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Control of Mitochondrial Ca^{2+} Retention by ADP-Stimulated Glutamic Dehydrogenase

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Abstract

The protective effect of ADP on unspecific Ca^{2+} release and collapse of the transmembrane potential was analyzed in mitochondria from kidneys of rats. The presence of ADP in the incubation mixture prevents Ca^{2+} leakage and collapse of $\Delta\psi$ in sucrose-containing medium, but fails to do so in KCl medium. The effect of the adenine nucleotide in sucrose media correlates with an increase in the level of reduced pyridine nucleotides; the increase was due to a stimulatory effect on the activity of glutamic dehydrogenase. It also was observed that in KCl media, in the presence and in the absence of ADP the rate of NADH oxidation through the respiratory chain was higher than in sucrose; in this latter medium a high level of reduced pyridine nucleotides was found, in comparison to KCl media. It is proposed that the role of ADP is to increase glutamic dehydrogenase activity and in consequence to provoke a higher rate of formation of NADH which in turn controls Ca^{2+} release.

Key Words: Mitochondria; calcium; mitochondrial Ca^{2+} transport; adenine nucleotides; glutamic dehydrogenase; kidney mitochondria; ADP-stimulated glutamic dehydrogenase.

Introduction

The influx and efflux of Ca^{2+} in mitochondria is a subject of intensive investigation. It is now established that Ca^{2+} uptake is supported by the membrane potential that derives from substrate oxidation or ATP hydrolysis (Lehninger *et al.*, 1967). Ca^{2+} transport is mediated through a membrane carrier sensitive to ruthenium red (Moore, 1971), lanthanides (Reed and Bygrave, 1974), and Cd^{2+} (Jarvisalo *et al.*, 1980; Chávez *et al.*, 1985). A number of mechanisms which alter the retention of accumulated Ca^{2+} have

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been identified. In mitochondria from electrically excitable tissues, Ca^{2+} is released by an electroneutral mechanism that involves a Na^+/Ca^{2+} exchange reaction (Crompton *et al.*, 1976), whereas in liver mitochondria, Ca^{2+} efflux takes place as a Ca^{2+}/H^+ antiport (Fiscum and Cockrell, 1978). In addition, Ca^{2+} release can also occur through an unspecific pathway that may be activated by several chemicals and metabolites whose common action is to diminish the ratio of matrix NAD(P)H/NAD(P). This has been postulated as a control mechanism for the opening of a gated pore for Ca^{2+} release (Lehninger *et al.*, 1978; Palmer and Pfeiffer, 1981; Bellomo *et al.*, 1982; Beatrice *et al.*, 1984).

Several factors that prevent unspecific Ca^{2+} leakage have been described, i.e., bongkrekic acid, Mg^{2+}, and ADP (Hunter and Haworth 1974; Peng *et al.*, 1977). With respect to the role of ADP, Harris (1979) proposed that ADP bound to mitochondria results in an occlusion of a pathway for calcium efflux. Toninello *et al.* (1983) showed that ADP is necessary for the maintenance of the transmembrane potential, an indispensable requirement for Ca^{2+} retention. In addition, Nicholls and Crompton (1980) reported that the adenine nucleotides stabilize the membrane against the deleterious effect of oxalacetate and other Ca^{2+}-releasing agents.

In the present study the role of ADP in mitochondrial Ca^{2+} retention was further studied. In agreement with published data (Haworth and Hunter, 1980; Jurkowitz *et al.*, 1983; Jurkowitz and Brierley, 1984), it was found that ADP promotes the accumulation of large quantities of Ca^{2+} in rat kidney mitochondria. It is also shown that ADP prevents Ca^{2+} leakage when mitochondria are incubated in a sucrose medium, but fails to do so in a KCl medium. In comparison to KCl media, the protective effect of ADP observed in sucrose-containing media is associated with an increase in the NAD(P)H/NAD(P) ratio by a mechanism which involves stimulation of mitochondrial glutamic dehydrogenase.

Materials and Methods

Rat kidney mitochondria were prepared as reported (Chávez *et al.*, 1985). Mitochondria were disrupted by sonication for 2 min at the maximum setting of an MSE sonicator apparatus. Calcium transport was measured by using the metallochromic indicator Arsenazo III, purified according to Kendrick (1976), in an Aminco dual spectrophotometer at 685–675 nm. Changes in the membrane potential were followed by a dual-wavelength spectroscopy using the dye safranine at 533 minus 511 nm, as reported by Akerman and Wikström (1976). Oxidation of NAD(P)H was monitored by difference absorbance measurements at 370–340 nm. Assay of glutamic dehydrogenase, pyruvate dehydrogenase, and malic dehydrogenase activities

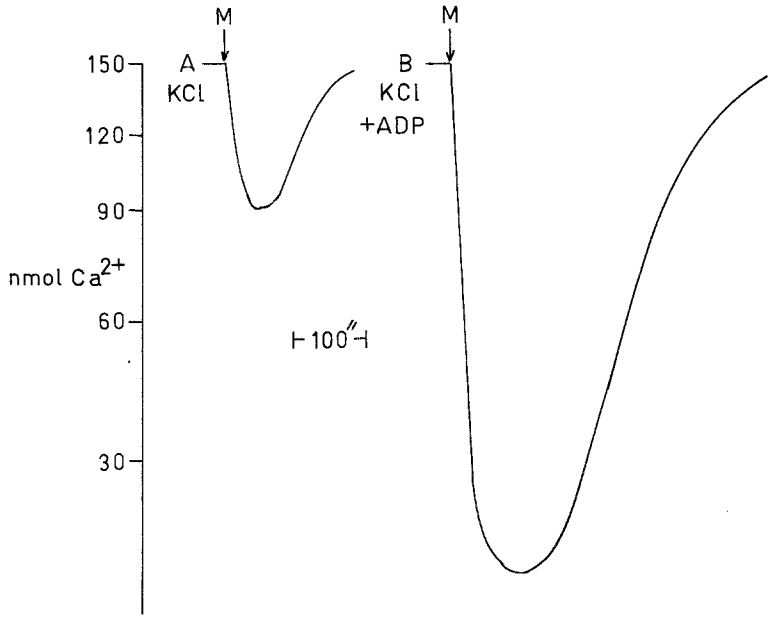

Fig. 1. The effect of ADP on mitochondrial Ca^{2+} movements in potassium-containing medium. Mitochondria (M) were added to media containing, in (A) and (B), 125 mM KCl, 10 mM Tris-chloride (pH 7.3), 10 mM acetate-Tris (pH 7.3), and 10 mM malate–10 mM glutamate; these substrates were adjusted with Tris base; in addition the media contained 50 μM $CaCl_2$ and 50 μM Arsenazo III. In (B) the medium was supplemented with 200 μM ADP.

were performed spectrophotometrically at 370–340 nm by using disrupted mitochondria. All mitochondrial incubations were conducted at 25°C, by using 2 mg mitochondrial protein per 3 ml media supplemented with 10 μg oligomicyn. Protein was determined by the method of Lowry *et al.* (1951).

Results

The ability of mitochondria to accumulate and retain high concentrations of Ca^{2+} has been firmly established (see Nicholls and Akerman, 1982, for review); however, when kidney mitochondria are added to a Ca^{2+}-containing medium with KCl as osmotic support and malate-glutamate as generators of the membrane potential, mitochondria accumulate Ca^{2+} at a level of 28 nmol/mg protein; this is followed of a rapid release (Fig. 1A). Trace B of Fig. 1 shows that the inclusion of 200 μM ADP markedly increased Ca^{2+} uptake (71 nmol/mg of protein); the above appears to be in agreement with early reports of Vasington and Murphy (1962) and Rossi and Lehninger (1964) who demonstrate an increase in mitochondrial Ca^{2+} uptake by the

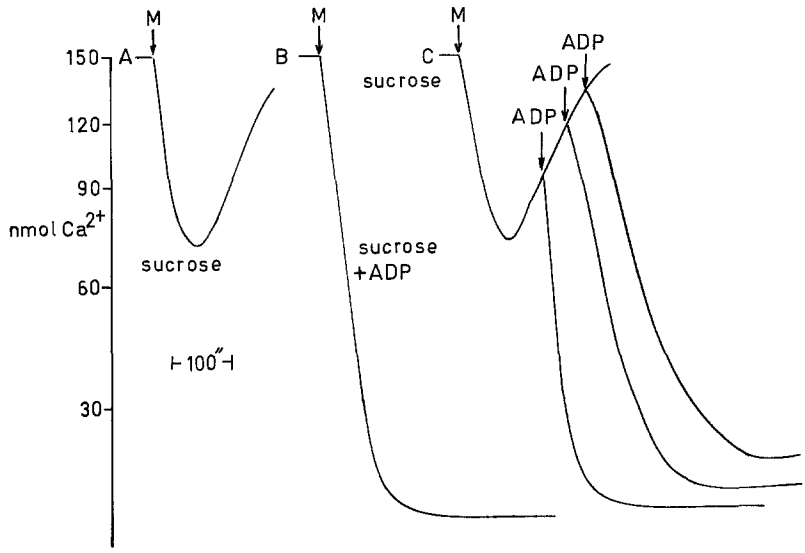

Fig. 2. The effect of ADP on mitochondrial Ca^{2+} movements in sucrose-containing medium. Mitochondria (M) were added to a similar incubation media as described for Fig. 1, except that KCl was replaced by 250 mM sucrose. Where indicated, 200 μm ADP was added.

addition of ADP. However, as observed (Fig. 1B) after a few seconds a rapid calcium efflux took place regardless of the presence of ADP.

When sucrose is used instead of KCl to maintain the osmolarity of incubation media, mitochondria took up an amount of Ca^{2+} similar to that observed in K^+-containing medium (38 nmol/mg of protein). Also in this medium there is a rapid Ca^{2+} efflux (Fig. 2A). Notwithstanding, when sucrose medium was supplemented by the addition of 200 μM ADP, mitochondrial Ca^{2+} load was increased to a level observed in the K^+-ADP medium (72 nmol per mg protein), but Ca^{2+} was retained (Fig. 2B). ADP added during the Ca^{2+} efflux phase promoted a massive accumulation of Ca^{2+} with values of about 70 nmol/mg protein (Fig. 2C).

The role of ADP in preventing unspecific Ca^{2+} efflux from mitochondria has been related to its ability to prevent a collapse of the transmembrane potential induced by external Ca^{2+} (Toninello *et al.*, 1983). Since the results indicated that ADP produces a different effect on Ca^{2+} retention in sucrose than in KCl medium, the action of ADP on Ca^{2+}-induced membrane depolarization was analyzed in mitochondria incubated in KCl or sucrose media. The results in Fig. 3A shows that the addition of 50 μM Ca^{2+} to mitochondria incubated in sucrose medium provoked a complete and rapid fall of the membrane potential, whereas only a diminution from 154 to 125 mV was observed when ADP was added to the sucrose medium (Fig. 3B).

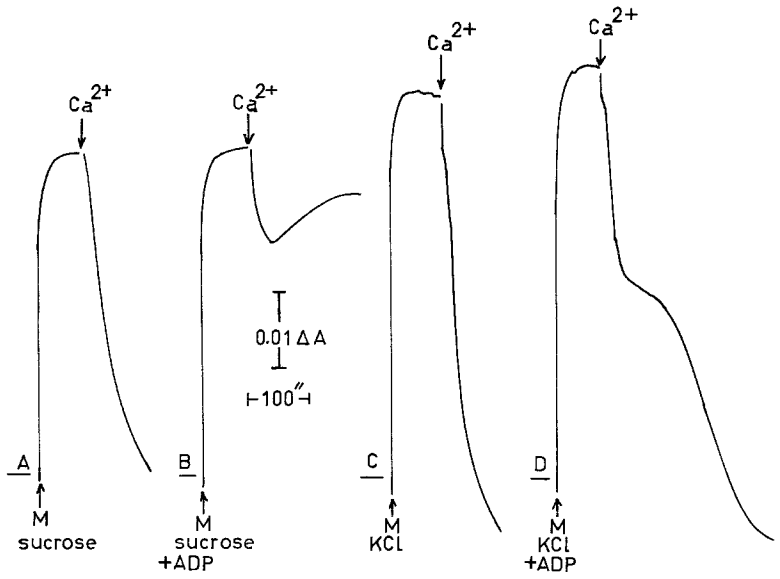

Fig. 3. Effect of ADP on the collapse of $\Delta\Psi$ induced by Ca^{2+} in sucrose and potassium medium. Mitochondria (M) were suspended in the standard media described in Figs. 1 and 2, with 10 μM safranine added. Where indicated, 50 μM $CaCl_2$ and 200 μM ADP were added.

In KCl medium ADP failed to maintain $\Delta\psi$ after the addition of Ca^{2+} (Fig. 3D).

It is also well established that the ability of mitochondria to retain Ca^{2+} is related to the level of reduced pyridine nucleotides (Lehninger *et al.*, 1978; Palmer and Pfeiffer, 1981; Bellomo *et al.*, 1982; Beatrice *et al.*, 1984). Therefore it appeared of interest to explore if ADP modified the mitochondrial NAD(P)H/NAD(P) ratio. Figure 4A shows that the addition of 50 μM Ca^{2+} to mitochondria incubated in sucrose medium inititated a rapid oxidation of NAD(P)H (18 nmol/mg protein). When ADP was added (Fig. 4B), the NAD(P)H/NAD(P) ratio shifted to a more reduced state. The high level of reduced NAD(P) promoted by ADP was not observed when mitochondria were incubated in potassium medium. Figure 4D shows that the redox steady state of pyridine nucleotides poised to a more oxidized state after the addition of Ca^{2+}. This result correlates with the lack of effect of ADP on the retention of Ca^{2+} by mitochondria in a K^+-containing medium (see Fig. 1B).

The finding that ADP increases the level of reduced pyridine nucleotides raised the possibility that ADP produced an inhibition of NADH dehydrogenase of the respiratory chain. In this respect, Weiner and Lardy (1973) reported that in Na^+-containing medium, ADP increases the intramitochondrial NAD(P)H pool by inhibiting electron transport at Site I of the

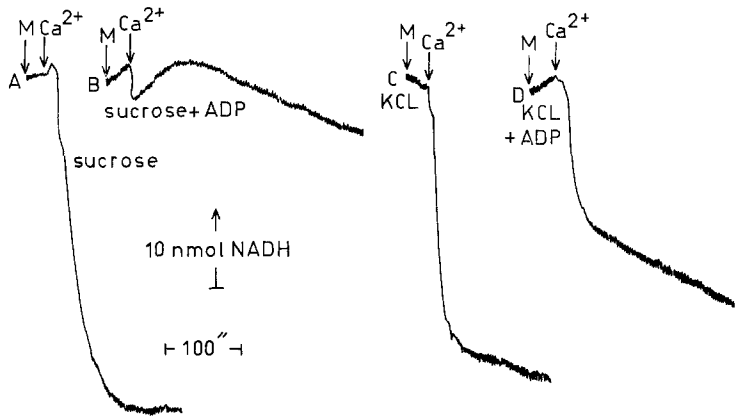

Fig. 4. The effect of ADP on Ca^{2+}-induced oxidation of pyridine nucleotides by mitochondria incubated in sucrose and K^+ media. Mitochondria (M) were added to a similar media as described in Figs. 1 and 2; 200 μM ADP and 50 μM Ca^{2+} were added where indicated.

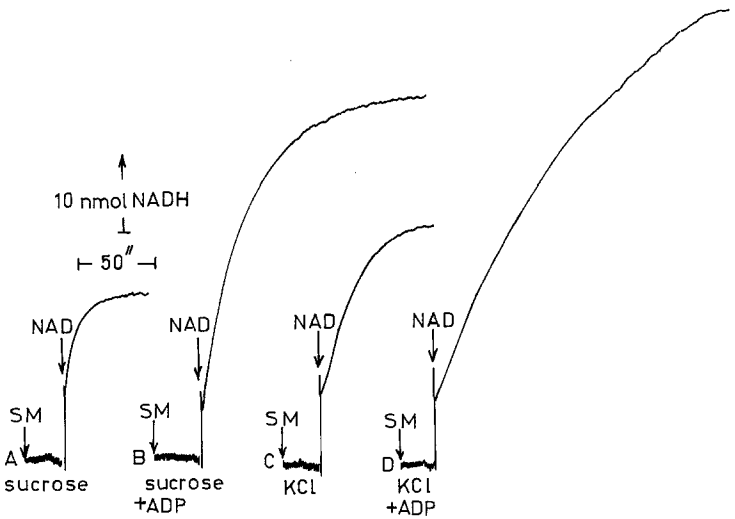

Fig. 5. ADP-dependent stimulation of glutamic dehydrogenase activity. Two milligrams protein of sonicated mitochondria (SM) was added to an incubation mixture containing 10 mM glutamate, 10 mM acetate-Tris (pH 7.3), and 10 μg rotenone; in addition media (A) and (B) contained 250 mM sucrose, and media (C) and (D), 125 mM KCl. Where indicated, 200 μM ADP and 184 μM NAD were added.

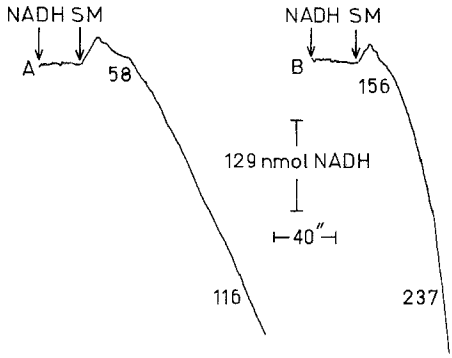

Fig. 6. The influence of KCl on NADH oxidation by sonicated mitochondria. Two milligrams protein of sonicated mitochondria (SM) was added to an incubation medium containing, in (A), 250 mM sucrose and 10 mM acetate-Tris (pH 7.3). In (B), the medium contained 125 mM KCl and 10 mM acetate-Tris (pH 7.3). Where indicated, 200 μM ADP and 215 μM NADH were added. The numbers at the side of the traces signify nmol NADH oxidized per min per mg protein.

respiratory chain. We studied the effect of ADP on NADH oxidation in submitochondrial particles. The results (not shown) indicated that in KCl or sucrose media, ADP failed to inhibit the oxidation of NADH.

Accordingly, it was considered that the ADP-dependent enhancement in the reduction state of pyridine nucleotides (Fig. 4B) was due to a stimulatory effect of ADP on some matrix NAD-dependent dehydrogenase enzymes, i.e., malate dehydrogenase or glutamate dehydrogenase. When the activity of malate dehydrogenase was assayed, no stimulatory effect of ADP was found (not shown). In contrast, as is seen in Fig. 5, ADP induced a marked increase of glutamic dehydrogenase activity, in agreement with early reports (Godinot and Gautheron, 1972; Bryla and Jolanta, 1977). These experiments were carried out in disrupted mitochondria in order to attain a higher enzymatic activity as was reported by Godinot and Gautheron (1972), and in the presence of rotenone to prevent NADH oxidation by the respiratory chain. It is important to note that the ADP-dependent stimulation was observed in both sucrose and KCl media (Figs. 5B and 5D). NADH formation reached a final higher level in K^+-containing medium, even though the initial rate of reduction of NAD was lower in this medium (14 nmol NADH produced $min^{-1} mg^{-1}$) than in sucrose medium (30 nmol NADH produced $min^{-1} mg^{-1}$).

When direct measurements of NADH oxidation were studied in sonicated mitochondria the results indicated that NADH was oxidized at a lower rate in sucrose (Fig. 6A) than in K^+ medium (Fig. 6B).

Discussion

Although the mechanisms controlling mitochondrial Ca^{2+} balance are not completely understood, the importance of an intact transmembrane potential for Ca^{2+} uptake is well established (Nicholls and Akerman, 1982). In addition there is a correlation between the redox state of mitochondrial pyridine nucleotides and the content of adenine nucleotides with the extent and rate of Ca^{2+} efflux; in this respect, Jurkowitz and Brierley (1984) showed that mitochondrial Ca^{2+} efflux as induced by hydrogen ions increases as an inverse function of adenine nucleotide content. Moreover, Toninello *et al.* (1983) concluded that the collapse of $\Delta\psi$ induced by Ca^{2+} addition is due to the release of endogenous Mg^{2+} and adenine nucleotides. In agreement with the latter authors Panov *et al.* (1980) suggested that the adenine nucleotide translocase operates as a gated pore regulated by the binding of ADP to specific sites, which could close the pathway for the loss of adenine nucleotides and, in consequence, prevent Ca^{2+} efflux. In addition, Siliprandi and co-workers (1983) confer to ADP a role in the maintenance of mitochondrial permeability barrier that can be modified by oxidation of –SH groups.

The results reported here provide evidence that one of the mechanisms involved in the protective effect of ADP on mitochondrial Ca^{2+} unspecific-efflux is to maintain a steady state of NAD(P)H/NAD(P) at a more reduced state. The redox state of pyridine nucleotides is controlled by several factors including substrate availability, activity of the substrate dehydrogenase, as well as the activity of NADH dehydrogenase of the electron transport chain. With respect to the role of mitochondrial substrates on Ca^{2+} movements, Wolkowitz and McMillin-Wood (1981) showed that the oxidation of pyridine nucleotides that results in Ca^{2+} efflux is due to NAD-dependent substrate limitation, i.e., glutamate.

In regards to the function of pyridine nucleotide-linked dehydrogenases on mitochondrial Ca^{2+} homeostasis, Beatrice *et al.* (1980) proposed that inhibition of glutathione reductase increases the GSSG/GSH ratio which in turn regulates the reduction state of membrane –SH groups involved in Ca^{2+} leakness.

The data presented in this work suggest that a plausible mechanism whereby ADP increases the NAD(P)H/NAD(P) ratio, and thereby Ca^{2+} retention, is through the stimulation of glutamate dehydrogenase activity. Although it is important to point out that when pyruvate-malate was used instead of glutamate-malate all the results described were reproduced (not shown), this is in line with the known ADP-dependent stimulation of pyruvate dehydrogenase (Krebs and Bravo, 1979).

A point that merits attention is the clear difference in the response to ADP observed when mitochondria are incubated in KCl in comparison

with those incubated in sucrose media. In sucrose media, ADP promotes Ca^{2+} retention (Fig. 2B), preserves the capability of mitochondria to maintain an energized state after the addition of Ca^{2+} (Fig. 3B), and maintains the steady state of NAD(P)H/NAD(P) in a more reduced level (Fig. 4B). Such effects are not reproduced when mitochondria are incubated in K^+-containing media, even though ADP induces an equal stimulation of glutamic dehydrogenase activity in KCl or sucrose medium (Fig. 5B and 5D). This would appear to be in contradiction with the finding that in the presence of ADP the steady state of pyridine nucleotides is in a more reduced state in sucrose than in potassium medium (Fig. 4); therefore the effect of K^+ on Site I of oxidative phosphorylation was considered. In this regard, it has been shown by Gómez-Puyou *et al.* (1972) that K^+ is a positive effector of electron flow in the NADH-CoQ span. In the presence of K^+, NADH originated from ADP-activated glutamic dehydrogenase enzyme is oxidized at a faster rate; apparently this higher rate of respiration causes the decrease of the NAD(P)H/NAD(P) ratio, and Ca^{2+} release. In sucrose medium NADH from glutamic dehydrogenation remains at a higher level due to the lower rate of NADH oxidation. This correlates with Ca^{2+} retention.

The effect of ADP on Ca^{2+} retention is well known (Hunter and Haworth, 1974; Nicholls and Crompton, 1980; Haworth and Hunter, 1980; Toninello *et al*, 1983; Jurkowitz *et al*, 1983; Jurkowitz and Brierley, 1984), but the mechanism through which ADP prevents Ca^{2+} efflux has not been defined. The data of this work show that modulation of the activity of matrix dehydrogenases by regulating the NAD(P)H/NAD(P) ratio controls the efflux of Ca^{2+} from the mitochondria. This would seem of interest since intramitochondrial Ca^{2+} affects the activity of matrix dehydrogenases (Denton and McCormack, 1980), but these activities in turn modify intramitochondrial Ca^{2+} levels.

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