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OXIDATIVE STRESS AND PARKINSON DISEASE

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10.1 INTRODUCTION

Parkinson disease (PD) is the most common neurodegenerative movement disorder, currently affecting more than 4 million predominantly elderly individuals worldwide [1]. Originally described as "the shaking palsy" in 1817 by the British physician James Parkinson, the disease is attended by a constellation of motoric deficits including bradykinesia (slowness in movements), postural instability, rigidity, and tremor that ultimately result in near total immobility. Although pathological changes are distributed in the PD brain [2], the principal neuropathology that underlies the characteristic motor phenotype of PD patients is unequivocally the loss of midbrain dopaminergic neurons in the substantia nigra pars compacta (SNpc), which results in a severe depletion of striatal dopamine (DA) and thereby an impaired nigrostriatal system that otherwise allows an individual to execute proper, coordinated movements. This specific pattern of neurodegeneration in PD is often accompanied by the presence of eosinophilic intracytoplasmic inclusions known as Lewy bodies (LBs) in surviving neurons in the SN as well as in other affected brain areas [2]. Accordingly, pharmacological replacement of brain DA via L-DOPA administration represents an effective symptomatic recourse for the PD patient (especially during the initial stages of the disease) and remains a clinical gold standard treatment for PD. However, neither L-DOPA nor any currently available therapies can slow or stop the insidious degenerative process in the PD brain. Furthermore, the major drawbacks with current therapies are the inevitable loss of effectiveness and increasing drug-induced side effects as the disease progresses. Invariably, the debilitating nature and morbidity of the disease present significant social, emotional, and economic problems. As the world population rapidly ages, these problems undoubtedly will also increase.

Despite nearly two centuries of research, the etiology of PD remains elusive. However, a broad range of studies conducted over the past few decades, including epidemiological, genetic and postmortem analysis, as well as in vitro and in vivo modeling, have contributed significantly to our understanding of the pathogenesis of the disease. In particular, the recent identification and functional characterization of several genes, including α-*synuclein*, *parkin*, *DJ-1*, *PINK1*, and *LRRK2*, whose mutations are causative of rare familial forms of PD have provided tremendous insights into the molecular pathways underlying dopaminergic neurodegeneration [3, 4]. Collectively, these studies implicate aberrant mitochondrial and protein homeostasis as key contributors to the development of PD, with oxidative stress likely acting as an important nexus between the two pathogenic events.

Notably, the brain is often thought to be particularly susceptible to oxidation-induced damage because of its high metabolic rate and its relatively reduced capacity to replenish its postmitotic neuronal populations compared with other organs. Moreover, the brain contains high

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levels of phospholipids and polyunsaturated free fatty acids, both of which are prone to modifications by oxidants. For SN dopaminergic neurons, the vulnerability toward oxidative stress is further enhanced by the abundance of redox-active iron in this region of the brain, as well as by the presence of DA, whose oxidation products are potentially cytotoxic [5, 6]. Although attractive, the hypothesis that oxidative stress plays a central role in PD pathogenesis continues to be keenly debated, particularly in view of recent findings in the clinic that antioxidant strategies have failed to produce convincing protection in PD patients. Furthermore, a major question that remains unresolved to this point is whether oxidative stress represents a cause or a consequence of neurodegeneration. Here, we review and discuss both historical evidence and current thoughts about the relationship between the oxidative stress and PD, which include our own views on the topic. For the benefit of the reader, we have also included a section on DA chemistry and oxidation that summarizes the oxidative pathways for catecholamines in the genesis of cytotoxic quinones.

10.2 REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

It is perhaps paradoxical to note that the production of reactive oxygen species (ROS), often regarded as the cellular "bad guys," is an inevitable consequence of aerobic (oxygen dependent) respiration—a process needed to keep all cells in our body, including postmitotic neurons, alive. During aerobic respiration, high-energy products (NADH and $FADH_2$) generated in the mitochondria via the Krebs cycle donate electrons to a series of electron carriers (complexes I–IV) on the electron transport chain (ETC) to produce a proton gradient across the inner mitochondrial membrane that is utilized by ATP synthase to drive oxidative phosphorylation of ADP to ATP (Fig. 10.1). Although molecular oxygen is typically reduced by electrons from Krebs cycle intermediates flowing through the ETC to produce H_2O, partial reduction of molecular oxygen to superoxide anion (O_2^{-}) occurs when electrons leak from the ETC (particularly at complex I). This free radical can be converted to the highly reactive hydroxyl radical ($OH^{\bullet}$) via an iron-catalyzed reaction known as the Fenton reaction, or to peroxynitrite ($ONOO^{-}$) upon reaction with nitric oxide (NO). Both hydroxyl radical and peroxynitrite are potent oxidants that can cause marked cellular damage by reacting with proteins, lipids, and nucleic acids. Further, these reactive species may also target the ETC, which results in a feedforward cycle of increasing oxidative stress and injury. It is noteworthy to mention that NO, which is generated by nitric oxide synthase (NOS), can itself contribute to oxidative damage through *S*-nitrosylation, a reaction whereby the cysteine residues of protein are modified to nitrosothiols, often resulting in altered protein function [7]. (The role of *S*-nitrosylation and nitrosative stress in PD has been covered extensively in several excellent recent reviews [8] and is only discussed briefly in this chapter.)

To protect against potential ROS-induced damage, aerobically respiring cells have developed over the course of evolution an effective antioxidant defense mechanism that consists of a plethora of antioxidant enzymes [such as superoxide dismutase (SOD), glutathione peroxidase, and catalase] as well as nonprotein antioxidants (such as glutathione, α-tocopherol, and ascorbic acid) to keep the levels of cellular free radicals in check. For example, ROS by-products produced by mitochondrial respiration are rapidly detoxified by SOD2 (which converts superoxide to hydrogen peroxide) so that their basal levels are low and nontoxic. As glutathione (GSH) is a major antioxidant in cells, the ratio between the reduced and oxidized form of glutathione (i.e., GSH/GSSG), is often taken as an indicator of cellular redox status [9]. When a state of imbalance between the production of ROS and their clearance by the antioxidant defense system occurs, oxidative stress ensues. However, it is important to highlight that although the overproduction of ROS leading to oxidative stress is usually bad, ROS may be beneficial to cells in some instances. For example, ROS generated by phagocytes during inflammation helps to kill invading pathogens [10]. Furthermore, by virtue of the reversibility of oxidation and reduction, ROS are also used as secondary messengers in redox-based intracellular signaling in response to internal and external cues. This includes neuronal signaling processes that influence synaptic neurotransmission, synaptic plasticity, and long-term potentiation [11, 12]. Likewise, the role of NO as a signaling molecule in the brain is well characterized [13]. Thus ROS [or reactive nitrogen species (RNS)] may act as signaling or stress molecules depending on their cellular levels.

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There is certainly ample support from postmortem studies to suggest that the redox state in the PD brain is in disequilibrium. For example, several groups have reported that markers for lipid peroxidation (including 4-hydroxynonenal and malondialdehyde), protein carbonyl modifications, and even DNA and RNA oxidation are markedly elevated in the SN of postmortem PD brains [14–17], and that these ROS-induced events are

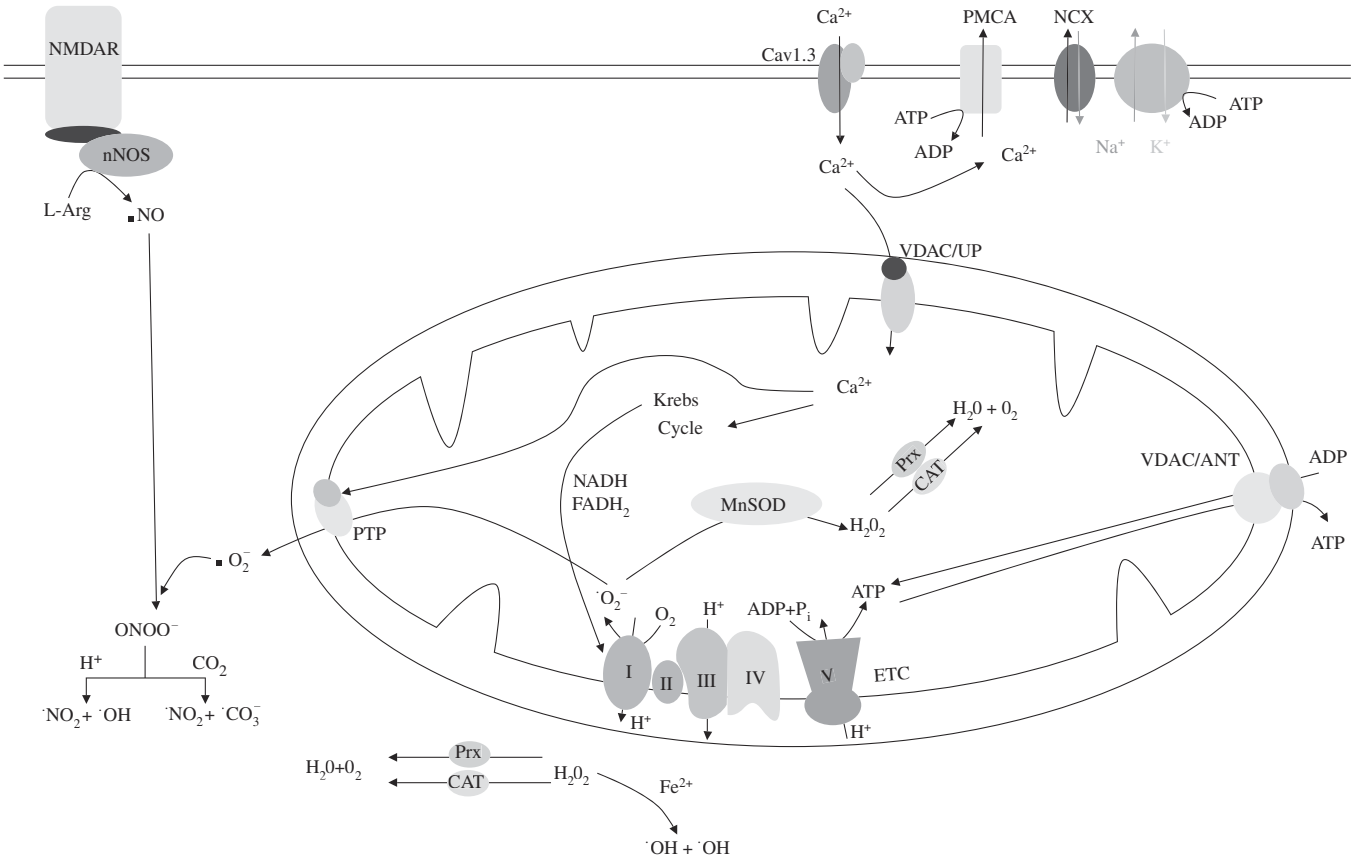

Fig. 10.1 Genesis of reactive oxygen and nitrogen species. NADH and $FADH_2$ produced via the Krebs cycle donate electrons to a series of electron carriers on the electron transport chain (ETC) to produce a proton gradient needed to drive the oxidative phosphorylation of ADP to ATP by ATP synthase. Leakage of electrons from the ETC (especially from complex I) occurs, leading to production of superoxide ($^{\bullet}O_2^-$), which is typically detoxified into hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD). H_2O_2 is then converted into H_2O and oxygen by catalase (CAT) and peroxiredoxin (Prx). H_2O_2 can also be converted to highly reactive hydroxyl radical ($^{\bullet}OH$) via the iron-catalyzed Fenton reaction. Activation of nitric oxide synthase (nNOS) as a result of *N*-methyl-D-aspartate receptor (NMDAR) over-stimulation leads to the production of nitric oxide (NO) that can react with $^{\bullet}O_2^-$ to form the reactive peroxynitrite ($ONOO^-$). $ONOO^-$ can react with H^+ or CO_2 in the cytosol to generate nitrogen dioxide (NO_2) and highly reactive $^{\bullet}OH$ or $^{\bullet}CO_3^-$. Dendritic influx of Ca^2, which apparently occurs via Cav1.3 Ca^2 channels in SN dopaminergic neurons during pace-making, can be taken up by the mitochondria via voltage-dependent anion channels (VDAC) and Ca^{2+} uniporter (UP). Ca^{2+} entering the mitochondrial matrix can stimulate enzymes of the Krebs cycle and thereby oxidative phosphorylation and concomitantly $^{\bullet}O_2^-$ production. The Ca^{2+} that entered through Cav1.3 channels is transported back across the plasma membrane at the expense of cellular energy through either the Ca^{2+}-ATPase (PMCA) or through a Na^+/ Ca^{2+} exchanger (NCX) that relies upon the Na^+ gradient maintained by the Na/K-ATPase.

accompanied by a dramatic depletion of reduced glutathione (presumably leading to a considerably weakened antioxidant defense system) [18]. Interestingly, the magnitude of GSH depletion appears to correlate well with disease progression, suggesting that GSH depletion may be taken as an (early) indicator of nigral degeneration. Changes in iron disposition also occur in the PD brain [19]. Notably, total iron is increased while ferritin is decreased in the diseased state, a condition that is expected to promote the availability of free iron capable of catalyzing the formation of ROS via the Fenton reaction. Correlating with the rise in iron content is an elevated expression of the divalent metal transporter 1 (DMT1) in the SN dopaminergic neurons of PD patients compared to age-matched control subjects [20]. DMT1 is a major transport protein responsible for the uptake of iron and other divalent metal ions into cells, and its expression has also been reported to increase with age [21]. Finally, these studies also revealed a significant reduction in the activity of mitochondrial complex I as well as ubiquinone (coenzyme Q_{10}) in the SN of PD brains [22–24].

The relevance of the above findings derived from postmortem diseased tissues has been supported by several toxin-induced models of PD. Indeed, parkinsonian

neurotoxins such as MPTP, paraquat, and rotenone that selectively destroy nigral dopaminergic neurons in experimental models are often inhibitors of mitochondrial complex I function, the impairment of which would enhance superoxide production and thereby the formation of highly reactive free radicals that can initiate neuronal death [25]. Importantly, in these models, there is apparently a temporal and causal relationship between oxidative damage and degeneration in nigral dopaminergic neurons [26, 27]. Interestingly, MPTP-induced dopaminergic neurodegeneration and associated motor deficits in treated animals can be mitigated by iron chelation either genetically via transgenic expression of ferritin or pharmacologically via administration of clioquinol [28]. Consistent with this, newborn mice administered iron display progressive midbrain neurodegeneration that is paralleled by an increase in markers of oxidative damage as they age [10]. Together, these studies suggest that iron accumulation is a key accelerator of oxidative stress that is relevant to dopaminergic cell loss.

Recent results generated from genetic models also support a role for oxidative stress in PD pathogenesis [25]. For example, several studies have linked oxidative stress to the aggregation of α-synuclein, a presynaptic protein that is also a major component of LBs. Consistent with this, LBs are enriched with nitrated α-synuclein [29], and the protein is similarly modified in MPTP-treated mice [30]. Importantly, whereas α-synuclein overexpression in mice leads to nigral mitochondrial abnormalities [31], its genetic ablation reduces ROS production in these animals and renders them markedly resistant to degeneration when challenged by MPTP and other mitochondrial toxins [32, 33]. These results suggest that the PD-associated protein may induce oxidative stress by promoting mitochondrial dysfunction. Related to this, several studies have also demonstrated the ability of iron-mediated oxidative stress to promote α-synuclein aggregation [34–36]. Separately, we and others have shown that parkin, a ubiquitin ligase associated with recessive parkinsonism that normally functions as a potent neuroprotectant, is also susceptible to oxidative modifications [37, 38]. After its modification by a wide spectrum of oxidative stress-inducing agents such as MPTP, paraquat, and iron, the protein exhibits altered solubility properties and thereby impaired protective function [37, 38]. It is now clear that parkin function is also linked to mitochondrial homeostasis, as is the case with another recessive parkinsonism-associated protein, PINK1, suggesting that the loss of parkin or PINK1 function can precipitate oxidative stress [39]. This notion is certainly well supported by numerous studies performed in different experimental models, including mouse models [40, 41]. Interestingly, parkin also helps to regulate oxidation level in DA-containing cells by limiting the expression of monoamine oxidases (MAO)-mitochondrial enzymes responsible for the oxidative deamination of DA (see next section), apparently by promoting the degradation of estrogen-related receptors (ERR), orphan nuclear receptors that play critical roles in the transcription regulation of many nuclear-encoded mitochondrial proteins [42, 43]. Perhaps the most direct genetic evidence supporting the role of oxidative stress in PD pathogenesis is the finding that mutations in the redox-sensitive protein DJ-1 cause an early-onset form of PD. DJ-1 is thought to operate as an antioxidant protein [44, 45], although a more recent study has classified DJ-1 as an atypical peroxiredoxin-like peroxidase that functions to scavenge mitochondrial H_2O_2 through oxidation of its cysteine at position 106 (a residue previously demonstrated by others via mutagenesis and structural analyses to be modified by oxidative stress) [45]. Notably, a pool of DJ-1 is known to be localized to the mitochondria [45, 46], and increased levels of H_2O_2 in mitochondria can be isolated from DJ-1 knockout mice [47]. Accordingly, the absence of DJ-1 may predispose dopaminergic neurons to oxidative stress-induced degeneration. Indeed, DJ-1-deficient animals are hypersensitive to pharmacological inducers of oxidative stress [48–53]. Taken together, the evidence from genetic studies supporting a relationship between oxidative stress and PD is rather compelling. However, a caveat is that none of these PD-associated genes exhibits a selective pattern of expression that could explain the vulnerability of SN dopaminergic neurons toward oxidation-mediated injury.

Why SN dopaminergic neurons are predisposed to degeneration in PD remains unclear, although the preferential accumulation of iron in this region of the brain may contribute to their vulnerability to endogenous and/or exogenous stress. Another obvious factor is DA itself. The sections below discuss the cytotoxic potential of DA reactions and address whether SN dopaminergic neurons are uniquely susceptible to degeneration by virtue of the neurotransmitter they carry.

10.3.1 DA Chemistry and Oxidation

There are two distinct pathways by which reactive metabolites can be generated from DA in the cell. The first pathway is driven by the MAO enzymes (located on the outer mitochondrial membrane) and aldehyde dehydrogenase where DA is converted to DOPAC (or dihydrophenylacetic acid) with the generation of H_2O_2 in the process. DOPAC can undergo further oxidation to form DOPAC quinones and ROS. Normally, DOPAC quinones will be conjugated to GSH. Those that fail to do so may undergo conversion to 5-*S*-cys-DOPAC, which can be further oxidized to other reactive species. The second

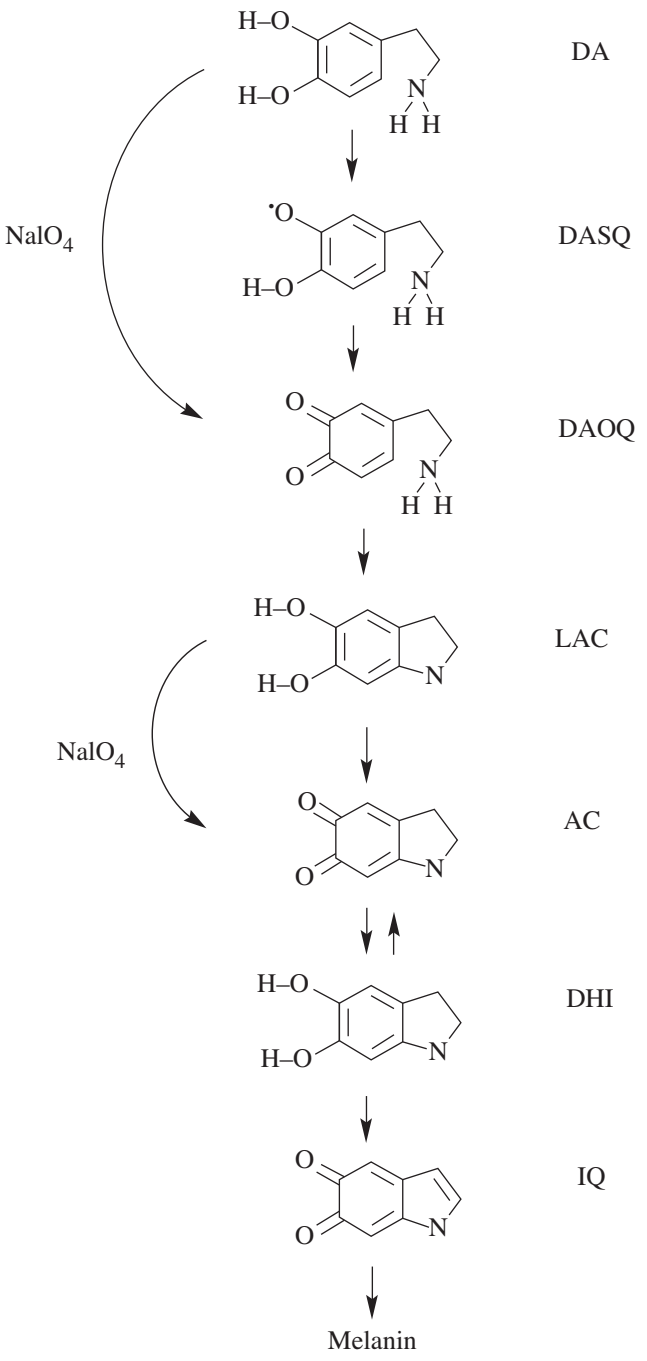

Fig. 10.2 Oxidative pathway for DA in the genesis of cytotoxic quinones. DA, dopamine; DASQ, dopamine semiquinone; DAOQ dopamine orthoquinone, LAC, leukoaminochrome; AC, aminochrome; DHI, dihydroxyindole; IQ, indole quinone.

pathway, which is described in detail below, involves the oxidation of the catechol ring of DA to form ROS and the electron-deficient quinone (Fig. 10.2).

It has been long appreciated that the neuromelanin that accumulates in the lysosomes of catecholaminergic neurons is the result of DA oxidation [54]. That this accumulation begins early in life and is progressive has been interpreted to mean that the process of oxidation of catecholamines to their quinone oxidation products is continuous throughout one's lifetime. Unlike the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) by the tyrosinase of melanosomes in melanocytes, the oxidation of catecholamines in neurons is not tyrosinase dependent and is termed "autooxidation," a process initiated by ROS as well as by molecular oxygen with transition metal catalysis. (A recent report demonstrating the presence of tyrosinase in the brain [55] and thereby supporting a role for tyrosinase in the oxidation of catecholamines should be interpreted with caution, as the observation may be a result of contamination by leptominingeal melanocytes.) The autooxidation of catecholamines is in addition to their oxidation by MAO. The process of autooxidation increases with increased pH and is countered by reducing agents, such as ascorbic acid. Thus the synaptic vesicle is an acidic and reducing environment where catecholamines are stable, whereas the higher pH of the cytosol enhances autooxidation [56].

In 1978 Graham described for the first time in complete form the autooxidative pathways for the catecholamines to the quinone species that polymerize to form neuromelanin [5]. As illustrated in Figure 10.2, the one-electron oxidation by O_2 can convert DA to its semiquinone radical (DASQ), which can be further oxidized to DA orthoquinone (DAOQ), reactions that generate ROS in addition. The electron-withdrawing carbonyls of DAOQ render the quinone ring electron-deficient and thus a potent electrophile that can react with external nucleophiles, such as the sulfhydryl functions of proteins and reduced glutathione. The amino function of DAOQ can then attack the quinone ring at the 6-position, an irreversible reaction that reduces the carbonyls to hydroxyl functions and yields leukoaminochrome (LAC). Once formed, LAC is then also vulnerable to autooxidation to yield the cyclized quinone aminochrome (AC). AC can then undergo tautomerization (an intramolecular rearrangement that is neither oxidation nor reduction) to 5,6-dihydroxyindole (DHI), and DHI in turn can undergo autooxidation to indole quinone (IQ) (Fig. 10.2). In parallel to the formation of eumelanin in melanosomes, it is likely that multiple species in this reaction sequence participate in the polymerization reactions that result in neuromelanin.

As one would expect, other catecholamines are also vulnerable to autooxidation to their corresponding quinone species. However, DA is much more vulnerable to autooxidation than either norepinephrine (NE) or epinephrine (E) [5]. Furthermore, the orthoquinone

products of NE and E cyclize, respectively, to leukonoradrenochrome and leukoadrenochrome much faster than the cyclization of DAOQ to LAC. The net result of these two observations is that while DA, NE, and E can all contribute to the neuromelanin polymer as the result of autooxidation, DA stands out with its greater potential for cytotoxicity, through both generation of ROS and its oxidation to DAOQ, with its greater availability for reaction with external nucleophiles. It should be appreciated that DAOQ, and especially the orthoquinones derived from NE and E, are transient species that cannot be observed during autooxidation, as the oxidations to the orthoquinones occur more slowly than the subsequent cyclization and oxidation steps. The only methods by which the orthoquinones can be observed are electrochemical oxidation [57] and through oxidation with periodate, as first described by Graham and Jeffs [58]. Indeed, the latter paper and Graham (1978) [5] established that catechols, like 1,2-glycols, form cyclic iodate intermediates that are then hydrolyzed to the orthoquinone. The use of periodate allows specific, rapid oxidation of catecholamines that is not confounded by additional products seen with other chemical oxidants [5, 59]. It is noteworthy to mention that orthoquinones have λ_{max} at 390 nm [5], not at 450 nm as recently reported [60], as this is the λ_{max} of AC at high pH through the dissociation of a proton (the λ_{max} of AC at neutral pH is 480 nm).

10.3.2 DA Oxidation and Neurotoxicity

Under normal conditions, DA is sequestered safely within synaptic vesicle. However, vesicular DA storage can be disrupted by various cellular stressors, resulting in increased cytosolic DA that can become toxic. The cytotoxicity of DA thought to play a role in neurodegeneration in PD is a product of ROS and reactive quinones generated during oxidation. Being electron deficient, DA quinones readily seek out cellular nucleophiles such as the cysteine residues on proteins. Quinone reactivity can thus deplete cellular stores of reduced glutathione. Indeed, elevated levels of 5-*S*-cys-DA and 5-*S*-cys-DOPAC are observed in the SN of PD brains [61]. Quinone reactivity can also result in the adduction and cross-linking of proteins, thereby altering their normal functions. For example, the modification of the PD-linked ubiquitin ligase parkin by the quinones derived from DA oxidation (DAQ) inactivates its enzymatic activity and promotes its aggregation [62]. Consistent with this, an increase in catecholamine-modified parkin was observed in the SNpc of postmortem PD brains [62]. Furthermore, parkin appears to be uniquely susceptible to DA-induced modifications compared to several related E3 members such as HHARI, Cbl, and CHIP [38, 62, 63]. Given that functional parkin plays an important neuroprotective role in the brain, its modification by DAQ provides a mechanism for its dysfunction that is relevant to the pathogenesis of sporadic PD. Notably, a recent proteomic study performed by the Hastings laboratory demonstrated that DJ-1 can also be modified by DAQ [64], although it is not known whether DJ-1 function is compromised as a result. In some cases, DA conjugates can be directly toxic. For example, modification of α-synuclein by DAQ stabilizes the PD-associated protein in its protofibrillar form that can permeabilize synaptic DA-containing vesicles [65]. The resulting vesicular DA leakage, in turn, would augment the pathogenic process. Moreover, DAQ-synuclein adducts can seed the formation of oligomeric complexes that not only inhibit the normal degradation of the protein by chaperone-mediated autophagy (CMA) but also block the degradation of other CMA substrates in the process [66]. The net result is the accumulation of α-synuclein (and other proteins) and an impaired CMA function. Interestingly, α-synuclein can also interact with DA transporters and facilitate their clustering at the cell surface, the consequence of which is an acceleration of DA uptake leading to increased ROS and sensitivity toward DA-induced apoptosis [67]. Finally, several groups have shown that DA oxidation products can promote mitochondrial dysfunction through the impairment of the activity of ETC, as well as inducing mitochondrial permeability transition pore (mPTP) opening leading to organelle swelling [68–71]. Irreversible opening of the mPTP is known to promote the release of proapoptotic factors into the cytosol.

Supporting a toxic role for DA, mice administered with exogenous DA via intrastriatal injection exhibit degeneration of SN dopaminergic neurons that begins in the terminal fields and progresses to eventual loss of cell bodies, the extent of which appears to correlate with the levels of quinone-modified proteins and can be reduced by antioxidant treatment [72, 73]. Furthermore, mice engineered to express a mere 5% of normal VMAT2, a transporter that normally sequesters cytosolic DA into vesicles, display progressive nigrostriatal dopamine dysfunction that ultimately results in neurodegeneration [74]. Alongside this, elevated cysteinyl adducts to L-DOPA and DOPAC are seen early and are followed by increased striatal protein carbonyl, 3-nitrotyrosine formation, and the accumulation of α-synuclein [74]. More recently, an elegant study in mice performed by Chen and colleagues demonstrated that striatal neurons engineered to take up extracellular DA exhibit degeneration within weeks, a phenotype that is accompanied by substantial oxidative protein modifications and decrease in glutathione level [56]. That forced uptake of DA by a neighboring cell (that

is normally GABAergic in nature) can result in its loss is a testimony to the cytotoxic role of DA.

10.3.3 Is DA Really the Culprit?

In view of the toxic potential of oxidative reactions involving DA, and the experimental evidence supporting the cytotoxic effects of DA oxidation products in vivo, it is curious that a considerable regional variability exists in the vulnerability of DA neurons toward degeneration in PD. Notably, the mesencephalon contains two major dopaminergic neuronal populations, namely, the A9 neurons of the SNpc that project to the striatum along the nigrostriatal pathway and the A10 neurons of the ventral tegmental area (VTA) that project to the limbic and cortical areas along the mesolimbic and mesocortical pathways [12]. Despite their anatomical proximity and biochemical and electrophysiological similarities, the A9 neurons are the ones that are selectively lost in PD. Furthermore, this subset of dopaminergic neurons are also lost at a significantly faster rate compared to other neuronal types under conditions of normal aging in the apparent absence of environmental toxin exposure [75]. Although the exact reason for this selectivity remains unclear, Chung and colleagues have recently demonstrated that the expression of antioxidant genes is intrinsically higher in A9 neurons than in A10 neurons [76], suggesting that A9 neurons may be constantly experiencing a sustained heightened level of oxidative stress. Given this, one would assume that the ability of A9 neurons to cope with sudden or chronic increase in ROS production may be more limited than that of their A10 counterparts. Supporting this, treatment of the two neuronal populations with ROS inducers such as MPTP, 6-OHDA, and paraquat (all of which are parkinsonian neurotoxins) results in the death of A9 but not A10 neurons [26, 77, 78]. Conversely, transgenic mice with increased activity of SOD1 or glutathione peroxidase, key ROS scavenging enzymes, are resistant to MPTP-induced dopaminergic neurodegeneration [57, 79].

Why the A9 neurons are in an apparent state of heightened oxidative stress is, however unclear, although a recent study by Mosharov and colleagues demonstrated that cytosolic DA concentrations in these neurons are significantly (2- to 3-fold) higher than in the more resistant VTA neurons [80]. Not surprisingly, cytosolic DA-enriched A9 neurons are found to be more sensitive than A10 neurons to the toxic effects of an acute L-DOPA challenge [80]. The difference in DA levels between these two groups of neurons may be attributed to differences in synthesis (as there is no significant difference with regard to precursor uptake, storage, or degradation of DA), which appears to be related to the use of distinct calcium (Ca^{2+}) channels by these neurons. Unlike the VTA neurons, A9 dopaminergic neurons use L-type Ca^{2+} channels to help maintain autonomous pacemaking [81]. By virtue of the activity and thereby opening of this type of channel in the absence of synaptic inputs, the A9 neurons experience a significantly larger magnitude and spatial extent of Ca^{2+} influx [82]. Importantly, antagonizing L-type Ca^{2+} channel diminishes the differences in cytosolic DA levels between A9 and A10 neurons and concomitantly reduces the toxic effects of acute L-DOPA treatment in the former group of neurons [80], suggesting a complex interplay between calcium and DA in the degeneration of A9 neurons in PD.

The reliance of SN dopaminergic neurons on voltage-dependent L-type Ca^{2+} channels obviously comes with a price, as intracellular Ca^{2+} concentration is under very tight homeostatic control by the actions of ATP-dependent pumps whose operations are metabolically expensive. A sustained entry of Ca^{2+} in these neurons would presumably work the mitochondria machinery harder and concomitantly raise the level of ROS that would predispose them to oxidative stress-induced degeneration. Supporting this, a recent elegant study conducted by Guzman et al. [83] in transgenic mice showed that the basal oxidation of a mitochondrially localized redox-sensitive form of GFP (mito-roGFP) is indeed significantly higher in SN dopaminergic neurons relative to their VTA counterparts. Furthermore, the level of mito-roGFP oxidation in SN (but not VTA) dopaminergic neurons can simply be lowered by the administration of L-type Ca^{2+} channel antagonists in these transgenic mice. Importantly, the ablation of DJ-1 expression results in the amplification of basal oxidant stress in SN dopaminergic neurons. Collectively, the findings by Guzman et al. emphasize the intimate relationship between L-type Ca^{2+} channels and mitochondrial oxidant stress. This "L-type Ca^{2+} hypothesis" [82] is certainly an attractive proposition, as neurons in the locus ceruleus (LC) that are also lost in the PD brain are similarly autonomous pacemakers dependent on the activity of L-type Ca^{2+} channels [84]. Furthermore, the majority of A10 neurons contain Ca^{2+}-binding proteins such as calbindin D-28K that are capable of buffering intracellular Ca^{2+} to prevent it from rising to damaging levels within the cell [85]. Comparatively, the A9 neurons express calbindin D-28K at significantly lower levels [76]. However, it is noteworthy that PD pathology according to the neuropathological staging proposed by Braak is not confined to the SN or LC but progressively extends from the caudal to the rostral brain regions, involving not just the dopaminergic system but also noradrenergic, cholinergic, and serotonergic systems that may or may not rely on L-type Ca^{2+} channels [86]. Thus, as attractive as the "L-type Ca^{2+} hypothesis" may seem, it does not

adequately explain all the predilection sites in the PD brain, although it remains an extremely attractive factor underlying the vulnerability of SN dopaminergic neurons to degeneration.

10.3.4 Extracellular Oxidative Stress

It is important to recognize that in addition to intrinsic factors, extracellular oxidative stress mediated by activated glial cells may also accelerate the degeneration of SN dopaminergic neurons in disease conditions. In their normal state, glial cells participate in the maintenance of neuronal homeostasis in several ways, including providing trophic support to neurons and helping to clear neurotransmitter released into synapses [87, 88]. Upon their activation during neuroinflammation, reactive astrocytes and microglia release various inflammatory cytokines and complement factors to repair or dispose of damage cells and thereby reestablish the regional microenvironment. Associated with this is the production of ROS and RNS [89]. Although beneficial, excess or chronic neuroinflammation can obviously exert unintended deleterious effects on neighboring dopaminergic neurons and contribute to disease progression [90]. The activation of glial cells is well documented in affected regions of the PD brain, as well as in genetic and toxin-induced models of PD [91]. Glial cell activation is usually accompanied by the upregulation of iNOS, which is either absent or expressed at very low level in the normal brain. Notably, iNOS null mice are less susceptible to MPTP-induced neurotoxicity compared to their wild-type counterparts and, accordingly, display reduced staining for markers of oxidative stress [80]. Similarly, inactivation of the inflammatory enzyme NADPH-oxidase, which catalyzes the production of superoxide from oxygen and NADPH, results in the attenuation of MPTP-induced neurotoxicity in mice [92]. The expression of both iNOS and NADPH-oxidase are elevated in the PD brain [92, 93]. Activated microglia is also known to upregulate the expression of cyclooxygenase-2 (Cox-2) and thereby the synthesis of prostaglandins that could, in turn, kill neurons directly through the activation of caspase-3 or indirectly via the excessive release of glutamate by astrocytes [94]. Interestingly, SN dopaminergic neurons express Cox-2, suggesting that dysregulated Cox-2 levels may lead to cell-autonomous suicide [95]. Inhibition of Cox-2 apparently mitigates the formation of the deleterious DA-quinone and reduced toxicity in a MPTP mouse model [95]. Notwithstanding the obvious damage to neighboring cells that chronic neuroinflammation could bring, the glial response is often taken to be a secondary cause of the neurodegenerative process. Furthermore, neuroinflammation is not limited to PD but a consistent feature of neurodegenerative diseases in general. Nonetheless, the role of non-cell-autonomous neuronal death is regaining its prominence in the field as more and more researchers are revisiting the cross talk between neurons and glial cells in neurodegeneration.

10.4 THERAPEUTIC IMPLICATIONS

Given the apparently compelling role of oxidative stress in PD pathogenesis, strategies aimed at reducing oxidative stress should in theory mitigate disease progression and provide clinical benefits. Indeed, the success of such strategies will provide the ultimate proof of concept that oxidative stress is a key factor underlying neuronal death in PD. The first controlled clinical trial to evaluate the potential of antioxidants as neuroprotective agents was the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) study [96]. Deprenyl (selegiline) is an irreversible MAO-B inhibitor that is expected to reduce the formation of DOPAC and hydrogen peroxide from oxidative deamination of DA. In so doing, deprenyl would decrease the formation of oxyradicals from hydrogen peroxide generated by DA metabolism. α-tocopherol is vitamin E, an established antioxidant. However, this study together with several other subsequent studies utilizing similar antioxidant strategies failed to produce convincing protection against PD. If oxidative stress is indeed the primary cause of PD pathogenesis, why have these trials apparently failed? There could be several explanations for the generally disappointing outcomes of antioxidant clinical trials in PD, including the study design involved, the availability of administered compounds in the brain, and the heterogeneity of PD patients. Furthermore, as much as we like to believe that oxidative stress is a central player in PD pathogenesis, it is obviously not the only player but one important factor among several others all interwoven in a tapestry of events underlying the degeneration process. To expect a singular therapy to work effectively for a complex disease like PD may just be too tall an order. Perhaps a combination of therapies targeted at major problem centers in the pathogenic cascade may be the way to go, although getting administrative approval for such cocktail drug trials would invariably pose a challenge. A "druggable" target related to the oxidative stress cascade is obviously the L-type Ca^{2+} channel, which can be antagonized by dihydropyridines (DHPs). DHPs have been approved for human use for the treatment of hypertension. Interestingly, a large Danish study conducted recently demonstrated that reducing Ca^{2+} influx in the brain via the administration of DHPs decreases the risk for PD [97]. Given the interplay between DA and calcium in

dopaminergic neurodegeneration, and the availability of FDA-approved Ca^{2+} channel antagonists, it may be worthwhile to explore the clinical benefits of combining antioxidants with Ca^{2+} channel blockers for the PD patient.

10.5 CONCLUSION

It is apparent from the discussion above that oxidative stress has a definite role in PD pathogenesis. Indeed, virtually all the epidemiological PD risk factors identified to date are directly or indirectly linked to oxidative stress. Similarly, the majority of the genetic causative factors of the disease are associated in one way or another with oxidative stress. Even normal aging, recognized as an unequivocal risk factor for PD, is accompanied by increased indices of oxidative stress. Coupling these phenomena to the knowledge that intrinsic pro-oxidant factors like iron, DA, and L-type Ca^{2+} channel all can confer neuronal vulnerability to degeneration, it is hard not to be persuaded by the oxidative stress hypothesis in PD pathogenesis. However, a nagging question that constantly surfaces is whether oxidative stress represents a cause or a consequence of PD. The "chicken and egg" argument here is that oxidative stress can be as much a promoter as it is a result of neuronal death. This is a longstanding debate that is difficult to resolve.

As discussed above, several studies have highlighted an unequal distribution of pro- and antioxidant factors in different regions of the brain [98]. Notably, the basal oxidant level in vulnerable neurons is typically higher than in neighboring neurons that are unaffected, as in the case between A9 and A10 dopaminergic neurons, even in seemingly healthy brain. The most logical assumption to make here is that neurons with high intrinsic oxidant levels are predisposed to degeneration but not necessarily already exhibiting signs of degeneration or undergoing degeneration. The corollary is that oxidative stress is unlikely to be a mere passive consequence of neuronal death, notwithstanding that dying neurons can further elevate pro-oxidative events. A related question that many often ask is whether oxidative stress represents a primary or secondary event in PD. Perhaps a more pertinent question to ask here is whether we should be taking a reductionist approach in pinpointing causative factors of PD. Many would agree that the PD pathogenesis involves an intricate network of interacting pathways rather than a linear sequence of events. For example, oxidative stress can cause mitochondrial dysfunction and vice versa. Likewise, oxidative stress can damage the proteasome, and, conversely, proteasome dysfunction can also lead to oxidative stress [99]. In this circle of events, oxidative stress can therefore be a primary cause as much as it can represent a secondary cause of the disease. The important thing to recognize is that while oxidative stress is a persuasive key player in PD pathogenesis, it is likely to be one among several other key players.

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