Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1706 (2005) 98–104

BIOCHIMICA ET BIOPHYSICA ACTA BBA

http://www.elsevier.com/locate/bba

Effects of extramitochondrial ADP on permeability transition of mouse liver mitochondria

Zemfira Z. Gizatullina*, Ying Chen, Stephan Zierz, Frank Norbert Gellerich

Muskellabor der Neurologischen Klinik der Martin-Luther-Universität Halle-Wittenberg, Ernst-Grube-Str. 40, D-06097 Halle (Saale), Germany

Received 16 April 2004; received in revised form 20 September 2004; accepted 22 September 2004
Available online 14 October 2004

Abstract

Carboxyatractylate (CAT) and atractylate inhibit the mitochondrial adenine nucleotide translocator (ANT) and stimulate the opening of permeability transition pore (PTP). Following pretreatment of mouse liver mitochondria with 5 μM CAT and 75 μM Ca^{2+}, the activity of PTP increased, but addition of 2 mM ADP inhibited the swelling of mitochondria. Extramitochondrial Ca^{2+} concentration measured with Calcium-Green 5N evidenced that 2 mM ADP did not remarkably decrease the free Ca^{2+} but the release of Ca^{2+} from loaded mitochondria was stopped effectively after addition of 2 mM ADP. CAT caused a remarkable decrease of the maximum amount of calcium ions, which can be accumulated by mitochondria. Addition of 2 mM ADP after 5 μM CAT did not change the respiration, but increased the mitochondrial capacity for Ca^{2+} at more than five times. Bongkrekic acid (BA) had a biphasic effect on PT. In the first minutes 5 μM BA increased the stability of mitochondrial membrane followed by a pronounced opening of PTP too. BA abolished the action about of 1 mM ADP, but was not able to induce swelling of mitochondria in the presence of 2 mM ADP.

We conclude that the outer side of inner mitochondrial membrane has a low affinity sensor for ADP, modifying the activity of PTP. The pathophysiological importance of this process could be an endogenous prevention of PT at conditions of energetic depression.

Keywords: Mitochondria; Permeability transition pore; Adenine nucleotide translocator; Carboxyatractyloside; Bongkrekic acid

1. Introduction

Mitochondrial adenine nucleotide translocator (ANT) changes between two conformations in which the ADP/ATP-binding site is either on the matrix side (m-state) or on the cytoplasmic side (c-state) of the inner membrane [1]. Bongkrekic acid (BA) binds at the m-state of ANT (low affinity state) and inhibits the mitochondrial permeability transition pore (PTP). Carboxyatractylate (CAT) and atractylate bind the c-state of ANT (high affinity state) and stimulate the PTP. It was suggested that the c-state conformation is required for PTP opening (see review in Ref. [2]). Based on the response of PTP to inhibitors and substrates of ANT, Halestrap proposed that the ANT is the main component of PTP, which can be opened by interaction with cyclophilin in the presence of Ca^{2+} and in the absence of adenine nucleotides [3–6]. Pore opening requires the binding of cyclophilin to the ANT, except when matrix [Ca^{2+}] is very high [2,3]. In this model cyclophilin is the trigger for pore opening, and its interaction with cyclosporine A (CsA) prevents the opening process [1–8]. Recently with transgenic mouse, it was shown that the ANT is not part of the PTP but has regulative properties for PTP [9].

The sensitivity of the mitochondrial PTP to Ca^{2+} is greatly increased by oxidative stress, adenine nucleotide depletion, elevated phosphate concentration, low membrane potential ($\Delta\psi$), and agents that stabilize the "c" conformation of the ANT. In contrast, the protection is afforded by low pH, high $\Delta\psi$, and agents that stabilize the "m" conformation of the ANT [2–6,10]. Matrix adenine nucleo-

Abbreviations: PTP, permeability transition pore; ANT, adenine nucleotide translocator; BA, bongkrekic acid; CAT, carboxyatractylate

* Corresponding author. Tel.: +49 345 557 3512; fax: +49 345 557 3505.

E-mail addresses: zemfira.gizatullina@rambler.ru (Z.Z. Gizatullina), frank.gellerich@medizin.uni-halle.de (F.N. Gellerich).

0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2004.09.013

tides decrease the sensitivity of the PTP to Ca^{2+} by binding to the ANT (see review in Ref. [2]).

In most assay procedures ADP acts as inhibitor of PTP [7]. Since ADP can modulate the sensitivity of PTP to CsA, the existence of a low affinity CAT-insensitive ADP binding site outside of ANT was assumed [7]. In other studies, where adenine nucleotides were used to prevent pore opening, only ATP was found to be effective [8]. 5 mM ATP, similar to that found physiologically in the cytosol, blocked permeability transition (PT) in intact heart mitochondria completely, whereas 1.5 mM ATP only partially inhibited PTP. 1 mM ADP, however, had no protecting effect on PT [2,8]. It was assumed that ATP could inhibit the pore by binding to some other component than ANT [2].

The present study was undertaken to define the influence of extramitochondrial ADP on the function of PTP under conditions of CAT- and BA-inhibited ANT. We established a pronounced ability of ADP to prevent the PT.

2. Materials and methods

2.1. Materials

Carboxyatractyloside, EGTA (ethyleneglycolbis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) were from Calbiochem. Oligomycin, bongkrekic acid, ADP (disodium salt), MOPS, succinate, FCCP (carbonyl cyanide *p*-[trifluoromethoxy] phenylhydrazone) and rotenone were from Sigma. Calcium Green-5N was from Molecular Probes. All reagents were of analytical grade.

2.2. Isolation of liver mitochondria

Mitochondria were isolated from mouse liver by differential centrifugation [11]. The isolation medium A contained 250 mM sucrose, 5 mM Tris, pH 7.4 and 1 mM EGTA. Immediately after decapitation, the liver was excised, chopped, and homogenized in 40-ml isolation medium with a Potter-Elvejem homogenizer. The homogenate was centrifuged at 800×*g* for 7 min. The supernatant was separated and recentrifuged at 6000×*g* for 15 min. The resulting pellet was resuspended with EGTA-free isolation medium B centrifuged at 6000×*g* for 15 min. The final pellet was resuspended again in medium B. All procedures were performed at 0–4 °C.

Mitochondrial protein concentration was determined by the bicinchoninic acid assay [12]. Bovine serum albumin was used as a standard.

2.3. Oxygen consumption

Oxygen consumption rates of mitochondria were monitored with a Clark-type oxygen electrode in an OROBOROS oxygraph [13]. Respiration of mitochondria at a final concentration 1 mg/ml was measured at 30 °C in incubation medium A consisting of 250 mM sucrose, 20 mM MOPS, 10 mM succinate, 5 μM rotenone, 10 mM KH_2PO_4, pH 7.2. Additions were made with a Hamilton syringes through a small hole in the cover of the chamber. The oxygen concentration in the air-saturated medium was considered to be 200 nmol O_2/ml at 95 kPa barometric pressures. The weight-specific oxygen consumption was calculated as the time derivative of the oxygen concentration (DATGRAPH Analysis software, OROBOROS).

2.4. Measurement of pore opening

The opening of the PTP was determined by swelling of mitochondria at 30 °C [10]. Mitochondrial swelling was measured by the decrease in light scattering at 520 nm, using a PC-supported spectrophotometer Lambda 18 in thermostated and stirred cuvettes. Liver mitochondria were incubated at 30 °C and a final concentration 0.7 mg protein/ml in incubation medium A.

2.5. Measurement of extramitochondrial Ca^{2+} concentration

Extramitochondrial Ca^{2+} concentration was measured fluorimetrically in the presence of 1 μM Calcium Green-5N with excitation and emission wavelengths set at 506 and 532 nm, respectively, with RF-5001PC spectrofluorimeter from Shimadzu [14].

3. Results

3.1. Swelling measurements

Addition of 75 μM exogenous Ca^{2+} to respiring mouse liver mitochondria induced the opening of the PTP as shown by a large amplitude swelling since the pore opened and the mitochondria became permeable to sucrose (Fig. 1A and B, trace 1). At first we investigated the influence of ADP on the PTP in the presence of both ANT inhibitors, CAT and BA.

Fig. 1A, trace 2, shows that addition of 5 μM CAT strongly accelerated the Ca^{2+} induced swelling of mitochondria in comparison to the control experiment without CAT. The rate of swelling was diminished by 150 μM ADP (Fig. 1A, trace 3) indicating a stabilization of mitochondrial inner membrane. In the presence of both effectors (150 μM ADP and 5 μM CAT), the stabilizing effect of ADP was clearly suppressing the pore opening effect of CAT (Fig. 1A, trace 4).

Fig. 1B demonstrates the influence of ADP additions to mitochondria, which are in the state of Ca^{2+}-induced swelling. If added before Ca^{2+}, 2 mM ADP completely suppressed Ca^{2+}-induced PTP (Fig. 1B, trace 3). Moreover, ADP is able to inhibit PTP, even after its activation by 5 μM CAT and 75 μM Ca^{2+} (Fig. 1B, trace 4) since the course of mitochondrial high amplitude swelling was abolished.

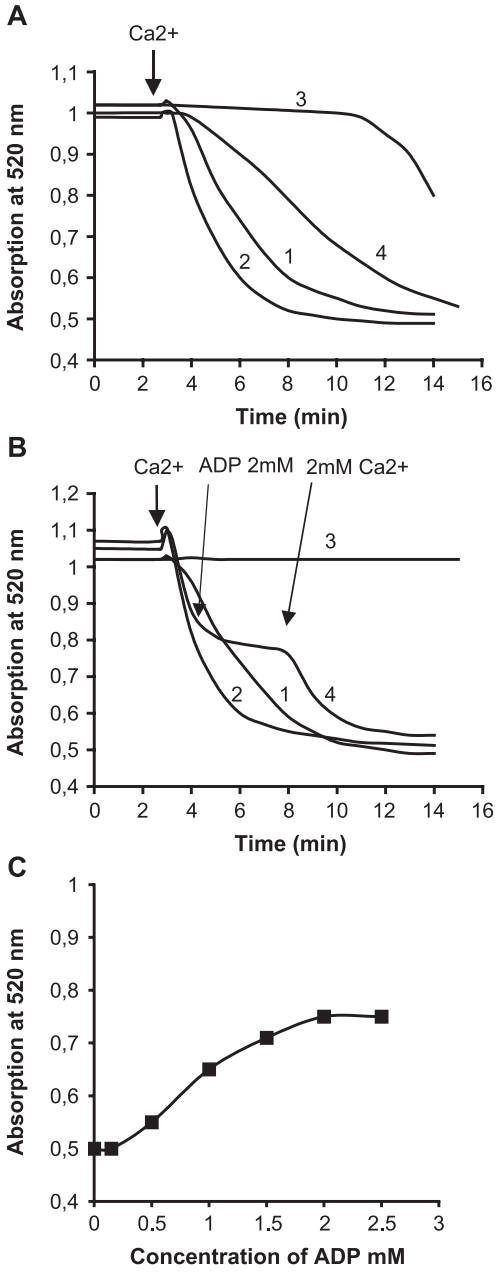

Fig. 1. Dose-dependent influence of extramitochondrial ADP on Ca^{2+}-induced swelling of mouse liver mitochondria in the presence of CAT. Sequential additions were done as follows: (A) trace 1—75 μM Ca^{2+}; trace 2—5 μM CAT, 75 μM Ca^{2+}; trace 3—150 μM ADP, 75 μM Ca^{2+}; trace 4—150 μM ADP, 5 μM CAT, 75 μM 75 μM Ca^{2+}. (B) Trace 1—75 μM Ca^{2+}; trace 2—5 μM CAT, 75 μM Ca^{2+}; trace 3—2 mM ADP, 75 μM Ca^{2+}; trace 4—5 μM CAT, 75 μM Ca^{2+}, 2 mM ADP, 2 mM Ca^{2+}. Mitochondria (0.7 mg/ml) were incubated in incubation medium A. The arrows mark the addition of 75 μM $CaCl_2$. The results of one of the five analogous experiments are presented. (C) Dose–response curve of the effect of ADP on the swelling of mouse liver mitochondria induced by CAT plus Ca^{2+}. Absorption at 520 nm was measured exactly 4 min after Ca^{2+} addition. The respective ADP concentration was added 2 s after Ca^{2+}.

Further addition of about 2 mM Ca^{2+} induced again the swelling of mitochondria (Fig. 1B, trace 4). Fig. 1C indicates that the extramitochondrial ADP effect on PTP was clearly dose-dependent with K_i of about 900 μM ADP.

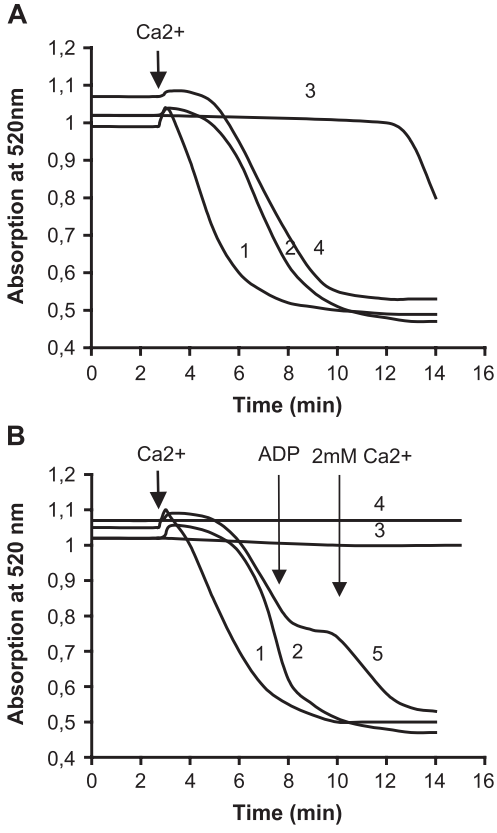

Fig. 2. Dose-dependent influence of extramitochondrial ADP on Ca^{2+}-induced swelling of mouse liver mitochondria in the presence of BA. Sequential additions were done as follows: (A) trace 1—75 μM Ca^{2+}; trace 2—5 μM BA, 75 μM Ca^{2+}; trace 3—150 μM ADP, 75 μM Ca^{2+}; trace 4—5 μM BA, 150 μM ADP, 75 μM Ca^{2+}. (B) Trace 1—75 μM Ca^{2+}; trace 2—5 μM BA, 75 μM Ca^{2+}; trace 3—2 mM ADP, 75 μM Ca^{2+}; trace 4—2 mM ADP, 5 μM BA, 75 μM Ca^{2+}; trace 5—5 μM BA, 75 μM Ca^{2+}, 2 mM ADP, 75 μM Ca^{2+}. Mitochondria (0,7 mg/ml) were incubated in incubation medium A. The arrows mark the addition of 75 μM $CaCl_2$. The results of one of the five analogous experiments are presented.

Since CAT sensitively inhibits the ANT in its c-state, it was important to know whether or not inhibition of the m-state has similar consequences for PTP. Fig. 2, trace 2,

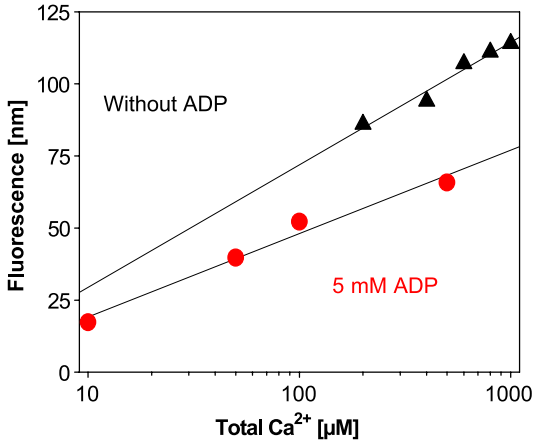

Fig. 3. Fluorimetric titration of free Ca^{2+} concentration in the presence and without ADP with Calcium Green-5N.

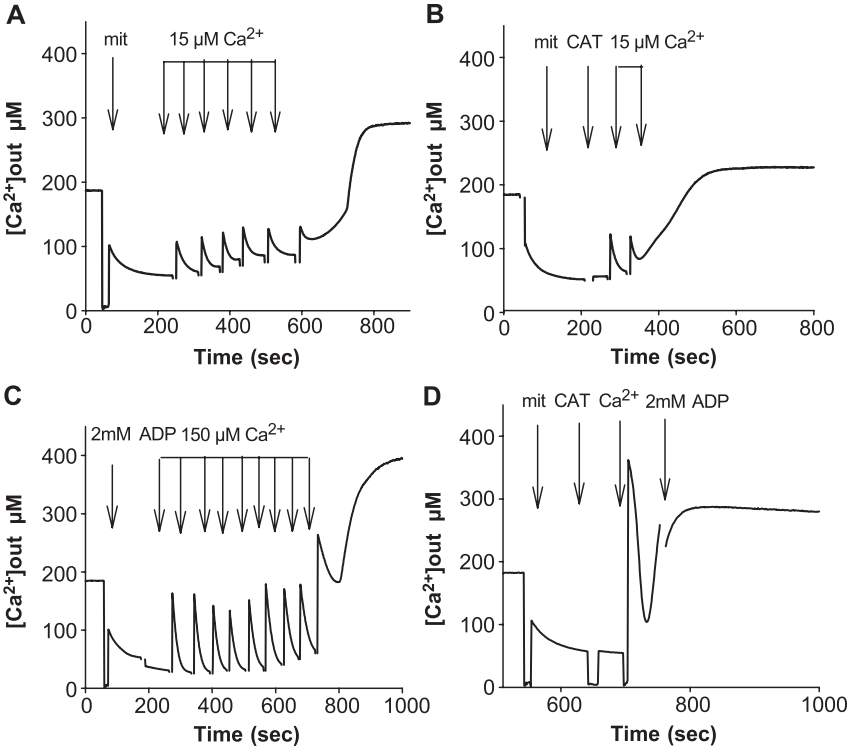

Fig. 4. Measuring of the extramitochondrial $[Ca^{2+}]_{out}$ in the presence of CAT in the mouse liver mitochondria. A—control (total 90 μM Ca^{2+}); B—5 μM CAT, 30μM Ca^{2+} (total); C—2 mM ADP, 1,35 mM Ca^{2+} (total); D—5 μM CAT, 1 mM Ca^{2+}, 2 mM ADP. Mitochondria (1.0 mg/ml) were incubated in incubation medium A. The results of one of the five analogous experiments are presented.

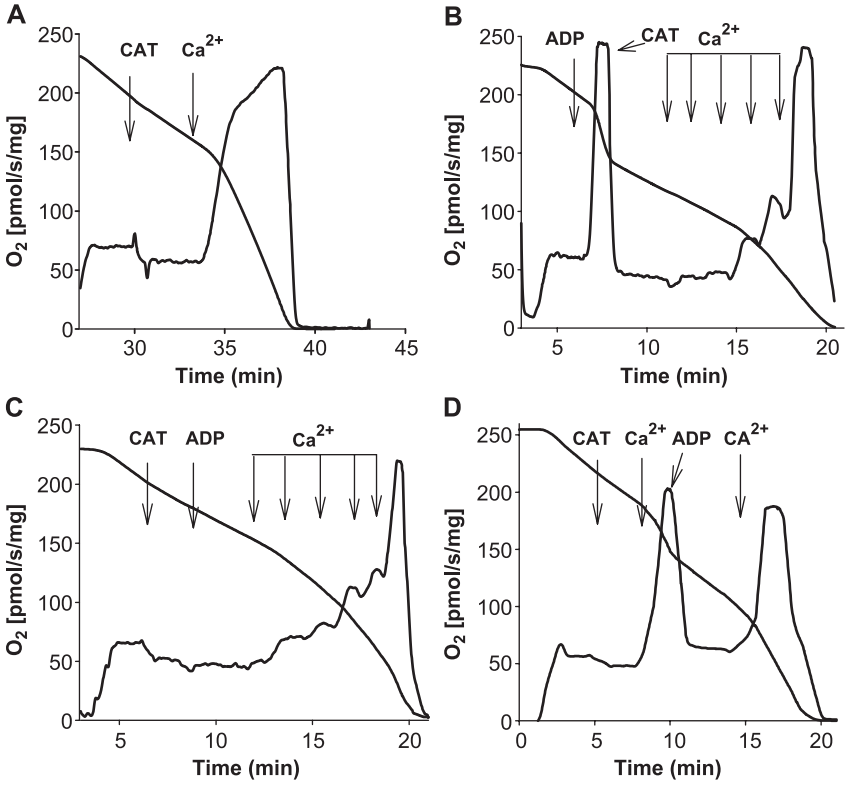

Fig. 5. ADP-dependent respiration at CAT-inhibited ANT in the presence of Ca^{2+} in mouse liver mitochondria. A—5 μM CAT, 50 μM Ca^{2+}; B—2 mM ADP, 5 μM CAT, 1 mM Ca^{2+} (total); C—5 μM CAT, 2 mM ADP, 1 mM Ca^{2+} (total); D—5 μM CAT, 50 μM Ca^{2+}, 2 mM ADP, 1 mM Ca^{2+}. Mitochondria (1 mg/ml) were incubated in incubation medium A. The results of one of the five analogous experiments are presented.

shows that the addition of 5 ìM BA prevented the Ca^{2+}-dependent swelling of mitochondria in the first minute of incubation but thereafter the pore was opened too, however, with a temporal delay compared to the control (Trace 1). The clear stabilization effect of 150 μM ADP (Trace 3) was diminished by the presence of 5 μM BA (Trace 4).

Fig. 2B, trace 3, shows again that 2 mM ADP completely prevented the Ca^{2+}-induced PTP (as in Fig. 1B, trace 3), and even the addition of 5 μM BA before or after ADP did not change the volume of mitochondria (Fig. 2B, trace 4). Similar to that shown above for CAT, the addition of 2 mM ADP was able to close again the PTP, which was already opened by BA and Ca^{2+} (trace 5). These data indicate that high extramitochondrial ADP concentration hindered the c- and m-conformation of ANT to open the PTP. This leads to the conclusion that extramitochondrial ADP is able to regulate the activity of PTP of mouse liver mitochondria.

3.2. Cytosolic Ca^{2+} and PT

It is known that ADP forms complexes with Ca^{2+} and other metal ions. Therefore, it could be possible that the preventing effect of ADP on Ca^{2+}-induced pore opening was simply due to diminishing of the free Ca^{2+}. We measured Ca^{2+} concentration fluorimetrically in the presence of 1 μM Calcium Green-5N. As shown in Fig. 3, the addition of 2 mM ADP decreased the free calcium concentration, but the remaining free Ca^{2+} was enough for pore opening.

In the next type of experiments we measured fluorimetrically the extramitochondrial Ca^{2+} concentration and checked the influence of ADP and of CAT on accumulation and release of Ca^{2+}. Mitochondria were loaded by repeated additions of 15 μM Ca^{2+} until a total amount of 90 μM Ca^{2+}/1.0 mg mitochondrial protein/ml was reached. Under these conditions mitochondria started to release about 250 μM Ca^{2+}, indicating the opening of PTP (Fig. 4A). Obviously the overload of mitochondria with Ca^{2+} finally caused PTP, which was accompanied by the release of Ca^{2+} from mitochondria into the extramitochondrial space. As shown in Fig. 4B, 5 μM CAT diminished the mitochondrial ability to accumulate Ca^{2+}, since pore opening occurred already after addition of 30 μM Ca^{2+}. 2 mM ADP (Fig. 4C), however, strongly increased Ca^{2+} accumulation and the release of Ca^{2+} was beginning not before the addition of 1.35 mM Ca^{2+}. Also the addition of 2 mM ADP to mitochondria, which are already in the state of PT induced by 5 μM CAT and 1 mM Ca^{2+}, caused a strong decrease of the swelling rate (Fig. 4D). This fast decrease of the Ca^{2+} release by 2 mM ADP in the presence of 5 μM CAT indicates again that extramitochondrial ADP increased mitochondrial capacity to keep extra amounts of Ca^{2+} (Fig. 4D).

Thus, it was established that ADP stabilized the mitochondrial inner membrane by preventing PT even under conditions where CAT inhibits ANT, which means under condition where ADP cannot enter the matrix space.

3.3. Ca^{2+}-induced mitochondrial respiration in the presence of ADP and ANT inhibitors

Finally, we investigated the influence of ADP on the Ca^{2+}-induced respiration of mitochondria in the presence of CAT or BA. Fig. 5A shows that addition of 50 μM of Ca^{2++} to respiring mitochondria in the presence of 5 μM CAT maximally increased mitochondrial respiration. Fig. 5B demonstrates that 5 μM CAT suppressed ADP-induced respiration but increased mitochondrial capacity to accumulate Ca^{2+}. Only after addition of 1 mM Ca^{2+} maximal respiration was observed again (Fig. 5B). Similar results were obtained in the next experiment, but here 2 mM ADP was added after 5 μM CAT. Under the conditions of this experiment, ANT was completely inhibited by CAT, because mitochondrial respiration did not increase after addition of 2 mM ADP. That means ADP was able to influence the mitochondria only from the outer side of mitochondrial outer membrane, but it increased the Ca^{2+} accumulation capacity (Fig. 5C). Moreover, addition of 2 mM ADP decreased the speed of CAT- and Ca^{2+}-induced respiration (Fig. 5D).

Fig. 6 shows the influence of ADP on Ca^{2+}-induced respiration of BA inhibited mitochondria. We obtained

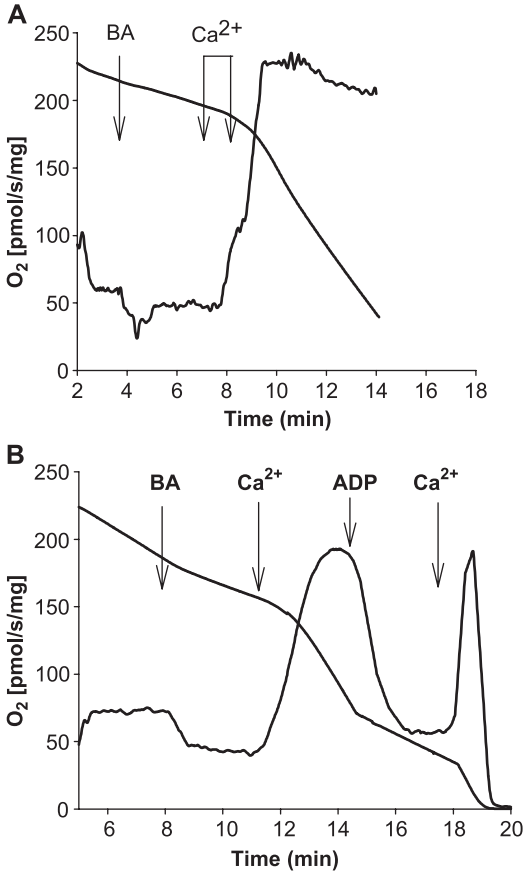

Fig. 6. ADP-dependent respiration at BA inhibited ANT inhibition in the presence of Ca^{2+} in mouse liver mitochondria. A—5 μM BA, two times 50 μM Ca^{++}; B—5 μM BA, 100 μM Ca^{2+}, 2 mM ADP, 1 mM Ca^{2+}. Mitochondria (1 mg/ml) were incubated in incubation medium A. The results of one of the five analogous experiments are presented.

similar results as in the presence of CAT. Addition of 100 μM Ca^{2+} after 5 μM BA maximally stimulated mouse liver mitochondria (Fig. 6A). Kinetic of this respiration was changed by addition of 2 mM ADP (Fig. 6B). In this case mitochondrial capacity to accumulate Ca^{2+} again was increased as it was shown above for CAT. Thus, ADP is able to stabilize mitochondrial membrane independently from the kind of inhibition of the ANT.

4. Discussion

Matrix ADP is an important modulator of the PTP and acts by decreasing the sensitivity of the calcium trigger side to Ca^{2+} [4,7,10]. With shrinking experiments, Halestrap et al. [4] detected that ADP inhibits PT in de-energized liver mitochondria through two binding sites with different affinities (K_i=1 and 25 μM ADP). CAT abolishes the high affinity site.

In the present study, we investigated the ADP-effect on energized mitochondria. Several lines of evidences demonstrate that extramitochondrial ADP is able to regulate the Ca^{2+}-dependent PTP even under conditions were its transport into mitochondria is inhibited. Since all experiments in this paper were performed in Mg^{2+}-free media, this effect was caused by Mg^{2+}-free ADP. Neither ATP nor AMP did act in this manner (data not shown) indicating that this is a specific ADP effect. About 2 mM extramitochondrial ADP reversibly diminished Ca^{2+}-dependent high amplitude swelling under conditions of uninhibited and inhibited ANT (Figs. 1 and 2). This decrease was not due to a total binding of free Ca^{2+} by ADP (Fig. 3). As it can be seen, in Fig. 4A and C, the Ca^{2+}-signals after addition of 150 μM Ca^{2+} in the presence of 2 mM ADP are clearly higher than the signals of 15 μM additions. Moreover, since the Ca^{2+} complexation by ADP is reversible, the mitochondrial accumulation of Ca^{2+} will continuously reduce the amount of complexed Ca^{2+} allowing so the mitochondrial accumulation of all added Ca^{2+}.

Direct measurements of extramitochondrial Ca^{2+} with Calcium Green-5N revealed that release of Ca^{2+} from loaded mitochondria could be immediately suppressed by addition of 2 mM ADP. ADP clearly increases the mitochondrial capacity to accumulate Ca^{2+} (Figs. 4 and 5). Finally, it was shown that ADP decreases Ca^{2+}-induced respiration under condition of CAT or BA-inhibited ANT (Figs. 5 and 6).

There are several pathological conditions as ischemia/reperfusion, intoxication of mitochondria, and sepsis where cellular metabolism is energetically depressed [15]. The main characteristics of energetic depression are strongly decreased cytosolic phosphorylation potentials and increasing Ca^{2+} concentrations. Results of our investigation indicate that, probably, for the survival of mitochondria under these extreme conditions, extramitochondrial ADP can play a role as membrane stabilizing factor. We have established that ADP drastically decreases the Ca^{2+}-sensitivity of mitochondrial PTP even if it cannot be transported by the ANT due to its inhibition by CAT or BA (Fig. 1B, trace 4; Figs. 4D and 5D). That means extramitochondrial ADP inhibits the interrelations between the PT and the ANT in its uninhibited state and if both the high (c-state) and the low (m-state) affinity sites of ANT are inhibited (Fig. 2B, trace 5; Fig. 6B).

All data of our investigation indicate that 2 mM extramitochondrial ADP remarkably increases the membrane stabilization of mitochondria even under condition of the interaction of ANT with cyclophilin in the presence of calcium. The affinity of this stabilizing effect is low (K_i=900 μM ADP), more than one order of magnitude higher than the high affinity site of 25 μM as described by Halestrap et al. [4]. This supports the conclusion that the binding site detected in this study is different from the CAT binding site [4]. The nature of this low affinity binding site for ADP is unknown. It is localized at the outer side of inner membrane and must be CAT-independent. It cannot be ruled out that ADP binds to the ANT outside of the CAT binding side but the binding to other inner membrane proteins cannot be disregarded as well. Recently it was demonstrated [16] for brain mitochondria that low molecular mass polypeptides in phosphorylated state could inhibit the PTP. It could be that high cytosolic ADP can alter the activity of these mitochondrial membrane peptides. Localized at the outer side of inner membrane, they could act as a low affinity sensor for ADP. Via this sensor, high extramitochondrial ADP could decrease the activity of PTP and diminish the Ca^{2+}-efflux from mitochondria. This could be a mechanism to stabilize mitochondrial function under some pathological condition such as ischemia/reperfusion or hypoxia.

Acknowledgements

This work was supported by DAAD and by DFG (GE 664/11, and SFB 598 Project B1). We thank Paolo Bernardi for stimulating discussions.

References

[1] T. Gropp, N. Brustovetsky, M. Klingenberg, V. Müller, K. Fendler, E. Bamberg, Kinetics of electrogenic transport by the ADP/ATP carrier, Biophys. J. 77 (1999) 714–726.
[2] M. Crompton, The mitochondrial permeability transition pore and its role in cell death, Biochem. J. 341 (1999) 233–249.
[3] A.P. Halestrap, A.M. Davidson, Inhibition of Ca^{2+}-induced large amplitude swelling of liver and heart mitochondria by cyclosporin A is probably caused by the inhibitor binding to mitochondrial matrix peptidylprolyl *cis–trans* isomerase and preventing it interacting with the adenine nucleotide translocase, Biochem. J. 268 (1990) 153–160.
[4] A.P. Halestrap, K.Y. Woodfield, C.P. Connern, Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase, J. Biol. Chem. 272 (1997) 3346–3354.
[5] A.P. Halestrap, G.P. McStay, S.J. Clarke, The permeability transition pore complex: another view, Biochimie 84 (2002) 153–166.

[6] S.J. Clarke, P. MsStay, A.P. Halestrap, Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A, J. Biol. Chem. 277 (2002) 34793–34799.
[7] S.A. Novgorodov, T.I. Gudz, Y.M. Milgrom, G.P. Brierley, The permeability transition in hart mitochondria is regulated synergistically by ADP and cyclosporin A, J. Biol. Chem. 267 (1992) 16274–16282.
[8] M.R. Duchen, O. McGuinnes, L. Brown, M. Crompton, On the involvement of a cyclosporin A sensitive mitochondrial pore in myocardial reperfusion injury, Cardiovasc. Res. 27 (1993) 1790–1794.
[9] J.E. Kokoszka, K.G. Waymire, S.E. Levy, J.E. Sligh, J. Cai, D.P. Jones, G.R. Macgregor, D.C. Wallace, The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore, Nature 427 (2004) 461–465.
[10] D.R. Hunter, R.A. Haworth, The Ca^{2+}-induced membrane transition in mitochondria. 1. The protective mechanisms, Arch. Biochem. Biophys. 195 (1979) 453–459.
[11] W. Schneider, Isolation of mitochondria from rat liver, J. Biol. Chem. 176 (1948) 250–254.
[12] K.J. Wiechelman, R.D. Braun, J.D. Fitzpatrick, Investigation of the bicinchoninic acid protein assay: identification of the group responsible for color formation, Anal. Biochem. 175 (1988) 231–237.
[13] T. Haller, M. Ortner, E. Gnaiger, A respirometer for investigating oxidative cell metabolism: toward optimization of respiratory studies, Anal. Biochem. 218 (1994) 338–342.
[14] L. Walter, V. Nogueira, X. Leverve, M.-P. Heitz, P. Bernardi, Three Classes of ubiquinone analogs regulate the mitochondrial permeability transition pore through a common site, J. Biol. Chem. 275 (2000) 29521–29527.
[15] F.N. Gellerich, S. Trumbeckaite, T. Müller, Y. Chen, M. Deschauer, Z. Gizatullina, S. Zierz, Energetic depression caused by mitochondrial dysfunction, Mol. Cell. Biochem. 265 (2003) 391–405.
[16] T. Azarashvili, O. Krestinina, I. Odinokova, Yu. Evtodienko, G. Reiser, Physiological Ca^{2+} level and Ca^{2+}-induced Permeability transition pore control protein phosphorylation in rat brain mitochondria, Cell Calcium 34 (2002) 253–259.