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Reversible Cycles of Mitochondrial Remodelling in Differentiated PC12 Cells: Implications for Neuronal Survival

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Mitochondria are dynamic organelles that rapidly move, change shape, and undergo fission and fusion. The physiological role of this continuous remodelling is unclear, but mitochondrial swelling and fission are associated with apoptosis, and changes in mitochondrial morphology are linked with numerous diseases. We used osmotic stress to trigger mitochondrial remodelling in differentiated PC12 cells transfected with a GFP fusion protein targeted to mitochondria (mtGFP) (see [1,2] for detailed methods). In response to hypotonic media, mitochondrial morphology rapidly changed; within 30 sec., the entire mitochondrial population fragmented, as formerly elongated mitochondria became spheroid. Reintroduction of norm-osmotic medium rapidly reversed these changes; within 10 sec., spheroid mitochondria began to fuse, and complete recovery of elongated mitochondria was observed within 120 sec. Time-lapse imaging showed that multiple cycles of remodelling were induced within the same cell (Fig. 1) by repeated media exchanges. Remodelling neither impaired mitochondrial function nor caused cell death. Mitochondrial remodelling also occurred in human embryonic kidney cells (HEK293), murine embryonic fibroblasts (MEF) (Fig. 2), astrocytes, and primary cortical neurons (not shown). Imaging in MEF cells was facilitated by the restriction of the mitochondria to a single focal plane. As an alternative to mtGFP, we used Rhodamine-123 to label mitochondria. Multiple cycles of remodelling were imaged without substantial photobleaching of Rhodamine-123 and, in primary neurons, the dye persisted in mitochondria for at least 7 days.

Prolonged exposures to other sublethal insults that evoked oxidative stress also induced mitochondrial remodelling, but on slower timescales (Fig. 3). Sublethal oxygen-glucose deprivation (OGD) for 5 hrs induced mitochondrial fission in differentiated PC12 cells. After reperfusion mitochondria recovered their elongated morphology within 24 hrs. Acute exposure to relatively high (10 μM) but not low (2 μM) levels of the uncoupling agent CCCP had a qualitatively similar effect. Beta amyloid peptide $A\beta_{1\text{-}42}$ (10 μM, 6 days [2]), MPP+ (250 μM, >48 hrs), or proteasome inhibitors (>24 hrs) resulted in irreversible fragmentation of mitochondria. Following such exposures, cell viability was impaired, but cell death did not occur. Our results indicate that, in neurons and other cells, rapid mitochondrial remodelling can occur throughout the mitochondrial population without compromising mitochondrial function or cell viability, and suggest that mitochondrial remodelling may play a key role in recovery from events (e.g. stroke, mechanical trauma) associated with neuronal swelling. They further suggest that chronic exposure to some neurotoxins can promote mitochondrial fission and impair mitochondrial fusion, and that these events may play a key role in mitochondrial dysfunction in neurodegenerative disease. This research was supported by [3].

References

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[2] D. Sirk et al., *J Neurochem.* 103 (2007) 1989.
[3] MSA to PK, NSERC, CIHR, and Krembil Foundation to LRM, NSERC to NS and JF.

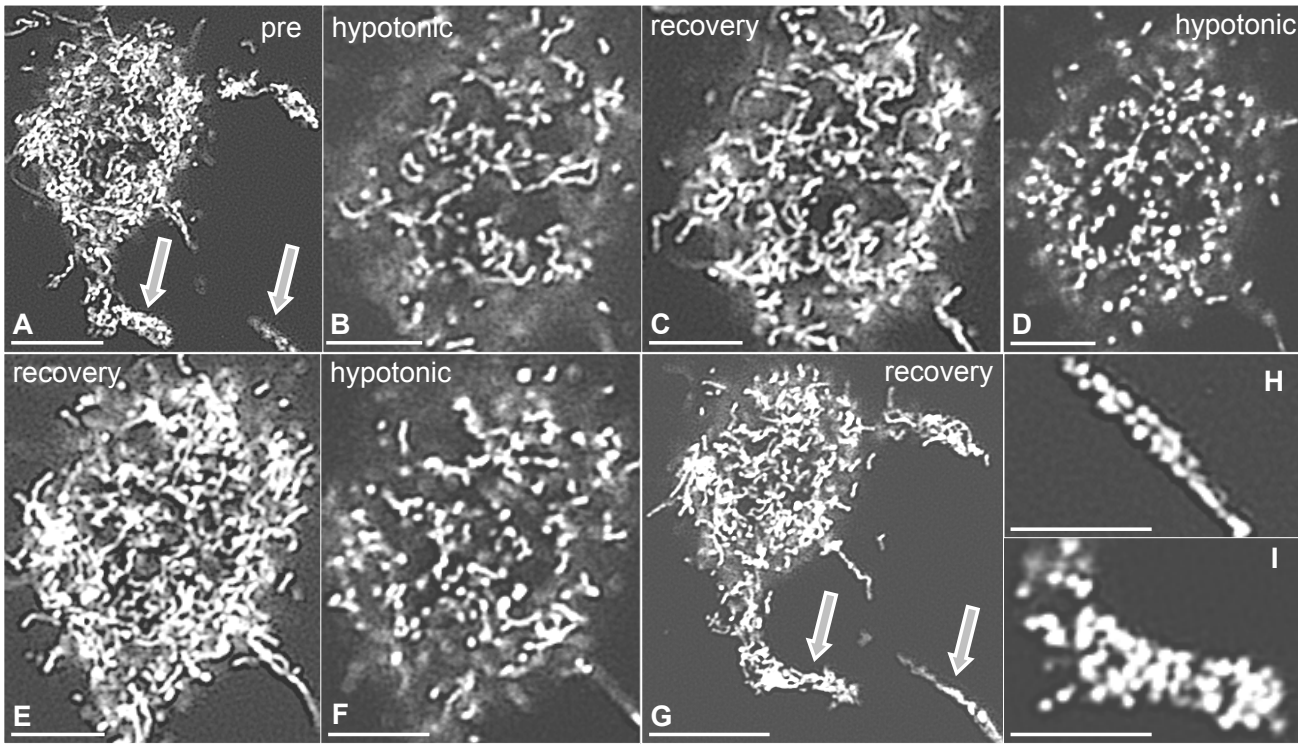

FIG. 1. Three cycles of osmotically induced mitochondrial remodelling in the same mtGFP-labeled PC12 cell. Images (A) through (G) were taken in the order indicated by the letters. (B) through (F) show changes in somatic mitochondria. (H) and (I) show rounded mitochondria in neurites, indicated by arrows in (A) and (G), under hypotonic conditions. Bars: 10 µm in (A) and (G); 5 µm elsewhere.

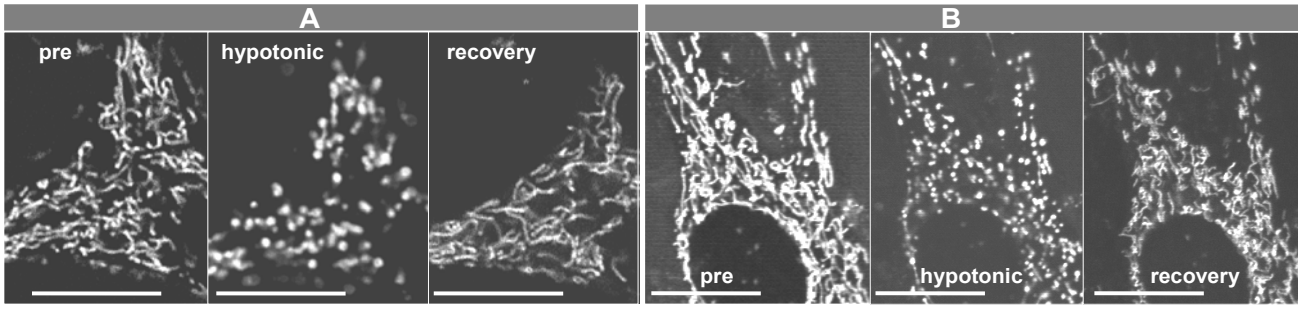

FIG. 2. (A) Osmotically induced mtochondrial remodelling in (A) an HEK293 and (B) a MEF cell. Note restriction of mitochondria to a single focal plane in (B). Bars: 10 µm in (A), 25 µm in (B).

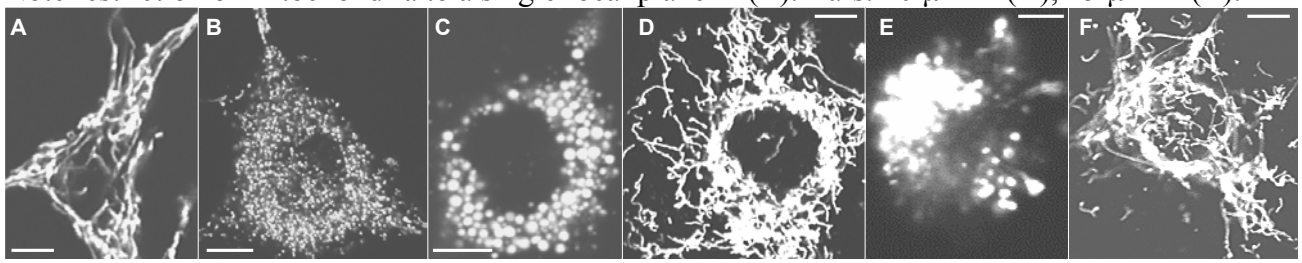

FIG. 3. Mitochondrial morphology in differentiated PC12 cells: (A) control, (B) 6 day exposure to $A\beta_{1\text{-}42}$, (C) 48 hr exposure to MPP+. Mitochondrial rounding induced by $A\beta_{1\text{-}42}$ or MPP+ was irreversible. (D) Pre and (E) post OGD. Remodelling induced by OGD was reversible (not shown) and prevented by treatment with the ROS scavenger MnTBAP during OGD (F). Bars: 10 µm.

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