Neuronal Nitric Oxide Synthase

Kourosh Masoumeh Arami, Behnam Jameie and Seyed Akbar Moosavi

Abstract

Nitric oxide synthase (NOS), a flavo-hemoprotein, regulates nitric oxide (NO) synthesis that has dual biological activities: as an important signalling molecule in vasodilatation and neurotransmission at low concentrations and at higher concentrations as a defensive cytotoxin. In central and peripheral nervous system, neuronal NOS (nNOS) produces NO that has been implicated in modulating physiological functions such as synaptic plasticity, learning, memory and neurogenesis as well as some pathological conditions in which overproduction of NO may lead to the generation of highly reactive species, such as peroxynitrite and stable nitrosothiols, which may cause irreversible cell damage in excitotoxicity, ischaemia, Parkinson, Alzheimer's disease (AD) and depression. NOS-derived NO also involves in regulation of blood pressure, smooth muscle relaxation and gut peristalsis via peripheral nitrergic nerves.

Keywords: neuronal nitric oxide synthase, nitric oxide

1. Introduction

After discovery of nitric oxide as a biological mediator many researchers have focused on the importance of nitric oxide in the physiology of the nervous system [1–3].

NO, as the smallest signalling molecule, is produced by three types of NO synthase arising from three different genes referred to as neuronal nitric oxide synthase (also known as nNOS, Type I, NOS-I and NOS-1) that is found in neuronal tissues, inducible nitric oxide synthase (also known as iNOS, Type II, NOS-II and NOS-2) that is synthesized after formation of pro-inflammatory cytokines or endotoxin and endothelial nitric oxide synthase (also known as eNOS, Type III, NOS-III and NOS-3) that is found in endothelial cells [4]. The nNOS and eNOS are constitutively expressed and considered to be calcium-dependent, but when the
activity of iNOS is fully activated at basal intracellular calcium concentration, it would be calcium-independent.

The main difference between three NOS isoform regarding the reactions achieved lies in the rate of the nicotinamide-adenine-dinucleotide phosphate (NADPH) oxidation, termed the uncoupled reaction. Moreover, nNOS carry on transferring electrons to the haem and, hence, oxidase NADPH at a high rate, while in eNOS and iNOS this reaction can happen at a much slower rate [5].

The nNOS constitutes the principal source of NO in distinct populations of neurons and synaptic spines in the brain and the peripheral nervous system while eNOS can occur in some neurons and iNOS may exist in microglia and astrocytes but usually not in neurons [6].

Interneurons expressing nNOS are involved in physiological procedures like neurovascular coupling to regulate neocortical blood flow [7], the homeostatic control of sleep [8], synaptic integration of adult neurons and balance of excitatory and inhibitory signalling in brain [9].

2. nNOS enzymology

The nNOS monomer with a molecular weight of 160.8 kDa and 1434 amino acids is inactive and can be activated after dimerization by tetrahydrobiopterin (BH₄), haem and l-arginine (L-Arg) binding [10]. Each nNOS monomer has two domains including a reductase domain (C-terminal) and an oxygenase domain (N-terminal) which can be separated by a calmodulin-binding motif [11]. The reductase domain which attaches the substrate NADPH comprises a binding site for flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) [12, 13]. An autoinhibitory loop within the FMN-binding domain regulates nNOS activity [10]. The oxygenase domain which binds the substrate l-arginine contains BH₄ binding site, cytochrome P-450-type haem active site and Zn binding site for nNOS dimerization facilitation (Figure 1).

Figure 1. Schematic demonstration of nNOS structure and the metabolic formation of nitric oxide by nNOS include an oxygenase domain (N-terminal) and a reductase domain (C-terminal) which can be separated by a calmodulin-binding motif. The reductase domain which binds NADPH includes a binding site for FMN, FAD and the oxygenase domain which binds l-arginine contains a tetrahydrobiopterin (BH₄) binding site and a cytochrome P-450-type haem active site. NADPH electrons (e⁻) via FMN and FAD transfer from the reductase domain to the oxygenase domain. nNOS catalyzes the oxidation of l-arginine to form l-citrulline and NO (Reproduced with permission from Dong-Ya Zhu).
All NOS proteins comprise a zinc–thiolate cluster formed by a zinc ion that is tetrahedrally coordinated to two Cys motifs (one donated by each monomer) at the NOS dimer interface. Zinc in NOS has a catalytic activity [10].

3. NOS-catalyzed reaction

All forms of NOS use L-arginine and molecular oxygen as the substrate and reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) as co-substrates to produce citrulline NO. FAD and FMN with BH$_4$ are cofactors of all isozymes. All NOS proteins are homodimers. A functional NOS transfers electrons from NADPH, via FAD and FMN in the carboxy-terminal reductase domain to the haem in the amino-terminal oxygenase domain. This flowing of electron (NADPH $\rightarrow$ FAD $\rightarrow$ FMN $\rightarrow$ haem) can be facilitated by Ca$^{2+}$/CaM binding. The oxygenase domain also binds the cofactor BH$_4$, molecular oxygen and L-arginine. At the haem site, the electrons are used to activate O$_2$ to oxidize L-arginine to L-citrulline and NO. Sequences near the cysteine ligand of the haem are also implicated in L-arginine and BH$_4$ binding. The NOS enzyme is monooxygenases, generating NO and citrulline from L-arginine (L-Arg), NADPH and O$_2$.

The NOS catalysis has two-step as follows: In first step, the substrate, L-Arg, is converted to N-hydroxy-L-arginine (NOHA), that in turn is converted to NO and citrulline in the second step. The nitrogen atom of NO is derived from the guanidino group of the L-Arg substrate, and the oxygen atom is derived from dioxygen [10, 14]. NO can activate some transduction pathways, such as, activation of guanylyl cyclase and conversion of Guanosine-5’-triphosphate (GTP) into Cyclic guanosine monophosphate (cGMP) and

Figure 2. Signal transduction of nNOS. nNOS is activated by a calcium-dependent calmodulin. nNOS produces NO from oxidation of arginine into citrulline. NO diffuses and act on pre-synaptic or post-synaptic targets. NO activates guanylyl cyclase (GC) that triggers a protein kinase G (PKG) resulting in Erk activation and the stabilization of TORC1 a CREB co-activator. PL. M, plasma membrane; CAT, cation and anion transporter. (Adapted from Gallo and Iadecola).
consequent activation of protein kinase G (PKG). PKG activity leads to Erk activation of early genes such as c-fos, Arc and Brain-derived neurotrophic factor (BDNF) (Figure 2). NO/cGMP pathway is implicated in various neurophysiological processes including neuronal synaptic modulation, development, learning and memory. Some effects of NO are cGMP-independent. For instance, several pre-synaptic targets such as SNAP25, synaptxin Ia, n-Sec 1, neurogranin as well as the post-synaptic targets ADP ribosyltransferase and NMDA receptors have been identified for NO [15].

4. Histological distribution

The nNOS has been found in neurons, astrocytes, the adventitia of brain blood vessels and cardiac myocytes. Besides brain tissue, nNOS has been distinguished by immunohistochemistry in the spinal cord, sympathetic ganglia and adrenal glands, peripheral nitriergic nerves, skeletal and cardiac myocytes, epithelial cells of different organs, kidney macula densa cells, pancreatic islet cells, parasympathetic ganglia, nonadrenergic noncholinergic peripheral autonomic nerve fibres and the vascular smooth muscle and endothelial cells [16]. In mammals, the largest origin of nNOS regarding tissue mass is the skeletal muscle [4, 16, 17].

Since NO cannot be stored in the cells, new synthesis is necessary to have its activities. Thus nNOS should be bonded to the plasma membrane directly or through adapter proteins. Fractionation studies have shown that brain nNOS occurs in particulate and soluble forms in cytosol far from membranes in a patch-like form. Furthermore, through the early six days in the cultured cerebral cortical astrocytes of rats, nNOS immunoreactivity mostly appeared in the cytoplasm. Nevertheless, at day 7, nNOS immunoreactivity was predominantly expressed in the nucleus, and this nuclear localization continued about 10 h. Then, nNOS immunoreactivity was mainly expressed in the cytoplasm again. Recently, some researchers showed nNOS nuclear localization without cytoplasmic staining of nNOS in some parts of neural and glial cells. Therefore, diverse functions of nNOS in the cell may arise from differential subcellular localization [10].

Adapter proteins are involved in transfer of nNOS to distinct sites. For instance, nNOS is anchored to membranes by binding to syntrophin, PSD95/SAP90 or PSD93. CAPON, another adapter protein for nNOS, comprises a C-terminal domain that binds to the PDZ domain of nNOS.

In rats and mice, five interneuron expressing nNOS have been found as follows: (1) neurogliaform cells, (2) Ivy cells (IvC), (3) interneurons expressing the vasoactive intestinal peptide (VIP) and calretinin (CR), (4) interneurons expressing PV and (5) projection cells close to the subiculum. PV cells expressing nNOS mainly exist in the dentate gyrus (DG) of hippocampus. Though species differences between rat and mouse have been noted that coexpression of nNOS and PV in rat DG is much lower than that in mouse. Somatostatin-expressing interneurons that express nNOS are resided in hippocampus.
5. Cofactors and prosthetic groups that impact on nNOS activity

5.1. Phosphorylation

Kinases increase nNOS activity by phosphorylation, whereas, phosphatases decrease nNOS activity by dephosphorylation [18]. nNOS phosphorylation is important for the enzyme activity that is regulated by some kinases and phosphatases, for example, calmodulin-dependent kinases, protein kinase C (PKC), protein kinase A (PKA) and phosphatase 1, which are in fact modulated by extracellular and intracellular factors [19]. Nonetheless, phosphorylation at different sites of nNOS affects its activity differently. The protein kinase CaMKII can phosphorylate nNOS at Ser847 that diminishes nNOS activity by Ca$^{2+}$–CaM binding inhibition. Ser847-PO$_4$ is found in the autoinhibitory loop which inhibits the movement of the loop even in the occurrence of high concentrations of Ca$^{2+}$–CaM, thus decreasing nNOS activity [20]. In contrast, the protein phosphatase 1 decreases phosphorylation level of nNOS at Ser847, leading to an increased nNOS activity. Another phosphorylation site of nNOS is at Ser1412 in endothelial nitric oxide synthase [21]. In addition, CaM-KI deregulates nNOS activity by phosphorylation at Ser741 in transfected cells. Since the expression of CaM-KI declines with the brain development, it still needs to be demonstrated whether nNOS is phosphorylated at Ser741 by CaM-KI in vivo [10].

5.2. Dimerization

Despite the ability of the reductase and oxygenase domains that function independently in some circumstances, NOS activity is accomplished by the homodimer [5].

Active nNOS is a dimer with two high-affinity binding sites for BH$_4$ and L-arginine. Two cysteine residues make a disulphide bridge or ligate a zinc ion to connect the two monomers. Furthermore, an ‘N-terminal hook’ domain sustains the dimer. BH$_4$ as well as haem and L-arginine make nNOS a stable dimer.

High-affinity binding sites for BH$_4$ and L-arginine and facilitating electron flow that occurs in dimerization raise nNOS activity. The electron seems to transfer from one monomer to another, which may be the main reason why the nNOS monomer is inactive. PIN (inhibitor of nNOS) destabilizes the nNOS dimer, thus inhibiting nNOS activity. Dimer stabilization preserves nNOS from proteolysis. Destabilization of dimeric nNOS makes it more vulnerable to be phosphorylated by protein kinase C and hydrolyzed by trypsin [5, 10]. nNOS monomers are able to catalyze the cytochrome c reduction. This shows that the electrons transfer within the reductase domain from NADPH by two flavins is independent of dimeric structure [5]. The haem plays a crucial role in dimerization. In absence of haem, NOS is monomer which is principally normal with respect to secondary structure [5].

5.3. Calcium and calmodulin

Dependence on Ca$^{2+}$ is the main characteristic between the constitutive and inducible isoforms. eNOS and nNOS are both triggered by an elevation in intracellular Ca$^{2+}$, followed by
the consequent binding of Ca\textsuperscript{2+}/CaM \[5\]. Calmodulin acts as an allosteric activator of all forms of NOS that facilitates electron flow transferring from NADPH to the reductase domain flavins and from the reductase domain to the haem center. Calmodulin binding is brought about by an increase in intracellular Ca\textsuperscript{2+} \[22, 23\].

When intracellular Ca\textsuperscript{2+} concentrations decline to basal levels, calmodulin detaches from nNOS, and it becomes inactive again. Hence, nNOS activity is primarily controlled by intracellular Ca\textsuperscript{2+} concentrations and so calmodulin-binding effect on nNOS activity.

5.4. Proteins binding to nNOS PDZ domain

PDZ (Post Synaptic Density proteins, discs-large, ZO-1) domain of the NH\textsubscript{2}-terminal involves in dimerization, activation and interaction of nNOS with many other proteins in specific areas of the cell \[24\]. These interactions determine the sub-cellular distribution and the function of the enzyme \[4, 10\].

nNOS PDZ domain contains two separate binding sites, one site binds to PDZ domains of other proteins and another site binds to COOH-terminal peptide ligands. Proteins containing PDZ domains are important in connecting constituents of signal transduction pathways in multiple complexes \[25\]. NO signalling is modified by nNOS attachment to membrane or cytosolic protein by direct PDZ–PDZ domain or C-terminal-PDZ interactions. PSD95 (post-synaptic density protein-95), a multivalent synaptic protein and main component of the post-synaptic density, can connect nNOS to N-methyl-d-aspartate receptor (NMDAR), and elucidate the NMDAR stimulation effect on nNOS activation. Post-synaptic targeting of nNOS is directed by binding to PSD95 (Figure 3). nNOS–PSD95 is a typical PDZ–PDZ binding, which needs the intact tertiary structure of both domains and a 30-amino acid extension.

CAPON, another adapter protein, comprises a C-terminal PDZ domain that binds to the N-terminal of nNOS PDZ domain and an N-terminal phosphotyrosine binding (PTB) domain. CAPON interacts with a component of the Ras family of small G proteins, Dexras1, which is induced by dexamethasone. Interaction of CAPON with nNOS delivers NO to Dexras1, leading to S-nitrosylation of Dexras1 on cysteine-11. Dexras1 binds to the peripheral benzodiazepine receptor-associated protein (PAP7), and PAP7 binds to the divalent metal transporter (DMT1), an iron channel mediating iron uptake in neurons \[26\]. Abnormally high cellular iron levels may lead to disordered neuronal function \[27\].

5.5. nNOS inhibitors

It has been shown that the N-terminus of nNOS could bind to a protein termed PIN which can inhibit nNOS activity. It is found that PIN destabilizes nNOS dimers and inhibits nNOS activity. Recently, it has been demonstrated that PIN inhibits production of NO and O\textsubscript{2}, not nNOS dimerization \[28\].

Another protein that inhibits NO productions is nitric oxide synthase interacting protein (NOSIP) \[29\]. NOSIP and nNOS co-localize in different areas of the central and peripheral nervous systems. NOSIP negatively affects nNOS activity in a neuroepithelioma cell line stably expressing nNOS. In addition, over-expression of NOSIP in cultured primary neurons
limits nNOS trafficking to terminal dendrites and direct nNOS to the soma. These findings suggest that NOSIP regulates NO production in the nervous system by regulating the activity and localization of nNOS. NOSIP upregulation by neuronal activity may prevent NO production in neurons [10].

6. Physiological and pathophysiological functions of nNOS

6.1. Physiological functions of nNOS

Even though nNOS-derived NO has many functions in neuronal signalling, it varies from a physiological neuromodulator to a neurotoxic factor when extra amount of NO is generated. Thus nNOS may play an important role in many physiological and pathological conditions [10]. Neuronal functions of nNOS are modulating physiological functions such as learning, memory and neurogenesis. In the central nervous system (CNS), nNOS-derived NO causes long-term regulation of synaptic transmission (long-term potentiation and long-term inhibition) [30], while in acute neurotransmission there is no involvement. NOS inhibitors diminish learning and produce amnesia in animal models, so it involves in memory formation. Also in the CNS, nNOS-derived NO is implicated in the central regulation of blood pressure. Blockade of nNOS activity in the medulla and hypothalamus makes systemic hypertension [31].

The nNOS-derived NO as an important neurotransmitter is participated in neuronal plasticity (especially in memory formation), peripheral and central transmission of pain signals,
regulation of central nervous system blood flow, neurotransmitter release from cholinergic nerve fibres and the functional regulation of organs with nitrergic innervation [32].

6.1.1. Pancreatic nNOS and insulin regulation

Pancreatic β-cells express nNOS that controls insulin secretion through two catalytic activities: nitric oxide production and cytochrome c reductase activity [33].

PIN is primarily incorporated with insulin secretory granules and co-located with nNOS. In addition, PIN overproduction increases glucose-induced insulin secretion, which is reversed by NO donor, sodium nitroprusside. In contrast, nNOS inhibitor increased insulin secretion induced by glucose. Therefore, PIN insulinotropic effect could be related to its co-localization with the actin-based molecular motor myosin and as such be involved in the physiological regulation of insulin secretion at the exocytotic machinery [33].

6.1.2. Cardiovascular regulation

Sarcoplasmic reticulum and mitochondria functions are regulated by nNOS through Ca²⁺ maintaining, which are directly related to myocardial injury. nNOS overexpression protects mouse hearts from injury and nNOS deficiency increases ventricular arrhythmia and mortality after myocardial infarction [34].

It is accepted that the local regulation of vascular tone in health is mainly regulated by eNOS-derived NO [35]. However, some studies have suggested that nNOS-derived NO may also be implicated in this process and plays an important role in the local regulation of basal microvascular tone as well as in the vasodilator response to mental stress [35]. nNOS can regulate vascular tone, independent of its effects in the central nervous system [35] and by direct effects on vascular smooth muscle. In the kidney S-methyl-L-thiocitrulline (SMTC) as a selective nNOS-inhibitor can decrease basal afferent and efferent arteriolar tone. These studies showed that macula densa (the main source of renal nNOS) removal eliminate the vasoconstrictor effects of SMTC.

In cerebral vessels, nNOS may regulate vascular tone reflex especially in response to hypoxia and/or hypotension. For example, Bauser-Heaton et al. have shown that selective inhibition of nNOS with N-(4S)-(4-amino-5-[aminoethyl]aminopentyl)-N’-nitroguanidine decreased basal cerebral arterial diameter and eliminated the vasodilator response to hypoxia.

In skeletal muscle nNOS is located at the cell membrane bound to the cytoskeletal protein dystrophin. Dystrophin absence in patients with Duchenne muscular dystrophy (DMD) makes a significant reduction in skeletal muscle nNOS expression and also in blood flow.

In coronary microvascular system, nNOS and eNOS have distinct local roles in the physiologic regulation. While nNOS may be principal factor in regulation of basal vasomotor tone and blood flow, eNOS-derived NO facilitates dynamic alterations in blood-flow distribution and has anti-atherosclerotic effects at endothelium.

Many smooth muscle tissues in the periphery are innervated by nitrergic nerves, that is, nerves that include nNOS that produce and release NO [4]. For example, relaxation of
corpus cavernosum and penile erection is occurred by nitrergic nerves. Phosphodiesterase-5 inhibitors (sildenafil, vardenafil and tadalafil) need at least a residual nNOS activity for their action [4].

Some studies indicated that nNOS-derived NO may change vascular tone by perivascular sympathetic nerve inhibition. Hatanaka et al. indicated that selective nNOS inhibitor, vinyl-1-5-(1-imino-3-butenyl)-1-ornithine (1-VNIO) raises arterial vasoconstriction and local norepinephrine concentration in perivascular nerve stimulation in isolated rat mesenteric arteries without endothelium. However, 1-VNIO could not alter vasoconstrictor response to exogenous norepinephrine, showing that nNOS-derived NO may affect release of neurotransmitter from perivascular sympathetic nerves.

In other studies nNOS-derived NO in central systems may change peripheral vascular tone. For instance, nitric oxidergic neurons in nucleus tractus solitaries influence on blood pressure in diabetic rats. Unilateral microinjection of sodium nitroprusside (100 mmol/60 nL) into the nucleus raised blood pressure in diabetic rats [36, 37]. In another study, it is revealed that nitric oxide in the nucleus raphe magnus modulates cutaneous blood flow in rats during hypothermia [38, 39].

6.1.3. Neurogenesis

NO as a paracrine messenger in newly produced neurons regulates the proliferation and differentiation of mouse brain neural progenitor cells. L-NAME, NO synthase inhibitor, raises cell proliferation and reduces neuronal differentiation [40].

The subventricular zone (SVZ) and the subgranular zone (SGZ) of dentate gyrus are two principal neurogenesis sites in the adult brain. In the adult mouse SVZ and olfactory bulb, NO has a negative control on the size of the undifferentiated precursor pool and enhances neuronal differentiation so acts as a physiological inhibitor of neurogenesis [41].

Mice treated with 7-NI display a rise in the number of mitotic cells in the SVZ, the olfactory bulb and the rostral migratory stream, but not in the DG. Though, a recent research established that nNOS inhibition elevated progenitor cells proliferation in the DG. Also, the anti-proliferative role of nNOS-derived NO on SVZ and DG has been shown in cerebral ischaemia. Thus, endogenous NO-derived nNOS can inhibit SVZ neurogenesis. Nevertheless, the role of nNOS in hippocampal neurogenesis is arguable. While nNOS-derived NO has anti-proliferative effect in adult animals, NO donor administration induces neurogenesis. It may be due to the different experimental protocols. Intravenous or hippocampal administration of a NO donor which can result in raised NO levels enhances cerebral blood flow, indirectly influencing neurogenesis [10].

6.1.4. Cerebral maps formation

NO has been involved in the cerebral map formation. In visual system, NO prompts synaptic refinement of immature synaptic connections at retinothalamic and retinocollicular levels. Normal organization of the somatosensory cortex and barrel field plasticity were found by daily
injection of nitroarginine before the period of ocular dominance column formation. However, NO may still contribute in establishing and refining neocortical connectivity. Definitely, when NADPHd activity is reformed in the barrel field, abnormal separation of thalamocortical axons happens. In these animals thalamocortical axons show fewer branch points in layer IV and abnormally expansive thalamocortical arbors. These results propose that NO could promote thalamocortical sprouting and participates in the consolidation of synaptic strength in layer IV of the primary somatosensory cortex [40].

6.1.5. Synchronization and coordination

Also, regulation of gap junctions is mediated by NO. Rorig and colleagues have shown that sodium nitroprusside (an NO donor) reduced the number of gap-junction-coupled neurons. Nonetheless, NO can affect electrical coupling, synchronization of metabolic states and coordination of transcriptional activity between connected neurons [40].

6.1.6. Neurotransmitter release and plasticity

Release of several neurotransmitters comprising acetyl choline, catecholamines, glutamate and gamma-Aminobutyric acid (GABA) are regulated by endogenous NO [40]. Furthermore, NO involves in balancing between GABAergic and glutamatergic synaptic transmission in early post-natal development. Disruption of this balance precipitates pathological disorders such as epilepsy, autism and schizophrenia [42, 43]. Moreover, NO is involved in fine-tuning synchronous network activity in the developing hippocampus [40].

NO plays an important role in memory formation in hippocampus [44] and NOS inhibition impedes learning and/or memory [45] while some studies failed to find any effect on learning and/or memory [10]. In mature hippocampus, NO regulates LTP at the Schaffer collateral/CA1 synapses and acts as a retrograde messenger. This occurs via the activation of post-synaptic NMDA receptors, synthesis of NO by NOS expressed in pyramidal cells and then retrograde activation of guanylate cyclase located in axon terminals. In contrast, in the cerebellum NO serves as an anterograde messenger that is produced in parallel fibre terminals or cerebellar interneurons and then diffuses to the post-synaptic Purkinje cell to induce long term depression (LTD) through a cGMP-dependent mechanism [40]. Additionally, NO involves in experience-dependent plasticity in the barrel cortex by reduction of bicuculine-induced activation of Erk and increment of c-Fos, Egr-1 and Arc.

In water maze, 8-arm radial maze, passive-avoidance and elevated plus-maze, 7-NI, at a dose inhibiting nNOS but not affecting blood pressure, induced amnesic effects. Before training in avoidance conditioning in goldfish anterograde amnesia was produced. However, immediately after training retrograde amnesia was formed. Moreover, genetic inhibition of nNOS indicated spatial performance impairment in the Morris water maze [46]. The hippocampus of nNOS knockout mice showed an abnormal expression of a synaptosomal-associated protein of the exocytotic machinery, glycolytic enzymes, T-complex protein 1, the signalling structure guanine nucleotide-binding protein G and heterogeneous nuclear ribonucleoprotein H of the splicing machinery. Therefore, in nNOS knockout mice spatial memory in the Morris water maze may impair by specific hippocampal protein derangements [10].
6.1.7. Gastrointestinal tract

The most obvious phenotype of nNOS knockout mice is stomach enlargement, several times the normal size, proving a role for nNOS in smooth muscle relaxation of the pyloric sphincter. Ablation of exon 6 in another nNOS knockout mice results in severe pyloric stenosis and reproductive endocrine abnormalities.

Citrulline measurements showed that nNOS activity in exon 2-deficient mice is 0.5% of that in wild-type, compared to 3% in exon 6-deficient mice [47].

6.2. Pathophysiological functions of nNOS

Abnormal NO signalling involved in some neurodegenerative pathologies that include excitotoxicity results in stroke, Parkinson’s and Alzheimer’s diseases and multiple scleroses. NO can involve in excitotoxicity, probably by peroxynitrite activation of poly-ADP-ribose polymerase (PARP) and/or mitochondrial permeability transition. High levels of NO can also produce energy reduction, caused by mitochondrial respiration inhibition and glycolysis. Some disorders of smooth muscle tone in the gastrointestinal tract (e.g. gastro-oesophageal reflux disease) may also drive from an excessive NO production by nNOS in peripheral nitrergic nerves [4].

An important mode of inactivation of NO is its reaction with superoxide anion (O₂⁻) to form the potent oxidant peroxynitrite (ONOO⁻). This can make oxidative damage, nitration and S-nitrosylation of biomolecules including proteins, lipids and DNA [48]. Nitrosative stress by ONOO⁻ has been involved in DNA single-strand breakage, followed by poly-ADP-ribose polymerase (PARP) activation [49].

6.2.1. Role of nNOS in neurodegeneration

Previous studies have shown that NO implicate in the pathogenesis of some neuroinflammatory/degenerative diseases. The constitutive NOS make the cuprizone-induced model of demyelination/remyelination [50]. Previous results demonstrate that demyelination was mainly prevented in mice lacking nNOS. In eNOS/io mice, demyelination increased to the same level as in wild type, but they showed a slight delay in spontaneous remyelination [50].

6.2.1.1. Role of nNOS in the pathophysiology of Parkinson’s disease

A progressive loss of dopaminergic input from the substantia nigra pars compacta leads to overactivity in Parkinson’s disease (PD) which creates extrapyramidal motor dysfunction, including bradykinesia, rigidity and tremor. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) yield Parkinson-like symptoms and has been used to assess the mechanism of PD pathogenesis.

Recent studies indicate that resistance to MPTP neurotoxicity in the nNOS knockout mice are more than wild-type ones. This was established by 7-NI that can protect against this neurotoxicity in experimental animals. Furthermore, a great expression of nNOS was reported in
basal ganglia and the respiratory burst of circulating neutrophils of PD patients. However, NO production and protein tyrosine nitration were considerably increased [10].

L-DOPA is the most used drug in Parkinson’s disease treatment. Nitric oxide is a satisfying goal for the decrease of L-DOPA-induced dyskinesia in PD [51]. Thus, nNOS in the pathogenesis of Parkinson’s disease is important.

6.2.1.2. Role of nNOS in the pathophysiology of Alzheimer’s disease

Disruption of neuronal nitric oxide synthase dimerization contributes to the development of Alzheimer’s disease (AD). nNOS-Ser293, a potential site of cyclin-dependent kinase-5 phosphorylation, may be participated in the nNOS dimerization reduction and, hence, the development of AD [52].

Extracellular accumulation of amyloid β-peptide (Aβ) causing the neuritic plaques and intracellular neurofibrillary tangles is due to the tau protein hyperphosphorylation considered to be an important feature of AD. Chronic infusion of Aβ(1-40) results in ONOO-formation and subsequent tyrosine nitration of proteins. Nitrotyrosine found in AD was highly co-localized with nNOS in cortical pyramidal cells. Moreover, all three isoforms of NOS are raised up in AD; therefore, NOS inhibitors could be useful for AD treatment [10].

6.2.1.3. Role of nNOS in the pathophysiology of multiple sclerosis

Multiple sclerosis (MS) is characterized by demyelination associated with an infiltration of mononuclear white blood cells within the CNS. The demyelination leads to diminishing conduction of the action potential in neurons.

nNOS can be induced in nerve injury. It has been revealed that nitric oxide deriving from nNOS may be toxic to oligodendrocytes and induce axonal degeneration. Additionally, it is shown that nitrate as NO degradation product is increased in cerebrospinal fluid of MS patients. Also, the oxidised agent, peroxynitrite is found within active MS lesions. Additionally, NO scavengers have been revealed to reduce the severity of an MS-like disease model [53].

6.2.2. Role of nNOS in the neurodevelopment

Previous studies indicated that nitric oxide derived from nNOS have been associated in social interaction. They have shown that nNOS deletion decreased anxiety-like behaviour, augmented general locomotor activity, reduced spatial learning and memory, and diminished preference for social novelty which are characteristics of autism spectrum disorder [54].

Attention deficit/hyperactivity disorder (ADHD) is a psychiatric disorder with inattention, hyperactivity and impulsivity as main signs, and is frequently associated with learning disability, substance abuse, epilepsy and other psychiatric disorders such as anxiety and disruption of circadian rhythm [55]. In other study, they reported that NOS1 Ex1f-VNTR is involved in impulsive and empathic personality traits and associated with self-rated impulsiveness and venturesomeness [56].
NOS1 KO exhibited higher locomotor activity than wild-type in a novel environment, as measured by open-field test. NOS1 KO mimics certain ADHD-like behaviours and could potentially serve as a novel rodent model for ADHD.

Recent results propose changes in NO-signalling pathways may be associated with ADHD in humans. Neuronal nitric oxide synthase (NOS1) is a main enzyme responsible for the neuronal creation of NO. Previous studies show that 28% of adult ADHD patients were found to be homozygous for a risk allele in the NOS1 promoter region (termed ex1f-VNTR) that diminishes NOS1 expression. This allele is apparently associated with developed prefrontal cortex and ventral striatal roles, which are involved in impulsive and aggressive behaviours associated with ADHD. Moreover, past findings showed that NOS1 knockout mice (NOS1 KO) could be a candidate model for ADHD. Behaviourally, NOS1 KO is apparently hyperactive and exhibits abnormal social, aggressive and impulsive behaviours as well as deficits in learning and memory [55].

6.2.3. Role of nNOS in excitotoxicity

Nitric oxide that occurs naturally in the brain without causing overt toxicity, implicated in cell death. One account is that ischaemia creates overproduction of NO, allowing it to react with superoxide to form the potent oxidant peroxynitrite [6].

Brain ischaemia is a great pre-synaptic glutamate release or post-synaptic stimulation of its membrane receptors, reproduce neuronal damage or death. Glutamate by binding to four major types of receptors, metabotropic receptors, NMDA receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainate receptors do its action post-synaptically. Following focal ischaemia, NMDA receptor activation causes more glutamate excitotoxicity and neuronal injury than other receptors owning to their high calcium permeability. Great stimulation of NMDAR results in Ca\(^{2+}\) overload in the cell, and therefore triggering the Ca\(^{2+}\)-sensitive enzymes. nNOS, as a Ca\(^{2+}\)-sensitive enzyme, indicates a principal role in excitotoxicity. In primary cortical neuronal cultures of nNOS\(^{-/-}\) mice, these neurons would be resistant to NMDA neurotoxicity and to oxygen-glucose deprivation compared with wild-type cultures. These in vitro studies indicate that nNOS-derived NO is the principal source of neurotoxicity in neurons [10]. nNOS\(^{-/-}\) mice have a reduced infarct size, under focal ischaemia. nNOS knockout mice are resistant to focal and global cerebral ischaemia, consistent with a role for nNOS-derived NO in cellular injury following ischaemia.

In addition, 7-nitroindazole and ARL 17477, as selective nNOS inhibitors, can also decrease the infarct size focal ischaemia. Recent studies demonstrate that nNOS expression and enzymatic activity in the hippocampus of mice was decreased under focal cerebral ischaemia. The nNOS reduction following ischaemia stimulated cell proliferation in the DG. Therefore, nNOS inhibition can improve ischemic injury [10].

Even though NMDA activates NOS, the NOS-containing neurons resist toxic effects of NMDA and form NO that is released to kill adjacent non-NOS neurons. The unique resistance of NOS neurons to NMDA toxicity seems to be associated with their very high content of manganese.
superoxide dismutase, which blocks interactions of NO with superoxide to form the peroxynitrite [10].

Since NO measuring and localizing is difficult, citrulline as a marker of NO synthase activity localized completely to nNOS-containing neurons and is eliminated following NOS inhibitors treatment. Additionally, no other enzyme capable of synthesizing citrulline has been found in the brain. Thus, citrulline staining supplies a useful approach to evaluate NO turnover [6].

Ischaemia triggers a pronounced enhancement in citrulline immunoreactivity but more so in a large population of the neuronal isoform of NO synthase (nNOS) in the peri-infarct than the infarcted tissue. In contrast, 3-nitrotyrosine (a marker for peroxynitrite formation) is confined to the infarcted tissue and is not present in the peri-infarct tissue.

6.2.4. Mood disorders

NO may play an important role in mediating the effect social interactions have on anxiety. Inhibition of nNOS diminishes anxiety-like responses to pair housing [57]. Also, anxiety-like behaviours in aged mice are recovered by modifying nNOS expression levels in the hippocampus or cerebellum [58].

Major depressive disorder is a mental disorder characterized by at least two weeks of low mood that is present across most situations. Paroxetine, a typical anti-depressant inhibited NOS in humans and animals. Moreover, imipramine (IMI, anti-depressant) significantly diminished NOS activity. IMI withdrawal significantly amplified NOS activity. Furthermore, plasma nitrate concentrations (indicator of NO production) were highly greater in depressed patients. This finding showed likely participation of NO in depression, in line with the observation with those 7-NI made anti-depressant-like effects in the Forced Swimming Test. Interestingly, immobilization-produced stress elevated nNOS mRNA and protein expression in hypothalamic-pituitary-adrenal axis in rats. Meanwhile, a recent research showed that chronic mild stress (CMS) augmented nNOS expression in the hippocampus. nNOS inhibition prevented CMS-produced depression. In addition, mice with targeted deletion of the genes encoding nNOS were resistant to the CMS-induced depression [10].

6.2.5. Pain modulation

It is revealed that central anti-nociceptive effect of tapentadolis augmented by 7NI. Neuronal NOS impact on the anti-nociceptive action of tapentadol at the spinal and supraspinal level [59].

Acknowledgements

The authors thank Neuroscience Research Center of Iran University of Medical Sciences for the support of this study.
Author details

Kourosh Masoumeh Arami1,2*, Behnam Jameie1,2 and Seyed Akbar Moosavi3

*Address all correspondence to: kourosharami.m@iums.ac.ir

1 Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran
2 Department of Basic Sciences, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
3 Department of Medical laboratory Sciences, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran

References

[1] Garthwaite J., Concepts of neural nitric oxide-mediated transmission. European Journal of Neuroscience, 2008. 27(11): pp. 2783–2802.

[2] Kang Y.C., et al., Regulation of programmed cell death in neuronal cells by nitric oxide. In Vivo, 2004. 18(3): pp. 367–376.

[3] Moncada S. and Bolaños J.P., Nitric oxide, cell bioenergetics and neurodegeneration. Journal of Neurochemistry, 2006. 97(6): pp. 1676–1689.

[4] Förstermann U. and Sessa W.C., Nitric oxide synthases: regulation and function. European Heart Journal, 2012. 33(7): pp. 829–837.

[5] Andrew P.J. and Mayer B., Enzymatic function of nitric oxide synthases. Cardiovascular Research, 1999. 43(3): pp. 521–531.

[6] Eliasson M.J., et al., Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. The Journal of Neuroscience, 1999. 19(14): pp. 5910–5918.

[7] Perrenoud Q., et al., Activation of cortical 5-HT (3) receptor-expressing interneurons induces NO mediated vasodilatations and NPY mediated vasoconstrictions. 2012; 6: 82.

[8] Kilduff T.S., Cauli B. and Gerashchenko D., Activation of cortical interneurons during sleep: an anatomical link to homeostatic sleep regulation?. Trends in Neurosciences, 2011. 34(1): pp. 10–19.

[9] Somogyi, P. and Klausberger T., Defined types of cortical interneurone structure space and spike timing in the hippocampus. The Journal of Physiology, 2005. 562(1): pp. 9–26.
[10] Zhou, L. and Zhu D.Y., Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. Nitric Oxide, 2009. 20(4): pp. 223–230.

[11] Cho, H.J., et al., Calmodulin is a subunit of nitric oxide synthase from macrophages. The Journal of Experimental Medicine, 1992. 176(2): pp. 599–604.

[12] Crane, B.R., et al., Structure of nitric oxide synthase oxygenase dimer with pterin and substrate. Science, 1998. 279(5359): pp. 2121–2126.

[13] Sagami, I., Daff S. and Shimizu T., Intra-subunit and inter-subunit electron transfer in neuronal nitric-oxide synthase effect of calmodulin on heterodimer catalysis. Journal of Biological Chemistry, 2001. 276(32): pp. 30036–30042.

[14] Feng, C., Mechanism of nitric oxide synthase regulation: electron transfer and interdomain interactions. Coordination Chemistry Reviews, 2012. 256(3): pp. 393–411.

[15] Gallo, E.F. and Iadecola C., Neuronal nitric oxide contributes to neuroplasticity-associated protein expression through cGMP, protein kinase G, and extracellular signal-regulated kinase. The Journal of Neuroscience, 2011. 31(19): pp. 6947–6955.

[16] Förstermann, U., et al., Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension, 1994. 23(6 Pt 2): pp. 1121–1131.

[17] Nakane, M., et al., Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle. FEBS Letters, 1993. 316(2): pp. 175–180.

[18] Rameau, G.A., et al., Biphasic coupling of neuronal nitric oxide synthase phosphorylation to the NMDA receptor regulates AMPA receptor trafficking and neuronal cell death. The Journal of Neuroscience, 2007. 27(13): pp. 3445–3455.

[19] El-Mlili, N., et al., Chronic hyperammonemia reduces the activity of neuronal nitric oxide synthase in cerebellum by altering its localization and increasing its phosphorylation by calcium-calmodulin kinase II. Journal of Neurochemistry, 2008. 106(3): pp. 1440–1449.

[20] Rameau, G.A., Chiu L.Y. and Ziff E.B., Bidirectional regulation of neuronal nitric-oxide synthase phosphorylation at serine 847 by the N-methyl-D-aspartate receptor. Journal of Biological Chemistry, 2004. 279(14): pp. 14307–14314.

[21] Adak, S., et al., Neuronal nitric-oxide synthase mutant (Ser-1412→ Asp) demonstrates surprising connections between heme reduction, NO complex formation, and catalysis. Journal of Biological Chemistry, 2001. 276(2): pp. 1244–1252.

[22] Li, H. and Poulos T.L., Structure–function studies on nitric oxide synthases. Journal of Inorganic Biochemistry, 2005. 99(1): pp. 293–305.

[23] Yamamoto, Y., et al., Ca²⁺, calmodulin and phospholipids regulate nitric oxide synthase activity in the rabbit submandibular gland. Journal of Comparative Physiology B, 2004. 174(8): pp. 593–599.
[24] Cui, H., et al., *PDZ protein interactions underlying NMDA receptor-mediated excitotoxicity and neuroprotection by PSD-95 inhibitors*. The Journal of Neuroscience, 2007. 27(37): pp. 9901–9915.

[25] Chanrion, B., et al., *Physical interaction between the serotonin transporter and neuronal nitric oxide synthase underlies reciprocal modulation of their activity*. Proceedings of the National Academy of Sciences, 2007. 104(19): pp. 8119–8124.

[26] Cheah, J.H., et al., *NMDA receptor-nitric oxide transmission mediates neuronal iron homeostasis via the GTPase Dextras1*. Neuron, 2006. 51(4): pp. 431–440.

[27] Moos, T. and Morgan E.H., *The metabolism of neuronal iron and its pathogenic role in neurological disease: review*. Annals of the New York Academy of Sciences, 2004. 1012(1): pp. 14–26.

[28] Xia, Y., Berlowitz C.O. and Zweier J.L., *PIN inhibits nitric oxide and superoxide production from purified neuronal nitric oxide synthase*. Biochimica et Biophysica Acta (BBA)-General Subjects, 2006. 1760(9): pp. 1445–1449.

[29] Dreyer, J., et al., *Nitric oxide synthase (NOS)-interacting protein interacts with neuronal NOS and regulates its distribution and activity*. The Journal of Neuroscience, 2004. 24(46): pp. 10454–10465.

[30] Izumi, Y. and Zorumski C., *Nitric oxide and long-term synaptic depression in the rat hippocampus*. Neuroreport, 1993. 4(9): pp. 1131–1134.

[31] Toda, N., Ayajiki K. and Okamura T., *Control of systemic and pulmonary blood pressure by nitric oxide formed through neuronal nitric oxide synthase*. Journal of Hypertension, 2009. 27(10): pp. 1929–1940.

[32] Böhme, G.A., et al., *Altered synaptic plasticity and memory formation in nitric oxide synthase inhibitor-treated rats*. Proceedings of the National Academy of Sciences, 1993. 90(19): pp. 9191–9194.

[33] Lajoix, A.D., et al., *Protein inhibitor of neuronal nitric oxide synthase (PIN) is a new regulator of glucose-induced insulin secretion*. Diabetes, 2006. 55(12): pp. 3279–3288.

[34] Hu, L., et al., *Ischemic postconditioning protects the heart against ischemia–reperfusion injury via neuronal nitric oxide synthase in the sarcoplasmic reticulum and mitochondria*. Cell Death & Disease, 2016. 7(5): p. e2222.

[35] Melikian, N., et al., *Neuronal nitric oxide synthase and human vascular regulation*. Trends in Cardiovascular Medicine, 2009. 19(8): pp. 256–262.

[36] Arami, M.K., et al., *The effect of hyperglycemia on nitric oxidergic neurons in nucleus tractus solitarius and blood pressure regulation in rats with induced diabetes*. Journal of Diabetes and Metabolic Disorders, 2005. 4: p. 106.

[37] Kourosh Arami, M., et al., *The effect of nucleus tractus solitarius nitric oxidergic neurons on blood pressure in diabetic rats*. Iranian Biomedical Journal, 2006. 10(1): pp. 15–19.
[38] Arami, M.K., *Nitric oxide in the nucleus raphe magnus modulates cutaneous blood flow in rats during hypothermia*. Iranian Journal of Basic Medical Sciences, 2015. 18(10): p. 989.

[39] Malakouti, S.M., et al., *Reversible inactivation and excitation of nucleus raphe magnus can modulate tail blood flow of male wistar rats in response to hypothermia*. Iranian Biomedical Journal, 2008. 12(4): pp. 237–240.

[40] Tricoire, L. and Vitalis T., *Neuronal nitric oxide synthase expressing neurons: a journey from birth to neuronal circuits*. 2012; 6: 82

[41] Moreno-López, B., et al., *Nitric oxide is a physiological inhibitor of neurogenesis in the adult mouse subventricular zone and olfactory bulb*. The Journal of Neuroscience, 2004. 24(1): pp. 85–95.

[42] Lewis, D.A., et al., *Perisomatic inhibition and cortical circuit dysfunction in schizophrenia*. Current Opinion in Neurobiology, 2011. 21(6): pp. 866–872.

[43] Marín, O., *Interneuron dysfunction in psychiatric disorders*. Nature Reviews Neuroscience, 2012. 13(2): pp. 107–120.

[44] O’Dell, T.J., et al., *Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger*. Proceedings of the National Academy of Sciences, 1991. 88(24): pp. 11285–11289.

[45] Koylu, E.O., et al., *Effects of nitric oxide synthase inhibition on spatial discrimination learning and central DA2 and mACh receptors*. Pharmacology Biochemistry and Behavior, 2005. 81(1): pp. 32–40.

[46] Akar, F.Y., et al., *7-Nitroindazole, a neuronal nitric oxide synthase inhibitor, impairs passive-avoidance and elevated plus-maze memory performance in rats*. Pharmacology Biochemistry and Behavior, 2007. 87(4): pp. 434–443.

[47] Liu, V.W. and Huang P.L., *Cardiovascular roles of nitric oxide: a review of insights from nitric oxide synthase gene disrupted mice*. Cardiovascular Research, 2008. 77(1): pp. 19–29.

[48] Mikkelsen, R.B. and Wardman P., *Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms*. Oncogene, 2003. 22(37): pp. 5734–5754.

[49] Ridnour, L.A., et al., *The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful biological situations*. Biological Chemistry, 2004. 385(1): pp. 1–10.

[50] Linares, D., et al., *Neuronal nitric oxide synthase plays a key role in CNS demyelination*. The Journal of Neuroscience, 2006. 26(49): pp. 12672–12681.

[51] Aquilano, K., et al., *Role of nitric oxide synthases in Parkinson’s disease: a review on the antioxidant and anti-inflammatory activity of polyphenols*. Neurochemical Research, 2008. 33(12): pp. 2416–2426.

[52] Kwon, K.J., et al., *Disruption of neuronal nitric oxide synthase dimerization contributes to the development of Alzheimer’s disease: Involvement of cyclin-dependent kinase 5-mediated phosphorylation of neuronal nitric oxide synthase at Ser293*. Neurochemistry International, 2016. 99.
[53] Tajouri, L., et al., Investigation of a neuronal nitric oxide synthase gene (NOS1) polymorphism in a multiple sclerosis population. Journal of the Neurological Sciences, 2004. 218(1): pp. 25–28.

[54] Walton, J.C., et al., Neuronal nitric oxide synthase and NADPH oxidase interact to affect cognitive, affective, and social behaviors in mice. Behavioural Brain Research, 2013. 256: pp. 320–327.

[55] Gao, Y. and Heldt S.A., Lack of neuronal nitric oxide synthase results in attention deficit hyperactivity disorder-like behaviors in mice. Behavioral Neuroscience, 2015. 129(1): p. 50.

[56] Retz, W., et al., Association of a functional variant of neuronal nitric oxide synthase gene with self-reported impulsiveness, venturesomeness and empathy in male offenders. Journal of Neural Transmission, 2010. 117(3): pp. 321–324.

[57] Workman, J.L., et al., Inhibition of neuronal nitric oxide reduces anxiety-like responses to pair housing. Behavioural Brain Research, 2008. 187(1): pp. 109–115.

[58] Tomiga, Y., et al., Effects of environmental enrichment in aged mice on anxiety-like behaviors and neuronal nitric oxide synthase expression in the brain. Biochemical and Biophysical Research Communications, 2016. 476.

[59] Bujalska-Zadrozny, M., et al., Central antinociceptive effect of tapentadol is increased by nitric oxide synthase inhibitors. Behavioural Pharmacology, 2016. 27.
