NITRIC OXIDE IN NEURODEGENERATION

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Abstract
Nitric oxide NO is an atypical neurotransmitter and is formed from L-arginine by the enzyme nitric oxide synthase (NOS), which is of three types, namely neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). nNOS and eNOS are constitutive, calcium-calmodulin dependent and release NO from neurons and endothelium, while iNOS is inducible, calcium independent and formed in activated immune cells. NO plays a modulatory role in brain, controlling release of neurotransmitters and is involved in synaptogenesis, synaptic plasticity, memory function and neuroendocrine secretion. Availability of animal models, use of NOS inhibitors and mutant mice lacking each NOS isoforms have provided evidence for the injurious effects of NO. NADPH-diaphorase staining is used to localise NOS containing neurons and 3-NT staining gives an idea of generation of NO. NO may also have some neuroprotective effects as NOS (NADPH-diaphorase positive) neurons are selectively resistant to NMDA mediated toxicity, ischaemic hypoxic insults and the degenerative processes of HD, AD, PD and ALS. Use of selective NOS inhibitors is beneficial in animal models, but has met with little or no success in the clinical treatment of neurodegenerative diseases per se.


Synthesis of Nitric Oxide
NO is formed from the guanidine nitrogen of L-arginine through an oxidative-reductive pathway that consumes five electrons and results in the formation of L-citrulline. The family of enzymes that catalyse NO synthesis are known as nitric oxide synthases (NOS). NOS requires three cosubstrates (L-arginine, NADPH and oxygen) and five cofactors or prosthetic groups (flavin adenine dinucleotide, flavin mononucleotide, calmodulin, tetrahydrobiopterin and heme) for the synthesis of NO. Purification and cloning of NOS has revealed the existence of at least three types of NOS: a neuronal NOS (nNOS) or type 1 NOS, an immunologic NOS (iNOS) or type 2 NOS and an endothelial NOS (eNOS) or type 3 NOS. Two of these (eNOS and nNOS) are constitutive, calcium-calmodulin dependent and release NO from endothelium and neurons while iNOS is inducible, calcium independent and formed in activated immune cells.

The release of NO by constitutive NOS (cNOS) is accelerated in response to stimulation of several membrane bound receptors by, for example glutamate, bradykinin, 5-HT, acetylcholine, histamine, endothelin 1, substance P and probably calcitonin gene related peptide. Increased flow velocity and subsequent increase in shear stress on endothelial cells may also stimulate cNOS. The eNOS isoforms are activated by small intracellular calcium transients. NOS is activated by glutamate neurotransmission acting on N-methyl-D-aspartate (NMDA) receptors. In a matter of seconds, the glutamate induced increase in intracellular calcium levels activates NOS via calmodulin. An increase in intracellular calcium concentration from 100 nM to 500 nM changes the rate of NO synthesis from less than 5% to more than 95% of the maximum rate, iNOS is not stimulated by calcium. It is not normally present in significant amounts but is expressed after stimulation by endotoxins and cytokines such as g-interferon and lipopolysaccharide which elicit new NOS production over 2 to 4 hours, and generates NO for prolonged
periods and in large amounts, which mediate destruction of microorganisms and tumours, and may also lead to pathological tissue damage.

NOS activity is present in many tissues of several species, including endothelium, brain, peripheral nerves, vascular smooth muscle, myocardium, macrophages, neutrophils and microglia. Apart from being expressed in neurons, nNOS has also been found to be constitutively present in some peripheral tissues and blood polymorphonuclear (PMN) leucocytes. nNOS is almost exclusively found in neurons, although there is some evidence of a calcium dependent NOS in astrocytes. There are at least three distinct physiologically active subclasses of nNOS (a, b and g), and the b subclass is functional in the striatum and the cortex, although the physiological difference between the different subclasses is unknown.

nNOS positive neurons stain for NADPH-diaphorase, and constitute only 1 to 2% of all cells in many areas, such as the cerebral cortex, the hippocampal formation, and corpus striatum. NOS positive neurons have extensive processes and ramify to influence the majority of neurons in culture and in intact animals. NO exerts its effects over a distance of 200 nm from its source, influencing an estimated 10^6 neurons. nNOS is located in microglia and in some neuronal populations in the brain, including basket and granule cells of the cerebellum, pedunculopontine nucleus, medium to large aspiny neurons within the striatum and cerebral cortex. Differences in NOS activity have been shown to be present in different regions of brain during aging. An increase in activity was shown in the cortex and hippocampus but not in the striatum of old animals.

Functions of Nitric Oxide

NO is an atypical messenger molecule in the brain that is involved in many central nervous system functions. Unlike conventional neurotransmitters, it is not stored in synaptic vesicles and released by exocytosis on membrane depolarisation; instead it is synthesized enzymatically on demand by nNOS. It does not act on typical extracellular receptor proteins on synaptic membranes, but it diffuses from one neuron to another to act directly on intracellular components. Its “receptor” is the heme moiety of soluble guanylate cyclase as well as other potential targets. The activity of conventional neurotransmitters is terminated by reuptake mechanisms or enzymatic degradation, whereas the activity of NO terminates when it chemically reacts with a substrate because the neurons cannot sequester and regulate the local concentrations of NO, thus the key to regulating NO activity is control of NO synthesis. Under conditions of normal release, NO is a neuronal messenger molecule. When NO is produced in excessive amount, it switches from a physiological neuromodulator to a neurotoxic effector.

The pharmacological effects and mechanisms of action of NO were well known before the discovery of endogenous formation of NO. The physiological functions of NO are,

- NO functions as the major inhibitory neurotransmitter of the intestine.
- NO also seems to be the “neurotransmitter” involved in penile erection.
- Since NO is freely diffusible, it has been suggested to serve as a retrograde messenger in long term potentiation (LTP) in the cornu ammonis –1 (CA1) layer of the hippocampus.
- Long term depression (LTD) in the cerebellum, may also involve NO.
- Indirect evidence suggests that NO participates in neuroendocrine secretion.
- NO appears to be a major regulator of vasodilator nerves of cerebral arterial smooth muscle. NOS inhibitors block neurally induced vasodilatation in cerebral arteries as well as cerebral vascular tone. Oxyhaemoglobin, a potent inhibitor of NO, may be one of the major factors responsible for vasospasm following SAH.
- Furthermore, NO is a potent inhibitor of platelet aggregation and in the absence of NO, platelets would be expected to aggregate and release further vasoconstricoster substances that would contribute to cerebral vasospasm.

Mechanism of Neurotoxicity of Nitric Oxide

NO is popularly regarded as being highly reactive but chemists point out that this is not in fact the case. There is little evidence in the chemical literature that it reacts with more than a small range of compounds. It is not destructive in the same way as the hydroxyl radical, and the suggestion that its cytotoxicity is simply the consequence of its radical nature can not be correct. The major source of NO that kills neurons seems to derive from NOS containing neurons. Microglia can function as macrophages, and macrophages as well as astrocytes can synthesise NO.

NO may play several roles in processes that lead to neurodegeneration. However, the mechanisms by which NO kills cells, particularly neurons are not fully understood.

There is strong experimental evidence that NO reacts with non-heme iron, for example, iron-sulphur (Fe-S) clusters. In contrast to the reaction of NO with heme-iron, which is reversible, the reaction of NO with iron-sulphur clusters often result in destruction of the clusters. This may be an important part of NO toxicity as several key enzymes, such as mitochondrial aconitase and complex I and II of the mitochondrial electron transport chain, have a catalytically active non-heme iron-sulphur complex. NO can displace iron from its binding site on ferritin, an iron storage protein, and consequentially promote lipid peroxidation.

NO can also damage DNA through nucleotide base deamination. DNA damaged by NO activates the nuclear enzyme poly ADPribosyl synthetase (PARS) which may impair energy metabolism and energy dependent processes by decreasing intracellular levels of NAD and ADP. NO has been shown to deplete intracellular glutathione levels through the formation of intracellular S-nitrosoglutathione. The depletion of intracellular glutathione renders cells vulnerable to subsequent oxidative stress, which may occur through peroxynitrite production.

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The majority of evidence indicates that toxic effects of NO occur through a non-enzymatic reaction of NO with the superoxide anion to form peroxynitrite (ONOO⁻) which is believed to be an extremely reactive molecule with potent oxidant properties. The rate of the reaction is three times faster than the rate of reaction of the enzyme superoxide dismutase (SOD) in catalyzing the dismutation of the superoxide anion to hydrogen peroxide. The peroxynitrite anion decomposes at physiologic pH to form a species with the reactivity of hydroxyl radical. Hydroxyl radicals rapidly react with and have a strong affinity for almost every molecular species found in living cells. Such reactions include breakage of single and double stranded DNA, chemical alterations of deoxyribose purine and pyrimidine bases, membrane lipids, carbohydrates and proteins, leading to a cascade of events that damage the mitochondrial electron transport system, decompartmentalize intracellular lipid peroxidation, prompt apoptosis and result in cell death.

NO has also been implicated in the cytotoxicity observed after excess stimulation of neurons by glutamate. Activation of NMDA receptors and the subsequent increase in intracellular calcium levels initiate most forms of glutamate excitotoxicity. Increased intracellular calcium levels can initiate a number of deleterious processes, including activation of NOS. NO is generated by NOS and nNOS or type 1 NOS is calcium dependent making it prone to activation by excitotoxic mechanisms such as glutamate excess.

Both inflammation and oxidative stress play a critical role in neurodegeneration associated with acute and chronic insults of the nervous system. Affected neurons are often responsive to and dependent on trophic factors such as nerve growth factor (NGF). In vitro model suggests that cells bearing both the high and low affinity NGF receptors may display increased sensitivity to TNFalpha in terms of iNOS expression and therefore be selectively at risk during acute (e.g. neurotrauma) or chronic (e.g. neurodegenerative diseases) conditions where high levels of pro-inflammatory cytokines in the nervous system occur pathologically. A recent study also suggests that modulation of NFkappaB-promoted transcription of selective genes could serve as a potential therapeutic target to prevent neuroinflammation-induced neuronal damage.

Experimental Evidence for Involvement of Nitric Oxide in Neurodegeneration

The involvement of NO in neurodegeneration has been studied by the use of substances which block the synthesis of NO by inhibiting NOS and the availability of animal models for various diseases. Transgenic mouse models exist for the major neurodegenerative diseases, including AD, tauopathy and ALS. Although many of the mice do not completely replicate the human disease they are intended to model, they have provided insight into the mechanisms that underlie disease etiology. The use of NOS inhibitors and mutant mice lacking each NOS isoform have provided evidence for the injurious effect of NO derived from neuronal or inducible isoforms. The most widely studied inhibitors of NOS are the substrates analogs of L-arginine, such as nitro-L-arginine methyl ester, nitro-L-arginine and monomethyl-L-arginine. These inhibitors affect both the constitutively expressed and inducible enzymes and do not discriminate between the neuronal and endothelial forms. In the case of AD and ALS models, mice have also provided a therapeutic testing ground for the testing of agents that have been shown to have considerable clinical promise. The slow progression of these diseases perhaps occurring over fifty years or more, complicates experimental approaches to modeling their pathophysiological mechanism.

It has been shown that several mitochondrial toxins produce lesions in animals which resemble distinct neurodegenerative diseases. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is used to produce a model of PD in primates and mice. The neurotoxic effects of MPTP are thought to be initiated by 1-methyl-4-phenylpyridinium (MPP⁺) which is a metabolite formed by the monoamine oxidase B-mediated oxidation of MPTP. MPTP causes pathology by targeting the destruction of nigrostriatal dopaminergic neurons, the same cells that are selectively lost in idiopathic PD. Treatment of experimental animals, including the mice and primates, with MPTP replicates the selective toxicity and results in accumulation of 3-nitrotyrosine (3-NT) in the nigrostriatal pathway. Interestingly MPTP toxicity could not be induced in nNOS knocked out mice. 7-Nitroindazole (7-NI), a specific NOS inhibitor, also dose dependently inhibited the MPTP induced dopamine depletion and prevented the increase in peroxynitrite induced 3-NT formation in mice without having any effect on the MAOB activity and dopamine uptake. Similar observations were also made in the cortical neuronal cultures from mice, in which the gene for nNOS has been deleted. Neuropathological changes such as dopamine depletion and degeneration of tyrosine hydroxylase positive neurons in MPTP induced Parkinsonism in baboons were also prevented by 7-NI. In an in vitro rat striatum slice preparation, prevention of MPP⁺ induced dopamine depletion by 7-NI has been observed. Recent experiments have shown that MPTP treatment led to peroxynitrite mediated nitration of tyrosine hydroxylase, which impaired the neuronal dopamine synthesis, and represents an early biochemical event in the MPTP toxicity. Definitive evidence that NO and peroxynitrite mediate toxicity in the MPTP model of PD again derives from studies of transgenic mice. Both nNOS knockouts and mice that overexpress Cu/Zn SOD are resistant to MPTP toxicity.

Striatal injections of malonate cause secondary excitotoxic lesions, ATP depletions, and age dependent increases in lactate that parallel age dependent increases in lesion size. Lesions show sparing of NADPH-diaphorase neurons in the striatum which is similar to the histology of HD. Lesions produced by malonate were significantly attenuated by treatment with 7-NI and the protection by 7-NI was completely abolished after co-administration of a high dose of L-arginine.

3-Nitropropionic acid (3-NP) is a naturally occurring plant toxin and mycotoxin that is an irreversible inhibitor of complex II. It produces disease in livestock, and illness occurred after ingestion of mildewed sugarcane in China. The illness results in delayed onset dystonia and putaminal necrosis. Systemic administration
of 3-NP to rats and nonhuman primates produces selective striatal lesions, which share characteristic histological features with HD.

After systemic 3-NP administration in rats there is an increased production of the peroxynitrite by-product 3-NT, of 8-hydroxy-2-deoxyguanosine (a marker of oxidative DNA damage) and of dihydroxybenzoic acids (markers of hydroxyl production) in the striatum. Treatment with 7-NI showed marked protection against striatal lesion volume, and the production of 3-NT, 8-hydroxy-2-deoxyguanosine, and dihydroxybenzoic acids. Furthermore mice overexpressing SOD were protected against 3-NP toxicity and the increase of 3-NT. These results provide evidence that NO and peroxynitrite production occurs in vivo in established animal models of neurodegenerative diseases, and that the NO production is of neuronal origin.

Experimental studies in rats exposed to methyl mercury suggest that NO produced by NOS in the granular layer neurons may be the initiator of neuronal degeneration in cerebellar ataxia due to methyl mercury intoxication. Rats exposed to methylmercury showed elevation of levels of NOS, NO$_2$/NO$_3$, and glutamate prior to the start of cerebellar degeneration. This suggests that NO produced by NOS in the granular layer neurons may be the initiator of neuronal degeneration in methyl mercury intoxication.

Continuous subcutaneous administration of polyanine modified catalase that has increased permeability of the blood brain barrier showed both a highly significant delay in onset and an increase in survival in a transgenic mouse model of FALS. This therapeutic benefit is due to the scavenger action of polyanine modified catalase against H$_2$O$_2$ and HO.

The inflammatory response is thought to be important for secondary damage following traumatic brain injury (TBI). The inducible nitric oxide synthase (iNOS) isoform is a mediator in inflammatory reactions and may catalyze substantial synthesis of NO in the injured brain. Studies have demonstrated neuroprotection by selective inhibition of iNOS after trauma. Selective (iNOS) inhibitor L-N-iminoethyl-lysine (L-NIL) appeared to protect the injured brain by limiting peroxynitrite formation. These findings also support a putative harmful role of iNOS induction early after TBI.

The enhanced production of nitric oxide (NO) via inducible nitric oxide synthase (iNOS) has also been implicated in the pathogenesis of neuronal apoptosis after acute traumatic spinal cord injury (SCI). In a recent study about the pathways mediating the synthesis and release of NO the authors have found activation of extra cellular signal regulated kinase ½ (ERK ½) and P-38 mitogen activated protein kinases (P-38 MAPK) in microglia/macrophages in the injured area of adults rats subjected to a complete transection at the T10 vertebra level was observed. The results indicate the ERK1/2 and p38 MAPK signaling pathway, especially the latter, play an important role in NO-mediated degeneration of neuron in the spinal cord following SCI. Strategies directed to blocking the initiation of this cascade may prove to be beneficial for the treatment of acute SCI.

Evidence from Human Studies for Involvement of Nitric Oxide in Neurodegeneration

NO is a very labile free radical with a half life of only a few seconds, so direct determination of NO is difficult. It is rapidly oxidized by tissue oxygen to the stable end products nitrate and nitrite, and may also form complexes with haemoglobin. In plasma, NO is converted to nitrite and nitrate in a ratio of 5:1. In the circulation, nitrate is almost totally converted to nitrite by haemoglobin. The measurement of plasma nitrite levels is reported to be a valid measure of NO generation in fasted human beings. The CSF nitrate and nitrite levels are significantly lower than serum levels which indicates that they do not freely pass the blood-brain barrier. Thus, indirect evidence for the involvement of NO can be obtained by the estimation of its stable end products in the blood or CSF. Direct evidence for the involvement of NO in various neurodegenerative diseases has been obtained by examining postmortem human brain and spinal cord using immunohistochemical techniques and NADPH and 3-NT staining. NADPH-diaphorase immunohistochemistry is used to localize NOS containing neurons in the brain. Cell bodies in normal healthy human brains exhibit no staining but capillaries or neural processes show faint staining in the substantia nigra.

Attempts have been made to measure blood and plasma levels of nitrate and nitrite as indices of altered NO metabolism in patients with PD, however, the results of various reports are conflicting. Molina et al. found no significant change in the CSF and plasma nitrate levels of PD patients. However, Quereshi et al. reported a significant increase in the CSF nitrite content. On the other hand Kuiper et al. found a significant decrease in the CSF nitrate content. Furthermore, the nitrate CSF/plasma ratio, arginine and citrulline levels in the CSF were significantly attenuated in the PD patients suggesting an alternation in the synthesis of NO in the brain. NO release from the neutrophils following PMA treatment, was increased by 61% and 57% in the newly diagnosed and treated PD patients respectively, in comparison to age matched controls. These evidences suggest that neutrophils express a primary alteration of NO release in PD patients, whereas H$_2$O$_2$ and oxidative stress parameters were probably related to the evolution of PD or due to L-dopa treatment. Consistent with this observation, nNOS was overexpressed in the neutrophils of PD patients. An increase in the basal nitrite content specific to the neutrophils of both treated and untreated PD patients has also been reported. There was no change in the plasma and platelet nitrite levels. Hence, the observations seem to be specific to nNOS, which is also expressed in the neutrophils. The increase, however, did not correlate with age, sex, duration of illness and severity of disease.

Post-mortem studies have thrown some light on the role of NO in PD. A reactive glosis occurs in the substantia nigra that is accompanied by an increase in the number of NADPH diaphorase
positive glial cells in the mesencephalon. Shergill et al. used electron paramagnetic resonance spectroscopy to detect NO radicals (measured by heme-NO) in post mortem PD nigra, which they suggested was associated with this reactive gliosis. Immunocytochemical studies have shown that antibodies to 3-NT show that there is an increased staining of the central core but not the halo of Lewy bodies in substantia nigra pars compacta (SNPC) of PD brain. Initial studies had indicated raised levels of 3-NT formed by the NO attack on free tyrosine and tyrosine in proteins in PD, although the validity of the evidence was questioned by Kaur et al. In a recent study Shukla et al., found no correlation between the CSF nitrite and MDA levels in PD patients.

The results of various studies on human subjects have shown that CSF nitrite levels are either normal or increased in patients with MND. The increased CSF nitrite levels seen by Tohgi et al. and Taskiran et al. are consistent with an increased generation of NO in the central nervous system due to augmentation of nNOS. Motor neuron survival is highly dependent on the supply of trophic factors. Deprivation of trophic factors results in induction of nNOS. Shukla et al., observed that there was no significant change in the CSF nitrite as well as malondialdehyde (MDA) levels in motor neuron disease as compare to normal control, however they have suggested involvement of NO in MND with positive family history.

Direct evidence for the involvement of NO, in patients with MND has been provided by immunohistochemical studies of the autopsy specimens of spinal cord. The expression of three different nNOS spliced variants, namely nNOS alpha, nNOS beta and nNOS gamma was investigated in the spinal cord of control and both familial and sporadic amyotrophic lateral sclerosis (FALS and SALS) patients. Increased immunoreactivity for nNOS beta and nNOS gamma was present in both FALS and SALS in the reactive astrocytes of the ventral horn and white matter. This finding indicates that nNOS beta and nNOS gamma spliced variants are upregulated in reactive astrocytes in ALS. Immunohistochemical investigation of the spinal cords of 15 patient with SALS, using antibodies to iNOS and 3-NT showed that the reactive astrocytes in the anterior horns and corticospinal tracts were more intensely immunostained for iNOS and 3-NT as compared with controls. These findings suggest that selective NO mediated oxidative damage in the motor system plays a part in the pathomechanism of neuronal degeneration in the spinal cord of SALS patients.

No change has been reported in the CSF nitrite, nitrate and cGMP levels in patients with spinocerebellar ataxia. The granular layer neurons are rich in nNOS and NO is known to be formed in the cerebellum. Fibroblasts of patients with ataxia telangiectasia are hypersensitive to killing by the NO donor, S-nitroso-gluthathione (GSNO). Cell killing by GSNO appears to be associated with the formation of nitrite rather than nitrate as the ultimate oxidation product of NO.

The results of CSF and plasma nitrite/nitrate levels in AD patients have shown contrasting findings. Two papers suggested no significant correlation between the levels of nitrate in the CSF and plasma and AD, while decreased levels of nitrate in CSF of AD patients were found in another study. In the brain of AD patients increased levels of NOS activity and reduced nitric oxide responsive soluble guanylyl cyclase activity in the superior temporal cortex has been demonstrated. In addition, the pattern of NAPDH-diaphorase in the neuropil of the hippocampus is changed dramatically in the brain of AD patients. NO synthase neurons are selectively spared in AD and particularly in the hippocampus.

3-Nitrotyrosine (3-NT) is formed by the peroxynitrite mediated nitration of tyrosine containing biomolecules and is a marker of NO formation. In various neurodegenerative diseases like PD, AD and ALS an increase in nitration of MnSOD in CSF has been observed.

The discrepancy observed in all these studies could be due to the controls which comprised of diseases like epilepsy, myopathy, myelitis, peripheral neuropathy, tension headache, vertigo which are not neurodegenerative. However, it is possible that the levels of NO in these diseases were not completely normal. CSF from normal healthy controls could not be obtained due to ethical reasons. It is also possible that the changes in the NO metabolism may be occurring in a localised area of the brain and spinal cord and may not be of a magnitude so as to be reflected in the CSF nitrite/nitrate levels. So, as with other attempts to find peripheral markers of central nervous system disorders, it appears that at present CSF/plasma nitrite and nitrate levels are not useful measures to determine changes in brain NO functions in neurodegenerative diseases.

Furthermore, the use of selective NOS inhibitors has however, met with little or no success in the clinical treatment of neurodegenerative diseases per se, where neurons continue to die, while in animal models the same drugs provide neuroprotection. This may indicate that either the animal models employed are not reflective of the events in neurodegenerative diseases or that because neuronal death involves a cascade of events, a single agent may not be affected and a combination of drugs acting on multiple mechanisms may be more useful.

A very recent study shows evidence that the small-conductance Ca2+/calmodulin-activated K+ channel KCNN4/KCa3.1/SK4/IK1 is highly expressed in rat microglia and is a potential therapeutic target for acute brain damage. Using a Transwell cell-culture system that allows separate treatment of the microglia or neurons, the authors have shown that activated microglia killed neurons, and this was markedly reduced by treating only the microglia with a selective inhibitor of KCa3.1 channels, triamethylene-34 (TRAM-34). These in vitro findings translated into in vivo neuroprotection, because the authors have also found that degeneration of retinal ganglion cells after optic nerve transaction was reduced by intraocular injection of TRAM-34. This study provides evidence that KCa3.1 channels constitute a therapeutic target in the CNS and that inhibiting this K+ channel might benefit acute and chronic neurodegenerative disorders that are caused by or exacerbated by inflammation.
Neuroprotective Effects of Nitric Oxide

NOS (NADPH diaphorase) neurons are selectively resistant to various types of neurotoxic insults including NMDA mediated neurotoxicity\(^7\), ischaemic hypoxic insults\(^8\), and the degenerative processes of HD\(^9\). The diaphorase activity of NOS may be associated with this neuroprotection, as induction of a similar enzyme, NAD(P)H:quinone reductase diaphorase, in a neuronal cell line protects these cells from glutamate toxicity\(^10\). In addition, NOS neurons are enriched in manganese superoxide dismutase (MnSOD)\(^11\) which could protect by removing superoxide anion that destroys NO\(^10\). Furthermore, NMDA neurotoxicity is attenuated by exogenous SOD, and transgenic mice overexpressing SOD are also resistant to NMDA neurotoxicity and focal cerebral ischaemic injury. Thus, the neuroprotective effect of NO is mainly due to its antioxidant property as evident by both in vitro and in vivo experiments where NO was found to protect against iron induced lipid peroxidation and DA depletion\(^11\). The neuroprotective effects of NOS may explain the selective motor neuronal death in MND with sparing of the sensory and autonomic fibres, because in the adult spinal cord, NOS is expressed mostly in the sensory and autonomic neurons which are unaffected in ALS\(^9\).

Nitric oxide generated by nitric oxide synthase or released from an endogenous S-nitrosothiol, S-nitrosoglutathione may up-regulate antioxidative thioredoxin system and antiapoptotic Bcl-2 protein through a cGMP-dependent mechanism. Moreover, nitric oxide radicals have been shown to have direct antioxidant effect through their reaction with free radicals and iron-oxgen complexes. In addition to serving as a stabilizer and carrier of nitric oxide, S-nitrosoglutathione may have protective effect through transnitrosylation reactions. Based on these new findings, a hypothesis arises that the homeostasis of nitric oxide, S-nitrosothiols, glutathione, and thioredoxin systems is important for protection against oxidative stress, apoptosis, and related neurodegenerative disorders\(^9\).

Conclusion

NO is a unique and ubiquitous biological effector molecule synthesized from L-arginine by the NO synthases (NOSs) that occur in many tissues and cells. NO is an unorthodox neurotransmitter as it diffuses freely through cell membranes, it activates soluble guanylate cyclase to convert GTP to cGMP and is deactivated within seconds to nitrates and nitrites. The L-arginine-NO metabolic pathway plays an important role in many biological processes. Besides its role as a mediator of several physiological functions, NO appears to be a neurotoxin under conditions of excessive production, which suggests a role for NO in neurodegenerative diseases.

Recent advances in both molecular genetics and neurochemistry have improved our knowledge of fundamental processes involved in cell death. There is a large body of evidence that excitotoxicity occurs in both acute and chronic neurologic diseases. NMDA receptor activation leads to an increase of intracellular calcium levels and thereby to an activation of calcium dependent nNOS. NO produced by these neurons mediates neurotoxicity in vitro and in vivo, which can be attenuated by inhibition of nNOS. In animal models of neurodegenerative diseases causing secondary excitotoxic lesions, peroxynitrite formation may be the key mediator of NO toxicity. The treatment with selective NO inhibitors for the neuronal or inducible isoforms or with compounds combining in one molecule selective nNOS inhibition and antioxidant properties may prove efficacious in the treatment of neurodegenerative diseases. Brain damage and disease involve activation of microglia and production of potentially neurotoxic molecules, but there are no treatments that effectively target their harmful properties.

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