Journal of Bioenergetics and Biomembranes, Vol. 36, No. 4, August 2004 (© 2004)

Protective Roles of CNS Mitochondria

Janet M Dubinsky,[1,3] Nickolay Brustovetsky,[1,2] and Reghann LaFrance[1]

Received March 15, 2004; accepted May 7, 2004

Mitochondria benefit their host cells by generating ATP, detoxifying oxygen, maintaining cellular redox potential, and detoxifying reactive oxygen species and xenobiotics. These beneficial roles are in stark contrast to mitochondrial participation in both necrotic and apoptotic degenerative pathways. However, cellular stresses do not always result in deleterious mitochondrial changes. Decreases in the calcium sensitivity of the permeability transition may be initial mitochondrial responses to stress that act to preserve mitochondrial function and prolong normal functioning of the host cell.

KEY WORDS: Permeability transition; calcium sensitivity; Huntington's disease; regional vulnerability; neurodegeneration.

With much focus upon the role of mitochondria in apoptotic signaling and the permeability transition in acute necrotic injury, the roles mitochondria play in generating energy, maintaining homeostasis and protecting cells from injury may appear as secondary or lesser functions. However, the role of mitochondria in preserving cell health is clearly the more dominant function. First and foremost is the principal role of mitochondria in generating ATP. Concomitant with generating ATP, reducing molecular oxygen through the electron transport chain prevents its spurious involvement in oxidation of other important cellular constituents, ie., proteins, lipids, or oligonucleotides (Skulachev, 1996). Electron transport and oxidative phosphorylation harness the high redox potential of O_2, transforming it into the high-energy phosphate bond of ATP. With O_2 as substrate, its flux becomes part of a highly regulated set of reactions, and thus it is less likely to participate in spurious, harmful oxidative activity. Residual reactive oxygen species are largely controlled either by transmutase reactions or by reducing their production by increasing O_2 utilization through the electron transport chain. Mild uncoupling, or even induction of the permeability transition, could serve to temporarily depolarize mitochondria and decrease production of reactive oxygen species. Thus, mitochondria benefit the cell by both producing energy efficiently and by largely detoxifying oxygen (Skulachev, 1996).

Mitochondria also function to maintain cellular homeostasis by sequestering calcium (Nicholls, 1985). Mitochondrial membrane potential is a potent driving force for moving Ca^{2+} up a concentration gradient into the mitochondrial matrix. Once inside, free Ca^{2+} is controlled by its solubility in association with phosphate, permitting temporary storage of Ca^{2+} in a relatively inactive form (Chalmers and Nicholls, 2003). Mitochondria act as a transient Ca^{2+} sink during rapid events that increase cytosolic Ca^{2+}, such as the neuronal action potential (Werth and Thayer, 1994), and during longer lasting events associated with Ca^{2+} waves (Landgraf *et al.*, 2004). Under stressful conditions, when neurons are challenged by overexposure to the excitotoxic neurotransmitter glutamate, mitochondrial sequestering of Ca^{2+} may figure critically in the ability of the neuron to prevent necrosis. Several recent studies of the often destructive mitochondrial permeability transition have revealed new ways in which mitochondria may actually be acting to preserve mitochondrial and cellular function.

In isolated mitochondrial preparations, bolus addition of a substantial Ca^{2+} load or repeated additions of smaller Ca^{2+} doses eventually leads to mitochondrial

[1] Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota.

[2] Current address: Department of Pharmacology & Toxicology, Indiana University Medical School, Indianapolis, Indiana.

[3] To whom correspondence should be addressed at Department of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church St SE, Minneapolis, Minnesota 55455; e-mail: dubin001@umn.edu.

depolarization, swelling, and an eventual inability of mitochondria to sequester the calcium. Evidence for the occurrence of this permeability transition *in situ* has been gathered from astrocytes, neurons, and a plethora of non-neuronal cells (Dubinsky and Levi, 1998; Jordan *et al.*, 2003; Kristal and Dubinsky, 1997; White and Reynolds, 1996). When neuronal cytosolic Ca^{2+} is elevated with an excitotoxic dose of glutamate, mitochondria depolarize in a manner that may or may not be sensitive to inhibitors of the permeability transition (Brustovetsky and Dubinsky, 2000b; Budd *et al.*, 2000; Reynolds, 1999).

Ambiguity surrounding a role of the permeability transition in excitotoxicity may lie in the variety of responses receiving that name and in the differential susceptibility of mitochondria from different neuronal populations. The conductance pathways opened by mPT may vary. In single-channel recordings, large conductance mitochondrial channels may be activated spontaneously or by peptides, Bcl-xL, or Ca^{2+} (Jonas *et al.*, 2003; Kushnareva *et al.*, 2001; Muro *et al.*, 2003). Any or all of these could contribute to events ascribed to permeability transition. In isolated mitochondrial preparations, Ca^{2+} may trigger a high conductance response, associated with simultaneous depolarization and swelling (Brustovetsky and Dubinsky, 2000a,b). While mitochondria may recover from such an event, it often results in mitochondrial disintegration and may be part of a regulated autophagic pathway (Lemasters *et al.*, 2002). Ca^{2+} may also activate a limited permeability that only depolarizes mitochondria, without causing swelling (Brustovetsky and Dubinsky, 2000a,b). Initiated under conditions of limited substrate availability, isolated CNS mitochondria respond to external Ca^{2+} with such a sustained depolarization. This depolarization drastically reduces the driving force for Ca^{2+} influx, limiting the mitochondria's ability to sequester Ca^{2+} (Brustovetsky and Dubinsky, 2000a,b). Such a response should protect mitochondria against more severe damage. Indirectly it protects the neuron from the eventuality of a compromised metabolism, preserving mitochondrial integrity for future ATP generation. Seen from the mitochondria's point of view, this limited permeability pathway, which may be a component of or precursor to the high conductance mPT, is an attempt to prevent the latter from occuring. Preventing Ca^{2+} entry precludes accumulation of matrix free Ca^{2+} to levels sufficient for induction of a high conductance mPT. Among the heterogeneous responses of cultured hippocampal neurons, mitochondrial depolarization accompanying glutamate-induced sustained increases in intracellular calcium may reflect opening of such low conductance pathways (Fig. 1). In such cases, FCCP addition to fully depolarize mitochondria and unload accumulated calcium results in no further increase in cytosolic Ca^{2+} (Brustovetsky and Dubinsky, 2000a,b). In a similar manner, chemically induced transient mitochondrial depolarization of cultured cortical neurons is neuroprotective (Stout *et al.*, 1998). In cerebellar cultures, similar mitochondrial depolarization can prevent glutamate-induced delayed calcium deregulation, a precursor to cell death (Nicholls and Budd, 1998). Admittedly, while depolarization via activation of a low conductance permeability may be protective of the mitochondria, it may not always result in endogenous neuroprotection. Elevated cytosolic Ca^{2+} can initiate a variety of other deleterious pathways, which results in cell death. However, preserving mitochondrial integrity may result in these other pathways, leading to a slower and more tidy apoptotic process, fueled by mitochondrial generated ATP, rather than an acute necrotic demise.

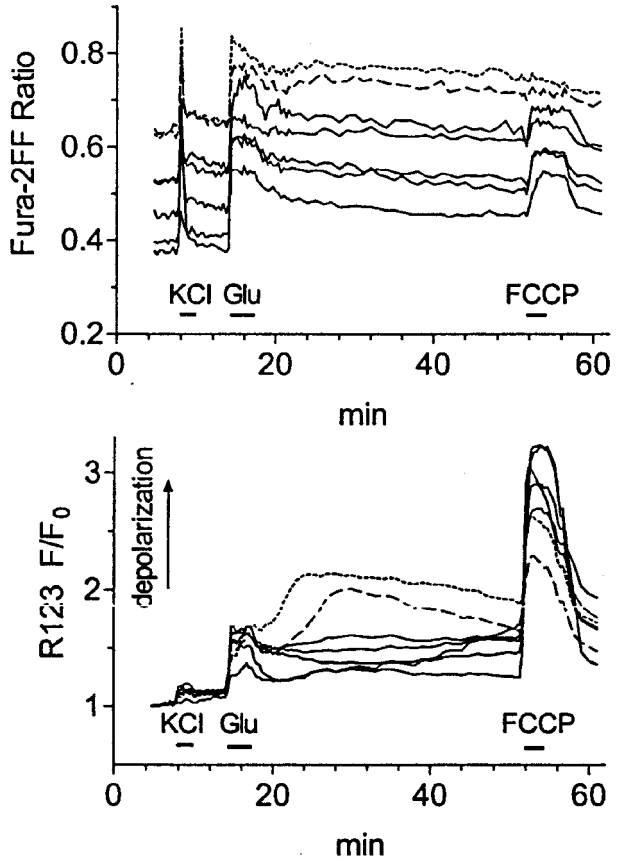

Fig. 1. Cultured hippocampal neurons loaded with the low affinity Ca^{2+} dye, fura-2FF, and the mitochondrial membrane potential sensitive dye, R123, display mixed reactions to 500 μM glutamate. Neurons with the most depolarized mitochondria display the highest sustained calcium levels. Mitochondrial depolarization with FCCP reveals an absence of mitochondrially stored calcium. (Reprinted with permission from *J. Neurosci.* **20**, 103)

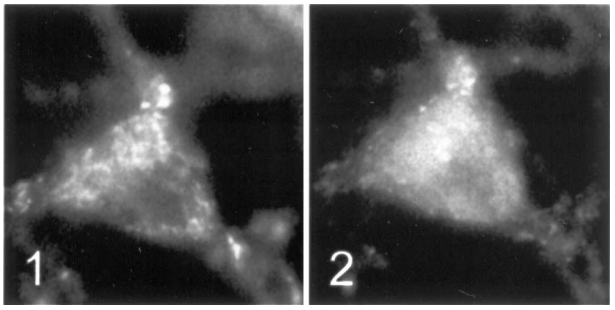

Fig. 2. Changes in mitochondrial morphology often associated with excitotoxicity. Cultured hippocampal neuron stained with mitotracker green (1) initially and (2) 20 min after 5 min of 500 μM glutamate.

Variability in regional brain mitochondria may also contribute to the controversy regarding whether or not the mPT participates in excitotoxicity. Strong arguments have been made against its participation in glutamate-induced delayed calcium deregulation in cultured cerebellar granule cells (Nicholls and Budd, 1998). However, isolated cerebellar mitochondria are less sensitive to Ca^{2+}-induced swelling than mitochondria from the cortex or hippocampus (Friberg *et al.*, 1999). Hippocampal neurons variably display morphological changes in mitochondria after excitotoxic glutamate exposure (Fig. 2) (Dubinsky and Levi, 1998), consistent with the high sensitivity of hippocampal mitochondria to Ca^{2+}-induced swelling (Friberg *et al.*, 1999). Similarly, isolated rat striatal mitochondria appear more susceptible to Ca^{2+}-induced depolarization and swelling than cortical mitochondria (Brustovetsky *et al.*, 2003). Such differential senstivity may contribute to the initially greater susceptibility of striatal neurons in degenerative diseases such as Huntington's disease. To address this question, we examined regional brain mitochondria from a very slowly progressing mouse model of HD in which long polyglutamine expansions have been placed in exon 1 of the mouse Huntington gene (Wheelar *et al.*, 2000). Indeed, when striatal and cortical mitochondria from Q111 mutant huntingtin knock-in mice of various ages were examined, the initially more susceptible striatal mitochondria changed with increasing age in a polyglutamine-dependent manner (Fig. 3). Striatal mitochondrial Ca^{2+} sensitivity decreased untill these mitochondria became equally sensitive to the cortical mitochondria. The shift in Ca^{2+} sensitivity occurred very early in the disease progression, at the time that nuclear localization of mutant huntingtin first occurred (Wheeler *et al.*, 2000). Thus, in the early stages of disease progression, the

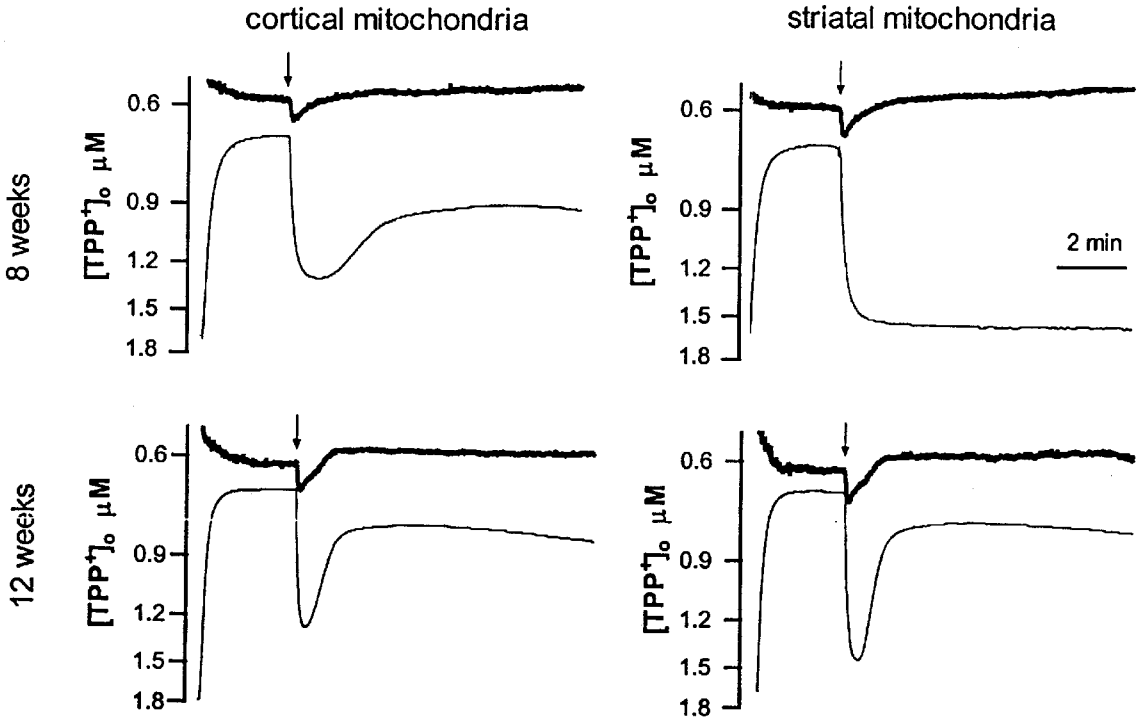

Fig. 3. Isolated cortical and striatal mitochondria from Q111 mice 8 and 12 weeks of age. Mitochondrial membrane potential was measured by the uptake of TPP^+ (thin lines) and swelling was monitored by light scattering (thick lines) (Brustovetsky *et al.*, 2003); 0.3 μmol Ca^{2+} per mg mitochondrial protein was added at the arrows. In older Q111 mice, striatal mitochondria retain the same Ca^{2+} sensitivity as observed at 12 weeks.

striatal mitochondria became more resistant to induction of permeability transition. This compensatory response again demonstrates a mitochondrial propensity for self-preservation. A higher Ca^{2+} threshold will make neurons more tolerant of increases in intracellular Ca^{2+}. However, this adaptive strategy may only work in the short term. If uncoupling and permeability transition are indeed safety valves or ways for cells to detoxify extra oxygen by maximally running electron transport (Skulachev, 1996), then by raising the threshold for onset of permeability transition, neurons may increase their risk for cumulative oxidative damage. As with any stressed tissue that mounts a compensatory response, overall susceptibility of the entire cell to other degenerative processes may be increased.

Similar adaptive mitochondrial behavior has been previously reported in precancerous liver cells of rats fed the carcinogen 2-acetylaminofluorene (Klohn *et al.*, 2003). Liver mitochondria harvested from animals on this diet for 3–4 weeks had a greatly decreased sensitivity to Ca^{2+} activation of mPT. While the epigenetic process leading to this increased resistance remains unknown, the mitochondrial response is clearly one of self-preservation, both for the mitochondria and for the immediate livelihood of the hepatocytes. Their eventual progression into neoplastic cells, resistant to cell death, may be a logical extension of the initially protective response.

Thus, in addition to their primary roles in energy generation, oxygen fixation, and calcium sequestration, mitochondria may adapt to locally stressful conditions with subtle protective behaviors. Depolarization resulting from uncoupling or Ca^{2+}-activated pathways may prevent mitochondrial disintegration and relieve the cell of excess oxygen (Skulachev, 1996). Shifting a cell's sensitivity to Ca^{2+} induction of a high conductance permeability transition may avoid acute necrosis, allowing cellular homeostasis to adapt to stressful conditions. Mitochondrial self-protection may be an early initial response to the stresses of elevated cytosolic calcium. Such adaptive behaviors may benefit the mitochondria themselves, their host cells, and the whole tissue.

REFERENCES

Brustovetsky, N., Brustovetsky, T., Purl, K. J., Capano, M., Crompton, M., and Dubinsky, J. M. (2003). *J. Neurosci.* **23**, 4858–4867.

Brustovetsky, N., and Dubinsky, J. M. (2000a). *J. Neurosci.* **20**, 103–113.

Brustovetsky, N., and Dubinsky, J. M. (2000b). *J. Neurosci.* **20**, 8229–8237.

Budd, S. L., Tenneti, L., Lishnak, T., and Lipton, S. A. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6161–6166.

Chalmers, S., and Nicholls, D. G. (2003). *J. Biol. Chem.* **278**, 19062–19070.

Dubinsky, J. M., and Levi, Y. (1998). *J. Neurosci. Res.* **53**, 728–741.

Friberg, H., Connern, C., Halestrap, A. P., and Wieloch, T. (1999). *J. Neurochem.* **72**, 2488–2497.

Jonas, E. A., Hoit, D., Hickman, J. A., Brandt, T. A., Polster, B. M., Fannjiang, Y., McCarthy, E., Montanez, M. K., Hardwick, J. M., and Kaczmarek, L. K. (2003). *J. Neurosci.* **23**, 8423–8431.

Jordan, J., Cena, V., and Prehn, J. H. (2003). *J. Phys. Biochem.* **59**, 129–141.

Klohn, P. C., Soriano, M. E., Irwin, W., Penzo, D., Scorrano, L., Bitsch, A., Neumann, H. G., and Bernardi, P. (2003). *Proc. Natl. Acad. Sci. U.S.A* **100**, 10014–10019.

Kristal, B. S., and Dubinsky, J. M. (1997). *J. Neurochem.* **69**, 524–538.

Kushnareva, Y. E., Polster, B. M., Sokolove, P. M., Kinnally, K. W., and Fiskum, G. (2001). *Arch. Biochem. Biophys.* **386**, 251–260.

Landgraf, G., Gellerich, F. N., and Wussling, M. H. (2004). *Mol. Cell. Biochem.* **256–257**, 379–386.

Lemasters, J. J., Qian, T., He, L., Kim, J. S., Elmore, S. P., Cascio, W. E., and Brenner, D. A. (2002). *Antiox. Redox. Signal.* **4**, 769–781.

Muro, C., Grigoriev, S. M., Pietkiewicz, D., Kinnally, K. W., and Campo, M. L. (2003) *Biophys. J.* **84**, 2981–2989.

Nicholls, D. G. (1985). *Prog. Br. Res.* **63**, 97–106.

Nicholls, D. G., and Budd, S. L. (1998). *Biochem. Biophys. Acta* **1366**, 97–112.

Reynolds, I. J. (1999). *Ann. N. Y. Acad. Sci.* **893**, 33–41.

Skulachev, V. P. (1996). *Q. Rev. Biophys.* **29**, 169–202.

Stout, A. K., Raphael, H. M., Kanterewicz, B. I., Klann, E., and Reynolds, I. J. (1998). *Nat. Neurosci.* **1**, 366–373.

Werth, J. L., and Thayer, S. A. (1994). *J. Neurosci.* **14**, 348–356.

Wheeler, V. C., White, J. K., Gutekunst, C. A., Vrbanac, V., Weaver, M., Li, X. J., Li, S. H., Yi, H., Vonsattel, J. P., Gusella, J. F., Hersch, S., Auerbach, W., Joyner, A. L., and MacDonald, M. E. (2000). *Hum. Mol. Genet.* **9**, 503–513.

White, R. J., and Reynolds, I. J. (1996). *J. Neurosci.* **16**, 5688–5697.