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FORUM REVIEW ARTICLE

To Eat or Not to Eat: Neuronal Metabolism, Mitophagy, and Parkinson's Disease

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Abstract

Neurons are exquisitely dependent upon mitochondrial respiration to support energy-demanding functions. Mechanisms that regulate mitochondrial quality control have recently taken center stage in Parkinson's disease research, particularly the selective degradation of mitochondria by autophagy (mitophagy). Unlike other cells, neurons show limited glycolytic potential, and both insufficient and excessive mitophagy have been linked to neurodegeneration. Kinases implicated in regulating mammalian mitophagy include extracellular signal-regulated protein kinases (ERK1/2) and PTEN-induced kinase 1 (PINK1). Increased expression of full-length PINK1 enhances recruitment of parkin to chemically depolarized mitochondria, resulting in rapid mitochondrial clearance in transformed cell lines. As *parkin* and *PINK1* mutations cause autosomal recessive parkinsonism, potential defects in clearing dysfunctional mitochondria may contribute to mitochondrial abnormalities in disease. Given the unique features of metabolic regulation in neurons, however, mechanisms regulating mitochondrial network stability and the threshold for mitophagy are likely to vary from cells that preferentially utilize aerobic glycolysis. Moreover, removal of the entire mitochondrial complement may represent part of a neuronal cell death pathway. Future work utilizing physiological injuries that affect only a subset of mitochondria would help to elucidate whether defective recognition of damaged mitochondria, or alternatively, inability to maintain or generate healthy mitochondria, play the major roles in parkinsonian neurodegeneration. *Antioxid. Redox Signal.* 14, 1979–1987.

Introduction

Mitochondrial dynamics, trafficking, turnover, and biogenesis play key roles in regulating the functional health of neurons. Mitochondria not only support the energy demands of neuronal electrophysiology, but also mediate calcium homeostasis, integration of cell death/survival signals, and fatty acid metabolism. Not surprisingly, perturbations in mitochondrial function have long been centrally implicated in the pathogenesis of Parkinson's disease (PD) (39, 74), a neurodegenerative disorder affecting sparsely myelinated projection neurons of the substantia nigra, brainstem, and cortex (4).

Recent discoveries that enzymes implicated in toxin and genetic PD models can regulate the sequestration and degradation of mitochondria by the autophagy-lysosomal system (see below), have triggered a new viewpoint. Rather than focusing solely on factors that promote mitochondrial damage, impairment of downstream quality control systems such as autophagy could also cause accumulation of dysfunctional mitochondria in chronic diseases such as PD and its model systems (7).

In subsequent sections, we review the literature on mitochondrial autophagy and PD, and discuss these findings in the context of unique features of neuronal metabolism and cell biology. The definition of "mitophagy" and important methodological issues in studying this dynamic phenomenon are highlighted, followed by analysis of divergent roles ascribed to kinases that traffic to mitochondria, and recent studies of parkin-mediated mitochondrial ubiquitination. There are many exciting new questions stimulated by these findings. What are the "eat-me" signals by which dysfunctional mitochondria are selected by the autophagic machinery? Do these mechanisms occur readily in neurons and with endogenous levels of proteins? What is the role of axonal transport in neuronal mitochondrial quality control? Is a decrease in endogenous parkin-associated mitophagy sufficient to cause neurodegeneration, or is increased mitochondrial damage also required? Is mitochondrial clearance ultimately beneficial or harmful to neurons? Further experimental studies employing neurons or model systems that simulate neuron-type metabolism represent important next steps in this rapidly growing area.

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Overview of Neuronal Metabolism

The brain is a highly metabolically demanding organ. Indeed, while constituting only 2% of total body weight, the brain consumes 25% of total body glucose utilization. Carbohydrates represent the primary source of brain oxidative metabolism, as brain oxygen consumption measurements approximately equal carbon dioxide production (32), using the nitrous oxide method for measuring cerebral arterial and venous concentration differences (25). While the brain can adapt to use the ketone bodies acetoacetate and D-3-hydroxybutyrate as energy substrates in nursing infants, during starvation, or with diabetic ketoacidosis, metabolism of glucose represents the primary energy source of the brain.

Glucose traverses the blood–brain barrier through GLUT1 transporters located on endothelial cells. GLUT1 transporters also mediate the uptake of glucose into astrocytes, while GLUT3 transporters mediate uptake into neurons. Astrocytes play a pivotal role in sensing hypoglycemia (33) and regulating neuronal glucose utilization in the context of excitatory synaptic transmission (36). In contrast to many other cell types that rely upon insulin signals to allow glucose utilization, neurons constitutively transport and utilize glucose. As insulin/Akt signaling plays a prominent role in regulating autophagy (37), differences in fundamental metabolic signaling responses between neurons and other cell types could extend to mitophagy regulation as well.

Glucose is metabolized to pyruvate through glycolysis, which, under aerobic conditions, progresses to the tricarboxylic acid (TCA) cycle. The TCA cycle transfers three pairs of electrons to NAD+ to form NADH and one pair of electrons to FAD to form $FADH_2$. NADH and $FADH_2$ are utilized by the electron transport chain during oxidative phosphorylation to reduce oxygen to water, producing ATP. This process is the most efficient pathway for generating ATP.

However, production of ATP by oxidative phosphorylation is also associated with generation of superoxide as a byproduct of single electron reductions of oxygen on the matrix side of complex I or both sides of complex III (41). The scavenging of these reactive oxygen species is accomplished by superoxide dismutases, glutathione peroxidase, and NADPH, the latter of which is produced by the pentose phosphate pathway of glucose metabolism. Two superoxide anions are converted into hydrogen peroxide by superoxide dismutases, and the hydrogen peroxide is converted into water and oxygen, oxidizing glutathione in the process. Glutathione reductase regenerates reduced glutathione in the presence of NADPH. Despite their higher mitochondrial respiratory activity, neurons contain significantly less glutathione and reducing potential than astrocytes. Thus, neurons are more vulnerable to oxidative stress and are dependent on astrocytes for protection (13). Of interest, a decreased level of reduced glutathione in the substantia nigra has been described in Parkinson's disease (58, 60).

It has further been demonstrated that neurons exhibit lower levels of glycolysis compared with astrocytes and fail to significantly upregulate glycolysis when respiration is blocked by nitric oxide (1). This may be because constant degradation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphate in neurons favors utilization of glucose through the pentose phosphate pathway rather than for glycolysis (23). During times of increased excitatory energy demand, however, the brain can utilize increased astrocytic glycolysis to fuel mitochondrial respiration in neurons. Under stoichoimetric conditions, astrocytes release two lactate molecules, in exchange for glutamate and three sodium ions, for consumption by the neuron (6).

The study of metabolic and biochemical parameters in primary neurons can be challenging, leading to the utilization of many types of transformed, immortalized cell lines to facilitate studies. While these are highly useful for delineating potential protein interaction and signaling pathways of relevance to neurodegenerative diseases, the influence of metabolic differences between neurons and transformed neuronal lines or non-neuronal lines should be kept in mind, and key findings verified in neurons themselves.

Mitophagy: Methods of Study

Mitophagy is defined as the relatively selective degradation of mitochondrial segments through the process of macroautophagy. While mitochondria are often included in the bulk cytoplasmic degradation stimulated by amino acid starvation, injuries targeting the mitochondria result in greater percentages of autophagic vacuoles containing mitochondrial constituents. The term "mitophagy," while often loosely used, is best applied to situations where disproportionate sequestration and/or degradation of mitochondria relative to other cytoplasmic constituents can be demonstrated.

There are a variety of assays used to monitor or infer the degree of mitochondrial autophagy. These include electron microscopy (7), fluorescence analysis of mitochondrial protein co-localization with markers of autophagosomes and lysosomes (9, 10), fluorescence or Western blot analysis for loss of mitochondrial proteins (43, 75), and in yeast, techniques to monitor the lysosomal processing of a mitochondrially targeted fusion protein (24). Particularly in cell injury or disease contexts, it is crucial to show dependence upon essential Atg gene products, in comparison with effects of proteasome or calpain inhibitors, since decreased levels of mitochondrial proteins could be due to autophagy, localized mitochondrial proteases, defects in mitochondrial import (which typically leads to proteasomal degradation of the mitochondrial protein) (69), and, depending on the time scale of the experiment, decreased biosynthesis.

For co-expression studies involving mitochondrially-targeted fluorescent proteins, the potential issue of plasmid competition or competition/saturation of mitochondrial translocases should be considered; particularly under stress conditions resulting in marginal mitochondrial membrane potentials to drive protein import, processing, or assembly. In general, confirmation of results using several endogenous markers of mitochondria is useful.

We utilize a combination of microscopy and biochemical experiments to demonstrate: 1) increased association of mitochondria with autophagosomes, and 2) degradative loss of mitochondria that is reversed by inhibiting the autophagolysosomal system. Both electron microscopy and fluorescence co-localization studies can employ bafilomycin A1 treatment to capture autophagosomes prior to fusion with lysosomes for analysis of content (7), or to demonstrate intact autophagic flux (9). Inhibiting the late stages of autophagosome maturation/degradation will cause further increases in the

level of cargo-co-localizing autophagosomes, indicating preserved lysosomal degradative flux, while inhibiting autophagy induction should reduce the number of co-localizing puncta. To measure mitochondrial content, Western blot analysis for proteins in different mitochondrial compartments (matrix, inner membrane, etc) (10, 75) is complemented by image analysis for the percent area of the cytoplasm occupied by mitochondria (9, 10), or by analysis of mitochondrial DNA content. The influence of new protein synthesis, mitochondrial DNA replication, and other proteolytic systems should also be addressed, particularly if only partial reversal is evident with Atg protein knockdown or lysosomal inhibitors.

Regulation of Selective Mitophagy in Parkinson's Disease

A critical role for PINK1 in mitochondrial function is now well established. PINK1 has been localized to the cytosol (3, 59, 62, 67), to the ER (67), and to the mitochondria (3, 59). While some studies implicate PINK1 localization to the intermembrane space (29, 59), another study showed outer membrane insertion with the kinase domain facing the cytosol (73). Loss of PINK1 has been associated with increased oxidative stress (9, 16, 18, 68), decreased membrane potential (15, 16, 54, 68), increased susceptibility to PD toxins (11, 22, 50, 54), and decreased ATP levels and respiration (18, 30, 31). Interestingly, aged PINK1 knockout mice demonstrate decreased complex I-, II-, or IV-supported mitochondrial respiration in the striatum but not in the cortex (17, 20). Higher calcium loads and decreased mitochondrial calcium buffering have also been observed (16). More recently, PINK1 has been implicated in regulating mitophagy.

Using PINK1 stable knockdown cells, loss of PINK1 was shown to increase levels of autophagy. The level of mitophagy was also increased, as measured as co-localization of GFP-LC3 with mitochondria, bafilomycin-reversible loss of mitochondrial area, and decreased levels of endogenous matrix and inner membrane proteins (9). Subsequent experiments utilized bafilomycin to demonstrate preferential sequestration of mitochondria in PINK1 knockdown cells compared to bafilomycin-treated control cells, and an increased rate of autophagosome formation (7). Mitophagy in PINK1-deficient cells is dependent upon mitochondrial fission and increased mitochondrial superoxide production (9). In contrast, expression of either full-length PINK1 or ΔN-PINK1 to mimic the N-terminal processed form, reverses the effects of PINK1 shRNA on autophagy as well as preventing toxin-induced autophagy (9).

Interestingly, overexpression of parkin restores mitochondrial morphology changes and male sterility seen in the PINK1 mutant *Drosophila* model (8), and reverses mitochondrial fragmentation in PINK1-deficient mammalian cells (17, 32). While Drosophila mutations in *PINK1* or *parkin* exhibit similar phenotypes, parkin appears to function downstream of PINK1, as PINK1 overexpression cannot reverse the parkin loss-of-function phenotypes (49). Indeed a physical interaction between PINK1 and parkin has been reported (26, 56, 64, 70). Alternatively, it is possible that parkin has additional functions independent of PINK1.

Narendra *et al.* demonstrated that parkin is recruited to depolarized mitochondria and subsequently promotes their degradation by mitophagy (42). Translocation to depolarized mitochondria was demonstrated with endogenous parkin in HEK293 cells and rat cortical neurons and overexpressed parkin in HeLa cells. Loss of mitochondria was also observed with overexpressed parkin in HeLa cells, resulting in decreased survival when cultured in glucose free, galactose-containing media (42), a growth condition that causes dependence upon mitochondrial ATP production. Parkin catalyzes mitochondrial ubiquitination, leading to autophagic protein recruitment and clearance, while disease-causing parkin mutants are defective in this function (28). In agreement with Drosophila studies, parkin increases cell survival in PINK1-deficient cells, through a mechanism dependent upon macroautophagy (9).

The role of PINK1 in parkin-associated mitophagy has been further investigated by several research groups. PINK1 expression appears to be necessary for recruitment of YFP-parkin to chemically uncoupled mitochondria in HeLa cells (66), as decreased translocation of overexpressed parkin to mitochondria is observed in PINK1 knockout MEFs (34, 43) and in SH-SY5Y neuroblastomas with knocked down PINK1 (19). Overexpression of PINK1 in N27 cells (66) or specifically outer mitochondrial membrane PINK1 in HeLa cells (43) was shown to recruit parkin to the mitochondria in the absence of depolarization. It has been suggested that PINK1 is constitutively degraded by well-polarized mitochondria, with lack of proteolysis serving as a signal for mitophagy (34, 43). Full-length PINK1, whether endogenous (43, 66) or overexpressed (34) in HeLa cells or overexpressed PINK1 in rat cortical neurons and N17 neuroblastoma cells (43), is stabilized by mitochondrial depolarization. Expression of PINK1 mutants is not able to restore parkin translocation to the mitochondria in chemically depolarized PINK1-deficient MEFs (34, 43) or SH-SY5Y neuroblastoma cells (66), although parkin mitochondrial translocation and degradation induced by hydrogen peroxide appear to be independent of mitochondrial PINK1 accumulation (52). A series of parkin mutants has been overexpressed to determine the regions of the protein necessary for mitochondrial translocation and promotion of mitophagy (19, 34). It has been reported that VDAC is necessary for parkin-mediated mitophagy and is directly ubiquitinated by parkin (19), although other targets likely exist as well.

It is interesting that PINK1-parkin co-overexpression studies yielded different results from experiments employing RNAi knockdown of endogenous PINK1 in terms of autophagy/mitophagy, suggesting multiple roles for PINK1 and parkin in maintaining mitochondrial quality control. Interestingly, parkin is not necessary for autophagy induced by mitochondrial uncouplers (12), serving instead to prime depolarized mitochondria for engulfment, while PINK1 appears to modulate both autophagy and mitophagy. While the major endogenous forms of PINK1 in neuroblastoma cells are 50 kDa or less (9), transfection with full-length PINK1, the ~66 kDa form that accumulates with chemical depolarization, was shown by several groups to be necessary for promoting parkin mitochondrial translocation. It is possible that PINK1 function with respect to mitophagy is regulated by post-translational processing rather than degradation. Further studies are needed to resolve the role of PINK1 in autophagy and mitophagy, and much remains to be learned about the post-translational regulation of PINK1 processing, localization, binding interactions, kinase activity, and substrates.

Mitophagy has also been implicated in toxin models of Parkinson's disease. The complex I inhibitor 1-methyl-4-phenylpyridinium (MPP+) increases autophagy in SH-SY5Y cells and in primary midbrain dopaminergic neurons (75). Interestingly, MEK inhibitors, or dominant negative ERK2, reverse the increased autophagy and mitochondrial loss observed in several model systems, implicating a role for ERK/MAPK signaling in mitochondrial function and/or clearance (75). Several reports have indicated ERK1/2 plays a key role in regulating mitochondrial function (40, 47). Moreover, wild-type and constitutively active ERK2 induce mitophagy in the absence of other injuries, whereas a kinase-deficient ERK2 mutant does not (10). The direct localization of constitutively active ERK2 on mitochondria, which correlates with increased mitophagy (10), suggests the possibility that ERK-mediated phosphorylation of unknown targets may serve to target mitochondria for degradation.

Mitochondrial Transport and Clearance of Dysfunctional Mitochondria

As additional studies on factors that modulate mitophagy emerge, it becomes important conceptually to separate the regulation of autophagic flux from regulatory mechanisms that mediate cargo specification (Fig. 1). In classic polygonal cells, the distances between dysfunctional mitochondria, endoplasmic reticulum, and lysosomes are relatively small, and similar factors could simultaneously regulate **autophagy induction** and **cargo specification**. For example, parkin functions only in cargo specification, but is not necessary for FCCP-induction of autophagy, while Nix regulates both general autophagy induction and mitophagy (12). While ERK1/2 induces both general autophagy and mitophagy, only the effects on mitophagy are correlated with the degree of mitochondrial ERK1/2 translocation (10). Due to the extreme cellular distances between the neuronal soma and mitochondria-rich axon terminals and dendritic spines, additional regulatory factors involving microtubule- and actin-dependent transport undoubtedly play important roles in neuronal mitophagy.

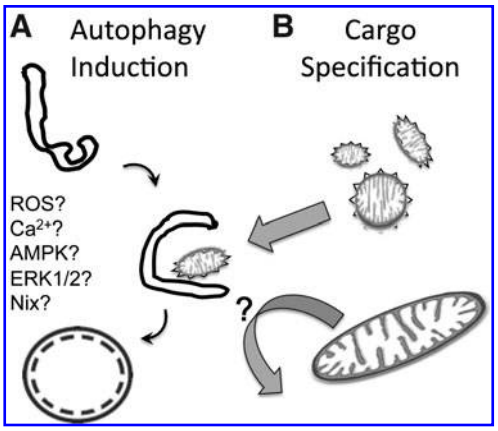

FIG. 1. Mitophagy depends upon coordinated upregulation of autophagy and specification of mitochondria as cargo. (A) Although it is unclear what triggers autophagy in response to mitochondrial injury, potential mediators known to induce autophagy include reactive oxygen species (ROS), calcium dysregulation, AMP kinase,and ERK1/2. The mitochondrial protein Nix has also been implicated in uncoupling-induced autophagy. These upstream signals engage the core autophagy machinery, which is necessary, but not specific for mitophagy. **(B)** Current studies are beginning to elucidate mechanisms by which damaged or dysfunctional mitochondria, schematized here by fission and surface alterations, may be selectively targeted for mitophagy. Whether there are also mechanisms to actively exclude functional mitochondria is unknown.

Hypothetically, there are at least two potential models for mitophagy of damaged distal mitochondria in neurons. Importantly, these models are not mutually exclusive. Selective autophagy of dysfunctional mitochondria could be accomplished through retrograde mitochondrial transport to perinuclear regions with high autophagic sequestration potential (Fig. 2). Indeed, use of the mitochondrial potential-sensing dye JC-1 revealed that mitochondria with higher potentials preferentially undergo anterograde transport, while those with lower potentials are transported towards the soma (38). While the complex III inhibitor antimycin also elicits retrograde transport, localized micropipette application of CCCP completely blocks mitochondrial transport in neurons (38). If axonal transport is important to autophagy of somatic or neuritic mitochondria, there may be differences in the ability of CCCP to induce mitophagy in neurons compared to other cell types. Further experiments in primary neurons may reveal whether selective axonal transport is important for neuronal mitophagy.

While the first model is consistent with the known role of the endoplasmic reticulum as a source for nascent autophagic membranes (35), prolonged transport of severely damaged mitochondria could present additional risks of apopotosome activation during transport through release of pro-death mediators. The PD-linked protein α-synuclein can prevent such retrograde death signals by scavenging released cytochrome c into crosslinked protein–lipid complexes similar to Lewy bodies (2). Nevertheless, more direct mechanisms to recognize and rapidly sequester damaged mitochondria would be beneficial to post-mitotic neurons. Indeed, early autophagosomes are often observed in neuritic compartments (46, 51). Thus, axonal or dendritic transport of autophagosomes may serve as alternative mechanisms to deliver dysfunctional mitochondria to the lysosome.

What then could be the source of autophagic membranes and/or the mechanism(s) by which damaged mitochondria could be recognized for autophagy in these distal neuritic and synaptic compartments? Recent data in yeast and in mammalian cells implicate mitochondria as alternative sources of autophagosome membranes (in situations where the mitochondria are not themselves undergoing autophagy) (21, 53). Through recruitment of p62, which binds ubiquitinated protein aggregates as well as LC3 itself (48), it is possible that parkin-mediated ubiquitination of mitochondrial surface constituents (19, 27) could serve as cargo recognition signals to directly facilitate selective mitophagy (Fig. 3), although the involvement of p62 in mitophagy has been questioned (44).

To Eat or Not to Eat: Biological Outcome(s) of Mitophagy in Neurons

Just as autophagy itself can fulfill multiple context-dependent roles, ranging from homeostatic housekeeping functions to either cell survival or cell death in the induced

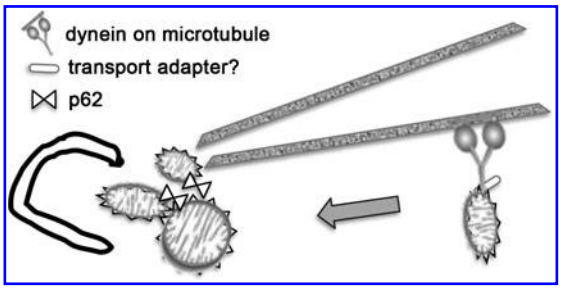

FIG. 2. Model 1: Cargo enrichment by selective axonal transport. In this model, damaged or dysfunctional mitochondria are recognized through an unknown adapter for retrograde dynein-dependent transport to the soma. Ubiquitinated mitochondria may also undergo p62-mediated aggregation. The increased concentration of damaged mitochondria in the perinuclear region in turn facilitates their relatively selective clearance by autophagy. PINK1, which is stabilized on depolarized mitochondria, has been reported to interact with Miro (55), a transport adapter involved in both anterograde and retrograde transport. Whether this interaction has functional effects on mitochondrial transport, and whether axonal transport regulates mitophagy, remain to be determined.

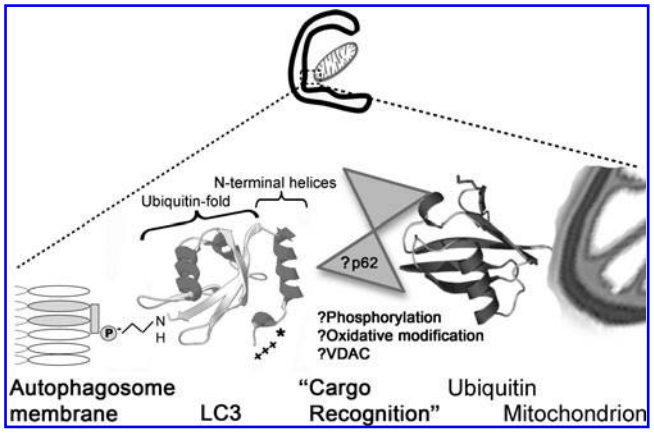

FIG. 3. Model 2: Localized cargo recognition via adapter proteins. In analogy to yeast mitophagy or autophagy of ubiquitinated protein aggregates (48), changes on the surface of damaged or dysfunctional mitochondria may be recognized by adapter proteins that link to components of the core autophagy machinery. Parkin-mediated ubiquitination of mitochondria, for example, could lead to recruitment of the ubiquitin-binding protein p62/SQSTM1, which is also capable of binding the N-terminal helical region of LC3 (57). While the roles of p62 and VDAC1 ubiquitination in FCCP/CCCP-induced mitophagy remain unresolved (19, 44), macromolecular components of the mitochondrial permeability transition pore and phospholipid oxidation represent attractive potential "eat-me" signals. The crystal structure of LC3 exhibits positively charged surface patches (61), which could hypothetically interact with phosphorylated or oxidatively modified macromolecules exposed on the mitochondrial surface. Interestingly, a recently described PKA phosphorylation site between the α1 and α2 helices of LC3 (*asterisk*) regulates induced, but not basal autophagy (5).

state (14), the biological outcome of increased mitophagy most likely varies, depending upon the intensity and nature of the stimulus, the cell type, and the context of induction. Certainly, it is beneficial to eliminate mitochondria that are unneeded due to unavailability of mitochondrial substrates in yeast, or during appropriate developmental stages in the lens cells or erythrocytes. Replacement of effete or impaired mitochondria could also reduce electron leaks or release of death mediators. However, what is the outcome of enhanced mitophagy in the context of the highly mitochondrially-dependent neuron? Can we separate effects attributable to the degree of mitochondrial damage itself, from the mitophagic response to such damage?

Even in a transformed neuronal cell line, we have found that the effects of inhibiting autophagy/mitophagy seem to differ, depending upon context. In the acute MPP+ toxicity model, decreasing or slowing the rate of autophagy leads to enhanced survival, although mitochondria are still clearly damaged (75). Interestingly, autophagy in this system proceeds independently of the Beclin 1/class III phosphatidylinositol-3 kinase system. In contrast, inhibiting Beclin 1-dependent autophagy in PINK1-deficient cells exacerbates cell death, whereas enhanced mitophagy via parkin overexpression confers protection (9). Additional studies are warranted to determine if the degree of mitochondrial damage is the determining factor, or if there are fundamental differences in the regulation of mitophagy under compensatory versus pro-death situations.

To further characterize metabolic responsiveness and reserves in neurons versus other cell types, we performed comparative metabolic analyses of primary mouse embryonic neuron cultures and the immortalized cervical cancer HeLa cell line (Figs. 4–6), using a Seahorse XF24 analyzer that allows simultaneous live cell measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Plotting OCR vs. ECAR allows comparison of the relative dependence on mitochondrial and glycolytic metabolism of different cell types under basal and stressed conditions. Primary neuron cultures exhibit significantly higher OCR and lower ECAR values compared with HeLa cells, indicating a greater dependence on oxidative phosphorylation (Fig. 4). OCR in both primary neuron cultures and HeLa cells is inhibited by oligomycin (Fig. 5). However, while primary neuron cultures exhibit a substantial mitochondrial reserve capacity revealed by the ionophore uncoupler p-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP), HeLa cells do not increase OCR over basal levels after the addition of FCCP (Fig. 5). This may indicate that mitochondria in HeLa cells are functioning close to their maximum oxygen consumption capacity under basal conditions with no detrimental effects. In contrast, the mitochondrial reserve capacity or spare capacity is important for neurons to survive periods of increased functional demand and stress (45, 72).

The basal ATP levels in primary neuron cultures were significantly reduced by inhibition of mitochondrial ATP production with oligomycin, or uncoupling of the electron transport chain with FCCP (Fig. 6), indicating a substantial dependence upon mitochondrial respiration for ATP generation, despite some degree of compensatory upregulation of glycolysis in the cultures that may be attributable to low levels of glia (not shown). In contrast, HeLa cells are able to fully maintain their basal ATP levels (Fig. 6) through

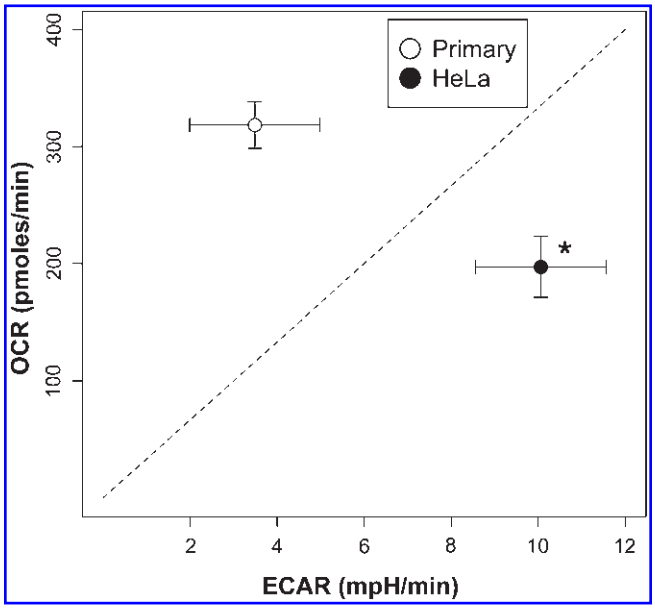

FIG. 4. Metabolic differences between HeLa cells and primary neuron cultures. Parameters of metabolic function were compared between primary cortical neuron cultures from C57BL/6 mice and HeLa cells cultured in glucose-containing media. Dissociated E15 cortical neurons were plated at 5×10^4/well in a poly-L-lysine coated Seahorse 24-well plate and studied at 7 DIV. The extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR) were measured using a Seahorse XF24 Flux Analyzer. Data was normalized to relative protein content by Coomassie dot blot performed at the end of the assay. $^*p<0.01$ vs. primary neurons for both OCR and ECAR.

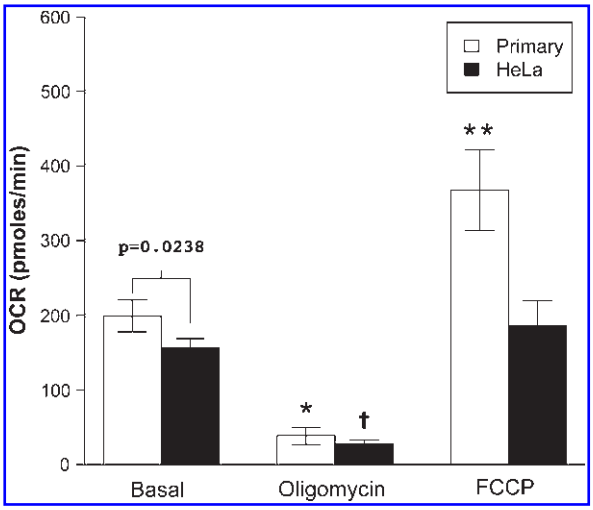

FIG. 5. OCR analysis reveals a robust spare respiratory (reserve) capacity in neurons, but not in HeLa cells. Following quantification of basal OCR, oligomycin (1 μ*M*), and then FCCP (300 n*M*) were added to each well. Mitochondrial-specific OCR was obtained by subtracting the non-mitochondrial OCR (in the presence of antimycin A) from total OCR. $^*p<0.01$ vs. basal primary; $^\dagger p<0.05$ vs. basal HeLa., $^{**}p<0.001$ vs. basal primary & vs. FCCP HeLa.

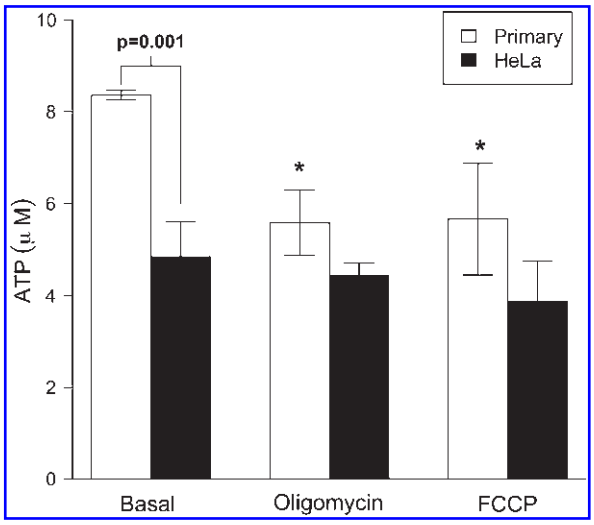

FIG. 6. Unlike primary neurons, ATP levels in HeLa cells are not affected by mitochondrial inhibitors. ATP levels were measured 30 min after treatment with the indicated inhibitors using a luminescence-based assay. All data are means ± standard deviation. $^*p<0.01$ vs. basal primary.

nonmitochondrial mechanisms, consistent with their preference for glycolytic metabolism (Fig. 4). These data suggest that survival of HeLa cells may not be dependent on mitochondrial function, whereas neurons exhibit metabolic compromise 30 min after addition of oligomycin or FCCP.

These fundamental differences in sensitivity to mitochondrial uncouplers may underlie potential differences in neuronal responses to mitophagy. While cells with high glycolytic potential can survive without functional mitochondria, and indeed seem to prefer glycolysis even under aerobic conditions (65), clearance of mitochondria commits neurons to programmed cell death even in the presence of apoptosis inhibitors (63, 71). Perhaps this is one reason that neurons exhibit robust respiratory reserve capacities, such that a portion of damaged mitochondria can be compensated through increased respiration of the remaining mitochondria. Furthermore, the ability or inability to maintain ATP levels may determine whether neurons are able to initiate energy-demanding processes such as autophagy/mitophagy, apoptosis, or regenerative responses. Given the importance of mitochondria to neuronal function, it is likely that safeguards and regulatory mechanisms to prevent excessive clearance of mitochondria will be identified in neurons and other highly aerobic cell types.

In summary, it is clear that all aspects of mitochondrial homeostasis, from biogenesis, through functional regulation, to turnover, are relevant to maintaining neuronal health and in forestalling neurodegenerative diseases. Exciting work in Parkinson's disease models implicate mitophagy regulation as a key factor. Future studies that address the regulation and functional impact of mitophagy in neurons, and in other model cell systems exhibiting neuron-like metabolic features, are needed to enhance our understanding of this fundamental process.

Acknowledgments

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Abbreviations Used

ECAR = extracellular acidification rate
ERK1/2 = extracellular signal-regulated protein kinases 1 and 2
FCCP = p-trifluoromethoxy carbonyl cyanide phenyl hydrazone
MPP+ = 1-methyl-4-phenylpyridinium
OCR = oxygen consumption rate
PD = Parkinson's disease
PINK1 = PTEN-induced kinase 1
TCA = tricarboxylic acid

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