Journal of Bioenergetics and Biomembranes, Vol. 28, No. 1, 1996

Original Article

Modulation of Matrix Ca^{2+} Content by the ADP/ATP Carrier in Brown Adipose Tissue Mitochondria. Influence of Membrane Lipid Composition

Edmundo Chávez,[1] Rafael Moreno-Sánchez,[1] Maria Eugenia Torres-Marquez,[1] Cecilia Zazueta,[1] Concepción Bravo,[1] Sara Rodríquez-Enríquez,[1] Cecilia García,[1] José S. Rodriguez,[1] and Federico Martinez[2]

Received October 16, 1995

The role of the adenine nucleotide translocase on Ca^{2+} homeostasis in mitochondria from brown adipose tissue was examined. It was found that in mitochondria incubated with 50 μM Ca^{2+}, ADP was not needed to retain the cation, but it was required for strengthening the inhibitory effect of cyclosporin on membrane permeability transition as induced by menadione. In addition, carboxyatractyloside was unable to promote matrix Ca^{2+} release, even though it inhibits the ADP exchange reaction. However, when the Ca^{2+} concentration was increased to 150 μM, carboxyatractyloside did induce Ca^{2+} release, and ADP favored Ca^{2+} retention. Determination of cardiolipin content in the inner membrane vesicles showed a greater concentration in brown adipose tissue mitochondria than that found in kidney mitochondria. It is suggested that the failure of the adenine nucleotide translocase to influence membrane permeability transition depends on the lipid composition of the inner membrane.

KEY WORDS: Matrix calcium; BAT mitochondria; membrane permeability transition; adenine nucleotide translocase; calcium transport.

INTRODUCTION

Mitochondrial calcium balance is maintained by uptake and release mechanisms (Gunter *et al.*, 1994). Calcium uptake is achieved electrophoretically, through a membrane uniport, which is sensitive to ruthenium red (Moore, 1971), lanthanum (Red and Bygrave, 1974), and cadmium (Jarvisalo *et al.*, 1980; Chávez *et al.*, 1985). At low matrix Ca^{2+} load, the route through which calcium is released may involve an interchange with H^+ (Fiskum and Cockrell, 1978; Fiskum and Lehninger, 1979), or Na^+ (Crompton *et al.*, 1977; Al-Shaikhaly *et al.*, 1979). However, at a high Ca^{2+} load, this cation can also be released via a nonspecific pore, activated by a wide variety of reagents (see Gunter and Pfeiffer, 1990 for a review). This process, called membrane permeability transition, is accompanied by a collapse of the membrane potential, swelling, and release of matrix solutes (Siliprandi *et al.*, 1983; Chávez and Holguín, 1988; Crompton *et al.*, 1988; Igbavboa *et al.*, 1989; Petronilli *et al.*, 1993). Modulation of the increased permeability has been associated with the orientation of the ADP/ATP translocase (ANT) across the inner membrane (Crompton and Costi, 1988; LeQuoc and LeQuoc, 1988; Rottenberg and Marbach, 1989; Novgorodov *et al.*, 1990). In this context, it is well established that atractyloside and carboxyatractyloside, which stabilize ANT in the

[1] Departamento de Bioquímica Instituto Nacional de Cardiología, Ignacio Chávez, México, D. F. 014080, México.

[2] Departamento de Bioquímica, Facultad de Medicina UNAM, México, D. F. 014080, México.

[3] Abbreviations used: BAT, brown adipose tissue; CAT, carboxyatractyloside; ANT, adenine nucleotide translocase; CCP, carbonyl cyanide *m*-chlorophenylhydrazone; CSA, cyclosporin A; DMO, 5,5-dimethyloxazolidine-2,4-dione; TPP, tetraphenylphosphonium; $\Delta\Psi$ transmembrane potential.

0145-479X/96/0200-0069$09.50/0

cytosol side, promote Ca^{2+} efflux (Asimakis and Sordahl, 1977; LeQuoc and LeQuoc, 1988; Chávez *et al.*, 1991). Conversely, ADP and bongkrekic acid induce Ca^{2+} retention by immobilizing ANT in the matrix side (Hunter and Haworth, 1979; Rottenberg and Marbach, 1990). Such a role of ANT has been demonstrated for mitochondria from a wide variety of sources, in which the main function of ANT is to export free energy to the cytosol. However, in thermogenic mitochondria from brown adipose tissue (BAT), the proton gradient is transduced into heat instead of being used to synthesize ATP (Nicholls, 1976; Himms-Hagen, 1990). In consequence, it appears that in these mitochondria ANT does not fulfill its usual physiological function. Therefore, a question arises: is the ADP/ATP translocase involved in the modulation of BAT mitochondrial Ca^{2+} content, as occurs in other types of mitochondria? The purpose of the present work was to attempt to answer such a question. The results show that when mitochondria were incubated in a medium containing 50 μM Ca^{2+}, ADP was not necessary for matrix calcium retention, but it was required to reinforce the protection by cyclosporin (CSA) of menadione-induced hyperpermeabilization. In addition, it was found that carboxyatractyloside did not induce Ca^{2+} release, despite its inhibitory effect on ADP exchange. An increase in Ca^{2+} concentration to 150 μM made evident the requirement of ADP for Ca^{2+} retention, and the ability of CAT to induce Ca^{2+} release.

MATERIALS AND METHODS

Mitochondria from brown adipose tissue were isolated from heat-adapted Wistar rats. When mitochondria from cold-adapted rats were used, the results were similar. The isolation medium and the centrifugation pattern were as described (Grav *et al.*, 1970). Kidney mitochondria were isolated as previously reported (Chávez *et al.*, 1985). Submitochondrial particles were prepared as described elsewhere (Lee and Ernster, 1965). Protein was determined by the method of Lowry (1951). Calcium movement was analyzed spectrophotometrically at 675–685 nm, using the dye Arsenazo III as an indicator (Kendrick, 1976). Alternatively, calcium uptake was estimated by using $^{45}Ca^{2+}$ (sp. act. 1000 cpm/nmol), following the filtration technique through Millipore filters of 0.45 μm pore diameter. The ADP exchange reaction was assayed as described (Winkler and Lehninger, 1968), using [^{14}C]-ADP (sp. act. 1220 cpm/nmol). Mitochondrial transmembrane potential was quantitatively determined by measuring the accumulation of [^{3}H]-tetraphenylphosphonium (sp. act. 8000 cpm/nmol) as described by Rottenberg (1984). The calculated values of membrane potential, using the Nernst equation, were corrected for unspecific TPP^+ binding to mitochondrial membranes, either by subtracting the label found in the presence of an excess of CCP plus antimycin, or using the partition coefficients calculated for rat liver mitochondria by Rottenberg (1984). Changes in transmembrane potential were also followed spectrophotometrically at 511–533 nm, using the dye safranine (Akerman and Wikström, 1976). The pH gradient was determined by following the distribution of [^{14}C]-DMO (sp. act. 800 cpm/nmol), [^{3}H]-H_2O (sp. act. 4800 cpm/nmol), and [^{14}C]-sucrose (sp. act. 900 cpm/nmol), as previously reported (Rottenberg, 1979). The mitochondrial membrane lipid composition was analyzed by thin layer chromatography, after extraction in chloroform–methanol (2:1). Organic phosphate was determined as described (Bartlett, 1959). Cyclophilin activity was followed spectrophotometrically at 390 nm by incubating mitochondrial matrix extract in a medium containing N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (125 μM) and 0.78 mg chymotrypsin (Halestrap and Davidson, 1990). The content of adenine nucleotide translocase in mitochondria was determined by titration of the rate of ATP synthesis with carboxyatractyloside (Moreno-Sánchez, 1985). ATP synthesis was measured in mitochondria incubated in a medium that contained 10 mM glucose; 10 units hexokinase; 1 mM $MgCl_2$; 3 mM GDP; 1 mM ADP; 250 mM sucrose; 10 mM HEPES; 1 mM EGTA; 5 mM phosphate; 5 mM succinate and 1 μM rotenone; pH 7.3 at 30°C. The reaction was started by addition of mitochondria, allowed to proceed for 3 min, and stopped with 3% perchloric acid. After neutralization, synthesized ATP was determined by generation of NADPH in a system of hexokinase and glucose-6-phosphate dehydrogenase. Other experimental conditions were as described in the corresponding captions to the figures.

RESULTS

The increased proton permeability in brown adipose tissue mitochondria is blocked by purine nucleotides (Nicholls, 1976; Lin and Klingenberg, 1982), which leads to the formation of a transmembrane potential. The experiment shown in Fig. 1A was carried out to assess, in our preparation, the GDP concentration

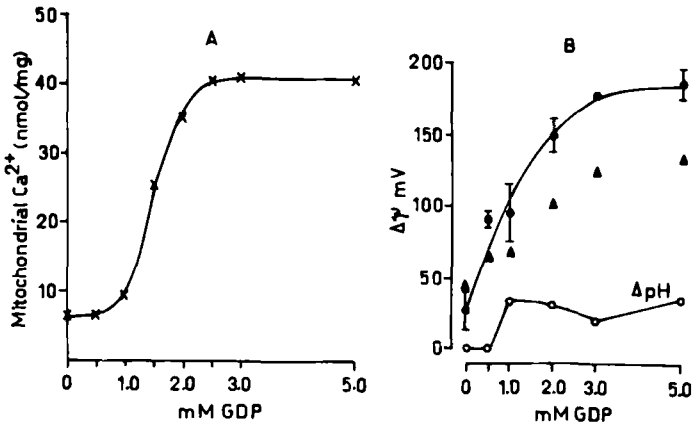

Fig. 1. Effect of increasing concentrations of GDP upon calcium uptake (A), and membrane H^+ electrochemical gradient (B) in BAT mitochondria. In A, 0.66 mg of mitochondrial protein were incubated in 1.0 ml of a medium containing 125 mM KCl; 10 mM succinate; 10 mM HEPES-KOH, pH 7.3; 2 mM phosphate, 5 μg rotenone; the indicated concentrations of GDP, and 50 μM $^{45}CaCl_2$ (sp. act. 1000 cpm/nmol). After 6 min of incubation at 25°C, aliquots of 0.2 ml were withdrawn and filtered through a Millipore filter of 0.45 μm pore diameter. The radioactivity in mitochondria retained in the filter was estimated in a scintillation counter. In B, BAT mitochondria (1 mg protein) were incubated with the indicated GDP concentrations during 3 min at 30°C with further addition of 1 μM [^{3}H]-TPP for the estimation of membrane potential, or with [^{14}C]-DMO or [^{14}C]-sucrose plus [^{3}H]-H_2O for the determination of pH gradient according to Rottenberg (1979, 1984). The values of $\Delta\Psi$ represent the mean ± standard deviation of three different mitochondrial preparations. The values of membrane potential were corrected for unspecific binding by means of a parallel experiment made in the presence of 1 μM CCP plus 50 μM antimycin (●) or following the partition coefficients for TPP^+ (▲) calculated for rat liver mitochondria. pH gradient was measured as described in Materials and Methods.

required to accumulate Ca^{2+}. GDP concentration dependence for Ca^{2+} uptake followed a sigmoidal pattern. A half maximal GDP concentration was attained at 1.5 mM, while saturation was achieved at 3 mM. In contrast, the GDP dependence of the transmembrane potential (Fig. 1B) showed a hyperbolic pattern; therefore, the experiments were performed by incubating mitochondria in media containing 3 mM GDP and, when indicated, 50 μM Ca^{2+}.

A number of reports have shown that ADP is an important prerequisite for Ca^{2+} retention in mitochondria prepared from different sources, i.e., liver, heart, brain, and kidney (Chávez and Jay, 1987; Halestrap and Davidson, 1990; Rottenberg and Marbach, 1990). It is interesting to note, however, that BAT mitochondria accumulated Ca^{2+} to a great extent, even in the absence of added ADP (Fig. 2A). Furthermore, it is shown that the addition of 10 μM CAT failed to promote loss of the accumulated Ca^{2+}, as previously shown for *Euglena* mitochondria (Uribe *et al.*, 1994). The latter result appears to be also in opposition to those found in mitochondria isolated from other tissues, in which CAT effectively induces Ca^{2+} release (Chávez *et al.*, 1991). Since BAT mitochondria require GDP to block the proton conductance, it would be reasonable to expect that GDP may act as a substitute for ADP, inhibiting membrane transition. On the one hand, it is well recognized that GDP does not replace ADP as a metabolite for the adenine nucleotide translocase (Klingenberg, 1985), and on the other hand, Panov *et al.* (1980) demonstrated that only ADP and ATP are able to inhibit nonspecific ion permeability; nevertheless, due to the high concentration of GDP that was used (3 mM), one could postulate an inhibition of the pore opening. Such a possibility was explored in kidney mitochondria, which require ADP for Ca^{2+} retention. Figure 2B shows that, in these mitochondria, Ca^{2+} was released regardless of the addition of 3 mM GDP.

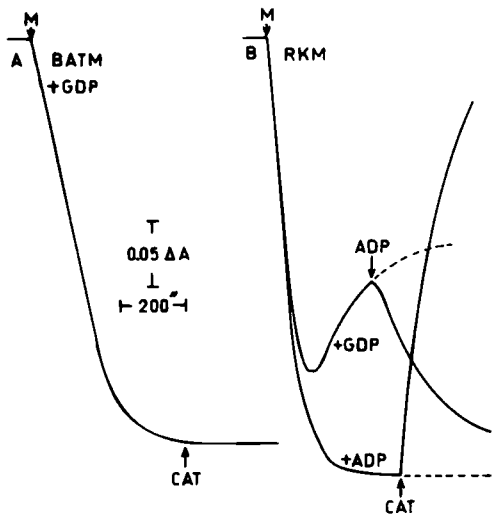

Fig. 2. Effect of ADP, GDP, and carboxyatractyloside in Ca^{2+} transport by BAT and kidney mitochondria. 2 mg protein from brown adipose tissue mitochondria (A) and kidney mitochondria (B) were added to 3 ml media containing 125 mM KCl; 10 mM succinate; 10 mM HEPES-KOH, pH 7.3; 2 mM phosphate; 50 μM $CaCl_2$; 50 μM Arsenazo III, and 5 μg rotenone. Where indicated, 3 mM GDP, 10 μM carboxyatractyloside (CAT), and 200 μM ADP were added. Calcium movements were followed spectrophotometrically at 675–685 nm. Temperature 25°C.

It is interesting to note that the addition of ADP, during the Ca^{2+} efflux phase, promoted a massive accumulation of the cation. The latter indicates that GDP does not hinder in any way the locus for the action of the adenine nucleotide. In addition it is shown that, in agreement with reported data (Chávez *et al.*, 1991), CAT induced Ca^{2+} depletion in kidney mitochondria.

A low content of ANT in BAT mitochondria could be the cause for the reduced requirement of ADP to retain mitochondrial Ca^{2+}. To test such a possibility a titration of this carrier was performed. The results indicate a value of 0.308 nmol/mg. Certainly such a value is lower than that found in rat kidney and rat heart mitochondria, i.e., 0.613 and 1.360 nmol/mg, respectively (Moreno-Sánchez and Torres-Marquez, 1991), but it was close to that obtained in rat liver mitochondria, i.e., 0.3 nmol/mg (Kunz *et al.*, 1988), in which ADP effectively inhibits membrane permeability transition (Lapidus and Sokolove, 1994).

Also, it was decided to analyze the effect of CAT and GDP on the ADP exchange reaction in BAT mitochondria. Table I shows that, in agreement with previous reports (Klingenberg, 1985), 10 μM CAT inhibited, by 80%, the equilibration of added ADP with the matrix adenine nucleotide pool, i.e., it was reduced from 4.4 to 0.9 nmol/mg. Furthermore, ADP was effectively exchanged in the presence of GDP (3.9 nmol/mg).

Table I. Inhibitory Effect of CAT on ADP Exchange in Mitochondria from BAT Adipose Tissue[a]

Additions	ADP exchanged (nmol/mg)
ADP	4.4
ADP + CAT	0.9
ADP + GDP	3.9
ADP + GDP + CAT	0.4

[a] Mitochondria (1 mg protein) were incubated in 1 ml of a medium containing 125 mM KCl; 10 mM HEPES, pH 7.3; 5 μg rotenone; 5 μg oligomycin and 20 nmol [^{14}C]-ADP. Where indicated, 10 μM CAT and 3 mM GDP were added. After 1 min of incubation at 24°C, an aliquot of 0.2 ml was filtered through a Millipore filter of 0.45 μm pore size and the radioactivity contained in the filter was estimated.

On the other hand, since the pioneer studies of Fournier *et al.* (1987), a growing body of evidence has shown that the immunosupressant cyclosporin A (CSA) inhibits the development of a hyperpermeable state (Crompton *et al.*, 1988; Broekemeier *et al.* 1989; Davidson and Halestrap, 1987; Petronilli *et al.* 1993). The protective action of CSA has been attributed to its binding to the enzyme cyclophilin (Halestrap and Davidson, 1990). Thus, the existence of this enzyme in BAT mitochondria was investigated. Table II shows the activity of the cis-trans isomerase in these mitochondria. Although such an activity was 25% lower than that found in kidney mitochondria, it should be noted that it was effectively inhibited, by nearly 65%, after the addition of 1 μM cyclosporin.

After establishing the presence of cyclophilin in BAT mitochondria, the effect of cyclosporin on mem-

Table II. Cyclophilin Activity in Brown Adipose Tissue Mitochondria as Compared with Kidney Mitochondria[a]

Source of mitochondria	Cyclophilin activity
BAT	94.5
BAT + CSA	33.2
KIDNEY	124.1
KIDNEY + CSA	38.4

[a] Protein (300 μg) of sonicated mitochondria were added to a cuvette containing 36 mM HEPES, pH 7.8; 0.78 mg chymotrypsin, and 125 μM N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Where indicated, 1 μM cyclosporin (CSA) was added. The results are given in nmol hydrolyzed substrate/30 sec.

brane permeability transition was analyzed. Due to the failure of CAT to induce Ca^{2+} release, menadione was used to induce membrane transition. Figure 3A illustrates that menadione (200 μM) successfully provoked mitochondrial Ca^{2+} release. This Ca^{2+} efflux was partially inhibited after the addition of 0.5 μM cyclosporin. Nevertheless, in agreement with Novgorodov *et al.*, (1990, 1991) and Zazueta *et al.* (1994), a total inhibition by cyclosporin was attained after the addition of 200 μM ADP (Fig. 3B). Furthermore, as observed in Fig. 4, CSA protected from the deleterious effect of menadione on membrane $\Delta\Psi$, provided 200 μM ADP was added.

Recently we have demonstrated that the effect of carboxyatractyloside on Ca^{2+} release is inversely proportional to the density of internal negative charges (Chávez *et al.*, 1991). In this respect, it has been shown that the negatively charged cardiolipin is located preferentially (around 80%) on the matrix side of the inner bilayer (Daum, 1985). As a result of the above, it was necessary to establish the amount of cardiolipin located in BAT submitochondrial particles. The results showed a significant difference between the values of cardiolipin found in kidney and those found in BAT mitochondria (Table III). In kidney mitochondria, of the total phospholipids, 8% corresponded to cardiolipin. This value agreed with that reported by Daum (1985). Interestingly, a higher value was found in BAT mitochondria: 24.5%. On the other hand, in agreement with previously reported data (Daum, 1985), the values for

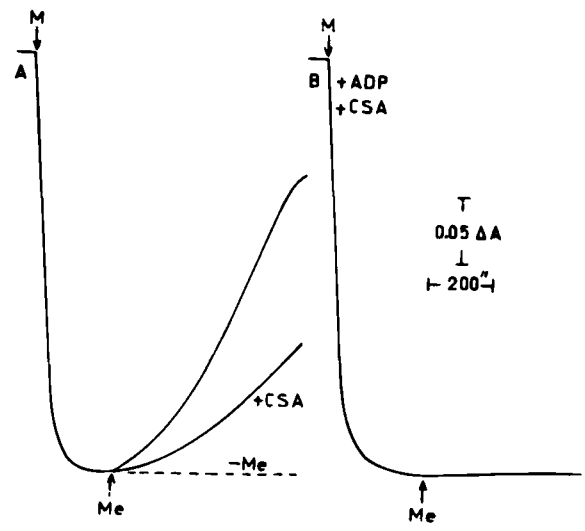

Fig. 3. Protective effect of cyclosporin plus ADP on Ca^{2+} release as induced by menadione. Experimental conditions were as described for Fig. 2. Where indicated, 200 μM menadione (Me), 200 μM ADP, and 0.5 μM cyclosporin (CSA) were added.

Table III. Percent of Cardiolipin and Phosphatidylcholine in the Inner Membrane of Kidney and Brown Adipose Tissue Mitochondria[a]

Phospholipid	Source of mitochondria	
	Kidney	BAT
Cardiolipin	8	24.5
Phosphatidylcholine	35.8	29.7

[a] 30 mg protein of both kidney and brown adipose tissue submitochondrial particles were used to analyze phospholipid content. After extraction in chloroform methanol (2:1), the analysis was carried out by thin layer chromatography, as described in Materials and Methods.

phosphatidylcholine content were similar: 35.8 for kidney and 29.7% for BAT mitochondria.

A high density of negative charges located in the matrix side of BAT mitochondria implies that, by increasing the external Ca^{2+} concentration from 50 to 150 μM, the ADP requirement for Ca^{2+} retention and CAT-induced Ca^{2+} release should be evident. Indeed, Fig. 5 shows that when mitochondria were incubated in a medium containing 150 μM Ca^{2+}, ADP was required to retain the cation, and 10 μM carboxyatractyloside promoted matrix Ca^{2+} release.

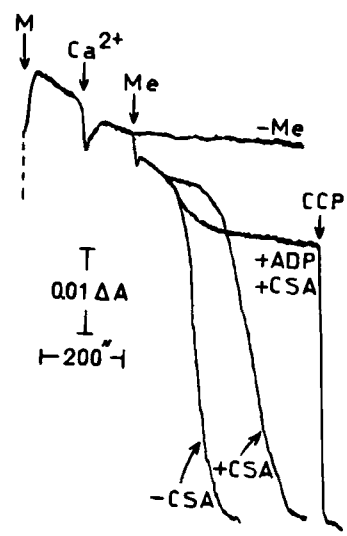

Fig. 4. Protective effect of cyclosporin plus ADP on membrane de-energization as induced by menadione. 2 mg of mitochondrial protein were added to media containing 125 mM KCl; 10 mM succinate 10; mM HEPES-KOH, pH 7.3; 2 mM phosphate; 5 μg rotenone and 10 μM safranine. Where indicated, 50 μM $CaCl_2$, 200 μM menadione (Me), 200 μM ADP, 0.5 μM cyclosporin, and 4 μM CCP were added. Changes in the transmembrane potential were followed in a double-beam spectrophotometer at 511–533 nm.

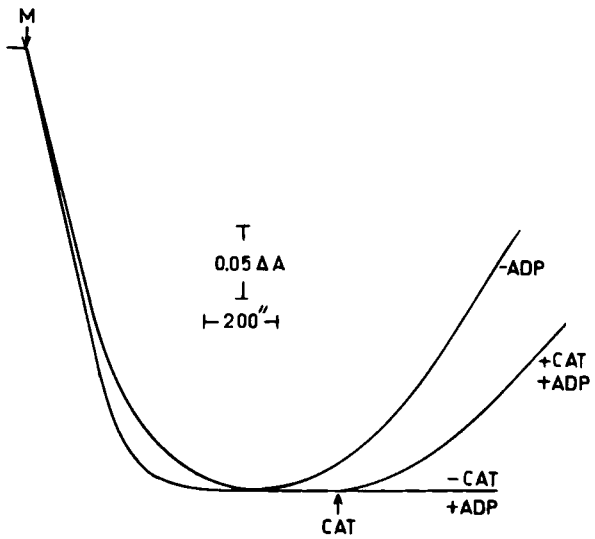

Fig. 5. Effect of ADP and carboxyatractyloside on BAT mitochondria incubated in higher concentrations of calcium. The experimental conditions were as described for Fig. 2, except that 150 μM, instead of 50 μM, and 70 μM Arsenazo III were added to the incubation media. In addition, where indicated, 200 μM ADP and 10 μM carboxyatractyloside (CAT) were added.

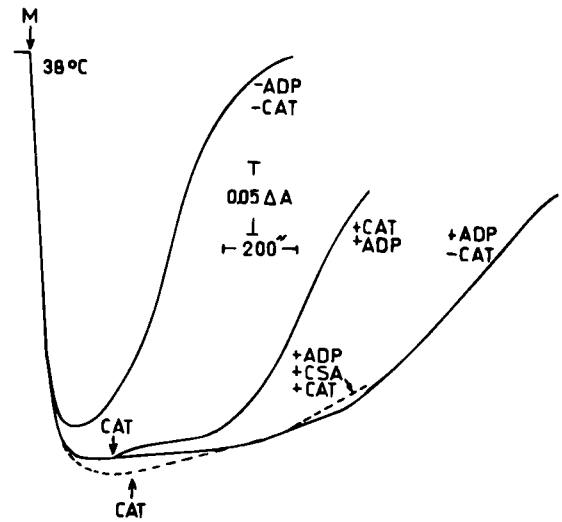

Fig. 6. Unmasking of CAT-induced Ca^{2+} release and ADP-dependence of Ca^{2+} retention by high temperature. The experimental conditions were as described for Fig. 2, except for the incubation temperature which was maintained at 38°C. Where indicated, 200 μM ADP, 0.5 μM CSA, and 10 μM CAT were added.

Changes in the conformation of ANT are required to open the nonspecific pore (Crompton and Costi, 1988; LeQuoc and LeQuoc, 1988; Rottenberg and Marbach, 1989; Novgorodov *et al.*, 1990); in turn, such changes are closely dependent on the incubation temperature. In fact, by diminishing the incubation temperature to 4°C, atractyloside became unable to induce Ca^{2+} release in kidney mitochondria (Chávez and Osornio, 1988). Accordingly, we decided to investigate whether, at a high temperature, ADP is required for Ca^{2+} retention, and also if CAT may induce an increase in membrane permeability for Ca^{2+} discharge. Indeed, at 38°C, the ability of BAT mitochondria to sequester and retain Ca^{2+} became highly dependent on ADP. Moreover, carboxyatractyloside was also able to induce mitochondrial Ca^{2+} efflux, and CSA plus ADP protected from such an effect (Fig. 6).

DISCUSSION

As previously reported by Panov *et al.* (1980) and Haworth and Hunter (1980), matrix Ca^{2+} retention depends on the sidedness of the ADP/ATP carrier in the membrane. In mitochondria from different sources the formation of a transmembrane ionic pore, for Ca^{2+} leakage, can be stimulated by carboxyatractyloside (Halestrap *et al.*, 1985; Davidson and Halestrap, 1987; Halestrap, 1989; Chávez *et al.*, 1991), and inhibited by ADP (Hunter and Haworth, 1979; Chávez and Jay, 1987; Rottenberg and Marbach, 1990). From the findings in this communication it appears evident that, in brown adipose tissue mitochondria, the adenine nucleotide translocase seems not to be involved in Ca^{2+} homeostasis. Such a conclusion was drawn from the observation that when mitochondria were incubated in media containing 50 μM Ca^{2+}, ADP was not required to maintain matrix Ca^{2+} concentrations, and carboxyatractyloside failed to induce mitochondrial Ca^{2+} release. The latter represents an atypical response, since a number of reports indicate that 50 μM Ca^{2+} suffices to induce membrane permeability transition (Bellomo *et al.*, 1982; Chávez and Holguín, 1988; Lenartowicz *et al.*, 1991; Petronilli *et al.*, 1994).

In agreement with Nicholls (1976) and Klingenberg (1985), this work shows that BAT mitochondria exchange ADP through a reaction sensitive to CAT. This result supports the notion that the adenine nucleotide translocase undergoes conformational changes from matrix (M) to cytosol (C) sides. Asimakis and Sordahl (1977) suggested that atractyloside, by immobilizing ANT in the cytosol side, transforms the carrier to a nonspecific gated pore. However, the results reported here indicate that such a conformational change might not be the unique event taking place in the membrane. In other words, in BAT mitochondria,

these conformational changes do not suffice to increase the permeability to Ca^{2+}; hence, for the transformation of ANT to a nonspecific pore an additional mechanism must be involved. In fact, Halestrap and Davidson (1990) have proposed a model in which the formation of the pore requires, in addition to CAT, the binding of Ca^{2+} to a specific site located in the translocase. Thus, the failure in the development of the nonspecific pathway in BAT mitochondria would be related to a diminution in the concentration of internal "active" Ca^{2+}. In a previous report (Chávez *et al.*, 1991) we demonstrated that in K-depleted mitochondria, in which the internal negative charges were increase, CAT failed to induce Ca^{2+} depletion. In BAT mitochondria, the high concentration of cardiolipin provides an increased pool of negative charges for unspecific Ca^{2+} binding (Rand and Sengupta, 1972; Krebs *et al.*, 1979), thus leading to a diminution of Ca^{2+} binding to the translocase, and the pore remains closed. However, the determination of matrix free Ca^{2+} content with Fluo 3 in BAT mitochondria showed similar values to those observed in kidney mitochondria (data not shown) under identical conditions of external free Ca^{2+} (50–1000 nM). A value of 400 nM for the dissociation constant (K_d) of the Fluo 3–Ca complex (Kao *et al.*, 1989) was used in the calculation of matrix free Ca^{2+}; the possibility remains that the K_d value may be lower in BAT mitochondria and, therefore, that the matrix Ca^{2+} concentration is indeed smaller that in kidney mitochondria. The assumption that the failure of ANT to regulate internal Ca^{2+} in BAT mitochondria is due to a lack of a binding site for Ca^{2+} in ANT, or to a diminution in the sensitivity of ANT for calcium ions, although highly speculative, has some support in the data of this study. BAT mitochondria requires ADP to retain Ca^{2+}, and the metal is now released by CAT when the external Ca^{2+} concentration is increased from 50 to 150 μM. The finding that menadione induced Ca^{2+} release and a drop in $\Delta\Psi$ in BAT mitochondria incubated with 50 μM is in conflict with the latter assumption. However, it should be pointed out that, in agreement with Bellomo *et al.* (1982), menadione promotes Ca^{2+} efflux by oxidation of NAD(P)H, a mechanism that stabilizes the translocase in the "c" conformation, opening the pore (LeQuoc and LeQuoc, 1989).

Finally, a finding that merits attention is the synergistic effect of ADP of increasing the inhibition of cyclosporin on membrane permeability transition. Such an effect of ADP was previously reported by Novgorodov *et al.* (1990, 1991) and Zazueta *et al.* (1994). However, their experiments were carried out in rat liver and rat brain mitochondria respectively, in which ADP is required to retain Ca^{2+}. In BAT mitochondria a paradoxical effect of ADP is present, i.e., on the one hand ADP was not needed to keep high internal Ca^{2+} concentrations, but, on the other hand, ADP was necessary to reach a complete inhibitory effect by cyclosporin. Thus, from our findings it can be speculated that the effect of ADP on CSA-induced protection is not achieved through the binding of the nucleotide to the translocase.

ACKNOWLEDGMENTS

This work was partially supported by grants 047N and F554 from CONACyT.

REFERENCES

Akerman, K. E., and Wikström, M. F. K. (1976). *FEBS Lett.* **68**, 191–197.
Al-Shaikhaly, M. H. M., Medegaard, J., and Cannon, B. (1979). *Proc. Natl. Acad. Sci. USA* **76**, 2350–2353.
Asimakis, G. K., and Sordahl, L. A. (1977). *Arch. Biochem. Biophys.* **179**, 200–210.
Bartlett, G. R. (1959). *J. Biol. Chem.* **234**, 466–468.
Bellomo, G., Jewell, S. A., and Orrenius, S. (1982). *J. Biol. Chem.* **257**, 11558–11562.
Broekemeier, K. M., Dempsey, M. E., and Pfeiffer, D. R. (1989). *J. Biol. Chem.* **264**, 7826–7830.
Chávez, E., and Holguín, J. A. (1988). *J. Biol. Chem.* **263**, 3582–3587.
Chávez, E., and Jay, D. (1987). *J. Bioenerg. Biomembr.* **19**, 571–580.
Chávez, E., Briones, R., Michel, B., Bravo, C., and Jay, D. (1985). *Arch. Biochem. Biophys.* **242**, 493–497.
Chávez, E., Moreno-Sánchez, R., Zazueta, C., Reyes-Vivas, H., and Arteaga, D. (1991). *Biochim. Biophys. Acta* **1070**, 461–466.
Chávez, E., and Osornio, A. (1988). *Int. J. Biochem.* **20**, 731–736.
Crompton, M., and Costi, A. (1988b). *Eur. J. Biochem.* **178**, 489–501.
Crompton, M., Kunzi, M., and Carafoli, E. (1977). *Eur. J. Biochem.* **79**, 549–558.
Crompton, M., Ellinger, H., and Costi, A. (1988). *Biochem. J.* **255**, 357–360.
Daum, G. (1985). *Biochim. Biophys. Acta* **822**, 1–42.
Davidson, A. M., and Halestrap, A. P. (1987). *Biochem. J.* **146**, 715–723.
Fiskum, G., and Cockrell, R. S. (1978). *FEBS Lett.* **92**, 125–128.
Fiskum, G., and Lehninger, A. L. (1979). *J. Biol. Chem.* **254**, 6236–6239.
Fournier, N., Ducet, G., and Crevet, A. (1987). *J. Bienerg. Biomembr.* **19**, 297–303.
Grav, H. J., Pedersen, J. L., and Christiansen, E. N. (1970). *Eur. J. Biochem.* **12**, 11–23.
Gunter, T. E., and Pfeiffer, D. R. (1990). *Am. J. Physiol.* **258**, C755–C786.
Gunter, T. E., Gunter, K. K., Sheu, S. S., and Gavin, C. E. (1994). *Am J. Physiol.* **C313–C339**.

Halestrap, A. P. (1989). *Biochim. Biophys. Acta* **973**, 355–382.
Halestrap, A. P., and Davidson, A. M. (1990). *Biochem. J.* **268**, 153–160.
Halestrap, A. P., Quinlan, P. T., Whipps, D. E., and Armstrong, A. E. (1985). *Biochem. J.* **236**, 779–787.
Haworth, R. A., and Hunter, D. R. (1980). *J. Membr. Biol.* **54**, 231–236.
Himms-Hagen, J. (1990). *FASEB J.* **4**, 2890–2898.
Hunter, D. R., and Haworth, R. A. (1979). *Arch. Biochem. Biophys.* **195**, 453–459.
Igbavboa, U., Craig, W. Z., and Pfeiffer, D. R. (1989). *Biochem. Biophys. Res. Commun.* **161**, 619–625.
Jarvisalo, J. O., Kilpio, J., and Saris, N. E. L. (1980). *Environ. Res.* **22**, 217–223.
Kao, J. P. Y., Harootunian, A. T., and Tsien, R. Y. (1989). *J. Biol. Chem.* **264**, 8179–8184.
Kendrick, N. C. (1976). *Anal. Biochem.* **76**, 487–501.
Klingenberg, M. (1985). *Ann. N.Y. Acad. Sci.* **456**, 279–288.
Krebs, J. J. R., Hauser, H., and Carafoli, E. (1979). *J. Biol. Chem.* **254**, 5308–5316.
Kunz, W., Gellerich, F. N., Schild, L., and Schoenfeld, P. (1988). *FEBS Lett.* **233**, 17–21.
Lapidus, R. G., and Sokolove, P. M. (1994). *J. Biol. Chem.* **269**, 18931–18936.
LeQuoc, K., and LeQuoc, D. (1988). *Arch. Biochem. Biophys.* **265**, 249–257.
LeQuoc, D., and LeQuoc, K. (1989). *Arch. Biochem. Biophys.* **273**, 466–478.
Lee, C. P., and Ernster, L. (1965). In Regulation of Metabolic Processes in Mitochondria, BBA Library, **7**, 218–234.
Lenartowicz, E., Bernardi, P., and Azzone, G. F. (1991). *J. Bioenerg. Biomembr.* **23**, 679–688.
Lin, C. S., and Klingenberg, M. (1982). *Biochemistry* **21**, 2950–2956.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.
Moore, C. L. (1971). *Biochem. Biophys. Res. Commun.* **42**, 298–304.
Moreno-Sánchez, R. (1985). *J. Biol. Chem.* **260**, 4028–4034.
Moreno-Sánchez, R., and Torres-Márquez, M. E. (1991). *Int. J. Biochem.* **23**, 1163–1174.
Nicholls, D. G. (1976). *Eur. J. Biochem.* **62**, 223–228.
Novgorodov, S. A., Gudz, T. I., Kushnareva, Y., Zorov, D. B., and Kudrashov, Y. B. (1990). *FEBS Lett.* **277**, 123–126.
Novgorodov, S. A., Gudz, T. I., Jung, D., and Brierley, G. P. (1991). *Biochem. Biophys. Res. Commun.* **160**, 33–38.
Panov, A., Filipova, S., and Lyakhovich, V. (1980). *Arch. Biochem. Biophys.* **199**, 420–426.
Petronilli, V., Cola, C., Massari, F., Colonna, R., and Bernardi, P. (1993). *J. Biol. Chem.* **268**, 21939–21945.
Petronilli, V., Costantini, P., Socorrano, L., Colonna, R., Passamonti, S., and Bernardi, P. (1994). *J. Biol. Chem.* **269**, 16638–16642.
Rand, R. P., and Sengupta, S. (1972). *Biochim. Biophys. Acta* **255**, 484–492.
Red, K. C., and Bygrave, F. L. (1974). *Biochem. J.* **140**, 143–155.
Rottenberg, H. (1979). *Methods. Enzymol.* **55**, 547–569.
Rottenberg, H. (1984). *J. Membr. Biol.* **81**, 127–138.
Rottenberg, H., and Marbach, M. (1989). *FEBS Lett.* **247**, 483–486.
Rottenberg, H., and Marbach, M. (1990). *Biochim. Biophys. Acta* **1016**, 87–98.
Siliprandi, D., Siliprandi, N., and Toninello, A. (1983). *Eur. J. Biochem.* **130**, 173–175.
Uribe, A., Chávez, E., Jiménez, M., Zazueta, C., and Moreno-Sánchez, R. (1994). *Biochim. Biophys. Acta* **1186**, 107–116.
Winkler, H. H., and Lehninger, A. L. (1968). *J. Biol. Chem.* **243**, 3000–3008.
Zazueta, C., Reyes-Vivas, H., Corona, N., Bravo, C., and Chávez, E. (1994). *Biochem. Mol. Biol. Int.* **33**, 385–392.