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Forum Review Article

Nitric Oxide in Health and Disease of the Nervous System

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Abstract

Nitric oxide (NO) is an important messenger molecule in a variety of physiological systems. NO, a gas, is produced from L-arginine by different isoforms of nitric oxide synthase (NOS) and serves many normal physiologic purposes, such as promoting vasodilation of blood vessels and mediating communication between nervous system cells. In addition to its physiologic actions, free radical activity of NO can cause cellular damage through a phenomenon known as nitrosative stress. Here, we review the role of NO in health and disease, focusing on its role in function and dysfunction of the nervous system. Substantial evidence indicates that NO plays a key role in most common neurodegenerative diseases, and, although the mechanism of NO-mediated neurodegeneration remains uncertain, studies suggest several possibilities. NO has been shown to modify protein function by nitrosylation and nitrotyrosination, contribute to glutamate excitotoxicity, inhibit mitochondrial respiratory complexes, participate in organelle fragmentation, and mobilize zinc from internal stores. In this review, we discuss and analyze the evidence for each of these mechanisms in different neurodegenerative diseases and propose future directions for research of the role of NO in neurodegeneration. *Antioxid. Redox Signal.* 11, 541–553.

Introduction

In recent years, studies have implicated nitric oxide (NO) as a key mediator of neurodegeneration in numerous diseases of the nervous system, including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and ischemic brain injury (stroke) (4, 7, 51, 67, 68, 73, 95, 115, 119, 124, 129, 140, 151). In addition to its many physiologic functions (for example, as a neurotransmitter, neuromodulator, and mediator of blood vessel dilation), NO can convert into highly reactive and toxic molecules that readily react with proteins, DNA, and lipids to alter their function (96). This dual action of NO, as both an important player in normal physiology and a contributor to pathophysiology, makes developing effective treatments to target NO toxicity particularly challenging.

Here, we review the current status of the NO-linked neurodegeneration field, focusing both on the progress that has been made and on identifying areas for future study. First, we present background information on NO, its reactions, its associated reactive species, and its normal physiologic functions. Next, we present the evidence of the participation of NO in neurodegenerative diseases and discuss the proposed mechanisms of NO-mediated neurotoxicity in different diseases. Because significant overlap exists between many protein targets of NO and different neurologic disorders, we organized this section by potential mechanism of pathology rather than by individual disease.

Nitric Oxide Production in Normal Physiology

Nitric oxide (NO) is a gas synthesized from L-arginine in mammals by enzymes known as nitric oxide synthases (NOSs) (88, 109, 116). NO and NOS have been identified in many organ systems including liver, lungs, vascular tissue, skeletal muscle, and smooth muscle (114). In addition, NOS produces NO in all brain cells—both neurons and glia (109, 116). Studies have confirmed the identity of three isoforms of NOS—neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS)—expressed in different cell types and under different cellular conditions (114). More recent studies also suggest the existence of a novel, fourth NOS, mitochondrial NOS (mtNOS) (61).

The NOS isozymes have both similarities and differences in expression pattern and characteristics of enzymatic activity (See Table 1). nNOS is predominantly active in central

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Table 1. Nitric Oxide Synthase (NOS) Isoforms

NOS isoform	*Location*	*Function*	*Cytosolic/membrane-associated?*	*Characteristics of enzymatic activity*
Endothelial NOS (eNOS)	Endothelial tissue of blood vessels	Vasodilation and relaxation of muscles and soft tissue	Membrane-associated	Produces low concentrations of NO over long periods
Neuronal NOS (nNOS)	Central and peripheral neurons	Cell-to-cell communication	Cytosolic	Produces low concentrations of NO over long periods
Inducible NOS (iNOS)	Immune cells and glial cells	Mediate cell death in response to pathogens	Cytosolic	Produces high concentrations of NO over short periods
Mitochondrial NOS (mtNOS)	Mitochondrial inner membrane	Ca^{2+} regulation	Membrane-associated	Part of Ca^{2+} feedback loop

and peripheral neurons, where production of NO is important for cell communication (114). eNOS produces NO mainly in endothelial tissue of blood vessels, where NO causes vasodilation and endothelial relaxation of muscles and soft tissue (107). iNOS is found primarily in immune cells and glial cells (astrocytes and microglia) and is activated in response to pathogen recognition and cytokine release (104, 107, 114). The primary function of iNOS is to use the oxidative stress of NO to defend against pathogens (114). For example, activated microglia in the nervous system express iNOS, causing neuronal cell death *in vivo* in mice (41) and *in vitro* in rat hippocampal cultures (93). Because the NOS isoforms have different physiologic functions, characteristics of their enzymatic activity and subcellular localization also differ. For example, nNOS and eNOS, constitutively active isoforms, produce low concentrations of NO over long periods and are activated by calcium ions (Ca^{2+}) through transient binding to the calcium-binding protein calmodulin, whereas iNOS, the inducible isoform, produces high concentrations of NO for short periods and is Ca^{2+} independent because calmodulin remains tightly bound to the protein (20, 38, 107). In addition, nNOS and iNOS are both cytosolic, whereas eNOS associates with the membranes of endothelial cells (28, 102, 106).

Although the existence of nNOS, iNOS, and eNOS is well documented, whether other NOS isoforms exist and are active in mammals remains a topic of debate. Because the link between mitochondria and NOS activity is well established (60), mitochondria have been an area of intense interest in the search for new NOS isoforms. Consequently, Ghafourifar *et al.* (61) first reported the presence of constitutively active mtNOS in mitochondria from rat liver. mtNOS associates with the matrix face of the mitochondrial inner membrane and seems to play a role in Ca^{2+} regulation (61). Ca^{2+} increases NO formation by mtNOS, which reversibly decreases mitochondrial membrane potential and oxygen consumption (61). Decreased membrane potential increases Ca^{2+} release from the organelle, which inactivates mtNOS, completing the feedback loop. Although studies have begun to clarify the functions of mtNOS, its identity and relation to other NOSs remain unclear (61).

Reactive Nitrogen Species (RNS) Formation and Nitrosative Stress

NO readily reacts with various molecules within the cellular environment, and the products of these reactions can damage the cell through a variety of mechanisms. The all-encompassing term *nitrosative stress* describes this ability of NO and its derivatives to damage cellular components such as proteins and DNA. A primary reaction in the production of RNS is the combination of NO and superoxide anions (O_2^-) to form peroxynitrite ($ONOO^-$) (See Table 2), a highly reactive neurotoxin (15, 43). Mitochondria provide both reactants necessary for the formation of $ONOO^-$, NO from mtNOS and O_2^- from the electron-transport chain. About 15% of superoxide produced by mitochondria goes toward the formation of peroxynitrite (the other 85% forms hydro-

Table 2. NO-Related Chemistry

Reaction name	*Reaction formula*
NO formation	L-Arginine + O_2 + (Calmodulin) $\xrightarrow{NOS}$ NO + (Citrulline)
Peroxynitrite formation	$NO + O_2^- \rightarrow ONOO^-$
Nitrosylation	$RS(H) + X\text{-}NO \rightarrow RS\text{-}NO + X^- + (H^+)$
Nitrotyrosination	$Tyr + ONOO\cdot \rightarrow Tyr\text{-}NO_2$

gen peroxide) (29, 118). Importantly, $ONOO^-$ formation in mitochondria induces cytochrome *c* release, an indicator of mitochondrial distress and potential inducer of cell death (62), and also irreversibly blocks the respiratory chain by competing with molecular oxygen (107).

Two other reactions that involve NO modification of proteins, nitrosylation and nitrotyrosination (See Table 2), are important both to the physiologic and pathophysiologic roles of NO. Nitrosylation is the reaction of NO with the amino acid cysteine to form nitrosothiols on interacting proteins (137, 138). Whereas the precise physiological mechanism of nitrosylation is unclear, it has been hypothesized that NO reacts with oxygen to form N_2O_3, which reacts with glutathione to form the *S*-nitrosoglutathione (GSNO) group, which is an important intermediate in *S*-nitrosylation signal transduction (35). Nitrotyrosination is the reaction of the amino acid tyrosine in target proteins with $ONOO^-$ to form 3-nitrotyrosine, which may impair cellular function through an as-yet-uncertain mechanism (76). Nitrotyrosination has been used as a marker in neurodegeneration models (9, 18, 135).

Physiologic Functions of NO

Although the primary focus of this review is the pathologic role of NO in neurologic diseases, it is important to understand how NO functions in normal physiology in healthy humans. Studies identifying NO as the endothelium-derived relaxing factor (EDRF) that mediates blood vessel dilation and relaxation were the first to ascribe a physiologic function to NO (55, 74, 75, 117). Studies have also linked NO to neurotransmission and immune cell response (57, 71, 72, 89, 136). These critical functions of NO make combating the negative effects of nitrosative stress difficult because, to be effective, potential treatments must selectively disrupt the pathologic actions of NO.

NO is an unconventional neurotransmitter because it is a gas, is not stored in synaptic vesicles, and is synthesized on demand by neurons (57, 89, 136). NO has many potential roles in the nervous system, some of which are not well understood. For example, NO is likely involved in nerve-mediated relaxation of the gut during digestion (136). Studies have shown that NO-producing agents mimic neuronal relaxation (11, 26), and NOS inhibitors prevent nerve-mediated gut relaxation (11, 26, 46). NO is also likely involved in innervating neural blood vessels, including cerebral arteries (19, 20) and penile arteries in males (27). Finally, modification of cysteine residues by NO in several key proteins, known as *S*-nitrosylation, enhances neuronal survival. For example, *S*-nitrosylation of NMDA (*N*-methyl-D-aspartate) glutamate receptors in neurons increases neuronal survival by locking the receptors in the "closed" position, thus preventing excitotoxicity (34, 82, 96, 97). NO also *S*-nitrosylates and inhibits caspases, cysteine proteases that play a critical role in apoptosis (83, 94, 100), suggesting that apoptosis may not be a major pathway of neuronal death in NO-linked neurodegenerative diseases.

Another important physiologic function of NO is as a toxic agent in the immune cell response to pathogens. Macrophages activated by bacterial endotoxin show high levels of NOS activity, which is necessary for tumoricidal and bactericidal actions of the macrophages (71, 72, 136). In addition, glial cells of the nervous system, both astrocytes and microglia, rely on NO to perform their immune-like activities (114). In both macrophages and glia, iNOS becomes active on pathogen-mediated activation, and the nitrosative stress created by NO helps defend against pathogens (104, 107, 114).

NO and Neurodegenerative Disease

Although NO has many important and beneficial physiologic functions, it can also play a role in neurodegenerative disease pathology. In this section, we present some of the evidence linking nitrosative stress to various neurodegenerative diseases, including PD, AD, ALS, HD, and stroke. We also discuss the potential mechanisms of NO-related neurotoxicity, such as protein nitrosylation and nitrotyrosination, excitotoxicity, mitochondrial respiratory complex inhibition, organelle fragmentation, and liberation of zinc (Zn^{2+}) from intracellular stores.

Evidence of NO-mediated neurodegeneration

Studies have provided a considerable link between NO and many prevalent neurodegenerative diseases, including PD, AD, ALS, HD, and stroke. First, animal models of 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity have shown that inhibition of NOS slows progression of disease pathology (67, 95, 119, 129). MPTP is a neurotoxin that inhibits complex I of the mitochondrial respiratory chain and mimics the symptoms of PD by killing substantia nigra neurons (92). Of note, 7-nitroindazole (7-NI), a selective inhibitor of nNOS, blocks MPTP-mediated decrease in striatal dopamine levels in mice (129) and baboons (67) and protects against MPTP-induced neuronal death in mice (119). 7-NI also protects against motor deficits and cognitive decline in the MPTP baboon model (67). In addition to the role of nNOS in MPTP-induced neurotoxicity, iNOS appears to play a role. MPTP treatment of mice causes massive gliosis, a proliferation of glial cells, and upregulation of iNOS, and iNOS-deficient mice are more resistant to MPTP (95). It is important to remember when evaluating the importance of the referenced studies that MPTP neurotoxicity is a phenotypic model of PD based on the clinical observation that MPTP contamination of the designer opioid 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP) caused PD-like symptoms in drug users (91). Thus, the MPTP model of PD does not necessarily have a mechanistic link to sporadic PD in humans. Evidence of nitrosative stress in human PD patients would of course be more convincing, and we highlight a few such studies in the mechanism part of this section.

In addition to the extensive research of NO in PD models, a proteomic study has found a correlation between β-amyloid deposition and nitration in a number of proteins in AD patients (140). Multiple proteins in the AD hippocampus, an area of intense β-amyloid deposition, are nitrated (140). In addition, familial ALS patients with a mutant superoxide dismutase-1 (mSOD1) gene, sporadic ALS patients, and mutant SOD1 transgenic mice show high rates of 3-nitrotyrosination in spinal cord motor neurons (7, 51, 124). Furthermore, mutant huntingtin (mtHTT), the pathologically mutated protein in HD, can complex with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and seven in absentia homolog 1 (SIAH1), a protein complex that reacts with NO (68), in cell

cultures (4). Finally, studies have shown that inhibition of nNOS activity is protective against ischemic stroke injury *in vivo* (73, 115, 151). Taken together, these findings suggest that NO may be involved in many neurodegenerative diseases.

Potential mechanisms of NO-mediated neurodegeneration

As we discussed earlier, two important properties of NO that may contribute to its pathologic functions are its ability to modify proteins through nitrosylation and nitrotyrosination and its ability to react with oxygen to form RNS. Accordingly, studies indicate that nitrosylation and nitrotyrosination of many different proteins play a role in the pathology of multiple neurodegenerative diseases. In addition, NO and RNS appear to play a role in glutamate-mediated excitotoxicity in nerve cells, mitochondrial respiratory complex inhibition, organelle fragmentation, and release of Zn^{2+} from intracellular stores, all of which have been linked to neurodegenerative disease.

S-Nitrosylation

Nitrosylation of proteins is a well-established mechanism of protein modification and regulation (137). Studies have identified dozens of proteins that become *S*-nitrosylated (137), several of which are associated with neurodegenerative diseases such as PD, AD, and stroke (See Fig. 1). First, Chung *et al*. (36) reported that Parkin, an E3 ubiquitin ligase mutated in some familial forms of PD, is *S*-nitrosylated in an MPTP *in vivo* mouse model of PD and in postmortem PD patient brain samples, but not in normal, age-controlled brains (36). However, whereas Yao *et al*. (149) also reported

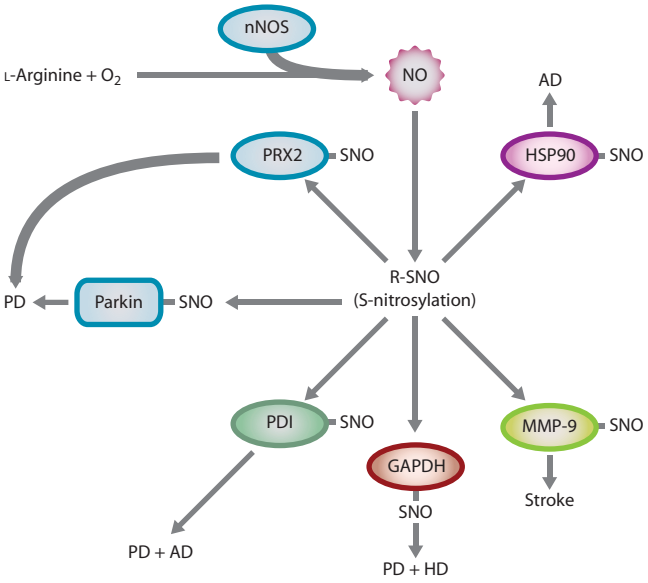

FIG. 1. NO *S*-nitrosylates multiple proteins in neurodegenerative diseases. NO *S*-nitrosylates Parkin and peroxiredoxin 2 (PRX2) in PD (shown in blue); protein-disulfide isomerase (PDI) in PD and AD (shown in green); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in PD and HD (shown in red); matrix metalloproteinase-9 (MMP-9) in stroke (shown in yellow); and heat-shock protein 90 (HSP90) in AD (shown in pink). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

nitrosylation of Parkin, they found an initial increase in E3 ubiquitin ligase activity before a significant decrease in activity. Thus, the ultimate effect of nitrosylation on Parkin and its role in PD pathogenesis remains a subject for further investigation.

Next, peroxiredoxin 2 (PRX2), the member of a family of abundant antioxidants most commonly found in neurons that reduce intracellular peroxides, becomes *S*-nitrosylated by reaction with NO (50). *S*-nitrosylation of PRX2 prevents its reaction with peroxides and inhibits its enzymatic activity and protective function against oxidative stress (50). Studies of human postmortem brains revealed an increase in *S*-nitrosylated PRX2 in human PD patients (50). A third nitrosylated protein linked to both PD and AD is protein-disulfide isomerase (PDI). PDI is an endoplasmic reticulum (ER)-associated chaperone protein that prevents neurotoxicity caused by ER stress and protein misfolding (143) and can also function as an NO receptor or donor, depending on the cellular context (134). Both PD and AD patient postmortem brains exhibit increased levels of nitrosylated PDI as compared with normal-aged brains (143). PDI nitrosylation prevents PDI-mediated ER stress reduction and allows protein misfolding (143). Heat-shock protein 90 (HSP90), a chaperone protein and coactivator of eNOS, is another protein associated with AD that undergoes nitrosylation (103). Postmortem brain samples from patients with AD exhibit increased levels of HSP90 (48, 80), and it has been suggested that inactivation of HSP90 may allow accumulation of tau and amyloid-β aggregates in the AD brain (113). Accordingly, *S*-nitrosylation of HSP90 abolishes ATPase activity that is necessary for its chaperone function (103).

GAPDH, another protein that may play a role in multiple neurodegenerative diseases, also undergoes *S*-nitrosylation in neurons (68). In addition to its well-known role in glycolysis, GAPDH contributes to nuclear signaling in apoptosis (77, 125). *S*-nitrosylation of GAPDH terminates its enzymatic activity and allows binding of GAPDH to SIAH1, an E3 ubiquitin ligase. SIAH1 has a nuclear localization signal and carries GAPDH to the nucleus. GAPDH stabilizes SIAH1 in the nucleus and allows degradation of nuclear proteins through ubiquitination (68). Interestingly, deprenyl, a drug that slows the progression of early-stage PD (113), prevents *S*-nitrosylation of GAPDH, binding of GAPDH to SIAH1, and nuclear translocation of GAPDH in an MPTP mouse model of PD (69). This action of deprenyl may account for its neuroprotective properties. Another potential link between GAPDH and neurodegenerative disease is the observed complex between mtHTT and GAPDH/SIAH1 in cell cultures (4). Furthermore, GAPDH accumulates in the nucleus of HD mice, suggesting a relation between the mtHTT–GAPDH–SIAH1 complex and the ability of mtHTT to enter the nucleus and kill neurons (131). Thus, the GAPDH/SIAH1 pathway of ubiquitination and cell death may be a common pathway in the pathology of multiple neurodegenerative diseases.

Finally, matrix metalloproteinase-9 (MMP-9), a protein involved in degradation of extracellular matrix proteins, is the target of *S*-nitrosylation during ischemic brain injury (stroke) (65). Studies have found increased expression of MMP-9 during ischemic stroke in the human brain (108, 123). MMP-9 colocalizes with nNOS in cerebral ischemia, facilitating activation of the enzyme activity (65). *S*-nitrosylation and fur-

ther irreversible oxidation of the protein thiol activates MMP-9 and activates its pathologic function in ischemic stroke (65).

3-Nitrotyrosination

When $ONOO^-$, formed from the reaction of NO and O_2^-, attacks proteins, it often results in 3-nitrotyrosination of tyrosine residues (8, 122). As discussed earlier, ALS patients and transgenic mice exhibit increased concentrations of 3-nitrotyrosine in spinal cord neurons (7, 51, 124). In addition, postmortem human AD brain samples show increased levels of 3-nitrotyrosinated proteins (135), and nitrotyrosination is a common event in the MPTP mouse model of PD (3).

Glutamate excitotoxicity

Glutamate excitotoxicity is caused by overstimulation of synaptic glutamate receptors that results in excessive Ca^{2+} influx and subsequent neuronal injury (98). Excitotoxicity is a common event in many neurodegenerative disorders, including ischemic stroke, HD, ALS, and perhaps AD (98).

As mentioned earlier, NO can protect cells from excitotoxicity by blocking NMDA-receptor opening (34, 82, 96, 97). This protective function of NO requires its conversion to the nitrosonium ion (NO^+) by the loss of one electron (96). (See Fig. 2) However, under different conditions, NO can also exacerbate neuronal injury resulting from excitotoxicity (44, 45). Increased intracellular Ca^{2+} levels, resulting from activation of glutamate receptors such as NMDA receptors, stimulate nNOS to produce more NO (44). (See Fig. 2) High concentrations of NO can interfere with *S*-nitrosylation of NMDA-receptor thiols (114). Thus, the continual influx of Ca^{2+} through the open NMDA receptors compounds the stress on mitochondria, which attempt to sequester and buffer Ca^{2+}. As mitochondrial membrane potential decreases because of the overflow of Ca^{2+} (2, 22, 23, 101), mitochondria reverse their ATP synthase in an attempt to restore it (2). Eventually, the excess Ca^{2+} uptake causes mitochondrial membrane potential loss, mitochondrial swelling, opening of the mitochondrial permeability transition pore, outer membrane rupture, and spill of Ca^{2+} and apoptogenic factors into the cytoplasm, which ultimately results in neuronal death (114).

A study by Marks and co-workers (101) recently showed that developmental differences can determine whether NO is protective or harmful to neurons after excitotoxic insults. (See Fig. 2.) In immature hippocampal neurons (from postnatal day 5 rats), Ca^{2+} influx after NMDA-receptor activation results in increased NOS activity—hence increased NO production and loss of membrane potential in mitochondria. Loss of membrane potential decreases Ca^{2+} uptake by mitochondria, which decreases mitochondrial NO production, salvages mitochondrial function, and protects neurons from NMDA toxicity (101). However, in mature hippocampal neurons (from postnatal day 19 rats), NO production after NMDA activation does not depend on mitochondrial membrane potential because the active NOS is localized to the cytosol rather than the mitochondria. Thus, the feedback effect of NO on mtNOS is not relevant and cannot protect the neurons from Ca^{2+} (101). These findings raise the question of

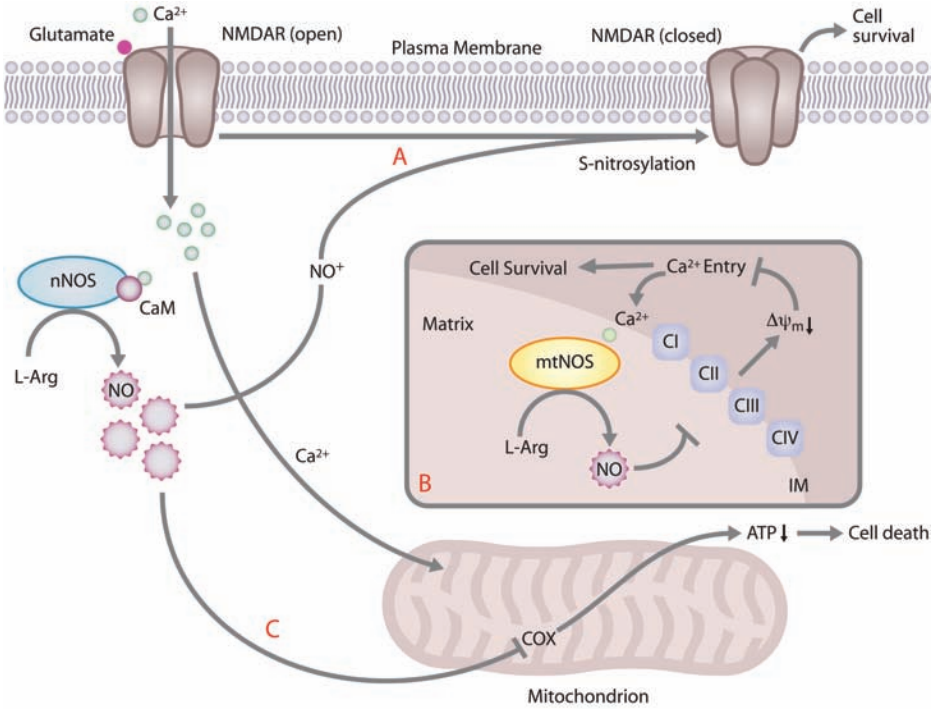

FIG. 2. NO can both protect and sensitize cells to excitotoxic cell death through a mitochondrial pathway. (**A**) NO plays a key role in excitotoxic pathways mediated by NMDA Ca^{2+} channels. Under normal physiologic conditions, NO (after converting to NO^+) *S*-nitrosylates NMDA receptors, blocks Ca^{2+} influx, and promotes cell survival. (**B**) When mtNOS is responsible for the majority of NOS activity (as is the case in immature neurons), Ca^{2+} enters mitochondria and stimulates NO production by mtNOS. NO inhibits the respiratory chain, which reduces the mitochondrial membrane potential, collapses the ion gradient, and decreases entry of Ca^{2+} into the mitochondria. Decreased Ca^{2+} influx causes a decrease in mtNOS activity and NO production, promoting cell survival. (**C**) When cytosolic nNOS is the primary producer of NO (as is the case in mature neurons), Ca^{2+} entry through overactive NMDA channels stimulates nNOS, and NO can then enter the mitochondria and directly inhibit complex IV (cytochrome *c* oxidase; COX) of the respiratory chain, which leads to a block of ATP production and eventual cell death due to energetic failure. In contrast to pathway B, no feedback loop is present to protect the cell from damage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

how cells regulate the different NOS enzymes at different stages of development and potentially support the existence of a mtNOS that is distinct from nNOS.

In sum, both the protective and detrimental effects of NO in glutamate signaling are well established. This suggests that, although blocking NOS activity (128) or selectively downregulating NMDA receptors (113) might be potential treatment options for neurodegenerative disease, the application of such treatments will be very complex and will require a careful balancing act.

Mitochondrial respiratory complex inhibition

Because neurons have high energy demands and do not readily use glycolysis, mitochondria must provide most of the required energy through oxidative phosphorylation (87). Thus, disruption of the mitochondrial respiratory chain that causes decreased ATP production can be very damaging to neurons. NO and $ONOO^-$ have been shown to inhibit mitochondrial respiratory complexes, particularly complex IV (also known as cytochrome *c* oxidase or COX), where NO competes with O_2 to bind at the enzyme active site, in many cell types including neurons (14, 24, 25, 37, 130). (See Fig. 3.) Interestingly, the cortex of human postmortem AD brains exhibits a loss of COX activity (42, 84, 110) and increased levels of iNOS (66, 70).

Studies also have shown inhibition of mitochondrial respiratory complex I in isolated brain mitochondria and intact neurons by $ONOO^-$ under certain conditions (12, 30, 99, 121). In these studies, $ONOO^-$ did not inhibit complex I unless mitochondrial integrity was disrupted (121) or levels of reduced glutathione (GSH) were low (5). This suggests a potential role for GSH, a major antioxidant in mammalian cells, in neuroprotection in PD (107). Interestingly, presymptomatic PD patients are deficient in both GSH in the substantia nigra (79) and complex I activity (126, 127).

Organelle fragmentation

Fragmentation of two important cellular organelles, the mitochondrion and the Golgi apparatus, may play an important role in neurodegeneration. To help maintain bioenergetic functionality and to facilitate equal energy transmission throughout neurons, mitochondria are dynamic organelles that actively migrate, divide, and fuse (86). A family of large, dynamin-related GTPases directs mitochondrial division (fission) and fusion. Three important members of the mitochondrial fission and fusion machinery are dynamin-related protein 1 (DRP1), a fission protein, and mitofusins 1 and 2 (MFN1, MFN2), outer membrane fusion proteins. (See Fig. 4)

To remain functional, cells must balance fission and fusion events. It is becoming increasingly clear that a shift in the fission/fusion balance toward continuous fission (fragmentation) can cause neurodegeneration, although the precise mechanism is unclear (16, 86). We recently showed that NO triggers mitochondrial fission and cell death in cortical neurons (6). In addition, NO causes spotlike cluster formation of the proapoptotic protein BAX on mitochondria at potential fission sites (81, 152). The p38 mitogen-activating protein (MAP) kinase stimulates BAX translocation to neurons (63). Occasionally, mitochondrial fission caused by NO was reversible, and neurons survived (6, 152). Interestingly, fission can be asymmetric, in which one division product is intact while its fission partner exhibits profound ultrastructural damage. Thus, mitochondrial fission caused by NO may resemble a normal stress response and repair mechanism. In support of this idea, we observed that autophago-

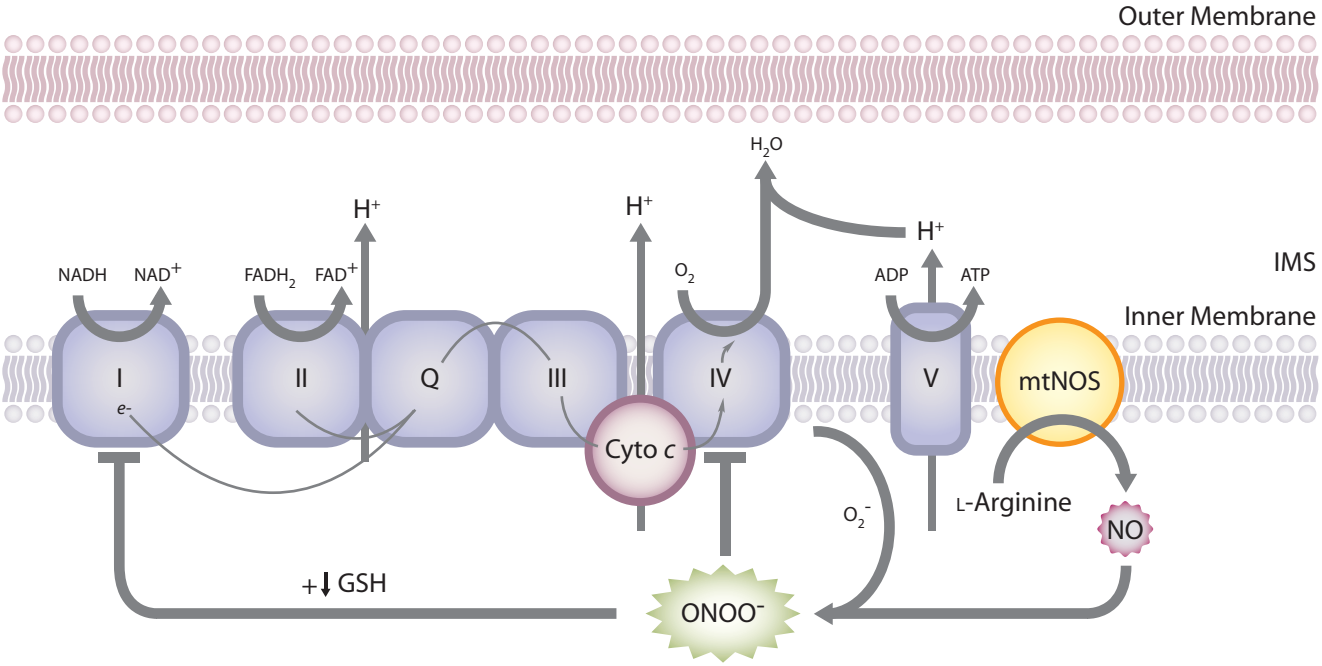

FIG. 3. $ONOO^-$ directly inhibits the mitochondrial respiratory chain. NO combines with O_2^- produced by the mitochondrial respiratory chain to form $ONOO^-$, which inhibits the respiratory chain at complex IV, and when reduced glutathione (GSH) levels are low, complex I. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

somes engulf damaged mitochondria after fission caused by NO. Whereas fission caused by NO alone did not cause cell death, we found that fission associated with BAX cluster formation on mitochondria triggered irreversible fission and cell death (110). Thus, NO-mediated mitochondrial fission and autophagy may be a neuroprotective mechanism, yet if the induction of additional signal-transduction pathways (such as BAX clustering to fission sites) overwhelms the fission and autophagy system, neuronal cell death can result. Interestingly, expression of MFN1 and dominant-negative mutant DRP1 inhibits fission and BAX foci formation on mitochondria (6, 152). This builds on another study that found that MFN2 protects cerebellar granule neurons against oxidative stress (78). Collectively, these studies suggest that mitochondrial fragmentation initiates NO-mediated neurodegeneration.

The precise mechanism of NO-mediated mitochondrial fission is presently unclear. Of note, dynamin, which is involved in endocytosis, is activated by NO (144). Thus, one can speculate that NO directly regulates DRP1 and/or MFN1/MFN2 activity. Alternatively, it is becoming increasingly clear that these large GTPases are regulated by phosphorylation and dephosphorylation (31, 39, 105, 142). Thus, NO may regulate kinases and phosphatases that control DRP1 and/or MFN activity. Studies have shown *S*-nitrosylation of G protein–coupled receptor kinases (148) and protein tyrosine phosphatases (32). Whether kinases and phosphatases, which regulate mitochondrial fission and fusion components, such as protein kinase A (PKA) and cyclin-dependent kinase 1 (Cdk1), are targets of *S*-nitrosylation is a subject for future investigation.

The Golgi apparatus is another cellular organelle that undergoes fragmentation under both physiologic and pathophysiologic conditions. Golgi disassembly and fragmentation is an important event in mitosis (as is mitochondrial fission) (141, 145) and has been observed *in vivo* in AD and ALS (64). In addition, NO induces Golgi fragmentation in cortical neuron cultures, and nitro-L-arginine, an NOS inhibitor, significantly inhibited NMDA-induced fragmentation (112). Interestingly, addition of MFN1 or dominant-negative mutant DRP1 decreased Golgi fragmentation after NMDA or NO exposure, suggesting that mitochondrial fragmentation occurs upstream of Golgi fragmentation (112). Collectively, these studies suggest that the roles of Golgi and mitochondrial fragmentation in NO-mediated neurodegeneration are important areas for further investigation.

Liberation of Zn^{2+} from intracellular ligands

Similar to NO, Zn^{2+} is a neuromodulator under normal physiologic conditions. Zn^{2+} is stored in presynaptic vesicles of glutaminergic neurons and is released on action potential firing. Zn^{2+} can modulate glutamate receptors by binding to the NR2A subunit (120) and thus plays a role in long-term potentiation and memory. Most of the intracellular Zn^{2+} is not free and is instead bound to high-affinity ligands, such as Zn^{2+} finger–binding proteins or metallothionein.

Also similar to NO, Zn^{2+} has been implicated in neurodegenerative disorders, in particular acute brain injuries such as ischemic stroke, prolonged seizures, and trauma (33, 53, 56, 90, 147). During these brain injuries, a dramatic increase occurs in free Zn^{2+}. It has been suggested that free Zn^{2+} then triggers neuronal death. Supporting this belief, Zn^{2+} was found to be particularly toxic to neurons (150). However, the mechanism responsible for the massive accumulation of Zn^{2+} is unknown. One possible mechanism is that Zn^{2+} released from presynaptic vesicles crosses from the synaptic cleft, through the plasma membrane, and enters the postsynaptic neuron *via* voltage-gated ion channels (133, 139, 146). However, we and others have provided another possibility for the accumulation of free Zn^{2+} ions during brain injury. Nitrosative stress or oxidative stress evokes the liberation of Zn^{2+} from its intracellular ligands, such as metallothionein, which in turn blocks the mitochondrial electron-transport chain, decreases mitochondrial membrane potential, increases free radical production, evokes cytochrome *c* release, and eventually leads to neuronal cell death (1, 17, 40, 52, 54). (See Fig. 5.) Thus, these studies have established an important connection between NO and Zn^{2+} pathways

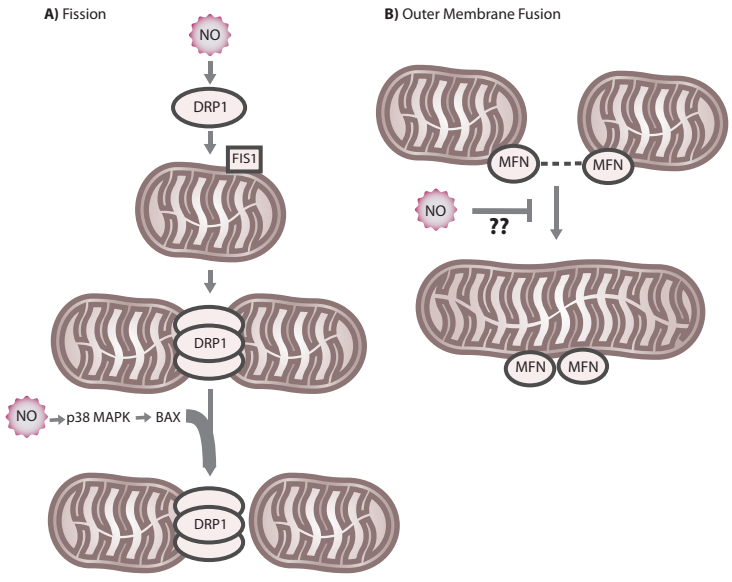

FIG. 4. Mitochondrial fission and fusion. (**A**) DRP1, in coordination with other factors including the protein FIS1 and possibly BAX, directs fission of mitochondria. NO can stimulate mitochondrial fission by an unknown mechanism. (**B**) MFNs mediate fusion of the mitochondrial outer membrane. NO might inhibit this process. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

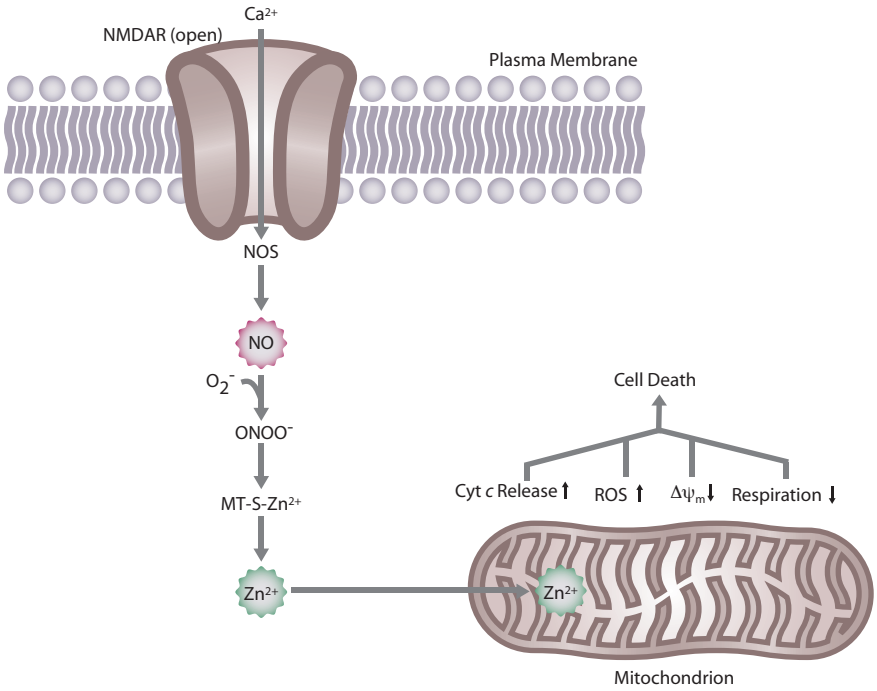

FIG. 5. Nitric oxide–mediated zinc (Zn^{2+}) causes mitochondrial dysfunction and cell death. NO, after conversion to $ONOO^-$, causes release of Zn^{2+} from metallothionein (MT). Free Zn^{2+} inhibits the mitochondrial respiratory chain, causing increased cytochrome *c* (Cyt *c*) release, increased reactive oxygen species (ROS) production, decreased mitochondrial membrane potential ($\Delta\Psi_m$), decreased respiration, and ultimately, cell death. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

in neuronal injury. In addition, Zn^{2+} is a potent inhibitor of proteins involved in energy production and defense against oxidative stress, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and the Rieske Fe-S protein complex (58, 59), and inhibition of these proteins may set off a vicious cycle of free radical production and bioenergetic failure. Thus, chelation of free Zn^{2+} might in part block NO-mediated neuronal injury.

Nonneuronal Cell–Type Involvement

Whereas neurodegenerative disease research typically focuses on neurons, and rightfully so, because neuronal injury is the ultimate cause of the disease symptoms, it is becoming increasingly clear that other cells of the nervous system, including astrocytes and microglia, also play important roles in disease pathology. For example, much of the recent research in ALS has focused on the role of astrocytes in disease onset and progression (85). Studies have reported that activated astrocytes expressing the mutant superoxide dismutase 1 (*mSOD1*) gene, which causes a form of familial ALS, release a soluble factor that contributes to motor neuron degeneration and death (47, 111). NO is one candidate factor that activated astrocytes may release. One important normal function of glial cells in the nervous system is to protect neurons from NO (13, 49). For example, concentrations of NO that are nontoxic to intact neurons (*i.e.*, with glial cells present) inhibit ATP synthesis in neurons cultured alone (*i.e.*, without glia) (21). Thus, the possibility of a link between NO and astrocyte-mediated neuronal injury in ALS is a question that deserves further exploration. More generally, how NO affects glial cells in other neurodegenerative diseases is a question that requires more research.

Outlook

As the field of NO research continues to expand, a growing appreciation exists of the many and varied functions of NO in human health and disease. Because NO activity is crucial for normal physiologic function, particularly of the nervous system where NO acts as a neurotransmitter and can protect against excitotoxicity and caspase activation under certain conditions, attempting to target selectively the deleterious actions of NO for treatment of neurodegenerative diseases is a difficult task. The NO system has many "moving parts," many of which have likely yet to be identified, and many of the reactions of NO and its reactive derivatives are nonspecific. Thus, attempting to understand how NO will behave, as a mediator of healthy function or as an inducer of neurodegeneration by nitrosylating and nitrotyrosinating proteins, increasing excitotoxic vulnerability, inhibiting mitochondrial respiration, fragmenting organelles, or mobilizing intracellular Zn^{2+} will require continued intensive and painstaking research. For example, two recent studies found that the antioxidant enzymes known as thioredoxins (TRX1 in the cytosol and TRX2 in mitochondria) denitrosylate caspase-3 (10, 132) and other low-molecular-weight proteins (132). Denitrosylation of caspase-3 activates the protease, and this activation is a key event in the apoptotic cascade. Although the link between caspase activation and neurodegenerative disease is controversial, this type of mechanistic insight is crucial for our general understanding of NO-mediated pathology. We hope that further progress in unraveling the mechanisms of NO reactions and identifying more of the players involved will allow us to develop more specific and effective ways to treat neurodegenerative diseases through the shared pathway of nitrosative stress.

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Abbreviations

7-NI; 7-nitroindazole; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; BAX, BCL-2–associated X protein; Ca^{2+}, calcium ion; COX, cytochrome *c* oxidase; DRP1, dynamin-related protein 1; EDRF, endothelium-derived relaxing factor; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione (reduced); GSNO, *S*-nitrosoglutathione; HD, Huntington disease; iNOS, inducible nitric oxide synthase; MAP, mitogen-activating protein; MFN1, mitofusin 1; MFN2, mitofusin 2; MMP-9, matrix metalloproteinase-9; MPPP, 1-methyl-4-phenyl-4-propionoxypiperidine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mSOD1, mutant superoxide dismutase 1; mtHTT, mutant Huntingtin; mtNOS, mitochondrial nitric oxide synthase; N_2O_3, dinitrogen trioxide; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NR2A, NMDA-receptor subunit 2A; O_2^-, superoxide radical; $ONOO^-$, peroxynitrite; PD, Parkinson disease; PRX2, peroxiredoxin 2; RNS, reactive nitrogen species; ROS, reactive oxygen species; SIAH1, seven in absentia homolog 1 (*Drosophila*); Zn^{2+}, zinc ion.

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