Oxidative Stress and Neurotoxicity

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There is increasing awareness of the ubiquitous role of oxidative stress in neurodegenerative disease states. A continuing challenge is to be able to distinguish between oxidative changes that occur early in the disease from those that are secondary manifestations of neuronal degeneration. This perspective highlights the role of oxidative stress in Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis, and multiple sclerosis, neurodegenerative and neuroinflammatory disorders where there is evidence for a primary contribution of oxidative stress in neuronal death, as opposed to other diseases where oxidative stress more likely plays a secondary or by-stander role. We begin with a brief review of the biochemistry of oxidative stress as it relates to mechanisms that lead to cell death, and why the central nervous system is particularly susceptible to such mechanisms. Following a review of oxidative stress involvement in individual disease states, some conclusions are provided as to what further research should hope to accomplish in the field.

Contents

1. Introduction

Oxidative stress is defined as the imbalance between biochemical processes leading to the production of reactive oxygen species (ROS)[1] and those responsible for the removal of ROS, the so-called antioxidant cascade. Research over the past few decades has revealed the widespread involvement of oxidative stress in a number of disease states, most notably those that have increased incidence with age. Indeed, the free radical theory of aging (*1*) is a central tenet that shapes our understanding of the biochemical changes that occur toward the end of life. ROS are known to damage all cellular biomacromolecules (lipids, sugars, proteins, and polynucleotides), and this damage can lead to secondary products that can be just as damaging as the initial ROS. The central nervous system is particularly vulnerable to oxidative insult on account of the high rate of O_2 utilization, the relatively poor concentrations of classical antioxidants and related enzymes, and the high content of polyunsaturated lipids, the biomacromolecules most susceptible to oxidation. In addition, there are regionally high concentrations of redox-active transition metals capable of the catalytic generation of ROS. Thus, it is not surprising that oxidative stress is a common discussion point for neurodegenerative disease, where damage to neurons can reflect both an increase in oxidative processes and a decrease in antioxidant defenses.

For three age-related neurodegenerative diseases, Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), in addition to the more common sporadic forms, there are rare familial forms, the hereditary bases of which continue to be defined by advances in molecular genetics. The sporadic forms of these diseases are predominately of unknown origin but are thought to reflect a complex combination of hereditary, environmental, and lifestyle factors. The same is true for multiple sclerosis (MS), a progressive autoimmune demyelinating disease. In contrast, Huntington's disease (HD) is a strictly autosomal, dominantly inherited, progressive neurodegenerative disorder. Regardless of etiology, there is irrefutable evidence for some component of oxidative stress in all of these neurodegenerative diseases, but the central question is whether oxidative stress is a consequence of degenerative processes initiated by some other factor, for example, genetic, or whether oxidative stress is an early event that contributes integrally to the etiology of the disease. Often both primary and secondary oxidative stress components occur simultaneously. Indeed, it has been relatively straightforward to document an association of oxidative stress with neurodegeneration, by finding increased levels of oxidative stress markers in tissues during disease progression or immunocytochemical evidence for oxidative damage to biomacromolecules in affected brain regions seen at

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[1] Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; Aβ, amyloid-β; AβPP, amyloid-β protein precursor; CSF, cerebrospinal fluid; DNPH, 2,4-dinitrophenylhydrazine; EAE, experimental autoimmune encephalomyelitis; GSH, glutathione; HD, Huntington's disease; HNE, (*E*)-4-hydroxy-2-nonenal; IRE, iron responsive element; IRP, iron regulatory protein; LDH, lactate dehydrogenase; mhtt, mutant huntingtin; mtDNA, mitochondrial DNA; MS, multiple sclerosis; NFT, neurofibrillary tangles; ONE, (*E*)-4-oxo-2-nonenal; PD, Parkinson's disease; PHF, paired helical filaments; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; SP, senile plaques.

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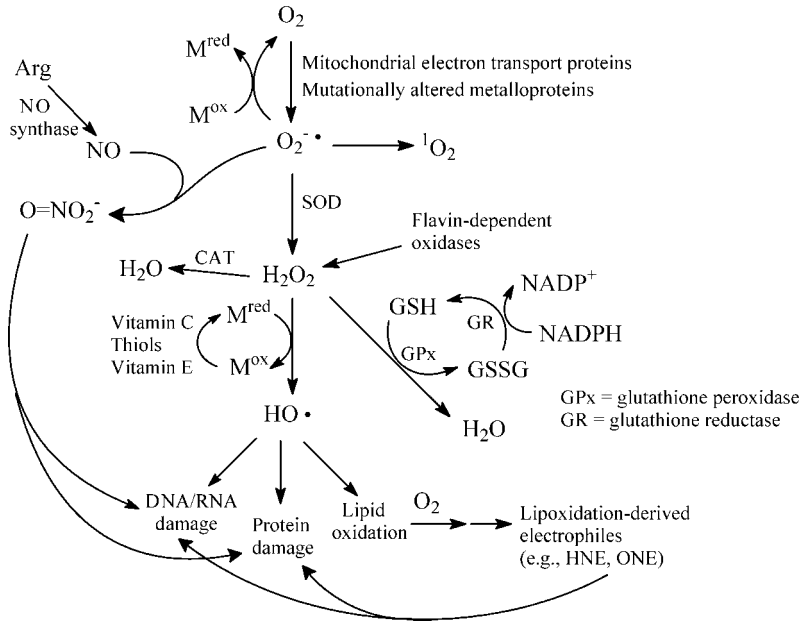
Scheme 1



autopsy. What is less clear is the nature of the relationship and mechanism between the state of oxidative stress and cell death. This perspective will both summarize the evidence for a primary role of oxidative stress in particular neurological diseases and offer insight into various mechanisms that tie oxidative stress to cell death.

2. Oxidative Stress and Neurodegenerative Disease—General Considerations

There are four key common threads that run across the spectrum of neurodegenerative disease, although not every disease has all features. First, there is increasing awareness of the interplay between a neuroinflammatory component and chronic oxidative stress. In recent years, the upsurge in research on nitric oxide (NO) as a common second messenger in inflammatory signaling has resulted in recognition of its enzymatic release from macrophages (or activated microglia in the CNS), along with superoxide. Accumulating levels of diffusible NO and superoxide give rise to peroxynitrite. Peroxynitrite and related reactive nitrogen species (RNS) are capable of both oxidation and nitration chemistries (*2*), resulting in a condition known as "nitrosative stress", and there is growing acceptance that ROS and RNS act together to mediate damage in degenerative disease (*3–6*). Not only do microglial-derived ROS and RNS create a stress upon ambient neurons, but conversely, oxidants can stimulate pro-inflammatory gene transcription in glia, creating a vicious cycle. A second common feature is the accumulation of unfolded or misfolded proteins in brain cells, leading some workers to refer to AD, PD, HD, and ALS as "protein conformational diseases". The third common feature, most prominent in AD, PD, and MS, is dyshomeostasis of both redox-active (e.g., copper and iron) and redox-inactive (e.g., zinc) metal ions (*7–9*). The fourth common feature is abnormal functioning of mitochondria (*10*), which play a critical role in metabolism and regulate the entire life cycle of the cell (e.g., in mediating apoptosis). These features are not independent; for example, that small-molecule products of oxidative stress can mediate protein misassembly (*11*).

Because oxidative phosphorylation in mitochondria is the major source of ROS, there is an intrinsic link between mitochondrial abnormalities in neurodegenerative disease and the involvement of oxidative stress. To protect itself under normal physiological conditions, the inner membrane of mitochondria incorporates a number of free radical scavengers and enzymatic ROS removal systems. However, in certain pathological states, it is apparent that mitochondrial defenses can become compromised, due to either genetic mutations or an increase in radical production. Although it is usually difficult to distinguish whether mitochondrial defects are the primary cause of toxicity or instead, reflect secondary collateral damage, a growing body of evidence seems to indicate that mitochondrial-derived oxidative stress is a primary event associated with neurodegeneration (*12*). Moreover, there is increasing awareness of the concept of mitochondrial ROS production causing self-inflicted damage to the respiratory chain, which can result in increased ROS production and a cycle of further mitochondrial protein damage. Also, the lack of protective histones in mitochondrial DNA (mtDNA) together with limited repair capacity render mtDNA an easy target for ROS.

AD and PD, in particular, are diseases for which there is the most compelling evidence for a role of mitochondrial aberrations, metabolic imbalance and resulting oxidative stress, both superimposed on hereditary factors and likely playing a larger role in the spontaneously occurring forms of these two diseases (*13–15*). Evidence for oxidative stress in AD and PD is consonant with the finding that the areas of the brain affected by these diseases contain abnormally high levels of redox-active metals, particularly iron. An excess of redox-active metals is presumed to be at least partially responsible for the oxidative damage seen to proteins, polyunsaturated lipids, and DNA/RNA in both AD (*16, 17*) and PD (*18*).

3. Overview of Oxidative Stress Biochemistry and Neurotoxicity

Mitochondrial production of ROS initially arises as superoxide anion radical from the side reaction of O_2 intercepting single electrons from the electron transport chain (Scheme 1). Superoxide can also arise from mutationally altered or damaged metalloenzymes involved in oxidative metabolism, and it is the principal source of defensive pro-oxidants generated in the respiratory burst of neutrophils. Superoxide can subsequently be transformed to H_2O_2, and the latter, through Fenton reaction

with reduced transition metal ions [usually Fe(II) or Cu(I)], can be transformed to hydroxyl radical. Rereduction of the resulting oxidized transition metal ions [Fe(III) or Cu(II)] can be effected by cellular reductants such as vitamin C or thiols and perhaps even vitamin E. This process is one type of what is known as "redox cycling", another being the short circuiting of the normal respiratory reduction of O_2 to water by one-electron organic redox agents that reduce O_2 instead to superoxide and are, in turn, re-reduced by flavin reductases at the expense of consuming cellular reducing equivalents.

Although copper, iron, manganese, and other trace redox-active transition metals are essential in most biological systems, their accumulation in tissues excess of the capacity of the cellular complement of metalloproteins (catalytic, transport, and storage) results in increased concentration of the free metal ions. Usually, it is these free metal ions, or equilibrium low-affinity complexes with amino acids, which mediate oxidative stress reactions (*19, 20*), and only trace levels are needed to catalyze redox cycling. Redox-inactive metal ions such as zinc may then be pathogenic by virtue of their ability to displace redox-active metal ions from sites where redox activity of the latter is sequestered. However, metal ions bound adventitiously to proteins or polynucleotides that retain free coordination sites can also contribute to ROS production. In the latter regard, it is important to keep in mind that because of numerous antioxidant defense mechanisms, neurotoxic levels of ROS would likely arise only through catalytic redox cycling processes rather than from stoichiometric reductions of metals by the biomolecules to which they bind. Regardless of redox potential, transition metals, through their coordination properties, may additionally contribute to neurodegeneration through their deleterious effects on protein and peptide structure, such as pathological aggregation phenomena. Thus, redox-active transition metals can sometimes exert dual neurotoxic properties.

The hydroxyl radical is considered the chief instigator of oxidative stress damage and reacts nondiscriminately with all biomacromolecules (Scheme 1) at diffusion-controlled rates, that is, within nanometer distances from its site of generation. In contrast to the hydroxyl radical, the superoxide radical is relatively unreactive, except at lower pH, where it exists as the hydroperoxy radical. Under normal conditions, damage by ROS is kept in check by an efficient antioxidant cascade, including both enzymatic and nonenzymatic entities. Important in the former regard are cytosolic copper-zinc superoxide dismutase (CuZnSOD, SOD1) and mitochondrial manganese superoxide dismutase (MnSOD), which convert superoxide to O_2 and H_2O_2. The latter, also the normal by-product of oxygen reduction by oxidases such as monoamine oxidase, is removed by catalase (CAT, Scheme 1) and peroxidases, which have ubiquitous tissue distribution. Given that the reaction catalyzed by SOD converts the less reactive superoxide to H_2O_2, the direct precursor of the more reactive hydroxyl radical, it is not immediately obvious why SOD is viewed as an antioxidant enzyme. The reasons appear to be that (i) in the absence of SOD, nonenzymatic dismutation of superoxide, although still fast, can result in generation of highly reactive singlet oxygen; (ii) superoxide can serve as the reductant of oxidized metal ions for the production of hydroxyl radical from H_2O_2, which, coupled with the Fenton reaction, is known as the Haber–Weiss process; (iii) superoxide can liberate redox-active free iron from iron sulfur proteins such as aconitase; and (iv) superoxide can react with NO to form highly reactive peroxynitrite. Although it is unclear whether superoxide could outcompete other cellular reductants (glutathione, NADPH, and ascorbate) in the reduction of oxidized transition metal ions (*21*), the enumerated factors together appear to explain why SOD overexpression is neuroprotective (*22, 23*).

It is important to consider the major chemical consequences of unchecked ROS production with respect to damage inflicted to biomacromolecules. An overarching principle is that the finding of oxidative markers in diseased tissues reflects a balance between the rate of their formation and the rate at which the damaged biomacromolecules undergo turnover (or repair). As such, increases in oxidative markers could represent a decreased efficiency of the ubiquitin-proteasome and other systems for removal of damaged biomolecules, as well as increased oxidative damage.

Oxidation of protein side chains (*24, 25*) by ROS and RNS species such as peroxynitrite usually results either in introduction of hydroxyl groups or in the generation of protein-based carbonyls detectable by 2,4-dinitrophenylhydrazine (DNPH) (*26, 27*). Oxidative damage to proteins can also occur due to alternate oxidants (e.g., HOCl) and circulating oxidized amino acids such as tyrosine radical generated by metalloenzymes such as myeloperoxidase (*28*). In addition, a substantial fraction of protein damage that occurs under conditions of oxidative stress may represent adduction of secondary products of the oxidation of sugars, termed glycoxidation, or of the oxidation of polyunsaturated lipids (*14*), termed lipoxidation. Considering the short diffusion distance of the hydroxyl radical, it seems likely that most H_2O_2-dependent protein oxidation reflects reaction of H_2O_2 with reduced metal ions coordinated adventitiously to the protein. Examples of such "site-specific" oxidations (*24, 29, 30*) include oxidation of His imidazole to its 2-imidazolone derivative, and the oxidative deaminations of Lys and Arg side chains, although mechanistic details have yet to be ascertained. Notwithstanding, besides cysteine (oxidized to its disulfide), the most susceptible residue to H_2O_2 is methionine, whose oxidation to methionine sulfoxide (MetO) is reversible in most cells through the action of stereospecific methionine sulfoxide reductases that catalyze the thioredoxin-dependent reduction of MetO back to Met (*31, 32*). Such cyclic oxidation–reduction of Met residues lead to consumption of ROS at the expense of cellular reducing agents and thus serve as a buffering mechanism that increases the resistance of proteins to oxidative damage.

Oxidative stress conditions and the occurrence of iron- or copper-mediated Fenton chemistry also result in oxidative damage to nucleic acids, in particular RNA. 8-Hydroxyguanosine (8OHG) is a marker of nucleic acid oxidation commonly observed in the cytoplasm of neurons (*33*). Polyunsaturated lipids in lipoproteins and membranes are also highly susceptible to oxidative stress damage. The availability of a particularly weak bis-allylic C–H bond in polyunsaturated lipids allows for propagation of a free radical chain autoxidation process known as lipid peroxidation, with alkylperoxy radicals as chain carriers. If there is extensive membrane oxidation, neurotoxicity could theoretically arise in part from compromises in membrane function, affecting maintenance of membrane potential, synaptic signaling, etc. Such a compromise has only been documented so far for mitochondria (*34, 35*). For whole cells, it will be difficult to distinguish whether altered membrane properties reflect ROS-mediated structural damage or a secondary effect of ROS-mediated intracellular signaling pathways (*36*).

The unsaturated hydroperoxides generated from peroxidation of polyunsaturated lipids undergo, in part, conversion to stable products such as isoprostanes that have been used as in vivo biomarkers of oxidative stress in neurodegeneration (*37, 38*).

However, the lipid hydroperoxides also undergo chain cleavage, mediated by reduced metals or ascorbate (*39*), to a host of mono- and bifunctional reactive aldehydes (*40, 41*), some containing the methyl terminus and thus freely diffusible, and others containing the carboxyl terminus (*42, 43*), either as the free acid or esterified to cholesterol or phospholipid. These aldehydes readily modify proteins (*14*) and DNA (*44*), and an intense effort over the past few decades has been directed at ascertaining the nature of these adducts. Although the latter are clearly at least candidate biomarkers of disease, evidence is also accumulating for their being causally involved in many pathophysiological effects associated with oxidative stress in cells and tissues in vivo (*45*). The greatest effort has been directed at 4-hydroxy-2-nonenal (HNE), a readily diffusible and selective electrophile (*40, 46*). There is now substantial evidence for increased HNE generation (and lipid peroxidation in general) in neurodegenerative disease, in particular AD and PD (see below). In addition to its ability to modify and cross-link proteins (*47, 48*), HNE readily modifies DNA bases (*49–53*). A cousin of HNE, 4-oxo-2-nonenal (ONE), is a more recently discovered direct product of lipid oxidation (*39, 54*), is more reactive with proteins (*47*) and DNA (*55, 56*), and is also more neurotoxic (*57*).

The α,β-unsaturation in many lipoxidation products, including 4-hydroxy-2-enals such as HNE, 4-oxo-2-enals such as ONE, simple 2-enals such as acrolein and 2-octenal, 2,4-dienals, and epoxyenals and epoxyenones, makes them particularly susceptible to conjugate addition reactions. The adducts thus formed, especially on Cys and His residues, become protein-bound aldehydes that can be derivatized by DNPH. Recent research suggests that DNPH-detectable protein-based carbonyls, a key benchmark of oxidative stress, may reflect mainly the consequence of covalent binding of α,β-unsaturated aldehydes emanating from lipid oxidation rather than metal-catalyzed oxidative degradation of protein side chains (*58*).

For those diseases where characteristic brain lesions represent deposition of specific proteins (see below), the immunoreactivity of these lesions to oxidative stress antibodies suggests that the irreversible deposition of protein reflects in part a "cementing" of possibly reversibly formed aggregates by covalent cross-linking associated with oxidative stress, including bifunctional lipid oxidation products (*59*). Because recent studies suggest that the small oligomeric intermediates in the aggregation process may be most neurotoxic, it will be important to ascertain how modification by lipoxidation-derived aldehydes may modulate the aggregation process and the resulting toxicity.

If one accepts a role of oxidative stress and ROS production as a causal contributor to neurodegenerative disease, the question arises as to what chemical steps initiate the biochemical cascade leading to neuronal death. This question is best addressed through studies on cells in culture, although the exact mechanism could vary somewhat with cell type. In general, although H_2O_2 could damage cells through direct oxidation of lipids, proteins, and DNA, and thus one might expect a generalized necrotic cell death at higher concentrations, most studies using lower concentrations of H_2O_2 have revealed a concentration-dependent induction of mitochondrial-driven apoptosis (*60, 61*). Recent research focused on identifying what upstream events might be involved in H_2O_2 toxicity has revealed (i) a marked reduction of Krebs cycle dehydrogenase activities (*62*), (ii) activation of JNK1/2 mainly via *N*-methyl-D-aspartate (NMDA) receptor-mediated influx of extracellular Ca^{2+} (*63*), and (iii) activation of the growth factor receptor/Ras/MEK/ERK signaling pathway (*64*). In cultured rat cortical neurons, H_2O_2-induced membrane depolarization and Ca^{2+} influx was shown to require activation of the 5-HT3 receptor (*65*).

The evidence that H_2O_2 at low concentration activates signaling pathways has broadened awareness that H_2O_2 may be involved in regulating a variety of physiological responses (*66*), including sensing of oxidative stress. There is much current interest in identifying the protein receptors involved in this signaling (*67*), and antioxidant enzymes may be playing a signal transduction role in addition to their function in removing ROS (*68*). Moreover, when one considers the potential role of other, mostly gaseous, small molecules that are endogenously produced, including O_3 (ozone), CO, CO_2, H_2S, and NO_2, as well as possible "cross-talk" among them through chemical interconversions, the coordinated regulation of these species suggests that they all may be used in cell signaling, at least at low, subtoxic concentrations (*69*). How all of these mechanisms identified in vitro translate into in vivo physiology will be an important subject of future research.

As far as lipid oxidation products such as HNE, ONE, and acrolein are concerned, most evidence suggests that low concentrations are also toxic to cells, including neuroblastoma cells (*57*), through an apoptotic pathway (*70, 71*). The chemical basis of action of the aldehydes that results in apoptosis is unknown, but recent research indicates that HNE is a signaling molecule at subtoxic concentrations (*70, 72–74*), modulating MAP kinases, PKC isoforms, cell cycle regulators, receptor tyrosine kinases, and caspases and activating the JNK-c-Jun/AP-1 pathway (*75*). Subtoxic concentrations of HNE were also observed to induce expression of various antioxidant/detoxification enzymes (*76*).

It is not unreasonable to think that some biological activities of HNE and other lipid oxidation products could reflect a noncovalent interaction with an appropriate receptor, and there is evidence that HNE is a ligand for at least the nuclear receptor peroxisome proliferator-activated receptor-β/δ (*77*). However, because HNE is capable of covalent protein modification, it seems likely that most signaling actions of HNE reflect covalent modification of proteins designed to sense oxidative stress, for example, through modification of particularly susceptible residues, such as Cys sulfhydryls and His imidazoles. Of the many reported observations of HNE signaling, some cases have now identified covalent adduct formation to accompany the signaling event (*78–80*), including one case where the modified residue was identified (*81*). This area of research is likely to be very active for the next several years, and because HNE will form adducts of any particular target protein examined, it will be important to determine whether the identified adduct is actually functionally related to the signaling event.

4. Neuropathology of AD

In addition to a selective neuronal degeneration, AD is characterized pathologically by the presence of two hallmark lesions in the brain, extracellular senile plaques (SP) and intraneuronal neurofibrillary tangles (NFT). SP contain amyloid-β (Aβ) peptide, primarily Aβ (1–42), whereas NFT are composed mainly of the microtubule-associated protein τ present as paired helical filaments (PHF). Research on the causes of AD over the past two decades has focused mainly on the hope of finding biochemical clues revealed by the multiplicity of genetic mutations that account for the familial cases of AD, although these are early onset, rapidly progressing forms of AD that could differ from the late onset sporadic disease. The familial AD cases involve mutations in the genes encoding the amyloid-β precursor protein (AβPP) and/or the presenilins,

proteolytic enzymes that process AβPP. It was initially presumed that these mutations would lead to either an overproduction of AβPP and/or altered AβPP proteolytic processing, leading to increased Aβ(1–42). Indeed, transgenic mice overexpressing AβPP or one or more of the human mutant AD-related proteins exhibit many of the neuropathologic and behavioral features of the human disease, including the development of SP, and, for a mouse also expressing mutant τ protein (*82*), NFT. Although these animal models have been, and continue to be, used to develop and test potential treatments for the disease, the absence of notable neurotoxicity has called into question the actual relevance to human AD. Adding to this controversy is recent data indicating that some pathogenic mutations lead to decreased rather than increased levels of Aβ (*83–85*). Thus, although the central theme in AD research for many years held that the onset and progression of AD is initiated by aggregation of Aβ into toxic fibrillar deposits within the extracellular space of the brain, much evidence suggests that the mature SP is nontoxic (*86*). More recent efforts to link Aβ and synaptic compromise in AD have instead focused on the soluble oligomers of Aβ, presumed intermediates in the aggregation process (*87–90*), but it is unclear, even for these, whether the neurotoxic effects observed in vitro are relevant to in vivo disease.

In contrast to SP, τ pathology and NFT do correlate with symptom presentation in patients (*91*). Although τ normally functions to regulate microtubule assembly (and transport) and thereby maintain normal axonal caliber, in AD τ is "hyperphosphorylated", at up to 22 different sites, at the stage where it loses its microtubule-binding and -stabilizing function and aggregates into PHF (*92*). τ hyperphosphorylation reflects both an abnormal action of kinases, as well as decreased phosphatase activity (*93*). Hyperphosphorylated τ not only fails in its normal function in stabilizing microtubules, but it also exhibits a "gain of toxic function" due to its sequestering normal τ, resulting in the disruption of microtubules (*94, 95*). Intraneuronal accumulation of PHF-τ could reflect inhibitory binding of oxidatively damaged protein to the proteasome, and dysfunction of the latter may be sufficient to induce neuronal degeneration and death in AD (*96*). Although it is unclear to what extent NFT deposition might potentially be reversible (assuming aggregation reflects only noncovalent interprotein interactions), it has been our contention that the persistent insolubilization and permanency of NFT aggregates represents, at least in part, cementing of the aggregates by processes associated with oxidative stress (*97, 98*).

Overall, there remains considerable debate not only as to whether neuronal loss in AD reflects more the appearance of SP or of NFT or indeed whether either of these late stage proteinacious deposits is intimately tied to neurotoxicity. SP and NFT may be more the effect than the cause of the disease (*99*). Indeed, a theory currently gaining increased favor is that deposition of SP and NFT is a compensatory response of the brain to counteract various toxic intermediates (*100*), possibly either by their sequestration into the deposits or by serving as a sacrificial trap for ROS or "buffer" for reactive carbonyl products of lipid and sugar oxidation (*101, 102*).

5. Role of Oxidative Stress in AD

Neuronal degeneration in the CNS of AD patients is associated with oxidative damage to all biomacromolecule types (*103*): (i) DNA and RNA oxidation is marked by increased levels of 8-hydroxy-2-deoxyguanosine (8OHdG) and 8-hydroxyguanosine (8OHG) (*16, 17*) and increased DNA oxidation and decreased repair in CSF (*104, 105*). (ii) Protein oxidation is marked by elevated levels of protein carbonyls and nitrotyrosine (*106–108*). (iii) Lipid peroxidation is marked by higher levels of malondialdehyde and isoprostanes, as well as protein modification by HNE (*38, 109–113*) and by acrolein (*114*). (iv) Sugar oxidation is marked by increased protein glycation and glycoxidation (*115–120*). The finding that most of the covalent modifications induced by products of oxidative stress are seen in apparently normal neurons in AD and at pre-NFT PHF-τ stages (*111, 121–123*) suggests that these modifications play at least partially a causative rather than merely by-stander role in the neurofibrillary pathology in AD. A state of oxidative stress underlying damage to vulnerable neurons in AD is further provided by immunocytochemical evidence for the upregulation in vulnerable neurons of antioxidant enzymes such as heme oxygenase-1 (HO-1) (*124–126*), SOD (*127, 128*), glucose-6-phosphate dehydrogenase (*129, 130*), and increased levels of reduced sulfhydryls (*130, 131*). Although increased oxidative damage in AD may thus represent an insufficient antioxidant response (*132*), there is evidence to suggest that oxidative markers are more prevalent in initial rather than later stages of the disease, possibly reflecting more successful compensating antioxidant effects of later events, for example, of SP deposition (see above) (*133*).

It has been known for some time that Aβ peptides are toxic to neurons in cell culture. Free radical mechanisms and ROS generation have been suggested to be responsible (*134*), and antioxidants protect against Aβ neurotoxicity (*135*). Human Aβ(1–42) was shown to be more neurotoxic than Aβ(1–40), whereas the reverse sequence was shown to be non-neurotoxic. This indicated that secondary and tertiary structural features played an integral role in toxicity and not just the nature of the amino acid side chains. An important question has been whether ROS generation derives chemically from pure Aβ peptide in solution or whether ROS generation might result from a cascade of cellular events following recognition of Aβ aggregates by cell-surface receptors, such as the receptor for advanced glycation end products (RAGE) (*136*). In the former case, it was considered at one extreme that the free peptide in solution might spontaneously generate radicals through a shear mechanism (*137*). However, the consensus of most subsequent studies focused on the involvement of redox-active transition metal ions in radical production (*138*) and Aβ neurotoxicity (*139*).

Any discussion surrounding the role of oxidative stress in AD is intimately tied to the findings of a major dyshomeostasis of metal ions in AD brain, in particular redox-active metals. Zinc, iron, and copper are significantly elevated in AD pathology (*140–144*). Histochemical analysis of AD brain reveals the presence of nonenzymatic redox activity that appears to represent copper as well as iron (*145*). Dysregulation of iron homeostasis in AD, consistent with the finding that the iron regulatory protein IRP-2 is specifically colocalized in AD pathology (*146*), may be a consequence of induction of HO-1 (*124–126*), the enzyme responsible for conversion of heme to iron and biliverdin. Thus, although HO-1 induction may reflect an effort to increase the generation of the antioxidant biliverdin, increased turnover of mitochondrial heme proteins with release of redox-active iron and copper (*147*) could actually increase rather than decrease oxidative stress (*148*). Such a scenario is consistent with the notion that mitochondrial abnormalities (*149–151*) and metal accumulations, likely acting in synergistic combination, are major producers of ROS possibly responsible for both local and global oxidative stress in AD that may underlie neuronal toxicity.

Data pointing to imbalances in trace metal homeostasis in AD has led over the years to efforts to identify disease-relevant metal ion interactions with both AβPP and Aβ. Initial studies

showed that metals could induce aggregation of Aβ peptides (*138, 152, 153*), possibly explaining the enrichment of these metals in SP (*154*). Thus, it was considered that metal chelators might not only prevent Aβ aggregation and deposition of Aβ senile plaques but might also solubilize Aβ aggregates by extracting out the metals (*155, 156*). However, studies to ascertain whether chelators might function in this regard revealed a complex interplay of effects on metal ion homeostasis. Thus, in the case of clioquinol, a drug that underwent initial clinical trials for treatment of AD, benefits appear to arise from counteracting the intracellular copper-depleting effects of AβPP (*157*).

The suggestion of redox metal interactions with proteins implicated in AD initially focused on AβPP. However, in studies claiming that AβPP could reduce Cu(II) to Cu(I) (*158, 159*), which could in turn reduce O_2 to ROS, the ligand used to detect Cu(I) was bathocuproine disulfonate, known to bind to Cu(II) and make it a much stronger than normal oxidizing agent (*160*). A high-affinity binding domain for copper (and zinc) was first described through NMR studies to be a tetrahedral "blue copper"-like site favoring reduction of Cu(II) to Cu(I) (*161*), but crystallography has more recently defined the site to be a typical type II "nonblue" site favoring Cu(II) (*162*). Although it thus seems unlikely that AβPP could reduce Cu(II) under physiological conditions, binding of copper to AβPP would no doubt modulate its redox properties (*163*), and Cu-mediated cleavage of AβPP occurs in the presence of H_2O_2 (*164*). At this point in time, the evidence for an oxidative stress role of AβPP-Cu redox chemistry is incomplete. Instead, evidence that AβPP overexpression causes increased Cu efflux suggests a role of AβPP in Cu homeostasis (*165*), where deficient intracellular Cu could potentially lead to insufficient SOD1 activity.

Recent focus on the interactions of the redox metal ions with proteins implicated in AD has shifted from AβPP to Aβ peptides. It was shown that Cu(II) markedly potentiated Aβ neurotoxicity in cell culture (*166*), presumably by promoting generation of H_2O_2 (*167*), and that redox-inert Zn(II) could suppress these effects by competing for Cu(II) binding sites (*168*). However, the source of reducing equivalents permitting aerobic generation of H_2O_2 by Cu(II)-Aβ in vitro (*167*) remained unclear, and if the Aβ peptide itself were the sacrificial reductant, H_2O_2 generation could be at best stoichiometric. It seems to have been determined later that buffer constituents were the source of the reducing equivalents and that Aβ-Cu(II) complexes of proper stoichiometry could catalyze reduction of O_2 to H_2O_2 at the expense of oxidizing cholesterol, vitamin C, L-DOPA, and dopamine (*169*).

Manipulations of the amino acids in Aβ, including His imidazole *N*-methylation, have been conducted to learn more about the redox control of Cu coordination and the role of particular side chains in ROS generation and neurotoxicity (*170–178*). At the same time, however, other studies have found evidence for a ROS detoxication role of Aβ (*100*). Thus, despite the plethora of studies, more work will be needed if the field is going to reach a consensus on the role of Aβ as being neurotoxic or neuroprotective (*179*), how either activity is modulated by metal complexation (*180*), and how dynamics of Aβ aggregation alter neurotoxic vs neuroprotective properties as a function of time course of the disease. It must be appreciated that Aβ-mediated ROS generation could be unique to in vitro cellular systems, since there is no evidence that Aβ causes oxidative stress in vivo (*17*). Nevertheless, oxidative stress is pervasive in AD, and proteomic-based identification of oxidized proteins in AD brain regions may offer insight to the molecular mechanisms involved (*181*).

6. Role of Oxidative Stress in the Pathogenesis of PD and Model Toxins

PD, a progressive neurodegenerative disorder characterized by movement and postural dysfunction, stems from a selective loss of catecholaminergic neurons of the substantia nigra pars compacta in the midbrain. The degeneration of the melanin-pigmented nigral neurons, accompanied by depletion of dopamine in the striatum, is the neuropathological basis of the movement disorders seen. Despite a well-described clinical and pathological phenotype, which is essentially identical for both the sporadic and the rare familial forms of PD, the molecular mechanisms of pathogenesis remain unknown; mitochondrial dysfunction, oxidative damage, environmental factors, and genetic predisposition might all be involved. Because oxidative stress is intimately linked to other components of the degenerative process, it is difficult to determine whether oxidative stress leads to, or is a consequence of, these events (*182, 183*).

There is substantial evidence that a defect in mitochondrial complex I, resulting in a 30–40% decrease in complex I activity in the substantia nigra, may be a central cause of sporadic PD (*184*). The decreased activity may reflect an underproduction of certain complex I subunits (*185, 186*), complex I misassembly, or self-inflicted oxidative damage (*187*). Evidence that a complex I deficiency and oxidative stress might underlie PD pathology is that selective inhibitors of complex I, such as rotenone and MPP+ (1-methyl-4-phenylpyridinium), recapitulate much of the pathology of PD (*188*). Further evidence for oxidative stress in PD is the finding of oxidative damage to DNA (*183, 189, 190*) and protein (*191, 192*) observed in the nigro-striatal region of PD brain, as well as immunocytochemical evidence for protein nitration (*193*), glycation (*194*), and HNE modification (*195, 196*).

Understanding the molecular mechanisms by which genetic mutations cause familial forms of PD holds great promise for unraveling the basis of neuronal degeneration in all forms of PD. Linkage analysis has led to the discovery of pathogenic mutations in six genes that may account for as many as 5–10% of the cases of PD: two autosomal dominant (coding for α-synuclein and dardarin), three autosomal recessive (including parkin (*197*)), and a sixth present in a single family with uncertain connection to PD (*198*). A possible role of subtle genetic factors in sporadic PD or misfunctioning of the same proteins for non-Mendelian reasons remains a point of consideration.

Besides the loss of pigmented nigral neurons, PD is characterized histopathologically by the presence of Lewy bodies, detergent-insoluble (*199*) eosinophilic intraneuronal filamentous inclusions found predominantly in the substantia nigra and locus coeruleus. Structurally similar Lewy bodies are also found in cortical neurons in PD and in diffuse Lewy body disease. The principal protein constituent of Lewy bodies is fibrillar α-synuclein. The physiological functioning of normal α-synuclein appears to involve synapse maintenance and plasticity, and overexpression of normal α-synuclein only modestly affects cell viability. On the other hand, most studies show that overexpression of mutant α-synuclein proteins is neurotoxic, most commonly by induction of apoptosis (*200, 201*). α-Synuclein fibrillization appears to be tied to τ fibrillization in vitro (*202*) and in diffuse Lewy body disease (*203*), and emerging evidence indicates that there is frequent disease overlap between classical

tauopathies (e.g., NFT formation in AD) and synucleinopathies (e.g., Lewy body formation in PD) (*204*).

Point mutations in α-synuclein that characterize the rare heritable forms of PD have been seen to increase the rate of formation of either fibrils or protofibril intermediates (*205*). Deposition of Lewy bodies in sporadic PD may then possibly reflect posttranslational modifications of α-synuclein by products of oxidative stress that affect peptide behavior in the same way as do the mutations. Oxidative stressors such as Cu(II) (*206*), Fe/H_2O_2 (*207*), cytochrome *c*/H_2O_2 (*208*), or nitrating reagents (*209*) induce aggregation/fibrillization of the protein, and human Lewy bodies and other α-synuclein inclusions are positive to antinitrotyrosine antibodies (*210*). However, oxidation of the four Met residues in α-synuclein to MetO can completely inhibit fibrillization of the peptide if there are no metals around (*211*). Evidence for a direct association of α-synuclein aggregation with neurotoxicity comes from a transgenic Drosophila model of PD, where (i) a deletion α-synuclein mutant unable to aggregate was nontoxic and (ii) an aggregation-prone truncation variant resulted in inclusions and enhanced neurotoxicity (*212*). The question remains, however, whether soluble misfolded forms or insoluble aggregates of α-synuclein are most tied to toxicity or, indeed, whether, like the NFT of AD, aggregates of α-synuclein are instead a protective adaptation to disease (*213*).

There is increased evidence in PD for dysfunction of the ubiquitin–proteasome system, which would exacerbate the pro-aggregatory effect of mutations in (or oxidative modifications of) α-synuclein due to cellular accumulation of indigestible misfolded or abnormal proteins, a condition known as "proteolytic stress" (*214, 215*). This relates to two of the other genetic defects associated with familial PD, E3 ubiquitin ligase (parkin) and ubiquitin C-terminal hydrolase L1 (*216*). The ubiquitin–proteasome process can also be impaired by products of oxidative damage, such as HNE (*182*), providing one mechanism for the occurrence of proteolytic stress in sporadic PD (*217*). Pharmacological inhibition of the proteasome in cultured catecholaminergic neurons (*218*) leads to apoptotic death and, in primary neurons, also to the formation of cytoplasmic ubiquitinated Lewy body-like inclusions that contain α-synuclein (*219*). Recent studies suggest that accumulation of unfolded and/or misfolded proteins in the ER lumen results in "ER stress" in PD (*220*). Although there are compensatory biochemical responses, in cases of severe and/or prolonged ER stress, cellular signals leading to cell death are activated.

PD is perhaps the most well-recognized neurodegenerative disease associated with elevated brain levels of metals. Abnormally high levels of iron seen at autopsy are associated with nigral degeneration in PD and increase with the severity of neuropathologic changes (*9, 221*). Although increased iron could signal a primary role of oxidative stress in PD pathology, iron overload could alternatively be a consequence of sequestration by eosinophilic protein aggregates. Iron has also recently been implicated in the promotion of α-synuclein aggregation either directly or via increasing levels of oxidative stress, suggesting an important role for iron in Lewy body formation (*222*). There is growing recognition of a hypothesis that oxidative stress, augmented iron deposition, and mitochondrial insufficiency constitute a single neuropathologic "lesion" (*223*). To whatever extent a labile pool of redox-active iron may be present, brain-permeable iron chelators (*224*) may ameliorate dopaminergic degeneration arising from iron's pro-oxidant or pro-aggregatory properties.

Assuming an oxidative stress-derived toxicity in PD, efforts to reveal what mechanisms could explain the selective demise of dopaminergic neurons have focused on the toxic consequences of dopamine oxidation. Indeed, dopamine is toxic to PC12 cells via oxidative stress, leading to apoptosis (*225*). It must be realized that metabolic deamination of dopamine and its *O*-methyl metabolite by MAO results in H_2O_2 as a by-product. Also, dopamine oxidation to dopamine quinone, stimulated by tyrosinase, is accompanied by the inactivation of tyrosine hydroxylase (*226*). Dopamine (aut)oxidation also probably explains the observed inactivation of parkin by dopamine (*227*), and dopamine quinone has been found to covalently modify Cys residues of the dopamine transporter (*228*). Dopamine quinone is a reactive electrophile but at physiological pH spontaneously cyclizes to an aminocatechol that readily autoxidizes to give dopaminochrome. The latter process occurs naturally in substantia nigral cells, where the aminochrome is a precursor of the characteristic pigment neuromelanin. However, dopaminochrome has also been shown to be responsible (*229*) for the inhibition of α-synuclein fibrillization seen for dopamine (*230*), suggesting that dopamine depletion in PD would enhance α-synuclein aggregation. Dopaminochrome and its one-electron-reduced semiquinone constitute an active redox cycling pair that is toxic to dopaminergic neurons in culture, resulting in hydroxyl radical production, mitochondrial damage, and necrotic cell death (*231*). Dopaminochrome-mediated redox cycling also plays a role in the selective catecholaminergic toxicity of copper (*232*) and iron (*233*), initiated by the uptake of the metal ion complexes of dopamine via the catecholamine transporter.

Dopamine oxidation can also lead to formation of 6-hydroxydopamine (6-OHDA), a known neurotoxin, from reaction of dopamine quinone with H_2O_2 (or alkyl hydroperoxides) mediated by peroxidase (*234*) or Fe(II) (*235*). Indeed, injection of 6-OHDA into rat substantia nigra produced the first toxin animal model of PD (*236*), where neuronal death reflects an apoptotic mechanism (*237*). 6-OHDA is an active redox-cycling agent (via its derived quinone and semiquinone), and studies on its biochemical mechanism of toxicity in PC12 cells have found catalase attenuation of neurotoxicity, suggesting that the latter is evoked by H_2O_2 (*238*). However, factors other than H_2O_2 also appear to be involved, because catalase did not inhibit the caspase activation contribution to 6-OHDA neurotoxicity (*239*). Overall, dopamine oxidation is a viable endogenous mechanism of toxicity likely to be important in PD. Such consideration points to the potential toxic role of dopamine accumulation [e.g., that could result as a consequence of mutant α-synuclein interfering with vesicular storage (*240*)] or of long-term treatment of PD patients with levodopa or other dopamine analogues (*241*).

Mechanistic features underlying oxidative stress in PD can also be revealed from studying related syndromes and animal models of the disease. The discovery in the early–mid 1980s that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an impurity present in the preparation of a demerol-like "designer drug", induces a PD condition in humans has led to the most well-accepted animal model of PD. The ultimate toxin, MPP+, generated from MAO-catalyzed oxidation of MPTP within the brain, is concentrated into dopaminergic cells by the dopamine reuptake pump (and is partly sequestered into dopamine vesicles), and it is further concentrated inside mitochondria and inhibits complex I of the respiratory chain (*242*). The mechanism leading to cell death in vivo is still debated (*243*) and may reflect a combination of energy deprivation, apoptosis, and oxidative

stress observed in various in vitro models (*244*). Because MPP+ is an analogue of the toxic herbicide paraquat, thought to kill cells by a redox cycling-mediated burst of ROS production, there was an early effort to demonstrate a paraquat-like neuronal death as the primary mechanism of MPTP dopaminergic neurotoxicity. Subsequent studies, however, showed that MPP+ is incapable of redox cycling (*245*), and a recent study demonstrated the obligatory role of inhibition of complex I in MPTP neurotoxicity in mice (*246*). How oxidative stress comes about is still incompletely defined but may arise from iron-catalyzed autoxidation of dopamine released from vesicle stores by MPP+, thereby connecting with PD pathogenesis (*247*). The MPTP animal model of PD has fueled continued suggestions of a possible environmental role [e.g., herbicide exposure (*248*)] in sporadic PD, a topic where there is a great need for further careful investigation.

Related to the role of iron in PD is that exposure to excessive levels of the essential trace metal manganese (present in Mn SOD and glutamine synthetase) results in extrapyramidal syndromes resembling PD. Recent data indicate that Mn-induced parkinsonism (termed manganism) differs from PD in that accumulation of Mn and damage occurs mainly in the basal ganglia (pallidum and striatum), rather than in the pars compacta of the substantia nigra (*249, 250*). Manganese neurotoxicity exhibits many signs of oxidative stress, playing a causal role in the neurotoxic syndrome, but whether Mn directly elicits ROS production remains unclear. Manganese(II) itself is essentially inactive in the Fenton reaction with H_2O_2 but can reduce superoxide to H_2O_2, which can then react with Cu(I) or Fe(II). Manganese(III) can exert toxicity to catecholaminergic neurons through oxidation of dopamine (*251, 252*). Although not easily explained, Mn(II) together with Mn(III) or Fe(III) appears to be especially active in ROS generation (*253*). However, because other data suggests only a minor involvement of ROS generation in Mn neurotoxicity (*254*), the main role of Mn may be interference with general redox-active metal homeostasis in tissues, for example, by displacement of redox-active iron from sites that limit its ROS-stimulating activity.

Lastly, oxidative stress has been demonstrated to occur in response to high doses of substituted amphetamines such as methamphetamine (METH) and 3,4-methlyenedioxymethamphetamine (MDMA), two stimulants with very high abuse liability in the United States that effect loss of both dopaminergic and serotonergic terminals in the brain. In addition to oxidative stress, excitotoxicity and mitochondrial dysfunction appear to play a major role in the neurotoxicity (*255, 256*). There is evidence that oxidative stress arises from peroxidative generation of dopamine quinone from dopamine released by METH into the cytoplasm from synaptic vesicles via reverse transport through the dopamine transporter (*257, 258*). RNS (peroxynitrite) also appear to play a major role in METH-induced dopaminergic neurotoxicity (*259*).

It is clear that PD results from a complex interplay among genetic and environmental factors, superimposed on which are aspects of mitochondrial dysfunction and oxidative stress. Future research will need to better address cause and effect aspects in the interdependence among these factors, to permit a mechanistic approach to therapeutic strategies.

7. Role of Oxidative Stress in ALS

Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative disease that occurs as both a minor familial form (fALS) and a sporadic form (sALS) accounting for 90% of the disease cases. The major genetic defect in fALS (accounting for 2% of all cases) is caused by mutations in the gene encoding the ubiquitous enzyme Cu/Zn-superoxide dismutase (SOD-1), more than 100 of which have been identified. Evidence for a role of oxidative stress comes from studies of postmortem tissue from sALS and fALS patients, showing an accumulation of oxidative damage to proteins, lipids, and DNA (*260–262*). Although the time course of accumulation of oxidative damage has not been determined, it has recently been shown that HNE levels are significantly elevated in the sera and spinal fluid of living sALS patients, correlating with the extent but not with the progression of the disease (*263*).

Most studies in the last decade, directed at elucidating mechanistic aspects of ALS pathogenesis, have focused on mouse models that express the mutant human SOD1 forms, since these animals experience age-dependent motor neuron degeneration with staged cellular and biochemical damage to nerve fibers and spinal cord tissue as well as increased protein and lipid oxidation. It was initially suggested that the toxicity of mutant SOD1 may be due to oxidative stress stemming from lack of sufficient SOD activity. However, because the different mutant enzymes were invariably found to retain normal SOD activity, it was instead proposed that the mutant enzymes exerted a "gain of function" activity, where some other deleterious copper-catalyzed enzymatic activity could be occurring in addition to SOD activity. Such copper-catalyzed reactions, for example, peroxidase-like activity or enhanced processing of NO to give RNS such as peroxynitrite, were demonstrated in vitro, but it is currently unclear whether these activities are responsible for toxicity in vivo. In fact, other studies suggest a lack of aberrant copper chemistry exerted by mutant SOD1 forms, including the finding that toxicity is not reduced in mutant SOD1 mice either lacking the chaperone for insertion of copper into SOD1 (CCS) (*264*) or where SOD1 binding of copper is negated by mutation of the key histidine ligands to alanine (*265*). A mutant SOD1 mouse model overexpressing CCS exhibited accelerated neurological deficits, but this appeared to reflect a mitochondriopathy not involving oxidative stress (*266*). Although the mitochondriopathy already evident in motor neurons of G93A-SOD1 mice does display features of a necrotic neuronal death associated with oxidative stress damage (*267*), this appears distinct from the apoptotic-like mechanism that appears to contribute to motor neuron degeneration in human sALS and fALS. Overall, studies of ROS generation and oxidative damage in vivo have produced mixed results, and trials of antioxidant therapies have been disappointing (*268*). It is thus unclear, as in AD, to what extent potential therapeutic strategies based on the mouse models will translate to treatment of the human disease.

Studies over the past 5 years have pointed to the multifactorial nature of pathogenesis in ALS. In addition to oxidative stress (*268*), these include excitotoxicity, aggregate formation, inflammation, growth factor deficiency, and neurofilament disorganization. This multitude of contributing factors indicates that ALS is a complex disease, possibly explaining why amelioration of only one factor (e.g., use of antioxidants to combat oxidative stress) might be ineffectual. The most popular current theory linking the mutant SOD1 forms to neurological deficits seen in mouse models expressing them is that the different point mutations create a misfolding defect (*269*), leading to small amyloid-like aggregates that appear in late stages of the disease. Neurotoxicity is now being considered to arise from a toxic effect of the aggregated misfolded protein, similar to the neurotoxicity that arises in other amyloidoses (*270*). On the basis of studies showing that a number of fALS SOD1 mutants have

increased affinity for copper (*271*), one must also consider the possibility that altered copper coordination may be associated with a misfolding tendency rather than with altered redox chemistry. In any event, it will be difficult to ascertain whether the final mutant SOD1 aggregates, or some soluble precursor, are responsible for toxicity to motor neurons. A "gain-of-interaction" between mutant SOD1 and other critical neuronal proteins such as dynein (*272*) may contribute to a defect in retrograde axonal transport that in turn may underlie motor neuron degeneration.

Mutations in SOD1 that lead to misfolding of the protein target it for degradation by proteasomes (*273*). Although proteasomal degradation of the mutant proteins is efficient, altered solubility and aggregation of mutant SOD1 could eventually impair and ultimately overwhelm this system (*274*). If misfolding is the cause of SOD1-ALS pathogenesis, a hypothesis-driven approach to drug design might be to find drugs that stabilize SOD1 against misfolding.

In summary of the data accumulated to date, it seems that there may be continued support for the possibility of a dual biochemical basis underlying ALS, oxidative stress, and oligomerization of misfolded proteins (presumably either mutant or posttranslationally modified wild-type proteins) (*275*). However, oxidative stress is most likely an indirect consequence of protein aggregation or cytopathic protein–protein interactions, rather than aberrant copper chemistry of SOD1. Indeed, the oxidative damage observed in ALS may reflect ROS and RNS that accompany a neuroinflammatory reaction, possibly arising in combination from mitochondrial dysfunction plus pathophysiologic activation of both astrocytes and microglia (*276*). As far as a potential link between fALS and sALS is concerned, it is possible that oxidative stress-induced modifications of SOD1 in sALS mimic the toxic properties of the mutant enzymes in fALS. In this regard, it is interesting to find that replacement of an oxidation-prone Trp residue in G93A-SOD1 with a Phe residue decreases cytotoxicity of the mutant protein in a motor neuronal cell culture model and decreases the propensity of the mutant to form cytoplasmic inclusions (*277*).

8. Role of Oxidative Stress in MS

MS is a chronic inflammatory demyelinating disease of the central nervous system that is generally believed to be of autoimmune origin (*278, 279*), although the underlying cause is still unclear. MS is characterized pathologically by selective and coordinated inflammatory destruction of the myelin sheath, with ensuing damage to the underlying axon (*280*). The fluctuating aspect of MS between periods of exacerbation and remission would suggest that this disease has little in common with progressive age-related neurodegenerative diseases. However, there is growing awareness that disease progression in MS is associated with axonal degeneration, and accumulating data indicate that oxidative stress plays a major role in the pathogenesis of MS (*281–284*). Increased levels of secondary products of oxidative stress and/or decreased levels of antioxidant enzymes and small molecule antioxidants are seen in blood and CSF during the active phases of MS (*285–289*).

Oxidative stress may arise from the increased levels of ROS and RNS attendant the inflammatory reaction, mostly reflecting the respiratory burst system of activated microglia. Activated mononuclear cells of MS patients produce high amounts of ROS (*290*) and RNS, and oxidative damage to DNA, including mtDNA (*291*), develops in association with inflammation in chronic active plaques (*290*). ROS and RNS generated by macrophages have been implicated as mediators of demyelination (*292*) and axonal injury in both experimental autoimmune encephalomyelitis (EAE—the generally accepted animal model for the study of MS) and MS (*293, 294*).

Independent of inflammation, evidence for mechanisms leading to neuronal degeneration in MS include mitochondrial dysfunction and an excitotoxic component. In EAE, nitration of mitochondrial proteins, which preceded infiltration of inflammatory cells, resulted in loss of mitochondrial membrane potential and apoptotic cell death (*295*). Also, excitotoxicity has been shown to be an integral aspect of neuronal compromise in both EAE (*296*) and MS (*297*), mostly leading to apoptotic cell death. Data showing the increased expression of glutamate transporters in MS (*298*) provides evidence that glutamate excitotoxicity may be a component in the etiology of the disease. There is some evidence for increases in iron and other metals in MS, especially in the vicinity of lesions. Evidence for the role of disrupted iron metabolism and iron-mediated oxidative stress in the pathogenesis of MS and EAE has been recently reviewed (*299*).

Evidence for a pathogenic role of ROS in MS pathology has led to the employment of several antioxidant strategies in an effort to ameliorate EAE (*293, 300, 301*). With the acceptance of the possibility that active oxidative stress contributes to the disease process, there is increasing focus on developing therapies directed at upregulating antioxidant enzyme systems (*302, 303*) or production of endogenous antioxidants (*304*). On the other hand, because compensatory ROS- and RNS-sequestering mechanisms are also upregulated in MS, it is unclear to what extent the increased ROS and RNS are causing a problem. Also, despite the encouraging results obtained with antioxidants, it has been claimed that some antioxidants may be acting through a nonantioxidant mechanism. Other recent data have raised questions about whether oxidative stress plays a functional role in MS pathology. For example, elevated levels of isoprostanes in CSF have been found in healthy siblings who never get the disease as well as in their MS brethren (*305*). Also, although plasma lipid peroxidation is elevated in MS patients as compared to controls, there may be no relation between the degree of oxidative stress in plasma and the progression of disability in MS (*306*). Although more epidemiological and clinical trials clearly need to be performed to corroborate a causal role of oxidative stress in MS, the possibility that antioxidant strategies might be efficacious in the fight against MS progression (*284*) should not be excluded. There is also increasing awareness that an antioxidant and other possible pleiotropic effects of statins may be beneficial in MS (*307, 308*).

9. Role of Oxidative Stress in HD

HD is an autosomal dominantly inherited progressive neurodegenerative disorder, affecting people in middle age. HD is characterized by the progressive development of involuntary choreiform movements, cognitive impairment, neuropsychiatric symptoms, and premature death. The etiology of HD is unknown, but increasing evidence suggests important roles of altered gene transcription, mitochondrial dysfunction, excitotoxicity, and oxidative stress. The protein product, huntingtin, of the mutant gene causing HD is widely distributed in both neurons and extraneuronal tissues. The mutation results in expansion of a polyglutamine repeat near the N terminus, leading to a conformational change of the protein and abnormal protein–protein interactions. Mutant huntingtin (mhtt) has been found to bind to numerous proteins, changing their behavior. It has been suggested that binding of mhtt to transcription factors results in reduced levels of acetylated histones and, in turn, a

decreased expression of genes that may play critical roles in neuronal survival (*309*). Also, huntingtin normally interacts with trafficking motors, and expression of mhtt results in disruption of microtubules and vesicular trafficking (*310*). In HD-affected areas of the caudate and cortex, mhtt has been immunochemically detected as a constituent of high molecular weight complexes and inclusion bodies. Thus, it is now generally accepted that in HD, alteration and/or sequestration of cellular targets by mhtt are likely to contribute to neuronal dysfunction and death, although the mechanism remains incompletely defined (*310*).

The mutant htt also impairs motility of mitochondria, and defects in mitochondrial trafficking are observed before other signs of toxicity. The induction by mhtt of mitochondria energy defects suggests a possible increased production of free radicals in vivo that could result in damage to predominantly mtDNA due to its proximity. Damage to DNA could in turn result in compromised defense and increased susceptibility to further damage. In asking whether mitochondrial damage is a primary or secondary event in toxicity (*310*), a large body of evidence supports an early and critical involvement of defects in mitochondrial energy metabolism as the initial disease trigger (*311*). Neuronal demise can then reflect a combination of downstream mechanisms, including excitotoxicity, apoptosis, and oxidative damage (*312*). Although evidence for oxidative stress in HD is less pronounced than in other neurodegenerative diseases, HD patients exhibit decreased activity of catalase in skin fibroblast cultures (*313*).

In summary, the best current theory to be addressed by potential therapeutic strategies holds that mhtt leads to abnormal protein–protein interactions including those that cause disruption of mitochondrial functioning, and that, in combination with consequential oxidative stress, there is proteasomal malfunction (*314*) and other downstream excitotoxic and inflammatory events that together result in neuronal death. Like other neurodegenerative disorders, HD thus appears to reflect operation of multiple different toxic mechanisms, which are confluent and depend on each other (*315*).

10. Conclusions

In the neurodegenerative diseases assessed above, AD, PD, ALS, HD, and MS, common issues are evident. First, is oxidative stress a cause or is it an effect? This is a complex academic issue, and unless oxidative stress is only a late-stage by-stander, the answer is of little relevance to asking the question of whether oxidative stress provides a therapeutic target for disease intervention. Second, what are the causes of oxidative stress? Prime suspects, common to all of these diseases, are mitochondrial abnormalities and redox metal ion dyshomeostasis. Third, what is the relationship between oxidative stress and other aspects of disease pathogenesis?

For most, if not all of the diseases, the answers to these questions are complex, mostly unresolved, and are evolving over time. Of prime importance is the growing awareness that all neurodegenerative diseases are to some extent multifactorial, and oxidative stress is inevitably intertwined with other disease mechanisms. For example, accumulation of self-aggregating proteins such as Aβ, τ, α-synuclein, and huntingtin may be involved both upstream and downstream of oxidative stress. The roles of inflammation, excitotoxicity, and genetics, including the possible role of more subtle genetic contributions to sporadic disease, are all important to keep in mind, and environmental contributions, including diet and lifestyle, should not be ignored (*316*).

Although oxidative stress is a common denominator of many disease states, the timing of maximum redox imbalance with regard to disease progression is likely quite different for different diseases. Oxidative stress is typically viewed as cytotoxic; yet, the mechanisms that underlie this toxicity are just beginning to be explored in a manner that pertains to normal pathophysiology. Most previously investigated cytotoxic paradigms or efforts to define oxidative stress-induced cell death have utilized supraphysiological excesses of an oxidant (or biologic induction of oxidants) that result in a rapid and predictable killing of cells. While useful data can be gleaned from such experiments, the relevance to disease is unclear, and often, investigators may be led to conclusions that should not be extrapolated beyond the limited experimental design. This defines a major challenge for further research.

In this perspective, it is concluded that the cell death process in neurodegenerative disease is associated with mechanisms involved at least in part with chronic oxidative stress. Unfortunately, oxidative damage seen in acute models of disease is likely more severe than the chronic (and perhaps steady state) levels of oxidative damage that likely characterize the actual disease processes (*317*). As such, while antioxidants appear to be effective in model systems, their effectiveness in treating disease has yet to be convincingly shown, although targeting oxidative stress, whether primary or secondary, should be expected to at least slow down disease processes.

Indeed, if oxidative stress is causally involved, it suggests possibilities for intervention into disease progression through the individual or combined (*318–320*) use of antioxidants, metal chelators, and agents designed to boost endogenous enzymatic and nonenzymatic defense processes. However, because most antioxidants considered for therapeutic intervention also have metal-reducing capacity, devising a successful regimen of antioxidants to retard the progression of these diseases remains a complicated goal.

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