Toxicology in Vitro 29 (2015) 1034–1041

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Tl^+ induces the permeability transition pore in Ca^{2+}-loaded rat liver mitochondria energized by glutamate and malate

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ARTICLE INFO

Article history:
Received 26 April 2014
Accepted 8 April 2015
Available online 21 April 2015

Keywords:
Rat liver mitochondria
Tl^+
Mitochondrial permeability transition
Mitochondrial swelling
Mitochondrial respiration
Mitochondrial complex I

ABSTRACT

It is known that Ca^{2+} and heavy metals more actively induce MPTP opening in mitochondria, energized by the I complex substrates. Thus, a rise in a Tl^+-induced MPTP was proposed in experiments on isolated rat liver mitochondria energized by the complex I substrate (glutamate and malate). Expose of the mitochondria to Ca^{2+} into a medium containing $TlNO_3$, glutamate, and malate as well as sucrose or KNO_3 resulted in a decrease in state 3, state 4, or DNP-stimulated respiration as well as an increase of both mitochondrial swelling and $\Delta\Psi_{mito}$ dissipation. The MPTP inhibitors, CsA and ADP, almost completely eliminated the effect of Ca^{2+}, which was more pronounced in the presence of the complex I substrates than the complex II substrate (succinate) and rotenone (Korotkov and Saris, 2011). The present study concludes that Tl^+-induced MPTP opening is more appreciable in mitochondria energized by glutamate and malate but not succinate in the presence of rotenone. We assume that the Tl^+-induced MPTP opening along with followed swelling and possible structural deformations of the complex I in Ca^{2+}-loaded mitochondria may be a part of the thallium toxicity mechanism on mitochondria in living organisms. At the same time, oxidation of Tl^+ to Tl^{3+} by mitochondrial oxygen reactive species is proposed for the mechanism.

1. Introduction

It is known that the calcium load of mitochondria favors the interaction of Ca^{2+} with high affine sites facing the matrix side and it may stimulate the opening of the mitochondrial permeability transition pore in the IMM (Zoratti and Szabò, 1995; Halestrap and Brenner, 2003; Halestrap, 2009). If the load is not substantial, the MPTP opens in the low conduction state, and consequently the IMM becomes permeable to inorganic cations (K^+, Na^+, H^+ and Ca^{2+}) (Ichas and Mazat, 1998). Some authors have suggested that the largest respiratory chain complex I might be involved in the regulation of the MPTP (Fontaine et al., 1998; Ichas and Mazat, 1998; Fontaine and Bernardi, 1999; Leverve and Fontaine, 2001; Halestrap and Brenner, 2003). The oxidation of PN and NAD(P)H in the matrix, and the production of ROS by the complex I have increased the MPTP opening that has caused Ca^{2+} release, mitochondrial swelling, and collapse of $\Delta\Psi_{mito}$ (Lê-Quôc and Lê-Quôc, 1989; Bindoli et al., 1997; Fontaine and Bernardi, 1999; García et al., 2005; Aldakkak et al., 2013). The opening of MPTP in the presence of acetoacetate, *t*BHP, or NAD(P)H oxidizers was due to ANT conversion in *c* conformation (Lê-Quôc and Lê-Quôc, 1989; Bindoli et al., 1997). A more notable decrease in calcium retention or state 4, state 3, or uncoupled respiration and increase in both swelling and $\Delta\Psi_{mito}$ dissipation were found in experiments with mitochondria energized by glutamate with malate but not succinate in the presence of rotenone (Fontaine et al., 1998; Fontaine and Bernardi, 1999; Leverve and Fontaine, 2001; Belyaeva et al., 2011). However, mild inhibition of the complex I by biguanides (metformin and phenformin) or a semi-synthetic tetracycline derivative (minocycline) decreased visibly MPTP opening in isolated mitochondria and cells, and the MPTP opening was manifested as an increase in Ca^{2+} retention and a decrease in mitochondrial Ca^{2+}-induced mitochondrial swelling (Fontaine and Bernardi, 1999; Gieseler et al., 2009; Li et al., 2012; Drahota et al., 2014). The involvement of the complex I and ROS production in the MPTP opening was shown for Cd^{2+}, Cu^{2+}, and Hg^{2+} (Zoratti and Szabò, 1995; Belyaeva et al., 2004, 2011). Heavy metals (Cd^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, As^{3+}) and selenite showed more visible MPTP opening because the inhibition of state 3 or uncoupled

Abbreviations: $\Delta\Psi_{mito}$, electrochemical potential; ANT, adenine nucleotide translocase; CsA, cyclosporine A; CypD, cyclophilin D; DNP, 2,4-dinitrophenol; EGTA, [ethylenebis(oxyethylene-nitrilo)] tetraacetate acid; IMM, inner mitochondrial membrane; 2-MPG, 2-mercaptopropionyl-glycine; MPTP, mitochondrial permeability transition pore; PEP, phosphoenolpyruvate; P_i, inorganic phosphate; PN, pyridine nucleotides; RLM, rat liver mitochondria; ROS, reactive oxygen species; *t*BHP, *tert*-butylhydroperoxide.

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http://dx.doi.org/10.1016/j.tiv.2015.04.006
0887-2333/

respiration as well as a simultaneous increase in PN oxidation and mitochondrial swelling were more substantial in experiments with these metals and a medium containing the complex I substrate (glutamate and malate) but not succinate and rotenone (Cameron et al., 1986; Skul'skiĭ et al., 1988; Lund et al., 1993; Miccadei and Floridi, 1993; Bates et al., 1994; Zoratti and Szabò, 1995; Brown et al., 2000; Belyaeva et al., 2002, 2011; Belyaeva and Korotkov, 2003; Shilo et al., 2003; Bustamante et al., 2005; Dineley et al., 2005).

Thallium belongs to a group of trace elements and is a toxic metal used in industry and medicine as ^{201}Tl (Mulkey and Oehme, 1993; Hsieh et al., 2012; Korotkov, 2013). Being a K^+ surrogate due to similarity in ion radii, Tl^+ can lightly penetrate plants and mammals and be readily absorbed through the skin and respiratory and digestive systems (Rodríguez-Mercado and Altamirano-Lozano, 2013). The toxicity in human was revealed as colorless, odorless and tasteless Tl^+ containing compounds are widely used as a component in manufacturing of cement, ceramic superconductors, and optical glasses, in coal combustion as well as a homicidal agent, a human depilatory, a rodenticide, and a suicidal poison (Mulkey and Oehme, 1998; Hoffman, 2003; Goel and Aggarwal, 2007; Rodríguez-Mercado and Altamirano-Lozano, 2013). Thallium injures renal, cardiovascular and central nervous systems as well as the gastrointestinal, liver and skin (Mulkey and Oehme, 1998, 2000; Leung and Ooi, 2000; Goel and Aggarwal, 2007; Al Hammouri et al., 2011). Considerable structural damages in mitochondrial and cell membranes were found in experiments on Tl-poisoned rats (Herman and Bensch, 1967; Woods and Fowler, 1986; Leung and Ooi, 2000; Kiliç and Kutlu, 2010). The mechanistic basis of thallium toxicity is explained by its ability to replace K^+ in K^+-dependent biochemical processes, to penetrate easily the inner mitochondrial membrane, to release Ca^{2+} from intracellular compartments, and to change cell cycle regulation (Saris et al., 1981; Mulkey and Oehme, 1993; Zierold, 2000; Korotkov, 2013; Rodríguez-Mercado and Altamirano-Lozano, 2013).

We previously found that the swelling of non-energized RLM has been permanently increased when $TlNO_3$ at 25–75 mM was augmented into the medium containing rotenone as well as KNO_3 or sucrose (Korotkov and Brailovskaya, 2001; Korotkov, 2009). This swelling did not depend on the concentration of Ca^{2+} in the medium (Korotkov and Saris, 2011). If the medium was free of rotenone, the Tl^+-induced swelling of non-energized RLM was influenced by both $TlNO_3$ and Ca^{2+} owing to the participation of endogenous mitochondrial substrates during calcium transport into the matrix (Korotkov and Saris, 2011). The injection of the complex II substrate (succinate) into the medium containing Ca^{2+} and rotenone caused massive swelling of RLM due to the opening of the Tl^+-induced MPTP in the inner membrane of Ca^{2+}-loaded RLM (Korotkov and Saris, 2011). In addition, the opening manifested both $\Delta\Psi_{mito}$ dissipation and the decrease in state 4, state 3, and DNP-stimulated respiration (Korotkov and Saris, 2011). On the other hand, formation of complexes of Tl^+ with soluble matrix proteins in experiments on RLM incubated in a medium containing $TlNO_3$ and KNO_3 was more substantial in the presence of glutamate and malate but not succinate and rotenone (Korotkov et al., 2014). The above mentioned findings on thallous salts impact on cells and mitochondria prompted us to investigate mechanism of Tl^+ toxicity in isolated RLM. However, a possible increase in the Tl^+-induced MPTP opening has not been investigated sufficiently in Ca^{2+}-loaded RLM, energized by the complex I substrates. The use of isolated mitochondria was earlier suggested as a model to evaluate deleterious effects of heavy metals and toxic chemicals on living organisms (Ogata et al., 1983). Here, we studied the Tl^+ impact on MPTP opening analyzing state 4, state 3, and DNP-stimulated respiration as well as mitochondrial swelling and $\Delta\Psi_{mito}$ dissipation in RLM energized by glutamate and malate after administration of Ca^{2+} into the medium containing $TlNO_3$, MPTP inhibitors (ADP, CsA, or Mg^{2+}), and sucrose or KNO_3.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were used in the research. The animals were kept at 20–23 °C under 12-h light/dark cycle with free access to the standard rat diet and water ad libitum. All treatment procedures of animals were performed in accordance with the Animal Welfare act and the Institute Guide for Care and Use of Laboratory Animals.

2.2. Isolation of mitochondria

Wistar adult male rats (200–250 g) were used in the standard isolation procedure of liver mitochondria (Korotkov and Saris, 2011). Liver mitochondria were suspended in a medium containing 250 mM sucrose, 3 mM Tris–HCl (pH 7.3), and 0.5 mM EGTA. Next mitochondria were twice washed by resuspension–centrifugation in a medium containing 250 mM sucrose and 3 mM Tris–HCl (pH 7.3), and finally suspended in 1 ml of the latter medium. The mitochondrial protein content was defined by Bradford method, and it was within the range of 50–60 mg/ml.

2.3. Oxygen consumption assay

The mitochondrial respiration rates (ng atom O/min per mg of protein) were estimated polarographically using Expert-001 analyzer (Econix-Expert Ltd., Russia) in a 1.3-ml closed thermostatic chamber with magnetic stirring at 26 °C (Figs. 1 and 2). Mitochondria (1.5 mg/ml of protein) were added into sucrose-adjusted 400 mOsm medium containing 0–75 mM $TlNO_3$, 250 mM sucrose (Fig. 1), 125 mM KNO_3 (Fig. 2), 5 mM Tris–NO_3 (pH 7.3), 3 mM $Mg(NO_3)_2$, 3 mM Tris–P_i, 5 mM glutamate, and 5 mM malate. Millimolar concentrations of $TlNO_3$ and Tl acetate were first applied in early studies being used swelling and polarographic technics in experiments with isolated mitochondria (Melnick et al., 1976; Saris et al., 1981) and this circumstance was previously discussed by us in more detail (Korotkov et al., 2007). Additions of Ca^{2+}, ADP, DNP, oligomycin, and CsA are detailed in Figs. 1 and 2 legends. The respiration, the swelling, and $\Delta\Psi_{mito}$ were tested in 400 mOsm media to check consistency and comparability between the results of different experiments.

2.4. Swelling of mitochondria

Swelling of mitochondria was tested as a fall in the apparent absorbance of mitochondrial suspension at 20 °C on an SF-46 spectrophotometer (LOMO, St. Petersburg, Russia) at 540 nm. Mitochondria (1.5 mg/ml of protein) were injected into a 1-cm cuvette with 1.5 ml of sucrose-adjusted 400 mOsm medium containing 0–75 mM $TlNO_3$, 250 mM sucrose (Figs. 3a and 4a), 125 mM KNO_3 (Figs. 3b and 4b and d), 5 mM glutamate plus 5 mM malate (Fig. 3), 5 mM succinate (Fig. 4d), 2 μM rotenone (Fig. 4c and d), 5 mM Tris–NO_3 (pH 7.3), and 1 μg/ml of oligomycin. Additions of Ca^{2+}, ADP, succinate, and CsA are specified in Figs. 3 and 4 legends.

2.5. Mitochondrial membrane potential

The inner membrane potential (Fig. 5), induced after administration of 5 mM glutamate and 5 mM malate in the medium, was