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NITRIC OXIDE NEUROTOXICITY IN NEURODEGENERATIVE DISEASES

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1. ABSTRACT

Nitric oxide (nitrogen monoxide; NO) is a simple molecule with diverse biological functions. NO and related reactive nitrogen oxide species (RNOS) mediate intricate physiological and pathophysiological effects in the central nervous system. Depending on environmental conditions, NO and RNOS can initiate and mediate neuroprotection or neurotoxicity either exclusively or synergistically with other effectors. The focus of this review is limited to the neuroprotectant / neurotoxic role of NO in Acquired Immune Deficiency Syndrome (AIDS) Dementia Complex (aka HIV – Associated Dementia; HAD) Amyotrophic Lateral Sclerosis (aka Lou Gehrig's Disease), Alzheimer's Disease, Huntington's Disease, Multiple Sclerosis and Parkinson's Disease. This review will shed light on the question: "How important is NO in neurodegenerative diseases?"

2. INTRODUCTION

Nitric oxide is a simple molecule with diverse biological functions. Owing to its relative stability (a biological half-life of several seconds under ideal conditions), a net ionic charge of zero and a high 1-octanol/water partition coefficient, NO can diffuse across lipid membranes to exert effects distal from its biosynthetic source (1-3). NO and related reactive nitrogen oxide species (RNOS) mediate complex physiological or pathological functions (4). As a signal transduction agent, NO is physiologically active in various organ systems, e.g., musculature, pulmonary, gastrointestinal, immune, renal, endocrinological and reproductive systems (5-10), in addition to modulation of regional and systemic circulation (11-13), and developmental neurosynaptic plasticity (14, 15). As a participant in oxidative stress, NO may serve as a protective antioxidant to reduce oxidative damage, or alternatively NO (and RNOS) may intensify oxidative stress via it's own oxidative and nitrative actions (16). Whether or not NO serves as an antioxidant or toxicant likely depends on the cellular source(s) and mode of NO synthesis, cellular targets, and the efficiencies of endogenous antioxidant defenses coupled with cellular repair mechanisms.

The NO literature is quite broad, owing to NO's ubiquitous presence and multi-faceted functions. There are many diverse, contemporary reviews of NO and nitric oxide synthase (NOS). For a succinct, global overview with a historical perspective, the reader may wish to consult the review by Salvador Moncada and Robert Furchgott (17), who have made seminal contributions to the discovery and identification of NO. Another excellent historical review is contained in the article by Law et. al. (18). Reviews of specific functions of NO, RNOS and NOS are readily retrievable from various scientific literature databases.

NO and related species exert intricate physiological and pathophysiological effects in the central

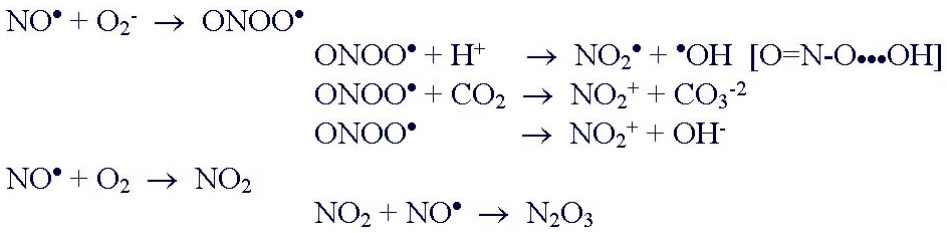

Figure 1. Generation of RNOS and Reactive Free Radical Derivatives from NO. (For illustrative purposes, NO and other free radicals are depicted with an unpaired electron.).

nervous system (CNS). Under the "right" conditions, NO and its derivatives can initiate and mediate neuroprotection or neurotoxicity either exclusively or synergistically with other effectors. As such, NO neurotoxicity represents only one mechanism of neurotoxicity (19); other mechanisms are operative, depending on the brain region, cell type and initial cellular insult. The focus of this review is limited to the neuroprotectant / neurotoxic role of NO in Acquired Immune Deficiency Syndrome (AIDS) Dementia Complex (aka HIV – Associated Dementia; HAD) Amyotrophic Lateral Sclerosis (aka Lou Gehrig's Disease), Alzheimer's Disease, Huntington's Disease, Multiple Sclerosis and Parkinson's Disease. Hopefully, this review will shed light on the question: "How important is NO in neurodegenerative diseases?"

3. NITRIC OXIDE BIOCHEMISTRY

The biochemistry and molecular biology of NO and related species have been lucidly presented in several excellent reviews (1-3, 20).

3.1. Nitric Oxide Synthase Isoforms

NO is synthesized by one of three isoforms of NOS, each of which are distinct gene products: eNOS for endothelial NOS, nNOS for neuronal NOS, and iNOS for immunoinducible NOS. Each isoform was characterized and cloned from many tissues from diverse animal species, including humans (17). Human NOS are gene products of chromosomes 12 (nNOS), 7 (eNOS) and 17 (iNOS) (18). nNOS is dynamically regulated during CNS development, plasticity and injury (21). Subsets of neuronal populations can express two to three NOS isoforms, e.g., hippocampal pyramidal CA1 - CA3 neurons express nNOS and eNOS, and cultured cerebellar granule neurons express all three isoforms (22, 23). While activated microglial cells clearly express iNOS, astrocytes express all three isoforms (24-27). Cerebrovascular endothelial cells express eNOS constitutively and can be induced to express iNOS following exposure to immunostimulatory agents.

3.2. Synthesis and Metabolism of Nitric Oxide

Synthesis of NO requires L-arginine, O_2, NADPH, flavin adenine dinucleotide, flavin mononucleotide and tetrahydrobiopterin in a five electron oxidation of the guanidino moiety of arginine. The biosynthesis of NO is highly regulated by intracellular calcium, immunoinducing agents and arginine availability (28). Constitutively expressed NOSs (eNOS and nNOS) are calcium/calmodulin dependent and thus sensitive to alterations in intracellular calcium. Constitutively expressed NOSs transiently produce NO for signal transduction processes, however, persistent receptor – ligand stimulation can lead to continual elevations of intracellular calcium and subsequent generation of NO, as might occur with glutamate neurotoxicity. Immunoinduced NOS (iNOS) is transcriptionally induced following cellular exposure to a variety of inducing agents: inflammatory cytokines (gamma interferon, tumor necrosis factor alpha, interleukin 6), lipopolysaccharides, beta-amyloid peptides, S100B and HIV coat proteins (29). iNOS is tightly bound by calcium / calmodulin such that it is calcium insensitive. The availability of intracellular arginine modulates NO production: sufficient arginine, either through available cellular pools or via increased arginine transport via cationic amino acid transporters, will ensure NO synthesis; however, insufficient arginine results in NOS generation of superoxide (30).

The biological fate of NO is influenced by a variety of factors: its local concentration, physiological milieu (redox environment, pO_2, pH, CO_2 concentration) and local concentrations of other bioreactants (metalloproteins, thiols, reactive oxygen species) (3, 31, 32). NO is chemically reactive towards O_2 or $O_2^{\bullet-}$, depending on the local concentrations of NO and oxygen tension, and on NO scavenging agents (e.g., glutathione or other thiols) or $O_2^{\bullet-}$ scavenging agents (e.g., superoxide dismutase (SOD)) (33). Oxidation, reduction or adduction of NO produces into a variety of nitrogen oxide species (e.g., NO^-, NO^+, $ONOO^-$, $ONOOCO_2^-$, NO_2, NO_2^-, NO_2^+, and N_2O_3) (Figure 1), collectively referred to as reactive nitrogen oxide species (RNOS) (19).

4. NEUROPROTECTIVE / NEUROTOXIC MECHANISMS OF NITRIC OXIDE AND REACTIVE NITROGEN OXIDE SPECIES

The issue of whether NO is a neuroprotective or neurotoxic in neurodegenerative diseases is the subject of much scientific discussion. Lipton *et al* proposed a NO redox based mechanism to explain NO's neuroprotective and neurotoxic effects (34). There are those who maintain that NO is a very modest effector of toxicity (3, 20, 31, 32), and may serve as a neuroprotectant under the right conditions (low local concentrations of NO and oxygen tension; presence of NO scavenging agents, such as glutathione or thiol compounds; superoxide scavenging agents, e.g. superoxide dismutase). The oxidant/ antioxidant balance of the central nervous system is also a

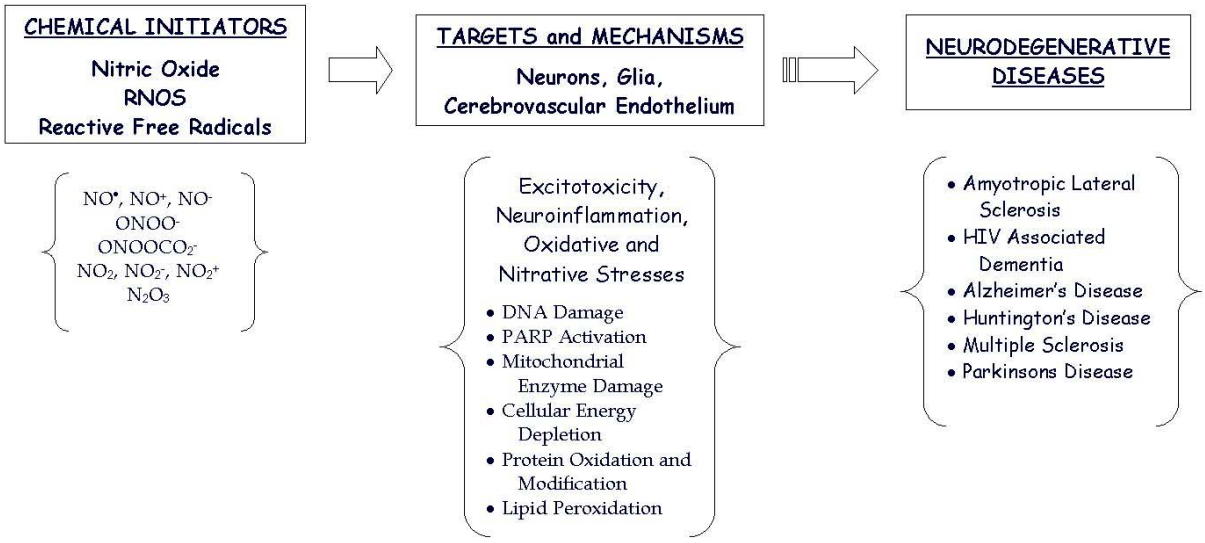

Figure 2. Mechanisms and Targets of Nitric Oxide – Mediated Neurotoxicity and Neurodegeneration. NO reacts with $O_2^{\bullet-}$ or O_2 to form RNOS and highly reactive free radical derivatives. These potent oxidizing compounds attack critical cellular targets in neurons, glia and cerebrovascular endothelium. Depending on the brain region and extent of toxic injury, these insults may initiate or promote the listed neurodegenerative diseases.

contributing factor in determining whether NO behaves as a neurotoxicant or neuroprotectant (35).

The protective effects of NO typically occur through inactivation of oxidants and reactive oxygen species known to evoke oxidant stress. The deleterious effects of NO result directly from its excitotoxicity; or through the generation of reactive nitrogen oxygen species (RNOS), resulting in neuroinflammatory, oxidative and nitrative stresses (19).

The sources and targets of NO are the cells on the CNS themselves: neurons, astrocytes, activated microglia and endothelial cells. Given the diffusivity of NO, neighboring cells are susceptible to RNOS toxicity. Neurons (25, 36), oligodendrocytes (37), choroid epithelium of the blood-cerebrospinal fluid barrier (38) and endothelial cells of the blood-brain barrier (39-41) are susceptible to RNOS toxicity. Neurons and oligodendrocytes, in contrast to astrocytes, are exquisitely vulnerable to RNOS toxicity owing to an inability to sustain a high level of glycolysis and lower reserves of intracellular antioxidants, such as glutathione and alpha-tocopherol (vitamin E) (42). Although nNOS neurons compose < 5% of the neuronal population (43), they are remarkably resistant to RNOS toxicity due to their high expression and activity of manganese SOD (44).

4.1. Mechanisms of Neuroprotection

Under certain environmental circumstances, NO can protect cells against oxidative stress. NO can scavenge oxidants such as hydroxyl (OH), alkoxyl ($RO^{\bullet}$) and alkyl hydroperoxyl ($ROO^{\bullet}$) radicals, thereby reducing oxidative stress mediated by these radicals (45) (1, 20, 46). NO can also limit lipid peroxidation through chain termination of the lipid free radical reaction (47, 48). The antiapopotic effects of NO occur through multiple mechanisms, including cellular elevations of cGMP, which interrupts the signal transduction process promoting apoptosis and/or through direct inhibition of caspase activity (47).

4.2. Mechanisms of Neurotoxicity

Neuronal death can occur through any number of potential mechanisms (19): derangements in intracellular calcium homeostasis and cellular energetics, oxidative and nitrative stresses, excitotoxicity, neuroinflammation and apoptosis. NO has major or minor roles in each of these mechanisms. The deleterious effects of NO are manifest as cytotoxic insults that contribute to the underlying pathology of various disease states (Figure 2) (49). Perhaps the major neurotoxic mechanism of NO is through the oxidative and nitrative effects of RNOS (16), although NO excitotoxicity subsequent to over activation of glutamate receptors is important as well (22).

4.2.1. Chemical Molecular Mechanisms

NO toxicity can be mediated via oxidative and nitrative stresses. The initial reaction of NO with superoxide ($O_2^{\bullet-}$) forms peroxynitrite ($ONOO^-$). Similarly, NO can react with O_2 to produce NO_2, which then reacts with NO to produce N_2O_3. While $ONOO^-$ is considered as the primary and most injurious effector of nitric oxide toxicity, NO_2 and N_2O_3 may also contribute substantial biological toxicity (1, 20). Targets of $ONOO^-$ and RNOS toxicity include amines, thiols, tyrosine and tryptophan residues, nucleic acids, iron-sulfur centers and metalloproteins.

$ONOO^-$ can effect toxicity through chemical reactions leading to the generation of other damaging species. At physiological pH, $ONOO^-$ also exists in equilibrium as its conjugate acid, ONOOH. Depending on

the cellular environment, ONOOH may decompose to cytotoxic species, i.e., hydroxyl radical (HO^-) and nitrogen dioxide (NO_2), or to the relatively inert nitrites/nitrates (NO_2^-, NO_3^-) via nitric acid (e.g. ONOOH $\rightarrow$ HNO_3) (1-3, 50). In addition, $ONOO^-$ rapidly and readily reacts with CO_2 to form $ONOOCO_2^-$, a far more potent nitrating specie than $ONOO^-$ (31).

Nitroxyl anion (NO^-) is a one-electron reduction product of NO, and is postulated to be physiologically synthesized by NOS, SOD and/or S-nitrosothiols (51-54). NO^- also reacts rapidly with NO to form reactive $N_2O_2^{\bullet-}$ or with O_2 to form $ONOO^-$ (2). NO^-, in the presence of hydrogen peroxide or transition metals, will react to form potent cytotoxic oxidants (55).

4.2.3. Cell Biological Mechanisms

Typically, high and sustained concentrations of NO and RNOS will indiscriminately attack critical macromolecules (lipids, proteins, DNA), subcellular targets (mitochondria) and cellular targets (neurons, glia, cerebrovascular endothelium) via the NO/ $O_2^{\bullet-}$ and NO/O_2 reaction pathways (20, 56). RNOS are capable of damaging macromolecules thereby creating cellular distress. When the number of damaging insults sustained by critical targets reaches an upper limit, cell death ensues via apoptotic or necrotic mechanisms (47, 57).

There are three mechanisms of DNA damage by RNOS (20): (1) direct interaction with DNA, resulting in mutagenic insults and DNA strand breakage; (2) inhibition of enzymes involved in DNA repair processes; and (3) chemical reaction with endogenous molecules to form DNA alkylating carcinogens, such as nitrosoamines. RNOS mediated nitrosative deamination of cytosine, adenine and guanine results in mutagenesis and DNA strand breaks (20, 58). This event sets in motion events that lead to apoptosis (47). RNOS inhibition of DNA ligase precludes the repair of RNOS – mediated DNA strand breaks (59-61). Lastly, RNOS – mediated DNA damage activates the DNA repair enzyme, poly(ADP-ribose) polymerase (PARP), which, ironically, undergoes RNOS nitrosylation. In the nitrosylated state, PARP activity is enhanced, which leads to excessive consumption and depletion of cellular energy reserves in compromised neurons (22, 62).

Another important mechanism of neurodegeneration is disruption of cellular energetics through mitochondrial dysfunction (63). RNOS can reversibly and irreversibly depress mitochondrial respiration by reversible inhibition of cytochrome c oxidase (complex IV) through low concentrations of NO (64); and by irreversible inhibition of mitochondrial complexes I-IV (e.g. cis-aconitase, NADH:ubiquinone oxidoreductase and succinate:ubiquinone oxidoreductase) through RNOS nitrosylation of the iron-sulfate centers (42, 65). RNOS inhibition of key respiratory enzymes promotes an intracellular mobilization and loss of iron. In addition, glycolysis is inhibited through RNOS nitrosylation of thiol residues on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (64, 66). The net effect of RNOS inhibition of mitochondrial complexes and impairment of GAPDH activity is the crippling of cellular ATP synthesis.

RNOS can nitrosylate and nitrate key amino acids in proteins, thereby altering protein function through alteration of active sites, conformation changes or blockade of phosphorylation sites. Nitrated proteins are typically targeted for degradation, which suggests a physiological mechanism for regulation of protein turnover. RNOS tyrosine nitration typically diminishes protein function, as observed with SOD and glutamine synthetase (56). RNOS - mediated S-nitrosylation of thiol groups may alter protein structure / function or promote oxidation of vicinal thiols or histidine residues (32, 67, 68). For example, the activities of G-proteins, ion channels and kinases are altered by S-nitrosylation (64, 67). RNOS also influence gene regulation through nitrosylation of cysteine residues of transcriptional regulators (24, 64), as exemplified by NO – mediated functional alterations of the transcription factors NF-kappaB and AP-1.

Lastly, while NO itself acts to quench lipid alkoxyl ($LO^{\bullet}$) or lipid hydroperoxyl ($LOO^{\bullet}$) radicals, RNOS can initiate and propagate lipid peroxidation (1, 48, 56). The reaction mechanisms are unclear, but may include a direct oxidant effect on fatty acids and/or oxidation of endogenous antioxidants, such as alpha-tocopherol (48).

4.2.3. Excitotoxicity

The N-methyl-D-aspartate (NMDA) glutamate receptor subtype is a ligand-gated ion channel that fluxes calcium upon ligand binding by the co-neurotransmitters glutamate and glycine. Under conditions of persistent synaptic glutamate, the subsequent chronic influx of calcium via the NMDA receptor complex promotes excitotoxicity through calcium-calmodulin interaction with nNOS, thereby triggering NO synthesis. NMDA receptor activation in non-nNOS neurons leads to mitochondrial synthesis of $O_2^{\bullet-}$. The products NO and $O_2^{\bullet-}$ diffuse from their sources to form $ONOO^-$ which is cytoxic to neighboring neurons (36, 69). nNOS neurons are uniquely resistant to NMDA toxicity due to over-expression of MnSOD (44). This hypothesis of NO-mediated NMDA excitotoxicity is not universally accepted, as the phenomenon has been inconsistently replicated by various laboratory groups using *in vitro* cell cultures (70).

S-nitrosylation of a redox modulatory site of the NMDA receptor complex (thought to be Cys-399 of the NR2A subunit) causes a down-regulation of the frequency of NMDA receptor channel opening (71, 72). Thus, NMDA receptor S-nitrosylation represents a feedback mechanism to provide neuroprotection through curtailment of excessive receptor activation under conditions of high NO levels. However, not all laboratory groups are convinced that NO acts exclusively at the redox modulatory site, as other evidence suggests that NO may act at multiple sites of the NMDA receptor complex (70).

4.2.4. Neuroinflammation

Neuroinflammation is an important process that promotes and contributes to neurodegeneration. Activated

glia (reactive astrocytes and microglia) and cerebrovascular endothelial cells are stimulated by and participate in neuroinflammatory processes. Activated astrocytes and microglia in regions of neurodegeneration were identified in Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, Huntington's Disease, HIV-dementia, and Amyotrophic Lateral Sclerosis (73-77). Microglial activation appears to be an early pathological event in neurodegenerative diseases, as suggested by observations from autopsy specimens from patients with early stage disease and *in vivo* imaging in patients with progressive disease (77). Neuroinflammatory activation of cerebrovascular endothelial cells initiates and contributes to blood-brain barrier disruption (see section 4.2.5.), permitting inward migration of activated T-cells and macromolecules normally excluded from brain parenchyma.

Neuroinflammation is typically marked by up regulated expression of major histocompatibility complex (MHC) molecules, elevations in proinflammatory cytokines (e.g. IL-1alpha, IL-1beta and TNF-alpha) and chemokines (e.g., MIP-1alpha, IL-5, and IL-8) (77), leukocyte - endothelial adhesion molecules and reactive oxygen intermediates (38, 39, 78). Elevated cytokines, in turn, promote the transcription of other a variety of inflammatory elements, including iNOS and cyclooxygenase-2 (COX-2; which is responsible for prostaglandin synthesis). In general, elevated concentrations of prostaglandins and nitrites/nitrates (end-products of NO metabolism) concurrent with increased COX-2 and iNOS expression are observed in experimental meningitis (79), Parkinson's Disease (80), Alzheimer's Disease (81), HIV-Associated Dementia (82, 83) and Amyotrophic Lateral Sclerosis (84).

COX-2 and iNOS are part of a family of primary inflammatory response genes, whereby COX-2 and iNOS expression are coordinately co-induced by lipopolysaccharides (LPS), bacterial endotoxins and various cytokines. Published reports suggest that NO and prostaglandins can modulate the activity of their own respective enzymes, and modulate each other's enzymatic counterpart. Pharmacological inhibition of one enzyme may also alter the activity and/or expression of the other enzyme. Based in an *in vivo* animal model of neuroinflammation (experimental meningitis) an empirical, biphasic, bell-shaped relationship between cerebrospinal fluid levels of prostaglandin E_2 (PGE_2) and NO was observed and mathematically validated, illustrating the complex behavior of the neuroinflammatory response (79).

Neuroinflammatory processes may activate excitotoxic mechanisms (19). Activated microglia release glutamate thereby initiating excitotoxicity (85). Moreover, prostaglandins trigger astrocytic release of glutamate (86), and the subsequent neuroinflammatory processes impair astrocytic glutamate reuptake.

4.2.5. Blood-Brain Barrier and Blood-Cerebrospinal Fluid Barrier Toxicity

The integrity of the blood-brain barrier and/or blood-cerebrospinal fluid barrier is often compromised during central nervous system infection or inflammation (19, 78). Barrier disruption leads to increased vascular permeability with subsequent influx of macromolecules, exudation of fluid and plasma proteins into cerebrospinal fluid (CSF) and brain tissue, and leukocyte recruitment and migration into the area of inflammation.

Altered barrier integrity can contribute to neurotoxic and neurodegenerative processes. For example, barrier disruption is often seen in multiple sclerosis (87, 88), HIV-1 dementia (89), cerebral ischemia (90), brain tumors (91) and meningitis (92). Given that excessive NO is an observed pathological process in these disease states (discussed in detail in following sections), it is tempting to hypothesize that NO is involved in evoking permeability changes of the blood-brain and blood-cerebrospinal fluid barriers.

The blood-brain barrier consists of astrocytic processes enveloping cerebral endothelial capillaries. Cerebrovascular endothelial cell production of physiological levels of NO is thought to maintain the integrity of the blood-brain barrier (93), whereas conversely, excessive NO levels disrupt the blood-brain barrier. Support for the hypothesis that excessive production of NO mediates blood-brain barrier disruption is derived from *in vitro* studies identifying iNOS induction in human astrocytes (94, 95), fibroblasts (96) and endothelial cells (97, 98). Preclinical evidence identified a role of NO in modulation of barrier integrity. In rats, intracisternal administration of lipopolysaccharides resulted in blood-brain and blood-cerebrospinal fluid barrier disruption, meningeal inflammation, and NO and prostaglandin E2 synthesis (38, 39, 79, 99-101). Treatment with a specific iNOS inhibitor, aminoguanidine, during meningeal inflammation significantly diminished meningeal NO production and preserved normal blood-brain and blood-cerebrospinal fluid barrier integrity (38, 39). Lastly, NO donor agents that released NO (as NO, NO- and NO+ redox forms) enhanced the blood-brain barrier permeability in normal rodents (41, 102). While NO itself provoked only modest elevations in permeability, the greatest enhancement of barrier opening occurred with NO donors that released the NO- and NO+ redox forms (41).

Clinical evidence supporting the deleterious effects of NO on blood-brain barrier integrity was provided by a study by Giovannoi *et al*, who observed a correlation of increased cerebrospinal fluid nitrite and nitrate concentrations (metabolites of NO) with increased cerebrospinal fluid albumin concentrations (103). Similarly, increased cerebrospinal fluid albumin ratios are a feature of HIV dementia (104), as is increased nitrotyrosine immunoreactivity in cerebrovascular capillary regions (105). Although these findings are observational and not proof of a NO cause and blood-brain barrier integrity effect, a compromised blood-brain barrier may contribute to disease pathogenesis.

5. NITRIC OXIDE IN NEURODEGENERATIVE DISEASES

Excitotoxicity, neuroinflammation and oxidative and nitrative stresses and are thought to contribute to the

neurodegenerative processes (Figure 2) in AIDS dementia, Parkinson's disease, Alzheimer's dementia, Huntington's disease, Multiple Sclerosis and Amyotrophic Lateral Sclerosis (ALS or "Lou Gehrig's Disease"). The disease clinical courses are likely due to initiating factors (environmental, genetic, and malfunctional biochemical processes) with a complex interplay of diverse secondary pathological mechanisms. It is difficult to determine if excitotoxicity, neuroinflammation and oxidative and nitrative stresses are primary causes, secondary effects or merely associated with disease progression. The general consensus is that NO neurotoxicity is a midstream pathological event, even though nitrated nervous system proteins are early markers of NO neurotoxicity in Alzheimer's, multiple sclerosis and ALS (16).

5.1. Acquired Immune Deficiency Syndrome (AIDS) Dementia Complex

AIDS dementia complex, aka HIV-Associated Dementia (HAD), is a neurological complication of human immunodeficiency virus type 1 (HIV-1) infection that affects about 1 in 5 patients in the late stages the disease. Neuronal loss is a prominent feature observed in autopsy specimens from AIDS patients (106).

Neurodegeneration occurs subsequent to release of viral proteins (tat, nef, vpr, rev, gp120 and gp41) (105-107), neurotoxins (excitatory amino acids, free radicals) and cytokines from reactive glia (105, 106, 108). A variety of mechanisms promote neurodestruction, including a contribution by NO and RNOS. HIV-1 viral proteins, notably gp 41and gp 120, trigger NO toxicity through microglial and astroglial induction of iNOS (108-110). Glial – derived glutamate triggers excitotoxicity via the NMDA receptor. Cytokine release triggers an inflammatory response, which includes induction of iNOS. NO and RNOS generated by reactive glia may contribute to the pathogenesis of AIDS dementia (111-115). Post-mortem examination of patients with AIDS dementia revealed nitrotyrosine immunohistochemical staining in perivascular regions, suggesting that NO and RNOS may contribute to blood-brain barrier dysfunction (105). Several reports observed iNOS immunoreactivity and increased nitrotyrosinated proteins were colocalized with gp41 (111-114). Moreover, iNOS mRNA levels in frontal white matter correlated with the severity of AIDS dementia complex (111, 116).

5.2. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a chronic and progressive paralytic disorder characterized by neurodegeneration of the large motor neurons in the brain and spinal cord. The disease prevalence is approximately 1 out of 100,000 people, typically striking people over the age of 50. The clinical presentation ranges from initial muscle weakness, tripping, abnormal fatigue of the limbs, slurred speech and muscle cramps and/or twitching. Disease progression involves muscle weakening and paralysis that eventually encompasses difficulty with speech, chewing, swallowing and breathing, followed by permanent ventilatory support in the late stages of the disease. ALS typically leads to an early death.

In the early 1990's, Beckman hypothesized that SOD-1 mutations found in 20% of patients with familial ALS may contribute to increased protein nitration and impaired phosphorylation due to ineffective scavenging of $O_2^{\bullet-}$ and subsequent formation of RNOS (117, 118). In support of this hypothesis, iNOS and nitrotyrosinated proteins are found in ALS patients and transgenic ALS mice (119-122). Moreover, patients with sporadic ALS have elevated CSF NO_2^- and NO_3^- (123, 124). However, the SOD mutation hypothesis remains controversial, with current hypotheses favoring reduced antioxidant defenses, impaired proteasome function, increased protein aggregation and NO neurotoxicity (16, 125).

5.3. Alzheimer's Disease

Alzheimer's disease is a progressive degenerative dementia, characterized by a gradual decline of memory, cognition (reasoning, judgment, disorientation, learning difficulties), loss of language skills, and decline in the ability to perform routine tasks. Sometimes agitation, anxiety, depression, delusions and/or hallucinations may also be clinically present. Approximately 5% of the elderly population over 70 years are afflicted with Alzheimer's, with that number doubling for each decade of life past 70.

The etiology of Alzheimer's is likely to be heterogeneous, multifaceted, complex and intricate. While several hypotheses on the etiology of Alzheimer's are advanced (disorders of beta-amyloid and/or tau protein processessing, neuroinflammation, genetic mutations, cardiovascular risk factors) (126, 127), all are the subject of much debate, and each likely offers a partial understanding of the disease etiology. Though far from perfect, the beta-amyloid hypothesis is currently favored (128).

Both epidemiological and basic research have established that inflammation and oxidative stress are major contributing mechanisms in the progression of Alzheimer's Disease. That NO and RNOS are contributory factors in the Alzheimer's disease process is supported by post mortem observations of increased nitrotyrosinated proteins and lipids in Alzheimer's patients (129-131). Beta-amyloid elicits NO - mediated excitotoxicity, neuroinflammation and oxidative stress (18). Beta-amyloid is directly neurotoxic in neuronal cell cultures (132, 133) and triggers excitotoxicity through elevations in intracellular calcium and subsequent activation of nNOS. Growing evidence suggests that beta-amyloid is critically involved in the induction of an inflammatory response through activation of astrocytes and microglia, eliciting subsequent production of inflammatory cytokines and neurotoxic factors, including NO (via iNOS), superoxide, prostaglandins (via COX-2) and glutamate. (For a detailed review, see (18).) Apolipoprotein E, thought to be a risk factor for Alzheimer's, elicits NO production from microglia, with isoform apoE4 as the most potent stimulator (134). Additional evidence supporting a neuroinflammatory process derives from immunohistochemistry findings of up regulated expression of cytokines (IL-1, TNF-alpha), iNOS and nNOS and Alzheimer's patients with Lewy body pathology (135).

5.4. Huntington's Disease

Huntington's disease (HD) is clinically characterized by a progressive deterioration in cognition, memory, mood and behavior, often accompanied by

choreic movements in late stage disease. Huntington's is a fatal autosomal hereditary neurodegenerative disease caused by a trinucleotide repeat disorder, involving an unusual expansion of CAG repeats coding for a polyglutamine tract of the huntingtin protein (136). Mutant huntingtin triggers interactions with multiple proteins to cause disruption of normal processes. Two mechanisms currently thought to be operative involve a role of mutant huntingtin in the disruption of normal transcriptional mechanisms and catabolism of misfolded proteins via the ubiquitin-proteasome system (137, 138). Mutant huntingtin also catalyzes downstream neurotoxic effects attributable to derangements in neuronal energetics and mitochondrial function. The resultant oxidative stress causes progressive neuronal degeneration in specific brain regions, e.g. the caudate nucleus and putamen in early disease, and the frontal cortex and other regions during disease progression (136).

The evidence for a role of NO in the neuropathology of Huntington's Disease is not nearly as convincing as it is for other diseases. NO is hypothesized to effect neurodegeneration in Huntington's by several hypothesized mechanisms. Glutamate excitotoxicity (via the NMDA receptor) and subsequent synthesis of NO (via nNOS) may occur due to excessive synaptosomal glutamate release / impaired glutamate reuptake secondary to oxidative stress. In support of this, animal models of Huntington's (transgenic mice and excitotoxic lesioned animals) suggest a participatory role of NO in neurotoxicity (136). Transcriptional dysregulation, secondary to altered mutant huntingtin – transcription protein binding, may alter nNOS expression and activity (136). Reduced nNOS activity may result in a loss of neuroprotection if NO serves as a neuroprotectant; alternatively, enhanced nNOS activity may promote neurodegeneration. In transgenic mice, age dependent alterations in nNOS activity (increased nNOS activity up to 19 weeks, followed by a progressive decline) suggest a neurotoxic role in the progression of Huntington's (139).

5.5. Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating neurological disorder with a relapsing and remitting clinical presentation. The disease affects approximately 1 out of 1,000, and tends to afflict women more commonly than men. Disease onset occurs usually between the ages of 20-40, but can occur at any age. The initial clinical presentation typically involves a range of symptoms, including muscle weakness, paralysis or tingling, fatigue, decreased coordination and loss of balance and visual disturbances. Because of the relapsing and remitting nature of the disease, patients may have a near normal lifespan with variable disabilites.

Data from multiple sclerosis patients and animal models of MS, i.e. experimental allergic encephalomyelitis (EAE) offer evidence for a contributing neurotoxic role of RNOS (140). iNOS and nitrotyrosinated proteins are prominent features found in the central nervous system lesions of patients and EAE animals (16, 32, 42, 141). MS patients with active disease also present with increased cerebrospinal fluid concentrations of NO metabolites, NO_2^- and NO_3^- (142-144). However, it is unresolved whether elevated cerebrospinal fluid NO metabolites correlate with the severity of active disease (145, 146).

5.6. Parkinson's Disease

Parkinson's disease is a chronic and progressive neurological disorder involving "shaking palsy" (paralysis agitans) with cardinal features of tremor, rigidity, bradykinesia and postural instability. The disease progresses to total disability, often accompanied by general deterioration of all brain functions, and may lead to an early death. The disease affects approximately 2 of every 1,000 people, usually afflicting people over the age of 50.

Nigrostriatal dopaminergic toxicity in idiopathic Parkinson's is thought to occur via oxidative stress, mitochondrial dysfunction and neuroinflammation mediated by NO, RNOS and hydroxyl radicals (147-150), secondary to environmental toxins (such as pesticides), altered iron metal homeostasis or genetic factors (mutations in alpha-synuclein). Excitotoxicity and altered catabolism of misfolded proteins via the ubiquitin-proteasome system (151) are additional mechanisms leading to neurodegeneration.

While some scientists reported a destructive role for NOS in the MPTP (1-methyl-4-pheny-1,2,3,6-tetrahydropyridine), animal model (152, 153), others suggest that NO is serves as an antioxidant and neuroprotectant through a scavenging action on toxic hydroxyl radicals (45). Evidence for a deleterious neuroinflammatory role of NO in Parkinson's is accumulating (150). Increased numbers of reactive glial cells expressing iNOS, COX-1 and COX-2 were observed in the substantia nigra of patients (76, 149, 150, 154). Oxidized and nitrated proteins were identified in Lewy bodies (protein inclusions), providing additional support for a RNOS contribution to the disease process (16, 155). In addition, elevated striatal tissue and cerebrospinal fluid levels of proinflammatory cytokines (150) and NO metabolites (NO_2^- and NO_3^-) (156) were detected in patient samples. The fact that nitric oxide displaces iron from ferritin supports hypotheses implicating deranged Fe(II) metabolism and Fe(II) – mediated oxidative damage in Parkinson's (151, 157).

6. PERSPECTIVE

During the "Decade of the Brain" in the 1990's, NO was named by Science as "Molecule of the Year" (158). While it came as no surprise when NO was first identified in the cerebellum (159-161), the breadth and depth of it's various roles continue to be absolutely amazing (162, 163).

The deleterious effects of NO and RNOS in *in vitro* and preclinical animal models of central nervous system diseases are well documented. The scientific literature provides a wealth of reports documenting the protective effects of pharmacologic modulation of NO meditated toxicities in *in vitro* and preclinical models. In

many instances, these preclinical pharmacology studies provide a "proof of concept" supporting a deleterious role of NO in various diseases states and an improvement in outcomes with a pharmacologic reduction of NO. In contrast, there are very few published clinical trials using pharmacologic agents that reduce NO levels. Concerns about drug side effects (such as severe hypertension), drug toxicities, poor pharmacokinetic characteristics (absorption, distribution across the blood-brain barrier, metabolism and elimination), and adverse effects on clinical outcomes are issues that have dogged clinical trials involving the systemic administration of non-selective NOS inhibitors (164).

There is considerable interest in developing effective pharmacologic agents that disrupt NO – mediated toxicities. Critical issues for the drug development of NOS inhibitors involve isoform selectivity and potency, as well as specificity for NOS over other arginine metabolizing or transport proteins. Drug discovery efforts have been focused not only on the development of NOS inhibitors but on other approaches, such as NO scavengers, NOS dimerization inhibitors, NOS cofactor antagonists and NOS transcriptional inhibitors (164). The stakes are high: Development of a successful NO modulating agent will have broad therapeutic indications for not just neurodegenerative diseases, but also for other systemic diseases where the deleterious effects of NO are evident, e.g. asthma, arthritis, diabetes, inflammatory bowel disease, inflammatory pain, other inflammatory conditions, septic shock and wound healing. However, just as intracellular calcium plays critical roles in homeostasis and disregulation, so too does NO. The question, though, is whether pharmacological modulation of NO will cause more harm than good, given the ubiquitous physiological roles of NO.

The composite perspective gleaned from the scientific literature offers a central, though not exclusive, role of NO in neurodegenerative diseases. While NO is one of several deleterious secondary mediators of neurodegeneration, its contributory effects should not and cannot be ignored. In the absence of a true cure for the neurodegenerative diseases discussed herein, the clinical management of these diseases will likely require the development of new therapeutic strategies that target not only NO but other neurotoxic mediators.

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