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Interaction of Sr^{2+} with Ca^{2+}-Induced Ca^{2+} Release in Mitochondria

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Abstract

Respiring rat liver mitochondria are known to spontaneously release the Ca^{2+} taken up when they have accumulated Ca^{2+} over a certain threshold, while Sr^{2+} and Mn^{2+} are well tolerated and retained. We have studied the interaction of Sr^{2+} with Ca^{2+} release. When Sr^{2+} was added to respiring mitochondria simultaneously with or soon after the addition of Ca^{2+}, the release was potently inhibited or reversed. On the other hand, when Sr^{2+} was added before Ca^{2+}, the release was stimulated. Ca^{2+}-induced mitochondrial damage and release of accumulated Ca^{2+} is generally believed to be due to activation of mitochondrial phospholipase A (EC 3.1.1.4.) by Ca^{2+}. However, isolated mitochondrial phospholipase A activity was little if at all inhibited by Sr^{2+}. The Ca^{2+}-release may thus be triggered by some Ca^{2+}-dependent function other than phospholipase.

Key Words: Ca^{2+} release; Ca^{2+} cycling; mitochondria; phospholipase A_2; Sr^{2+}; swelling.

Introduction

Energized rat liver mitochondria are able to accumulate Ca^{2+}, Sr^{2+}, and Mn^{2+} but release the accumulated Ca^{2+} when a threshold of accumulated Ca^{2+} has been exceeded with concomitant oxidation of reduced pyridine nucleotides, swelling, and uncoupling (Saris 1963; for reviews see Saris and Åkerman, 1980; Nicholls and Åkerman, 1982). The threshold is lowered by inorganic phosphate and elevated by Mg^{2+} and ADP or ATP (Saris, 1963). A number of laboratories have shown that accumulation of Sr^{2+} or Mn^{2+} does not result in swelling and release of the accumulated cations (Chappell and Crofts, 1965; Pfeiffer *et al.*, 1978; Hunter and Haworth, 1979a; Beatrice

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et al., 1980; Coelho and Vercesi, 1980; Palmer and Pfeiffer, 1981; Pezzi, 1984). The mechanism of the release is still obscure. The model that there is an unspecific increase in the permeability of the inner membrane due to unsaturated fatty acids formed by phospholipase A_2 that is activated by elevated matrix Ca^{2+} has gained wide support (Siliprandi *et al.*, 1979; Beatrice *et al.*, 1980, 1982; Harris and Cooper, 1981). This is supported by the protecting effects of inhibitors of phospholipase A_2 on mitochondrial damage and swelling (Scarpa and Lindsay, 1972; Scherphof *et al.*, 1972). However, activation of phospholipase A_2 may be a late phenomenon and not the cause of Ca^{2+} release. Thus, several laboratories are in favor of specific Ca^{2+} efflux mechanism regulated by the oxidation state of membrane thiol groups of NADPH (Harris *et al.*, 1979; Lötscher *et al.*, 1979; Rugolo *et al.*, 1981; Baumhüter and Richter, 1982; Moore *et al.*, 1983; Vercesi, 1984). It has also been proposed that an increased H^+ permeability causes a collapse of the membrane potential and efflux of Ca^{2+} through the uptake pathway, the uniporter (Bernardi and Pietrobon, 1982; Al Nasser and Crompton, 1986). The picture is further complicated by the transition in mitochondrial configuration from a state of condensed matrix to an expanded matrix volume brought about by Ca^{2+} (Hunter and Haworth, 1979b). This can be prevented or reversed by ADP (Stoner and Sirak, 1973; Hunter and Haworth, 1979a). The stimulation of Ca^{2+} efflux is also inhibited by ADP or ATP and Mg^{2+} (Saris, 1963; Harris *et al.*, 1979; Zoccarato *et al.*, 1981). The mechanism of action of these agents is still obscure.

The interaction of Sr^{2+} with the Ca^{2+} release from mitochondria has been little studied. Saris and Bernardi (1983) have shown that Sr^{2+} inhibits the ruthenium-red-insensitive Ca^{2+} efflux. In an early study Caplan and Carafoli (1965) found that Sr^{2+} prevents both the spontaneous swelling of mitochondria and that induced by various means, including Ca^{2+}. In this study we found that Sr^{2+} may both stimulate and inhibit the Ca^{2+}-induced release, depending on whether it is added before Ca^{2+} or later.

Experimental Procedure

Rat liver mitochondria were prepared as described elsewhere (Allshire *et al.*, 1985). Uptake and release of Ca^{2+} or Ca^{2+} *plus* Sr^{2+} was followed by dual-wavelength photometry using the wavelength couple 685–665 nm with the aid of 50 μM arsenazo II (Scarpa 1979) obtained from Sigma Chemical Co., and recrystallized (Scarpa 1979). Changes in $\Delta\Psi$[3] were monitored at

[3]Abbreviations: $\Delta\Psi$, membrane potential; Mops, 3-morpholinepropane sulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

554–524 nm by the safranine technique (Åkerman and Wikström, 1976). Incubations were carried out at 23°C using a mitochondrial suspension of 1 mg protein/ml in a medium containing 140 mM sucrose, 40 mM choline chloride, 10 mM Mops neutralized with Tris to pH 7.0, 2 mM primary potassium phosphate neutralized with Tris, 4 mM succinate, neutralized from succinic acid with Tris, 6 μM rotenone. Mitochondrial phospholipase A_2 was purified and assayed with the aid of 1-acyl-2[1-^{14}C]linoleoylphosphatidylethanolamine (specific radioactivity 300 dpm/nmol) as described previously (de Winter *et al.*, 1982, 1984).

Results

The Ca^{2+}-induced Ca^{2+} release occurs when a certain amount of Ca^{2+} has been taken up per mitochondrial protein. The effect of various agents on the threshold can also be studied by following the time course of Ca^{2+} release. Thus, protecting agents will inhibit or delay the release while stimulating agents will accelerate it. In Fig. 1 the adition of 40 μm Ca^{2+} was not enough to trigger a release, but accumulation of an additional 10 μM Ca^{2+} induced the release after about 10 seconds (trace a). The release was clearly biphasic. If Sr^{2+} was added together with the second addition of Ca^{2+} (trace b and c), the lag before the release set in was clearly prolonged and the biphasicity became more pronounced with increasing amounts of Sr^{2+}. Figure 2 shows experiments in which enough Ca^{2+} was added to trigger the release (trace a).

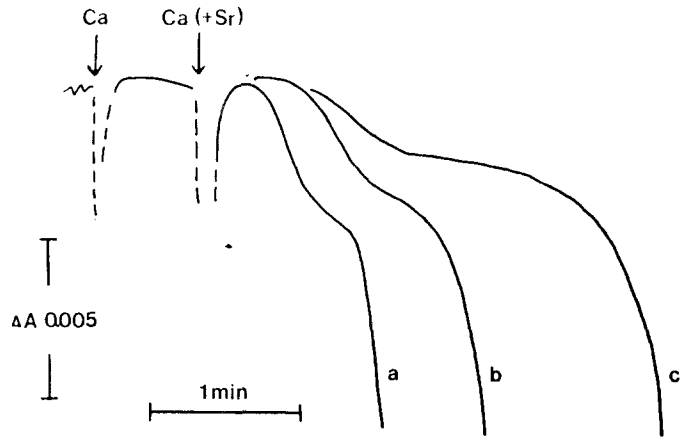

Fig. 1. The effect of Sr^{2+} on the retention of Ca^{2+} by mitochondria. The first addition of Ca^{2+} was 40 μM, the second one was 10 μM. With the second addition was also added Sr^{2+} to a final concentration of (a) 0 μM; (b) 20 μM; (c) 30 μM. For details, see Experimental Procedure.

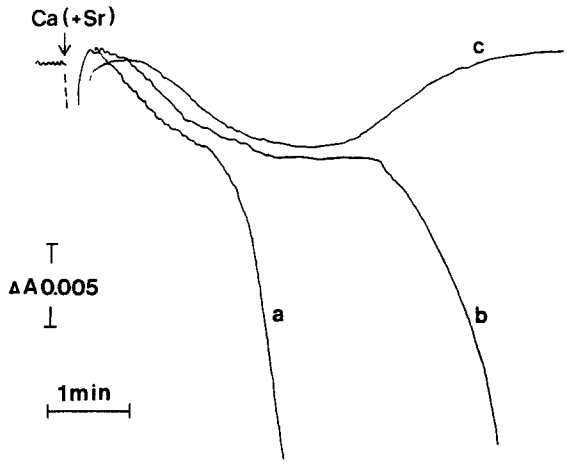

Fig. 2. The effect of Sr^{2+} added with Ca^{2+} on the retention of Ca^{2+}. At the arrow 60 μM Ca^{2+} was added with (a) 0 μM Sr^{2+}; (b) 30 μM Sr^{2+}; (c) 60 μM Sr^{2+}.

If enough Sr^{2+} was added with the Ca^{2+} (trace c), Ca^{2+} release was prevented altogether, and the Ca^{2+} that had appeared in the medium during the initial phase of the biphasic release was reaccumulated.

Figure 3 shows that Sr^{2+} was effective in postponing the Ca^{2+} release when added during the first phase of Ca^{2+} efflux, and was able even to induce a partial reuptake of Me^{2+}. The downward deflection at the addition of Sr^{2+} indicates the formation of a Sr^{2+}-arsenazo III complex (broken lines), and the return of the trace an accumulation of mainly Sr^{2+}. However, when Sr^{2+} was added before Ca^{2+} (Fig. 4), the mitochondria became more sensitive to Ca^{2+}.

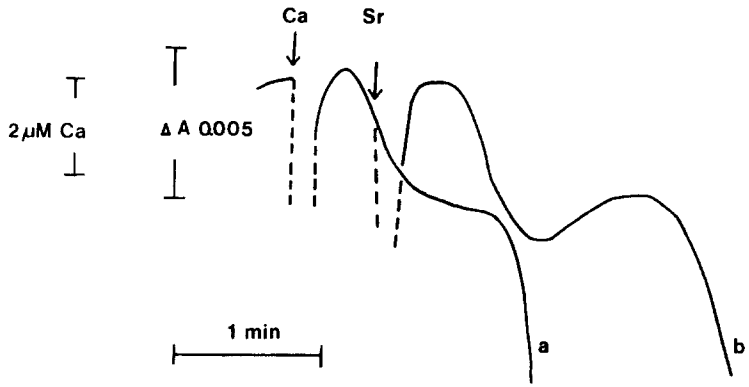

Fig. 3. The effect of Sr^{2+} added after Ca^{2+} on the retention of Ca^{2+}. At the arrows 60 μM Ca^{2+} and 30 μM Sr^{2+}, respectively, were added. Sr^{2+} was added where indicated in trace b.

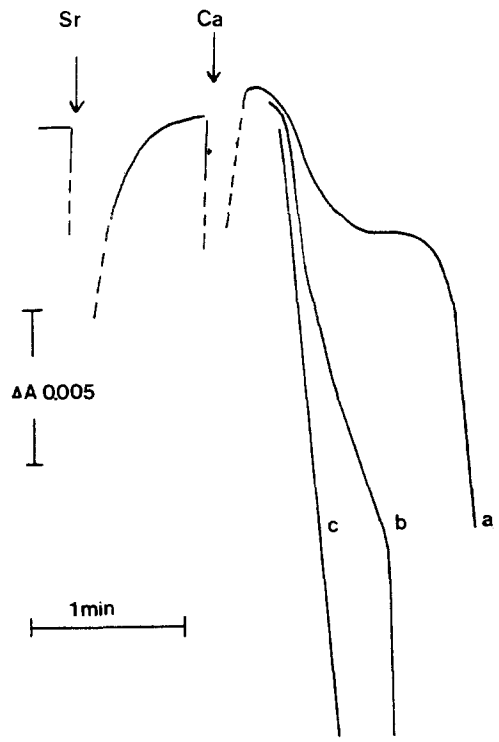

Fig. 4. The effect of Sr^{2+} added before Ca^{2+} on the retention of Ca^{2+}. 60 μM Ca^{2+} and (a) 0 μM Sr^{2+}; (b) 25 μM Sr^{2+}; (c) 50 μM Sr^{2+} were added at the arrows.

Figure 5 shows the changes in $\Delta\Psi$ in experiments analogous to those in Fig. 2 (traces a and b). Addition of Ca^{2+} alone caused a drop of $\Delta\Psi$ during the phase of Ca^{2+} uptake and then a partial repolarization, corresponding to slowing down of efflux in the biphasic curve. This was followed by an extensive depolarization at the onset of the Ca^{2+} release. When Ca^{2+} was added together with 30 μM Sr^{2+} (broken line), there was a full repolarization and a delayed depolarization.

Figure 6 depicts the stimulation of mitochondrial phospholipase A_2 activity by Ca^{2+}, Sr^{2+}, and Ca^{2+} *plus* Sr^{2+}. Since in this connection only fairly low concentrations of the cations were of interest, up to 100 μM concentrations were used. The activities at these concentrations were low and close to the lower limit of the assay. As noted previously (de Winter *et al.*, 1984), Sr^{2+} stimulated the enzyme substantially, up to a third of the stimulation obtained with the corresponding concentrations of Ca^{2+}. A possible inhibition of the Ca^{2+}-stimulated activity by Sr^{2+} was therefore compensated for by stimulation by the added Sr^{2+}. Little if any effect of

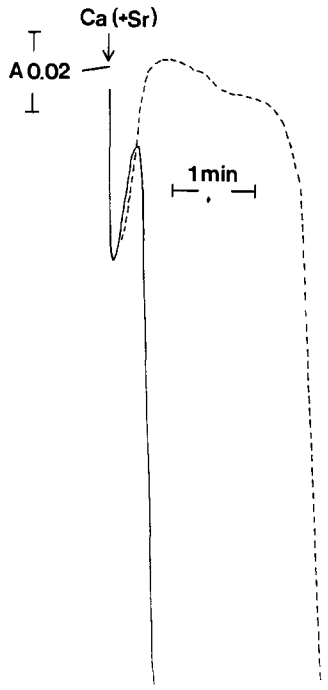

Fig. 5. Changes in $\Delta\Psi$ during Ca^{2+} uptake and release. The experimental conditions were as in Fig. 2a and b. Downward deflection corresponds to a decrease in $\Delta\Psi$. The continuous line represents the trace with Ca^{2+} alone. For details, see Experimental Procedure.

added Sr^{2+} upon the phospholipase A_2 activity was seen in the presence of Ca^{2+}.

Discussion

In this study it was found that Sr^{2+} exerts a protective effect on rat liver mitochondria by decreasing their sensitivity toward Ca^{2+} overload. This effect was to be expected, since already Caplan and Carafoli (1965) found that Sr^{2+} inhibited mitochondrial swelling induced by various means. Furthermore, accumulation of Sr^{2+} does not induce an overload state with release of the accumulated cations and small molecules (Chappell and Crofts, 1965; Pfeiffer *et al.*, 1978; Beatrice *et al.*, 1980; Coelho and Vercesi, 1980; Palmer and Pfeiffer, 1981; Pezzi, 1984) nor a transition in the mitochondrial configuration (Hunter and Haworth 1979a, b). It is of interest that the protective action was evident not only when Sr^{2+} was added at the same time as Ca^{2+} but also when it was added later, before an extensive Ca^{2+} release

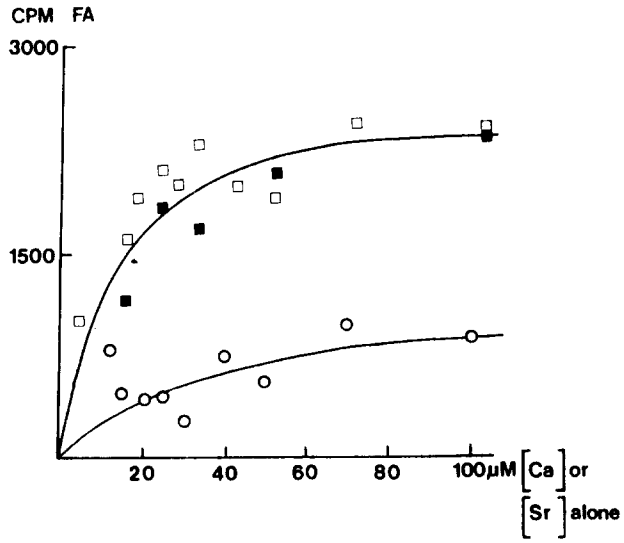

Fig. 6. Activity of mitochondrial phospholipase A_2 in the presence of Ca^{2+} and Sr^{2+}. FA, fatty acid released; ○, Sr^{2+}; □, Ca^{2+}; ■, Ca^{2+} *plus* 25 μM Sr^{2+}. For details, see Experimental Procedure.

had occurred. This is not readily reconciled with Sr^{2+} effects on specific Ca^{2+} efflux pathways in spite of the fact that Saris and Bernardi (1983) have shown that Sr^{2+} indeed does inhibit ruthenium red-insensitive Ca^{2+} efflux. Rather the occurrence of a lag before Ca^{2+} release sets in, during which Sr^{2+} still is effective, is compatible both with the model that the release is associated with a configurational transition (Hunter and Haworth, 1979a, b) and with the phospholipase A_2 hypothesis (Siliprandi *et al.*, 1979; Beatrice *et al.*, 1980, 1982; Harris and Cooper, 1981). Hunter and Haworth (1979a) showed that the mitochondrial suspension contained a heterogeneous population. Some mitochondria underwent a configurational transition with a limited Ca^{2+} load, lost their content of the cation which then caused the remaining mitochondria to accumulate the Ca^{2+} and exceed their tolerance threshold until the whole population was affected. Sr^{2+} could thus exert its protective function when added after the Ca^{2+} as long as a substantial proportion of the mitochondria were still in good shape. In the phospholipase A_2 model there would be a steady state in the concentration of lysophospholipids and fatty acids influenced by hydrolysis of phospholipids and the reacylation of lysophospholipids, which could be tipped in favor of accumulation of fatty acids by stimulation of the phospholipase A_2 activity by Ca^{2+} and in favor of reacylation by inhibition of the enzyme (Beatrice *et al.*, 1980, 1982). However, in this study little if any inhibition of purified mitochondrial

phospholipase A_2 by Sr^{2+} was seen using phosphatidylethanolamine as substrate. This data does not support the phospholipase A_2 model, although there is certainly an activation of the enzyme during Ca^{2+} release (Siliprandi *et al.*, 1979; Beatrice *et al.*, 1980, 1982; Harris and Cooper, 1981). This may, however, be a late development. On the other hand, the phospholipase A_2 activity is very sensitive to the state of its substrates and therefore some caution must be exercised in extrapolating the data on the purified enzyme to the one *in situ*.

The finding that Sr^{2+} added prior to Ca^{2+} may sensitize rather than protect the mitochondria raises some interesting questions. It seems that part of the accumulated divalent cations are removed in a time-dependent fashion from the matrix to sites where they are no longer in equilibrium with the free cations. This could occur by precipitation as phosphates or by binding to some membrane sites where the cation then is made inaccessible. After accumulation of Sr^{2+} and its precipitation or binding, less sites would be available to Ca^{2+} taken up later. As a result, the matrix concentration of Ca^{2+} would be increased, and the Ca^{2+}-dependent activity leading to the overload would be enhanced. The relatively early effects on the membrane potential make a Ca^{2+}-dependent increase in H^+ conductance and a fast efflux through the uniporter (Bernardi and Pietrobon, 1982; Al Nasser and Crompton, 1986; Crompton *et al.*, 1987) an attractive hypothesis.

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