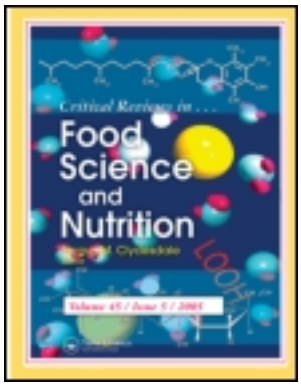

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L-Arginine and Nitric Oxide in CNS Function and Neurodegenerative Diseases

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L-Arginine and Nitric Oxide in CNS Function and Neurodegenerative Diseases

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One of the main functions of L-arginine (ARG) is the synthesis of nitric oxide (NO). NO is an important regulator of physiological processes in the central nervous system (CNS). NO promotes optimal cerebral blood flow, consolidates memory processes, facilitates long-term potentiation, maintains sleep-wake cycles, and assists in normal olfaction. However, at pathological levels, NO adversely affects brain function producing nitroxidative stress and promoting development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and other disorders of the CNS. This review summarizes current knowledge of the role of NO in the CNS and the role of diet in regulating the levels of NO.

Keywords L-arginine, nitric oxide, neurodegenerative disease, Alzheimer's disease, Parkinson's disease

INTRODUCTION

L-Arginine (ARG) is converted to nitric oxide (NO) by the nitric oxide synthase (NOS) family of enzymes. Both ARG and NO have vital functions throughout the body, including the central nervous system (CNS). Intensive ARG consumption as a nutritional supplement or eating specific arginine-rich foods may affect NO production and NO-dependent activities. Since its discovery as an endothelium-derived relaxing factor, NO has been shown to be a regulator of important physiological processes in addition to vasodilation, including effector functions in the cardiovascular, immune, and nervous systems. In the CNS, NO functions as a diffusible chemical messenger and its actions are associated with cognitive function, synaptic plasticity, pain perception, as well as the regulation of sleep-wake cycles, appetite, body temperature, and neurosecretion (Guix et al., 2005; Calabrese et al., 2007). However, under specific conditions, NO appears to be produced in excess and in such cases may play a causative role in the development of inflammatory neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and other disorders of the CNS. Since physiologic levels of NO appear to be neuroprotective whereas higher concentrations are decidedly neurotoxic, it is of particular importance to understand the potential dietary regulation of the levels of ARG and NO levels in the circulation and in the CNS.

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DIETARY ARG

ARG is a conditionally essential amino acid. It is widely accepted that healthy adults synthesize adequate amounts of ARG for basic biochemical functions, and variable ARG content in the diet may be of only variable consequence. However, under conditions of stress such as pregnancy, rapid growth, injury, and perhaps lesser appreciated conditions, supplemental ARG must be acquired from the diet in order to maintain sufficient circulating and cellular ARG for essential bodily functions. For children, ARG is usually considered essential to the diet in order to maintain normal growth.

ARG is enriched in animal and vegetable foods such as dairy products, beef, pork, poultry, seafoods, grains, nuts, seeds, and soy. Estimates from the Third National Health and Nutrition Examination Survey (NHANES III) are that mean ARG intake for the US adult population is 4.40 $\pm$ 0.05 g/d, although approximately 25% of people consume less than 2.6 g/d, a level considered inadequate, and a significant fraction of people consume more than 7.5 g/d (King et al., 2008). There is an association between low ARG intake and inflammatory markers associated with cardiovascular disease (Wells et al., 2005).

Approximately 10–12% of newborns exhibit ARG deficiency, a condition which, if left untreated, inhibits normal growth and development (Wu et al., 2009). Levels of plasma ARG are also reported to be insufficient in low birth weight and preterm infants and these developing individuals are susceptible to intestinal inflammation and necrotizing enterocolitis (Shah and Shah, 2007). Controlled studies have shown a benefit of ARG supplementation in these infants. ARG is used also as a dietary supplement in subgroups of healthy individuals (e.g., bodybuilders, in whom standard supplemental doses are 12–18 g/d) as well as patients with all forms of malnutrition (Cynober, 2007).

Several factors affect the bioavailability of dietary ARG in infants and adults. Such factors are the levels of ARG, lysine, manganese, and n-3 fatty acids in the diet and circulating hormones including cortisol, growth hormone, leptin, cytokines, endotoxins, as well as other biomolecules such as creatine, lactate, ornithine, and methylarginine (Wu et al., 2009). Citrulline is of particular importance as it is converted in the body to ARG. Pharmacokinetic studies indicate that citrulline, at oral doses of 5 to 10 g, is better absorbed and shows greater systemic bioavailability than ARG (Cynober, 2007). Increased dietary citrulline significantly increases plasma levels of citrulline, ARG, and NO without affecting urea output (Rouge et al., 2007). Consumption of foods rich in citrulline such as watermelon results in similar outcomes (Figueroa et al., 2010; 2011; Wu et al., 2007). Other citrulline-rich foods include meats, fish, eggs, milk, and legumes.

An essential question is whether the dietary changes in ARG affect activity of NOSs and NO production. The concentrations of ARG in extracellular fluids and within cells are considered to be in excess of the saturation points of the NOS enzymes (Pollock et al., 1991; Bruckdorfer, 2005). Serum ARG concentrations in normal humans are approximately 100 μM (Hong et al., 2010) and ARG is actively transported into cells, so that cellular concentrations may be even higher. These concentrations are far in excess of the Kms for ARG of purified eNOS and nNOS (1–2 μM) (Pollock et al., 1991; Furfine, 1994). Yet, in one study of an individual with a rare genetic disorder affecting dietary uptake and metabolism of ARG, plasma ARG concentration was reduced by 79% and serum levels of nitrogen oxides (NOx) as well as brachial artery vasodilatory responses were approximately 70% lower than controls (Loscalzo, 2001). Significantly, after intravenous ARG supplementation, NOx levels were increased and vasodilatory responses were restored to normal, suggesting physiologic regulation of cellular NOS activities by upper micromolar concentrations of serum ARG. These are complex physiologic systems, for instance, with variations in circulating levels of ADMA (asymmetric dimethylarginine), the naturally occurring competitive inhibitor of eNOS, and thus it is difficult to make definitive conclusions about the effects of serum ARG concentrations on NO levels. In addition, studies of purified NOSs have shown complex catalytic mechanisms dependent on electron transfers through FAD and FMN with tetrahydrobiopterin and NADPH acting as other electron donors, rendering the regulatory role of ARG concentrations in NO synthesis difficult to define (Daff, 2010).

Despite these caveats, studies support a link between dietary ARG and plasma levels of ARG and NO. In healthy human subjects instructed to drink juice supplemented with ARG (9g/day) for 1 week, mean serum ARG increased from 101 μM to 169 μM and mean total urinary nitrates plus nitrites as well as urinary cGMP increased 34% and 30%, respectively, although the latter two increases were not statistically significant (Evans et al., 2004). Lucotti et al (Lucotti et al., 2009) demonstrated that 6.4g daily ARG supplementation for six months to nondiabetic patients resulted in increased serum NO levels and ARG/dimethylarginine ratios along with improvement in insulin sensitivity and decreased inflammation as judged by serum levels of IL-6 and MCP-1. Similar results were observed in type 2 diabetes patients (Wascher et al., 1997; Lucotti et al., 2006). In animal studies, cholesterol-fed rabbits provided drinking water supplemented with 2.25% ARG for 12 weeks, showed an average rise in plasma ARG from 89 μM to 205 μM and a 43% increase in urinary nitrate, an index of NO levels (Bode-Boger et al., 1996).

DIETARY EFFECTS ON NITRIC OXIDE

NO can be generated both enzymatically from dietary ARG by NOSs as well as nonenzymatically through nitrite reduction (Figure 1). Nitrite from nitrate-foods including celery, beets, spinach, and other leafy types, as well as salivary-derived nitrite enters the bloodstream and accumulates in principal organs such as heart, kidney, and brain. Nitrite is thus a principal intravascular and tissue storage form of NO. Orally-ingested nitrite, at

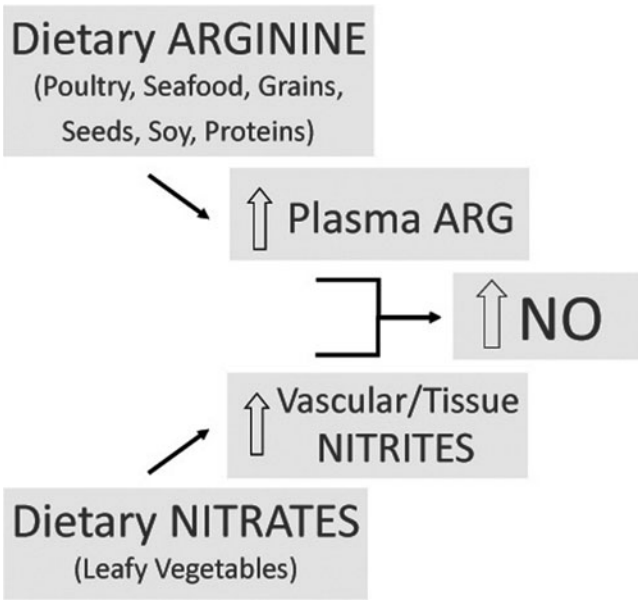

Figure 1 Effects of dietary arginine and dietary nitrates on nitric oxide production.

elevated doses, has been demonstrated to be a source of NO in vivo (Kanematsu et al., 2005). Nitrate from drinking water (regulated to be not more than 44 and 50 mg nitrate ion/liter in the USA and Europe, respectively) as well as nitrate from vegetable foods is converted in the body to NO plus other nitrogenous compounds (Gilchrist et al., 2010). NO formation by nitrite reduction is accelerated by food components including polyphenols. Under hypoxic states, nitrite is converted to NO throughout the body via NOS-dependent and NOS-independent reactions including reactions with cytochrome P450, carbonic anhydrase, xanthine oxidase, hemoglobin, myoglobin, and neuroglobin (Ngb) (Lundberg and Weitzberg, 2010). Ngb is preferentially expressed in neurons of the central and peripheral nervous system and it binds gaseous ligands including O2 and NO. Ngb promotes neuroprotection and resistance to neurodegenerative disease.

NOS ENZYMES

NO is produced from the five electron oxidation of the terminal guanidine-nitrogen of the amino acid ARG and the reaction is catalyzed by the NADPH-dependent enzyme, nitric oxide synthase (NOS). The reaction, as illustrated below, requires the co-factors flavin, heme, and tetrahydrobiopterin. After its formation, NO diffuses outside the cell.

L-ARGININE + NADPH + O2 → L-CITRULLINE + NO

Three isoforms of NOS have been identified: two constitutive enzymes, neuronal NOS (nNOS) endothelial NOS (eNOS), and one inducible enzyme (iNOS). A review on the structures and mechanisms of NO synthases has been published recently (Daff, 2010). Neuronal NOS, encoded by the human gene NOS1, is predominant in neurons but is also highly expressed in skeletal, cardiac, and smooth muscles. Hypoxic conditions promote expression of nNOS in aorta, mesenteric arterioles, pulmonary arteries, brain, and diaphragm (Ward et al., 2005). Expression of nNOS in the brain is especially high in the cortex, hippocampus, hypothalamus, olfactory bulb, claustrum, amygdala, and thalamus. It is also present in astrocytes and cerebral blood vessels. Endothelial NOS is expressed in endothelial cells (Zhou and Zhu, 2009) and the viscous drag of blood flowing over the endothelial layer is the most significant stimulus for NO formation through eNOS. eNOS is also found in the cerebral endothelial cells. Inducible NOS, originally cloned as the macrophage NOS, is induced in these cells by inflammatory stimuli including TNFα, lipopolysaccharide, and interferon-γ. It functions to kill or inhibit the growth of tumor cells, bacteria, fungi, and parasites. The levels of iNOS in the brain are low, but evidence of iNOS induction has been observed experimentally in astrocytic and microglial cells (Zweier et al., 2010; Luiking et al., 2010).

NO stimulates the soluble guanylyl cyclase (sGC) causing an increase in cGMP (Nakamura et al., 2007), resulting in smooth muscle relaxation and neurotransmission. Other regulatory proteins influenced include the Akt/PKB (protein kinase B), CREB (cAMP Response Element Binding Protein), and haemoproteins such as cyclooxygenase and haemoxygenase-1 (Edwards and Rickard, 2007).

NO activates sGC through its specific interaction with the heme center of the enzyme. cGMP is generated in response to natriuretic peptides and NO released from membrane bound GC and cytosolic sGC, respectively. NO activation by sGC causes initiation of cGMP cascades. NO diffuses either to the post-synaptic density or to the presynaptic terminal resulting in both anterograde and retrograde signaling. eNOS in the endothelial cells of the adjacent vessels is an additional source of NO. Studies conducted using PDE inhibitors and sGC stimulators have identified these NO release pathways (Menniti et al., 2006). The signals are transmitted through cGMP-dependent protein kinases (cGKs), cyclic nucleotide gated channels, and hyperpolarization-activated channels. While the presynaptic transmission is through Ca2+ signaling and cytoskeleton dynamics, the postsynaptic transmission is presumed to be due to disrupted transmission and changes in subunit composition of AMPA receptors. In the hippocampus and amygdala, retrograde signaling takes place via cGK11 and in the cerebellum, anterograde signaling is mediated by cGK1 (Kleppisch et al., 2009). Activation of cGKs result in the down regulation of cGMP (He et al., 2007).

EFFECTS ON CNS FUNCTION

Cerebral Blood Flow

Cerebral blood flow is an important factor in the regulation of brain functions. The regulation of the smooth muscle tone in vessels as well as blood flow is regulated by NO, which is produced by the adrenergic neurons and endothelial cells (Table 1). NOS inhibitors, NO scavengers, and sGC inhibitors reduce cerebral blood flow and promote vasoconstriction (Toda

Table 1 Effects of L-Arginine and nitric oxide on CNS functions

CNS Function	Effects of ARG and/or NO	Mediators	References
Cerebral blood flow	Vasodilation and increased blood flow	eNOS	(Toda et al., 2009; Toda et al., 2009)
Learning and memory	LT potentiation and memory processing	NO, peroxynitrite	(Rickard et al., 1998; Monfort et al., 2002; Edwards and Rickard 2005)
Sleep	Sleep promotion and REM sleep	ARG, iNOS, NO	(Chen et al., 2003; Gautier-Sauvigne et al., 2005)
Olfaction	Tonic NO production in olfactory bulb	ARG, nNOS, NO	(McQuade et al., 2010)

et al., 2009). NO influences the cerebral blood flow under conditions of hyperoxia, hypoxia, hypercapnia, and carbon monoxide inhalation (Iwanishi et al., 2009). Basal release of NO induces increased cerebral blood flow in various mammals and is mediated by eNOS. This role for NO has been supported by recent studies using NOS inhibitors and measuring the plasma asymmetric dimethylarginine (ADMA) (Kielstein et al., 2006) and dimethylarginine methylaminohydrolase (DDAH-1) levels (Toda et al., 2009).

Learning and Memory

NO is necessary for memory consolidation. Activities affected by NO include delayed recall in mature monkeys (Prendergast et al., 1997) and a distinct form of long-term memory in honeybees (Muller, 1996). Though these actions were originally thought to be mediated by NO-activated sGC, further research has brought into light new pathways of NO action, specifically those mediated by reactive nitrogen species, such as activation of ion channels and stimulation of mono-ADP-ribosyltransferase (Edwards and Rickard, 2007). The NO/GC pathway plays a memory processing role in long-term potentiation (Monfort et al., 2002). This contention has been supported by studies in which cGMP was increased or decreased and then memory retention was assessed (Monfort et al., 2002). The NO/GC pathway has been implicated in various memory-related activities like avoidance of learning (Izquierdo et al., 2000), memory formation (Allweis et al., 1984), temporary/transient retention (Edwards and Rickard, 2002), discrimination tasks like olfactory sensation (Kendrick et al., 1997), Y maze tasks (Yamada et al., 1996), and spatial working memory (Zhang et al., 2010 ; Diaz et al., 2010) Peroxynitrite is formed when NO reacts with superoxide. It has been shown that genetic disruption of this superoxide production results in poor spatial memory, emphasizing the role for peroxynitrite in memory processing (Edwards and Rickard, 2005). The activation of ion channels by NO occurs by both S-nitrolyation of cysteine residues and through activation of GC. This results in increased activity of large conductance potassium activated channels (Lang et al., 2003), cyclic nucleotide-gated channels (Broillet and Firestein, 1997), NMDA receptors (Manzoni and Bockaert, 1993) and p21RAS (Hart and Dulhunty, 2000) while inhibiting the actions of caspases (Li et al., 1997), glyceraldehyde 3-phosphate dehydrogenase (Molina y Vedia et al., 1992) and ryanodine receptors (Aghdasi et al., 1997). These activities implicate NO in the signal mechanisms of memory processing (Rickard et al., 1998; Edwards and Rickard, 2002).

Sleep

NO has been largely implicated in the sleep-wake cycle. Results from polygraphic readings, pharmacological and animal research, single reading, and voltammetric detection have further confirmed this hypothesis. Intracerebral inoculations of ARG have shown changes in the sleep-wake pattern of rats (Monti et al., 2004). The intraperitoneal administration of 7-nitroindazole, an nNOS inhibitor, caused a decrease in the duration of rapid eye movement (REM) and sleep-wave-sleep (Burlet et al., 1999). This supports a facilitative role of NO in promoting sleep. In a study involving transgenic mice, it was shown that the REM sleep was shorter in nNOS-knockout mice in contrast to the iNOS-knockouts where the REM sleep was longer, indicating opposing roles for nNOS and iNOS in REM sleep (Chen et al., 2003). NO is also found to regulate the REM sleep by modulating the release of acetylcholine (Leonard and Lydic, 1997), serotonin (Jouvet, 1972), gamma-amino butyric acid (GABA), and adenosine (Hars, 1999). The changes in sleep cycle in elderly animals have been shown to be mediated by NO, although the evidence is limited (Butterfield et al., 1999). In degenerative diseases like Parkinson's disease (Abdelgabar and Sharma, 2003; Garcia-Borreguero et al., 2003), Alzheimer's disease (Autret et al., 2001), epilepsy (Buisson et al., 1993), sleeping sickness (Buguet et al., 1989), and others, there is evidence of a relationship between sleep and NO production (Gautier-Sauvigne et al., 2005). The rhythmic changes that are present in the cortical electroencephalogram are from the cholinergic neurons in the basal part of the forebrain, suggestive of a possible role for NO (Marino et al., 2003).

Olfaction

More than twenty years ago, endogenous NO was found to be in the exhaled air of humans and other animals like rabbits and guinea pigs. Recently, NO production in the mouse olfactory bulb has been documented (McQuade et al., 2010). Olfactory NO is thought to have three main functions: protection, mucociliary clearance, and airborne messengership. Interestingly, a study on the role of nasal NO in the inflammatory process found an improvement in nasal polyposis (Delclaux et al., 2008). Nasal NO has been proposed to be used as a diagnostic tool and also for treatment of various respiratory diseases. Since NO levels are affected by many drugs and conditions (Landis, 2003), further research is needed to it document basal concentrations of the gas.

EFFECTS ON NEURODEGENERATIVE DISEASES

Alzheimer's Disease

The pathogenesis of Alzheimer's disease (AD) involves increased vascular oxidative stress and brain hypoperfusion (Markesbery and Carney, 1999; Edwards and Rickard, 2007; Nakamura et al., 2007). Lower levels of antioxidants and aberrant expression of enzymes, especially nNOS, are found in mild cognitive impairment and early AD disease (Luth et al., 2002; Malinski, 2007). Immunohistochemical analysis showed co-localization of nitrotyrosine with nNOS in cortical pyramidal cells and with eNOS and iNOS in astroglial cells

Table 2 Effects of L-Arginine and nitric oxide on neurodegenerative and on other CNS pathophysiological disorders

CNS disorder	Effects of ARG and/or NO	Mediators	References
	Neurodegenerative Diseases		
Alzheimer's disease	Peroxynitrates, citrullinated proteins	PAD enzymes	(Souza et al., 2000; Ali et al., 2005)
Parkinson's disease	Nitrative and oxidative stress	nNOS	(Murray et al., 2003; Pridgeon et al., 2007)
Multiple sclerosis	Elevated NO in MS patient CSF	iNOS, NO	(Calabrese et al., 2002)
	Other CNS pathophysiological disorders		
Migraine	NO and cortical spreading depression	sGC, NO	(Ashina et al., 2000; Gupta et al., 2007)
Seizures	Proconvulsant and anticonvulsant	ARG, iNOS, NO	(De Sarro et al., 1993; Przegalinski et al., 1996)
Subarachnoid SAH	Increased basilar artery diameter	ARG, iNOS	(Hirose et al., 1995)
Stress and behavior	Restrain sexual and aggressive behavior	nNOS	(Nelson et al., 1995)
Ischemia-inflammation	Reduces stroke lesion volume	NO donors, eNOS	(Willmot et al., 2005)

(Luth et al., 2002). These observations imply that there is an enzymatic imbalance in both these conditions resulting in increased amounts of oxidation and nitrosylation products found in the blood and cerebrospinal fluid of AD patients (Riviere et al., 1998; Markesbery and Carney, 1999; Pratico and Pratico, 2002). The mitochondria in the vascular wall cells appear to be the likely target in such cases. Brain biopsy samples from AD patients have a pattern similar to that seen in patients after long-term reperfusion of ischemic tissue, especially with regards to the mitochondrial structure, the extent of neuronal degeneration and the nuclear damage. There is also an increase in the lipid nitration product, 5-nitro-gamma-tocopherol, in the affected regions of the brain, suggesting an important role for NO in lipid oxidation in these pathologic states (Luth et al., 2001; Luth et al., 2002). Higher levels of enzymes involved in the NO metabolism, such as dimethylargininase and argininosuccinate synthetase, have been noted in AD patients along with the disruptions in tetrahydropterin (BH4) metabolism (Barford et al., 1984). Co-localization of dimethylargininase and argininosuccinate synthetase with iNOS is associated with increased production of NO (Heneka et al., 2001).

In postmortem analysis of brains from AD patients, specific morphological changes have been observed in the blood-brain barrier, along with deposition of amyloid deposits in the cortex, subcortex, and blood vessels (Stewart et al., 1992; Hachinski and Munoz, 1997). The deposition of Aβ-amyloid in the tunica media and collagen deposition in tunica adventitia of brain blood vessels causes narrowing of the vessels (Suo et al., 2000). This decreases cerebral perfusion causing mitochondrial dysfunction leading to increased production of reactive oxygen species (ROS) (de la Torre, 2000). The accumulation of oxidative products like peroxynitrite further damages the blood-brain barrier (de la Torre, 2000) (Table 2).

Direct evidence for an influence of dietary ARG on the pathogenesis of Alzheimer's disease is limited. Dietary patterns characterized by intakes of poultry, fish, nuts, and dark and green leafy vegetables are strongly associated with lower AD risk (Gu et al., 2010). Lean cuts of meat, including poultry, as well as fish and eggs, and especially peanuts and other nuts, all contain high amounts of ARG.

However, excess levels of ARG in the CNS may not be entirely beneficial. In one controlled study measuring cerebrospinal fluid (CSF) markers, it was observed that ARG was significantly elevated in the CSF of patients with AD compared to those with only mild cognitive impairment (Kaiser et al., 2010). Whether this is a cause or an effect is unclear. It has been strongly suggested that aberrations in metabolic pathways involving ARG are involved in the onset and progression of AD (Ishigami et al., 2005). Thus, investigation into these pathways may reveal therapeutic approaches. Indeed, peptidyl arginine deiminase (PAD) enzymes posttranslationally modify proteins, converting protein-arginines into protein citrullines (Knuckley et al., 2010). Increased activity levels of PAD have been associated with the neurodegenerative disease AD and multiple sclerosis, as well as other human diseases such as rheumatoid arthritis (Ishigami et al., 2005; Knuckley et al., 2010). PAD type 2 has also been shown to bind amyloid-type peptides and promotes their degradation with formation of insoluble fibrils (Mohlake and Whiteley, 2010).

Parkinson's Disease

Parkinson's disease (PD), the second most common neurodegenerative disease after AD, is caused by loss of dopaminergic neurons in the substantia nigra (Danielson et al., 2008). Postmortem samples from brain tissues have shown that nitrative and oxidative stress contributes to the neurodegeneration of the substantia nigra in PD patients (Jenner et al., 1992). Peroxynitrite, produced by peroxidase activity or from NO reacting with superoxide radical, is found to cause oxidative damage to proteins with 3-nitrotyrosine being an in vivo marker of peroxynitrite production (Souza et al., 2000). Inhibition of nNOS or iNOS in baboon has resulted in development of resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin causing permanent symptoms of PD, thus confirming the need for NO in MPTP-induced cell death (Hantraye et al., 1996). Similarly, use of 7-nitroindazole, the selective inhibitor of nNOS, or analysis with nNOS knockout mouse models prevented both the formation of 3-nitrotyrosine and the depletion of striatal dopamine in models of dopaminergic neurodegeneration (Ali et al., 2005). Furthermore, posttranslational protein modifications relevant to PD suggest the involvement of oxidative and nitrative stress

in the pathogenesis of the disease. Exposure to peroxynitrite results in the nitration and stabilization of alpha-synuclein filaments (through di-tyrosine formation), leading to the formation of alpha-synuclein aggregates (Souza et al., 2000). Nitrated alpha-synuclein has been detected in brain inclusions of PD patients and within the substantia nigra and ventral midbrain of mice treated with MPTP (Przedborski et al., 2001). The nitrated alpha-synuclein contributes to increased microglial activation, degeneration of dopaminergic neurons, and decreased number of T-cells. The nitrosative stress manifested by S-nitrosylation of proteins including parkin (Palacino et al., 2004), DJ-1 (Bonifati et al., 2003), PTEN-induced putative kinase 1 (PINK1) (Pridgeon et al., 2007), and tyrosine hydroxylase have been found in the brains of PD patients (Murray et al., 2003). The nitrosative stress leads to increased ROS production, decreased ATP production and increased cell death (Murray et al., 2003). Thus, the identification of ever increasing numbers of such proteins suggests that oxidative and nitrosative stress plays a crucial role in PD initiation and progression.

Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

The first reports implicating NO in the pathogenesis of experimental autoimmune encephalomyelitis (EAE) came in 1992 (MacMicking et al., 1992; Willenborg et al., 1999). Experiments on Lewis rats found a gender difference in NO production whereby an increased number of macrophage was found in the spleen of the female rat suggesting high levels of NO being released (Willenborg et al., 1999). NO inhibits T effector cell proliferation and clonal expansion and it also inhibits mobility and migration of other T-cells (Willenborg et al., 2007). In cuprizone toxicity (a condition where a copper chelator inhibits monoamine oxidase and causes liver and brain damage), NO causes non-immune mediated inflammation and demyelination (Willenborg et al., 1999; Willenborg et al., 2007).

Multiple sclerosis (MS) affects the gray matter of the brain. Glutamate has been shown to play a role in the pathogenesis of this demyelinating disorder (Newcombe et al., 2008; Pampliega et al., 2008; Westall, 2008). When specific glutamate receptor inhibitors were used, neurological symptoms were found to be decreased and damage to the blood-brain barrier was inhibited (Hawkins, 2009). Peroxynitrite formation is triggered by glutamate, causing serious damages in the brain. A study conducted using brain endothelial cell line, bEnd.3, confirmed the generation of peroxynitrite after glutamate exposure (Scott et al., 2007). In clinical studies, MS patients showed levels of NO in the cerebral spinal fluid which were significantly increased compared to healthy controls, and the CSF nitrite levels correlated with disease activity (Danilov et al., 2003). Also, the presence of biochemically active iNOS protein, suggested to be from activated astrocytes or microglia, was demonstrable in the CSF of MS patients in relapse but not in control subjects (Calabrese et al., 2002). Accordingly, the data support an involvement of NO in the pathogenesis of MS.

EFFECTS ON OTHER CNS PATHOPHYSIOLOGICAL DISORDERS

Migraine

Migraine, with or without aura, is a form of primary headache. NO has shown to be involved in the pathogenesis of migraine. It has been shown that NO is not only initiating but is also involved in the entire attack of migraine (Olesen et al., 1994). L-nitromono-methylarginine (L-NMMA), an NOS inhibitor, is effective in the treatment of acute episodes of migraine (Lassen et al., 1997). Nitroglycerin (GTN), a nitrate, produces a throbbing migraine-like headache and has been extensively studied to find the association between NO and migraine. In one study, intravenous infusion of GTN was given and it was shown to cause headache and that increasing the dosage resulted in increased frequency of migraine attacks (Iversen et al., 1989). The aura, the subjective feeling felt prior to the headache, has been found to be associated also with NO production and the phenomenon is called cortical spreading depression (CSD) (Olesen et al., 1990). The probable location of action of NO in CSD is nitroxidergic endothelium nerves in the neurovascular junction (Toda and Okamura, 1990), vascular endothelium (Fozard, 1995), or in the central nervous system (Tassorelli et al., 2004). The primary mechanism of action of NO in migraine induction is activation of sGC (Olesen et al., 1995). Other mechanisms have been suggested to include interactions with calcitonin gene-related peptide (Olesen et al., 2004), serotonin receptors (5-HT 1B/1D), and female hormones like estrogens (Gupta et al., 2007). This relation has many therapeutic potentials and can be used in prevention of migraine attacks. NOS inhibitors and scavenging methods are used for prevention of migraine attacks. Administration of NO scavengers like hydroxycobalamin (van der Kuy et al., 2002), superoxide (Ikeda, 2002), and 5-HT 1B/1D receptor antagonists like sumatriptan (Read and Parsons, 2000), decrease NO levels. NO has also shown to play an important role in the other types of primary headache like tension headache (Ashina et al., 2000) and cluster headache (May et al., 1998).

Seizure

A number of studies have investigated the role of NO in the pathophysiology of epilepsy, although the strongest evidence is still lacking. Confounding influences include factors such as animal species studied, mode of administration of drug, type of seizure, and inhibitors used. While, administration of nitro-L-arginine methyl ester (L-NAME) caused an increase in pentetrazol (noncompetitive GABA antagonist)-induced seizures in Sprague Dawley rats, it decreased or had no effect on such seizures in Wistar rats (Osonoe et al., 1994; Kirkby et al., 1996). ARG, the NO precursor, decreased convulsions caused by kainate (Penix et al., 1994) and increased those caused by NMDA (De Sarro et al., 1993). 7-Nitroindazole, the selective

iNOS inhibitor, has been used to study the effect of NO on different types of seizures. 7-Nitroindazole acts as proconvulsant in clonic seizures (Lallement et al., 1996) and decreases the severity of NMDA-induced convulsions (Przegalinski et al., 1996). Thus, NO-independent and NO-dependent pathways for the pro- and anti-convulsant actions have been postulated and are topics of active research interest.

Subarachnoid Hemorrhage

Subarachnoid hemorrhage (SAH) is a common medical condition among the elderly. The most common cause is the rupture of an intracranial berry aneurysm at the anterior communicating artery at the circle of Willis. SAH and the liberated hemoglobin released from the lysed erythrocytes decrease NO production (Kanamaru et al., 1987). Despite the decrease, the functionality of NO is preserved. SAH also causes induction of iNOS. In an experiment, the diameter of the basilar artery was found to be increased by the addition of ARG (Hirose et al., 1995). In animals, administration of aminoguanidine, an iNOS inhibitor, decreased the occurrence of induced aneurysm (Fukuda et al., 2000). The vasospasm is thought to be mediated by the imbalance between constricting and relaxing factors (Hanggi and Steiger, 2006). This action of NO in vasospasms is an important finding and adds to its therapeutic potentials.

Stress and Behavior

NO has been shown to have implication in stressful conditions. Physiological stress factors such as food deprivation (Ueta et al., 1995) and lactation (Ceccatelli and Eriksson, 1993) as well as psychological stress factors such as restraints (Calza et al., 1993) have been shown to cause an increase in NOS1 mRNA (encoding nNOS) in the paraventricular nucleus (PVN) and an increase in NOS2 mRNA (encoding iNOS) expression in the brain after the administration of injection of cytokines. NO has also been studied regarding its possible role in sexual and aggressive behaviors (Nelson et al., 1995). The NOS1 knockout mice have shown an increase in sexual and aggressive behavior (Hirose et al., 1995). Administration of NOS inhibitors resulted in decreased food intake implying a possible role for NO in feeding behavior (Morley, 1996). High amounts of cortical hormones found in depressed patients are associated with decreased NOS enzyme levels (Lopez-Figueroa et al., 1998).

Ischemia and Inflammation

NO production during ischemia and inflammation has been studied and researched for its therapeutic potential. Animals subjected experimentally to ischemic conditions showed increased expression of all three NOS isoforms. Immunoreactive nNOS and eNOS peaked within 24 hrs (Holtz et al., 2001), while iNOS peaked later, possibly causative in late injury (Murphy, 2000). Additional studies have shown that levels of iNOS are increased by pro-inflammatory conditions including infection but are decreased by anti-inflammatory drugs (Galea and Feinstein, 1999). Given this, it is significant that NOS inhibitors have been shown to decrease the infarct size in animals (Willmot et al., 2005). Protection against future invasion can be produced by exposure to sub-threshold injury, a phenomenon called preconditioning. NOS has been found to help in preconditioning (Nandagopal et al., 2001). This has been shown by administering NOS inhibitors that cause reversal of the phenomenon and NO donors which can induce it. This relationship should be further studied for its possible implication in prevention of stroke.

CONCLUDING REMARKS

Evidence is now clear that the NO system plays a major role in CNS health and disease. At physiologic levels, NO appears to be neuroprotective. NO affects optimal cerebral blood flow, plays a role in consolidating memory and in facilitating long-term potentiation, maintains sleep-wake cycles, and assists in normal olfaction. However, excessive NO production can result in nitroxidative stress. Nitroxidative stress is implicated in the pathogenesis of neurodegenerative disease such as AD, PD, and other CNS disorders. In AD, elevated levels of nitrosylation products are found in the cerebrospinal fluid and in PD excess nitrosylation of neuroprotective proteins have been observed. Thus, it may be significant that altering the consumption of ARG-containing and nitrate-rich foods can affect circulating ARG and thus potentially affect the levels of NO in the circulation and the CNS.

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