ORIGINAL ARTICLE

TISSUE SPECIFIC SENSITIVITY OF MITOCHONDRIAL PERMEABILITY TRANSITION PORE TO CA^{2+} IONS

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Summary: Ca^{2+}-induced opening of the mitochondrial permeability transition pore (MPTP) is involved in induction of apoptotic and necrotic processes. We studied sensitivity of MPTP to calcium using the model of Ca^{2+}-induced, cyclosporine A-sensitive mitochondrial swelling. Presented data indicate that the extent of mitochondrial swelling (dA_{520}/4 min) induced by addition of 25 μM Ca^{2+} is seven-fold higher in liver than in heart mitochondria (0.564 ± 0.08/0.077± 0.01). The extent of swelling induced by 100 μM Ca^{2+} was in liver tree times higher than in heart mitochondria (0.508±0.05/ 0.173±0.02). Cyclosporine A sensitivity showed that opening of the MPTP is involved. We may thus conclude that especially at low Ca^{2+} concentration heart mitochondria are more resistant to damaging effect of Ca^{2+} than liver mitochondria. These finding thus support hypothesis that there exist tissue specific strategies of cell protection against induction of the apoptotic and necrotic processes.

Key words: *Mitochondria; MPTP; Swelling; Cell death; Apoptosis; Necrosis*

Introduction

Recent data in the literature have shown that mitochondria play an important role in induction of apoptotic and necrotic processes (9) and that mitochondrial membrane permeability transition pore (MPTP) is involved in cell death processes (3, 10). Opening of this pore is induced by impaired intracellular homeostasis of calcium ions; sensitivity to calcium is modified by many factors from which oxygen radicals are the most important (2, 8, 13, 14, 16). When the pore is opened mitochondrial membrane potential is discharged and aerobic ATP generation is depressed. Mitochondrial swelling due to equilibration of ion and water gradient between cytosol and mitochondrial matrix is accompanied by rupture of outer mitochondrial membrane and release of cytochrome c which activates caspases and apoptotic reactions (12, 17, 18, 19, 20).

Two criteria are used as evidence that MPTP is responsible for mitochondrial swelling and various accompanying reactions: calcium activation of particular reactions and their inhibition by EGTA, cyclosporine A (4, 11), or sanglifehrin A (7). In spite of its importance in pathogenesis of many diseases in which apoptotic and necrotic processes are involved, knowledge about the structural organization, functional activity and regulation of MPTP is not sufficient (3, 14).

In our work we focused on problems concerning tissue specific regulation of MPTP function because it has been shown that there exists physiological diversity of mitochondrial processes in various tissues (1). Recently, Panov et al. (16) observed specific differences in tolerance to calcium-induced swelling between liver and brain mitochondria. We present complementary additional data showing that also heart mitochondria are more resistant to calcium-induced opening of membrane permeability transition pore when compared with liver mitochondria.

Methods

Chemicals. All chemicals used were of the highest commercially available purity from Sigma (Sigma Aldrich Co. Germany).

Animals. Male Wistar rats (BioTest, Konarovice, Czech Republic) with body weight 220–250 g were used for experiments. The rats were housed at 23 °C with 12-h light-dark cycle periods. The animals have free access to standard laboratory diet (ST-1, Velaz, Czech Republic) and tap water. All animals received care according to the guidelines set out by the Institutional Animal use and care committee of the Charles University, Prague, Czech Republic. Animals were sacrificed by exsaquination from aortic bifurcation in ether narcosis and liver and heart were washed in cold isolation medium.

Isolation of mitochondria. Liver or heart mitochondria were isolated by differential centrifugation essentially as described by Bustamante et al. (5). The tissue was cut into

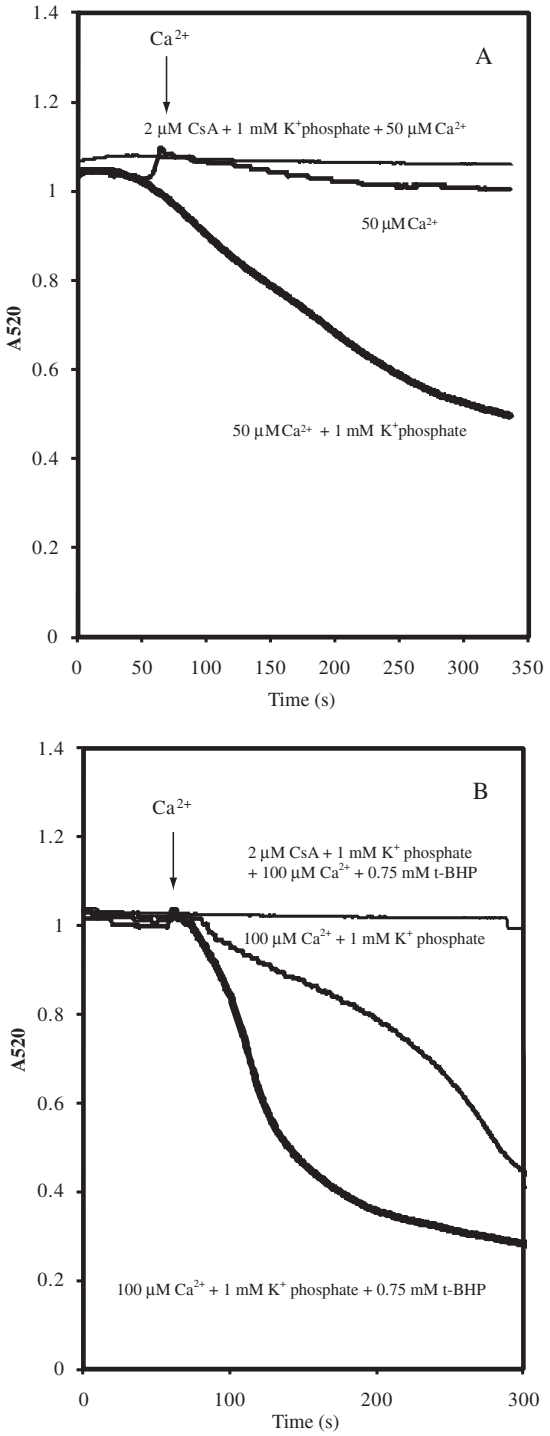

Fig. 1: Ca^{2+} swelling of rat liver mitochondria. Activation by phosphate (A) and by t-butyl hydroperoxide (B). Mitochondria (0.4 mg protein/ml) were incubated in the swelling medium at 30 °C and calcium was added after 60 s. Other additions present in the medium, 2 µM cyclosporine A (CsA), 1 mM K-phosphate (K-phos.), 0.75 mM *tert*-butyl hydroperoxide (t-BHP) and 50 or 100 µM $CaCl_2$ (Ca) are indicated in Figs. 1A and B.

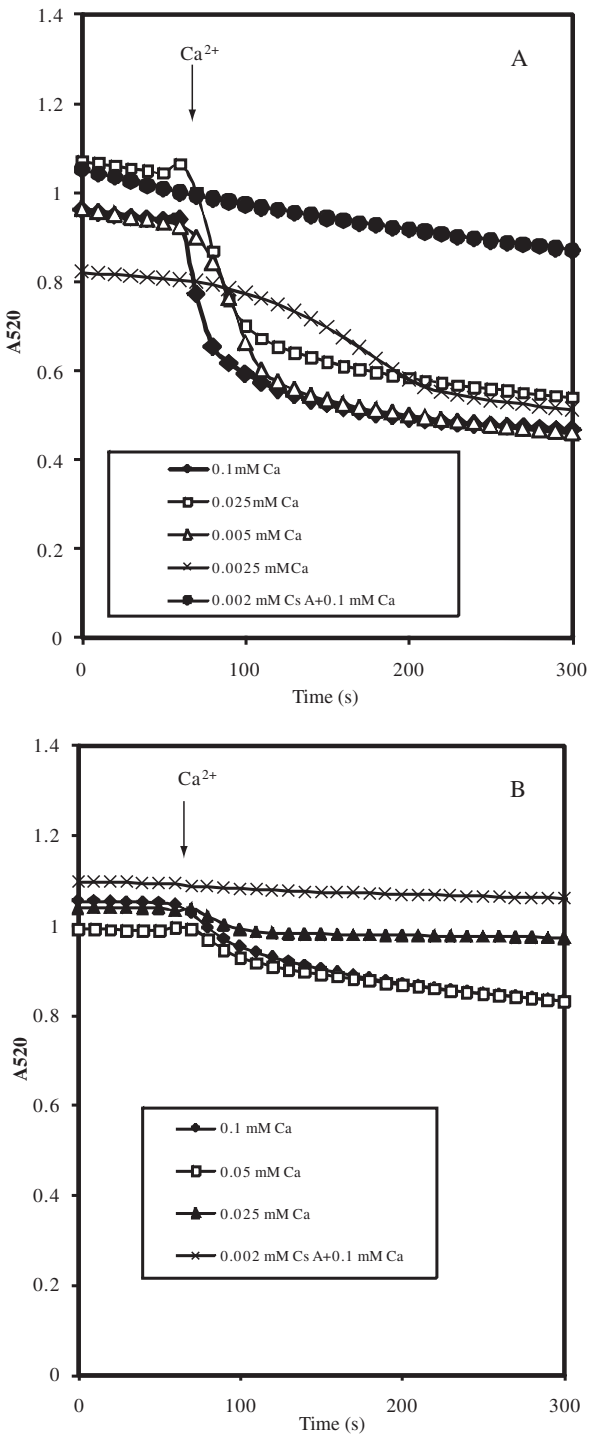

Fig. 2: Concentration dependence of Ca^{2+} swelling by rat liver (A) and rat heart (B) mitochondria. Mitochondria (0.4 mg protein /ml) were incubated in the swelling medium in the presence of different calcium concentrations as indicated in Fig. 2A and B. Ca was added after 60 s of preincubation.

small pieces and homogenized at 0 °C by a teflon-glass homogenizer in the medium containing 220 mM D-Mannitol, 70 mM sucrose, 2 mM HEPES, 0.2 mM EGTA, 0.5 mg fatty acid free bovine serum albumin (BSA) per ml, pH 7.2. The homogenate was centrifuged for 10 min at 600 g and resulting supernatant for 10 min at 6 800 g. The mitochondrial sediment was washed twice in the isolation mannitol-based medium without EGTA and BSA and suspended in the same solution.

Determination of mitochondrial swelling. Mitochondrial swelling was estimated from the decrease in the absorbance at 520 nm measured in a spectrophotometer (Shimadzu UV-1601) at 30 °C. The swelling medium contained 125 mM sucrose, 65 mM KCl, 10 mM HEPES, pH 7.2, 5 mM succinate and 1 mM K-PO_4 (6) and other additives as indicated in figures. Mitochondria were added to give the absorbance about 1.0. After one minute of preincubation of mitochondria, $CaCl_2$ solution was added and the decrease of absorbance was detected in 10 s intervals for further 5 or 10 min.

Determination of proteins. Protein content was determined by the method of Lowry et al. (15) using bovine serum albumin as standard.

Statistical analysis. All measurements were made five times, representative traces are shown in the figures. Values in the table are expressed as a mean ± SD, after testing of normality, the statistical significance was analysed using one-way ANOVA test followed by Tuckey post hoc test for comparison between control group and the others, (GraphPad Prism 4.03 for Windows, GraphPad Software, USA), $p < 0.05$ was considered as significant.

Results and Discussion

Calcium-induced swelling of mitochondria depends on various factors. Mitochondrial swelling measured in medium without phosphates was less sensitive to calcium ions. Addition of 1 mM phosphate leads to pronounced increase of mitochondrial swelling (Fig. 1A). Fig. 1B demonstrates that calcium-induced swelling in the presence of phosphates is significantly activated by prooxidant tert-butylhydroperoxide (tBHP). Because we were interested to study the effect of low calcium concentrations on MPTP opening we used in further experiments medium with 1 mM K-phosphate.

When the calcium concentration dependence of liver mitochondria was tested in the presence of succinate and phosphate, calcium-induced swelling could be detected at low (2.5–25 μm) calcium concentrations (Fig. 2A). The swelling was completely inhibited by cyclosporine A what confirms that opening of the mitochondrial permeability transition pore is involved (Fig. 2A).

Under the same experimental conditions swelling of heart mitochondria was measured. The extent of swelling, during 4 min of incubation after addition of calcium, was much lower in rat heart mitochondria than in liver mitochondria. The difference between these two mitochondrial preparations was significantly more pronounced at 25 μM (0.564 ± 0.08 vs 0.077 ± 0.01) than at 100 μM (0.508 ± 0.05 vs 0.173 ± 0.02) calcium concentrations (Tab. 1). Fig. 3 depicts that the maximal induction of swelling in liver mitochondria appears in the concentration range of 2.5–25 μM instead of in heart mitochondria the extent slowly increases up to 100 μM calcium concentration.

Our data are thus in full agreement with recent findings of Panov et al. (16) who found differences in calcium-dependent swelling between brain and liver tissue. Brain mitochondria were more resistant to calcium-induced opening of the membrane permeability transition pore. They suggest that evidently liver tissue having higher regenerative capacity (16) is less protected against opening of MPTP and initiation of apoptotic and necrotic processes than brain.

Tab. 1: The extent of Ca^{2+} swelling of rat liver and heart mitochondria.

	25 μM Ca^{2+}	100 μM Ca^{2+}
RLM – rat liver mitochondria	0.564 ± 0.08	0.508 ± 0.05
RHM – rat heart mitochondria	0.077 ± 0.01***	0.173 ± 0.02***
RLM/RHM	7.32	2.93

Data indicate average of the extent of mitochondrial swelling during 4 min after addition of Ca^{2+}; experimental conditions are the same as in Fig. 1; n = 5, *** $p<0.001$.

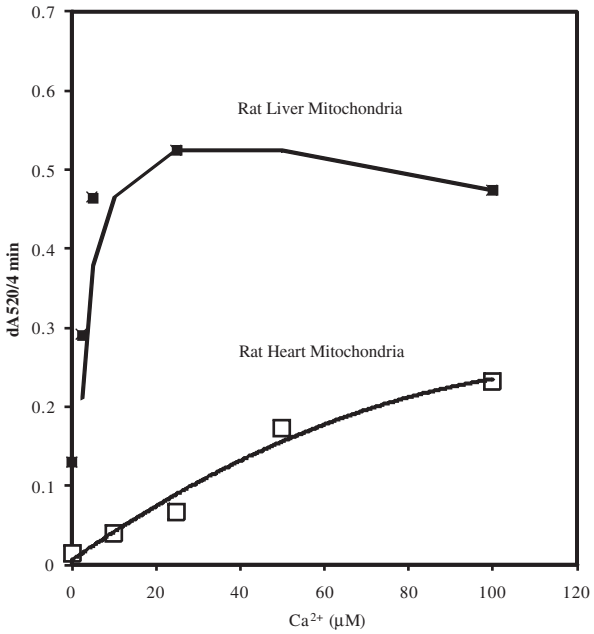

Fig. 3: The calcium concentration dependence of the extent of mitochondrial swelling during 4 min of incubation. Data are calculated from the swelling curves presented in Fig. 2. as a difference between the absorbancy before addition of calcium and absorbancy at 4 min after calcium addition.

This could be interpreted as different strategy how to compete with factors inducing necrotic and apoptotic processes (16). Our results support this hypothesis and extend previous data. Similarly as brain, heart is less sensitive to deleterious action of increased concentration of calcium ions.

To elucidate mechanisms participating on these defense reactions requires more experimental data. There are many endogenous factors e.g. nucleotides, phosphate, magnesium, fatty acids, thyroid hormones or pH that could modulate calcium-induced opening of MPTP. Effects of all these endogenous factors can be further influenced by various xenobiotics that induce oxidative stress.

Conclusion

The results of our study show tissue specific sensitivity of mitochondrial permeability transition pore to calcium-induced ions. We proved that heart mitochondria are more resistant to Ca^{2+} opening of mitochondrial membrane permeability transition pore in comparison with liver mitochondria.

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References

1. Bernard G, Faustin B, Passerieux E, et al. Physiological diversity of mitochondrial oxidative phosphorylation. Am J Physiol Cell Physiol 2006;291:1172–82.
2. Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol Rev 1999;79:1127–55.
3. Bernardi P, Krauskopf A, Basso E, et al. The mitochondrial permeability transition from in vitro artifact to disease target. FEBS J 2006;273:2077–99.
4. Broekemeier KM, Carpenter-Deyo L, Reed DJ, Pfeiffer DR. Cyclosporin A protects hepatocytes subjected to high Ca^{2+} and oxidative stress. FEBS Lett 1992; 304:192–4.
5. Bustamante E, Soper JW, Pedersen PL. A high-yield preparative method for isolation of rat liver mitochondria. Anal Biochem 1977;80:401–8.
6. Castilho RF, Kowaltowski AJ, Vercesi AE. 3,5,3` – triiodothyronine induces mitochondrial permeability transition mediated by reactive oxygen species and membrane thiol oxidation. Arch Biochem Biophys 1998;354:151–7.
7. Clarke SJ, McStay GP, Halestrap AP. 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. 2002;277:34793–9.
8. Crompton M, Ellinger H, Costi A. Inhibition by cyclosporin A of a Ca^{2+} – dependent pore in heart mitochondia activated by inorganic phosphate and oxidative stress. Biochem J 1988;255:357–60.
9. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J 1999;341:233–49.
10. Desangher S, Martinou JC. Mitochondria as the central control point of apoptosis. Trends Cell Biol 2000;10:369–77.
11. Fournier N, Ducet G, Crevat A. Action of cyclosporine on mitochondrial calcium fluxes. J Bioenerg Biomembr 1987;19:297–303.
12. Kadenbach B. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. Biochim Biophys Acta 2003;1604:77–94.
13. Kim JS, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2006; 290:2024–34.
14. Leung AW, Halestrap AP. Recent progress in elucidating the molecular mechanism of mitochondrial permeability transition pore. Biochem Biophys Acta 2008;1777:946–52.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
16. Panov A, Dikalov S, Shalbuyeva N, et al. Species- and tissue-specific relationships between mitochondrial permeability transition and generation of ROS in brain and liver mitochondria of rats and mice. Am J Physiol Cell Physiol 2007;292:708–18.
17. Tsuimoto Y, Nakagawa T, Shimizu S. Mitochondrial membrane permeability transition and cell death. Biochim Biophys Acta 2006;1757:1297–300.
18. Wallace KB, Eells JT, Madeira VMC, Cortopassi G, Jones DP. Mitochondria-mediated cell injury. Fundam Appl Toxicol 1997;38:23–37.
19. Wallace DC. Mitochondrial diseases in man and mouse. Science 1999;283: 1482–8.
20. Zhang JG, Tirmenstein MA, Nicholls-Grzemski FA, Fariss MW. Mitochondrial electron transport inhibitors cause lipid peroxidation-dependent and –independent cell death: protective role of antioxidants. Arch Biochem Biophys 2001; 393:87–96.

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