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Interaction of peroxidized cardiolipin with rat-heart mitochondrial membranes: Induction of permeability transition and cytochrome *c* release

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Abstract **Cardiolipin peroxidation plays a critical role in mitochondrial cytochrome *c* release and subsequent apoptotic process. Mitochondrial pore transition (MPT) is considered as an important step in this process. In this work, the effect of peroxidized cardiolipin on MPT induction and cytochrome *c* release in rat heart mitochondria was investigated. Treatment of mitochondria with micromolar concentrations of cardiolipin hydroperoxide (CLOOH) resulted in a dose-dependent matrix swelling, $\Delta\Psi$ collapse, release of preaccumulated Ca^{2+} and release of cytochrome *c*. All these events were inhibited by cyclosporin A and bongkrekic acid, indicating that peroxidized cardiolipin behaves as an inducer of MPT. Ca^{2+} accumulation by mitochondria was required for this effect. ANT (ADP/ATP translocator) appears to be involved in the CLOOH-dependent MPT induction, as suggested by the modulation by ligands and inhibitors of adenine nucleotide translocator (ANT). Together, these results indicate that peroxidized cardiolipin lowers the threshold of Ca^{2+} for MPT induction and cytochrome *c* release. This synergistic effect of Ca^{2+} and peroxidized cardiolipin on MPT induction and cytochrome *c* release in mitochondria, might be important in regulating the initial phase of apoptosis and also may have important implications in those physiopathological situations, characterized by both Ca^{2+} and peroxidized cardiolipin accumulation in mitochondria, such as aging, ischemia/reperfusion and other degenerative diseases.**

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Keywords: Peroxidized cardiolipin; MPT; Cytochrome *c* release; Rat heart mitochondria

1. Introduction

Mitochondria play a central role in the apoptosis induction. Release of cytochrome *c* from mitochondria is considered a critical, early event in the induction of apoptosis cascade that ultimately leads to programmed cell death [1,2]. Calcium is considered as an important trigger for the release of cytochrome *c* from mitochondria [3,4]. The currently recognized mechanism for Ca^{2+}-induced cytochrome *c* release from mitochondria involves opening of the permeability transition pore [5,6]. Pore transition results in a dramatic drop in the mitochondrial transmembrane electrical potential, osmotic swelling of the mitochondrial matrix, rupture of the outer mitochondrial membrane and release of intermembrane proapoptotic proteins, such as cytochrome *c*, apoptosis inducing factor (AIF) and Smac-DIABLO. The MPT is thought to occur after the opening of a putative channel complex, located at the mitochondrial site and consisting of modified and assembled inner and outer membrane components, including the ADP/ATP translocator, cyclophillin D, the voltage dependent anion channel (VDAC) and hexokinase [5,6]. Recently, doubt has been cast on the role of adenine nucleotide translocator (ANT) as an essential component of MPT, because mitochondria isolated from livers of ANT-knock-out mice appear capable of undergoing MPT [7]. However, these data have been questioned by Halestrap [8].

Cardiolipin, a phospholipid localized almost exclusively within the inner mitochondrial membrane, is emerging as an important factor in the regulation of many mitochondrial bioenergetic processes, including electron transport, inner membrane supermolecular assembly, anion transport, efficient ATP synthesis, binding of cytochrome *c* and the function of multiple other mitochondrial inner membrane enzymes (for review see [9]). Cardiolipin is also emerging as an important player in the control of the mitochondrial phase of apoptosis [10–14].

MPT appears to be accompanied by a burst of ROS and this phenomenon is proposed to be involved in the autoamplification phase of MPT. ROS production can cause oxidative damage of cardiolipin changing its physical and functional properties. In fact, either because of its content of unsaturated fatty acids (around 90% represented by linoleic acid) or because of its location in the inner mitochondrial membrane, near to the sites of ROS production (mainly complexes I and III of the respiratory chain), cardiolipin molecules are particularly susceptible to peroxidative attack by oxyradicals [15]. Oxidative damage to cardiolipin would negatively impact the biochemical function of the mitochondrial membranes, altering membrane fluidity, ion permeability, surface charge, passive electric properties, membranous enzyme activity in

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Abbreviations: ANT, adenine nucleotide translocator; Atr, atractyloside; BKA, bongkrekic acid; CSA, cyclosporin A; CLOOH, cardiolipin hydroperoxide; RR, ruthenium red

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various physiopathological conditions [16–18]. Involvement of peroxidized cardiolipin in the mitochondrial permeability transition was recently suggested [19].

The present study was undertaken to provide a more definitive understanding of the role of peroxidized cardiolipin as a possible inducer of MPT and to gain information of its mechanism of action. The results indicate that micromolar concentrations of peroxidized cardiolipin could enhance Ca^{2+}-dependent permeability transition and cytochrome *c* release in rat heart mitochondria. ANT appears to be involved in this processes, as suggested by the modulation exerted by ANT ligands and inhibitors.

2. Materials and methods

2.1. Preparation of mitochondria

Rat heart mitochondria were isolated in an ice cold medium containing 250 mM sucrose, 10 mM Tris–HCl, 1 mM EGTA, pH 7.4, by differential centrifugation of heart homogenates essentially as previously described [17]. Mitochondrial pellet was resuspended in 250 mM sucrose, 10 mM Tris–HCl pH 7.4 and stored in ice.

Mitochondrial protein concentration was measured by the biuret method, using serum albumin as standard.

2.2. Standard incubation procedure

Mitochondria were incubated at 25 °C in a standard reaction medium containing 150 mM sucrose, 50 mM KCl, 5 mM Tris (pH 7.4), 10 μM EGTA, 1 mM Pi, 2 mM pyruvate and 5 mM malate, with continuous stirring. CL and CLOOH were added to mitochondria dissolved in methanol. Other additions are indicated in the figure legends.

2.3. Measurement of Ca^{2+} flux

Changes in the concentration of free extramitochondrial Ca^{2+}, due to movement of the ion across the inner mitochondrial membrane, were followed spectrophotometrically at 675–685 nm in the presence of 50 μM Arsenazo III [20], as a free Ca^{2+} indicator, with an HP 8453 diode array spectrophotometer.

2.4. Measurement of mitochondrial membrane potential

The membrane potential of intact heart mitochondria was measured following the safranine O fluorescence quenching at 525 nm (excitation), 575 nm (emission) with a Jasco FP-750 spectrofluorometer. Freshly isolated mitochondria (0.5 mg protein) were suspended in 3 ml of the standard reaction medium in the presence of 8 μM safranine O and the formation of membrane potential was induced by the addition of 2 mM pyruvate and 5 mM malate as substrates.

2.5. Mitochondrial swelling

Changes in absorbance of rat heart mitochondria were monitored spectrophotometrically at 540 nm with an HP 8453 diode array spectrophotometer.

2.6. Detection of cytochrome c release

Cytochrome *c* content in the supernatant was determined by using a 5 mm C4 reverse-phase column (150 × 4.6 mm) on an HP series 1100 HPLC chromatograph. A gradient of 20% acetonitrile in water with trifluoroacetic acid (0.1% vol:vol) to 60% acetonitrile in water with trifluoroacetic acid (0.1% vol:vol) over 12 min with a flow rate of 1 mL/min was used. Absorption at 393 nm was used. To improve the sensitivity of the method, the supernatants were supplemented with bovine serum albumin (BSA) to a final concentration of 25 μM [21]. The amount of cytochrome *c* was quantitated by the peak area derived from integrating the chromatographic peak.

2.7. Preparation of peroxidized cardiolipin

Cardiolipin oxidation was carried essentially as reported by Parinandi [22]. Bovine heart cardiolipin (25 mg) was suspended in 5 ml of buffered saline medium (0.15 M NaCl, pH 7.4) with vigorous vortexing. The milky suspension was sonicated with a Branson sonifier (mod. 250) for 5 min. The resultant vesicular preparation was flushed for 2 min with 100% oxygen gas, tightly sealed and kept at 37 °C for 20 h. Cardiolipin was extracted, dissolved in methanol and phosphorus content was estimated as described previously [23].

2.8. Analysis of peroxidized cardiolipin

Peroxidized cardiolipin was analyzed by the HPLC method [17] and monitored at 206 and 233 with a diode array detector. Non-oxidized cardiolipin was used as internal standard. Normal non-oxidixed cardiolipin showed a single specific absorbance peak at 206 nm, without absorbance at 233, while peroxidized cardiolipin showed a small peak at 206 nm and a large peak a 233 nm, indicating the formation of conjugated dienes. The amount of conjugated dienes was calculated using $\varepsilon 233 = 25.2\ mM^{-1}\ cm^{-1}$ as described by Buege and Aust [24].

2.9. Cardiolipin hydroperoxide (CLOOH) assay

The level of CLOOH was measured by the FOX2 method using the peroxide-mediated oxidation of ferrous ion in the presence of xylenol orange [25]. The FOX2 reagent was composed of 100 μM xylenol orange, 4.4 mM butylated hydroxytoluene, 25 mM sulfuric acid and 250 μM ammonium ferrous sulfate. Peroxidized cardiolipin was solubilized in 100 μl of methanol and incubated with 900 μl of FOX2 reagent for 30 min at room temperature. The amount of CLOOH was calculated using ε_{560} of $4.52 \times 10^4\ M^{-1}$.

3. Results

In the first series of experiments, the response of isolated rat heart mitochondria to peroxidized cardiolipin was examined (Fig. 1A). Addition of micromolar concentrations of CLOOH to heart mitochondrial suspension, respiring with pyruvate + malate and supplemented with 30 μM Ca^{2+}, caused a concentration-dependent change in the apparent absorbance that is indicative of large amplitude swelling (traces b–d). The maximal rate and extent of swelling was obtained at 2 μM CLOOH (trace d). This effect of peroxidized cardiolipin could not be replaced by intact non-oxidized cardiolipin (trace a). No osmotic alteration was observed in the control curve obtained in the presence of 30 μM Ca^{2+} or 2 μM CLOOH alone (not shown).

As observable in Fig. 1B, the CLOOH-dependent swelling was almost completely blocked by cyclosporin A (CSA) (trace c) as well as by BKA (trace b), two typical inhibitors of MPT, indicating that the CLOOH-dependent mitochondrial absorbance changes are indicative of MPT induction. The addition of ruthenium red (RR), an inhibitor of mitochondrial Ca^{2+} uptake, also prevented the CLOOH-induced absorbance changes (trace d), suggesting that Ca^{2+} entry into mitochondria is necessary for the induction of MPT by CLOOH. The addition of dithiothreitol (DTT) had practically no protective effect against CLOOH-induced swelling. This minimizes the possibility that CLOOH-dependent MPT induction resulted from an oxidative reaction at the level of critical thiol groups.

The effect of ANT ligands and inhibitors on the CLOOH-dependent mitochondrial swelling was examined (Fig. 1C). Both ATP (trace c) and ADP (trace b) exerted a strong inhibitory effect on the CLOOH-dependent swelling (trace a). Atractyloside (Atr), an inhibitor of the ANT carrier, at concentration at which, per se, did not affect the permeability of the mitochondrial membrane, stimulated the rate of the CLOOH-dependent swelling (trace d).

To further demonstrate the induction of MPT by peroxidized cardiolipin, experiments were carried out recording the changes in the mitochondrial membrane potential ($\Delta\Psi$)

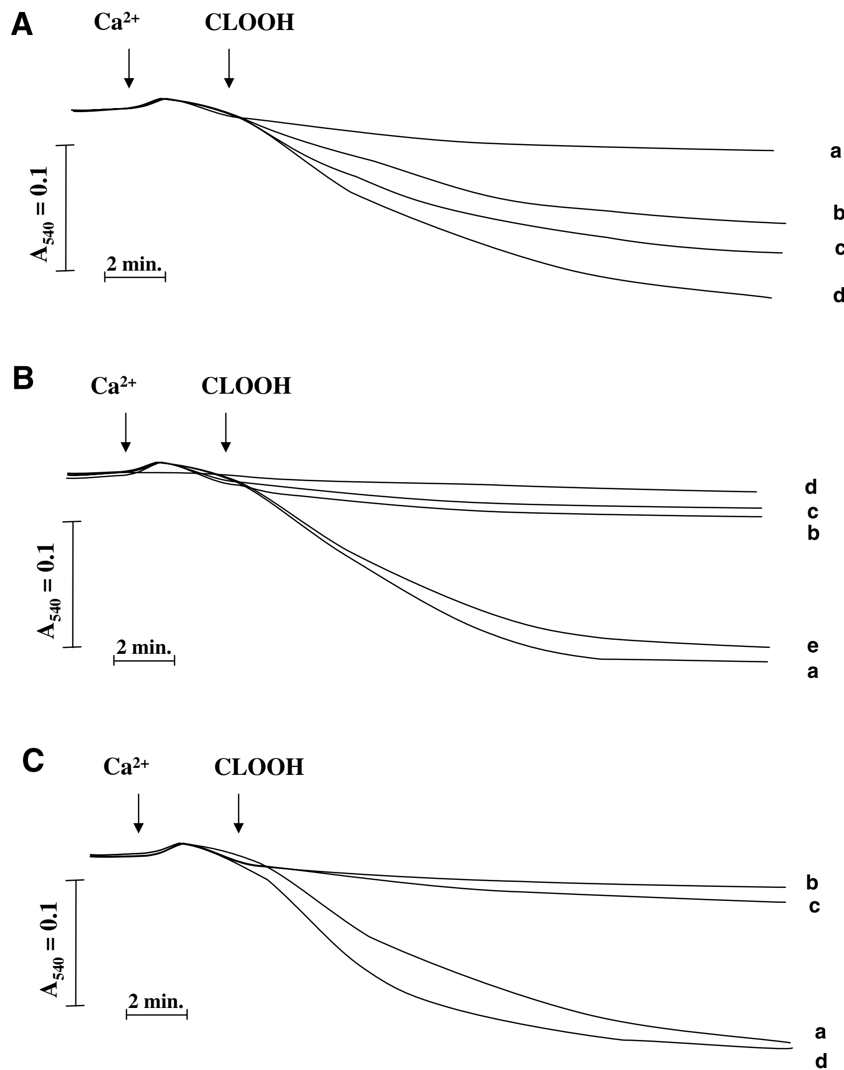

Fig. 1. Mitochondrial swelling induced by peroxidized cardiolipin in rat heart mitochondria. Mitochondria (0.5 mg protein/3 ml) were incubated in the standard reaction medium and supplemented with 30 μM Ca^{2+}. (A) CLOOH, dissolved in methanol, was added at concentrations 0.5, 1, 2 μM (traces b–d, respectively). Non-oxidized cardiolipin, dissolved in methanol, was added at 2 μM (trace a). Changes in mitochondrial volume were monitored at 540 nm. (B) CLOOH was added at concentrations 2 μM in the absence (trace a) and in the presence of CSA 2 μM (trace c) or BKA 5 μM (trace b) or RR 1.5 μM (trace d) or dithiothreitol 1 mM (trace e). (C) CLOOH was added at concentrations 2 μM in the absence (trace a) and in the presence of ADP 200 μM (trace b) or ATP 200 μM (trace c). Atr 30 μM was added 1 min before CLOOH (trace d). The traces shown are typical of at least five similar experiments.

(Fig. 2A). Mitochondrial $\Delta\Psi$ was generated by the addition of pyruvate + malate as substrates. The addition of 30 μM Ca^{2+} to mitochondria (trace a) resulted in a transient reduction in $\Delta\Psi$, as Ca^{2+} uptake occurs via an electrophoretic uniporter that draws upon the $\Delta\Psi$ for active Ca^{2+} accumulation. Approximately 3 min after the addition of Ca^{2+}, $\Delta\Psi$ recovered back to its control level and remained stable for at least 10 min. The addition of CLOOH, 3 min after that of Ca^{2+}, caused a decrease in membrane potential (trace b), while the addition of equimolar concentration of non-oxidized cardiolipin had practically no effect (trace e). When CLOOH was added to mitochondria in the absence of Ca^{2+}, no decrease in membrane potential was observed (trace c). The mitochondrial depolarization induced by CLOOH + Ca^{2+} was prevented by the addition of CSA (trace f) or BKA (trace d).

By using the Ca^{2+} indicator arsenazo III, we tested the ability of heart mitochondria to accumulate and retain Ca^{2+} in the presence or in the absence of peroxidized cardiolipin (Fig. 2B). Heart mitochondria, energized with pyruvate + malate and incubated in the presence of 30 μM Ca^{2+}, were able to accumulate and retain for at least 10 min almost all the $Ca^{2+}$of the medium (trace a). The addition of CLOOH, 3 min after Ca^{2+} addition, caused the release of the accumulated Ca^{2+} (trace b). Non-oxidized cardiolipin could not replace peroxidized cardiolipin in this effect (trace c). Inclusion of CSA (trace d) or BKA (trace e) in the incubation medium, protected mitochondria against the CLOOH-induced decrease in mitochondrial Ca^{2+} accumulation.

As an additional index of MPT opening by peroxidized cardiolipin, the release of cytochrome *c* from heart mitochondria supplemented with oxidizable substrates pyruvate + malate, was measured. For direct evaluation of cytochrome *c* release from heart mitochondria, extramitochondrial cytochrome *c* was analyzed by a new, very rapid and sensitive method based

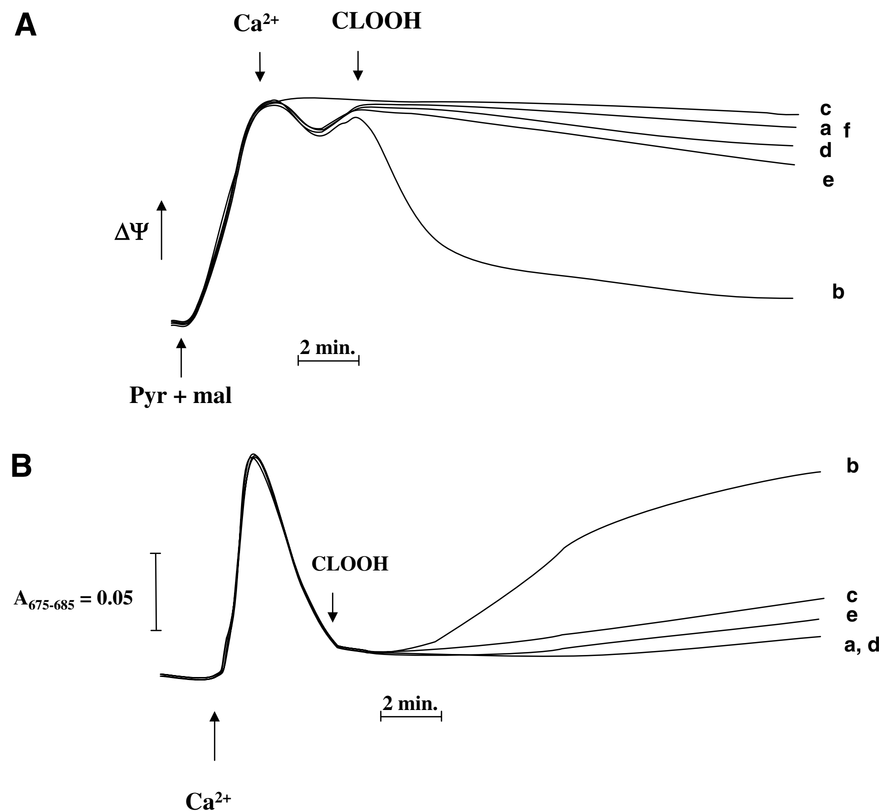

Fig. 2. Changes in mitochondrial membrane potential (A) and in extramitochondrial Ca^{2+} concentrations (B) induced by CLOOH. (A) Mitochondria (0.5 mg protein/3 ml) were incubated in the standard reaction medium and supplemented with 30 μM Ca^{2+} (trace a). CLOOH was added at concentrations 2 μM in the absence (trace b) and in the presence of CSA 2 μM (trace f) or BKA 5 μM (trace d). Non-oxidized cardiolipin 2 μM in the presence of Ca^{2+} 30 μM (trace e). CLOOH in the absence of Ca^{2+} (trace c). (B) Mitochondria were incubated as described in (A) in the presence of 50 μM of arsenazo III and supplemented with 30 μM Ca^{2+} (trace a). CLOOH was added at concentrations 2 μM in absence (trace b) and in the presence of CSA 2 μM (trace d) or BKA 5 μM (trace e). Non-oxidized cardiolipin 2 μM in the presence of Ca^{2+} 30 μM (trace c).

on reverse phase HPLC [21,26]. As shown in Fig. 3, the addition of 30 μM Ca^{2+} or 2 μM CLOOH alone, did not induce significant cytochrome *c* release compared to control samples. However, when Ca^{2+} and CLOOH were added together, the amount of cytochrome *c* released from mitochondria was significantly higher than that of the control sample. Similar release of cytochrome *c* was not observed in the presence of non-oxidized CL and Ca^{2+} (not shown). The Ca^{2+}/CLOOH-dependent release of cytochrome *c* from mitochondria was completely abolished by CSA, an inhibitor of MPT, as well as by RR, which prevents Ca^{2+} influx into the mitochondrial matrix by inhibiting Ca^{2+} uniporter.

In Fig. 4, the results of the effect of ANT ligands and inhibitors on Ca^{2+}/CLOOH-induced release of cytochrome *c* in rat heart mitochondria, are reported. ADP, ATP and BKA almost completely inhibited this process, while Atr had no effect.

4. Discussion

An important mediator in the apoptotic cascade is the release of cytochrome *c* from the mitochondrion, leading to the assembly of the apoptosome and activation of caspase. Cytochrome *c* release is regulated by the Bcl2 family proteins, and one possible target of these proteins in the cell is the mitochondrial permeability transition pore. The molecular nature of this MPT and the precise mechanism by which it is linked to cytochrome *c* release is still a matter of debate.

Cardiolipin is a significant player in the apoptotic cell death program [27] and one of the major factor in the t-Bid-induced destabilization of mitochondrial bioenergetics [28]. Cardiolipin is believed to significantly contribute to the outer membrane permeabilization and cytochrome *c* release during apoptosis. A growing body of evidences indicate that oxidized cardiolipin rather than non-oxidized cardiolipin is the real player in mitochondrial cytochrome *c* release [11,13,14,19]. The mechanism by which oxidized cardiolipin may trigger cytochrome *c* release from mitochondria and subsequent apoptotic process is still not well understood.

The results presented in this study demonstrate that treatment of rat heart mitochondria with micromolar concentrations of peroxidized cardiolipin results in a concentration-dependent matrix swelling, $\Delta\Psi$ collapse, release of preaccumulated Ca^{2+} and release of mitochondrial cytochrome *c*. These effects were not observed with non-oxidized cardiolipin. All these events were inhibited by the endecapeptide immunosuppressant CSA, a ligand of mitochondrial cyclophillin and BKA, an inhibitor of the ADP/ATP carrier, both typical inhibitors of MPT induction. Together these results indicate that peroxidized cardiolipin behaves as an inducer of MPT.

Peroxidized cardiolipin alone did not induce pore opening, nor cytochrome *c* release from mitochondria. Ca^{2+} accumula-

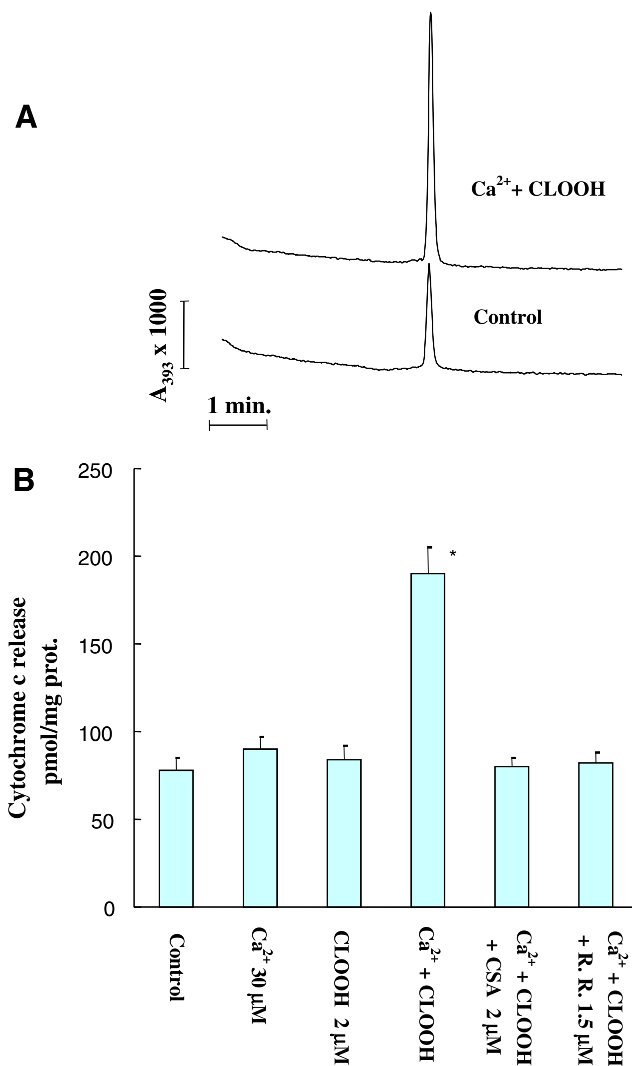

Fig. 3. CLOOH-induced release of cytochrome *c* from rat heart mitochondria. Mitochondria (0.5 mg protein/3 ml) were incubated in the standard reaction medium. Twenty minutes later, mitochondria were centrifuged and supernatant was withdrawn, filtered and then injected into the HPLC to determine the cytochrome *c* content as described in Section 2. (A) Representative HPLC traces for cytochrome *c* release from control and 30 µM Ca^{2+} + 2 µM CLOOH treated mitochondria. (B) Quantitation of cytochrome *c* released from control and treated mitochondria. All values are expressed as means ± S.E. of five separated experiments. $^{*}P < 0.01$ vs. control.

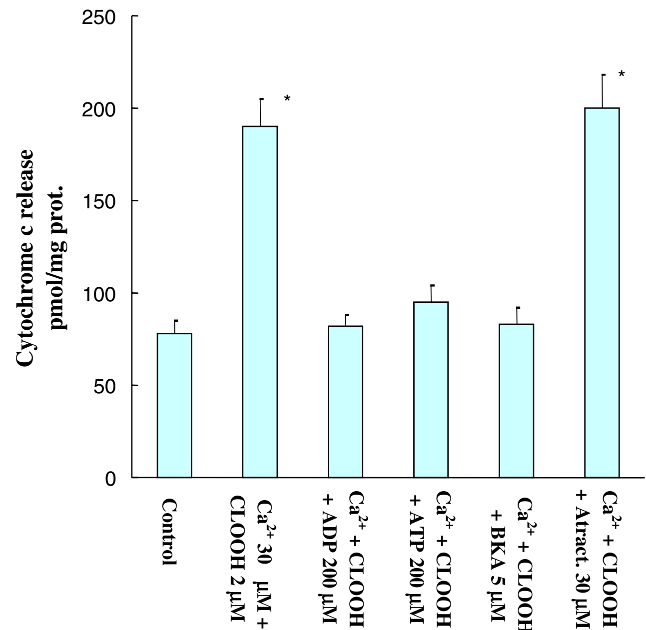

Fig. 4. Effect of ANT ligands and inhibitors on the CLOOH-induced release of cytochrome *c* from rat heart mitochondria. Experimental condition are similar to those reported in Fig. 3. All values are expressed as means ± S.E. of five separated experiments. $^{*}P < 0.01$ vs. control.

tion by mitochondria was required for both these processes to occur, as indicated by the inhibitory effect exerted by RR, an inhibitor of the Ca^{2+} uniporter. It should be noted that the level of Ca^{2+} used in these experiments did not, per se, induce pore opening. Thus, it is conceivable that peroxidized cardiolipin lowers the threshold of Ca^{2+} for inducing MPT and/or potentiates the effect of this cation in MPT opening. Interestingly, the induction of pore opening in a similar manner, has also been reported for other reactive lipid peroxidation products, including 4-hydroxynonenal [29] and 15-deoxy-$\Delta^{12\text{-}14}$-prostaglandin J_2 [30] and is another example of the "two hit" hypothesis leading to pore opening [31].

The synergistic effect of Ca^{2+} and peroxidized cardiolipin on the MPT induction, suggests that both these compounds play a coordinated role in this process, by interacting with components of MPT. The fact that the effect of peroxidized cardiolipin on the MPT induction is modulated by ligands and inhibitors of ADP carrier, points to an involvement of this protein system in this process. Cardiolipins were shown to be tightly associated with ANT with a stoichiometry of 3 cardiolipins per protein monomer [32]. It was suggested that interactions between two monomers of the ANT is mediated by cardiolipins, which could stabilize the dimer and structure, by controlling the conformational changes and participate in triggering a concerted ADP/ATP exchange [33]. Oxidized cardiolipin, by interacting with ANT, would destabilize the appropriate conformation of this protein, favouring a conformation of ANT for opening of MPT pore. Bivalent cation calcium could be involved in this transition. Indeed, it was proposed by Brustovetsky and Klingemberg that the channel opening in ANT is caused by binding of Ca^{2+} to the cardiolipin tightly bound to ANT [34].

Besides inducing MPT, oxidized cardiolipin, in the presence of Ca^{2+}, promotes also cytochrome release from mitochondria. It is well established that cardiolipin molecules bind cytochrome *c* to the outer surface of the inner mitochondrial membrane [35]. Oxidized cardiolipin would induce cytochrome *c* release from mitochondria, by disturbing the interaction of this hemoprotein with non-oxidized cardiolipin, thus promoting cytochrome *c* detachment from mitochondrial inner membrane. Our results are compatible with the two-steps model for cytochrome *c* release from mitochondria, whereby cardiolipin oxidation is required for both cytochrome *c* detachment from the inner mitochondrial membrane and for MPT opening, followed by the release of cytochrome *c* [13,15].

The synergistic effect of Ca^{2+} and oxidized cardiolipin in the induction of MPT and in the release of cytochrome *c* from mitochondria, might be important in regulating, together with

other factors, the initial phase of apoptosis and also may have implications in those physiological situations, characterized by Ca^{2+} influx and accumulation of oxidized cardiolipin in mitochondria, such as aging, ischemia and reperfusion and other degenerative diseases.

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References

[1] Liu, X., Kim, C.N., Yang, J. and Jemmerson, R. (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. Cell 86, 147–157.
[2] Kroemer, G. and Reed, J.C. (2000) Mitochondrial control of cell death. Nat. Med. 6, 513–519.
[3] Hajnoczky, G., Davies, E. and Madesh, M. (2003) Calcium signaling and apoptosis. Bioch. Biophys. Res. Commun. 304, 445–454.
[4] Szabadkai, G. and Rizzuto, R. (2004) Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighborhood? FEBS Lett. 567, 111–115.
[5] Bernardi, P. (1999) Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol. Rev. 79, 1127–1155.
[6] Halestrap, A.P., Doran, E., Gillespie, J.P. and O'Toole, A. (2000) Mitochondria and cell death. Biochem. Soc. Trans. 28, 170–177.
[7] Kokoszka, J.E., Waymire, K.G., Levy, S.E., Sligh, J.E., Cai, J., Jones, D.P., MacGregor, G.R. and Wallace, D.C. (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 427, 461–465.
[8] Halestrap, A.P. (2004) Mitochondrial permeability: dual role for ADP/ATP translocator? Nature 430, 1.
[9] Schlame, M., Rua, D. and Greenberg, M.L. (2000) The biosynthesis and functional role of cardiolipin. Prog. Lipid. Res. 39, 257–288.
[10] Lutter, M., Fang, M., Luo, X., Nishijima, M., Xie, X. and Wang, X. (2000) Cardiolipin provides specificity for targeting of t-Bid to mitochondria. Nat. Cell Biol. 2, 754–761.
[11] Petrosillo, G., Ruggiero, F.M., Pistolese, M. and Paradies, G. (2001) Reactive oxygen species generated from the mitochondrial electron transport chain induce cytochrome *c* dissociation from beef-heart submitochondrial particles via cardiolipin peroxidation. Possible role in the apoptosis. FEBS Lett. 509, 435–438.
[12] Ostrander, D.B., Sparagna, G.C., Amoscato, A., McMillin, J.B. and Dowhan, W. (2001) Decreased cardiolipin synthesis corresponds with cytochrome *c* release in palmitate-induced cardiomyocyte apoptosis. J. Biol. Chem. 276, 38061–38067.
[13] Iverson, S.L. and Orrenius, S. (2004) The cardiolipin-cytochrome *c* interaction and the mitochondrial regulation of apoptosis. Arch. Biochem. Biophys. 423, 37–46.
[14] Kagan, V.E., Tyurin, V.A., Jiang, J., Tyurina, Y.Y., Ritov, V.B., Amoscato, A.A., Osipov, A.N., Belikova, N.A., Kapralov, A.A., Kini, V., Vlasova, I.I., Zhao, Q., Zou, M., Di, P., Svistunenko, D.A., Kurnikov, I.V. and Borisenko, G.G. (2005) Cytochrome *c* acts as a cardiolipin oxygenase required for release of proapoptotic factors. Nat. Chem. Biol. 4, 223–232.
[15] Petrosillo, G., Ruggiero, F.M. and Paradies, G. (2003) Role of reactive oxygen species and cardiolipin in the release of cytochrome *c* from mitochondria. The FASEB J. 17 (15), 2202–2208.
[16] Paradies, G., Petrosillo, G., Pistolese, M., Di Venosa, N., Serena, D. and Ruggiero, F.M. (1999) Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free. Radical Biol. Med. 27, 42–50.
[17] Paradies, G., Petrosillo, G., Pistolese, M., Di Venosa, N., Federici, A. and Ruggiero, F.M. (2004) Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: involvement of reactive oxygen species and cardiolipin. Circ. Res. 94, 53–59.
[18] Petrosillo, G., Ruggiero, F.M., Di Venosa, N. and Paradies, G. (2003) Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin. FASEB 17 (6), 714–716.
[19] Imai, H., Koumura, T., Nakajima, R., Nomura, K. and Nakagawa, Y. (2003) Protection from inactivation of the adenine nucleotide translocator during hypoglycaemia-induced apoptosis by mitochondrial phospholipid hydroperoxide glutathione peroxidase. Biochem. J. 371, 799–809.
[20] Scarpa, A. (1979) Measurements of cation transport with metallochromic indicators. Meth. Enzymol. 56, 301–352.
[21] Crouser, E.D., Gadd, M.E., Julian, M.W., Huff, J.E., Broekemeier, K.M., Robbins, K.A. and Pfeiffer, D.R. (2003) Quantitation of cytochrome *c* release from rat liver mitochondria. Anal. Biochem. 317, 67–75.
[22] Parinandi, N.L., Weis, B.K. and Schmid, H.H. (1988) Assay of cardiolipin peroxidation by high-performance liquid chromatography. Chem. Phys. Lipids 49, 215–220.
[23] Ruggiero, F.M., Landriscina, C., Gnoni, G.V. and Quagliariello, E. (1984) Lipid composition of liver mitochondria and microsomes in hyperthyroid rats. Lipids 19, 171–178.
[24] Buege, J.A. and Aust, S.D. (1978) Microsomal lipid peroxidation. Meth. Enzymol. 52, 302–310.
[25] Jiang, Z.Y., Hunt, J.V. and Wolff, S.P. (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem. 202, 384–389.
[26] Petrosillo, G., Ruggiero, F.M., Pistolese, M. and Paradies, G. (2004) Ca^{2+}-induced reactive oxygen species production promotes cytochrome *c* release from rat liver mitochondria via mitochondrial permeability transition (MPT)-dependent and MPT-independent mechanisms: role of cardiolipin. J. Biol. Chem. 279, 53103–53108.
[27] McMillin, J.B. and Dowhan, W. (2002) Cardiolipin and apoptosis. Biochim. Biophys. Acta 158, 97–107.
[28] Gonzalvez, F., Pariselli, F., Dupaigne, P., Budihardjo, I., Lutter, M., Antonsson, B., Diolez, P., Manon, S., Martinou, J.C., Goubern, M., Wang, X., Bernard, S. and Petit, P.X. (2005) tBid interaction with cardiolipin primarily orchestrates mitochondrial dysfunctions and subsequently activates Bax and Bak. Cell Death Differ. 6, 614–626.
[29] Kristal, B.S., Park, B.K. and Yu, B.P. (1996) 4-Hydroxyhexenal is a potent inducer of the mitochondrial permeability transition. J. Biol. Chem. 271, 6033–6038.
[30] Landar, A., Shiva, S., Levonen, A.L., Oh, J.Y., Zaragoza, C., Johnson, M.S. and Darley-Usmar, V.M. (2006) Induction of the permeability transition and cytochrome *c* release by 15-deoxy-Delta12,14-prostaglandin J_2 in mitochondria. Biochem. J. 394, 185–195.
[31] Brookes, P.S., Yoon, Y., Robotham, J.L., Anders, M.W. and Sheu, S.S. (2004) Calcium, ATP, and ROS: a mitochondrial love–hate triangle. Am. J. Physiol. Cell Physiol. 287, C817–C833.
[32] Beyer, K. and Klingemberg, M. (1985) ADP/ATP carrier protein from beef heart mitochondria has high amounts of tightly bound cardiolipin, as revealed by 31P nuclear magnetic resonance. Biochemistry 24, 3821–3826.
[33] Nury, H., Dahout-Gonzalez, C., Trezeguet, V., Lauquin, G., Brandolin, G. and Pebay-Peyroula, E. (2005) Structural basis for lipid-mediated interactions between mitochondrial ADP/ATP carrier monomers. FEBS Lett. 579, 6031–6036.
[34] Brustovetsky, N. and Klingemberg, M. (1996) Mitochondrial ADP/ATP carrier can be converted into a large channel by Ca^{2+}. Biochemistry 35, 8483–8488.
[35] Tuominen, E.K., Wallace, C.J. and Kinnunen, P.K. (2002) Phospholipid–cytochrome *c* interaction: evidence for the extended lipid anchorage. J. Biol. Chem. 277, 8822–8826.