Nitric Oxide in Neuronal Degeneration

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The role of nitric oxide (NO) as a biological messenger molecule has been established through elegant investigations in the fields of immunology, cardiovascular pharmacology, toxicology, and neurobiology (1–4). The discovery of NO as a messenger molecule in the nervous system has changed the conventional concepts of neuronal communication. NO is an unorthodox neurotransmitter. It is not stored in synaptic vesicles and released by exocytosis upon membrane depolarization but is synthesized on demand. NO does not mediate its biological actions by binding to membrane-associated receptor proteins, but diffuses from one neuron to another to act directly on intracellular components. The activity of conventional neurotransmitters is terminated by either reuptake mechanisms or enzymatic degradation. The activity of NO terminates when it chemically reacts with a substrate. There are multiple points at which biological control can be exherted over the production and activity of conventional neurotransmitters. None of these classical biological mechanisms are exploited by the nervous system to regulate the activity of NO. Since the cell cannot sequester and regulate the local concentration of NO, the key to regulating NO activity is to control NO synthesis.

Putative cellular targets of NO as well as its potential physiologic and pathophysiologic roles in the nervous system are rapidly being discovered. NO may regulate neurotransmitter release, it may play a key role in morphogenesis and synaptic plasticity, it may regulate gene expression, and it may mediate inhibitory processes associated with sexual and aggressive behavior. Under conditions of excessive formation, NO is emerging as an important mediator of neurotoxicity in a variety of disorders of the nervous system.

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Nitric Oxide Synthase Isoforms

Although as early as 1916 it was noted that mammals excrete more nitrate than is consumed in the diet, it was not until the 1980s that nitrogen oxides were recognized as normal products of mammalian metabolism (5, 6). Attempts to understand the vasodilatory effects of organic nitrates (7, 8) and the role of actylcholine-induced relaxation of vascular smooth muscle (9) led to the discovery that NO, produced in endothelial cells, is critical in the regulation of vascular hemodynamics (10, 11). Parallel investigations identified a role for NO synthase (NOS) and NO in macrophages (3, 12) in which an L-arginine–dependent pathway that produced nitrogen byproducts contributed to the cytotoxic action of macrophages on tumor cells, protozoa, and other microorganisms (13–16). The NOS isoform in endothelial cells is constitutively expressed and briefly activated by increases in intracellular calcium while the NOS isoform in macrophages, which normally do not contain detectable levels of NOS protein, requires protein synthesis before the enzyme is expressed. Once NOS is expressed in macrophages, it produces large quantities of NO for sustained periods. These initial observations led to the classification of NOS enzymes as constitutive or inducible. However, this nomenclature is not entirely accurate. Recent studies have shown that following traumatic or pathologic insult all NOS isoforms can be "induced", in that new enzyme protein synthesis occurs (1).

Molecular cloning experiments have led to the identification of three NOS genes, neuronal NOS (nNOS), endothelial NOS (eNOS), and immunologic NOS (iNOS), which were named by the tissue from which they were first cloned. All three isoforms have been identified in numerous tissues and all three isoforms can be expressed in the central nervous system (CNS) nNOS is present not only in neurons but also in skeletal muscle, neutrophils, pancreatic islet cells, endometrium, and respiratory and gastrointestinal epithelia (17). eNOS has also been localized to a small population of neurons in the central nervous system (18). iNOS can be expressed in most tissues including neurons, astrocytes, and endothelial cells (12, 17, 19,

20). iNOS produces NO for sustained periods resulting in neuronal damage (21–23). NO produced by astrocytes and microglia may contribute to neurologic damage in several disease states (24).

Regulation of NOS Catalytic Activity

NO is formed by the stoichiometric conversion of L-arginine to L-citrulline and NO in the presence of oxygen and NADPH through an oxidative-reductive pathway which requires five electrons (25). The mechanism and order in which these cofactors and reactants interact with NOS differs between the nNOS and eNOS isoforms and iNOS. nNOS and eNOS are expressed as noninteractive monomers which stoichiometrically bind the flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) (4, 12, 25). After binding heme, tetrahydrobiopterin and L-arginine the NOS monomers dimerize (26). Subsequent increases in intracellular calcium are required, resulting in calcium/calmodulin complexes which bind to nNOS or eNOS dimers and induce a conformational change allowing the flow of electrons from the reductase portion of the enzyme which contains the flavoprotein binding domains to the oxidative portion of the enzyme containing the heme and arginine binding domains (27). The inactive monomers of iNOS, on the other hand, bind calmodulin even at very low concentrations of intracellular calcium (28). Dimerization and activation then occurs on the binding of heme, tetrahydrobiopterin, and L-arginine, which are usually abundantly present in most cells. Therefore, most iNOS is expressed as functionally active dimers. While the catalyst for nNOS and eNOS activation is intracellular calcium, the regulation of iNOS activity is at the level of mRNA and protein synthesis and degradation.

The multiple sites for cofactors and substrate binding on NOS provides multiple opportunities for pharmacologic regulation of catalytic activity. Analogs of L-arginine, such as nitro-L-arginine, compete for binding to the catalytic site and subsequently decrease NO formation. Flavoprotein inhibitors such as diphenyleneiodonium prevent the shuttling of electrons essential for the conversion of L-arginine to L-citrulline and NO. Calmodulin provides the last link necessary in the shuttling of electrons from the flavoproteins in the reductase domain of the enzyme to the heme moiety and catalytic site. Calmodulin antagonists, such as W-7, calmidazolium, and some gangliosides are effective inhibitors of nNOS catalytic activity (29, 30). Consensus sites for phosphorylation are evident from the predicted protein sequences of nNOS. *In vitro*, biochemical studies indicate that neuronal NOS can be phosphorylated by calcium/calmodulin-dependent protein kinase, cyclic AMP-dependent proteins kinase, cyclic GMP-dependent protein kinase, and by protein kinase C. Phosphorylation of NOS by all these enzymes decreases NOS catalytic activity *in vitro* (1, 31, 32). Calcineurin, a protein phosphatase, dephosphorylates NOS and subsequently increases its catalytic activity (33). Multiple levels of regulation of nNOS are thus possible by phosphorylation.

Nitric Oxide Biochemistry

The combination of a nitrogen atom with an oxygen atom yields a species with an unpaired electron. Therefore, NO is by definition a free radical. Due to the free electron, free radicals are chemically very reactive. While neurochemists regard NO as highly reactive due to its short half-life ($t_{1/2}$) in comparison to other traditional neuronal messenger molecules, chemically, NO is not considered a highly reactive free radical because it has a limited number of chemical species that it can react with (34).

NO can react with molecular oxygen (O_2), yielding nitrogen dioxide (NO_2). This reaction occurs rapidly in the gas phase at high concentrations of reactants. However, in solution at concentrations of reactants that exist in cells, the $t_{1/2}$ is several hours, suggesting that under normal physiological conditions O_2 is not the primary chemical target of NO (34). NO rapidly reacts with nitrogen dioxide to yield nitrite ions (NO_2^-), the basis of the colormetric Greiss reaction (35). The Greiss reaction is a commonly used indirect measure of NO formation from activated cells expressing iNOS such as macrophages and astrocytes in which high concentrations of both nitrogen and oxygen intermediates are rapidly and continuously being formed (35). Thiols are commonly assumed to be a major targets for NO. Nitrosothiols with biological relevance have been isolated and characterized including, S-nitrosoglutathione and the nitrosothiol of serum albumin (36). However, the biosynthetic pathway under which this occurs is not understood, in that at physiologic pH, NO does not readily react with thiols (37).

Recently, a plethora of often conflicting biological roles have been assigned to NO. To address this issue investigators have examined the various valence states of NO and their biological function (38, 39). In addition to the free radical form there are the oxidized and reduced forms (NO^+ and NO^-) of NO. All three valence states may exist in the brain and the different valences states may be, in part, responsible for some of the conflicting activities attributed to NO. NO^+ is an active nucleophile which is unlikely to be formed at neutral pH or react with any other nucleophile other than water (34). However, NO^+ can readily be transferred from a nitrosothiol to a second thiol or another nucleophile in a process called transnitrosation (34). The work of Stamler and colleagues (36, 38) indicate that NO^+ does not exist in the free form, but rather NO^+ is donated from complex carrier molecules to

exert its biologic activity. The biologic significance of NO^- is as yet unknown, but it also does not exist in the free form and is donated from complex carrier molecules. NO free radical can exist in the free form in solution and most likely is the form of NO that is produced on stimulation of nNOS.

The biologic role for NO in the S-nitrosylation of many proteins is emerging as an important regulatory system (36, 38). For instance, the NMDA receptor is inactivated by nitrosylation. Thus, NO may physiologically modulate glutaminergic neurotransmission (38). However, recent work suggests that NO inhibits NMDA currents through an interaction with cations rather than the redox modulatory site of the NMDA receptor channel (39). NO also stimulates the apparent auto-ADP ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (40). NO may react with an active site cysteine resulting in inhibition of GAPDH catalytic activity and depression of glycolysis. The mechanism of apparent ADP ribosylation is not clear but may reflect direct binding of NAD to the cysteine catalyzed by S-nitrosylation (41). Through the formation of intracellular S-nitrosoglutathione, NO can deplete intracellular glutathione levels resulting in a rapid and concomitant activation of the hexose-monophosphate shunt pathway (42). NO also inhibits thioester-linked long chain fatty acylation of neuronal proteins possibly through a direct modification of substrate cysteine thiols in the neuronal growth cone. Thus, NO might reversibly inhibit the growth of rat dorsal root ganglion neurites (43).

Transition metals are a significant biological target of NO resulting in NO-metal complexes. A particularly important and well-characterized transition metal target of NO is the iron in the heme moiety of guanylyl cyclase (44). The NO-iron interaction in guanylyl cyclase induces a conformational change in the heme moiety which activates the enzyme and results in increased cGMP levels. NO increases prostaglandin synthesis through activation of another heme-containing enzyme, cyclooxygenase (45). Interaction of NO with a heme moiety can also inhibit the enzymatic activity of NOS and indoleamine 2,3-dioxygenase. Additional heme-containing proteins that may be targets for NO include catalase, cytochrome c, hemoglobin, and peroxidase.

NO also reacts with the nonheme iron of iron-sulfur clusters, in numerous enzymes including, NADH-ubiquinone oxidoreductase, *cis*-aconitase, and NADH:succinate oxidoreductase (3). Recent reports however, suggest that peroxynitrite, a reaction product of NO and superoxide anion, reacts with iron-sulfur clusters rather than NO directly (46, 47). These reactions readily occur after macrophage activation. However, in contrast to the reversible reaction of NO with heme, the reaction of NO with iron-sulfur cluster results in the desolution of the cluster (48). Through these interactions NO may inhibit DNA synthesis by binding to the nonheme iron of ribonucleotide reductase. NO can liberate iron from ferritin, an iron-storage protein. NO can influence iron metabolism at the post-transcriptional level by interacting with cytosolic aconitase. NO disrupts aconitase activity and exposes the RNA binding site permitting binding of the iron-responsive element binding protein to the iron-responsive element (49, 50). Production of NO following stimulation of NMDA receptors in rat brain slices activates the RNA binding function of the iron-responsive element binding protein while diminishing aconitase activity. The iron-responsive element binding protein has a discrete neuronal localization in the brain, suggesting that it may be an important molecular target for NO action in the brain (51).

NO will react with the superoxide anion ($O_2^{\cdot -}$) to produce the potent oxidant, peroxynitrite ($ONOO^-$) (52) at a rate three times faster than the reaction rate of superoxide dismutase (SOD) in catalyzing the dismutation of the $O_2^{\cdot -}$ to hydrogen peroxide. Therefore, when present at appropriate concentrations, NO can effectively compete with SOD for $O_2^{\cdot -}$. $ONOO^-$ is chemically complex. It has the activity of the hydroxyl radical and the nitrogen dioxide radical although it does not readily decompose into these entities (53). $ONOO^-$ is also a potent oxidant which reacts readily with sulfhydryls (52) and with zinc-thiolate moieties (54). Additionally, it can directly nitrate and hydroxylate aromatic rings on amino acid residues (55), oxidize lipids (52), proteins (56), and DNA (57).

Nitric Oxide–Mediated Neurotoxicity

NO has emerged as an important neuronal messenger in both the central and peripheral nervous systems. Models of the potential diffusion of NO suggest that even with brief bursts of NO (100 msec) and a short half-life (0.5–5 sec) the physiologic sphere of influence could extend as far as 100 μm from the point of origin (encompassing approximately 2 million synapses) (58). Given the potential sphere of influence of NO it is not surprising that NO has been implicated in a variety of functions including the nonadrenergic noncholinergic (NANC) neurotransmission in several peripheral systems, in the control of local regulation of cerebral blood flow and in the control of synaptic plasticity and sensory signaling (1, 59). However, when present in higher concentrations, NO, like glutamate, can initiate a neurotoxic cascade (29, 60) (Fig. 1). Substantial evidence indicates that excess glutamate acting via NMDA receptors mediates cell death in focal cerebral ischemia. Glutamate neurotoxicity has been implicated in neurodegenerative diseases such as Alzheimer's disease and Huntington's disease (61, 62). The activation of NMDA receptors and the subse-

quent increase in intracellular calcium levels presumably initiates most forms of glutamate neurotoxicity. NMDA applied only for a brief (5 min) period of time sets in motion irreversible processes which result in cell death 12 to 24 hr later. This type of toxicity is exquisitely dependent upon calcium. Since NOS is a calcium-dependent enzyme, we wondered whether activation of NOS could be involved in NMDA neurotoxicity. Treatment of cortical cultures with NOS inhibitors or removal of L-arginine from the media prevents NMDA neurotoxicity (29, 60). Additionally, reduced hemoglobin, which binds NO and prevents it from reaching its target cells, completely prevents NMDA neurotoxicity. Blockade of NMDA neurotoxicity by NOS inhibitors is also observed in cultures of caudate-putamen and hippocampus. If NO is the mediator of NMDA neurotoxicity, then compounds which release NO directly should also be neurotoxic. Cortical cultures exposed to the NO donors, sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP), or SIN-1 exhibit a delayed neurotoxicity which follows the same time course as NMDA neurotoxicity (29). Finally, lesion of the NOS neurons from culture with low dose quisqualate, results in a culture that is resistant to NMDA neurotoxicity indicating that neurons are the source of neurotoxic NO in this culture model (29).

NO has been implicated in glutamate neurotoxicity in a variety of model systems and prolonged application of NOS inhibitors, after the initial exposure to excitatory amino acids, leads to enhanced neuroprotection. Others have failed to confirm NO's role in this phenomenon (1). NO may also protect neurons from glutamate neurotoxicity when cells are exposed to NO prior to exposure to glutamate. Recent studies indicate that NO may posses both neurodestructive and neuroprotective properties depending upon the redox mileau. The NO radical ($NO^{\cdot}$) is neurodestructive, while NO^{+} (i.e., NO complexed to a carrier molecule) is neuroprotective (38). finally, some of the *in vitro* studies were performed in serum-free conditions. Neuronal NOS is not initially expressed in primary cultures of cortex, caudate, and hippocampus but develops between 14 and 21 days in culture (29). We have not observed significant neuronal expression of NOS in serum-free cultures. It is possible that glutamate stimulated elevations in cGMP in serum-free cultures reflects activation of NOS in glial cells or a small population of neurons that is not sufficient to significantly contribute to neurotoxicity.

Most NOS inhibitors are not useful in the study of nNOS function in the CNS of intact animals because inhibition of eNOS results in hypertension and alterations in cerebral blood flow. Even concentrations of NOS inhibitors that do not cause systemic hypertension result in potent cerebral vasoconstriction and up to an 80% increase in cerebral vascular resistance (63). Results may also be confounded by a potential interaction of arginine analog NOS inhibitors with other arginine dependent cellular processes. L-arginine is an essential amino acid involved in the urea cycle. Disturbances in the urea cycle can affect the TCA cycle and ultimately the energy balance in the cell. Additionally, L-arginine metabolism feeds into the polyamine pathway. Alterations of this pathway can have profound effects on cellular function and survival.

The potential involvement of NO in stroke, the third leading cause of death and morbidity in the United States, has prompted the search for selective nNOS inhibitors. Besides arginine analog NOS inhibitors which compete with L-arginine at the catalytic site, NOS can be inhibited indirectly at several regulator sites, thus providing alternative strategies for protection against NO-mediated cell death. The flavoproteins, FAD and FMN, are critical for the necessary shuttling of electrons involved in the conversion of L-arginine to NO and L-citrulline. The flavoprotein inhibitor, diphenyleneiodonium, is potently neuroprotective against NMDA neurotoxicity, although it is unlikely to be therapeutically useful. Calmodulin is an essential co-factor for the activation of NOS. Agents that inhibit or bind calmodulin, such as calmidazolium, or W7 and some gangliosides can decrease NOS catalytic activity and provide neuroprotection (29, 30). Neuroprotection provided by gangliosides in a variety of animal models may be through inhibition of NOS as the potency of NOS inhibition closely parallels the ganglioside's affinity for binding calmodulin and providing neuroprotection (30). Phosphorylation of NOS and the attendant reduction of its catalytic activity also provides another potential approach to neuroprotection. The immunosuppressants, FK506 and cyclosporin A, which bind small, soluble receptor proteins designated FK506-binding proteins (FKBP) and cyclophilins respectively, inhibit NOS and are neuroprotective. This neuroprotection is due to the interaction of the immunosuppressant with its respective binding protein to inhibit the calcium-activated phosphatase, calcineurin. Inhibition of calcineurin prevents the dephosphorylation and activation of NOS, thus NOS remains in the inactive phosphorylated state (33). The physiologic relevance of this observation was recently confirmed by the report that FK506 is profoundly neuroprotective in a rat model of focal ischemia (64).

Another potential strategy for developing neuroprotective agents is to identify the targets of NO that participate in neurotoxicity. Guanylyl cyclase is a well known target of NO and mediates many of the physiologic actions of NO (59). However, neither inhibitors of guanylyl cyclase nor cell permeable analogs of cGMP have any effect on NMDA or NO neurotoxicity (29, 65). Therefore, it is unlikely that activation of gua-

nylyl cyclase and elevation of intracellular cGMP levels participates in the genesis of neurotoxicity. The majority of evidence indicates that NO's toxic effects occur through an interaction with the superoxide anion to form peroxynitrite. The toxic effects of NO or $ONOO^-$ may occur through multiple mechanisms. NO can damage DNA with the subsequent activation of the nuclear enzyme poly(ADP-ribose) synthetase (PARS). Once activated, PARS catalyzes the attachment of ADP ribose units to nuclear proteins, such as histone and PARS itself. For every mole of ADP ribose transferred, 1 mole of NAD is consumed and four free energy equivalents of ATP are consumed to regenerate NAD (66). Thus, activation of PARS can rapidly deplete energy stores (Fig. 1). Consistent with this possibility is that cortical cell cultures are protected from glutamate and NO neurotoxicity by a series of PARS inhibitors (67). The neuroprotective effects of these agents parallels their potency as PARS inhibitors.

Following cerebrovascular infarction, release of excitatory amino acids in the extracellular space stimulates NMDA receptors, increasing NOS activity and NO levels. Marked increases in NO production in the brain occur during focal ischemia (68). Once formed, NO can react with the superoxide anion, levels of which are also increased during transient ischemia, to form peroxynitrite. If peroxynitrite is the toxin of physiologic importance, then one would expect that inhibiting accumulation of superoxide anion, or decreasing production of NO, would be associated with decreased brain injury after focal ischemia. Consistent with this notion is the observation that animals treated with SOD before focal ischemia and in transgenic mice which overexpress SOD, the infarct volume following focal ischemia is markedly attenuated (69). In a similar manner, NOS inhibitors reduce infarct volume following middle cerebral artery occlusion in mice, rats, and cats (1). Recent studies show that low doses of NOS inhibitors are neuroprotective while high doses are ineffective and suggest that partial inhibition of neuronal NOS is sufficient to obtain an optimal neuroprotective effect (70). Exacerbation of injury occurs at high doses of NOS inhibitors through adverse vascular effects from inhibition of endothelial NOS, resulting in decreased regional cerebral blood flow and increased infarction volume. Supporting this hypothesis is the recent study in transgenic mice which lack nNOS. These mice have reduced infarct volumes compared with age-matched wild-type controls following focal permanent middle cerebral artery occlusion (71). In rat ischemia models, 7-nitroindozole consistantly reduces infarct volume while the nonselective NOS inhibitors exacerbate injury. L-arginine increases cerebral blood flow and decreases infart volume through enhancement of endothelially derived NO (72). Thus, neuronally derived NO plays an important role in mediating neuronal cell death following focal ischemia and endothelially derived NO plays an important protective role by regulating and maintaining proper cerebral blood flow.

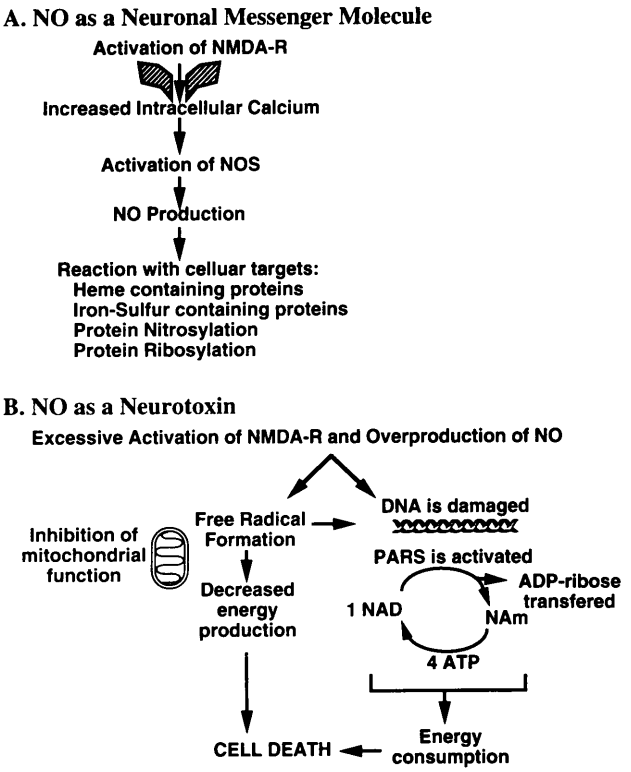

Figure 1. Dual roles of nitric oxide as a neuronal messenger (A) or neurotoxin (B). Under normal physiologic conditions production of NO results in local modulation of cellular activity through modification of cellular proteins. A well-studied target is the heme-containing enzyme, gualylate cyclase, which is involved in cerebral blood flow, neurotransmitter release, and synaptic plasticity. Other NO targets may also participate in physiologic signaling by NO. Under pathophysiologic conditions, such as ischemic damage, excessive glutamate is released, NOS is overactivated and other free radicals are formed. NO has the opportunity to compete for the superoxide anion (O_2^-) and peroxynitrite ($ONOO^-$) may be formed. It is likely that $ONOO^-$ is a major mediator of neuronal cell death. Both NO and $ONOO^-$ can react with DNA resulting in DNA damage. Damaged DNA activates poly(ADP-ribose) synthetase (PARS). For every mole of NAD that PARS consumes in the transfer of ADP ribose to nuclear proteins, four free energy equivalents of ATP are required to regenerate NAD from nicotinamide (NAm). Overactivation of PARS can lead to the rapid and permanent depletion of NAD and ATP. Additionally, mitochondrial function can be impaired by free radicals reducing the cells ability to replace energy stores. Eventually, energy-dependent processes fail and the cell dies.

Because glutamate acting via NMDA receptors stimulates NO formation, it would be logical to expect that NOS neurons would be the first cells to succumb to excess NMDA receptor stimulation. However, NOS neurons are resistant to NMDA and NO neurotoxicity (29, 73). It is unknown why NOS neurons are resistant to NMDA and NO neurotoxicity but they probably possess protective "factors" which render them relatively resistant to the toxic NO environment

they create. NOS neurons within the striatum are enriched in manganese SOD (Mn-SOD), and Mn-SOD in these neurons may prevent the local formation of toxic peroxynitrite rendering NOS neurons resistant to the toxic actions of NO (74). Another possibility may be that NO diffuses rapidly away from NOS neurons along a concentration gradient in a manner that prevents the generating neuron from seeing excessive concentrations of NO. More likely, NOS neurons possess other protective mechanisms which have yet to be identified.

Although nNOS is constitutively expressed, under some pathologic insults nNOS can be induced in certain cells through new protein synthesis (75). In spinal cord motorneurons, nNOS is expressed after avulsion or lesion of the C6 or C7 root (75). The expression of nNOS corresponds with the subsequent death of the motorneurons. Consistent with the concept that NO plays a role in neuronal cell death, treatment with a NOS inhibitor dramatically increases the number of surviving neurons (76).

Potential Roles for NO in Neurodegenerative Disorders

NO may play a role in neurodegenerative disorders and other forms of neurotoxicity. NO is an important mediator in CNS oxygen toxicity since inhibitors of NOS protect mice against CNS oxygen toxicity (77). NO may play a role in the pathogenesis of AIDS dementia as the neurotoxicity in primary cortical cultures induced by the HIV coat protein, gp120, (78), is due in part, to activation of NOS (79). Additionally, gp120 has been shown to induce iNOS in human monocyte-derived macrophages and astrocytoma cell lines (80). Using competitive reverse-transcriptase polymerase chain reaction (RT-PCR) we have observed a 3-fold increase in iNOS in cortical tissue from patients infected with HIV and a 7.5-fold increase in iNOS in patients infected with HIV who developed severe dementia (Wildemann B *et al.*, manuscript in preparation).

Overproduction of NO may also play a pathologic role in inflammatory disorders of the central nervous system. In primary rat cultures, induction of iNOS results in neuronal cell death 72 hr later (21, 22). Induction of human iNOS in demyelinating regions of multiple sclerosis brains has been observed (81), and NO may be directly toxic to the myelin-producing oligodendrocytes (82). Increased levels of NO production have also been observed in bacterial meningitis (83). Recent studies suggest that migraine sufferers have supersensitivity to NO (84). NO may also contribute to the pathogenesis of Alzheimer's disease. In primary cortical cultures, NOS inhibitors provide neuroprotection against toxicity elicited by fragments of human β-amyloid (Resink A *et al.*, manuscript in preparation). NOS neurons are also relatively spared in Alzheimer's disease (85).

The nNOS null transgenic mice provide a unique opportunity to investigate the potential role of NOS in behavior. The nNOS null mice exhibit excessive mounting behavior to nonesterus females when compared with the wild-type mice implicating a role for NO in sexual behavior. Additionally, the nNOS mice are markedly more aggressive than the wild-type mice. In a neutral arena, nNOS null mice will initiate more attacks in a shorter amount of time than wild-type mice and do not assume a submissive posture like wild-type mice (86). Thus, neuronally derived NO may play an important inhibitory function in normal sexual and social encounters. Derangements in NO formation or disposition may account for similar disorders in humans.

Summary

NO has clearly revolutionized our thinking about aspects of neurotransmission and neuronal signaling. NO is emerging as an important regulator of a variety of physiologic processes; however, under conditions of excessive or inappropriate formation, NO is also emerging as an important mediator of pathologic nervous tissue damage. Uncovering and understanding the targets of NO that contribute to the neuropathologic process will hopefully lead to the development of selective therapeutic agents and to a better understanding of basic processes underlying normal and pathological neuronal functions.

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