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Forum Review

Mitochondrial Inhibition and Oxidative Stress: Reciprocating Players in Neurodegeneration

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ABSTRACT

Although the etiology for many neurodegenerative diseases is unknown, the common findings of mitochondrial defects and oxidative damage posit these events as contributing factors. The temporal conundrum of whether mitochondrial defects lead to enhanced reactive oxygen species generation, or conversely, if oxidative stress is the underlying cause of the mitochondrial defects remains enigmatic. This review focuses on evidence to show that either event can lead to the evolution of the other with subsequent neuronal cell loss. Glutathione is a major antioxidant system used by cells and mitochondria for protection and is altered in a number of neurodegenerative and neuropathological conditions. This review also addresses the multiple roles for glutathione during mitochondrial inhibition or oxidative stress. Protein aggregation and inclusions are hallmarks of a number of neurodegenerative diseases. Recent evidence that links protein aggregation to oxidative stress and mitochondrial dysfunction will also be examined. Lastly, current therapies that target mitochondrial dysfunction or oxidative stress are discussed. *Antioxid. Redox Signal.* 7, 1117–1139.

INTRODUCTION

Neurodegenerative diseases, many environmental neurotoxicants, and neuropathological conditions such as hypoxia and ischemia affect distinct brain regions and neuronal populations. For many of these, the underlying reason for the selective vulnerability is unknown, although the concept of pathoclisis, *i.e.,* that intrinsic differences in cellular metabolism underlie the selective cellular response to insult, first proposed by Vogt in 1937 (248) remains a tenable hypothesis. Although the regions and cells that degenerate in various illnesses and insults are distinct, several features are common to many of these conditions and include mitochondrial dysfunction, altered antioxidant defenses, and oxidative damage. Mitochondrial dysfunction has been described in Parkinson's disease (PD) (193, 212), Huntington's disease (HD) (237), Alzheimer's disease (AD) (180), amyotrophic lateral sclerosis (ALS) (50), hereditary spastic paraplegia (HSP) (43), Friedreich's ataxia (FA) (35), and parasupranuclear palsy (PSP) (192). In these diseases, as well as in the neurodegenerative synucleinopathies, diabetic neuropathy, hypoxia, and ischemia, abundant evidence exists for oxidative damage to tissue marked by oxidized proteins, lipids, and DNA (3, 17, 36, 57, 59, 84, 113). With a few exceptions, the molecular cause for the perturbation of mitochondrial function is not known. Also unknown is if disruption of mitochondrial function is a primary event that leads to oxidative stress and damage or, conversely, if oxidative stress lies upstream of and contributes to mitochondrial dysfunction. Although it will be important to determine the initiating event in any disease process, an understanding of how these events interact to ultimately damage neurons will help to elucidate potential targets for intervention.

PD is typical of may neurodegenerative diseases in that the sporadic form of the disease, which represents ~90% of the Parkinson population, is characterized by mitochondrial dysfunction (193, 212) and oxidative damage (for review, see 117). In PD, dopamine neurons in the substantia nigra and their terminal projections to the striatum degenerate. Because of the central finding of mitochondrial deficits and oxidative damage to so many degenerative conditions, this review will focus on the interactive nature and cyclical fueling of deleterious events brought on by these forces, with an emphasis on

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the dopamine system. As glutathione is a major antioxidant system used by cells and mitochondria for neuroprotection and is perturbed in a number of neurodegenerative and neuropathological conditions, this review will also address the multiple defensive roles played by glutathione during mitochondrial inhibition or oxidative stress. Several neurodegenerative diseases are characterized by protein aggregation and inclusions. Evidence that links this to oxidative stress and mitochondrial dysfunction will also be examined. Lastly, past and current antioxidant approaches to the treatment of neurodegenerative diseases will be discussed. The review will begin with an overview of reactive oxygen species (ROS) production by mitochondria and the antioxidant systems used by mitochondria to defend against oxidative attack.

ROS GENERATION BY MITOCHONDRIA

Mitochondria established a residence in eurkaryotic cells over a billion years ago and, in so doing, provided the means by which oxygen utilization could be coupled to efficient large-scale energy production (94). Mitochondria provide a passageway for reducing equivalents derived from fuel molecules to molecular oxygen. Nicotine adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) are the electron messengers for these fuel molecules passing their electrons to subsequent carriers known as the electron transport chain (ETC) proteins found as complexed aggregates housed in the inner membrane of the doubled membrane mitochondrion. Mammalian mitochondria contain at least 20 electron carriers, most of which are grouped into four major ETC complexes plus an ATP synthase complex (Fig. 1). ATP production is coupled to electron transfer through complexes I, III, and IV via a proton gradient generated at these three sites. Translocation of four protons is needed for the generation of 1 ATP. Given this stochiometry, it has been determined that NADH oxidation would yield 2.5 ATPs, whereas $FADH_2$ oxidation would yield 1.5 ATPs (182). The introduction of aerobic metabolism of fuel molecules by mitochondria greatly enhanced energy production, thus providing a major biological advantage to cells. The aerobic metabolism of glucose increased the yield of ATP per glucose molecule oxidized severalfold as compared with its metabolism via anaerobic pathways.

The acquisition of power in the form of energy production that was provided by mitochondria did not come without its cost to the cell. Free radicals such as superoxide ($O_2^{\cdot -}$) and hydroxyl radicals ($HO^{\cdot}$), as well as ROS such as hydrogen peroxide (H_2O_2), are known to damage cellular proteins, lipids, and DNA and are thought to be the major contributors to cellular aging (24). In the 1970s, pivotal studies by Britton Chance and colleagues (34, 45) demonstrated that mitochondria were a major source of free radical production in cells. The passage of electrons down the chain to molecular oxygen does not occur with 100% integrity, and 1% to as much as 5% of the total amount of O_2 consumed can form $O_2^{\cdot -}$ (45). This low-level leakage of electrons to O_2 is not trivial. It has been esti-

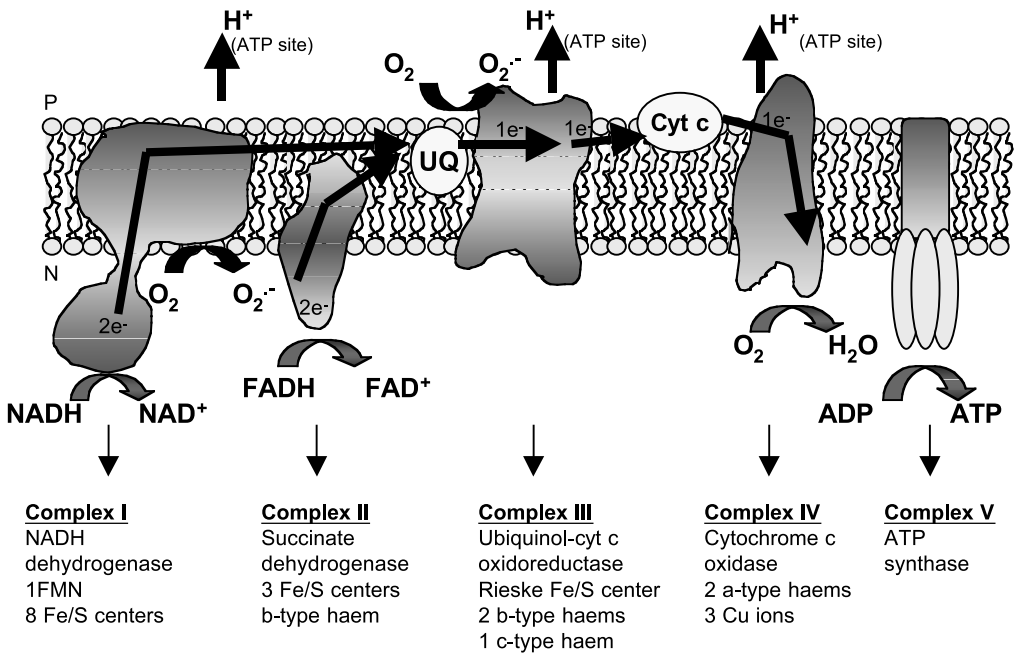

FIG. 1. The mitochondrial electron transport carriers. Mammalian mitochondrial electron carriers are grouped into four complexes plus an ATP synthase complex. Pairs of electrons travel from either NADH through complex I or $FADH_2$ through complex II to ubiquinone to reduce it to ubiquinol. In addition, ubiquinone can receive electrons from $FADH_2$ through enzyme complexes other than complex II, *i.e.,* glycerol-3-phosphate dehydrogenase and electron-transferring flavoprotein-ubiquinone oxidoreductase (not shown). Beyond ubiquinol, electrons are transferred individually through complex III, cytochrome *c*, and complex IV to reduce O_2 to H_2O. ATP synthesis at complex V is coupled to electron transfer at complexes I, III, and IV via a proton gradient. Leakage of electrons to O_2 to form the free radical superoxide ($O_2^{\cdot -}$) occurs at complexes I and III.

mated that with a leakage of 1–2%, a 60-kg woman would produce 160–320 mmol of $O_2^{\cdot -}$ per day and an 80 kg man 215–430 mmol of $O_2^{\cdot -}$per day (40).

Although thermodynamically all of the electron carriers in their reduced form have the potential of passing electrons to O_2, evidence indicates that there are only two free radical-forming sites within the mitochondrial ETC (41); the ubiquinone (UQ) site of complex III and a still disputed site in complex I. Ubiquinol (UQH_2), the fully reduced form of UQ of complex III, falls victim to the necessity of passing its pair of electrons to downstream carriers one electron at a time. Cytochromes and iron-sulfur non-heme iron proteins accept a single electron. The oxidation of UQH_2 and passage of individual electrons to the bc_1 complex of III, known as the Q cycle, result in the formation of the free radical ubisemiquinone ($UQ^{\cdot -}$). During Q cycle electron transfer, a molecule of UQH_2 diffuses to the cytoplasmic face of the inner mitochondrial membrane (P face). At this site, one electron from UQH_2 is transferred to the Rieske protein, 2Fe/2S of complex III, leaving a $UQ^{\cdot -}$ near the P face of the membrane. Usually the unpaired electron of $UQ^{\cdot -}$ is then transferred to cytochrome *b* of complex III to continue the Q cycle and the ultimate passage of electrons to O_2 (see 182 for a complete description of electron transfer through the Q cycle). If the $UQ^{\cdot -}$ has access to molecular oxygen at the P side of the inner mitochondrial membrane prior to its electron transfer to cytochrome *b*, the electron can pass to O_2 to form $O_2^{\cdot -}$. Much of the Q cycle of electron transfer within complex III and the production of ROS at this site were deduced with the use of pharmacological agents, such as antimycin A and myxothiazol, that inhibit various specific sites in complex III. Antimycin A blocks the transfer of electrons through the *b* cytochromes, promoting the presence of $UQ^{\cdot -}$ at the P side of the membrane and enhancing ROS production. In contrast, myxothiazol blocks the initial transfer of electrons from UQH_2 to the Rieske protein, preventing $UQ^{\cdot -}$ formation and abrogating ROS formation. The rate of $O_2^{\cdot -}$ production at complex III is dependent on the metabolic state. The more reduced the electron carriers in the ETC, the greater the formation of $O_2^{\cdot -}$. Thus, active respiration (state 3) is characterized by a low rate of $O_2^{\cdot -}$, whereas resting respiration (state 4) is associated with a high rate of $O_2^{\cdot -}$ formation.

The UQ site of complex III is generally accepted as the source of ROS formation within complex III. The site of electron leakage in complex I, however, is less certain. Evidence exists for electron leakage from Fe-S cluster N2 (82) (the terminal Fe-S cluster prior to UQ), from the flavin mononucleotide of complex I (155) (the direct electron acceptor from NADH), or from a complex I-associated $UQ^{\cdot -}$ (247). Although the exact site(s) of electron leakage from complex I needs further elucidation, it is clear that this is a site distinct from that in complex III. A number of investigators have shown that inhibition of complex I by the pesticide rotenone increases ROS production (18, 149, 219, 262) at a site upstream of the rotenone binding site (109). In addition, in the absence of ADP, succinate oxidation via complex II produces a high rate of $O_2^{\cdot -}$ production that is associated with increased reduction of NADH and can be abolished by rotenone (155). These findings are consistent with reversed electron flow from complex II, through complex I and a site of ROS production upstream of the rotenone binding site.

In addition to ROS production by electron transport carriers, the inner mitochondrial membrane and matrix have been shown to contain nitric oxide synthase (NOS) (19, 138). Nitric oxide (NO), produced as a product of NOS activity, can reversibly inhibit complex IV by competing for binding with O_2 (37, 85). Mitochondrial NO has been proposed to serve a physiological role in modulation of O_2 uptake (201), and in regulation of matrix pH (83). On the other hand, NO can increase $O_2^{\cdot -}$ production in mitochondria (200). NO reacts with $O_2^{\cdot -}$ to form peroxynitrite, which can nitrate proteins and alter function. Nitration of mitochondrial proteins (244) including complex I (179) has been demonstrated. Accumulation of nitrated proteins in mitochondria can lead to mitochondrial dysfunction (58). In addition, the formation of NO has been implicated in triggering mitochondrial permeability transition and apoptosis (14). Recent studies suggest that α-ketoglutarate dehydrogenase, a Krebs cycle enzyme (Adam Vizi, this issue), and mitochondrial uncoupling proteins (Kim-Han and Dugan, this issue) may also contribute to ROS formation, and the reader is referred to these reviews for details. Which of the different avenues of ROS production by mitochondria represent the more relevant source during physiological or pathophysiological states remains enigmatic. It is clear, however, that in relation to other cellular constituents, mitochondria generate substantially more free radicals. The steady-state concentration of $O_2^{\cdot -}$ in the mitochondrial matrix is approximately five- to 10-fold higher than in the cytosol or nuclear spaces (40). This poses a significant challenge for the organelle to eliminate reactive species and prevent damage.

MITOCHONDRIAL DEFENSE MECHANISMS

Aerobic metabolism with its consequent potential for oxidative damage to the cell necessitated the evolution of antioxidant mechanisms to neutralize free radical attack. Mitochondria contain a number of antioxidant defense strategies to remove $O_2^{\cdot -}$, H_2O_2, and $HO^{\cdot}$, nitrosating species, and peroxynitrite (Table 1). As indicated in the prior section, the immediate free radical generated by the ETC at complex I and complex III is $O_2^{\cdot -}$. In an intact cell, $O_2^{\cdot -}$ formed from $UQ^{\cdot -}$ of complex III occurs at the cytoplasmic face of the inner mitochondrial membrane, and it is thought that the $O_2^{\cdot -}$ formed is directed to the intermembrane space and cytoplasm (182), as well as mitochondrial matrix (100). In contrast, $O_2^{\cdot -}$ formed in complex I predominately enters the matrix (100). Although not a strong oxidant, $O_2^{\cdot -}$ can be cytotoxic. Unopposed $O_2^{\cdot -}$ formation can disrupt enzyme activities by forming unstable complexes with transition metals of prosthetic groups, produce peroxynitrite through interaction with NO, reduce ferric iron to its ferrous state to promote Fenton chemistry, and abstract protons from weak organic acids to form hydroperoxides and other oxidizing products (see 47 for discussion).

The disproportionation of $O_2^{\cdot -}$ to H_2O_2 serves as an important first line of defense. Superoxide dismutases (SOD) are metalloenzymes that catalyze the disproportionation of $O_2^{\cdot -}$ to H_2O_2 and O_2. In eurkaryotic cells, two classes of SOD exist based on the transition metal present at the active site of the

Table 1. Antioxidant Defense Systems in Mitochondria

Antioxidant	*Function*	*Location*
Mn-SOD	Disproportionation of $O_2^{\cdot -}$ to H_2O_2	Matrix
Cu,Zn-SOD	Same as Mn-SOD	Intermembrane space
Glutathione (GSH), (nonenzymatic)	Directly removes hydroxyl and carbon-based radicals	Matrix
	Protein reduction	
	Tocopherol, ascorbate reductant	
GSH (enzymatic) with glutathione peroxidase	Catalyzes removal of H_2O_2 with glutathione	Matrix
		Contact points in intermembrane space
GSH (enzymatic) with glutathione reductase + NADPH	Catalyzes reduction of oxidized glutathione using NADPH as reductant	Matrix
		Contact points in intermembrane space
GSH (enzymatic) with glutaredoxin	Catalyzes reversible glutathionylation of protein cysteine sulfhydryl groups	Grx-2 in matrix
	Catalyzes reduction of ascorbate using glutathione	Grx-1 in intermembrane space
Peroxiredoxin	H_2O_2 scavenger using thioredoxin or glutathione	
	Possible peroxynitrite scavenger	
Thioredoxin/thioredoxin reductase	Reduction of mixed disulfides	Matrix
	Reduction of H_2O_2 via peroxiredoxin	
	Reduction of ascorbate	
	Thioredoxin reductase and NADPH used for recycling of oxidized thioredoxin	
Ascorbate	Directly scavengers hydroxyl, alkoxyl, peroxyl radicals, superoxide radicals, and nonradical species: hyperchlorous acid, ozone, singlet O_2, nitroxide, peroxynitrite	Matrix
	Reductant for tocopherol and thiyl ($GS\cdot$) radicals	
α-Tocopherol	Reduction of peroxyl radicals to prevent propogation of lipid peroxidation	Lipid bilayer of inner and outer mitochondrial membranes
Coenzyme Q10	Reduction of peroxyl radicals	Inner mitochondrial membrane
	Reduction of tocopherol radical	

enzyme. Cu,Zn-SOD is the predominate form in the cytosol, whereas Mn-SOD is the form in mitochondrial matrix. Cu,Zn-SOD was also recently identified in the membrane space between the inner and outer mitochondrial membranes (186). Although H_2O_2, the product of the SOD reaction, is not a free radical as it lacks an unpaired electron, it is capable of mediating oxidative damage. H_2O_2 can readily diffuse across membranes and is capable of producing damage at sites distant from its origin. In the presence of free transition metals, H_2O_2 can react to form highly reactive $HO^{\cdot}$ or alkoxyl radicals (Fenton chemistry) (126). H_2O_2 removal is, therefore, another critical line of defense that is served by glutathione in association with glutathione peroxidase and glutathione reductase.

Reduced glutathione (GSH) is a tripeptide consisting of glutamate, cysteine, and glycine and is the most abundant nonprotein thiol in cells. GSH is synthesized in the cytosol and transported into mitochondria. Glutathione peroxidase, a selenoprotein, catalyzes the two-electron reduction of H_2O_2 using GSH as the hydrogen donor. Glutathione peroxidase activity is predominately in the soluble matrix fraction; however, a small portion (10–15%) is present near contact sites between the inner and outer mitochondrial membranes (190). The reduction of H_2O_2 by glutathione produces oxidized glutathione (GSSG): two molecules of glutathione linked together by a disulfide bond at their cysteine sulfhydryl moieties. GSSG requires recycling back to GSH for its continued function as an antioxidant. This is accomplished by glutathione reductase at the expense of NADPH. The distribution of glutathione reductase in mitochondria is similar to that of the peroxidase (190). Cytosol contains catalase as an additional mechanism for removal of H_2O_2. In general, with the exception of mitochondria from heart, little catalase is present in mitochondria from other tissues. H_2O_2 removal can also occur via peroxiredoxin (78, 184). The peroxiredoxins are a family of enzymes that use thioredoxin-2 to reduce H_2O_2. Peroxiredoxin-3 is specific to mitochondria (121). At present, there has been little work done on this family of enzymes in the brain, although a few reports suggest that it may be important in neurodegenerative disease and pathology. Altered peroxiredoxin activity was reported in AD, PD, and Downs syndrome (136). Interestingly, bacterial peroxiredoxin can directly remove peroxynitrite (38). In rodent hippocampus, overexpression of mitochondrial peroxiredoxin-3 protected cells from excitotoxic injury and prevented nitration of proteins, suggesting that peroxiredoxin may directly remove peroxynitrite in mammalian cells as well (104).

Protein oxidation occurs during oxidative stress, and it is important to maintain proteins in a reduced state. Thioredoxin plus thioredoxin reductase can serve as a hydrogen donor to

reduce a variety of mixed disulfides (glutathione, cysteine, cystamine, etc.) (112). Oxidized thioredoxin is then recycled back to its reduced form with NADPH and thioredoxin reductase. Thioredoxin can also donate its protons via peroxiredoxin to reduce peroxides (44). Another member of the thioredoxin family, glutaredoxin, was recently identified in the CNS (15). A mitochondrial form of the enzyme was cloned (86, 157), and a functionally active form of glutaredoxin was demonstrated in brain and liver mitochondria (73). Unlike thioredoxin, glutaredoxin is specific for protein-mixed disulfides containing glutathione (93). Glutaredoxin can catalyze both the attachment of glutathione to a cysteine residue on a protein to form a glutathione-protein-mixed disulfide (thiolation) or remove the glutathione to return the protein to its reduced state (dethiolation) (275). Although, both thioredoxin and glutaredoxin can dethiolate proteins containing glutathione, glutaredoxin carries this out more effectively (119) and is thought to be the principal thiolating/dethiolating enzyme for mixed disulfides containing glutathione in the cell.

In addition to the enzymatic removal of free radicals, glutathione and ascorbate can nonenzymatically remove free radicals in the aqueous phase of cells and organelles. GSH can react directly with free radicals, predominately $HO^{\cdot}$ and carbon-based radicals. The unstable thiyl radical $GS^{\cdot}$ that is formed is reduced back to GSH by ascorbate or alternatively may form GSSG (62). Ascorbate, because of its low reduction potential, is an excellent scavenger of radical species, *i.e.,* $HO^{\cdot}$, alkoxyl, and peroxyl radicals, $O_2^{\cdot -}$, as well as nonradical species such as hypochlorous acid, ozone, singlet oxygen, nitrosating species, nitroxide, and peroxynitrite (42). Ascorbate is also used to regenerate other small-molecule antioxidants such as glutathione and α-tocopherol (99). Ascorbate can compete with SOD for removal of $O_2^{\cdot -}$ (116) and can account for upwards of 50% of $O_2^{\cdot -}$ removal (238). Removal of free radicals by ascorbate leads to its oxidation to dehydroascorbate. Both thioredoxin and glutaredoxin have dehydroascorbate reductase activity (167, 253) for the recycling of ascorbate.

Protection against lipid peroxidation requires that an antioxidant be accessible to the hydrophobic milieu of the lipid bilayer. Vitamin E and UQH_2 are both lipophilic and capable of preventing lipid peroxidation. Vitamin E is part of a family of lipophilic phenolic antioxidants called tocopherols. Vitamin E is incorporated into the lipid bilayer of both the inner and outer mitochondrial membranes (242). Tocopherols interact primarily with peroxyl radicals. Ascorbate, glutathione, and UQH_2 can reduce the tocopherol radical that results from this interaction so that tocopherol can continue to serve to prevent propagation of lipid peroxidation.UQH_2, an integral part of the chain of electron carriers in the inner mitochondrial membrane, not only functions as a prooxidant and major source of $O_2^{\cdot -}$, but can act as an antioxidant by directly preventing lipid peroxidation and indirectly recycling vitamin E (for review, see 115). The antioxidant battery of defenses within mitochondria insures that, in most instances, maintenance and repair are upheld. When perturbed, however, excess ROS in mitochondria can lead to cytochrome *c* release and the triggering of apoptotic events, as reviewed by Jemmerson *et al.* in this issue. Alternatively, excess ROS can further impair mitochondrial function, leading to severe ATP depletion and necrosis.

IMPAIRED MITOCHONDRIA CAN GENERATE ROS BY DIRECT AND INDIRECT MECHANISMS

Direct ROS formation

During normal mitochondrial respiration, 1–5% of the total O_2 consumed is partially reduced to $O_2^{\cdot -}$ by electron leakage from components of complexes I and III. Impairment of mitochondrial function results in a further enhancement of ROS production (40). This has been borne out in many *in vitro* and *in vivo* experiments involving toxins and toxicants that inhibit complex I (149, 219, 262), complex II (140, 215, 270), complex III (204), and complex IV (97, 172). Restriction of electron flow results in the reduction of electron carriers upstream of the site of impairment and increases the probability of leakage of electrons to molecular oxygen. In addition, through activation of mitochondrial NOS, NO production can directly impede electron flow to O_2 at complex IV, subsequently increasing $O_2^{\cdot -}$ formation and setting the stage for peroxynitrite generation. Mitochondrial dysfunction can, therefore, lead to enhanced ROS production through direct generation by mitochondria (Fig. 2). As mentioned above, many neurodegenerative diseases are associated with defects in mitochondrial function and oxidative damage. Investigators have been successful in recapitulating many of the features of specific neurodegenerative diseases with mitochondrial toxins. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a miperedine analogue, used unwittingly in the 1980s by drug users, produced a rapid advanced parkinsonism characterized by loss of nigral dopamine neurons (16). MPTP, via its metabolite, 1-methyl-4-phenylpyridinium (MPP^+), was found to be an inhibitor of complex I (183) and has since been used extensively to model PD. The pesticide rotenone, a potent complex I inhibitor, was recently shown to not only reproduce the relatively selective loss of dopamine neurons, but also recapitulate the protein aggregates and inclusion bodies observed in the disease (27) when administered chronically and at low doses. 3-Nitropropionic acid (3-NPA), a mycotoxin found in the plant, *Indigofera endecaphylla*, and fungus, *Arthrinium,* is a suicide inactivator of succinate dehydrogenase (complex II) (8). Consumption of *Indigofera* by grazing animals and sugarcane spoiled by *Arthrinium* in humans has led to dystonia and other neurological problems (156, 176). 3-NPA, when administered peripherally to rats over several days, causes selective damage to subpopulations of γ-aminobutyric acid (GABA) neurons in the striatum characteristic of HD (20). Direct infusion of 3-NPA into the rat striatum, however, produces less selective damage (22). Malonate, a competitive, reversible inhibitor of succinate dehydrogenase (complex II), at low concentrations, selectively damages rat dopamine neurons (4, 230, 265, 267), thus modeling PD. At higher concentrations of malonate, GABA neurons are also affected (6, 159, 267). A common feature of all of the mitochondrial poisons mentioned above is that the damage can be ameliorated by either enhancing antioxidant mechanisms (23, 129, 203, 216, 266) or preventing or eliminating free radicals (141, 165, 213, 268). It is very clear from the abundant literature that oxidative stress is a downstream mediator of mitochondrial dysfunction and a major contributing factor to neuronal loss.

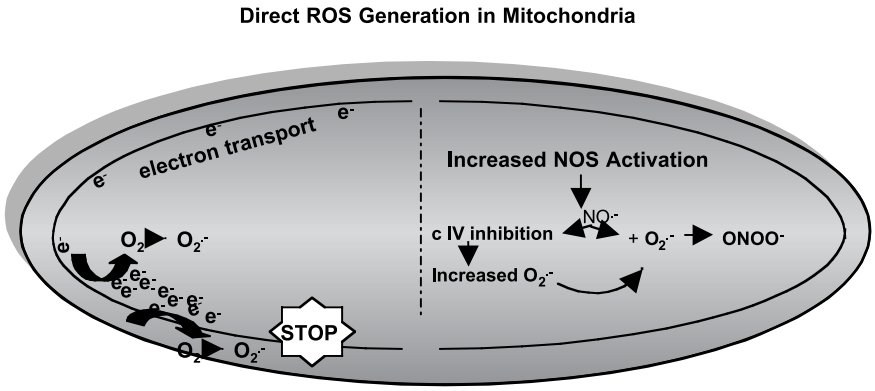

FIG. 2. Direct generation of ROS by mitochondria during mitochondrial dysfunction. Two major pathways can generate ROS in mitochondria during mitochondrial dysfunction: leakage of electrons to O_2 to form $O_2^{\cdot-}$ via the electron transport chain and activation of mitochondrial NOS. During normal respiration, there is some leakage of electrons to O_2 at complexes I and III (see Fig. 1). More leakage occurs when electron carriers are more fully reduced as occurs during resting state 4 respiration. When electron flow is impeded through genetic mutation, mitochondrial toxin exposure, or oxidative damage, carriers upstream of the site of impairment become more reduced. This increases the probability of leakage of electrons to O_2. Mitochondrial impairment may also result in Ca^{2+} dysregulation and activation of NOS. The NO formed acts competitively with O_2 for binding to cytochrome oxidase of complex IV. This can subsequently increase $O_2^{\cdot-}$ formation. NO and $O_2^{\cdot-}$ can react to form peroxynitrite ($ONOO^-$), which in turn can nitrate mitochondrial proteins.

Indirect ROS formation

In addition to the direct production of ROS by impaired mitochondria, extramitochondrial generation of ROS can be triggered by mitochondrial dysfunction (Fig. 3). One major contributor to this indirect source of ROS by impaired mitochondria is via the secondary activation of glutamate receptors. A number of studies have shown that neuronal damage caused by impairment of metabolism can be attenuated by glutamate receptor antagonists (55, 145, 243, 265, 276). In most situations of imposed mitochondrial blockade by exogenous poisons, the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor appears to be exclusively involved. NMDA receptor activation secondary to mitochondrial inhibition may come about from glutamate release into the extracellular environment, but can also occur in the absence of any net increase in extracellular glutamate (263, 264). The former tends to occur with more severe and complete inhibition of metabolism, whereas the latter predominates when impairment is incomplete or mild. Under incomplete inhibition of mitochondrial function, the weak or alternative excitotoxic hypothesis predicts that a partial reduction in energy stores (ATP/ADP), compromised Na^+,K^+-ATPase activity, and a deteriorating membrane potential may contribute to loss of regulation at the NMDA receptor via loss of the voltage-sensitive Mg^{2+}-channel blockade (5, 95, 108, 264). In the absence of the Mg^{2+} blockade, the EC_{50} for glutamate is shifted and lower extracellular concentrations of glutamate can activate the receptor (185). Excitotoxic involvement has been implicated in PD, HD, AD, and ALS, (for review, see 166). As the mitochondrial defects in these conditions are incomplete, for example, a 30–40% loss of complex I activity in PD (193, 212), the weak or alternative excitotoxic hypothesis may be more relevant to events occurring in slow progressive neurodegenerative diseases. An exception to this is in ALS, where the AMPA-kainate subtype of glutamate receptor and increased extracellular

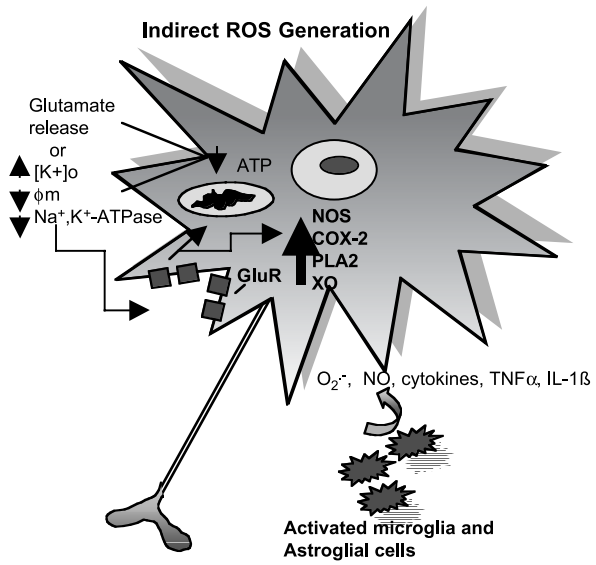

FIG. 3. Indirect generation of ROS by mitochondria during mitochondrial dysfunction. One major pathway in neurons to generate ROS during mitochondrial dysfunction is via secondary activation of glutamate receptors. Glutamate receptor activation can directly impact mitochondria, alter mitochondrial Ca^{2+} homeostasis, and increase ROS production. In addition, a number of cytosolic enzymes, including NOS, cyclooxygenase-2 (COX-2), phospholipase A_2 (PLA_2), and xanthine oxidase (XO), increase activity following glutamate receptor stimulation to subsequently increase ROS production. In response to neuronal injury or as a direct effect of toxin exposure, microglia and astroglial cells can become activated and produce a number of potentially toxic substances including $O_2^{\cdot-}$, NO, cytokines, tumor necrosis factor α (TNFα), and interleukin 1β (IL-1β) that can feedback and damage neurons.

glutamate are thought to contribute to excitotoxic damage. AMPA-kainate receptors predominate in spinal motor neurons. In ALS, an increase in extracellular glutamate is due to a loss of the glial glutamate transporter EAAT2 (151), which functions to clear glutamate from the extracellular space.

Overstimulation of both NMDA and AMPA-kainate receptors can increase ROS production in cells (67, 70, 139, 207). Mitochondria are responsible for some of the observed increases in ROS production (67, 150, 207) caused by excitotoxicity, but cytosolic sources resulting from activation of NOS (2, 214), phospholipase A_2 (PLA_2) (142), cyclooxygenase-2 (COX-2) (173, 196), and xanthine oxidase (69, 70) have also been demonstrated. A variety of antioxidants (71, 181, 268), as well as modulation of antioxidant defenses (32, 79, 233), can alter glutamate receptor-mediated excitotoxicity. In addition, there is evidence for the involvement of NO (163), PLA_2 (1, 75), COX-2 (131, 240), and xanthine oxidase (91) in damage due to mitochondrial dysfunction.

As oxidative stress is a common mediator of damage with both mitochondrial dysfunction and excitotoxicity, and as a secondary excitotoxicity is a contributing mechanism to damage due to mitochondrial inhibition, the possibility is raised that glutamate receptor activation is a major contributor to oxidative stress when mitochondrial function is perturbed. This, however, appears not to be the case, at least for midbrain dopamine neurons. Mesencephalic cultures treated with the complex II inhibitor malonate or the excitotoxin glutamate increase ROS production (269, 270). Toxicity with either challenge can be prevented by the NMDA antagonist MK-801. However, malonate and glutamate produce differing effects on cellular redox status as indicated by GSH and GSSG levels (270), whereas MK-801 blocks ROS production by glutamate, but not malonate (76, 270). These findings indicate that the secondary activation of glutamate receptors associated with mitochondrial dysfunction does not contribute significantly to the oxidative stress that is observed. The amount of ROS generated by glutamate receptors may be minor compared with that produced by mitochondrial inhibition *per se.* The underlying message is that although glutamate receptors may be important contributors to overall events, they are but one of many contributing mechanisms that result in irreversible neuronal damage. Consistent with this, ROS generation is an important mechanism to damage non-CNS cells during energy impairment and occurs independent of glutamate receptor involvement (33). In the CNS, the secondary involvement of glutamate receptors may serve to make neurons as compared with other cell types more susceptible to systemic mitochondrial defects such as occurs in PD (193) and AD (194).

Nonneuronal sources are also important contributors to ROS generation during mitochondrial dysfunction. Activated microglia through production of $O_2^{\cdot -}$, NO, cytokines, tumor necrosis factor α, and interleukin 1β (see 153) and activated astroglial cells through production of NO and interleukin 1β (7) can damage surrounding neurons (30, 46, 168). Evidence for activated microglia and an inflammatory response exists for PD, AD, and multiple sclerosis (for review, see 135). In animal models of MPTP toxicity, NADPH oxidase activity by microglial cells significantly contributes to $O_2^{\cdot -}$ production upon activation of the microglia (81, 257). Mice deficient in NADPH oxidase activity or pharmacological inhibition of microglial activation (81, 256, 257) can partially protect against MPTP toxicity. Activation of microglial cells with lipopolysaccharide in rodents is sufficient to selectively damage dopamine neurons (80). It is not known at present, though, if microglial involvement is an initiating event or a consequential response to damaged neurons. Temporal studies in the MPTP model (257) suggest that, with MPTP, initial ROS production is independent of microglial activation, placing microglia downstream and in the role of amplifying the oxidative insult.

MITOCHONDRIAL DAMAGE CAN OCCUR DOWNSTREAM OF ROS GENERATION

Abnormal mitochondrial function, rather than being a cause of oxidative stress in neurons, could come about secondary to ROS generation. A clear example of this is in the neurodegenerative disease FA. FA is caused by a GAA trinucleotide repeat on chromosome 9 that causes a decrease in transcription and, therefore, loss of function of the gene for frataxin (189). Pathology includes degeneration of spinocerebellar tracts of the spinal cord and loss of dorsal root ganglion neurons. Work on a gene homologue in yeast indicates frataxin to be important for regulation of iron content by mitochondria (11). The increase in mitochondrial iron coupled with H_2O_2 production during normal mitochondrial respiration sets the stage for Fenton chemistry and production of $HO^{\cdot}$. $HO^{\cdot}$ radicals are one of the most damaging free radicals in cells. A consequence of the increased iron in mitochondria in patients with FA is oxidative damage to mitochondrial proteins and DNA (35). Thus, in FA, a systemic nuclear encoded genetic defect can lead to increased oxidative stress and downstream mitochondrial dysfunction and selective damage to certain populations of neurons.

Several mitochondrial enzymes or enzyme complexes have been shown to be sensitive to inhibition by ROS and include aconitase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, and complexes I, II, and III. All of these proteins or protein complexes contain important sulfhydryl groups required for activity and are subject to possible oxidation or covalent modification. In addition, complex IV is inhibited by NO. Interestingly, the deficiencies in mitochondrial function for PD, HD, FA, PSP, AD, and HSP involve one or more of these enzymes or enzyme complexes (Table 2). This would suggest mitochondrial dysfunction secondary to ROS production as opposed to a primary genomic defect directly involving a respiratory enzyme. In contrast, cybrid technology, in which blood platelets from patients containing mitochondrial DNA are fused with rho cells contributing nuclear DNA, but lacking mitochondrial DNA, demonstrates the stable transfer of defects in mitochondrial prodigy in PD (234), AD (235), but not HD (236), raising the possibility of a primary genetic mitochondrial mutation in some neurodegenerative diseases. Note in the case of FA, a mutation in a nuclear encoded gene for a nonrespiratory mitochondrial protein can lead to ROS production and secondary defects in respiratory mitochondrial proteins. Defects in mitochondrial metabolism may, therefore, be a result of a primary genetic mutation involving mitochondrial DNA or be secondary either to a genetic mutation involving a nuclear en-

Table 2. Mitochondrial Proteins Affected in Neurodegenerative Diseases

Neurodegenerative disease	*Protein and/or activity altered*	*References*
Parkinson's	Complex I	193, 212
	Complex II/III	98
	α-Ketoglutarate dehydrogenase	175
	Coenzyme Q10	223
Huntington's	Aconitase	237
	Complex II/III	
Alzheimer's	α-Ketoglutarate dehydrogenase	29
	Pyruvate dehydrogenase	
	Complex IV	
Parasupranuclear palsy	Aconitase	192
	α-Ketoglutarate dehydrogenase	
Friedreich's ataxia	Aconitase	35
	α-Ketoglutarate dehydrogenase	

coded protein or to a nongenetic external event such as exposure to an environmental toxin. The latter two situations may result in oxidative stress that, in turn, leads to mitochondrial dysfunction.

Although the temporal hierarchy of events and underlying cause in most neurodegenerative diseases with regard to mitochondrial dysfunction and oxidative stress are unknown, the evidence is overwhelming that these factors are important players that contribute to the loss of neurons during disease progression. Regardless of which is the initiating event, each can potentially lead to the evolution of the other and set into motion a self-sustaining and amplifying reciprocation between ROS generation and mitochondrial impairment (Fig. 4).

CELL-SPECIFIC FACTORS MAY CONTRIBUTE TO ROS GENERATION AND CELLULAR VULNERABILITY

Although the cellular sequelae upstream of mitochondrial dysfunction in FA are becoming clear, they are much less understood for other neurodegenerative diseases in which mitochondrial dysfunction has been observed, *i.e.,* PD, HD, AD, ALS, and PSP. It is reasonable to postulate that as yet understood events may occur to promote oxidative stress in these other conditions that, in turn, leads to oxidative damage to mitochondrial proteins or conversely promotes mitochondrial dysfunction that results in oxidative stress. In sporadic ALS,

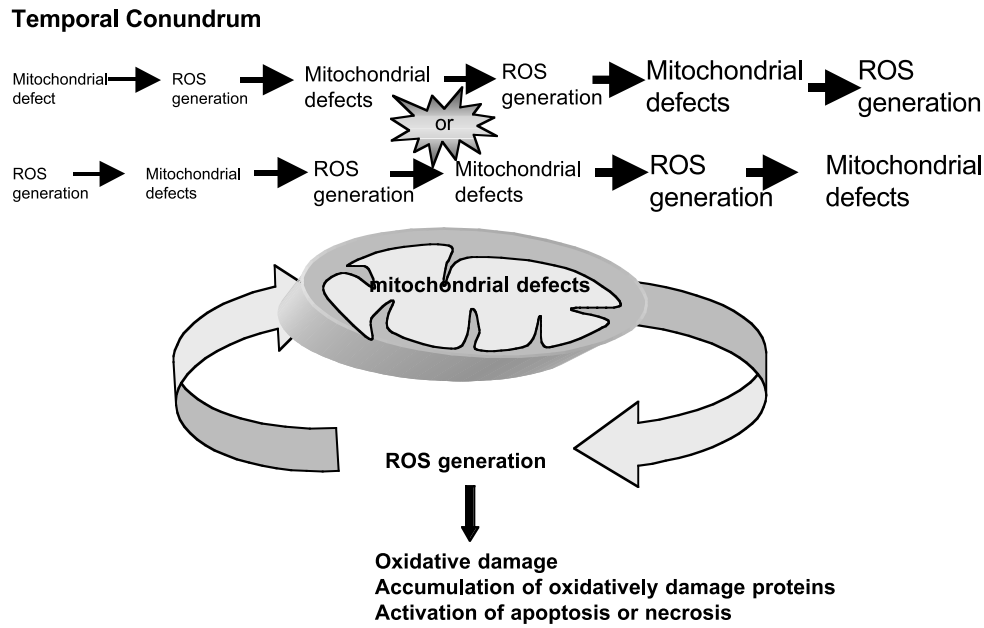

FIG. 4. The temporal association between mitochondrial dysfunction and ROS generation. Mitochondrial defects and oxidative stress are common findings for many neurodegenerative diseases. What constitutes the primary event, *i.e.,* mitochondrial dysfunction or ROS generation, is unknown for most of the neurodegenerative diseases. Evidence shows that either one can lead to the evolution of the other and set into motion a self-sustaining and amplifying cycle that can ultimately trigger activation of cell death processes.

for example, decreases in the glutamate transporter EAAT2 and triggering of glutamate receptors may lead to ROS production (67, 139, 207), dysregulation of mitochondrial Ca^{2+} (39, 199), and mitochondrial damage (68, 170), whereas in familial ALS, mutant SOD1 can accumulate in mitochondria (154) and can bind to mitochondrial Bcl-2 (195) to possibly promote mitochondrial dysfunction. In HD, mutant huntingtin protein may directly interact with mitochondria to lower membrane potential and alter mitochondrial Ca^{2+} homeostasis (49, 191) or can increase ROS production (259). In PD, dopamine itself has been postulated to contribute to cell damage and vulnerability (26, 53, 54, 103, 178). Enzymatic and nonenzymatic metabolism of dopamine can generate H_2O_2 and dopamine-quinones, capable of modifying proteins through 5-cysteinyl dopamine covalent adduct formation (92, 101, 102). In relation to mitochondrial dysfunction, the effects of dopamine can be either upstream or downstream of mitochondrial perturbation. Free dopamine in the cytosol or extracellular space is capable of menacing the oxidative state of cells. A safeguard that minimizes this is the sequestration of dopamine into vesicles via the vesicular monoamine transporter 2 (VMAT2). Mitochondrial impairment may lead to loss of dopamine homeostasis through disturbance of vesicular dopamine storage. Uptake of dopamine into vesicles via VMAT2 requires a proton electrochemical gradient that is generated by a vesicle H^+-ATPase (77). Reduced levels of ATP diminish monoamine uptake into vesicles (77), which will increase levels of cytosolic dopamine. An increase in cytosolic or extracellular dopamine can be damaging to dopamine neurons and non-dopamine neurons, respectively. Ischemia, a physiologically imposed mitochondrial inhibition due to lack of substrate and O_2, causes dopamine release (88, 228). Elimination of striatal dopamine prior to ischemia or pharmacologically evoked mitochondrial inhibition abrogates damage to striatal neurons (76, 87, 159, 178). Low concentrations of the complex II inhibitor malonate selectively damage dopamine neurons *in vitro* (265) and *in vivo* (267), whereas higher concentrations in striatum *in vivo* damage GABA neurons as well (6, 22, 267, 271). This latter effect has been shown to be secondary to dopamine release (76, 158). At concentrations of malonate that are selectively toxic to striatal dopamine terminals, prior depletion of vesicular stores of dopamine with the VMAT2 inhibitors tetrabenazine or Ro 4-1284 is protective (178). In total, these findings support the view that mitochondrial inhibition can lead to loss of dopamine homeostasis that, in turn, can contribute to oxidative stress and cellular vulnerability.

Loss of dopamine homeostasis, however, may not only be a downstream consequence of mitochondrial impairment, but may contribute to mitochondrial dysfunction. Dopamine can inhibit mitochondrial metabolism (26, 53, 90) by both monoamine oxidase (MAO)-dependent and -independent mechanisms (89, 90). Dopamine released into the cytosol is rapidly metabolized by MAO to 3,4-dihydroxyphenylacetic acid (DOPAC) and H_2O_2. The readily diffusible H_2O_2 can enter mitochondria to undergo Fenton chemistry and $HO^{\cdot}$ formation to inhibit complexes I and II (90, 273). As recently demonstrated in PC12 cells with intracellular patch electrochemistry, the catechol product of dopamine metabolism, namely, DOPAC, is not readily cleared from the cytosol (177). This could have important pathophysiological relevance because DOPAC can also contribute to mitochondrial inhibition via catechol oxidation and quinone formation with a potency similar to that of dopamine (89). Thus, loss of dopamine homeostasis may add to an oxidative burden caused by extant mitochondrial deficiencies to contribute further to mitochondrial malfunction or, alternatively, may initiate a process that leads to mitochondrial impairment.

MITOCHONDRIAL IMPAIRMENT AND DISRUPTION OF GLUTATHIONE HOMEOSTASIS

Inhibition of mitochondrial function caused by stroke/ischemia and reperfusion or by mitochondrial toxins can result in loss of glutathione (174, 219, 220, 270). It might, therefore, be expected that neurodegenerative diseases in which there is an underlying mitochondrial deficit would show derangements in GSSG or GSH levels. This has not been clearly evident except in the case of PD (see 217 for discussion of evidence for glutathione involvement in AD and ALS). Autopsied material from the substantia nigra of patients with PD show a 40% loss of GSH (118, 208, 225, 229). This loss cannot be explained by a compensatory increase in GSSG (229) or in a decrease in the synthetic enzymes for glutathione (226), although there is evidence for an increase in the glutathione catabolizing enzyme γ-glutamyltranspeptidase (226). The loss of glutathione need not be downstream of mitochondrial impairment and, indeed, evidence argues that this event occurs early in the disease process. Glutathione levels are decreased significantly in the substantia nigra from patients with incidental Lewy body disease (118). This disease is thought to represent a preclinical stage of PD. Mitochondrial function in incidental Lewy body disease brain, however, is normal (63, 118), suggesting that glutathione loss precedes decreased mitochondrial function. Whether glutathione deficits contribute to loss of mitochondrial function in PD is not known, although evidence suggests this possibility. Depleting glutathione results in damage to complexes I, II, and IV (105, 171). The decreases in complexes I and II may come about as a result of unrestricted generation of H_2O_2 due to loss of this function of GSH and subsequent $HO^{\cdot}$ formation. Complexes I and II have been shown to be sensitive to these reactive species (90, 273). Loss of complex IV or complex I activity could result from enhanced NOS activity and NO production as GSH depletion can result in increased NOS activity in the brain (106) and NO competes with O_2 for binding to this complex. At high NO concentrations, complex I is also affected (51, 209).

Regardless of the temporal conundrum with respect to disturbance in the glutathione system and mitochondrial defects, it is reasonable to postulate that perturbation of this system will likely contribute to the ongoing progressive damage that accompanies neurodegenerative diseases such as PD or to the evolution of damage that accompanies neuropathological situations such as stroke/ischemia. The glutathione system is unique in that it serves a number of different roles in cells (Fig. 5), and understanding all the facets of its contribution to cell homeostasis is essential to understanding the consequences of its loss. A well studied function of glutathione is H_2O_2 re-

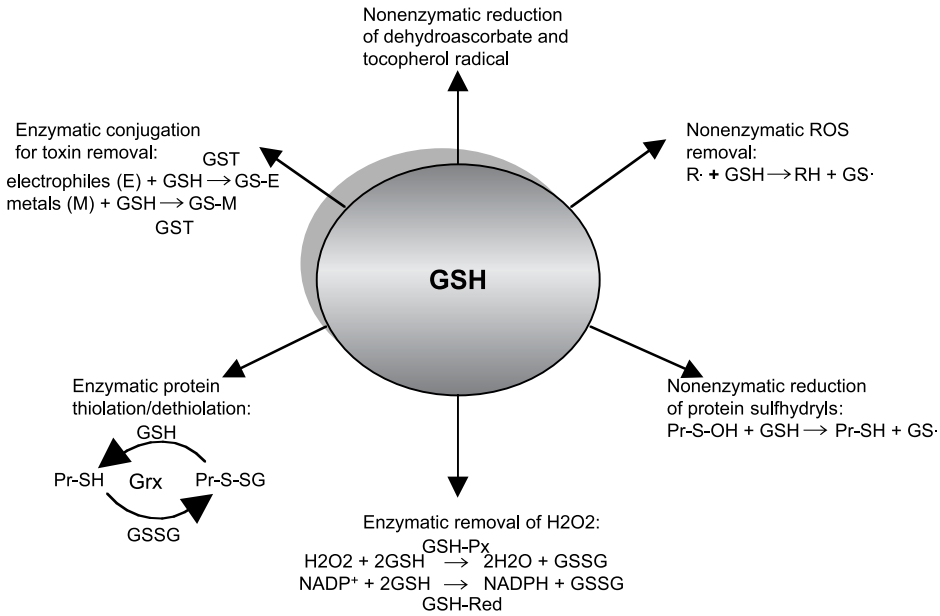

FIG. 5. Varied roles for glutathione. Glutathione participates in a number of nonenzymatic reactions to remove hydroxyl and carbon-based radicals, maintain protein (Pr) sulfhydryls in a reduced state, and reduce other small-molecule antioxidants, such as ascorbate and tocopherol. Glutathione also participates in a number of enzymatic reactions to remove H_2O_2 via glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Red.), thiolate and dethiolate proteins with glutathione via glutaredoxin (Grx), and remove toxic electrophiles and metals via a family of glutathione *S*-transferases (GST). The regulation of redox status, protein glutathionylation, and protein reduction are postulated to underlie a role for glutathione in actin polymerization, DNA synthesis and repair, protein synthesis, amino acid transport, apoptotic signaling, and redox modulation of glutamate receptor currents.

moval via glutathione peroxidase and reductase. This function for glutathione serves a primary role in protecting neurons from mitochondrial impairment. Glutathione depletion greatly potentiates damage during inhibition of mitochondrial respiration (258, 268). Consistent with this, enhancing glutathione peroxidase activity ameliorates damage (266), whereas loss of glutathione peroxidase potentiates damage (130) due to mitochondrial dysfunction. Catalase provides a secondary source of H_2O_2 removal only when glutathione levels are severely depleted (71). Glutathione is also important in toxin removal in a reaction catalyzed by one of several isoforms of glutathione *S*-transferase. A polymorphism in glutathione *S*-transferase P1 was found to differ in PD patients versus control subjects exposed to pesticides (169), suggesting that this aspect of the glutathione system may be a susceptibility factor in PD.

Glutathione is also important in maintaining protein sulfhydryl groups in a reduced state. As the oxidation state of proteins can influence protein activity, this role of glutathione is critical for maintaining normal homeostatic function. In brain, a more recently identified function of glutathione is the glutathionylation and deglutathionylation of sulfhydryl groups of cysteine residues on proteins. Thiolation and dethiolation of proteins with glutathione occur under both physiological (232, 250) and pathophysiological situations (25, 53). The process is catalyzed by glutaredoxin (Grx-1), an enzyme that was recently identified in brain cytosol (15). Mitochondria contain a unique glutaredoxin (86, 157), Grx-2. Brain mitochondria show functional Grx-2 activity, with a specific activity double that of liver mitochondria (73). This is likely due to the high rate of oxidative metabolism in brain and suggests an important role for glutaredoxin in brain mitochondrial function. Mice exposed to the excitotoxin BOAA up-regulate Grx-1 protein and activity, and this corresponds to recovery from loss of mitochondrial complex I activity (125). The exact role(s) for protein glutathionylation and its reversal remain a source of debate at present. Postulated roles include regulation of protein function (128), source of a 'hidden' pool of glutathione to be used for repair and recovery once an oxidative stress is removed (134), protection of vulnerable SH groups on proteins from irreversible oxidation (52, 152), and a means to safely remove GSSG from the cellular environment to maintain GSSG/GSH status during oxidative stress (72, 272). With regard to the latter two postulated roles, the dual action of glutaredoxin as a thioltransferase (93) and dehydroascorbate reductase (252) appears to couple a cooperative interaction between glutathione and ascorbate in carrying out these beneficial functions for the cells (72). In addition, evidence exists for glutathione's involvement in DNA synthesis and repair, protein synthesis, amino acid transport, apoptotic signaling, redox modulation of glutamate receptor currents, as well as a role as a potential neurotransmitter (see 12 for discussion). Given the eclectic nature of the many roles served by glutathione, it may be simplistic to assume that its depletion under certain conditions affects only ROS removal. In those conditions in which glutathione

status is compromised, antioxidant therapy with a substitute antioxidant may not be fully efficacious as many of the roles served by glutathione would not be restored.

MITOCHONDRIAL IMPAIRMENT, OXIDATIVE DAMAGE, AND PROTEIN ACCUMULATION

In addition to the common findings of mitochondrial dysfunction and oxidative stress in many neurodegenerative diseases, protein aggregation is now recognized as a shared feature in PD, AD, ALS, and the CAG trinucleotide repeat diseases spinobulbar muscular atrophy, dentatorubropallidoluysian atrophy, several of the spinocerebellar ataxias, and HD (for reviews, see 210, 255). Genetic clues from familial PD point toward inadequate clearance of oxidatively damaged proteins through the ubiquitin proteasomal pathway as underlying protein accumulation and aggregation. Loss or diminished function of parkin, an E3 ligase of the ubiquitin proteasomal system, results in early onset autosomal recessive PD (127). Mutations in UCHL-1, a ubiquitin C-terminal hydrolase and ligase, also lead to familial PD (146). The propensity for protein aggregation increases with increasing protein concentration and, in some instances, conformational change. Increased expression of α-synuclein caused by a triplication of the α-synuclein gene or mutations in α-synuclein lead to its aggregation and early onset PD (137, 202, 227, 261). Proteins in Lewy body inclusions include, among others, α-synuclein, parkin, as well as proteasomal elements. Recent findings in familial PD of genetic mutations in DJ-1, a protein that participates in the oxidative stress response (31), and PINK1, a mitochondrial targeted kinase (245), provide a bridge to link familial and sporadic forms of the disease and tie together the mitochondrion, oxidative stress, and protein misfolding.

In the absence of a genetic mutation, sporadic forms of PD may be caused by environmental factors (144, 239), but it is likely that environmental and genetic factors interact to predispose neurons to damage. Pesticides such as rotenone, at low chronic doses, can lead to increased free radical production with aggregation of α-synuclein and inclusion body formation (27, 219). Increased ROS production can impair ubiquitin proteasomal function (114, 206), a somewhat counterintuitive finding as this pathway is a major pathway for the clearance of oxidatively damaged proteins (96, 188). This, however, may only be applicable to robust generation of ROS as low-level oxidative stress stimulates, rather than impairs, proteasomal activity (64, 262). The degree of impairment of mitochondrial function is also important in determining whether there is involvement of the ubiquitin pathway. As the ubiquitin pathway is ATP-dependent (110, 246), severe mitochondrial inhibition that collapses energy status produces cell death in the absence of ubiquitin proteasomal involvement and without accumulation of ubiquitinated proteins (262). Only low-dose, chronic complex I impairment that still partially preserves ADP/ATP status leads to increases in protein ubiquitination and accumulation (262). Dopamine may also participate in the process of protein aggregation through promotion of oxidative damage to proteins and through cysteinyl-dopamine-protein adduct formation. Cysteinyl-dopamine-protein adducts are increased in PD brain (231) and following MPTP exposure (241). In cell-free systems, dopamine can form adducts with α-synuclein and promote its aggregation (56). Further, dopamine induces proteasome inhibition in PC12 cells (124). These findings indicate that, like primary genetic mutations, nongenetic triggers can initiate a deleterious series of events similar to those induced by genetic mutations to produce pathology (Fig. 6). The challenge now is in understanding how these different pieces of the puzzle, *i.e.,* the mitochondrion, oxidative stress, and clearance of damaged proteins, come together to produce selective neuronal damage in the various neurodegenerative diseases and in the familial and sporadic forms of the diseases.

DIRECTIONS IN TARGETING MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASE

The common features of mitochondrial dysfunction and oxidative stress found in numerous neurodegenerative diseases suggest that, regardless of the peculiarities that render certain populations of neurons vulnerable in a specific neurodegenerative disease, there may be a limited number of mechanisms that are evoked to passage a neuron to its point of degeneration. There is still much work to be done before the series of events that constitute cause and effect in most neurodegenerative conditions can be laid out in tandem. The "glass half-full" interpretation, however, is that these common features provide optimism that successful neurotherapeutic strategies discovered in the treatment of one neurodegenerative disease may have beneficial application to a number of neurodegenerative diseases. There are at present a number of agents that either augment mitochondrial function or possess antioxidant properties that are being looked at in animal models or in the treatment of neurodegenerative disease.

Coenzyme Q10, a component of the ECT, has shown some promise in a limited clinical trial (224) in PD and may be of promise in HD (133) and FA (57), although it is uncertain if this compound produces its beneficial effects by enhancing mitochondrial function or by acting as an antioxidant. Creatine can serve as a repository for high-energy phosphate in the form of phosphocreatine and has been used as a nutritional supplement to boost energy reserves (197). Creatine has shown promise in animal models of PD (164), HD (10), and ALS (132). Selegiline, a MAO inhibitor, is in clinical use and was found to have modest beneficial effects in PD (187, 222). Through MAO inhibition, selegiline may block conversion of a protoxin to its toxin form, such as in the case of conversion of MPTP to MPP^+, preserve dopamine levels, or reduce ROS production by limiting dopamine metabolism. MAO-independent actions of selegiline are also indicated and are thought to involve apoptotic pathways through modulation of proapoptotic and antiapoptotic genes (161, 162). The antibiotic, minocycline, prevents microglial activation and thereby limits ROS production by these cells. Its efficacy has been shown in animal models of PD and ALS (66, 256, 274), although this has not been universally found (260). Minocycline can also inhibit

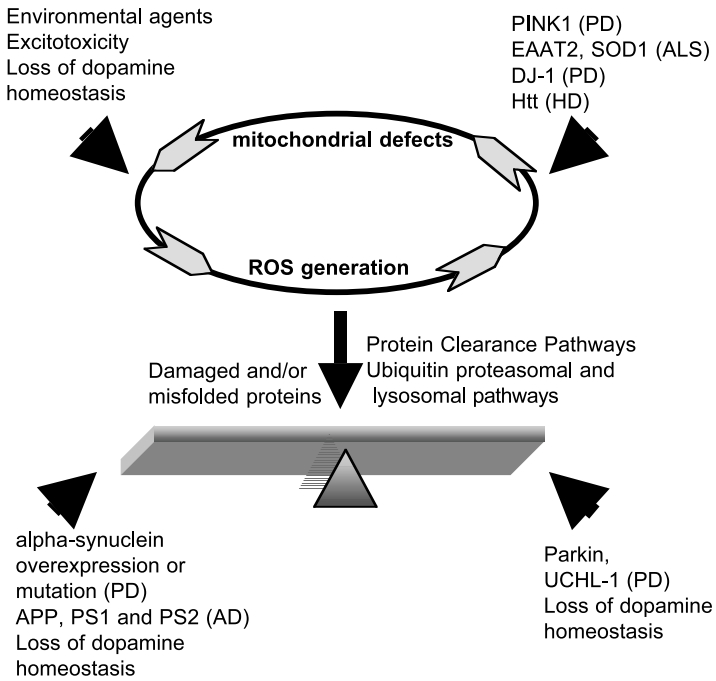

FIG. 6. Genetic and nongenetic influences can trigger similar cellular events that lead to neurodegeneration. The central players in many neurodegenerative diseases, *i.e.,* mitochondrial dysfunction, oxidative stress, and buildup of damaged or misfolded proteins, can be triggered by either genetic or nongenetic events. Environmental agents, such as rotenone, excitotoxicity, or loss of dopamine homeostasis can perturb mitochondrial function and increase ROS generation. Similarly, mutated huntingtin protein (htt) can interact with mitochondria, perturb Ca^{2+} homeostasis, and increase ROS. Aberrant mRNA for EAAT2, as found in sporadic ALS, can lead to overstimulation of glutamate receptors and produce downstream effects on mitochondrial function and ROS generation, whereas mutant SOD1 was recently reported to accumulate in mitochondria from spinal cord and to bind to the antiapoptotic mitochondrial protein Bcl-2. Recently described mutations in PINK1, a mitochondrial kinase, and DJ-1, a protein that participates in the oxidative stress response, provide a link between mutations found in familial PD and the mitochondrial deficits observed in the sporadic forms of the disease. A consequence of the self-sustaining cycle of mitochondrial dysfunction and ROS generation is an increase in the rate of production of damaged proteins. The pathways to clear unwanted proteins, namely, the ubiquitin proteasomal and lysosomal pathways, are challenged to maintain a balance between the rate of production and rate of clearance of damaged or misfolded proteins. Cell survival may hang in the balance. Genetic and nongenetic factors may also influence events at this later stage. Overexpression of normal α-synuclein or mutations in α-synuclein may produce damaged proteins at rates that exceed their clearance or directly impact proteasomal or lysosomal function. Likewise, mutations in amyloid precursor protein (APP), presenilin 1 (PS1), or presenilin 2 (PS2) can lead to accumulation of β-amyloid peptide and hyperphosphorylated tau. On the other hand, other familial Parkinson mutations, Parkin and UCHL-1, may interfere with the proper functioning of the ubiquitin proteasomal pathway. Cellular factors, such as increased cytosolic dopamine or ROS production, may also directly impede protein clearance pathways.

caspases 1 and 3 activities and preserve mitochondrial permeability transition (48, 211, 251). Cyclooxygenase activity can be a source of $O_2^{\cdot -}$ production in cells. COX-2 inhibitors have shown neuroprotection in animal models of PD and ALS (65, 132, 241). As dopamine can serve as a substrate for the peroxidase activity of COX-2 to form dopamine-quinones (101), neuroprotection by inhibition of COX-2 may result from this action as well. Epidemiological studies suggested the usefulness of COX-2 inhibitors in the treatment of AD (28). Their success in clinical trials, however, has been disappointing (205). The dopamine agonists, ropinirole and pramipexole, have shown modest effects in slowing progression of PD (160, 254). Among the many potential mechanisms of action of this class of drugs, the dopamine agonists may directly serve as antioxidants or up-regulate endogenous antioxidants (143). Other agents of potential benefit include nicotinamide, a precursor for NAD, acetyl-L-carnitine, which facilitates transport of long-chain fatty acids into mitochondria, α-lipoic acid, a fatty acid with antioxidant properties, and riboflavin, a flavin precursor (for reviews, see 13, 21).

In addition to the strategy of directly impacting oxidative stress or mitochondrial function, indirect approaches to preserve mitochondrial integrity are being developed. Mitochondria are integral players in inducing apoptosis, and a number of compounds that interfere with this process are being examined (for review, see 249). Agents that interfere with the accumulation of protein aggregates are also being looked at as potential therapeutics. Transglutaminase, an enzyme that catalyzes cross-links between glutamate and lysine residues in proteins to promote insoluble protein aggregates, plays a role in the CAG trinucleotide repeat diseases (122, 147) and may play a role in PD (120). Small molecules that inhibit this process,

such as cystamine, have shown improvement in animal models of HD (61, 123), although this protective benefit may involve antioxidant activity or inhibition of caspase activity (148).

Although many antioxidant approaches have proven successful in animal models of several neurodegenerative diseases, it has been a formidable challenge to translate this to clinical success. A clinical trial of high-dose ascorbate and vitamin E delayed the need for L-Dopa therapy by 2–4 years in 75% of PD patients (74). The prooxidant properties of ascorbate, however, require caution when considering its use as a therapeutic agent. The DATATOP study failed to find any benefit for vitamin E treatment in PD (221). Inadequate dosage and improper biodistribution could account for the negative findings. It should also be considered that vitamin E is a downstream line of defense to control propagation of lipid peroxidation and would control only one aspect of oxidative attack. In addition, vitamin E requires GSH, ascorbate, or ubiquinone for its regeneration, and the depleted state of GSH (118, 208, 225, 229) and coenzyme Q10 (223) in PD may hamper the effectiveness of vitamin E supplementation.

As indicated previously, the loss of GSH may result in a diversity of aberrantly affected processes, in addition to simple failure to remove ROS. Thus, augmentation of GSH levels *per se* may be required for effective therapy in neurodegenerative conditions where GSH is diminished. One study in PD patients has reported intravenous application of GSH for 1 month with improvement in motor ability (218). Brought to question is whether this benefit is a direct result of brain elevation of GSH because GSH does not readily cross the blood–brain barrier and is not taken up by neurons. Other strategies, such as intercerebral ventricular administration of γ-glutamylcysteine (198), a precursor of GSH, or treatment with procysteine (60), *N*-acetyl-L-cysteine (107, 111), or the ethyl ester of glutathione (9), are being tried in animal models to elevate brain glutathione levels. Whether this will effectively impact disease progression in humans remains to be evaluated. Ultimately, it may prove necessary to combine various therapies that are directed at different targets implicated in disease etiology, *i.e.,* enhancement of mitochondrial function, GSH, and/or other antioxidant supplementation, inhibition of microglial activation, or transglutaminase and apoptotic inhibitors, in order to stabilize neurons and slow or halt disease progression. Such an achievement would be a giant step forward and second only to disease prevention. The abundant new information gathered with regard to our understanding of the cellular processes leading to neurodegeneration provides optimism that this achievement is within reach.

ABBREVIATIONS

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; COX-2, cyclooxygenase-2; DOPAC, dihydroxyphenylacetic acid; ETC, electron transport chain; FA, Friedreich's ataxia; FAD, flavin adenine dinucleotide; GABA, γ-aminobutyric acid; Grx-1, cytosolic glutaredoxin; Grx-2, mitochondrial glutaredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; HD, Huntington's disease; $HO^{\cdot}$, hydroxyl radical; H_2O_2, hydrogen peroxide; HSP, hereditary spastic paraplegia; MAO, monoamine oxidase; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAD, nicotinamide adenine dinucleotide; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; 3-NPA, 3-nitropropionic acid; $O_2^{\cdot -}$, superoxide; PD, Parkinson's disease; PLA_2, phospholipase A_2; PSP, parasupranuclear palsy; ROS, reactive oxygen species; SOD, superoxide dismutases; UQ, ubiquinone; $UQ^{\cdot -}$, ubisemiquinone; UQH_2, ubiquinol; $VMAT_2$, vesicular monoamine transporter 2.

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