Oxidative Stress, Glutamate, and Neurodegenerative Disorders

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There is an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders, as well as in stroke, trauma, and seizures. At the same time, excessive or persistent activation of glutamate-gated ion channels may cause neuronal degeneration in these same conditions. Glutamate and related acidic amino acids are thought to be the major excitatory neurotransmitters in brain and may be utilized by 40 percent of the synapses. Thus, two broad mechanisms—oxidative stress and excessive activation of glutamate receptors—are converging and represent sequential as well as interacting processes that provide a final common pathway for cell vulnerability in the brain. The broad distribution in brain of the processes regulating oxidative stress and mediating glutamatergic neurotransmission may explain the wide range of disorders in which both have been implicated. Yet differential expression of components of the processes in particular neuronal systems may account for selective neurodegeneration in certain disorders.

The selective vulnerability of neuronal systems is a remarkable characteristic of age-related degenerative disorders of the brain—including Parkinson's disease, (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Over the last decade, considerable circumstantial evidence has accumulated that implicates excessive stimulation of glutamate (Glu)–gated cation channels in triggering neuronal degeneration in experimental models of these disorders as well as in epilepsy, stroke, and hypoglycemia (*1*). A troubling conundrum, however, in linking the neurotoxic action of Glu to age-related neurodegenerative disorders is the mismatch between the millisecond dynamics of Glu-gated ion channels and the years involved in the gradual process of neuronal loss.

A separate line of investigation has focused on the role of oxidative stress as the proximate cause of several of these degenerative disorders. Oxidative stress refers to the cytotoxic consequences of oxygen radicals—superoxide anion ($\cdot O_2^-$), hydroxy radical ($\cdot OH$), and hydrogen peroxide (H_2O_2)—which are generated as byproducts of normal and aberrant metabolic processes that utilize molecular oxygen (O_2). The attractive feature of the oxidative stress hypothesis is that it can account for cumulative damage associated with the delayed onset and progressive nature of these conditions (*2*, *3*). Evidence is now emerging that activation of Glu-gated cation channels may be an important source of oxidative stress and that these two mechanisms may act in a sequential as well as a reinforcing manner, leading to selective neuronal degeneration. Understanding the relation between oxidative stress and Glu neurotransmission could lead to the development of pharmacologic interventions that disrupt this chain of pathological events without impairing excitatory neurotransmission.

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Oxidative Stress

The brain consumes a disproportionate amount of the body's O_2, as it derives its energy almost exclusively from oxidative metabolism of the mitochondrial respiratory chain. Mitochondria (mt) are found in neuronal cell bodies but are also distributed throughout the neuritic processes—dendrites, axons, and synaptic boutons—where adenosine triphosphatases (ATPases) maintain ion gradients across the neuronal membrane. Neuronal oxidative phosphorylation, which generates adenosine triphosphate (ATP) while reducing O_2 to H_2O by the sequential addition of four electrons and four H^+, varies in proportion to neuronal firing. The "leakage" of high energy electrons along the mt electron transport chain causes the formation of $\cdot O_2^-$ and H_2O_2 (*2*). The genes encoding the components of the mt respiratory chain are located both on nuclear (Nu)DNA and mtDNA. MtDNA has a mutation rate that is 10 times greater than that of NuDNA and has less effective repair mechanisms (*4*). Consistent with the proximity of mtDNA to a major source of cellular oxidants, there is remarkable 15-fold increase in oxidized nucleotides in brain mtDNA with age (*4*). Recent evidence that hereditary mtDNA mutations and deletions are associated with syndromes characterized by neurodegeneration and stroke (*5*) may indicate that mutations acquired with aging may disrupt the efficiency of electron transport and augment oxidative stress (*4*).

Several enzymes expressed in the brain, including monoamine oxidase (MAO), tyrosine hydroxylase, and L-amino oxidase, produce H_2O_2 as a normal byproduct of their activity (Fig. 1). Auto-oxidation of endogenous substances, for example, ascorbic acid and catecholamines, which are differentially concentrated in certain neuronal systems, yields H_2O_2 (*6*). The activity of some neuronal enzymes yields oxidants. The Ca^{2+}-dependent activation of phospholipase A_2 (PLA_2) releases arachidonic acid (AA), which yields $\cdot O_2^-$ through its subsequent metabolism by lipoxygenases and cyclo-oxygenases to form eiconasoids (*7*). The formation of nitric oxide (NO), the diffusible second-messenger, endothelial-derived relaxing factor (EDRF), is catalyzed by NO synthetase (NOS), a form of which is concentrated in certain neurons, is activated by Ca^{2+}, and is regulated by neurotransmitter receptors (*8*). NO reacts rapidly with $\cdot O_2^-$ to yield the peroxynitrite anion, which decomposes to $\cdot OH$ (*9*). Under conditions of energy failure and elevated intracellular Ca^{2+}, xanthine dehydrogenase is converted to xanthine oxidase by Ca^{2+}-dependent proteases (*10*). The conversion of accumulated hypoxanthine and xanthine by xanthine oxidase to uric acid yields $\cdot O_2^-$ (*2*). The hydroxyl radical ($\cdot OH$), the most reactive species, is not generated directly by any known enzymatic reaction. However, H_2O_2 slowly decomposes to $\cdot OH$, a process that is markedly accelerated in the presence of Fe^{2+} by the Fenton reaction (*2*).

Arrayed against these sources of cellular oxidants in the brain are defense mechanisms dedicated to reducing the levels of oxidants. Ascorbic acid (vitamin C) is a hydrophilic antioxidant, whereas α-tocopherol (vitamin E) is a hydrophobic antioxidant that is concentrated in membranes. Vitamin E deficiency causes neurologic symptoms (*11*), and large doses of

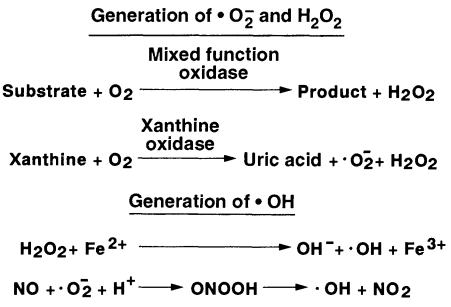

Fig. 1. Free radical reactions that may lead to the production of H_2O_2, $\cdot O_2^-$, and $\cdot OH$. [Adapted from (*92*)]

vitamin E protect against atherosclerosis, in which oxidation of cholesterol is a critical feature of atheroma formation (*12*). Although α-tocopherol can to react with singlet oxygen and ·OH, the major antioxidant action of α-tocopherol is due to its ability to donate labile hydrogens to peroxy and alkoxy radicals, thereby preventing lipid peroxidation (*2*). Thus, α-tocopheral has been classified as a "chain-breaking" antioxidant. Glutathione, a tripeptide synthesized intracellularly, in its reduced state nonenzymatically scavenges both singlet oxygen and ·OH.

Superoxide dismutase (SOD) (E.C. 1.15.1.6), catalase (E.C. 1.11.1.6), and glutathione peroxidase (E.C. 1.11.1.9) are enzymes responsible for degradation of $\cdot O_2^-$ and H_2O_2. Three forms of SOD, encoded by three separate genes, are expressed in eukaryotic cells. Copper-zinc superoxide dismutase (CuZnSOD) is found in the cytosol, whereas manganese-containing superoxide dismutase (MnSOD) is localized to the mt matrix (*13*). An extracellular form of CuZnSOD is expressed at low levels in plasma and extracellular fluids, where it may in part protect NO by reducing the $\cdot O_2^-$ concentration (*14*). All three forms of SOD catalyze the dismutation of $\cdot O_2^-$ to H_2O_2, thereby reducing the risk of ·OH formation. Catalase and glutathione peroxidase remove H_2O_2 from the intracellular environment by reducing it to H_2O and O_2. Relative to glutathione peroxidase, which is found in high concentrations in the brain, there is little catalase in both gray and white matter. In addition to eliminating H_2O_2, glutathione peroxidase participates in pathways responsible for the detoxification of lipid peroxy radicals. Finally, quinone reductase, a cytosolic enzyme first noted for its role in protecting against carcinogens, catalyzes a two-electron reduction of quinones to more stable and less reactive hydroquinones (*15*).

Oxygen radicals can attack proteins, deoxynucleic acids, and lipid membranes, thereby disrupting cellular functions and integrity. But the brain contains large amounts of polyunsaturated fatty acids (PUFA), which are particularly vulnerable to free radical attack, because the double bonds within membranes allow easy removal of hydrogen atoms by reactive oxygen species such as ·OH (*16*). The carbon radical formed in the PUFA undergoes molecular rearrangement to form more stable conjugated dienes, which can cross-link fatty acids within cellular membranes. Under aerobic conditions, lipid peroxidation continues as conjugated dienes combine with O_2 to form additional organic peroxy radicals. Peroxy radicals abstract hydrogen from adjacent fatty acid chains, thereby propagating the lipid peroxidation process. Furthermore, peroxy radicals can combine with an abstracted hydrogen atom to form lipid hydroperoxides which, in the presence of Fe^{2+}, decompose to alkoxy radicals and aldehydes. Thus, like a brush fire, the action of a single ·OH can initiate a chain reaction that generates numerous toxic reactants that rigidify membranes by cross-linking, disrupt membrane integrity, and damage membrane proteins.

Glutamate Toxicity

Although multiple factors can precipitate oxidative stress in cells, the neurotransmitter Glu is the major effector of this process in brain, primarily through activation of its ionotropic receptors. Glutamate and related excitatory amino acids (EAAs) account for most of the excitatory synaptic activity in the mammalian central nervous system and are released by an estimated 40% of all synapses (*17*). The ionotropic receptors mediating the depolarizing action of Glu have been named after their most potent agonists and can be distinguished by their pharmacological and electrophysiological properties: the NMDA (*N*-methyl-*D*-aspartate), the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxasole-proprionic acid), and the KA (kainic acid) receptors. The best characterized of the three, the NMDA receptor complex, consists of a membrane-spanning channel, which is highly permeable to both Na^+·K^+ and Ca^{2+} in a voltage-dependent manner and possesses several regulatory sites, including glycine, Zn^{2+}, polyamine, and phencyclidine binding sites, all of which allosterically affect Glu-mediated channel opening (*18*). Both AMPA and KA receptors are linked to Na^+ permeable channels. Each exhibits significant affinity for the other's recognition site, and no antagonists selectively differentiate KA from AMPA receptors. Thus, they are often designated as "non-NMDA receptors". Finally, a fourth class of Glu receptors, the metabotropic receptors, exert their effects by means of G protein–initiated biochemical events and not ion channels. Although the metabotropic Glu receptors do not directly mediate the neurotoxic effects of Glu, activation of subtypes of these receptors may potentiate or attenuate neurotoxicity (*19*).

Over a dozen genes encode the subunits of the Glu receptors (*20*). There are three structural classes of non-NMDA receptor subunits genes: (i) GluR1–GluR4, which have high affinity for AMPA but low affinity for KA; (ii) GluR5–GluR7, which have moderate affinity for KA; (iii) and KA-1 and KA-2, which bind KA with high affinity but do not form active homomeric channels. Although non-NMDA receptor-channel complexes were not originally thought to be permeable to Ca^{2+}, heteromeric complexes comprised of both AMPA- and KA-sensitive receptor subunits can actually form channels that sustain Ca^{2+} currents (*20*). Ca^{2+} permeability appears to be determined by the substitution, by means of mRNA editing, of an arginine for a glutamine in the transmembrane 2 segment of GluR2, GluR5, and GluR6. Two different gene families are responsible for encoding five different NMDA receptor subunits. The NMDAR1 is a receptor subunit found ubiquitously throughout the brain and has at least seven RNA splice variants. NMDAR1 can assemble into active homomeric channels, which display both the pharmacological and electrophysiological properties of the native NMDA receptor. The NMDAR2 (A through D) subunits do not form active channels alone but have been demonstrated to potentiate NMDAR1 responses. The heteromeric configuration of NMDAR1 with different members of the NMDAR2 subunits determines the functional variability in electrophysiological and pharmacological properties of the NMDA receptors in brain.

Thirty-five years ago, Lucas and Newhouse (*21*) observed that systemic administration of either Glu or aspartate caused degeneration of the neural retina in neonatal mice. Subsequently, Olney and coworkers (*22*) demonstrated in the arcuate nucleus of the hypothalamus a correlation between the neurotoxic effects and the neuroexcitatory potency of a number of peripherally administered acidic amino acids. A striking characteristic of these lesions was that it selectively killed neurons with cell bodies in the region of high Glu concentration, spared axons passing through the region from distant neurons, and did not damage glial cells (*23*). Accordingly, Olney coined the term "excitotoxins" to denote this group of EAAs, which appeared to selectively kill neurons by their depolarizing action. Subsequent studies involving intracerebral injection of Glu analogs revealed that agonists for all three classes of Glu ionotropic receptors caused the same cell body–specific, axon-sparing lesion, although subtle but important differences in neuronal vulnerability were observed among the agonist subtypes (*24*).

Because of the excitatory and neurotoxic potential of Glu, it is not surprising that its concentration in the extracellular space of the brain is quite low, with cerebral spinal fluid (CSF) concentrations of approximately 0.3 μM and average brain parenchyma concentration of 3 mM, representing a 10,000-fold gradient. The primary mechanism for clearing extracellular Glu is the action of a family of Na^+-dependent high-affinity transporters on glutamatergic neuronal processes as well as on glia (*17*).

Energy failure due to anoxia or hypoglycemia results in a marked efflux of Glu to achieve concentrations in the extracellular space compatible with neurotoxic effects (*25*).

Although the activation of Glu receptors is a key step in the sequence of events leading to neuronal degeneration, it is by no means all that is necessary. This caveat is supported by the following observations: (i) Glu receptor density correlates poorly with neuronal sensitivity (*26, 27*); (ii) in spite of the presence of Glu receptors on axons and terminals, these structures are generally spared from a direct degenerative response (*24, 28*); (iii) deafferentation of brain regions, especially of glutamatergic innervation, markedly attenuates the neurotoxic action of KA and NMDA receptor agonists (*29, 30*); and (iv) neuronal degeneration evolves over hours after the brief activation of the receptors (*28*). These incongruities have motivated investigators to focus on post-receptor mechanisms responsible for Glu receptor–induced neuronal degeneration.

Studies in tissue culture indicate that Glu receptor–mediated neuronal degeneration can be separated into two distinct forms, acute and delayed, distinguished by the time course and ionic dependence of neuronal degeneration (*27, 31*). The acute form of neurotoxicity is characterized by neuronal swelling in the presence of agonist, which leads to osmotic lysis of the neurons, and can be prevented by eliminating from the culture medium Na^+ or Cl^-, two ions responsible for the massive influx of water when Glu-gated cation channels are open. In contrast, delayed neuronal degeneration caused by NMDA and, in most cases, KA agonists is Ca^{2+}-dependent and transpires over several hours after a brief exposure to a high concentration of agonist or prolonged exposure to a low concentration of agonist (*27, 31, 32*). Neither delayed NMDA- nor KA-induced toxicity is dependent upon acute neuronal swelling since blockade of the acute toxicity does not prevent the progression of delayed neurotoxicity. Thus delayed neurotoxicity in vitro, which more closely resembles Glu receptor–mediated neurodegeneration in vivo, has been effectively dissociated from neuronal "excitation" (*31, 32*). The elevation of intraneuronal Ca^{2+} need not be prolonged to produce neuronal damage (*33*). Furthermore, depending upon the type of neuron, this increase in intracellular Ca^{2+} may be mediated by the activation of both voltage-dependent Ca^{2+} channels and NMDA receptor–gated Ca^{2+} influx (*34*). The role of Ca^{2+} in non-NMDA receptor–mediated neurotoxicity is more complex. Consistent with recent molecular findings that post-transcriptional editing of KA/AMPA receptor subunit transcripts allows the resulting channels to conduct Ca^{2+} currents (*19*), non-NMDA receptor–mediated influx of Ca^{2+} has been demonstrated in neurons in primary cultures of striatum, cerebellum, and hippocampus (*35*). Nevertheless, others have found that Ca^{2+}-deficient medium does not prevent KA-induced neurotoxicity (*27, 36*).

The Ca^{2+}-mediated effects of Glu receptor activation leading to neuronal degeneration may involve a number of different pathways that cause oxidative stress. NMDA receptor–mediated stimulation of PLA_2 and the subsequent release of AA leads to the generation of oxygen radicals (*37, 38*). AA and oxygen radicals enhance the release of Glu and inhibit its uptake inactivation by neuronal and glial transporter processes, thus promoting a vicious cycle (*39, 40*). The relation between non-NMDA receptor activation and AA metabolism has been less clear-cut. Dumuis and co-workers (*37*) could not demonstrate AA release from striatal neurons in culture with non-NMDA receptor agonists, although Sun and co-workers (*41*) reported AA release from brain slices with KA. Elevated intraneuronal Ca^{2+} activates peptidases, such as calpain I, which can catalyze the enzymatic conversion of xanthine dehydrogenase to xanthine oxidase; the catabolism of purine bases by xanthine oxidase yields $\cdot O_2^-$ (*10*). This reaction may become quite prominent since NMDA and KA receptor agonists cause a depletion of ATP and elevation of AMP within 2 hours (*42*). Furthermore, as lactic acid increases markedly at the same time (*42*), the acidic conditions favor the liberation of cellular stores of Fe^{2+}, which promotes the Fenton reaction to yield ·OH from H_2O_2 (*2*).

Dawson and co-workers (*43*) have proposed that NO generated by NMDA receptor–mediated activation of NOS by Ca^{2+} in certain neurons causes the degeneration of surrounding neurons. They have demonstrated protection against NMDA neurotoxicity in tissue culture by treatment with NOS inhibitors or with reduced hemoglobin, which binds NO; by elimination of arginine, the substrate for NOS, from the culture medium; or by selective destruction of NOS-expressing neurons by prior treatment with quisqualic acid, to which they are differentially sensitive. Although NO interferes with vital cellular processes, including mt oxidative phosphorylation and ribonucleotide reductase (*8*), NO reacts with $\cdot O_2^-$ to form the peroxynitrite anion, which decomposes to ·OH (*9*). Notably, NOS-expressing neurons are resistant to both NMDA-mediated as well as NO-induced degeneration, indicating that they, like NOS-expressing macrophages, possess some protective factor; the high concentrations of MnSOD in these cells might account for their resistance (*44*). However, other laboratories have not confirmed protection against NMDA- or Glu-induced neurotoxicity by inhibition of NOS (*45*); and a complete dissociation between KA toxicity and NOS activity has been demonstrated in rat cerebellar granule cell cultures (*46*). Furthermore, systemic treatment with NOS inhibitors has been reported to reduce or to exacerbate the brain damage caused by middle cerebral artery ligation, a lesion mediated by NMDA receptors (*47*). These disparities may reflect the salience of the redox state of the cellular environment. As recently reported by Lipton and co-workers (*48*), elevation of $\cdot O_2^-$ favors the formation of peroxynitrite, whereas reducing conditions supports *S*-nitrosylation of the NMDA receptor thiol, which down regulates the receptor and confers protection against neurotoxicity.

The fact that stimulation of Glu ionotropic receptors may activate a variety of processes leading to oxidative stress does not prove that oxidative stress, in fact, is the proximate cause of neuronal degeneration. Three lines of evidence are critical in distinguishing between oxidative stress as an epiphenomenon and a causal event: (i) oxygen radicals are generated during the period of irreversible damage; (ii) there is evidence of oxidative damage; and (iii) free radical scavengers or inhibitors of processes generating oxygen radicals prevent the neuronal degeneration. All three criteria have been satisfied for both NMDA and non-NMDA receptor agonists, although the multiple sources of oxygen radicals and variation in intrinsic mechanisms of protection may account for apparent contradictory results in different experimental systems.

Using cultured cerebellar granule cells, Lafon-Cazal and co-workers (*49*) have demonstrated by electron paramagnatic resonance with spin adducts that NMDA receptor stimulation, but not KA receptor stimulation, produces marked elevations in $\cdot O_2^-$ and ·OH. Furthermore, co-incubation of cells with NMDA and the spin traps provided nearly complete protection against delayed neurodegeneration. In this system, the primary source of oxidants was likely AA metabolism as a consequence of PLA_2 activation and not peroxynitrite anions generated by NOS. Using the U74500A, a 21–amino steroid with antioxidant effects, Monyer and co-workers (*50*) found significant, albeit incomplete, protection against delayed NMDA receptor–mediated neuronal degeneration in primary cultures of murine cortical cultures. Mice transgenic for CuZnSOD that express elevated SOD activity are less vulnerable than controls to ischemic brain damage (*51*), which is mediated in part by NMDA recep-

tors (52). Furthermore, neurons cultured from these transgenic mice are less vulnerable to Glu toxicity (53). Nevertheless, systemic treatment with a variety of free radical scavengers did not protect against striatal lesions produced by intracerebral injection of NMDA receptor agonists (54, 55).

Convincing evidence of the role of free radicals in non-NMDA receptor–mediated neurotoxicity is also mounting. Results in vivo and in vitro have demonstrated the accumulation of two products of lipid peroxidation concurrent with KA-induced neurotoxicity (41, 56); both lipid peroxidation and neurodegeneration were attenuated by treatment with antioxidants (56). Sun and co-workers (41) have detected free radicals in vivo in brain after systemic administration of KA by means of spin-trapping techniques. Furthermore, the neurotoxic effects of intracerebrally administered KA or quisqualic acid, an AMPA receptor agonist, are blocked by the centrally active antioxidant, idebenone, which did not affect NMDA receptor–mediated neurotoxicity (54). Xanthine oxidase has been implicated as a source of oxygen radicals, because allopurinol, a xanthine oxidase inhibitor, as well as the combination of SOD and catalase protect against KA neurotoxicity in mouse cerebellar granule cell cultures (57). Protein kinase C may also serve as a modulator by its effects on intracellular Ca^{2+} since treatment with gangliosides that inhibit its translocation attenuate KA-induced neurotoxicity in primary cultures of cerebellar granule cells (58).

A well-characterized form of Glu-induced neurotoxicity resulting from oxidative stress is not mediated by a Glu-gated ion channel but rather by a cystine transporter to which Glu binds (59). Utilizing the N18-RE-105 cell line, a neural retinaglioma hybridoma, Murphy and co-workers (60) demonstrated that elevated Glu in the culture medium caused a degeneration of these neuron-like cells after approximately 8 hours of continuous exposure (55). Cell lysis was heralded by blebbing of the cytoplasmic membrane, breaks in the neuritic processes, and condensation of the nucleus. Although cytotoxicity required Ca^{2+} in the medium, intracellular electrical recordings revealed that Glu caused only modest depolarization (+5 mV), inconsistent with activation of Glu-gated cation channels. The cytotoxic potency of Glu inversely correlated with concentration of cystine in the culture medium. The resulting cystine deprivation causes a progressive decline in cellular glutathione; as the nadir is reached, oxidants accumulate intracellularly as revealed by the conversion of dichlorofluorescin to its oxidized fluorescent product. The addition of antioxidants such as α-tocopheral or idebenone, even late in the exposure period, protects against Glu toxicity, prevents the intracellular accumulation of oxidants, but does not reverse glutathione depletion (61). Inhibition of PLA_2 or the subsequent metabolism of AA by lipoxygenases, but not by cycloxygenases provide protection against Glu toxicity, suggesting that the former pathway is a significant source of oxidants (62). Prior induction of quinone reductase, an enzyme that catalyzes the two-electron reduction of quinones, decreases vulnerability to Glu whereas inhibition of its activity potentiates Glu toxicity (63). Notably, degeneration is forestalled by inhibition of de novo protein synthesis and endonuclease cleavage of DNA in an agonal event, consistent with apoptosis, which is responsible for programmed cell death (64).

Immature neurons in culture degenerate as a consequence of Glu inhibition of cystine transport before they have expressed substantial levels of Glu-gated ion channels (65). Although astrocytes appear to be resistant to the cytotoxic effects of cystine deprivation, perhaps due to high glutathione levels, Oka and co-workers (66) have demonstrated that immature oligodendroglia are vulnerable. Their experiments, carried out in cystine-free medium, indicated that elevated extracellular concentrations of Glu markedly stimulated the efflux of intracellular cystine by a carrier-mediated process, which results in glutathione depletion. This mechanism may account for periventricular leukomalacia of prematurity, in which ischemia causes extensive and selective degeneration of oligodendroglia, thereby disrupting the subsequent formation of white matter.

Research on the role of Glu in neurodegenerative disorders is constrained by experimental design, which selects for effects observable within hours to days. This time frame may not reveal to pathologic processes that transpire over years. Chronic intracerebral infusion of low doses of Glu receptor agonists produces a more selective pattern of neuronal loss, reminiscent of human neurodegenerative diseases, than do acute injections (67). Although massive depolarization due to high doses of agonists causes "acute" osmotic damage, persistent superphysiologic stimulation of these receptors would increase oxidative metabolism, thereby augmenting mt generation of $\cdot O_2^-$ (2). This scenario is plausible because drugs that uncouple mt oxidative phosphorylation cause selective neuronal degeneration mediated primarily by Glu receptor stimulation (68). Repeated depolarization would also favor activation of NMDA receptor channels, in addition to AMPA/KA channels, as the depolarization block is removed, thereby further disturbing Ca^{2+} homeostasis (69). Together, these Glu-mediated mechanisms would present the neuron with sustained oxidative stress, resulting in cumulative damage to DNA, proteins, and lipids as antioxidant defenses are compromised with age (Fig. 2). Glu-mediated oxidative stress alone or in combination with other sources of oxidants has been implicated in the neurodegeneration of PD, ALS, and HD.

Neurodegenerative Disorders

Idiopathic PD is a chronic, progressive disorder of late life, which is characterized by rigidity, unintentional tremor, and bradykinesia. There is a selective degeneration of neuromelanin-containing neurons, especially the nigral dopaminergic neurons projecting to the caudate-putamen. As reviewed by Fahn and Cohen (70), several biochemical features of the dopaminergic system render it at high risk for oxidative insult. MAO catabolizes intraneuronal dopamine, which is present in high millimolar concentrations within the neuron, yielding H_2O_2 stoichiometrically. The activity of MAO increases with age. Pharmacological manipulations that enhance dopamine turnover cause an increase in oxidized glutathione, which can be suppressed by simultaneous treatment with the MAO inhibitors clorgyline and deprenyl, indicating that metabolism by MAO is a source of oxidative stress (71). In postmortem studies of PD, the activity of glutathione peroxidase and amounts of glutathione are reduced in the substantia nigra but they are not reduced in an allied disorder, progressive supranuclear palsy (72). In addition, a several-fold increase in lipid hydroperoxides and decline in PUFA in the substantia nigra have been reported to accompany PD (73). Catecholamines also react with oxygen nonenzymatically to form highly cross-linked quinones, and to form neuromelanin, yielding reactive hemiquinones, H_2O_2, and oxyradicals (6). Furthermore, the substantia nigra, especially the pars reticulata, contains high concentrations of iron, which binds to neuromelamin and enhances $\cdot OH$ formation (16).

Several models for PD have been developed over the last 20 years, although none completely reproduces the neuropathology of PD in that they do not show Lewey bodies, an abnormal neuronal inclusion unique to PD. When injected intracerebrally, 6-hydroxydopamine (6-HODA) is concentrated in dopaminergic neurons by the high affinity dopamine transporter. It then reacts with molecular oxygen to produce H_2O_2, $\cdot O_2^-$, and $\cdot OH$, molecules that disrupt the structural and metabolic integrity of the dopaminergic neurons (74).

High systemic doses of amphetamine or its analogs also cause destruction of central dopaminergic neurons, presumably by releasing dopamine into the neuronal cytoplasm from the storage vesicles, thereby markedly augmenting oxidative catabolism via MAO (*75*). Finally, 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP), a side-product in the synthesis of a "designer" meperidine, causes dopaminergic neurodegeneration and the symptoms of PD when injected systematically in humans and subhuman primates (*76*).

MPTP is catabolized by MAOB in glia to 1-methyl-4-phenyl pyridine (MPP+), which is actively accumulated in dopaminergic neurons by the high affinity reuptake transporter for dopamine. MPP+ is then concentrated by the electrochemical gradient in the mt and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and generating oxygen radicals (*77*). Notably, selective reduction in complex I has been described in the substantia nigra, platelet, muscle, and brain mt in PD, although contrary results have been reported (*5*). That oxidative stress is the proximate cause of MPTP-induced dopaminergic neuronal degeneration is indicated by the demonstration that mice transgenic for and overexpressing CuZnSOD are resistant to the neurotoxic action of MPTP (*78*). Treatment with NMDA receptor antagonists protect against the dopaminergic degeneration induced by both amphetamine and MPP+ (*75, 79*), indicating a critical link between oxidative stress and Glu neurotransmission in this system.

ALS is a disorder with onset in mid-life, which is characterized by a selective and progressive degeneration of the lower motor neurons in spinal cord and the upper motor neurons in the cerebral cortex. Approximately 0.05% of the population is affected by ALS, which invariably leads to death due to the complications of paralysis. EAA metabolism is disturbed in patients with ALS: CSF concentrations of Glu, aspartate, and their presumed neuronal storage form *N*-acetylaspartyl glutamate (NAAG) are elevated, while Glu, aspartate, and NAAG concentrations are reduced in spinal cord and motor cortex (*80*). Selective decreases in Glu uptake by synaptosomes prepared from spinal cord, motor cortex, and somatosensory cortex have been also shown in postmortem tissue (*81*). Similar disturbances of EAA disposition are observed in the spinal cord of Brittany spaniels that suffer from an hereditary form of ALS (*82*). β-*N*-oxalylamino-L-alanine, a toxin in the chickling pea *Lathyrus sativus* responsible for lathyrism, or cycad toxin, β-*N*-methylamino-L-alanine, which has been implicated in Guamian ALS-PD-dementia complex, cause selective degeneration of upper and lower motor neurons (*83*). In vitro studies indicate that these two EAA analogs preferentially act on the KA/AMPA subtype of glutamate receptors (*1*).

Rosen and co-workers (*84*) have recently demonstrated 11 missense mutations in the gene encoding CuZnSOD in families suffering from an autosomal dominant form of ALS (FALS). The sporadic form of ALS and FALS have similar clinical symptoms and neuropathology, although FALS accounts for only 10% of ALS cases. Notably, the missense mutations affect amino acid substitutions that do not directly involve the catalytic site of CuZnSOD, but rather alter conserved regions critical for the tertiary structure and dimerization of the enzyme. Measurement of SOD activity in postmortem frontal cortex and in blood cells of FALS patients with missense mutations revealed highly significant reductions in SOD activity, but these reductions did not occur in patients with sporadic ALS or in a FALS case without a missense mutation of CuZnSOD (*85*). Notably, protein carbonyl content, a measure of protein oxidation, was elevated by 85% in patients with sporadic ALS as compared to controls, suggesting that oxidative stress is a common feature of ALS whether the disease is due to loss of CuZnSOD activity or to other causes. Although the status of EAA in FALS remains to be determined, a reasonable interpretation of these findings is that the CuZnSOD mutations sensitize motor neurons to "normal" Glu neurotransmission by KA/AMPA receptors, whereas elevated extracellular EAA provides the oxidative stress for motor neurons in the sporadic forms of ALS. Consistent with this hypothesis, primary cultures of the rat motor neurons exposed chronically to agents that inhibit the transport of Glu (thereby increasing its extracellular concentration) exhibit a delayed motoneuron degeneration (*86*); this motor neuron loss could be prevented by treatment with KA/AMPA but not NMDA receptor antagonists.

HD is an autosomal dominant disorder with onset typically in mid-life that is characterized by disturbances in movement, psychiatric symptoms, and a progressive dementia. As reviewed by DiFiglia (*87*), neuropathologic studies indicate that neurons intrinsic to the striatum bear the brunt of the degenerative process, especially the γ-aminobutyric acid (GABA)–enkephalin containing spiny neurons projecting to the globus pallidus and the GABA–Substance

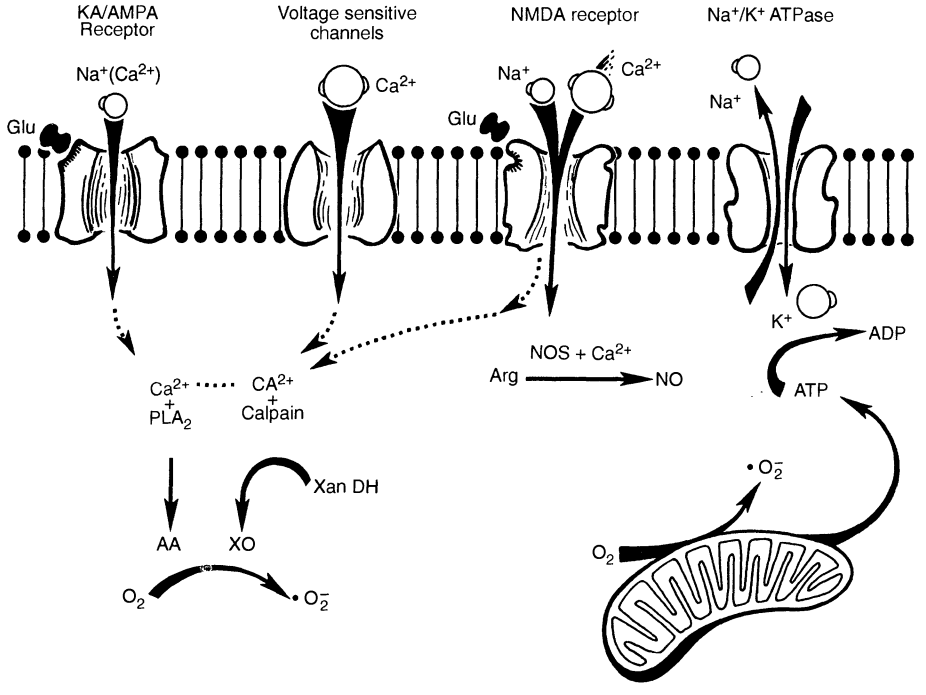

Fig. 2. Schematic representation of the glutamate receptor–mediated processes that promote oxidative stress. Activation of the KA/AMPA receptor by Glu opens channels through which Na^+ and, under certain circumstances, Ca^{2+} flow. Activation of the NMDA receptor, under partially depolarizing conditions, permits Na^+ and Ca^{2+} to flow through its channels. Depolarization activates voltage-dependent Ca^{2+} channels, permitting the influx of Ca^{2+}. Elevated intraneuronal Ca^{2+} may activate phospholipase A_2 (PLA_2) initiating the arachidonic acid metabolic cascade, activate proteases such as calpain to convert xanthine dehydrogenase (Xan DH) to xanthine oxidase (XO), and stimulate NO synthetase. Depolarization also increases ATP consumption by the Na^+/K^+ ATPase, which increases oxidative phosphorylation, yielding $\cdot O_2^-$ as a by-product.

P neurons projecting to the substantia nigra; striatal medium and large aspiny neurons, internal capsule fibers, and dopaminergic afferents are spared. Since the demonstration that intrastriatal injection of KA could reproduce several aspects of the neuropathology of HD (*88*), dysfunction of EAA disposition has been implicated in HD. In fact, the CSF levels of Glu are elevated in HD (*89*). Agonists that act at the NMDA receptors, however, more faithfully reproduce the neurochemical selectivity observed in HD (*90*). Pharmacologic inhibition of mt complex I or complex II causes the same selective pattern of striatal degeneration, which is mediated by NMDA receptors (*67, 68*). Germane to the delayed onset of neurodegeneration in HD, neuronal susceptibility to complex II inhibition increases with age of the animal (*68*). Disruption of the respiratory chain should lead to impaired oxidative phosphorylation and the elevated indices of oxidative stress. Recent NMR spectroscopy studies have disclosed elevated lactic acid in the brains of living HD patients (*91*), and ultrastructural studies have revealed abnormal mt structures and the accumulation of lipofusion in HD (*92*). A novel, expanded, unstable trinucleotide repeat on chromosome 4 has been demonstrated to be the genetic defect responsible for HD, although the function of the affected gene has not yet been identified (*93*).

Dysfunction of Glu mechanisms or evidence of oxidative stress have been implicated in an increasing number of conditions involving acute damage to brain tissue, including stroke (*94*), hypoxia-reperfusion, trauma, and epilepsy (*95*). Although the actual insult may be brief and limited in time, neuronal degeneration, especially in the penumbra around the primary lesion, evolves over hours to days.

Conclusion

It would be incorrect to conclude that oxidative stress is the sole mechanism responsible for Glu-induced neuronal degeneration in vivo, because the delayed effects of acute monovalent ionic shifts (Na^+, Cl^-, H^+) and Ca^{2+} activation of proteases, kinases, and nucleases likely contribute. Thus, in most models studied thus far, antioxidants provide only partial protection or merely shift the dose response to Glu. Furthermore, a host of mediators such as acetylcholine, which activates NOS via muscarnic receptors; bradykinin, which activates PLA_2; and pathologic events such as reperfusion injury can generate oxidants independent of Glu (*94*). Unrelated primary pathologic processes may compromise neuronal function, thereby enhancing the vulnerability to Glu such as amyloid A4 peptide in Alzheimer's disease (*96*). Finally, oxidative stress from other sources such as catecholamine metabolism in PD may place neurons in jeopardy to Glu related stressors. Nevertheless, the evidence, while still largely circumstantial, is convincing that oxidative stress represents an important pathway, initiated in part by Glu, that leads to neuronal degeneration in a manner consistent with the course and pathology of several degenerative disorders of the brain.

There remains, however, a substantial gap in our knowledge between Glu receptor activation and the specific metabolic processes that promote oxidative stress at the neuronal level. To close this breach, future studies should provide a more refined molecular characterization of Glu receptor subunits that are expressed in vulnerable systems; a more precise linkage between the biophysical characteristics of these receptors and the intracellular mechanisms generating oxidants; and a more detailed understanding of oxidant defense mechanisms as they relate to the unique microenvironments of specific neuronal systems. Molecular strategies, especially transgenic methods to alter the expression of specific components involved in this complex sequence of events, will be important in these studies. Understanding the relation between Glu and oxidative stress is important, because it offers a pharmacological strategy for blocking a pathway significantly involved in neurodegeneration without interfering directly with excitatory neurotransmission. Nevertheless, the terms "excitotoxicity" and "oxidative stress" are no longer sufficient as explanations, but rather serve as guideposts in the search for precise mechanisms responsible for degenerative disorders of the brain.

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Programmed Cell Death and the Control of Cell Survival: Lessons from the Nervous System

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During the development of the vertebrate nervous system, up to 50 percent or more of many types of neurons normally die soon after they form synaptic connections with their target cells. This massive cell death is thought to reflect the failure of these neurons to obtain adequate amounts of specific neurotrophic factors that are produced by the target cells and that are required for the neurons to survive. This neurotrophic strategy for the regulation of neuronal numbers may be only one example of a general mechanism that helps to regulate the numbers of many other vertebrate cell types, which also require signals from other cells to survive. These survival signals seem to act by suppressing an intrinsic cell suicide program, the protein components of which are apparently expressed constitutively in most cell types.

Although the death of neurons (and other cell types) was first recognized as a regular feature of vertebrate development almost 70 years ago (*1*, *2*), it is only in the last 20 years that the scale and general importance of normal neuronal death have gradually become appreciated (*3*–*5*). The neurotrophic theory has provided a useful conceptual framework for an understanding of this massive cell death (*4*–*7*). The theory grew out of the pioneering studies of Levi-Montalcini, Hamburger, and Cohen on normal neuronal death, neuron–target-cell interactions, and the prototypic neurotrophic factor nerve growth factor (NGF), although it was many years after the discovery of NGF that its connection to normal neuronal death was recognized (*8*). The theory is based on two main suppositions: (i) The survival of developing vertebrate neurons depends on specific neurotrophic factors secreted by the target cells that the neurons innervate, and (ii) many types of neurons are produced in excess, so that only a proportion get enough neurotrophic support from their target cells to survive. This neurotrophic mechanism is thought to have at least three advantages for the nervous system, facilitating both its evolution and development (*4*–*7*). First, it ensures that neurons that project to an inappropriate target are automatically eliminated, because they fail to receive the neurotrophic factors they require for survival. Second, it increases the likelihood that all target cells become innervated. Third, it helps ensure that the number of neurons is appropriately matched to the number of target cells they innervate.

The strongest evidence for the neurotrophic theory has come from experiments on developing NGF-dependent sympathetic and sensory neurons, about half of which normally die during development. If perinatal animals are treated with exogenous NGF, this normal cell death is largely prevented (*9*), whereas if they are treated with neutralizing antibodies to NGF, almost all of these neurons die (*10*). Moreover, the target neurons produce NGF in small amounts that are correlated with the density of innervation (*11*); if a target tissue is removed, the developing neurons that should innervate it die (*3*, *12*). A similar dependence on target-derived survival factors is displayed by many types of vertebrate neurons (*4*–*7*). In addition, NGF is now known to be only one member of a family of homologous neurotrophic proteins called neurotrophins (*6*, *13*, *14*), which bind to complementary members of the Trk family of receptor tyrosine kinases (*14*, *15*). Like NGF, the other known neurotrophins—brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5)—have been shown to promote the survival of specific developing neurons in vitro (*6*, *13*, *14*) and (for BDNF) in vivo (*16*). Developing neurons, however, do not depend exclusively on signals from their targets for survival (*17*): Many require signals from the neurons that innervate them (*7*, *18*), some require specific hormones (*19*), and it seems likely that many require signals from neighboring glial cells. Thus, the control of neuronal survival

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