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Mitochondrial Trafficking in Neurons: A Key Variable in Neurodegeneration?

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Mitochondria are the proximate target of a number of different neurotoxins. Typically, impairing of the key bioenergetic function of mitochondria by toxins is considered as the main mechanism of action. However, the effective maintenance of energy generation in neurons depends on the biogenesis, trafficking, and degradation of mitochondria in addition to the traditional bioenergetic functions. We have recently demonstrated that glutamate alters both the trafficking and morphology of mitochondria in primary neurons. In addition, several other potential neurotoxins, including nitric oxide and zinc, inhibit mitochondrial movement and, in some cases, alter morphology too. This suggests that some part of the action of neurotoxins might include the impairment of mitochondrial trafficking in neurons, with the resultant failure of local ATP delivery.

KEY WORDS: Mitochondrial movement; excitotoxicity; zinc; fluorescence imaging; nitric oxide.

INTRODUCTION

Mitochondria have emerged as key targets of a number of different types of neurotoxin. In excitotoxic neuronal injury, activation of *N*-methyl-D-aspartate (NMDA) receptors activates a massive calcium entry that is initially buffered by mitochondria (Budd and Nicholls, 1996; White and Reynolds, 1995). However, this mitochondrial calcium loading disrupts mitochondrial function by depolarizing the mitochondrial membrane potential ($\Delta\Psi_m$) (Khodorov *et al*., 1996; Schinder *et al*., 1996; White and Reynolds, 1996), stimulating oxidant production (Dugan *et al*., 1995; Reynolds and Hastings, 1995), and causing cytochrome c release (Budd *et al*., 2000). Which of these events, if any, kill neurons in excitotoxicity is unclear, but preventing mitochondrial calcium accumulation effectively protects neurons from injury (Budd *et al*., 2000; Stout *et al*., 1998). Mitochondria are also the target of a number of neurotoxins that work more slowly than NMDA-mediated excitotoxicity. Toxins that target complex I of the electron transport chain, such as MPP^+ and rotenone, cause injury closely resembling Parkinson's disease in rodent models (Betarbet *et al*., 2000; Singer *et al*., 1987). Systemic administration of 3-nitroproprionic acid, a complex II poison, selectively kills neurons in the striatum, and produces a syndrome like Huntington's disease (Beal *et al*., 1993). Again, the critical impairment responsible for committing neurons to die following exposure to these toxins is not clear. However, a combination of energetic impairment and enhanced production of oxidants may well account for the injury.

In attempting to link mitochondrial failure to neuronal injury, the range of mitochondrial dynamics to consider in neurons extends well beyond simply ATP and oxidant production. In all cells, effective mitochondrial function depends on an intact mitochondrial life history, from biogenesis through delivery of mitochondria to appropriate cellular targets and ultimately the correct retrieval and degradation of the organelles at the end of their effective lifetime. Although the details of mitochondrial life history in neurons have not been clearly established, it is easy to appreciate the additional challenges faced by neurons because of the distances over which mitochondria must travel in order to supply ATP to regions of the cell that have high energy demands. An additional variable is that of mitochondrial morphology. It has been appreciated for some time that the shape of mitochondria varies

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considerably between cell types. Intriguingly, recent studies have suggested that morphology is a key variable of mitochondria within cells, and one that may govern the outcome from injury (Frank *et al*., 2001; Karbowski and Youle, 2003). Here we will review recent data that show that both movement and morphology of mitochondria are affected by neurotoxins.

NEURONAL INJURY CHANGES MITOCHONDRIAL MORPHOLOGY

Several types of morphological change in mitochondria can be anticipated on the basis of current knowledge. Perhaps the most obvious is the swelling that should be associated with the induction of permeability transition, an event associated with excitotoxic neuronal injury (Reynolds and Hastings, 2001; Schinder *et al*., 1996). In addition, the regulation of mitochondrial fission and fusion can result in a net alteration in mitochondrial shape, and it has been proposed that fission of mitochondria is associated with apoptotic cell injury (Frank *et al*., 2001). We recently described effects of glutamate on the morphology and movement in neurons in culture (Rintoul *et al*., 2003). Acute application of glutamate and the subsequent influx of calcium through NMDA receptors results in a cessation of movement (discussed later), a profound alteration in morphology and also an occasional fragmentation of mitochondria (Fig. 1) that occurs within minutes of glutamate stimulation. The change in morphology is calcium dependent, and is associated with a disruption of the cytoskeleton. This morphological change could be attributed to mitochondrial swelling. However, the lack of sensitivity to cyclosporin A makes it difficult to conclude that permeability transition is the cause of the swelling. Interestingly, the glutamate-induced morphological change recovers within 1–2 h, well before neurons die from this stimulus.

Zinc is an important endogenous neurotoxin that is mobilized during ischemic brain injury (Koh *et al*., 1997).

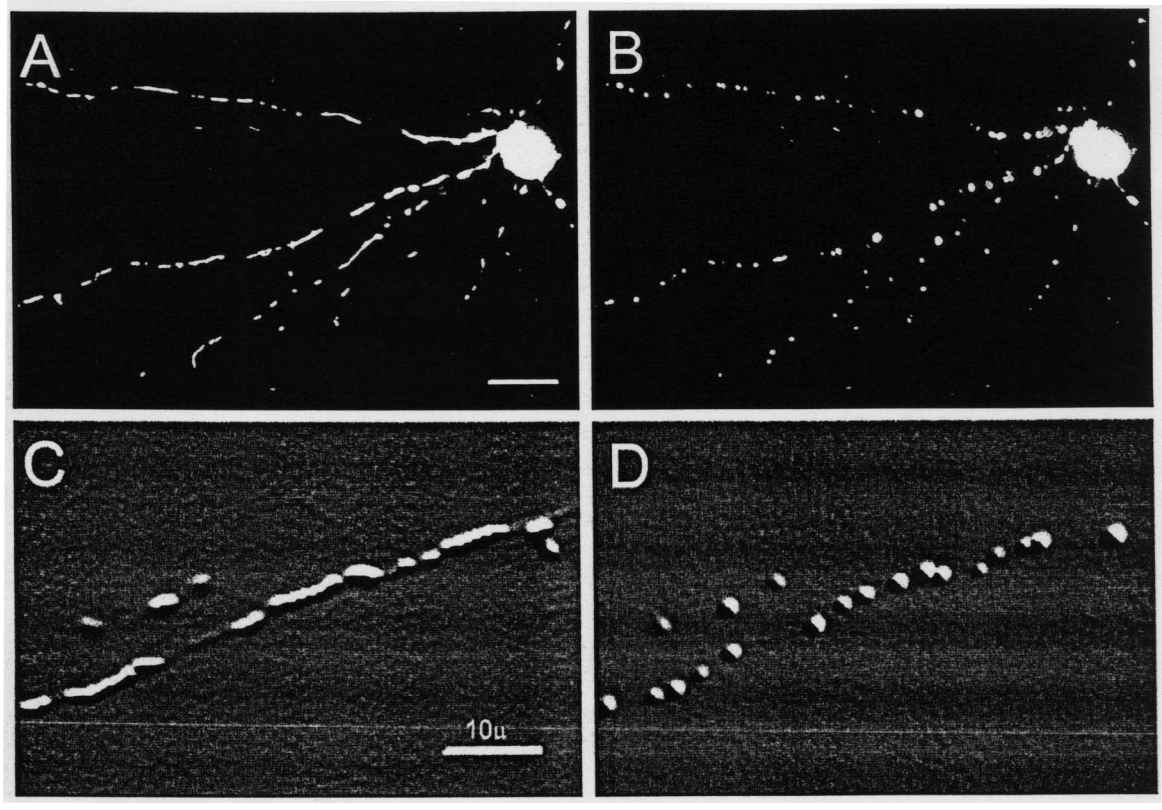

Fig. 1. Glutamate-induced changes in mitochondrial morphology. These images were obtained from a neuron in primary culture. The neuron expressed mitochondrially targeted enhanced yellow fluorescent protein. Panel A shows mitochondria before, while panel B was taken after, a 10-min exposure to 30 μM glutamate. Panels C and D show the same effect with enhancement of the image to illustrate that fragmentation occurs in addition to the swelling of the mitochondria.

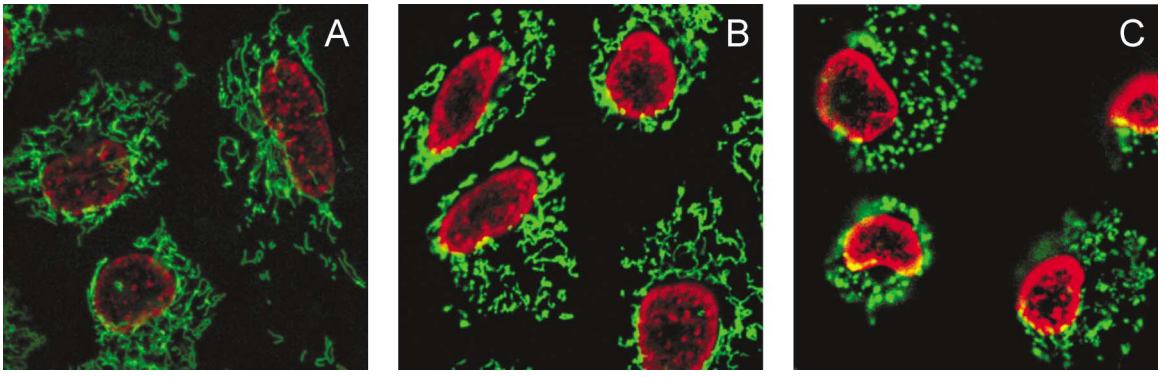

Fig. 2. Zinc alters mitochondrial morphology. This experiment was performed in HT22 cells that were stably transfected with mt-eYFP (green fluorescence) and also stained with Hoechst 33342 (red fluorescence) to label the nucleus. Panel A shows control cells, while the cells in B and C were exposed to 3 μM zinc in the presence of 20 μM pyrithione. Panel B was imaged 1 h after zinc exposure, while panel C was obtained after 2 h. Note the loss of filamentous structure of mitochondria as a consequence of zinc treatment.

We have recently found that zinc produces a different form of morphological change in mitochondria. We exposed HT22 cells that express mitochondrially targeted enhanced yellow fluorescent protein (mt-eYFP) to zinc in the presence of an ionophore, pyrithione. Over the next 2 h, zinc exposure resulted in nuclear condensation and a progressive fragmentation of mitochondria that precedes loss of cytochrome c (Fig. 2). This indicates that cytochrome c release is not simply a consequence of the mitochondria being shredded during the execution of apoptosis. The molecular basis for the fragmentation is not clear, but it has been shown that cells can be protected from apoptosis with a dominant negative form of the fission-promoting protein DRP1 (Frank *et al.*, 2001), and it is tempting to speculate that this response reflects an induced imbalance between mitochondrial fission and fusion that makes an important contribution to the fate of the cell.

NEURONAL INJURY CHANGES MITOCHONDRIAL MOVEMENT

As already noted, neuronal mitochondria may have to travel considerable distances to reach the site of ATP demand. Processes that disrupt the delivery of mitochondria may negatively impact neuronal viability by essentially imposing a local starvation because of the absence of necessary mitochondria. We have recently found a number of conditions that impair mitochondrial movement. The first of these was NMDA receptor activation with glutamate. As described above, glutamate alters mitochondrial morphology, but also produces a profound inhibition of mitochondrial movement (Rintoul *et al.*, 2003). In an effort to determine potential mechanisms for this effect, we compared the actions of glutamate to the uncoupler FCCP, which depolarizes $\Delta\Psi_m$, and oligomycin, which inhibits ATP synthesis. Both of these agents inhibited movement, but did not alter morphology. This suggests that movement critically depends on ATP production by mitochondria, and further implies that the morphological change is a consequence of the calcium entry produced by glutamate but not FCCP or oligomycin (Fig. 3).

The latter conclusion suggested an additional series of experiments. It has been well established that nitric oxide is an effective inhibitor of complex IV of the electron transport chain at relatively low concentrations (Brorson *et al.*, 1999; Brown, 1999). We speculated that NO would impair mitochondrial movement. Exposing neurons to the NO donor PAPA nonoate confirmed this to be the case, and NO appears to decrease movement without altering mitochondrial morphology. We have found that several other neurotoxins decrease mitochondrial movement in neurons, including elevated intracellular zinc and oxidant exposure, but the mechanisms by which these effects occur are still under investigation.

CONCLUSIONS

These studies illustrate the point that neurotoxins can acutely alter mitochondrial movement and morphology in addition to the known effects on ATP synthesis and oxidant generation. It seems likely that inhibiting movement will result in the impairment of the delivery of mitochondria to relevant sites within neurons, although it remains to be established whether there is a specific link between the cessation of movement and injury to neurons. It should also be noted that nerve growth factor inhibits mitochondrial

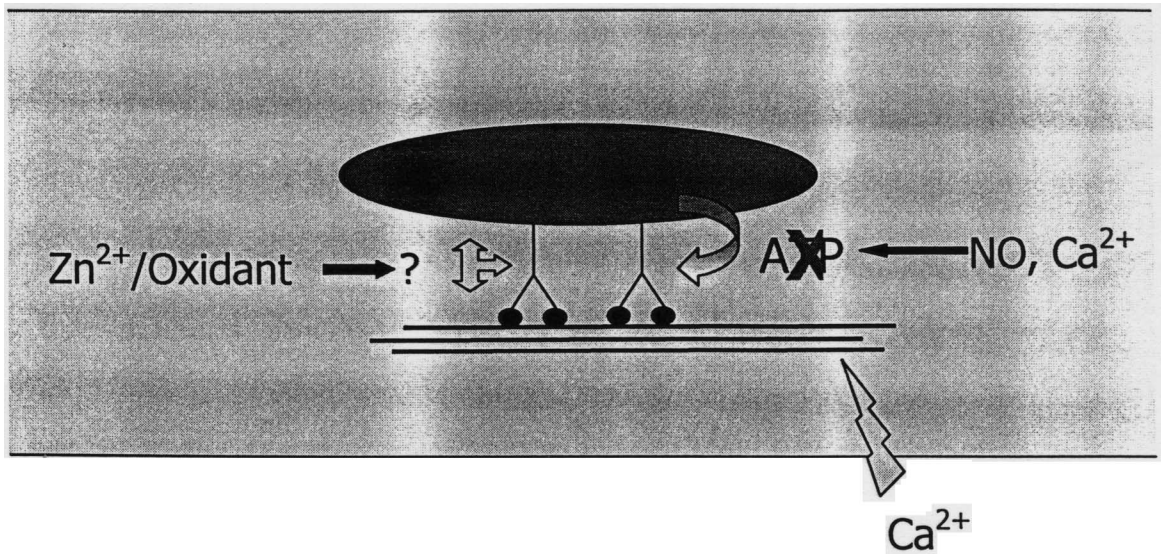

Fig. 3. Mechanisms for altering mitochondrial movement in neurons. There are likely to be three or more mechanisms for altering trafficking. Calcium can act by disrupting cytoskeletal structures like microtubules. Agents like NO and calcium can also impair movement by inhibiting ATP synthesis by mitochondria. Other neurotoxins, including zinc and some oxidizing agents inhibit mitochondrial movement without obviously depolarizing mitochondria, so this is likely to be the result of a separate mechanism.

movement in peripheral neurons (Chada and Hollenbeck, 2003). Nerve growth factor would not be considered neurotoxic in these cells, and the transient docking of mitochondria proposed as an action of the trophic factor might be considered to be a beneficial effect. It is more difficult to discern the consequence of morphological alterations for the function of neuronal mitochondria. Gross swelling as a consequence of calcium overload is probably a reflection of mitochondrial impairment, rather than a specific indicator of altered function. The more subtle fragmentation presumably yields mitochondria that are functionally intact, so that the specific hazard resulting from the decrease in mitochondrial size remains to be elucidated. However, it does raise the interesting possibility that the mechanisms that control fission or fusion might represent a novel target for neuroprotection.

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REFERENCES

Beal, M. F., Hyman, B. T., and Koroshetz, W. (1993) *Trends Neurosci.* **16**, 125–131.

Betarbet, R., Sherer T. B., Mackenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000) *Nat. Neurosci.* **3**, 1301–1306.

Brorson, J. R., Zhang, Z. H., and Vandenberghe, W. (1999) *J. Neurosci.* **19**, 9149–9159.

Brown, G. C. (1999) *Biochim. Biophys. Acta Bio-Energetics* **1411**, 351–369.

Budd, S. L., and Nicholls, D. G. (1996) *J. Neurochem.* **66**, 403–411.

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

Chada, S. R., and Hollenbeck, P. J. (2003) *J. Exp. Biol.* **206**, 1985–1992.

Dugan, L. L., Sensi, S. L., Canzoniero, L. M. T., Handran, S. D., Rothman, S. M., Lin, T.-S., Goldberg, M. P., and Choi, D. W. (1995) *J. Neurosci.* **15**, 6377–6388.

Frank, S., Gaume, B., Bergmann-Leitner E. S., Leitner, W. W., Robert, E. G., Catez, F., Smith, C. L., and Youle, R. J. (2001) *Dev. Cell* **1**, 515–525.

Karbowski, M., and Youle, R. J. (2003) *Cell Death Differ.* **10**, 870–880.

Khodorov, B., Pinelis, V., Vergun, O., Storozhevykh, T., and Vinskaya, N. (1996) *FEBS Lett.* **397**, 230–234.

Koh, J.-Y., Suh, S. W., Gwag, B. J., He, Y. Y., Hsu, C. Y., and Choi, D. W. (1997) *Science* **272**, 1013–1016.

Reynolds, I. J., and Hastings, T. G. (1995) *J. Neurosci.* **15**, 3318–3327.

Reynolds, I. J., and Hastings T. G. (2001). *In Mitochondria in Pathogenesis* (Lemasters, J. J., and Nieminen, A.-L. eds.), Kluwer Academic/Plenum Publishers, New York, pp. 301–316.

Rintoul, G. L., Filiano, A. J., Brocard, J. B., Kress, G. J., and Reynolds, I. J. (2003) *J. Neurosci.* **23**, 7881–7888.

Schinder, A. F., Olson, E. C., Spitzer, N. C., and Montal, M. (1996) *J. Neurosci.* **16**, 6125–6133.

Singer, T. P., Castagnoli, N., Ramsay, R. R., and Trevor, A. J. (1987) *J. Neurochem.* **49**, 1–8.

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

White, R. J., and Reynolds, I. J. (1995) *J. Neurosci.* **15**, 1318–1328.

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