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The Role of Glial Nitric Oxide in Neurotoxicity and Central Nervous System Diseases

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1. IMMUNE REGULATORY FUNCTION OF GLIAL CELLS

Neuroglia ("nerve glue") were first identified in the late 1800s, and were so named because these cells surrounded neurons. Today it is recognized that these cells are not simply "packing material" for neurons, but serve in many important capacities. Glia facilitate neuronal migration during development, assist in the maintenance of the neuronal milieu for normal neurotransmission, produce neurotrophic factors, participate in immunological responses within the central nervous system (CNS), and surround the brain microvasculature to constitute the blood–brain barrier *(1–3)*. Glia differ from their neuronal counterparts in that glia compose 20–50% of the total brain volume, possess nonsynapsing cellular extensions, and retain the ability to replicate. Glia can be classified into three broad categories: astrocytes (both fibrous and protoplasmic), microglia, and oligodendroglia. Each of these types can be subdivided into finer distinctions, depending on morphological and functional specifications *(1,2,4)*.

Historically, it was believed that the CNS was immunologically isolated from the systemic immune system. This antiquated notion was inferred from observations that the brain lacked a lymphatic drainage system, and that systemic lymphocytes were usually excluded from the CNS by the blood–brain and blood–cerebrospinal fluid (CSF) barriers *(5–7)*. Contemporary research has revised our understanding of the immune response within the brain. Under certain inflammatory conditions, the blood–brain barrier is less restrictive to the migration of activated monocytes, T- and B-lymphocyte cells, natural killer cells, and granulocytes *(8)*. Moreover, in vitro and in vivo studies have clearly established that astrocytes and microglia can initiate an immune response within the CNS *(1,5,9,10)*. Although both cell types are sharing overlapping immunological functions, there are important functional distinctions *(5)*.

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Astrocytes are immunologically activated following challenge by drugs, injuries, diseases, and infectious pathogens *(10)*. Activated astrocytes respond to inflammatory mediators in a typical pleiotropic fashion: namely, activation of early response genes, expression of various adhesion proteins, and elaboration of various cytokines, eicosanoids, proteases, and cytotoxic molecules (e.g., reactive nitrogen and oxygen intermediates) *(10)*. Astrocytes can transform into a state known as "reactive astrocytes," whereby astrocytic swelling, hypertrophy, hyperplasia, and gliosis occur during the progression of CNS disease *(1,11)*. On in vitro exposure to interferon-γ (IFN-γ), lipopolysaccharides (LPS), or various viruses, astrocytes are induced to express major histocompatibility complex (MHC) class II antigens, thereby functioning as antigen-presenting cells *(2,6)*. Companion in vivo studies confirm the in vitro results, but with the cautionary note that astrocytes *in situ* do not respond to these inflammatory mediators as robustly as previously thought *(10)*.

Microglia are often referred to as the "resident macrophages" of the brain, as they share a lineage to circulating monocytes and macrophages. Similar to astrocytes, microglia normally exist in a quiescent, resting state. CNS injury or disease elicits microglial transformation to an immunologically responsive state. Activated microglia can rapidly proliferate, migrate to the site of injury, express MHC class II antigens, phagocytose pathogens or damaged tissue, and elaborate cytokines and cytotoxic agents *(5,7)*. Microglia typically possess tumor cytotoxicity and antigen-presenting abilities, which are superior to astrocytes *(7,10)*.

2. GLIAL CELL SYNTHESIS OF NITRIC OXIDE (NO)

Many exciting research issues have emerged in the quest to understand the roles of activated astrocytes and microglia in neurodegenerative diseases. An exceptionally prominent finding was the discovery that neurotoxic quantities of NO are produced by activated astrocytes and microglia as part of their repertoire of immunologic responses *(12–16)*. Therefore, the era of NO neurotoxicity was heralded into "The Decade of the Brain" (a.k.a., the 1990s).

NO serves as nature's versatile biological emissary, either modulating essential physiological functions or promoting pathological disturbances. NO plays many roles in the cardiovascular, pulmonary, gastrointestinal, immune, renal, endocrinological, or CNS/peripheral nervous system *(17,18)*. Understanding the diversified functions of NO will guide the development of new therapeutic drugs aimed at managing or curing various diseases.

The primary focus of this chapter is restricted to a discussion of the role of glial-derived NO in CNS neurological afflictions. The scientific literature is richly endowed with contemporary reviews of NO: the reader is urged to consult various comprehensive analyses for an expanded appreciation *(17–22)*.

2.1. Characteristics of Glial Nitric Oxide Synthase (NOS)

2.1.1. Biochemical Characteristics

Many tissues and cells express one or more isoforms of NOS. To date, there are three distinct isozymes: NOS I, originally identified as a constituitive isozyme present in neurons; NOS II, an inducible isoform expressed in murine macrophages; and NOS III, a distinct constituitive isozyme localized to endothelial cells *(19)*.

Table 1
Modulation of CNS NOS I Expression by Various Agents or Pathologies

	Specie/tissue/cell type
Enhanced expression	
In vivo	
Brain lesion (mechanical, chemical or thermal)	Rodent cerebellar Purkinje cells, brainstem precerebellar neurons *(184)*
Cerebral ischemia transient focal	Rodent neurons *(185)*, cerebral endothelia *(186)*, rodent supraoptic nucleus, paraventricular nucleus *(187)*
Xhronic salt loading	Guinea pig cerebellum *(188)*
Estradiol	Rodent anterior pituitary gland *(189)*
Gonadectomy	Rodent dorsal root ganglia *(189)*
Nerve injury (transection, ligation or avulsion)	Guinea pig cerebellum *(188)*
Pregnancy	Guinea pig cerebellum *(188)*
Testosterone	Rodent cerebellar astrocytes *(190)*
In vitro	
Dexamethasone	No published data
Diminished expression	
In vivo	Bovine cerebellar homogenates *(50)*
In vitro	
NO	

The original NOS literature identified each isoform by its expression characteristics (tissue source, constituitive or inducible). Unfortunately, this simplistic nomenclature created substantial ambiguity by understating the subtle complexities of the NOS isoforms. For example, the neuronal isoform is present in skeletal muscle *(23)*, neurons express both neuronal and endothelial forms *(24)*, endothelial cells express both constitutive and induced forms *(25)*, and the expression of the constituitive isoforms can be upregulated (*see* Table 1). To reduce this confusion, this chapter will use nomenclature proposed by Förstermann et al. *(19)* and endorsed by Nathan and Xie *(22)*.

Glia express two forms of NOS, similar to those found in other cell types: NOS I, a constituitive form *(26–30)* and NOS II, an inducible form *(13–16,31)*. Synthesis of NO by both isoforms consumes L-arginine, molecular O_2, and NADPH, and requires flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin in a five-electron oxidation of the guanidino moiety of arginine *(14,19,22)*. Depending on the cell type, NOS I is distributed in the soluble or particulate fractions *(23,32–36)*. In general, NOS II is a cytosolic enzyme *(37,38)*, although microglial NOS II is localized to the cytosolic and particulate fractions *(39)*.

The biosynthesis of NO is highly regulated *(40)*. The activity of NOS I is controlled by calcium and calmodulin, whereby cellular exposure to certain agents will elicit an increased intracellular Ca^{2+} and provoke transient NOS I activation. Kinase-mediated phosphorylation of NOS I provides additional mechanisms of regulatory control *(41,42)*. This contrasts sharply to the gene-mediated regulation

of NOS II, whereby *de novo* protein synthesis occurs following cellular exposure to a variety of immunostimulatory agents. Once expressed, NOS II biosynthesizes prodigious quantities of NO for hours to days. Although NOS II is tightly associated with calmodulin, NOS II is unlike NOS I in that its catalytic activity is independent of elevated intracellular Ca^{2+} *(43)*. It is for this reason that NOS II activity is universally regarded as "Ca^{2+}-independent."

2.1.2. Molecular Biological Characteristics

Both NOS I and NOS II were first cloned from rodent cerebella and macrophages, respectively *(41,42)*, and from astrocytes as well *(44)*. The protein sequence homology between NOS I and II is ~50%, confirming that expression is governed by distinct genes. Cloned rodent astrocyte NOS II is >92% homologous to mouse macrophage NOS II *(44)*. NOS II appears to be conserved across species, as rodent macrophages, rodent smooth muscles, rodent hepatocytes, and human hepatocytes share an ~80–90% protein sequence homology. Of note is that a recent report suggests the existence of multiple NOS II subtypes *(45)*.

Molecular cloning studies identified an unexpected, yet limited sequence homology between the Cterminus of NOS and NADPH cytochrome P450 (cP450) reductase (a member of a supergene family responsible for the biotransformation of drugs and xenobiotics) *(41)*. Furthermore, NOS possessed the unique UV-visible spectral signature of cP450, a characteristic of the heme chromophore contained in both enzyme systems. Although this information intimates that NOS and cP450 are of the same supergene family, the current perspective is that these enzymes are distantly related. The limited homology between these enzymes merely suggests that NOS evolved with the incorporation of the cP450 heme chromophore for efficient catalysis *(46)*.

2.1.3. Modulation of NOS Expression

A plethora of agents or diseases modulate NOS expression through transcriptional or posttranscriptional mechanisms (Tables 1 and 2). Although many compounds activate the appropriate signal transduction machinery for *de novo* NOS, numerous other agents inhibit NOS transcription or translation, diminish NOS mRNA stability, or enhance NOS protein degradation *(19)*. Also of interest are those pharmacologic agents that can inhibit NOS activity *(20)*. A number of L-arginine analogs (e.g., aminoguanidine, N^G-monomethylarginine, N^G-nitroarginine) irreversibly inhibit NOS by a suicide substrate mechanism *(47–49)*. Of note, NO itself impairs NOS activity by product inhibition *(50)* or reduced activation of the nuclear transcriptional factor, NFκB *(51)*.

2.2. NOS II Within the CNS

NO biosynthesis evolved as a primitive immunologic response against invading pathogens. Immunological production of NO is a nonspecific host defense mechanism that indiscriminately attacks both host and pathogen targets. However, the mechanisms and extent to which NO mediates CNS toxicity depend on the cell type, the biochemical status of the cellular microenvironment, and the molecular target *(18,52–54)*.

2.2.1. *Cell Targets of NO Toxicity*

Neurons *(12,55)*, oligodendrocytes *(56)*, choroid epithelium of the blood–CSF barrier *(57)*, and endothelial cells of the blood–brain barrier *(58,59)* are susceptible to NO toxicity evoked by inflammatory mediators. Microglia and astrocytes, the sources of NOS II activity, are themselves surprisingly more resilient to NO cytotoxicity for reasons not fully understood *(60,61)*.

2.2.2. *Biochemical Mechanism(s) of NO Toxicity*

It is presently thought that endogenous NO is biosynthesized as a free radical with one unpaired electron, i.e., $NO^{\bullet}$. In this form, $NO^{\bullet}$ possesses sufficient hydrophobicity to diffuse across biological membranes either to modulate signal transduction pathways or disable invading pathogens *(54,62)*. $NO^{\bullet}$ is chemically reactive toward molecular oxygen (O_2) or superoxide anion ($O_2^{\bullet -}$), depending on the concentrations of NO, oxygen tension, and superoxide dismutase (SOD) activity of the local milieu *(54)*. Alternatively, the oxidative-reductive status of the cellular microenvironment may facilitate a one-electron redox reaction of $NO^{\bullet}$, producing either nitrosonium ion (NO^+) or nitroxyl anion (NO^-) *(62–64)*. All three forms of NO ($NO^{\bullet}$, NO^+, and NO^-) can biochemically react with transition metals, free sulfhydryl residues, or the nucleophilic centers of deoxyribonucleic acids and tyrosine residues either through direct $NO^{\bullet}$ attack or by NO group transfer involving NO^+ or NO^- *(62–65)*.

The major mechanism of NO toxicity is through the reaction of $NO^{\bullet}$ with $O_2^{\bullet -}$ to form $ONOO^-$ (peroxynitrite anion). Under conditions of normal cellular function, the intracellular concentrations of $NO^{\bullet}$ and $O_2^{\bullet -}$ are relatively low. $O_2^{\bullet -}$ is effectively scavenged by SOD, and NO is constituitively produced at concentrations sufficient for modulation of second-messenger pathways. However, during an inflammatory response, microglia elaborate prodigious quantities of both $NO^{\bullet}$ and $O_2^{\bullet -}$ *(66–68)*, such that NO effectively competes with SOD for reaction with $O_2^{\bullet -}$ *(53,54,69)*. This results in a major fraction of $NO^{\bullet}$, which is transmuted into cytotoxic levels of $ONOO^-$ *(53,54,69,70)*. $ONOO^-$ is sufficiently stable to diffuse across several cell membranes to effect toxicity through a variety of mechanisms (*see* Section 2.2.3.). $ONOO^-$ can be "detoxified" through protonation to peroxynitrous acid and subsequent decomposition to the relatively less toxic species, hydroxyl radical ($HO^{\bullet}$) and nitrogen dioxide (NO_2), or to nitrate via nitric acid (e.g. $ONOOH \rightarrow HNO_3 \rightleftharpoons NO_3^- + H^+$) *(53,54,69)*.

2.2.3. *Molecular Targets of NO Toxicity*

The extent of $NO^{\bullet}$ toxicity is highly dependent on the number of insults sustained by critical molecular target(s). NO depresses mitochondrial respiration by nitrosylation of the iron-sulfate centers of several key enzymes, e.g., mitochondrial aconitase, NADH:ubiquinone oxidoreductase, and succinate:ubiquinone oxidoreductase *(71–74)*. This subsequently promotes an intracellular mobilization and loss of iron. Nitrosylation of thiol residues stimulates auto-ADP ribosylation of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), leading to a loss of functional activity *(75)*. NO can impair protein synthesis *(76)*, DNA synthesis *(77)*, and elicit DNA mutagenesis through nitrosative deamination *(78)*.

Table 2
Modulation of CNS NOS II Expression by Various Agents or Pathologies

	Specie/tissue/cell type
Enhanced expression	
In vivo	
Borrelia burgdorferi spirochetes	Rodent mixed glia *(191)*
Borna disease virus	Rodent brain *(179)*
Cerebral ischemia	
Transient global	Rodent hippocampal astrocytes *(153)*
Transient focal	Rodent striatal astrocytes, microglia/ monocytes, endothelia *(154)*
CMV	Human retinal glia *(164)*
Excitotoxic lesions	Rodent astrocytes and microglia *(152)*
EAE	Rodent spinal cord—infiltrating macrophages *(155)*, rodent brain *(179)*
IL-2	Human *(101)*
IL-12	Rodent astrocytes and microglia *(182)*
LPS	Rodent meninges and choroid epithelia *(57,168)*
Lymphocytic choriomeningitis virus	Rodent meninges and choroid epithelia *(169)*
Neurotropic coronavirus (JHM hepatitis strain)	Rodent spinal cord astrocytes *(181,192)*
Nerve injury (trauma, transection, ligation, or avulsion)	Rodent astrocytes *(151,193)*
Rabies virus	Rodent brain microglia and infiltrating macrophages *(157,179)*
In vitro	
β amyloid protein + IFN-γ	Rodent microglia *(134,135)*
Glutamate	Rodent astrocytes *(116)*
IL-1β	Human fetal astrocytes *(194)*
+ IFN-γ	Human fetal astrocytes *(194)*, rodent astrocytes *(195,196)*
IFN-γ + TNFα	Human fetal astrocytes *(194)*
LPS	Rodent cerebellar neurons *(197)* astrocytes and microglia *(14,117)*
+ TFNα	Human fetal microglial cells *(115)*
+ IFN-γ	Rodent cerebral endothelia *(59,146)* microglia and meningeal fibroblasts *(56,57)*
+ IFN-γ + TNFα	Rodent retinal Müller glia *(198)*
Mycoplasma	Mixed rodent glia *(199)*
Pneumococcal cell wall components	Rodent astrocytes *(200)*
Protein kinase C activators	
Phorbol 12-myristate 13-acetate (PMA) + IFN-γ	Rodent microglia *(56)* Rodent astrocytes *(201)*

(*continued*)

Table 2 (Continued)

	Specie/tissue/cell type
S100β (glial-derived growth factor)	Rodent astrocytes *(202)*
TNFα	Meningeal fibroblasts *(117)*
Diminished expression	
In vivo	No published data
In vitro	
β-adrenergic agonists (norepinephrine, isoproterenol, dibutyryl cyclic AMP)	Rodent astrocytes *(203)*
Angiotensin II	Rodent astrocytes (but not microglia) *(204)*
ATP	Rodent astrocytes *(205)*
Basic fibroblast growth factor (b-FGF)	Human fetal microglia *(206)*
2,4-Diamino-6-hydroxypyrimidine (inhibitor of tetrahydrobioptern synthesis)	Fibroblasts, endothelial cells, macrophages *(207)*
Dexamethasone	Rodent astrocytes *(199,201)* and meningeal fibroblasts *(117)*
Ethanol	C_6 glioma cells *(208)*
Glutamate	Rodent astrocytes *(205)*
IL-4	Human fetal astrocytes *(194)*, rodent astrocytes *(209)*, rodent macrophages *(210)*
IL-10	Human fetal astrocytes *(194)* rodent astrocytes *(209)*, rodent macrophages *(211)*
NO	Human fetal microglia *(51)*, rodent astrocytes *(212)*
Transforming growth factor β1 (TGF-β1)	Human fetal astrocytes *(194)*, rodent astrocytes *(195)*, rodent microglia *(56)*, rodent peritoneal macrophages *(213,214)*
Tyrosine kinase inhibitors (genistein, tyrphostin-25)	Rodent astrocytes *(201,215)*

Additionally, NO-mediated DNA damage also involves nitrosylation of poly(ADP-ribose) synthetase (PARS), thereby increasing PARS activity with a consequent decrease in cellular energy stores in compromised neurons *(79)*.

Compared to $NO^{\bullet}$ or $O_2^{\bullet -}$, $ONOO^-$ is a much more potent oxidant, possessing substantially greater reactivity toward critical cellular components *(80–82)*. $ONOO^-$ is far more lethal to neurons than to astrocytes, in part because of the limited neuronal supply of glutathione, a thiol that affords limited protection against $ONOO^-$ toxicity *(71)*. $ONOO^-$ can wreak cellular havoc by indiscriminate oxidation of tissue sulfhydryls *(83)* or lipid peroxidation of membranes *(84)*. SOD can catalyze $ONOO^-$ to form an intermediate complex (SOD-NO^+) capable of nitrating tyrosine residues *(70,85,86)*. Superfluous nitration of proteins may alter function

through conformational changes or blocked phosphorylation/dephosphorylation sites. It is hypothesized that the mutations in SOD observed in patients with amyotrophic lateral sclerosis may contribute to an increased nitration of proteins (especially neurofilaments) and impaired phosphorylation *(69,87)*. Also tantalizing is the possible antigenicity of nitrosylated or nitrated proteins in provoking autoimmune disease processes *(54)*. Without a doubt, excessive amounts of NO• or $ONOO^-$ are cellular poisons.

2.3. The NOS II Expression Conundrum: Rodent vs Human

2.3.1. NOS II Expression and Activity in Humans

The controversy surrounding the expression (or lack thereof) of NOS II in humans has reached an intensity of polemical proportions. With the appropriate stimuli, NOS II is faithfully expressed in a diversity of rodent cell types, especially macrophages. However, NOS II is not reliably expressed in human monocytes, the counterparts of rodent macrophages. In spite of this species difference, human NOS II expression is restricted to fewer cell types than the rodent (e.g., human hepatocytes *[88]*, chondrocytes *[89]*, mesangial cells *[90]*, keratinocytes *[91]*, pulmonary epithelial cells *[92,93]*, endothelia *[94]*, retinal cells *[95]*, islets of Langerhans *[96]*, and astrocytes—*see* Section 2.3.2.). Indirect support for NOS II activity in humans derives from early reports of elevated NO_2^- and NO_3^- (stable degradation products of NO) in patients with systemic sepsis *(97,98)*, meningitis *(99,100)*, or cancer patients receiving interleukin-2 (IL-2) therapy *(101,102)*. Unequivocal evidence of human NOS II expression was obtained with the molecular cloning of NOS II from human hepatocytes *(88)* and chondrocytes *(89)* and by the localization of the human NOS II gene to chromosome 17 *(103)*.

2.3.2. Glia NOS II: Similarities and Differences in Rodent and Humans

As is the case with human vs rodent macrophages, the expression of NOS II in the human CNS is just as controversial, and depends on the cell type, stimulatory agent, and culture conditions. NOS II is robustly induced in rodent astrocytes and microglia by a variety of agents (Table 2). This sustained production of glial NO is sufficient to cause oligodendroglial or neuronal cell death in cocultures *(12,13,56, 104)*. However, human glia present a slightly different scenario. NOS II can be convincingly induced in human fetal astrocytes, whereas human microglia weakly express NOS II (if at all). The addition of HIV1 envelope proteins or inflammatory cytokines (IL-1β or IL-1β plus IFN-γ or TNFα) to cultured human fetal astrocytes vigorously induced NOS II *(105–109)*, as measured by culture media concentrations of NO_2^- and NO_3^-, NOS II mRNA, NADPH diaphorase histochemistry (a relatively specific NOS protein marker *[110,111]*) and pharmacologic inhibitors of NOS activity. Unlike rodent glia, LPS failed to evoke NOS II in human glia.

The data for NOS II expression in human microglia are not nearly as persuasive as that for human fetal astrocytes. Primary cultures of human microglia (either fetal or postmortem adult) fail to respond to any known inducer of NOS II *(108, 109,112,113)*. A slight, but unconvincing increase in culture media NO_2^- and NO_3^- was observed for fetal and adult microglia stimulated with LPS IFN-γ *(114)*. How-

ever, a recent report detected statistically significant amounts of NO_2^- and NOS II mRNA in ramified human fetal microglia on stimulation with LPS + TFNα *(115)*. The discrepancy among these studies may be attributable to the use of primary microglial cultures passaged one to three times vs older, ramified microglia passaged 48–50 times. Unquestionably, more work is needed to understand NOS II expression, or lack thereof in human microglia.

3. EVIDENCE OF DELETERIOUS NOS ACTIVITY IN CNS DISEASES

Excessive NOS activity is implicated in many human neurological diseases and animal disease models. The following subsections discuss the in vitro or in vivo involvement of NO toxicity in a variety of CNS diseases.

3.1. In Vitro Studies of Glial NO Neurotoxicity

3.1.1. NOS II-Mediated Neuronal and Oligodendroglial Cell Death

Neurons and oligodendrocytes are the primary targets of glial-derived NO *(12, 13,55,56)*. Several studies utilizing rodent glial-neuronal coculture systems document the neurotoxic effects of NO derived from microglia *(12,13)*, astrocytes *(55,116)*, or meningeal fibroblasts *(117)* (although one study does not support these findings *[118]*). Although human microglia generally fail to express NOS II reliably *(112)* (*see* Section 2.3.2.), cytokine-stimulated human fetal astrocytes do faithfully express NOS II, with consequent cytotoxicity to fetal neurons *(119)*.

There is considerable debate regarding whether NO toxicity occurs by necrosis or apoptosis. Cortical neurons undergo apoptosis or necrosis, depending on the concentration and duration of NO exposure *(120)*. Although oligodendrocytes (but not microglia or astrocytes) undergo a necrotic death *(61)*, macrophages *(121, 122)* and undifferentiated PC12 cells *(123)* die by apoptotic mechanisms on exposure to $NO^\bullet$ or $ONOO^-$. Moreover, murine macrophages mediate apoptosis in target cells through the elaboration of NO *(124)*.

3.1.2. NOS II Enhancement of N-Methyl-D-Aspartate Neurotoxicity (NMDA)

NMDA receptors are a subset of glutamate receptors that serve as important regulators of physiological CNS functions. In cell-culture studies, excessive NMDA receptor activation causes neuronal toxicity *(125,126)*. In vivo, NMDA receptors are suspected to have a significant, deleterious role in cerebral ischemia, epilepsy, hypoxia, hypoglycemia, traumatic brain injury and possibly Huntington's disease *(127,128)*.

Although excessive NOS I activity is implicated as an important mediator of NMDA neurotoxicity *(18,129)*, recent evidence identified a contributing role of NOS II in delayed neuronal death. Glutamate application to astrocytic-neuronal cocultures stimulated the time-dependent development of intense glial NADPH diaphorase staining with attendant neuronal death *(116)*. Certain brain insults, e.g., ischemia and trauma, provoked both cytokine expression and overactivation of NMDA receptors *(130)*. In glial-neuronal cocultures, cytokine-induced NOS II expression augmented the neurotoxic effects of NMDA *(130,131)*. Furthermore, the extent of neuronal death was attenuated by NOS inhibitors. Postulated mechanisms for NOS II potentiation of NMDA toxicity involves:

1. The generation of $ONOO^{\bullet -}$;
2. Inhibition of astrocyte glutamate reuptake transporters; and
3. Inhibition of astrocyte glutamine synthetase *(130,131)*.

3.1.3. NOS II and Amyloid-β-Induced Neurotoxicity

Although the etiology of Alzheimer's disease is unknown, it is suspected that amyloid-β plaques are somehow involved in neuronal loss. What is unclear is whether the plaques contribute to or are a consequence of the underlying pathology. Although some studies have demonstrated that amyloid-β is directly neurotoxic in cell cultures *(132,133)*, other reports identify an indirect mechanism, involving amyloid-β induction of NOS II *(134,135)*. IFN-γ and amyloid-β fragments stimulate NOS II expression and activity in rodent microglia *(134,135)* or the neuroblastoma cell line MES 23.5 *(136)*. However, one study does not support an NO neurotoxic effect initiated by amyloid-β *(137)*. Companion studies utilizing human astrocytes are presently lacking.

3.2. In Vivo Studies Implicating Glial NO Toxicity

3.2.1. Permeability Alterations in the Blood–Brain Barrier

Alterations in blood–brain barrier function are evident in multiple sclerosis (MS) *(138,139)*, HIV-1 dementia *(140)*, cerebral ischemia *(141)*, brain tumors *(142)*, and meningitis *(143)*. Transient loss of blood–brain barrier integrity is a putative initiating event in Rasmussen's epilepsy *(144,145)*. Given that excessive NO synthesis may be a pathological process involved in a number of these disease states (*see* following sections), it is tempting to speculate that NO might be involved in permeability changes of the blood–brain barrier.

Morphologically, the blood–brain barrier consists of astrocytic processes enveloping cerebral endothelial capillaries. Support for the hypothesis that excessive production of NO mediates blood–brain barrier disruption is derived from in vitro studies identifying NOS II induction in human astrocytes *(105,106)*, fibroblasts *(117)*, and endothelial cells *(94,146)*. In rats, intracisternal administration of LPS provoked blood–brain and blood–CSF barrier disruption, accompanied by meningeal inflammation and NO synthesis *(47,57,58,147)*. Treatment with a specific NOS II inhibitor, aminoguanidine, during meningeal inflammation significantly diminished meningeal NO production and preserved normal blood–brain and blood–CSF integrity *(57,58)*.

3.2.2. Brain Tumors

Immunohistochemistry techniques were used to identify increased NOS I and II expression in human glioma specimens *(148)*. The authors speculate that NOS expression may promote tumor survival.

3.2.3. Brain Insult: Cerebral Ischemia, Excitotoxic Lesioning, and Trauma

Increased NOS II and NOS I activity (*see* Section 3.1.2.) *(149,150)* is thought to contribute to neuronal death caused by brain insults. Brain lesions caused by stab wounds *(151)* or injection of the excitotoxin quinolinic acid *(152)* were heavily populated with NOS II-reactive astrocytes and microglia. Reactive astrocytes, but not microglia, expressed NOS II in an in vivo rodent model of ischemic insult *(153)*. In another study of focal cerebral ischemia, the time-course of NADPH-

diaphorase staining indicated the *de novo* expression of NOS II in endothelia, astrocytes, and microglia as well *(154)*. Aminoguanidine, a specific NOS II inhibitor, significantly attenuated the infarct region caused by focal ischemia *(150)*.

3.2.4. *Experimental Autoimmune Encephalomyelitis (EAE)*

EAE is thought to represent an animal model of MS (*see* Section 3.2.7.). EAE animals demonstrated NOS II-positive cells in perivascular regions (most likely infiltrating macrophages), increased NOS II mRNA *(155)*, and elevated NO *(156)* and NO_2^-/NO_3^- *(155,157–159)*. The severity of EAE in mice was related to the increased expression of NOS II *(155)*. Administration of aminoguanidine delayed the onset and development of the disease *(160)*.

3.2.5. *Nervous System Complications of Human Immunodeficient Virus (HIV-1) Infection*

3.2.5.1. NO and HIV-1-Associated Dementia

Continuing debate surrounds a potential role of NO in AIDS dementia *(140, 161,162)*. In vivo evidence for NOS activity is scant and is generally derived from the detection of increased NOS II mRNA transcripts in postmortem brains infected with HIV-1 *(163)*. Using in vitro cell culture, Koka et al. established that human glia produce NO and NOS II mRNA in response to HIV-1 envelope proteins *(107)*.

3.2.5.2. NO and Retinitis in HIV-1 Disease

Cytomegalovirus (CMV) infection of the eye is a prevalent complication of HIV disease. Dighiero et al. observed that Müller glial cells of CMV-infected retinas were positive for NOS, as detected by NADPH-diaphorase staining and NOS immunohistochemistry *(164)*.

3.2.6. *Meningitis*

Excessive NO synthesis during meningitis *(47,57,165)* is intimately involved in disturbances of cerebrovascular permeability *(147)*, cerebral blood flow *(165,166)*, and pial arteriolar dilatation *(165)*. Many lines of evidence suggest that NO may contribute to these permeability alterations. Elevated CSF concentrations of NO_2^- / NO_3^- were detected in patients diagnosed with bacterial meningitis *(99,100,167)*. Immunohistochemical evidence of NOS II expression was observed in the epiplexus cells and choroid epithelium of postnatal rats previously treated with intracisternal LPS *(168)*. In a rodent model of bacterial meningitis, the synthesis of NO by rat meningeal and choroid plexi tissues was linked to permeability increases in the blood–CSF and blood–brain barriers *(58,147)*. The administration of NOS inhibitors (e.g., aminoguanidine or N^G-nitroarginine) during meningeal inflammation significantly diminished meningeal NO production, attenuated white blood cell migration into the CSF, and prevented barrier disruption *(57,58,165)*. Similarly, the neuropathology and clinical course of meningeal inflammation elicited by lymphocytic choriomeningitis virus were correlated with the expression of NOS II *(169)*.

3.2.7. *Multiple Sclerosis*

MS is a chronic, inflammatory demyelinating disease of unknown etiology. A characteristic of this autoimmune disease is the development of demyelinated plaques associated with perivascular infiltrates, activated astrocytes, microglia, and

cytokines (IL-1, TNFα, and IFN-γ) *(56,170)*. Evidence for NO toxicity during the inflammatory, destructive process derives from observations of:

1. Elevated CSF NO_2^- and NO_3^- *(171)*
2. Circulating antibodies against S-nitrosocysteine *(172)*;
3. Intense staining of active lesions with NADPH-diaphorase activity (a marker of NOS —*see* Section 2.3.2.) *(170,173)*; and
4. Increased human NOS II mRNA in patients with active MS *(173)*.

Moreover, MS patients have an abnormal disruption of the blood-brain barrier *(174)*, which could be owing to NO toxicity of the cerebral vasculature (*see* Section 3.2.1.). In vitro studies with rodent astrocytes, microglia, and oligodendrocytes strongly implicate toxicity owing to NOS II activity *(56,60)*.

3.2.8. Viral, Parasitic, and Fungal CNS Infections

Bacterial (*see* Section 3.2.6.), fungal, parasitic, and viral infections elicit NOS II expression and activity. Although excessive NO elaboration provokes neurotoxicity, NO also inhibit viral replication *(175)*. NO is prolifically synthesized by rodent microglia and rodent or human astrocytes infected with *Cryptococcus neoformans (106,113), Toxoplasma gondii (112,176–178)*, Borna viral disease *(179, 180)*, coronavirus *(181)*, rabies *(157,179)*, and vesicular stomatitis virus *(182,183)*.

4. CONCLUSIONS AND FUTURE DIRECTIONS

This chapter presents an overview of the role of glial-derived NO in the etiology of CNS diseases. Convincing evidence supports the hypothesis that NO is an important inflammatory product of animal and human neurological damage. More in vivo research is needed to underscore a link between neurotoxicity and NO. An equally important issue is the potential utility of NOS inhibitor therapy in humans. Pharmacologic intervention holds great promise for persons suffering from CNS afflictions. However, the biomedical research community and pharmaceutical industry must respond to the challenge of NOS inhibitor therapy: the need for selective, potent NOS inhibitors that effectively cross the blood-brain and blood-CSF barriers targeted to the diseased region. Clearly, many tantalizing questions persist, highlighting the need for more clinical and basic research.

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