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H^+-Dependent Efflux of Ca^{2+} from Heart Mitochondria

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Abstract

A rapid loss of accumulated Ca^{2+} is produced by addition of H^+ to isolated heart mitochondria. The H^+-dependent Ca^+ efflux requires that either (a) the NAD(P)H pool of the mitochondrion be oxidized, or (b) the endogenous adenine nucleotides be depleted. The loss of Ca^{2+} is accompanied by swelling and loss of endogenous Mg^{2+}. The rate of H^+-dependent Ca^{2+} efflux depends on the amount of Ca^{2+} and P_i taken up and the extent of the pH drop imposed. In the absence of ruthenium red the H^+-induced Ca^{2+}-efflux is partially offset by a spontaneous re-accumulation of released Ca^{2+}. The H^+-induced Ca^{2+} efflux is inhibited when the P_i transporter is blocked with *N*-ethylmaleimide, is strongly opposed by oligomycin and exogenous adenine nucleotides (particularly ADP), and inhibited by nupercaine. The H^+-dependent Ca^{2+} efflux is decreased markedly when Na^+ replaces the K^+ of the suspending medium or when the exogenous K^+/H^+ exchanger nigericin is present. These results suggest that the H^+-dependent loss of accumulated Ca^{2+} results from relatively nonspecific changes in membrane permeability and is not a reflection of a Ca^{2+}/H^+ exchange reaction.

Key Words: Mitochondrial Ca^{2+} efflux; mitochondrial Ca^{2+} buffering; intracellular pH; mitochondrial membrane permeability.

Introduction

Several recent studies have concluded that isolated mitochondria can take up and retain Ca^{2+} less effectively as the pH of the suspending medium is decreased (Nicholls, 1978; Struder and Borle, 1980; Wolkowitz and McMillan-Wood, 1981) and that a rapid drop in pH or "H^+-pulse" results in release of accumulated Ca^{2+} (Akerman, 1978; Tsokos *et al.*, 1980). This H^+-dependent release of Ca^{2+} from liver mitochondria has been explained in terms of either a Ca^{2+}/nH^+ exchange reaction (Akerman, 1978) or a

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simultaneous loss of Ca^{2+} and P_i (Wolkowitz and McMillan-Wood, 1981). Such results have led to the suggestion that alterations in intracellular pH may lead to release of Ca^{2+} from mitochondria which would affect a number of Ca^{2+}-dependent reactions in the cytosol (Struder and Borle, 1980; Tsokos *et al.*, 1980). In this regard, recent studies with isolated adult rat heart myocytes have shown remarkable alterations in morphology following anaerobic to aerobic transitions (Altschuld *et al.*, 1981; Hohl *et al.*, 1982) and these changes may have a basis in altered intracellular Ca^{2+} metabolism related to the declining intracellular pH. With these possibilities in mind, we have examined the effects of H^+ pulses on the ability of isolated heart mitochondria to retain accumulated Ca^{2+}. The present communication reports that decreased pH promotes the loss of Ca^{2+} from heart mitochondria when mitochondrial pyridine nucleotides are oxidized or when endogenous adenine nucleotides are depleted. The H^+-induced Ca^{2+} efflux appears to result from nonspecific increases in the permeability of the membrane to ions. The results do not support a direct Ca^{2+}/nH^+ exchange mechanism for release of Ca^{2+} from these mitochondria.

Materials and Methods

Beef heart mitochondria were prepared using Nagarse and EGTA as previously described (Jung *et al.*, 1977). Where indicated, these mitochondria were depleted of endogenous adenine nucleotides by washing with 1–5 mM PP_i at 0–3°C (Asimakis and Sordahl, 1981). The uptake and release of Ca^{2+} by mitochondria was followed in an Aminco DW-2 at 720–790 nm in the presence of the metallochromic Ca^{2+} indicator antipyrylazo III (40 μM) as described by Scarpa *et al.* (1978). The absorbance records were calibrated by addition of known amounts of Ca^{2+} for each pH and medium change examined. Control studies using K^+ + valinomycin-dependent reactions established that swelling and contraction of the mitochondria or changes in redox state of respiratory carriers did not affect the Ca^{2+}-dependent absorbance at these wavelengths. In addition, many of the responses indicated by the antipyrylazo III absorbance records were verified using a Radiometer F2112 Ca-sensitive electrode (records not reproduced).

The redox state of the mitochondrial NAD(P)H was monitored at 340–370 nm under the same conditions as Ca^{2+} movements in parallel incubations in which antipyrylazo III was omitted. Swelling was followed at 540 nm in the DW-2 spectrophotomer. The adenine nucleotide composition and content was estimated by fluorimetry (Adam, 1955). The composition of the suspending medium and other experimental details are given with the individual experiments presented.

Results

Release of Ca^{2+} from Heart Mitochondria Induced by H^+

Beef heart mitochondria respiring with succinate + rotenone in a KCl–P_i medium take up virtually all of an added charge of Ca^{2+} (280 nmol · mg mg^{-1} protein) at pH 7.15 (Fig. 1). The heart mitochondria used in this study, like other such preparations in the literature (Vercesi *et al.*, 1978), are capable of accumulating large amounts of Ca^{2+} without apparent damage to energy-coupling mechanisms. In the absence of rotenone these mitochondria release accumulated Ca^{2+} in a P_i-dependent reaction accompanied by extensive NAD(P)H oxidation (see Coelho and Vercesi, 1980), but when rotenone is present to prevent oxidation of pyridine nucleotides, Ca^{2+} is retained until anaerobiosis. In the present study Ca^{2+} loads of over 250 nmol · mg^{-1} are retained even when NADH is oxidized by addition of acetoacetate when the mitochondria are respiring with succinate in the presence of rotenone (Fig. 1A).

When the pH is dropped rapidly to pH 6.5 by addition of HCl, there is little loss of Ca^{2+} in the absence of acetoacetate, but a precipitous loss from mitochondria in which the pyridine nucleotides have been oxidized (Fig. 1). The H^+-dependent release of accumulated Ca^{2+} is insensitive to ruthenium red. In the presence of ruthenium red, which selectively blocks re-uptake of Ca^{2+} by the Ca^{2+} uniporter, the release of Ca^{2+} proceeds to completion (Fig. 1A), whereas in the absence of this reagent there is a partial re-accumulation of Ca^{2+} prior to anaerobiosis (Fig. 1B). The H^+-induced release of Ca^{2+} is usually biphasic with a slow rate of release for about 30 sec preceding the maximum rate of efflux (Fig. 1).

The rate of H^+-induced Ca^{2+} release increases with the amount of Ca^{2+} accumulated (Fig. 2). Mitochondria which have accumulated 280 nmol Ca^{2+} per mg protein (140 μM in Fig. 2) show high rates of Ca^{2+} efflux when HCl is added to produce a final pH of 7.0 or below. Much lower rates of Ca^{2+} loss occur when the mitochondria are exposed to 70 or 35 μM Ca^{2+} (Fig. 2). In agreement with other studies (Wolkowitz and McMillan-Wood, 1981), the rate of H^+-dependent Ca^{2+} release also depends on the P_i concentration (K_m of 1.2 mM P_i) and is strongly inhibited when the phosphate transporter is blocked by addition of *N*-ethylmaleimide (records not shown; see Tsokos *et al.*, 1980).

The H^+-dependent Ca^{2+} efflux from mitochondria treated with aceto-acetate is also strongly inhibited by oligomycin (Fig. 3) and by adenine nucleotides (Fig. 3). Low levels of ADP in the absence of oligomycin effectively retard the H^+-dependent reaction (K_i for ADP of 35 μM), and the combination of ADP with oligomycin nearly abolishes Ca^{2+} release under these conditions (Fig. 3).

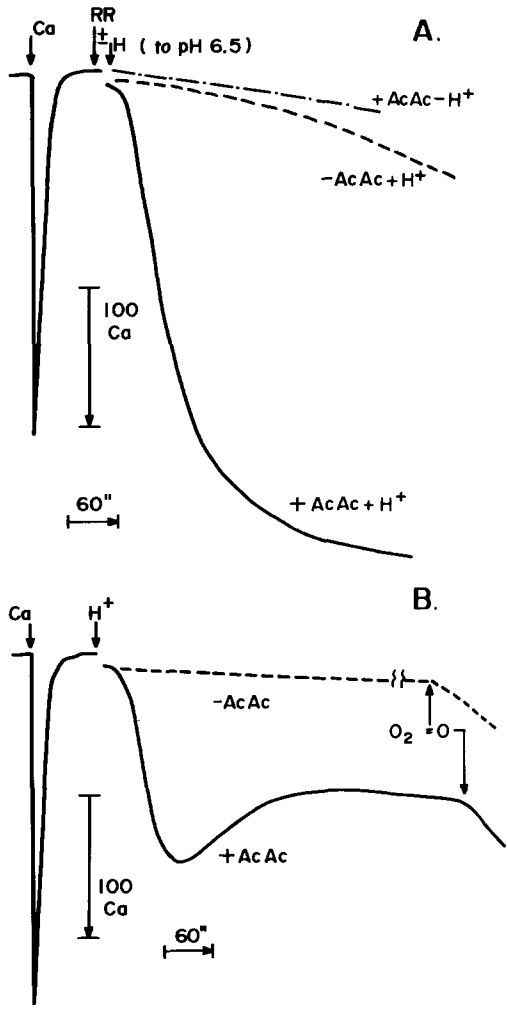

Fig. 1. Release of accumulated Ca^{2+} from heart mitochondria following a rapid pH decrease (H^+ pulse). Beef heart mitochondria (0.5 mg/ml) were incubated at 25°C in a medium of KCl (125 mM) and the K^+ salts of 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES, 4 mM), P_i (2 mM), and succinate (5 mM) and rotenone (5 μg/mg). The accumulation and release of Ca^{2+} (140 μM, 280 nmol/mg) was followed at 720–790 nm in an Aminco DW-2 spectrophotometer in the presence of antipyrylazo III (40 μM). Where indicated, Li^+ acetoacetate (AcAc, 1 mM) was also present. In (A), ruthenium red (RR, 1 μM) was added just prior to addition of a pulse of HCl (1 mM) sufficient to bring the pH from 7.15 to 6.5. In (B) ruthenium red was omitted. The deflection produced by 100 nmol Ca^{2+} is indicated.

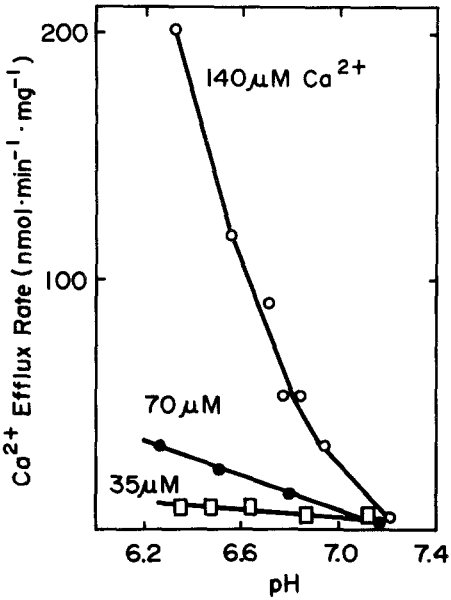

Fig. 2. Rates of H^+-induced Ca^{2+} efflux as a function of amount of Ca^{2+} accumulated. Maximum rates of Ca^{2+} efflux in the presence of acetoacetate and ruthenium red were estimated from records obtained under the conditions of Fig. 1A and are plotted as a function of the final pH of the mitochondrial suspension in the experimental cuvette. The initial pH was 7.25 in all cases. Ca concentrations: (○) 280 nmol/mg; (●) 140 nmol/mg; (□) 70 nmol/mg.

The H^+-dependent Ca^{2+} efflux is accompanied by loss of endogenous Mg^{2+} and by swelling of the mitochondria. Both of these parameters indicate that nonspecific changes in membrane permeability may accompany the H^+-dependent release of Ca^{2+}, and both increase in magnitude roughly in parallel with the loss of accumulated Ca^{2+} as the magnitude of the imposed pH drop is increased (Table I). Neither loss of Mg^{2+} nor swelling occurs when the pH is decreased to 6.4 in the absence of acetoacetate. There is also no indication of Ca^{2+} loss, Mg^{2+} loss, or swelling at pH 6.4 in the presence of acetoacetate when oligomycin and ADP are also added (Table I).

H^+-Dependent Release of Ca^{2+} from Mitochondria Depleted of Adenine Nucleotides

In the course of these investigations it was noted that occasional preparations of beef heart mitochondria released Ca^{2+} spontaneously or on addition of the H^+ pulse without oxidation of the pyridine nucleotide pool by

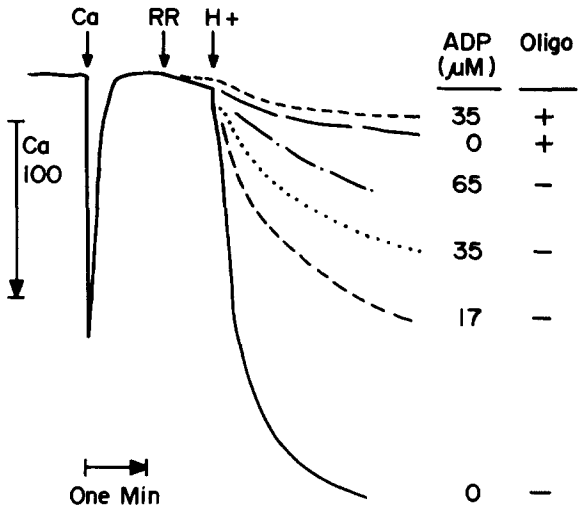

Fig. 3. Effect of ADP and/or oligomycin on H^+-dependent Ca^{2+} efflux from heart mitochondria. The experiments were carried out under the conditions of Fig. 1A using acetoacetate-oxidized mitochondria and the indicated concentration of ADP in the presence or absence of oligomycin (3 μg/mg). The sequence of addition was as follows: oligomycin immediately following addition of mitochondria, ADP 15 sec later followed by acetoacetate at 25 sec, and finally Ca^{2+} at 1 min. The initial pH was 7.15 and H^+ was added to pH 6.6 in each case.

Table I. Loss of Mg^{2+} and Swelling of Mitochondria Accompanying H^+-Induced Ca^{2+} Efflux[a]

	Ca^{2+} lost	Mg^{2+} lost	Swelling
	(% total in 1 min)		($\Delta A_{540} \cdot min^{-1}$)
pH 7.1	13	3	—
pH 6.9	23	14	0.023
pH 6.65	40	24	0.040
pH 6.4	64	45	0.053
pH 6.4, no acetoacetate	0	0	0.006
pH 6.4 + oligomycin + ADP	0	1	0.002

[a]Heart mitochondria were incubated and loaded with Ca^{2+} under the conditions described in Fig. 1A. Except where indicated, acetoacetate was present and ruthenium red and H^+ added (to the indicated final pH) as shown in Fig. 1A. The loss of Ca^{2+} was monitored in the presence of antipyrylazo III at 720–790 nm; loss of Mg^{2+} was determined by atomic absorption after rapid centrifugation; swelling was followed at 540 nm in the DW-2. Where indicated, oligomycin (3 $\mu g \cdot mg^{-1}$) and ADP (35 μM) were also present.

addition of acetoacetate. Analysis of these preparations of mitochondria revealed that they contained significantly less total adenine nucleotide than the more typical preparations shown in Figs. 1–3. Beef heart mitochondria depleted of adenine nucleotide by treatment with PP_i (Asimakis and Sordahl, 1981) to a level of 3.8 ± 1.1 ($n = 4$) nmol · mg^{-1} showed a pattern of Ca^{2+} release identical to that of these spuriously depleted preparations (Fig. 4A). Adenine nucleotide-depleted mitochondria are usually capable of holding less

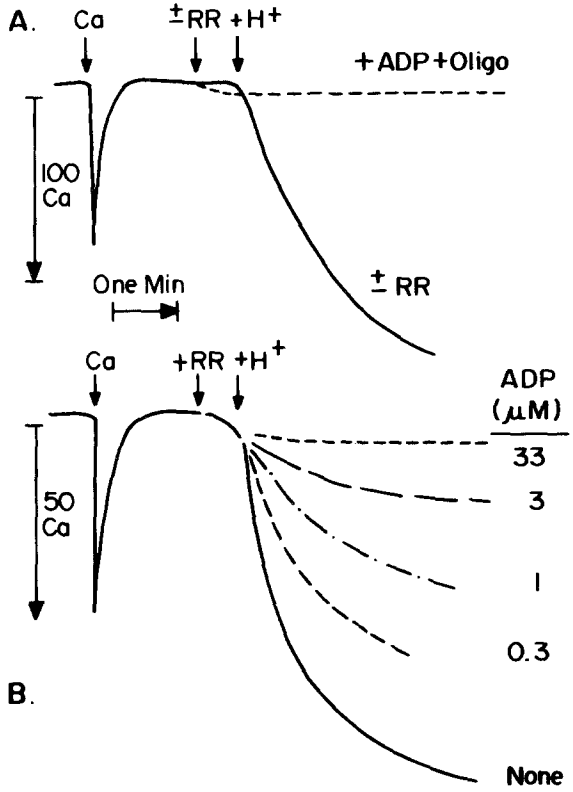

Fig. 4. H^+-induced Ca^{2+} efflux from heart mitochondria depleted of adenine nucleotides. Experimental conditions were identical to those of Fig. 1 except that the Ca^{2+} added was 70 μM (140 nmol · mg^{-1}), no acetoacetate was added, and the heart mitochondria used were treated with PP_i (Asimakis and Sordahl, 1981) to reduce their total adenine nucleotide content from 8.7 to 3.8 nmol · mg^{-1}. (A) Where indicated, ruthenium red (1 μM), ADP (33 μM), and oligomycin (3 μg · mg^{-1}) were added. (B) Oligomycin (3 μg · mg^{-1}) was present from the beginning of the experiment with the indicated concentration of ADP. Sequence of additions was as described for Fig. 3. The initial and final pH were 7.15 and 6.6, respectively.

Ca^{2+} than preparations which contain the normal compliment of adenine nucleotide of 8.7 ± 1.2 ($n = 7$) nmol · mg^{-1} and are therefore challenged with only 140 nmol Ca^{2+} · mg^{-1} in the study shown in Fig. 4A. A pulse of H^+ to pH 6.45 causes an immediate release of accumulated Ca^{2+} with no indication of ruthenium red-sensitive re-uptake (Fig. 4A). This H^+-dependent release of Ca^{2+} from adenine nucleotide-depleted heart mitochondria, in contrast to mitochondria with the normal adenine nucleotide complement, does not require addition of acetoacetate (compare Fig. 4 with Fig. 1). The release of Ca^{2+} from these heart mitochondria is also sensitive to addition of ADP in the presence of oligomycin (Fig. 4A). Addition of 1 μM ADP (or less) in the presence of oligomycin produces inhibition of the H^+-dependent Ca^{2+} release in these mitochondria (Fig. 4B). In the presence of oligomycin ATP is also an effective inhibitor of H^+-induced Ca^{2+} efflux (50% as effective as ADP on a molar basis; records not shown).

The rate of H^+-dependent Ca^{2+} efflux is inversely proportional to the endogenous total adenine nucleotide content in heart mitochondria not treated with acetoacetate (Fig. 5A). Endogenous total adenine nucleotide levels also appear to determine the extent to which H^+-dependent Ca^{2+} efflux will be inhibited by oligomycin (Fig. 5B). Preparations in which total adenine nucleotide content is greater than about 4 nmol · mg^{-1} show considerable sensitivity to oligomycin in the absence of exogenous ADP or ATP (Fig. 5B). Heart mitochondria in which the H^+-dependent Ca^{2+} efflux is 50% sensitive to oligomycin are further inhibited by ADP (K_i of 0.5 μM) and ATP (K_i of 2.5μM) in protocols identical to those of Fig. 4B. Addition of 0.5 μM ADP corresponds to only 1.5 nmol · mg^{-1} protein under these conditions.

H^+-Induced Ca^{2+} Efflux Is Inhibited by Na^+ and by Nigericin

The rate of H^+-induced efflux of Ca^{2+} from heart mitochondria suspended in 125 mM NaCl is considerably slower than that produced by an identical pH drop in 125 mM KCl (Fig. 6A). There is also less oxidation of the pyridine nucleotide pool following addition of the H^+ pulse to mitochondria in NaCl than is seen in KCl (Fig. 6B), and less H^+-dependent swelling when the mitochondria in NaCl are compared with those in KCl (Fig. 6C). It appears that the effects of the H^+ pulse are minimized in an all-Na^+ medium. Since mitochondria contain a component which promotes Na^+/H^+ exchange (see Cromptom and Heid, 1978, and Brierley *et al.*, 1977, for example), it seems likely that the availability of this pathway for equilibration of ΔpH and monovalent cation gradients may modify the H^+-dependent Ca^{2+} efflux reaction. Support for this concept comes from the observation that addition of an exogenous K^+/H^+ exchanger, nigericin, just prior to the H^+-pulse in the KCl medium produces a strong inhibition of H^+-dependent Ca^{2+} efflux (Fig.

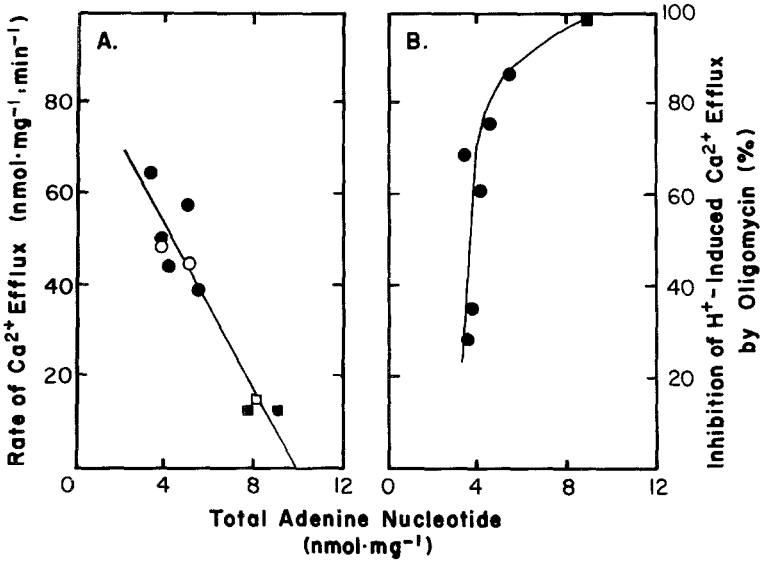

Fig. 5. Dependence of rate (A) and oligomycin sensitivity (B) of H^+-induced Ca^{2+} efflux on endogenous adenine nucleotide levels. Experimental conditions were as for Fig. 4 (no acetoacetate added) except that 70 μM Ca^{2+} (140 nmol · mg^{-1}) was used. The squares are the rate of efflux of untreated heart mitochondria; circles are results with mitochondria treated with PP_i (Asimakis and Sordahl, 1981) to decrease total adenine nucleotide (sum of ATP, ADP, and AMP) to the level shown. Open symbols represent a single mitochondrial preparation, untreated mitochondria (□), and mitochondria depleted to two different levels by varying PP_i concentration (○). In (B), oligomycin was present at 3 μg · mg^{-1}. The mitochondria treated to reduce endogenous adenine nucleotides showed no decline in ADP:O or respiratory control index and no change in uncoupler-dependent respiration rates in the absence of added adenine nucleotides when compared to controls.

7A). Nigericin virtually abolishes the swelling which normally follows the H^+ pulse in the KCl medium (Fig. 7B) and makes a given pH drop much less effective in producing Ca^{2+} efflux (Fig. 7C).

The H^+-dependent Ca^{2+} efflux is also inhibited by nupercaine (K_i = 90 μM, records not shown), a reagent known to inhibit Ca^{2+} efflux from liver or heart mitochondria induced by a variety of "Ca^{2+}-releasing agents" including P_i, oxaloacetate, acetoacetate, diamide, and ruthenium red (Pfeiffer *et al.*, 1979; Palmer and Pfeiffer, 1981; Dawson and Fulton, 1980).

Discussion

These studies have established that a decrease in pH can produce a rapid and extensive loss of accumulated Ca^{2+} from isolated beef heart mitochondria.

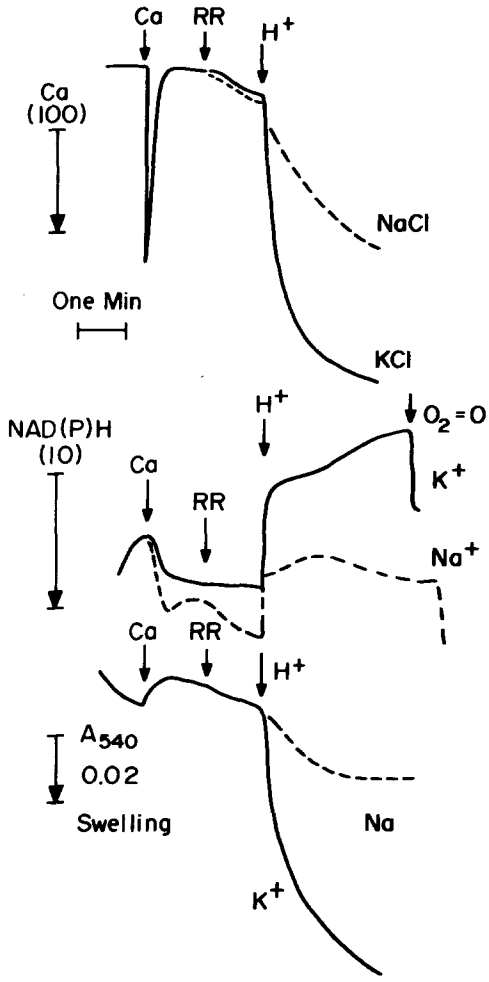

Fig. 6. The effect of replacing KCl with NaCl (125 mM) on the H^+-induced Ca^{2+} efflux, the redox state of NAD(P)H, and mitochondrial swelling. Except for the change of monovalent cation the conditions were identical to those of Fig. 1 for acetocetate-treated heart mitochondria. The final pH was 6.6 in each case.

This H^+-induced Ca^{2+} efflux occurs when either (a) the matrix NAD(P)H pool is oxidized or (b) endogenous adenine nucleotides are depleted. The rate of H^+-induced Ca^{2+} efflux is a function of the pH drop imposed, the level of Ca^{2+} accumulated, and the presence of P_i.

It is now well established that mitochondria can take up and release Ca^{2+}

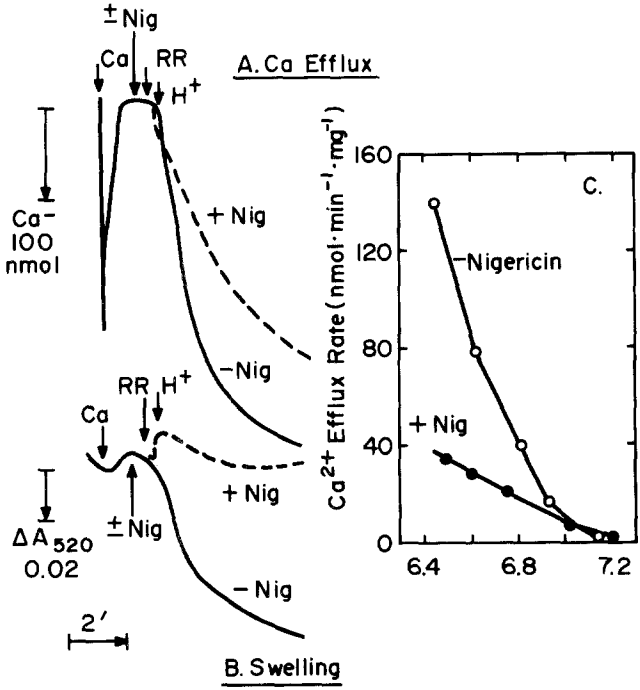

Fig. 7. The effect of nigericin on H^+-induced Ca^{2+} efflux (A), mitochondrial swelling (B), and the rate of Ca^{2+} efflux as a function of pH (C). The nigericin (1.3 μM) was added just prior to ruthenium red and the experimental conditions were those described for Fig. 1 for acetoacetate-treated heart mitochondria.

by opposing electrophoretic and electroneutral pathways (see Nicholls and Crompton, 1980, Carafoli, 1981, or Saris and Akerman, 1980, for recent reviews). The uptake of Ca^{2+} depends on a specific, high-affinity uniporter and occurs in response to the negative mitochondrial membrane potential ($\Delta\psi$). Influx is balanced by an electroneutral efflux mechanism which is presently thought to be a Ca^{2+}/Na^+ exchange reaction in mitochondria from heart and many other tissues and a Ca^{2+}/H^+ exchange in liver mitochondria.

The release of accumulated Ca^{2+} is accelerated by conditions that result in oxidation of pyridine nucleotides (Lehninger *et al.*, 1978), and Fiskum and Lehninger (1979) have concluded that the Ca^{2+}/H^+ antiport process is regulated by the redox state of mitochondrial pyridine nucleotides. In contrast, Nicholls and Brand (1980) contend that the acetoacetate added to oxidize pyridine nucleotides sensitizes the mitochondria to damage from accumulation of Ca^{2+} and P_i and that the efflux of Ca^{2+} occurs by reversal of the uniporter after collapse of the membrane potential. A number of studies (reviewed by Nicholls and Crompton, 1980) indicate that high levels of Ca^{2+}

in the presence of P_i may produce such structural changes leading to loss of $\Delta\psi$ and subsequent release of Ca^{2+} and that these effects are potentiated by reagents such as phosphoenolpyruvate, atractylate, oxalacetate, and thiol-group reagents. These structural changes are opposed by membrane-stabilizing reagents, such as adenine nucleotides and oligomycin (Nicholls and Crompton, 1980).

It should be noted that the heart mitochondria used in the present study do not release Ca^{2+} even when the pyridine nucleotide pool is extensively oxidized by addition of acetoacetate in the presence of rotenone (Fig. 1). However, it has been established that acetoacetate oxidizes NADH, but not NADPH, in these mitochondria (Jung and Brierley, 1981) and there is evidence that oxidation of the latter component is the crucial factor in Ca^{2+} release from liver mitochondria (Prpic and Bygrave, 1980). A rapid decrease in pH under these conditions results in extensive release of Ca^{2+} (Fig. 1A), a biphasic oxidation of pyridine nucleotides (Fig. 6), loss of endogenous Mg^{2+} (Table I), and swelling (Table I). Each of these responses is strongly opposed by oligomycin and by added adenine nucleotides (Fig. 3 and Table I). These findings, plus the fact that elevated levels of Ca^{2+} (Fig. 2) as well as P_i and a functional P_i transporter are required for H^+-dependent Ca^{2+} efflux, suggest that the reaction occurs as a result of structural changes (cf. Nicholls and Crompton, 1980) and does not depend on a Ca^{2+}/H^+ exchange process as has been suggested for an analogous H^+-dependent release of Ca^{2+} from liver mitochondria (Akerman, 1978). In addition, the biphasic rate of Ca^{2+} release often observed following a pulse of H^+ (Fig. 1) suggests that Ca^{2+} release is not directly coupled to a Ca^{2+}/H^+ exchange.

The spontaneous re-accumulation of the released Ca^{2+} in the absence of ruthenium red (Fig. 1B) indicates that with continued respiration at least a portion of the mitochondria are able to restore $\Delta\psi$ and reverse the events leading to Ca^{2+} release. Transient effects of an H^+ pulse on NAD(P)H redox state and energy coupling have been reported previously by Lowenstein and Chance (1968).

The present studies also suggest that the rate and extent of H^+-induced Ca^{2+}-efflux depend on whether or not the mitochondria are able to dissipate a portion of the imposed ΔpH via monovalent cation/H^+ exchange. It is well known that Na^+ promotes the loss of Ca^{2+} from heart mitochondria by Ca^{2+}/Na^+ exchange (cf. reviews by Carafoli, 1981; Saris and Akerman, 1980). Despite the presence of this Na^+-dependent Ca^{2+}-efflux component at neutral pH, the H^+-dependent efflux of Ca^{2+} is more rapid and extensive in a K^+ medium than in a Na^+ medium (Fig. 6). In this regard, Roman *et al.* (1979) have reported that a Na^+ medium offers kidney and liver mitochondria considerable protection against Ca^{2+} loss and swelling produced by unsaturated fatty acids. The protective effect of a Na^+ medium may be related to the

ability of the mitochondrial Na^+/H^+ exchanger to dissipate ΔpH, thus decreasing the driving force for phosphate entry. Secondly, the action of the Na^+/H^+ exchanger would result in the extrusion of Na^+ ions, and hence diminish osmotic swelling (Brierley *et al.*, 1977). It should be noted that the putative endogenous K^+/H^+ exchanger appears to be strongly inhibited by matrix Ca^{2+} (Shi *et al.*, 1980) so that a similar modification of an imposed ΔpH by K^+/H^+ exchange would not be available in the K^+ medium. Nigericin, which provides an exogenous K^+/H^+ exchange pathway, strongly inhibits H^+- and Ca^{2+}-dependent oxmotic swelling in the K^+ medium (Fig. 7B) and considerably diminishes the ability of an H^+ pulse to produce efflux of Ca^{2+} (Figs. 7A and 7C). Nigericin also inhibits the uptake of P_i by minimizing ΔpH (see Wolkowitz and McMillan-Wood, 1981, for example). These considerations lead to the conclusions that Ca^{2+}-dependent increases in monovalent cation permeability and osmotic swelling are important vectors for inducing or sustaining H^+-dependent Ca^{2+} release and that monovalent cation/H^+ exchange can oppose the effects of such changes.

The exact sequence of events leading to release of Ca^{2+} from heart mitochondria after a H^+ pulse cannot be specified at present. There is considerable evidence that the conditions that promote Ca^{2+} loss can be related to increased phospholipase activity (see Pfeiffer *et al.*, 1979, Palmer and Pfeiffer, 1981, and Harris and Cooper, 1981, for example). The present studies have shown that nupercaine, an inhibitor of phospholipase activity, inhibits H^+-dependent Ca^{2+} release, a result that is in line with an involvement of this activity in the generalized permeability increase associated with the H^+ pulse.

There are also numerous indications that the efflux of Ca^{2+} from mitochondria is closely related to the availability of adenine nucleotides. Effects of ATP and oligomycin have been ascribed to a generalized increase in membrane stability (Nicholls and Scott, 1980), but the low levels of ADP (ca. 1 μM) that prevent H^+-induced Ca^{2+} efflux from adenine nucleotide-depleted heart mitochondria (Fig. 4) suggest that a more specific process may be involved. In this regard, it has been suggested that the loss of Ca^{2+} may occur via a gated pore, controlled specifically by ADP (Haworth and Hunter, 1980; Zoccarato *et al.*, 1981). Adenine nucleotides may also act to limit the uptake of P_i indirectly by decreasing the permeability to K^+ (Panov *et al.*, 1980) or by decreasing phospholipase activity (Waite *et al.*, 1969). The fact that a minimal internal adenine nucleotide content is necessary in order for oligomycin to inhibit H^+-induced Ca^{2+} efflux (Fig. 5B) suggests that oligomycin may act by preserving a favorable matrix adenine nucleotide composition. This possibility is currently being examined and the studies will be reported elsewhere.

The present studies provide support for previous suggestions (Tsokos *et*

al., 1980) that H^+-dependent membrane changes could prevent mitochondrial buffering of Ca^{2+} under physiological conditions. Such effects could be especially important in ischemic cells in which increased Ca^{2+} and P_i levels accompany acidosis and in which mitochondrial adenine nucleotides can be depleted (Hohl *et al.*, 1982; Asimakis and Sordahl, 1981).

Acknowledgments

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