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Preventing Ca²⁺-mediated nitrosative stress in neurodegenerative diseases: Possible pharmacological strategies

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Abstract

Overactivation of the NMDA-subtype of glutamate receptor is known to trigger excessive calcium influx, contributing to neurodegenerative conditions. Such dysregulation of calcium signaling results in generation of excessive free radicals, including reactive oxygen and nitrogen species (ROS/RNS), including nitric oxide (NO). In turn, we and our colleagues have shown that these free radicals trigger pathological production of misfolded proteins, mitochondrial dysfunction, and apoptotic pathways in neuronal cells. Here, we discuss emerging evidence that excessive calcium-induced NO production can contribute to the accumulation of misfolded proteins, specifically by S-nitrosylation of the ubiquitin E3 ligase, parkin, and the chaperone enzyme for nascent protein folding, protein-disulfide isomerase. Additionally, excessive calcium-induced NO generation leads to the formation of S-nitrosylated dynamin-related protein 1, which causes abnormal mitochondrial fragmentation and resultant synaptic damage. In this review, we also discuss how two novel classes of pharmacological agents hold promise to interrupt these pathological processes. Firstly, the NMDA receptor antagonists, Memantine and NitroMemantine, block excessive extrasynaptic glutamate excitation while maintaining synaptic transmission, thereby limiting excessive calcium influx and production of ROS/RNS. Secondly, therapeutic pro-electrophiles are activated in the face of oxidative insult, thus protecting cells from calcium-induced oxidative stress via the Keap1/Nrf2 transcriptional pathway.

Keywords

Reactive Nitrogen Species; S-Nitrosylation; Molecular Chaperone; Ubiquitin-Proteasome System; Protein Misfolding; Mitochondrial Fission; Neurodegeneration; Memantine

1. Introduction

A prominent feature in many neurodegenerative diseases is excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including nitric oxide (NO), which contribute to neuronal cell injury and death [1–3]. *N*-Methyl-D-aspartate (NMDA)-type glutamate receptors have been linked to ROS and NO production in the nervous system. Overactivation of NMDA receptors causes excessive influx of Ca²⁺ ions, which generates ROS and activates neuronal NO synthase (nNOS) [4]. Reaction of the NO group with critical

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cysteine thiols of target proteins results in the formation of S-nitrosoproteins (SNO-Ps) and can thus regulate protein function [5]. Lipton and Stamler initially discovered and characterized this biochemical process on the NMDA receptor itself although the activity of potentially hundreds or thousands of other proteins are modulated in this fashion, and coined the term “S-nitrosylation.” S-Nitrosylation can mediate either protective or neurotoxic effects depending on the action of the target proteins. Additionally, mitochondrial respiration produces free radicals, principally ROS, in response to excessive calcium influx, and one species of ROS, superoxide anion (O_2^-), reacts rapidly with free radical NO to form the very toxic product peroxynitrite ($ONOO^-$) [5] (Fig. 1).

In recent work, we have shown that S-nitrosylation and further oxidation of critical cysteine residues can lead to protein misfolding. Misfolded proteins form aggregates in many neurodegenerative diseases, and soluble oligomers of these aberrantly folded proteins are thought to adversely affect cell function by interfering with normal cellular processes or initiating cell death signaling pathways [6]. As examples, α -synuclein and synphilin-1 in Parkinson's disease (PD), and amyloid- β ($A\beta$) and tau in Alzheimer's disease (AD) form toxic oligomers of non-native secondary structures. The formation of larger aggregates may be an attempt of the cell to wall off these toxic proteins. Protein aggregation is also a signature of Huntington's disease (a polyQ disorder), amyotrophic lateral sclerosis (ALS), and prion disease [7].

Sporadic forms of neurodegenerative diseases, rather than single gene mutation, constitute the vast majority of cases, and pathologic protein misfolding in these diseases may be the result of posttranslational changes to the protein engendered by nitrosative and/or oxidative stress, which can thus mimic the more rare genetic variants of the disease [8]. Here we focus on specific examples that show the critical roles of S-nitrosylation of ubiquitin E3 ligases, e.g., parkin, and endoplasmic reticulum (ER) chaperones, such as protein-disulfide isomerase (PDI), in accumulation of misfolded proteins in neurodegenerative diseases [9–11]. We also review recent findings on S-nitrosylation of dynamin-related protein 1 (Drp1), which can contribute to the pathological fragmentation of mitochondria. We further discuss therapeutic applications of the NMDA receptor open-channel blockers memantine and its newer NO-derivatives for preventing excessive production of ROS and NO. Finally, we discuss the use of a class of pro-electrophiles for neuroprotection from neurodegenerative disorders [3,12].

2. Generation of ROS/RNS by Ca^{2+} influx through NMDA receptor channels in response to glutamatergic signaling

The amino-acid glutamate functions as the major excitatory neurotransmitter and is present at millimolar concentrations in the adult central nervous system (CNS). Ca^{2+} stimulates release of glutamate from the presynaptic nerve terminal into the synaptic cleft where it diffuses to postsynaptic receptors on an adjacent neuron. Normal excitatory neurotransmission is essential for synaptic development and plasticity as well as learning and memory. In contrast, excessive glutamate excitation plays a role in a variety of neurological disorders ranging from acute hypoxic-ischemic brain injury to chronic neurodegenerative diseases. Survival pathways appear to be mediated via NMDA receptor synaptic activity, whereas neuronal damage may be mediated by excessive extrasynaptic activity [13,14]. Severe overstimulation of excitatory receptors can cause necrotic cell death, while less fulminant or chronic overstimulation can cause apoptotic or other forms of cell death [15–17].

Glutamate receptors in the nervous system are divided into two groups, ionotropic (representing ligand-gated ion channels) and metabotropic (coupled to G-proteins). Ionotropic glutamate receptors are represented by three separate classes, NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate; each receptor type is named for the

synthetic ligands that selectively activate them. NMDA receptors, unlike most other types, are highly permeable to Ca^{2+} . Depolarization relieves blockade of NMDA receptor-coupled ion channels by Mg^{2+} [18]. Ca^{2+} influx promotes many normal intracellular signaling pathways, but excessive influx promotes pathological signaling, contributing to cell injury and death via production of free radicals such as ROS and NO as well as other enzymatic processes [2,5,16,17,19] (Fig. 1 and Fig. 2). “Excitotoxicity” is defined as neuronal damage caused by excessive activation of glutamate receptors [20] and is at least partly mediated by excessive Ca^{2+} influx through NMDA receptor-associated ion channel [2,3,21]. Increased levels of neuronal Ca^{2+} , in conjunction with the Ca^{2+} -binding protein CaM, trigger the activation of nNOS and subsequent generation of NO from the amino acid L-arginine [22,23] (Fig. 2).

A connection between ROS/RNS and mitochondrial dysfunction in neurodegenerative diseases, especially in PD, has recently been postulated [1,24]. Pesticides and other environmental toxins specifically inhibit mitochondrial complex I, generating excessive ROS/RNS, thus contributing to aberrant protein accumulation [9–11]. In animal models, administration of complex I inhibitors, such as MPTP, 6-hydroxydopamine, rotenone, paraquat and maneb, recapitulates many features of sporadic PD, including degeneration of dopaminergic neurons, overproduction and aggregation of α -synuclein, accumulation of Lewy body-like intraneuronal inclusions, and impairment of behavioral functions [1,24]. Studies such as these, strongly suggest a relationship between ROS/RNS and protein misfolding. Each may be the pathogenic trigger for the other in neurodegenerative diseases, but the mechanism has not yet been determined.

Alternatively, accumulation of ROS can trigger caspase activation resulting in synaptic damage and apoptosis [22]. This process can be exacerbated by the action of endogenous “neurotoxic” electrophiles such as the prostaglandin derivative 15d-PGJ2 and catecholamine metabolites (including dopamine) [22]. Such electrophiles compromise the reductive capacity of the cell by binding reduced cysteine residues, such as in glutathione (GSH), through a reaction called S-alkylation.

3. Nitrosative stress, protein misfolding, synaptic injury, and neuronal cell death

NO participates in cellular signaling pathways that regulate broad aspects of brain function, including synaptic plasticity, normal development, and neuronal cell death [19]. These effects were thought to be largely achieved by activation of guanylate cyclase to form cyclic guanosine-3',5'-monophosphate (cGMP), but emerging evidence suggests that a more prominent reaction of NO is S-nitros(yl)ation of regulatory protein thiol groups [4,5,25]. S-Nitrosylation is the covalent addition of an NO group to a cysteine thiol/sulfhydryl (RSH or, more properly, thiolate anion, RS^-) to form an S-nitrosothiol derivative (R-SNO). Such regulatory modifications are broadly found in mammalian, plant, and microbial proteins. We and our colleagues have found that a consensus motif of nucleophilic residues (generally an acid and a base) surround a critical cysteine, increasing the susceptibility of the sulfhydryl to S-nitrosylation [26]. This process is counterbalanced by denitrosylation by means of thioredoxin/thioredoxin reductase, class III alcohol dehydrogenase, protein-disulfide isomerase (PDI), intracellular GSH, and other mechanisms. We first identified the physiological relevance of the redox-based mechanisms by which NO and related RNS exert seemingly paradoxical effects in the CNS [5]. NO is neuroprotective through S-nitrosylation of NMDA receptors and caspases, yet is neurodestructive through formation of peroxynitrite or S-nitrosylation of matrix metalloproteinase (MMP)-9, GAPDH, and other targets, as discussed below [5,27,28] (Fig. 2).

Accumulating evidence suggests that S-nitrosylation is analogous to phosphorylation in regulating the biological activity of many proteins [5,9–11,26–29]. However, the chemistry of NO is much more complex. NO is often a good “leaving group,” resulting in further oxidation of the thiol to a disulfide bond between neighboring (vicinal) cysteine residues. Alternatively, as NO “leaves” for another reaction partner, the cysteine residue can react with ROS to yield sulfenic (–SOH), sulfinic (–SO₂H) or sulfonic (–SO₃H) acid derivatives of the protein [10, 11,27]. S-Nitrosylation may possibly produce a nitroxyl disulfide, in which the NO group is shared by proximate cysteine thiols [30].

What is the consequence of these many oxidative/nitrosative reactions? Recent evidence suggests that NO-related species may play a significant role in the process of protein misfolding. Increased nitrosative and oxidative stress are associated with chaperone and proteasomal dysfunction, resulting in accumulation of misfolded aggregates [8,25]. However, until recently little was known regarding the molecular and pathogenic mechanisms underlying contributions of NO to the formation of aggregates such as amyloid plaques in AD or Lewy bodies in PD. We and others recently presented physiological and chemical evidence that S-nitrosylation modulates the ubiquitin E3 ligase activity of parkin [9,10,31]. Additionally, we found that S-nitrosylation regulates the chaperone and isomerase activities of PDI [11], contributing to protein misfolding and neurotoxicity in models of neurodegenerative disorders.

Nitrosative stress can also result in defects in mitochondrial function. For example, NO affects mitochondrial respiration by reversibly inhibiting complexes I and IV [32,33]. Mitochondria thus compromised will release ROS, and this in turn could contribute to brain aging and/or pathological conditions associated with neurodegenerative diseases. Additionally, increased nitrosative and oxidative stress can elicit dysfunction of mitochondrial dynamics (fission and fusion events) [34–36]. Although the exact mechanism whereby NO contributes to excessive fragmentation of mitochondria remains enigmatic, our recent findings have shed light on the molecular events underlying this relationship, particularly in AD. Specifically, we have recently discovered (patho)physiological and chemical evidence that S-nitrosylation modulates the GTPase activity of the mitochondrial fission protein, Drp1 (dynamin related protein 1), thus contributing to mitochondrial fragmentation. We found that excessive mitochondrial fragmentation results in bioenergetic impairment, synaptic damage, and eventually frank neuronal loss in models of AD [37].

4. Protein misfolding and aggregation in neurodegenerative diseases

Healthy neurons generally show no accumulation of protein aggregates, indicating that the appearance of such structures is a response to pathological stresses. Considerable evidence suggests that misfolded or otherwise abnormal proteins are produced even in healthy cells. The difference can largely be accounted for by cellular mechanisms for quality control, such as molecular chaperones, the ubiquitin-proteasome system (UPS), and autophagy/lysosomal degradation. A reduction in molecular chaperone or proteasome activities can result in deposition and accumulation of aberrant proteins either within or outside of cells in the brain under pathological conditions. Several mutations in molecular chaperones or UPS-associated enzymes are known to contribute to neurodegeneration [6,38]. For example, a reduction in proteasome activity was found in the substantia nigra of PD patients [39], and overexpression of the molecular chaperone HSP70 prevented neurodegeneration *in vivo* in models of PD [40]. Aggregated proteins were first considered to be pathogenic. However, recent evidence suggests that macroscopic aggregates are an attempt by the cell to sequester aberrant proteins, while soluble (micro-) oligomers of such proteins are the most toxic forms [23].

5. S-Nitrosylation of Parkin and the UPS

Studies of rare mutations have revealed key components of the mechanism for protein aggregation and pathology in PD, including sporadic forms of the disease. Such studies revealed that mutated α -synuclein is a major constituent of Lewy bodies in PD patient brains, and that mutant forms of the ubiquitin E3 ligase parkin or the ubiquitin carboxy-terminal hydrolase UCH-L1 (a deubiquinating enzyme) may result in UPS dysfunction and also result in hereditary forms of PD. Formation of polyubiquitin chains on a peptide constitutes the signal for proteasomal degradation. The cascade of activation (E1), conjugation (E2), and ubiquitin-ligase (E3)-type enzymes catalyzes the conjugation of the ubiquitin chain to the proteins marked for degradation. Individual E3 ubiquitin ligases play a key role in the recognition of specific peptide substrates [41].

Parkin is a member of a large family of E3 ubiquitin ligases. Parkin contains a total of 35 cysteine residues, many of which coordinate structurally important zinc atoms, which are often involved in catalysis [42]. Parkin recruits substrate proteins as well as an E2 enzyme (e.g., UbcH7, UbcH8, or UbcH13). Interestingly, mutations in the gene encoding parkin have been associated with Autosomal Recessive Juvenile Parkinson's disease. In this case, mutations underlying this disorder usually do not produce Lewy bodies. However, other mutations in parkin resulting in adult onset PD have been associated with Lewy body formation. Mutations in both alleles of the parkin gene will cause dysfunction in its activity, although not all mutations result in loss of parkin E3 ligase activity [38]. Additionally, wild-type parkin can mediate the formation of non-classical and "non-degradative" lysine 63-linked polyubiquitin chains [43, 44]. Parkin can also mono-ubiquitinate Eps15, HSP70, and itself, possibly at multiple sites. These activities may explain why some parkin mutations result in the formation of Lewy bodies while others do not. Synphilin-1 (α -synuclein interacting protein) is a well-characterized substrate for parkin ubiquitination, and is found in Lewy body-like inclusions in cultured cells when co-expressed with α -synuclein. Accumulation of these proteins portends a poor prognosis for the survival of dopaminergic neurons in familial PD and possibly also in sporadic PD.

PD is the second most prevalent neurodegenerative disease and is characterized by the progressive loss of dopamine neurons in the substantia nigra pars compacta. Aberrant protein accumulation is observed in patients with genetically-encoded mutant proteins, and recent evidence from our and other laboratories suggests that nitrosative/oxidative stress acts as a potential causal factor for protein misfolding in the much more common sporadic form of PD. Nitrosative/oxidative stress can mimic hereditary PD by promoting protein misfolding in the absence of a genetic mutation [9,10,31]. In fact, S-nitrosylation and further oxidation of parkin result in a dysfunctional enzyme and disruption of UPS function [9,10]. We found that nitrosative stress produces S-nitrosylation of parkin (forming SNO-parkin) in rodent models of PD and in brains of human patients with PD and the related α -synucleinopathy, DLBD (diffuse Lewy body disease). Initially, S-nitrosylation of parkin stimulates its ubiquitin E3 ligase activity, which may contribute to Lewy body formation. Subsequently, with time we found that the E3 ligase activity of SNO-parkin decreases, resulting in UPS dysfunction [10, 31]. Importantly, S-nitrosylation of parkin on critical cysteine residues also compromises its neuroprotective activity [9]. It is likely that S-nitrosylation influences the enzymatic functions of similar ubiquitin E3 ligases, suggesting that this process may be involved in a number of degenerative disorders.

6. S-Nitrosylation of PDI mediates protein misfolding and neurotoxicity in cell models of PD and AD

In the endoplasmic reticulum (ER), PDI facilitates proper protein folding by introducing disulfide bonds into proteins (oxidation), breaking disulfide bonds (reduction), and catalyzing

thiol/disulfide exchange (isomerization), thus facilitating disulfide bond formation, rearrangement reactions, and structural stability [45]. During oxidation of a target protein, oxidized PDI catalyzes disulfide formation in the substrate protein, resulting in the reduction of PDI. In contrast, the reduced form of the active-site cysteines can initiate isomerization by attacking the disulfide of a substrate protein and forming a transient intermolecular disulfide bond. As a consequence, an intramolecular disulfide rearrangement occurs within the substrate itself, resulting in the generation of reduced PDI. Several mammalian PDI homologues, such as ERp57 and PDIp, also localize to the ER and may manifest similar functions [46]. Increased expression of PDIp in neuronal cells under conditions mimicking PD suggests the possible contribution of PD to neuronal survival [46]. In many neurodegenerative disorders and cerebral ischemia, the accumulation of immature and denatured proteins results in ER dysfunction [46], but up-regulation of PDI represents an adaptive response promoting protein refolding and may offer neuronal cell protection [46]. We recently reported that S-nitrosylation of PDI (to form SNO-PDI) disrupts normal protein folding and this neuroprotective role [11].

The ER normally manifests a relatively positive redox potential in contrast to the highly reducing environment of the cytosol and mitochondria. This redox environment in the ER can influence the stability of protein S-nitrosylation and oxidation reactions [47]. Excessive NO is known to create ER stress by disruption of Ca^{2+} homeostasis. One possible mechanism is the increased activity of the ER Ca^{2+} channel-ryanodine receptor through S-nitrosylation [48]. Interestingly, we have recently reported that excessive NO, as well as rotenone exposure, which is known to lead to PD, can lead to S-nitrosylation of the active-site thiols of PDI, inhibiting its isomerase and chaperone activities [11]. S-Nitrosylation of PDI prevented its attenuation of neuronal cell death triggered by ER stress, misfolded proteins, or proteasome inhibition. Also, PDI was S-nitrosylated in the brains of virtually all cases we examined of sporadic AD and PD. These results suggest that SNO-PDI can mediate protein misfolding and consequent neuronal cell death or injury.

The activity of the UPS and molecular chaperones normally decline with age [49]. Since we have not found detectable levels of SNO-parkin and SNO-PDI in normal aged brain but only in disease states [9–11], it is likely that S-nitrosylation of these and similar proteins is a key mechanism contributing to neurodegenerative conditions.

7. Mitochondrial fission/fusion machinery in nerve cells

Neurons are particularly vulnerable to mitochondrial defects because they require high levels of energy for their survival and specialized function. Mitochondrial biogenesis, an event that produces new mitochondria, is required in regions that demand high concentrations of ATP, especially the synapse. The distribution of mitochondria at the nerve terminal can control synaptic transmission and structure [50–52].

In healthy neurons, the fission/fusion machinery proteins maintain mitochondrial integrity and insure their presence at critical locations. These proteins includes Drp1 and Fis1, acting as fission proteins, and Mitofusin (Mfn) and Opa1, operating as fusion proteins [53]. In both familial and sporadic neurodegenerative conditions, abnormal mitochondria regularly appear in the brain as a result of dysfunction in the fission/fusion machinery. Genetic mutations in Mfn2 can cause Charcot-Marie-Tooth (CMT) disease, a hereditary peripheral neuropathy that affects both motor and sensory neurons [54,55]. Mutations in Opa1 cause Autosomal Dominant Optic Atrophy (ADOA), characterized by the loss of retinal ganglion cells and the optic nerve, representing their axons [56]. Recently, Waterham et al. described a heterozygous, dominant-negative mutation of Drp1 in a patient whose symptoms were broadly similar to those of CMT neuropathy and ADOA [57]. Drp1 includes four distinct structural domains: an N-terminal GTPase domain, a dynamin-like middle domain, an insert B domain, and a C-terminal GED

domain. The mutation (Ala 395 to Asp) was found in the middle domain of Drp1. This case study further suggested that a defect in mitochondrial fission may have more severe consequences than those of fusion defects, since the Drp1 mutation caused a much earlier onset (prenatal) and fatal outcome. Additionally, it is apparent that the balance between fission and fusion is critical for normal function of mitochondria and determination of phenotype in disease. These fission/fusion proteins are widely expressed in human tissues, clearly supporting the notion that neurons are particularly sensitive to mitochondrial dysfunction.

8. S-Nitrosylation of Drp1 mediates mitochondrial fission and neurotoxicity in cell models of AD

Recent studies have demonstrated that posttranslational modification of mitochondrial fission or fusion proteins can contribute to altered mitochondria dynamics. For example, phosphorylation, ubiquitination, sumoylation, and proteolytic cleavage of Drp1 regulate mitochondrial fission by affecting Drp1 activity, at least in cell culture systems [58–65]. Excessive activation of mitochondrial fission or fusion proteins by posttranslational modification was posited to contribute to neurodegeneration by compromising mitochondrial function. Interestingly, along these lines, we recently reported that excessive NO can lead to S-nitrosylation of Drp1 at Cys644 [37]. Cys644 resides within the GTPase effector domain (GED) of Drp1, which influences both GTPase activity and oligomer formation of Drp1 [66–69]. S-Nitrosylation of Drp1 (forming SNO-Drp1) induces formation of Drp1 dimers, which function as building blocks for tetramers and higher order structures of Drp1, and activates Drp1 GTPase activity; however, substitution of Cys644 to Ala (C644A) abrogated these effects of NO.

We further demonstrated that exposure to oligomeric A β peptide results in formation of SNO-Drp1 in cell culture models. Moreover, we and our colleagues have observed that Drp1 is S-nitrosylated in the brains of virtually all cases of sporadic AD [37,70]. In order to determine the consequences of S-nitrosylated Drp1 in neurons, we exposed cultured cerebrocortical neurons to the physiological NO donor, S-nitrosocysteine (SNOC), or to A β oligomers and found that both induced SNO-Drp1 formation and led to the accumulation of excessively fragmented mitochondria. Moreover, mutation of a specific cysteine residue in Drp1 (C644A) prevented these effects of SNOC or A β on mitochondrial fragmentation, consistent with the notion that SNO-Drp1 triggered excessive mitochondria fission or fragmentation. Finally, in response to A β , SNO-Drp1—induced mitochondrial fragmentation caused synaptic damage, an early characteristic feature of AD, and eventually apoptotic neuronal cell death. Importantly, blockade of Drp1 nitrosylation (using the Drp1(C644A) mutant) prevented A β -mediated synaptic loss and neuronal cell death, suggesting that SNO-Drp1 may represent a potential therapeutic target to protect neurons and their synapses in AD. Thus, the posttranslational modification of S-nitrosylation can mimic the effect of rare genetic mutations in contributing to the AD phenotype.

9. Potential protection of neurons from NMDAR-induced excessive Ca²⁺ and oxidative/nitrosative stress

9.1 Memantine and Derivatives

Oxidative/nitrosative stress mediates, at least in part, glutamate-induced neuronal cell injury and death, and several antioxidant molecules have been reported to protect neurons against such assaults [15,71]. One mechanism that could potentially curtail excessive Ca²⁺ influx and the resultant formation of neurotoxic ROS and RNS is inhibition of NMDA receptors. Until recently, however, drugs in this class (e.g., MK-801) blocked virtually all NMDA receptor activity, including physiological activity, and therefore manifested unacceptable side effects

by inhibiting normal functions of the receptor. For this reason, many previous NMDA receptor antagonists have disappointingly failed in advanced clinical trials conducted for a number of neurodegenerative disorders. However, In contrast, we have demonstrated that the adamantane derivative, memantine, preferentially blocks excessive (pathological/extrasynaptic) NMDA receptor activity while relatively sparing normal (physiological/synaptic) activity [14]. Memantine effectively blocks only excessively open channels through an uncompetitive mechanism of action in conjunction with a relatively fast off-rate, resulting in a low affinity for the NMDA receptor. Despite its low affinity for the NMDA receptor, memantine is still relatively selective for this receptor. An uncompetitive antagonist can be distinguished from a noncompetitive antagonist, which acts allosterically at a noncompetitive site, i.e., at a site other than the agonist-binding site. Additionally, the action of an uncompetitive antagonist is contingent upon prior activation of the receptor by the agonist. Hence, a fixed low-dose of memantine will block higher concentrations of agonist to a relatively greater degree than lower concentrations of agonist, providing greater protection when more glutamate is present. Furthermore, the apparent affinity of a drug is determined by the ratio of its “off-rate” divided by its “on-rate” for the target. The on-rate is not only a property of drug diffusion and interaction with the target, but is also influenced by the drug’s concentration. In contrast, the off-rate is an intrinsic property of the drug-receptor complex and diffusion, unaffected by drug concentration. A relatively fast off-rate is a major contributor to memantine’s low affinity for the NMDA receptor as well as its clinical tolerability because this property insures that once excessive activity is normalized, the drug will leave the channel and not disrupt subsequent physiological neurotransmission. Thus, the critical features of memantine’s mode of action are its Uncompetitive mechanism and Fast Off-rate, or what we call a UFO drug – a drug that is present at its site of inhibitory action only when you need it and then quickly disappears (Fig. 3). Many studies *in vitro* and in animal models of stroke and neurodegenerative disease showed that memantine protects neurons from NMDA receptor-mediated excitotoxic damage [72]. In fact, memantine has been approved for human use by the FDA for moderate-to-severe AD and is currently being studied for other neurodegenerative disorders, including HIV-associated dementia and Huntington’s disease.

Therapeutic results with memantine are meaningful, but as with most first generation drugs, improvements are possible through creation of derivatives. The reaction of NO with the sulfhydryl groups of critical cysteine residues of the NMDA receptor, especially C399 on the external domain of the NR2A subunit, down-regulates (but does not completely shut off) receptor activity [3,12]. Therefore, we have developed combinatorial drugs called NitroMemantines that use memantine to target NO to the nitrosylation sites of the NMDA receptor in order to avoid the systemic side effects of NO, while still utilizing the uncompetitive channel blocking properties of memantine. Preliminary studies show NitroMemantines to be more effective neuroprotectants *in vitro* and in animal models than memantine and at lower concentrations [3]. Though much research remains to be done on these second generation NitroMemantine drugs, the combination of memantine with NO has created a new, improved class of UFO drugs that should be both clinically tolerated and neuroprotective.

9.2 Electrophiles

A complementary approach is needed to deal with residual oxidative stress triggered by excessive calcium influx or other mechanisms. One strategy for finding neuroprotective drugs is to search for low-molecular-weight compounds that can regulate the redox state of the cell and thereby block oxidative damage [73]. We and others found electrophilic compounds that transcriptionally induce protective antioxidative enzymes in neurons [74,75]. The activity of these compounds is attained by inducing the transcription of so-called “phase 2 enzymes,” such as heme oxygenase-1 (HO-1) and sulfiredoxin, that regulate the intracellular redox state of the cell [73,76,77]. The Keap1/Nrf2 transcriptional pathway is key in regulating the activity of

phase 2 enzymes. Keap1 facilitates ubiquitination of Nrf2, but electrophiles or NO reacting with a critical cysteine on Keap1 can cause the dissociation Nrf2 from Keap1. Nrf2 thus stabilized can translocate to the nucleus where it binds to the antioxidant-responsive element (ARE) in upstream promoters of phase 2 enzymes to activate their transcription [76–78].

The specific nature of the electrophile is important. Chemically, enones, such as curcumin [79] and neurite outgrowth-promoting prostaglandin (NEPP11) [75], are true electrophiles. Some enone-type neuroprotective electrophilic compounds that we have studied, such as NEPP11, accumulate in neurons, exerting a direct protective action through induction of HO-1 via the Keap1/Nrf2 pathway [75]. In contrast, however, we advocate the use of catechols that only become electrophiles upon oxidative conversion to quinones [80]. Thus, catechol-type compounds can function as prodrugs that exert their effects only under oxidative conditions [73,81]. In this manner, catechols, unlike enones, avoid reaction with other cysteine groups, e.g., those on glutathione, that could paradoxically decrease the antioxidative capacity of the cell. Some catechol-type neuroprotective pro-electrophilic compounds act preferentially in astrocytes, protecting neurons by a paracrine mechanism [74,82]. Carnosic acid (CA) is a naturally occurring catechol-type poly-phenolic diterpene obtained from *Rosmarinus officinalis* (the herb rosemary) [83]. Prior work had suggested that CA could exert free radical-scavenging activity [84], but we have recently found that CA, after being converted by oxidative stress from a catechol/pro-electrophile into a true quinone/electrophile, exerts its primary action by activating the Keap1/Nrf2 pathway at sites of oxidative insult [85]. In Parkinson's disease, oxidative stress has a crucial role in disease progression [86] but also can be used to activate such pro-electrophilic compounds to provide neuroprotection where it is needed. This approach represents a novel strategy against neurodegenerative disorders in which pro-electrophilic drugs are activated to electrophiles through pathological oxidative activity.

10. Conclusions

Sporadic forms of neurodegenerative diseases can be caused by excessive NMDA receptor activation and/or mitochondrial dysfunction that results in excessive nitrosative and oxidative stress. These pathological processes can result in malfunction of the UPS and/or molecular chaperones and contribute to abnormal protein accumulation and neuronal damage. Here we have described a mechanistic link between free radical production, abnormal protein accumulation, and neuronal cell injury in neurodegenerative disorders such as PD and AD. The elucidation of NO-mediated S-nitrosylation of parkin, PDI, and Drp1 in neurodegenerative disease may promote development of new drugs to prevent aberrant protein misfolding or excessive mitochondrial fission by targeted disruption of this process. Additionally, we describe the action of new memantine derivatives, NitroMemantines, that not only address the excitotoxicity damage caused by excessive Ca^{2+} influx via uncompetitive antagonism of the NMDA receptor, but also through their abilities to S-nitrosylate the NMDA receptor. We have also discovered a new class of electrophilic drugs that activate the endogenous protective mechanisms of the cell in response to oxidative stress. Both of these types of drugs, memantine and electrophiles, are preferentially activated by pathological conditions while being relatively inert during normal homeostasis of the cell. Thus, these next generation CNS drugs should be better tolerated clinically, making them both safe and effective.

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References

1. Beal MF. Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2001;2:325–334. [PubMed: 11331916]
2. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 1994;330:613–622. [PubMed: 7905600]
3. Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov* 2006;5:160–170. [PubMed: 16424917]
4. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 1988;336:385–388. [PubMed: 2904125]
5. Lipton SA, Choi YB, Pan ZH, et al. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 1993;364:626–632. [PubMed: 8394509]
6. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* 2005;6:11–22. [PubMed: 15611723]
7. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* 2003;40:427–446. [PubMed: 14556719]
8. Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. *Neurology* 2006;66:S102–S109. [PubMed: 16432136]
9. Chung KK, Thomas B, Li X, et al. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* 2004;304:1328–1331. [PubMed: 15105460]
10. Yao D, Gu Z, Nakamura T, et al. Nitrosative stress linked to sporadic Parkinson's disease: S-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. *Proc Natl Acad Sci USA* 2004;101:10810–10814. [PubMed: 15252205]
11. Uehara T, Nakamura T, Yao D, et al. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 2006;441:513–517. [PubMed: 16724068]
12. Lipton SA. Pathologically activated therapeutics for neuroprotection. *Nat Rev Neurosci* 2007;8:803–808. [PubMed: 17882256]
13. Papadia S, Soriano FX, Leveille F, et al. Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat Neurosci* 2008;11:476–487. [PubMed: 18344994]
14. Okamoto S, Pouladi MA, Talantova M, et al. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat Med*. 2009 *in press*.
15. Ankarcrona M, Dypbukt JM, Bonfoco E, et al. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* 1995;15:961–973. [PubMed: 7576644]
16. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* 1995;92:7162–7166. [PubMed: 7638161]
17. Budd SL, Tenneti L, Lishnak T, Lipton SA. Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons. *Proc Natl Acad Sci USA* 2000;97:6161–6166. [PubMed: 10811898]
18. Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature* 1984;309:261–263. [PubMed: 6325946]
19. Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* 1991;88:6368–6371. [PubMed: 1648740]
20. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 1969;164:719–721. [PubMed: 5778021]
21. Chen HS, Lipton SA. The chemical biology of clinically tolerated NMDA receptor antagonists. *J Neurochem* 2006;97:1611–1626. [PubMed: 16805772]

22. Abu-Soud HM, Stuehr DJ. Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. *Proc Natl Acad Sci USA* 1993;90:10769–10772. [PubMed: 7504282]
23. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991;351:714–718. [PubMed: 1712077]
24. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000;3:1301–1306. [PubMed: 11100151]
25. Isaacs AM, Senn DB, Yuan M, Shine JP, Yankner BA. Acceleration of amyloid beta-peptide aggregation by physiological concentrations of calcium. *J Biol Chem* 2006;281:27916–27923. [PubMed: 16870617]
26. Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 2005;6:150–166. [PubMed: 15688001]
27. Gu Z, Kaul M, Yan B, et al. S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. *Science* 2002;297:1186–1190. [PubMed: 12183632]
28. Hara MR, Agrawal N, Kim SF, et al. S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol* 2005;7:665–674. [PubMed: 15951807]
29. Stamler JS, Lamas S, Fang FC. Nitrosylation. the prototypic redox-based signaling mechanism. *Cell* 2001;106:675–683. [PubMed: 11572774]
30. Houk KN, Hietbrink BN, Bartberger MD, et al. Nitroxyl disulfides, novel intermediates in transnitrosation reactions. *J Am Chem Soc* 2003;125:6972–6976. [PubMed: 12783550]
31. Lipton SA, Nakamura T, Yao D, Shi ZQ, Uehara T, Gu Z. Comment on "S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function". *Science* 2005;308:1870. author reply 1870. [PubMed: 15976289]
32. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett* 1994;345:50–54. [PubMed: 8194600]
33. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci USA* 1998;95:7631–7636. [PubMed: 9636201]
34. Barsoum MJ, Yuan H, Gerencser AA, et al. Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. *EMBO J* 2006;25:3900–3911. [PubMed: 16874299]
35. Bossy-Wetzel E, Lipton SA. Nitric oxide signaling regulates mitochondrial number and function. *Cell Death Differ* 2003;10:757–760. [PubMed: 12815458]
36. Yuan H, Gerencser AA, Liot G, et al. Mitochondrial fission is an upstream and required event for bax foci formation in response to nitric oxide in cortical neurons. *Cell Death Differ* 2007;14:462–471. [PubMed: 17053808]
37. Cho DH, Nakamura T, Fang J, et al. S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science* 2009;324:102–105. [PubMed: 19342591]
38. Cookson MR. The biochemistry of Parkinson's disease. *Annu Rev Biochem* 2005;74:29–52. [PubMed: 15952880]
39. McNaught KS, Perl DP, Brownell AL, Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann Neurol* 2004;56:149–162. [PubMed: 15236415]
40. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* 2002;295:865–868. [PubMed: 11823645]
41. Ross CA, Pickart CM. The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases. *Trends Cell Biol* 2004;14:703–711. [PubMed: 15564047]
42. Marin I, Ferrus A. Comparative genomics of the RBR family, including the Parkinson's disease-related gene parkin and the genes of the ariadne subfamily. *Mol Biol Evol* 2002;19:2039–2050. [PubMed: 12446796]

43. Lim KL, Chew KC, Tan JM, et al. Parkin mediates nonclassical, proteasomal-independent ubiquitination of synphilin-1: implications for Lewy body formation. *J Neurosci* 2005;25:2002–2009. [PubMed: 15728840]
44. Lim KL, Dawson VL, Dawson TM. Parkin-mediated lysine 63-linked polyubiquitination: a link to protein inclusions formation in Parkinson's and other conformational diseases? *Neurobiol Aging* 2006;27:524–529. [PubMed: 16213628]
45. Lyles MM, Gilbert HF. Catalysis of the oxidative folding of ribonuclease A by protein disulfide isomerase: dependence of the rate on the composition of the redox buffer. *Biochemistry* 1991;30:613–619. [PubMed: 1988050]
46. Conn KJ, Gao W, McKee A, et al. Identification of the protein disulfide isomerase family member PDip in experimental Parkinson's disease and Lewy body pathology. *Brain Res* 2004;1022:164–172. [PubMed: 15353226]
47. Forrester MT, Benhar M, Stamler JS. Nitrosative stress in the ER: a new role for S-nitrosylation in neurodegenerative diseases. *ACS Chem Biol* 2006;1:355–358. [PubMed: 17163772]
48. Xu L, Eu JP, Meissner G, Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 1998;279:234–237. [PubMed: 9422697]
49. Paz Gavilan M, Vela J, Castano A, et al. Cellular environment facilitates protein accumulation in aged rat hippocampus. *Neurobiol Aging* 2006;27:973–982. [PubMed: 15964666]
50. Chen H, Chan DC. Critical dependence of neurons on mitochondrial dynamics. *Curr Opin Cell Biol* 2006;18:453–459. [PubMed: 16781135]
51. Li H, Chen Y, Jones AF, et al. Bcl-xL induces Drp1-dependent synapse formation in cultured hippocampal neurons. *Proc Natl Acad Sci USA* 2008;105:2169–2174. [PubMed: 18250306]
52. Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 2004;119:873–887. [PubMed: 15607982]
53. Youle RJ, Karbowski M. Mitochondrial fission in apoptosis. *Nat Rev Mol Cell Biol* 2005;6:657–663. [PubMed: 16025099]
54. Kijima K, Numakura C, Izumino H, et al. Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum Genet* 2005;116:23–27. [PubMed: 15549395]
55. Zuchner S, Mersiyanova IV, Muglia M, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet* 2004;36:449–451. [PubMed: 15064763]
56. Delettre C, Lenaers G, Griffoin JM, et al. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet* 2000;26:207–210. [PubMed: 11017079]
57. Waterham HR, Koster J, van Roermund CW, Mooyer PA, Wanders RJ, Leonard JV. A lethal defect of mitochondrial and peroxisomal fission. *N Engl J Med* 2007;356:1736–1741. [PubMed: 17460227]
58. Wasiak S, Zunino R, McBride HM. Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. *J Cell Biol* 2007;177:439–450. [PubMed: 17470634]
59. Breckenridge DG, Kang BH, Kokel D, Mitani S, Staehelin LA, Xue D. *Caenorhabditis elegans* drp-1 and fis-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. *Mol Cell* 2008;31:586–597. [PubMed: 18722182]
60. Karbowski M, Neutzner A, Youle RJ. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. *J Cell Biol* 2007;178:71–84. [PubMed: 17606867]
61. Nakamura N, Kimura Y, Tokuda M, Honda S, Hirose S. MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep* 2006;7:1019–1022. [PubMed: 16936636]
62. Yonashiro R, Ishido S, Kyo S, et al. A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *EMBO J* 2006;25:3618–3626. [PubMed: 16874301]
63. Chang CR, Blackstone C. Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J Biol Chem* 2007;282:21583–21587. [PubMed: 17553808]
64. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep* 2007;8:939–944. [PubMed: 17721437]

65. Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem* 2007;282:11521–11529. [PubMed: 17301055]
66. Low HH, Lowe J. A bacterial dynamin-like protein. *Nature* 2006;444:766–769. [PubMed: 17122778]
67. Zhu PP, Patterson A, Stadler J, Seeburg DP, Sheng M, Blackstone C. Intra- and intermolecular domain interactions of the C-terminal GTPase effector domain of the multimeric dynamin-like GTPase Drp1. *J Biol Chem* 2004;279:35967–35974. [PubMed: 15208300]
68. Ramachandran R, Surka M, Chappie JS, et al. The dynamin middle domain is critical for tetramerization and higher-order self-assembly. *EMBO J* 2007;26:559–566. [PubMed: 17170701]
69. Pitts KR, McNiven MA, Yoon Y. Mitochondria-specific function of the dynamin family protein DLP1 is mediated by its C-terminal domains. *J Biol Chem* 2004;279:50286–50294. [PubMed: 15364948]
70. Wang X, Su B, Lee HG, et al. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci* 2009;29:9090–9103. [PubMed: 19605646]
71. Murphy TH, Schnaar RL, Coyle JT. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. *Faseb J* 1990;4:1624–1633. [PubMed: 2180770]
72. Chen HS, Pellegrini JW, Aggarwal SK, et al. Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J Neurosci* 1992;12:4427–4436. [PubMed: 1432103]
73. Satoh T, Lipton SA. Redox regulation of neuronal survival mediated by electrophilic compounds. *Trends Neurosci* 2007;30:37–45. [PubMed: 17137643]
74. Kraft AD, Johnson DA, Johnson JA. Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *J Neurosci* 2004;24:1101–1112. [PubMed: 14762128]
75. Satoh T, Okamoto SI, Cui J, et al. Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophilic [correction of electrophilic] phase II inducers. *Proc Natl Acad Sci USA* 2006;103:768–773. [PubMed: 16407140]
76. Itoh K, Tong KI, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. *Free Radic Biol Med* 2004;36:1208–1213. [PubMed: 15110385]
77. Padmanabhan B, Tong KI, Ohta T, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. *Mol Cell* 2006;21:689–700. [PubMed: 16507366]
78. Li CQ, Kim MY, Godoy LC, Thiantanawat A, Trudel LJ, Wogan GN. Nitric oxide activation of Keap1/Nrf2 signaling in human colon carcinoma cells. *Proc Natl Acad Sci USA* 2009;106:14547–14551. [PubMed: 19706542]
79. Yazawa K, Kihara T, Shen H, Shimmyo Y, Niidome T, Sugimoto H. Distinct mechanisms underlie distinct polyphenol-induced neuroprotection. *FEBS Lett* 2006;580:6623–6628. [PubMed: 17118359]
80. Nakamura Y, Kumagai T, Yoshida C, et al. Pivotal role of electrophilicity in glutathione S-transferase induction by tert-butylhydroquinone. *Biochemistry* 2003;42:4300–4309. [PubMed: 12680784]
81. Lipton SA. Turning down, but not off. *Nature* 2004;428:473. [PubMed: 15057811]
82. Ahlgren-Beckendorf JA, Reising AM, Schander MA, Herdler JW, Johnson JA. Coordinate regulation of NAD(P)H:quinone oxidoreductase and glutathione-S-transferases in primary cultures of rat neurons and glia: role of the antioxidant/electrophile responsive element. *Glia* 1999;25:131–142. [PubMed: 9890628]
83. Kosaka K, Yokoi T. Carnosic acid, a component of rosemary (*Rosmarinus officinalis* L.), promotes synthesis of nerve growth factor in T98G human glioblastoma cells. *Biol Pharm Bull* 2003;26:1620–1622. [PubMed: 14600414]
84. Aruoma OI, Halliwell B, Aeschbach R, Loligers J. Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica* 1992;22:257–268. [PubMed: 1378672]
85. Satoh T, Kosaka K, Itoh K, et al. Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. *J Neurochem* 2008;104:1116–1131. [PubMed: 17995931]

86. Jenner P. Oxidative stress and Parkinson's disease. *Handb Clin Neurol* 2007;83:507–520. [PubMed: 18808931]

Fig. 1. Possible mechanisms whereby Ca^{2+} signaling contributes to NO generation in neurodegenerative conditions. Hyperactivation of NMDA receptors (NMDARs) by glutamate (Glu) and glycine (Gly) induces excessive Ca^{2+} influx and activation of neuronal NO synthase (nNOS). nNOS produces NO from L-arginine. In Alzheimer's disease (AD), soluble oligomers of A β peptide, thought to be a key mediator in AD pathogenesis, can facilitate neuronal NO production in both NMDAR-dependent and -independent manners. In Parkinson's disease (PD), mitochondrial dysfunction caused by pesticides or other environmental toxins can trigger NO production possibly via mitochondrial and NMDAR/ Ca^{2+} influx pathways. Note that in addition to RNS, ROS are also produced in response to A β and pesticides

Fig. 2.

Overstimulation of NMDA receptors (NMDARs) by glutamate (Glu) and glycine (Gly) induces excessive Ca^{2+} influx, activation of neuronal NO synthase (nNOS), and subsequent formation of SNO proteins. nNOS produces NO from L-arginine, and NO reacts with sulfhydryl groups to form S-nitrosylated proteins. Physiological levels of NO mediate neuroprotective effects, at least in part, by S-nitrosylating the NMDAR and caspases, thus inhibiting their activity. In contrast, we postulate that overproduction of NO can be neurotoxic via S-nitrosylation of Parkin (forming SNO-PARK), PDI (forming SNO-PDI), GAPDH, MMP-2/9, PrxII, and COX-2. S-Nitrosylated parkin and PDI contribute to neuronal cell injury by triggering

accumulation of misfolded proteins. S-Nitrosylation of Drp1 (forming SNO-Drp1) causes excessive mitochondrial fragmentation in neurodegenerative conditions.

Fig. 3.

Memantine and NitroMemantine preferentially block excessive extrasynaptic NMDA receptor activity, while relatively sparing synaptic receptors. (*Top*) Normal (physiological/synaptic) activity of the NMDAR is required for synaptic function and neuronal survival. Many NMDAR antagonists, such as MK-801, completely block receptor activity, including physiological synaptic activity, and thus result in severe side effects and clinical intolerability. (*Bottom*) Excessive activation of the NMDAR, predominantly at extrasynaptic sites, is thought to induce neuronal cell injury and death, and is associated with the accumulation of misfolded proteins. Memantine (Mem) and the newer NitroMemantine drugs (NitroMem) preferentially block

excessive (pathological) extrasynaptic NMDA receptor activity, while relatively sparing normal (physiological) synaptic activity [14].