rotenone) causes a decrease in the endogeneous Mg^{2+} content with a concomitant increase in extramitochondrial Mg^{2+} (Fig. 1). A23187, a bivalent cation ionophore, known to cause Ca^{2+} and Mg^{2+} efflux from mitochondria (Reed and Lardy, 1972), causes a much more significant reduction in mitochondrial Mg^{2+} (Fig. 1). The amount of Mg^{2+} remaining in the mitochondria is the same if the ionophore is added after the succinate-induced efflux or in the absence of succinate. In different preparations of mitochondria only 15–20% of the mitochondrial Mg^{2+} remains after A23187 addition.

La^{3+}, a competitive inhibitor of both Ca^{2+} uptake (Mela, 1968, 1969) and efflux (Crompton *et al.*, 1978 Åkerman, 1978), in mitochondria, has no significant effect on Mg^{2+} efflux (Fig. 2) at low concentrations (10 μM). Higher La^{3+} concentrations (100 μM) cause a slight inhibition of efflux. This slight inhibition could be repeatedly seen with different mitochondrial preparations. Ruthenium red, a noncompetitive inhibitor of Ca^{2+} uptake (Moore, 1971; Vasington *et al.*, 1972), has, on the other hand, no effect on the succinate-induced Mg^{2+} release at similar concentrations (not shown), in

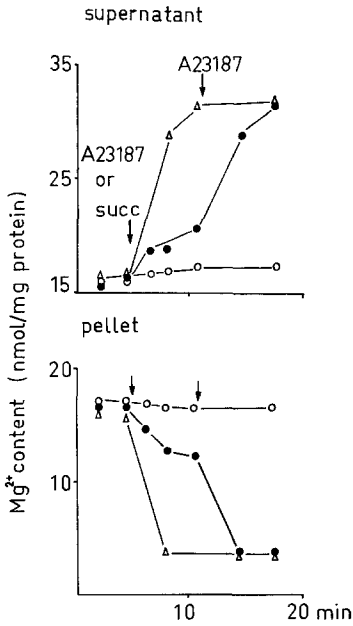

Fig. 1. Effect of succinate and A23187 on the Mg^{2+} content in heart mitochondria. Mitochondria (1mg protein/ml) were added to the basal incubation medium (at time = 0) containing 5 μM rotenone (○, ●). Addition of 5 mM succinate and 8 μM A23187 subsequently (●) or 8 μM A23187 alone (△) as indicated.

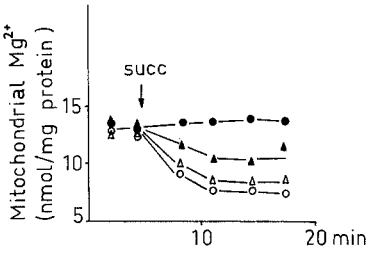

Fig. 2. Effect of La^{3+} on Mg^{2+} efflux from heart mitochondria. Conditions as in Fig. 1 (●, ○) except that 10 μM (△) or 100 μM $LaCl_3$ (▲) was present initially. Addition of 5 mM succinate (○, △, ▲) as indicated (succ).

agreement with earlier studies (Crompton *et al.*, 1976b). Note that the concentrations of La^{3+} and ruthenium red used in the present study are two to four orders of magnitude higher than those needed to inhibit mitochondrial Ca^{2+} transport completely (cf. Reed and Bygrave, 1974).

Nigericin, which exchanges monovalent cations for protons, prevents respiration-linked Mg^{2+} efflux (Fig. 3). No effect of nigericin is, however, observed if this ionophore is added after the succinate-induced response is completed (not shown). A proton conductor, FCCP, which prevents Mg^{2+} efflux from heart mitochondria (Crompton *et al.*, 1976b), also has no effect on mitochondrial Mg^{2+} if added after the succinate-induced release is completed (not shown).

Valinomycin, a monovalent cation conductor, on the other hand, significantly stimulates Mg^{2+} efflux if added after the succinate-induced efflux has occurred (Fig. 3), but has no significant effect on Mg^{2+} efflux if present initially before succinate (not shown). Valinomycin also does not affect mitochondrial Mg^{2+} when added in deenergized conditions (not shown).

Discussion

It has earlier been proposed that a main part of mitochondrial Mg^{2+} (about 50%) is present in the intermembrane space (Bogucka and Wojtczak, 1971), possibly bound to some protein species (Bogucka and Wojtczak, 1976). It is evident from the present study (Fig 1) that a significant amount

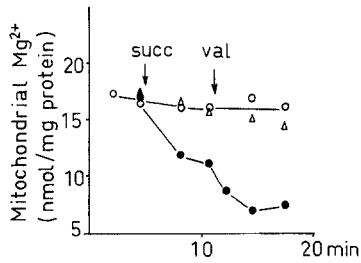

Fig. 3. Effect of nigericin and valinomycin on Mg^{2+} efflux from heart mitochondria. Conditions as in Fig. 1 (○, ●) except that 0.5 μg/ml nigericin (△) was initially present. Addition of 5 mM succinate (●, △) and 50 μg/ml valinomycin (●) subsequently (val) as indicated.

of Mg^{2+} is released into the extramitochondrial medium upon dilution and that changes in the mitochondrial Mg^{2+} content are reflected directly in changes in the extramitochondrial compartment. A23187, which exchanges bivalent cations for protons, almost completely depletes mitochondrial Mg^{2+}. Only about 20% of the initial mitochondrial Mg^{2+} content remains after addition of the ionophore. The ionophore should only affect bulk phase distribution of Mg^{2+} across membranes and not Mg^{2+} bound to proteins directly. Therefore it appears that the main part of mitochondrial Mg^{2+} in the present conditions of incubation is present in a membrane-bounded compartment, probably in the matrix space. The fact that the Mg^{2+} content of the mitochondria remaining when both succinate and A23187 have been added is the same would indicate that A23187 and succinate release Mg^{2+} from the same pool.

Neither ruthenium red (Crompton *et al.*, 1976b) nor La^{3+}, which both significantly inhibit Ca^{2+} transport specifically (Mela, 1968, 1969; Moore, 1971; Vasington *et al.*, 1972; Reed and Bygrave, 1974), have any effects on Mg^{2+} efflux at low concentrations. This would indicate that the transport system(s) involved in Ca^{2+} uptake and release are not involved in Mg^{2+} efflux. Mg^{2+} causes some inhibition of Ca^{2+} uptake (Vainio *et al.*, 1970; Sordahl, 1974; Crompton *et al.*, 1976a; Hutson *et al.*, 1976; Åkerman *et al.*, 1977; Åkerman, 1977; Vial *et al.*, 1978), and therefore it has been suggested that Mg^{2+} might interact with the Ca^{2+} transport system (Vainio *et al.*, 1970; Crompton *et al.*, 1976b). However, the inhibition by Mg^{2+} occurs at very high concentrations of this cation and is probably due only to an interference with the surface binding of Ca^{2+} (Åkerman *et al.*, 1977; Åkerman, 1977), rather than to an interaction with a Ca^{2+} translocator (Åkerman *et al.*, 1977; Åkerman, 1977), since similar effects on Ca^{2+} transport are also observed with a spermine, a tetravalent bulky molecule, which is not expected to interfere with specific Ca^{2+} transport sites (Åkerman, 1977).

The driving force of Mg^{2+} efflux in heart mitochondria is worth some consideration. Since the Mg^{2+} extrusion is dependent on respiration and inhibited by uncouplers or respiratory chain inhibitors (Crompton *et al.*, 1977b), some link to the energy coupling in mitochondria is expected. It is of interest that valinomycin, which in the present conditions is expected to completely abolish the mitochondrial membrane potential and increase the proton gradient (cf Mitchell, 1966), significantly enhances Mg^{2+} efflux. Nigericin, which, on the other hand, would abolish the proton gradient without affecting the membrane potential, inhibits Mg^{2+} extrusion almost completely. This together with the fact that proton conductors, which abolish both the proton gradient and membrane potential, also inhibit Mg^{2+} efflux (Crompton *et al.*, 1977b), suggest that the proton gradient created by mitochondrial respiration is the driving force of Mg^{2+} efflux. The existence of

a proton/cation antiport mechanism in the mitochondrial membrane has previously been proposed for monovalent cations (reviewed in Brierley, 1976) as well as for Ca^{2+} (Åkerman, 1978; Fiskum and Cocrell, 1978; Nicholls, 1978; Nicholls and Crompton, 1980). According to such models the membrane potential would drive cation uptake and the proton gradient would determine efflux. The net retention of cations would thus depend on the relative kinetic properties of both translocators. The finding (Crompton *et al.*, 1976b) that efflux of Mg^{2+} occurs below 2mM of extramitochondrial Mg^{2+} and that uptake occurs above this value is also in agreement with the above proposal. Even if nigericin and uncouplers inhibit Mg^{2+} efflux, when added prior to succinate, no reversal can be observed when these agents are added after the efflux has occurred. This would indicate that in such conditions the extramitochondrial Mg^{2+} concentration is below that required for an effective function of the transport system or that the system works only in one direction.

Since the free cytosolic Mg^{2+} concentration is on the order of 0.1–1 mM (VanRossum, 1970; Veloso *et al.*, 1973; Endo, 1975) in various tissues, Mg^{2+} fluxes accross the mitochondrial membrane might indeed occur also *in vivo* as a response to changes in cellular Mg^{2+}.

Acknowledgments

This work was supported by a grant from the Sigrid Jusélius Foundation. The author wishes to thank Ms. Kaija Niva for technical assistance, Ms. Annikki Saulamo for typing the manuscript, and the staff of the department of Public Health, University of Helsinki, for instructive help and for the opportunity to use their atomic absorption equipments.

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