

Review

Nitric oxide, a biological double-faced janus- Is this good or bad?

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Summary. Nitric oxide (NO) is a biological messenger molecule produced by one of the essential amino acids L-arginine by the catalytic action of the enzyme NO synthase (NOS). The dual role of NO as a protective or toxic molecule is due to several factors, such as; the isoform of NOS involved, concentration of NO and the type of cells in which it is synthesised, the availability of the substrate L-arginine, generation of guanosine 3,5'-cyclic monophosphate (cGMP) from soluble guanylate cyclase and the overall extra and intracellular environment in which NO is produced. NOS activation as a result of trauma (calcium influx) or infection leads to NO production, which activates its downstream receptor sGC to synthesise cGMP and/or leads to protein nitrosylation. This may lead to one or more systemic effects including altered neurotransmission which can be protective or toxic, vaso/bronchodilatation in the cardiovascular and respiratory systems and enhanced immune activity against invading pathogens. In addition to these major functions, NO plays important role in thermoregulation, renal function, gastrointestinal motility, endocrine function, and various functions of the urogenital system ranging from renin secretion to micturition; spermatogenesis to penile erection; and ovulation to implantation and parturition. A schematic summary of the functions of NO and the various isoforms of NOS expressed in body systems is shown in figure 1. In this review, the historical background, biochemistry and biosynthesis of NO and its enzymes together with the mechanism of NO actions in physiology and pathophysiology are discussed.

Key words: Nitric oxide, Good or bad?

Introduction

NO-historical background

Nitroglycerine, the organic nitrate, has been known since 1878 as a therapeutic drug of choice for angina and also as a destructive compound in the hands of terrorists. A review of the literature reveals equal mention of the use of nitroglycerine for destructive activities and for new therapies in diverse conditions ranging from insect bites to restless leg syndrome. Nitroglycerine (later named as glyceryl trinitrate-GTN) was first discovered in 1846 and has been used continuously for the treatment of angina since 1867 (Brunton, 1967; Murrell, 1979; Thatcher et al., 2004). GTN decreases cardiac hypertension by vasodilatation and it is almost universally assumed that this mechanism is mediated by release of nitric oxide (NO). However, the precise mechanism of biotransformation of GTN to NO is still poorly understood despite many years of clinical use.

NO was identified as a biological signaling molecule more than two decades ago, and many of its functions are still controversial or not fully established. During the late 1970s, NO was shown to be a ligand that activates sGC to cause vascular smooth muscle relaxation (Arnold et al., 1977; Craven and de Robertis, 1978). However, NO came into the limelight when Drs. Furchgot, Ignarro and Murad received the Nobel Prize for the identification of 'endothelial derived relaxing factor (EDRF)' as NO. It was additionally noted that NO and EDRF produce a comparable relaxation of vascular smooth muscle and proposed that EDRF is NO or a labile compound releasing NO (Ignarro et al., 1987; Ignarro, 1991; Moncada et al., 1988, 1991). NO research is also well known for having changed the lives of thousands of impotent men around the world through the development of the anti-impotence drug, Viagra, which inhibits the breakdown of cGMP (Luke and Gerald, 2004; Sussman, 2004), thus facilitating prolonged vasodilator action of NO. In 1988, NO was recognized as a messenger molecule in the central nervous system

(CNS) (Garthwaite et al., 1988, 1993). However, NO has actions beyond the CNS and cardiovascular system and these have been the focus of considerable research in recent years (Dejam et al., 2004).

NO- biosynthesis and biochemistry

NO is a simple but unique gaseous molecule and free radical that can serve many diverse functions. NO is produced from L-arginine by NOS. Although NOS enzymes are differentially regulated and found in different cell types (see later), they exhibit similar biochemical properties. Biosynthesis of NO involves oxidation of the guanidino group of L-arginine and gives rise to NO and equal amounts of L-citrulline. This reaction is accompanied by an NADPH-dependent reduction of molecular oxygen (Mayer et al., 1991), which is incorporated into both NO and L-citrulline suggesting a mono- or dioxygenase-like reaction. Two of the three NADPH-derived reducing equivalents seem to be utilized for the initial hydroxylation of L-arginine into N^G-hydroxy-L-arginine. Hence, NADPH can be used as a marker for biological NO activity. The reaction catalyzed by different NOS isoforms is thought to be identical as N^G-hydroxy-L-arginine (L-HOArg) is a substrate for both the inducible and neuronal NOS (Stuehr et al., 1991a; Zembowicz et al., 1991).

The biological actions of NO depend on which reactions it undergoes with the target molecules in the cells, membranes, and the extracellular environment. There are three major forms of nitrogen monoxide (collectively called NO): the nitrosium ion (NO⁺), the neutral free radical (NO^{*}), and the nitroxyl ion (NO⁻), which all have different properties and biological reactivity (Stamler et al., 1992; Stamler, 1994). It is possible that the particular NO form depends on the overall redox (reduction and oxidation) state and may differ between NOS isoforms and perhaps between tissues and organ systems. At neutral pH, NO is likely to be predominantly in the free radical form (NO^{*}). The dominant reactions of NO will be with another free radical such as super oxide ion (O₂⁻), a metal (heme iron) or O₂. These with their secondary reaction products, and products of NO oxidation and reduction are capable of reacting with metals or thiols to give further products, often with specific biological activity (Stamler, 1994). Thus, the biochemistry of NO is complex because of the reactions of NO itself, the interactions of secondary products of NO and the overall chemical environment under which NO is produced.

Nitric oxide synthases

Although various cell types of the mammalian body may express several isoforms of NOS, the commonly expressed three major isoforms are discussed in this review. *Neuronal NOS* {nNOS, also known as NOS I or type I or brain (b) NOS} is constitutively expressed in the cytoplasm of central and peripheral neurons (Bredt et al., 1990; Mayer et al., 1990; Schmidt and Murad 1991;

Thippeswamy and Morris, 1997a,b; Thippeswamy et al., 2001a) and other cell types including kidney macula densa cells (Wilcox et al., 1992), β -pancreatic cells (Schmidt et al., 1992b), skeletal muscle (Nakane et al., 1993), and epithelial cells lining the airways (Kobzik et al. 1993), stomach and uterus (Schmidt et al., 1992b); endothelial NOS (eNOS, also called NOS II or type II) is membrane associated and is expressed constitutively in endothelial cells (Pollock et al., 1991); and inducible NOS (iNOS, also known as NOS III or type III) is induced in immune cells in response to infection (lipopolysaccharide) or cytokines (Hevel et al., 1991; Stuehr et al., 1991a; Yui et al., 1991) and by glia cells of CNS (Khan et al., 2005). Depending on calcium requirement for their activation, these enzymes are further classified as calcium-dependent (nNOS and eNOS) and calcium-independent (iNOS). Molecular cloning of NOS from various sources has revealed considerable sequence similarities between the different isoforms (Xie et al., 1992; Geller et al., 1993).

NO-mechanism of action

As shown in figure 2, there are two major pathways for NO actions: a) autocrine pathway- as an intracellular messenger either by activating sGC thus generating a second messenger, cGMP or by S-nitrosylation of intracellular proteins (Jaffrey et al., 2001; Stamler et al., 2001); b) paracrine pathway- as an intercellular messenger via cGMP and its downstream pathways to regulate neighbouring or more distant cells and tissues. In general, cGMP and its downstream targets mediate the overall physiological functions of NO. Guanylyl cyclase (GC) exists in two major forms; the membrane-bound and the soluble GC (for further details on GC read reviews by Mayer, 2000; Bellamy et al., 2000, 2001, 2002; Gibb et al., 2003). The membrane-bound GC consists of a membrane spanning region, an extracellular domain for binding of atrial natriuretic peptides and an

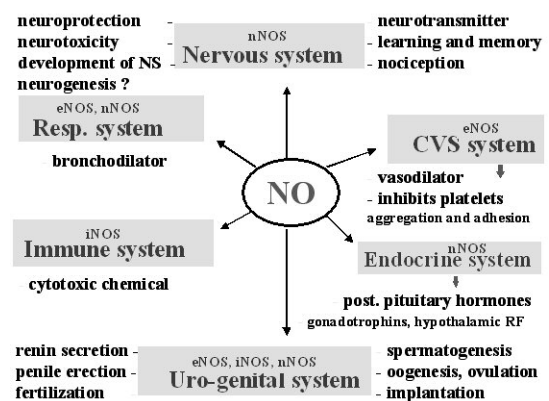


Fig. 1. A schematic representation of the summary of NO function. CVS, cardiovascular system; NO, Nitric Oxide; nNOS, neuronal NO synthase; eNOS, endothelial NO synthase; iNOS, inducible NO synthase; RF, releasing factor.

intracellular catalytic domain (Koesling et al., 1991; Currie et al., 1992; Garbers, 1992). Soluble GC is cytosolic (Koesling et al., 1991) and is the principal target for NO and its progenitors such as sodium nitroprusside, SIN-1 and organic nitrates. As mentioned above, sGC is considered as a receptor for NO. Binding of NO to the heme segment of sGC catalyzes the formation of the second messenger, cGMP from GTP. Cyclic GMP in turn binds to- and activates a cGMP-dependent protein kinase (G-kinase), which phosphorylates specific proteins on serine or threonine residues and also activates phosphodiesterase and ion channels (Mayer, 2000; Gibb et al., 2003; Sager, 2004).

There are three principal downstream targets for cGMP: protein kinase-G (PKG/cGK), cyclic-nucleotide gated ion channels (CNGC) and cyclic-nucleotide phosphodiesterase (PDE). Of these, PKG controls the vast majority of ion channels since both contain many consensus phosphorylation sites (Fischmeister and Mery, 1996; White, 1999; Soderling and Beavo, 2000). These are further discussed under 'NO-functions' in the context of pathophysiology.

In recent years, the cGMP-independent mechanism of NO and protein S-nitrosylation of cysteine thiols have been shown to play vital roles in health and disease (reviewed by Foster et al., 2003). This gave a new twist to the view that the concentration of NO and the isoform of NOS involved determine the toxicity/ protective role of NO. However, it is important to emphasise here that the modification of thiols by NO requires other reactive oxygen species (ROS) or redox species such as O_2 , transition metal and electron acceptors, which all induce conformational changes of protein/s required for downstream action of NO (Lai et al., 2001; Stamler et al., 2001). Although ROS are potent inducer of apoptosis, the apoptotic machinery is subject to more complicated redox regulation. NO prevents apoptosis by the suppression of caspases through a cGMP-dependent mechanism (Kim et al., 1997; Thippeswamy et al., 2001b) and by direct inhibition of caspase by protein S-nitrosylation (Mohr et al., 1997; Dimmeler et al., 1997; Ogura et al., 1997; Ahern et al., 2002). A surprising new dimension to NO signaling is the direct (cGMP-independent) action of NO on a number of calcium regulating channel proteins through S-nitrosylation. These include NMDA/AMPA receptors, Ca^{++} -activated K^+ channels, Na^+ channel, L- or N-type Ca^{++} channels depending on the cell types (reviewed by Ahern et al., 2002). By regulating the calcium level, this could be the self-limiting mechanism of NOS inactivation that prevents further NO production.

NO-functions

Nervous system

Neuroprotection or neurotoxicity?

The role of NO in mediating the functions of the

nervous system is widely researched and many issues are still controversial. NO production via nNOS and e-NOS is largely protective while cytokine-induced NO via iNOS is toxic. Despite its highly diffusible nature, NO can exert specific effects within the CNS and PNS. Under physiological conditions, NO produced by neurons (nNOS) facilitates neurotransmitter release and uptake via neuron-glia communication. Many studies relate the effects of NO to its ability to modulate the release of neurotransmitters, such as glutamate, gamma amino butyric acid (GABA), substance P (SP) and acetylcholine (Kiss and Vizi, 2001; Ohkuma and Katsura, 2001; Dinh et al., 2005; McMahon et al., 2005). NO is also considered as an atypical neurotransmitter for three reasons; a) NO cannot be stored in membrane-bound vesicles like classical neurotransmitters, instead has to be produced as and when required, b) NO has no

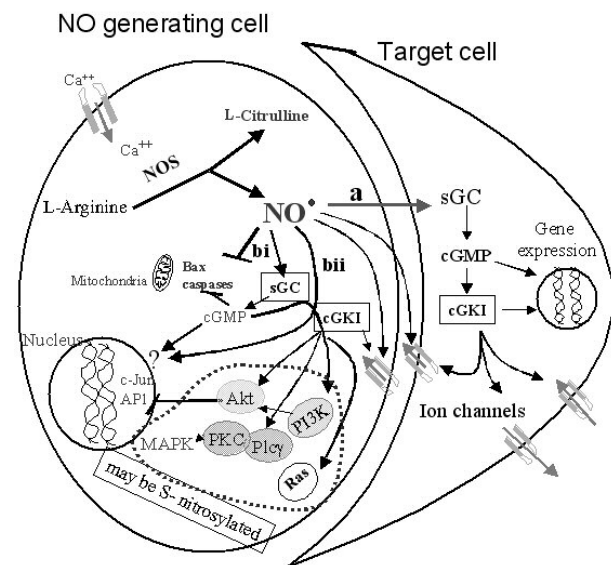


Fig. 2. Diagrammatic representation of the overall mechanism of the NO-cGMP pathway. NO produced from the generator cell (may be a neuron (nNOS) or an endothelium (eNOS) or a macrophage (iNOS)) acts in two major pathways: **a)** Paracrine pathway: NO diffuses from the generator cell and activates sGC in the neighbouring cell (target cell may be glia or smooth muscle) or may be a distant cell in case of neurons via synapses to generate cGMP. Cyclic GMP and/or its downstream cGK modulate gene expression or ion channels depending on the type of cells/tissues/organs. **b)** Autocrine pathway: NO might act within the generator cell via; i) sGC-cGMP pathway, for example, by inhibiting bax and caspases in neurons and/or via ii) s-nitrosylation of ion channel receptors or intracellular proteins (indicated by dots), for example, in the absence/insufficient trophic factor/s (e.g. nerve growth factor for neurons- NO mediates the activation of downstream of NGF signalling pathway to promote cell survival e.g. inhibiting the transcription factors c-Jun/AP1 via Akt). And/or NO might directly influence gene expression for survival or might affect other intracellular messenger molecules such as phospholipase C β (Plc β), protein kinase C (PKC), phosphotydyl triphosphate kinase (PI3K).

specific receptors although the sGC and NMDA receptor are considered as indirect targets, c) NO is not only released by nerve terminals (at synapses), but also from the cell body and axons suggesting non-vesicular release. The binding of glutamate activates NMDA receptors in the post-synaptic neuron that result in Ca^{++} influx, which activates nNOS. Then NO either via cGMP or by S-nitrosylation suppresses NMDA receptor activity (Lei et al., 1992; Manzoni et al., 1992; Ahern et al., 2002) thereby limiting further Ca^{++} influx and thus protecting neurons.

The neurotoxicity of NO in inflammatory and degenerative diseases, including ischemic hypoxia is mainly due to excessive iNOS production by activated microglia, glutamate induced-NO from neurons and excessive ROS production from an inflammation. Overproduction of NO and ROS seems to be responsible for cell death, and conversely, selective inhibitors of NOS decrease neurotoxic damage (Iadecola et al., 1997; Cardenas et al., 1998, 2000). Severe neuronal death occurs in cerebral ischemia as a result of increases in intracellular Ca^{++} . This is considered a critical event in neuronal damage in a wide variety of other pathological conditions, including oxidative stress and glutamate-induced neurotoxicity. Impairment of mitochondrial function with decreased ATP synthesis and induction of apoptosis have been suggested as sequence of events that occurs under these circumstances (Madl and Burgesser, 1993; Simpson and Isacson, 1993; Sundkvist et al., 2003).

The precise mechanisms of NO-mediated neuroprotection or neuronal cell death are becoming clearer. These include i) direct activation or inhibition of signalling or metabolic pathways (Stamler, 1994; Zhang et al., 1994; Thippeswamy et al., 2001b, 2005a), ii) reaction of NO with super oxide to form the highly reactive peroxynitrite anion which blocks cell's respiratory chain (Lipton et al., 1993; Estevez et al., 1995; Mitrovec et al., 1995; Troy et al., 1996). Several studies have focused on the involvement of caspases (cystein-aspartate-proteases), which form a complex family of enzymes. Caspase 1 (interleukin-1 β converting enzyme, ICE) and caspase 3 (CPP32, apopain, YAMA) have been implicated in neuronal apoptosis (Deshmukh et al., 1996; Stefanis et al., 1996; Yakovlev et al., 1997; Thippeswamy et al., 2001b, 2005a). Recent literature also suggests the involvement of other caspases in neuronal apoptosis, for example, caspase 7 is dramatically up regulated in both neurons and astrocytes within 5 days following traumatic brain injury (Larner et al., 2005). Furthermore, nNOS gene knockout mice are resistant to focal and transient global ischemia (Huang et al., 2002) suggesting a protective role for nNOS in CNS. The NO role in the ceramide-Jun kinase pathway (Park et al., 1996; Willaime-Morawek et al., 2003) and the NF- κ B-Jun kinase pathway that activates a large number of genes in both neuroprotection and neurotoxicity (O'Neill and Kaltschmidt, 1997; Barger et al., 2005) has also been reported.

Development and postnatal nerve injury

Given the unconventional properties of NO, it is possible that NO diffuses from the generator cell to regulate the growth, development and possibly function of neighbouring cells or tissues. If NO produced by a group of cells, act additively within a limited volume, this could contribute to the synchronization in development of precursor cells (Penunova and Enikolopov, 1995). This also may explain the crucial role played by NO in development of the nervous system. NO-regulated mitogenesis leads to initial rapid proliferation of neural and glial stem cells. However, this has to be followed by controlled cessation in order to prevent excessive growth. Immunohistochemical studies have revealed transient expression of nNOS in discrete areas of the developing rat nervous system (Bredt and Snyder, 1994). In the brain, nNOS expression occurs selectively in the majority of cells in the cerebral cortical plate at E15-19. The cells in the cortical plate send their processes to the thalamus and this innervation gradually decreases nNOS after birth and is absent in adults. Similarly, in the olfactory epithelium and sensory ganglia, nNOS expression occurs prominently in neurons from E12 to early postnatal life. In embryonic sensory ganglia virtually all neurons express nNOS, whereas in adults only 5% express nNOS (Bredt and Snyder, 1994; Thippeswamy et al., 2005b). Injury to peripheral sensory fibres or nerve growth factor deprivation in culture causes increased nNOS expression in sensory ganglion neurons and the subsequent NOS block causes apoptosis of neurons suggesting the protective role of NO in nerve injury (Verge et al. 1992; Fiallos-Estrada et al. 1993; Thippeswamy and Morris, 1997a,b; Thippeswamy et al., 2001b; 2005b). Similarly, following olfactory bulbectomy of adult rats, newly developing olfactory neurons express nNOS as they extend processes to replace those lost by bulbectomy (Bredt and Snyder, 1994). After three weeks of target innervation, however, these neurons lose their NOS expression. Thus, in the olfactory system and in other parts of the nervous system, transient NOS expression is a feature both of ontogeny and of the response to injury.

Being a diffusible gas, NO may also be suited to signaling and maintaining the ganglion/nuclei in a cluster. It may also act as a neuron-glia signaling molecule causing the glioblasts/glia to produce substances locally, for example epidermal growth factor which aids neuronal survival (Nelson et al., 2004). It is also known that NO switches or arrests dividing cells and initiates differentiation (Fayad et al., 1997; Hemish et al., 2003). It has been shown that nNOS is involved in the differentiation of neural precursor cells derived from the neural tube in the mouse, and in the human neuroblastoma cell line (Ogura et al., 1997). A NO donor compound, sodium nitroprusside induced differentiation of PC12 cells (Penunova and Enikolopov, 1995). Hence, NO might be involved in terminal differentiation of neuroblasts in the developing dorsal

root ganglion (DRG) neurons. In addition, Hindley et al. (1997) and Yamazaki et al. (2004) have demonstrated the involvement of NO and cGMP in neurite growth. Our preliminary data also supports NO induced neurite growth in NGF-deprived cultures and in DRG cultures treated with physiological concentrations of exogenous NO. At present, one can only speculate about the roles of NO in prenatal development. However, nNOS knockout mice develop normally which could be due to the compensatory mechanism of NO produced by other isoforms of NOS (Thomas et al., 2003).

Neurodegenerative diseases

Although there are several forms of neurodegenerative disorders, only classical diseases in which NO has a major role are discussed here. Alzheimer's disease (AD) is the most common chronic progressive neurodegenerative condition originally described by Alzheimer about a century ago. Clinically, AD is characterized by progressive loss of memory, intellectual and emotional malfunction (Selkoe, 1994; Terry, 1994) associated with changes in neuroendocrine and autonomic functions (Raskind et al., 1982; Separ and Gerner, 1982; Balldin et al., 1983; Christie et al., 1987; Iacono and Sandyk, 1987). Morphological changes in AD include: cerebral atrophy and astrogliosis with plaques containing β -amyloid protein (often called neurofibrillary tangles, NFT) (Bondareff et al., 1990; Caputo et al., 1992; Novak et al., 1993). These plaques cause neuronal cytoskeleton and synapse disruption and neuronal death leading to cortico-cortical and cortico-spinal disconnection (Selkoe, 1994; Terry, 1994; Jellinger and Bancher, 1998; Malchiodi-Albedi et al., 2001).

Several studies suggest that NOS containing neurons are resistant to neurodegenerative processes in the caudate putamen (one of the basal nuclei of the brain) in Huntington disease and in the hippocampal formation in AD (Hyman et al., 1992). It has been shown that plaque mediated β -amyloid induces NOS in neurons (Luth et al., 2000; Hartlage-Rubsamen et al., 2001) suggesting a possible protective role played by nNOS. However, Sohun et al. (1999) have shown that eNOS is responsible for the major abnormalities in several important neurodegenerative diseases, including AD. Overall the increased NO from different sources could contribute to progressive neurodegeneration, through protein nitration by reactive peroxynitrite. Peroxynitrite alters the physical and chemical nature of membrane-bound and cytosolic proteins leading to mitochondrial/oxidative damage observed in most neurodegenerative disorders (Koppal et al., 1999).

Parkinson's disease (PD) is another common senile neurodegenerative condition associated with selective loss of dopaminergic neurons in the substantia nigra of the midbrain region. As in other classical neurodegenerative diseases, oxidative stress and mitochondrial dysfunction cause the death of

dopaminergic neurons. It has been shown that NO generated by both iNOS and nNOS appears to contribute to this pathology. Experimental models such as NOS inhibition in wild type mice or mice in which the iNOS or nNOS gene has been knocked out protect dopaminergic neurons following challenge of these mice with a PD-inducing drug, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Klivenyi et al., 2000; Antunes et al., 2005).

Learning and memory

Synaptic plasticity is an important process involved in learning and memory and includes remodelling of existing synapses and generation of new synapses to replace old or non-functional synapses, and long term potentiation (LTP) (Benson et al., 2001; Garner et al., 2002; Yamagata et al., 2003). Though several endogenous molecules are involved in these processes, NO is particularly well suited for these type of functions. As a diffusible gas, NO acts as 3-D messenger molecule to influence a larger area including pre- and postsynaptic neurons and the surrounding glia cells. Kirchner et al. (2004) have also demonstrated that the absence of the neuronal source of NO in nNOS knockout mice severely affects their cognitive functions such as learning and memory. In several other studies using different learning models and species, it is becoming clear that nNOS-mediated NO at physiological concentrations promotes learning and memory function (Monfort et al., 2004; Prickaerts et al., 2004; Weitzdoerfer et al., 2004). However, it has recently been shown that during synaptic remodeling in the adult CNS, neuronal NO causes synaptic detachment via S-nitrosylation-mediated mechanism and also inhibits synapse formation by cGMP-dependent mechanism (Sunico et al., 2005).

Epilepsy

The importance of NO in learning, memory and dementia imply that NO signalling influences the functions of hippocampus and associated brain regions. Since these brain regions are also linked to motor function, understanding the role of NO in epilepsy is also gaining prominence. Regarding the role of NO in epileptogenesis, there have been contradictory reports that NO acts as an anti-convulsant or as a pro-convulsant, depending on seizure stimulus, the cellular source of NO, and the activation of specific NOS isoforms (Kaneko et al., 2002). However, these conclusions are controversial (De Sarro et al., 1991; Herberg et al., 1995; Kaputlu and Uzbay, 1997; Przegalinski et al., 1996; Bashkatova et al., 2000; de Vasconcelos et al., 2000). The controversy is due to the complex interaction between the excitatory and inhibitory neurotransmitter pathways of the CNS. In CNS, release of the excitatory amino acid, glutamate, by presynaptic neurons is regulated by GABAergic (GABA-gamma amino butyric acid) inhibitory input to

presynaptic neurons. The binding of glutamate activates N-methyl-D-aspartate (NMDA) receptors of the postsynaptic neuron leading to the activation of Ca^{++} -calmodulin-dependent nNOS to generate NO. It has been hypothesized that the downstream effects of NO modulate NMDA receptor function to prevent further calcium influx and/or suppress further glutamate release from the presynaptic neuron thus preventing seizures (Klamer et al., 2005; Takata et al., 2005). The other hypothesis is that NO could also regulate GABAergic input to presynaptic neuron thus enabling NO to function indirectly as a proconvulsant (Proctor et al., 1997; Rajasekaran, 2005).

Sleep

Recent studies using specialised techniques such as Volta metric measurement of NO and single cell recording, combined with animal models in which NOS isoforms have been either deleted or mutated, all suggest that NO plays a crucial role in sleep (Brabely and Ackerman, 1999; reviewed by Gautier-Sauvigne et al., 2005). Studies from transgenic mice, in which either nNOS or iNOS gene has been deleted, confirmed that it is the concentration of NO and its associated regulators (possibly directly influenced by the isoform of NOS involved) that determine the duration of rapid eye movement (REM) sleep (Chen et al., 2003). REM sleep was significantly shorter in nNOS knockout mice while it was higher in iNOS knockouts compared with appropriate wild type control mice suggesting the complexity of NO's role in sleep and awake (Burllet et al., 1999).

Pain

Pain, is an important symptom of inflammation, which is a major clinical problem in several chronic diseases such as arthritis and cancer-related pain syndromes. For a decade, several studies have shown that the central release of glutamate and substance P induces nitric oxide release that in turn modulates the pain processing mechanism in central neurons (McMahon et al., 2005). In rats treated with carrageenan or prostaglandin, hyperalgesia is induced, and this could be blocked by NOS and sGC inhibitors (Duarte et al., 1990; Ferreira et al., 1991; Ventura-Martinez et al., 2004) suggesting the anti-nociceptive role of NO and cGMP. It has also been shown that the NO-cGMP pathway mediates systemic anti-nociceptive effects, which are independent of opioid receptors (de Moura et al., 2004).

Behaviour- Aggression, depression and psychiatric disorders

NO modulates behaviour including depression and anxiety, cognition and emotional status. This is being supported by increased plasma nitrate (NO-metabolic

end product) contents in depressed patients (Suzuki et al., 2001). NOS inhibitors, such as 7-nitroindazole and 1-(2-trifluoromethylphenyl)-imidazole (TRIM) have been tested in animal models as anti-depressants and anti-anxiolytics (Volke et al., 1997, 2003; Yildiz et al., 2000). It has also been shown that anti-depressant drugs such as Trazodone, a potent 5-HT_{2C}-receptor agonist, acts by inhibiting the N-methyl-D-aspartate/nitric oxide/cyclic GMP pathway in rat cerebellum (Maura et al., 1995; Marconi et al., 1998).

Cardiovascular system

For more than a century, nitroglycerine has been used as a drug of choice for angina patients. Nitroglycerine releases NO that leads to vasodilatation. In the past two decades, scientists have discovered eNOS expressed by the endothelial cells lining the blood vessels. The NO-cGMP mediated mechanism of vascular relaxation is being utilised as the basis of new treatments to promote vasodilatation. Influx of calcium in endothelial cells activates eNOS to produce NO that diffuses and activates sGC in smooth muscles to generate cGMP. The concentration of cGMP in a cell is determined by the presence of phosphodiesterase (PDE), which breaks down cGMP (Conti and Jin, 1999). The intracellular action of cGMP, i.e. smooth muscle relaxation, is primarily mediated by cGMP-dependent protein kinase type Ia (PKG/cGK Ia), which phosphorylates myosin light chain. In addition to PKG/cGK several types of cyclic nucleotide-activated ion channels also appear to be involved (Finn et al., 1996; Lincoln et al., 2001). A similar mechanism is proposed for NO-cGMP mediated anti-hypertensive effect and also for pulmonary vascular relaxation, which is essential for neonatal lung development and function (Moreno et al., 2004). Viagra (sildenafil), which hit the headlines of many journals and newspapers a couple of years ago, is a PDE type 5 inhibitor that delays the hydrolysis of cGMP thus facilitating vasodilatation, erectile tissue congestion and prolonged penile erection (Castro et al., 2004).

NO plays an important role in platelet aggregation and adhesion. Platelets are activated in diabetes and hypertension and they contain all components of the L-arginine-nitric oxide pathway (McGrath et al., 2002). In diabetic patients, activated platelets produce excess amounts of super oxide that results to increased susceptibility of these patients to thrombosis (Yamagishi et al., 2001). Dixon et al. (2003) have shown that iNOS uncoupling in platelets causes congestive cardiac failure, thus demonstrating a functional relationship between endothelium and NO-mediated vasodilatation. NO on its own (in the absence of super oxide ions) has an anti-thrombotic property, including inhibition of the production of tissue factor and of plasminogen activator inhibitor by endothelial cells, as well as suppression of platelets aggregation (Yang and Loscalzo, 2000; Fiorucci et al., 2002b; Perez-Ruiz et al., 2002).

Respiratory system

NO has a major role in the pathophysiology of respiratory functions. Asthma and chronic obstructive pulmonary disease patients tend to have bronchoconstriction, airway hyper reactivity (AHR), increased exhaled NO and pulmonary inflammation characterized by eosinophil and neutrophil influxes (Saleh et al., 1997; Ichinose et al., 2000). NO appears to have a bronchodilator effect especially in the upper airways in animal models (Prado et al., 2005), which appears to be mediated by cGMP (Toward et al., 2004). At physiological concentration, NO functions as anti-inflammatory agent by suppressing leukocyte activation and vascular permeability and thus oppose AHR and maintain smooth muscle tone (Barnes et al., 1999; Trophy et al., 2000). High concentrations of NO, derived from iNOS, however, favour inflammation via the development of Th2-lymphocyte responses (for example, priming of mast cells by eosinophils and IgE) and by increasing microvascular leakage (Trophy and Page, 2000). Inflammation may also be exacerbated through the formation of cytotoxic peroxynitrite from excessive NO and inflammatory-derived super oxide (Nijkamp and Folkerts, 1995; Ichinose, et al., 2000). Therapeutic interventions would include suppression of pro-inflammatory mediators responsible for iNOS induction or by promoting elevated cGMP levels (for example, PDE inhibitors) that will have negative feedback for NO overproduction. Endothelial NOS also plays a major role in maintaining pulmonary vascular tone and lung development during later stage of foetal development and early neonatal period (Miller et al., 2005; Zhao et al., 2005).

Immune system

Immune cell-mediated NO synthesis has a central role in defensive mechanisms, for example, inflammation mediated by cytokines and immunomodulatory activity including tumour immune rejection (Bogdan, 2001). NO is involved in immune regulation by limiting T-helper cell type 1 (Th1) responses and inducing expression of Th2-derived cytokines IL-4 and IL-10 (Wei et al., 1995). In the immune system, high concentrations of NO induce apoptosis as a result of formation of peroxynitrite (Brown and Broutaite, 2002; Radi et al., 2002). This is also thought to be important for tumour cell killing processes during tumour immune rejection. The immunosuppressive role of NO has been demonstrated *in vivo* in different experimental models of immune-mediated diseases, such as autoimmune nephritis (Gabbai et al., 1997), autoimmune encephalomyelitis (Van der Veen, 2001), and graft-vs-host (Bobe et al., 1999). The role of NO in regulating intestinal inflammation, however, is controversial, and both protective and detrimental effects have been reported (Hatoum, et al., 2003; Lamine et al., 2004). In recent

years, several studies have shown that the addition of an NO-releasing moiety to conventional drugs, such as aspirin and other anti-inflammatory analgesic drugs, confers new and potent immunomodulatory activities on Th1 function, configuring a new family of drugs (Cicala et al., 2000; Fiorucci et al., 2000, 2002). For example, an NO derivative of aspirin, in contrast to the standard molecule, is able to protect mice against concanavalin A-induced hepatitis by inhibiting Th1 function (Fiorucci et al., 2000).

Endocrine system

Several studies in recent years suggest that the NO-cGMP signaling pathway plays an important role in gonadotrophin-releasing hormone (GnRH) from the hypothalamus. The combination of estradiol and progesterone increases the expression of nNOS protein in the pre-optic area and hypothalamus; however, these hormones had little effect on the abundance of sGC (Chu et al., 2004). NO inhibits basal and ACTH (adrenocorticotrophic hormone)-or angiotensin II-stimulated aldosterone synthesis in glomerulosa cells from bovine adrenal gland (Sainz et al., 2004). NO is also implicated in the regulation of energy metabolism, possibly through the enhancement of mitochondria formation (Nisoli et al., 2003) or via the regulation of metabolic processes such as adipose tissue lipolysis (Fruhbeck et al., 2001) and glucose transport (Tanaka et al., 2003). The expression of eNOS, and to a lesser extent of iNOS, in omental and subcutaneous adipose tissues in adults, and a significant increase in eNOS expression in the omental tissues of obese patients suggest the positive role of NO in obesity (Ryden et al., 2001).

Recent studies have demonstrated that under certain circumstances, iNOS can become enzymatically uncoupled from its cofactors to produce super oxide rather than NO. This phenomenon has been described in animal models of hypertension (Landmesser et al., 2003) and in diabetic human endothelial cells (Guzik et al., 2002). Overall, events leading to activation or deregulation of macrophages triggers series of events such as the development of type I diabetes, which is insulin dependent (IDDM). Activated macrophages produce cytokines such as IL-1 β and TNF- α and iNOS mediated NO, all cause inflammation of cells in the Islets of Langerhans of pancreas, thus affecting the insulin production leading to IDDM (Corbett and McDaniel, 1992; Rabinovich, 1993; El-Mahmoudy et al., 2005).

Urogenital System

Urinary system

All three NOS isoforms are expressed in the renal system. In renal cortex, macula densa cells express nNOS; mesangial and proximal tubule cells express iNOS; afferent and efferent arterioles and glomerular

capillaries express eNOS. NO and its interaction with super oxide ion regulate renal blood pressure and renal functions such as glomerular filtration rate. NO has protective effects against ischemia/reperfusion induced renal dysfunction and tissue damage, probably through the suppression of endothelin-1 overproduction in post-ischemic kidneys (Kurata et al., 2004). Recently rodent models of nNOS and eNOS deficient mice have demonstrated that NO has a permissive role in the macula densa and in the control of renin secretion (Castrop et al., 2004).

The urine storage ability of the urinary bladder is markedly impaired following inflammation of the urinary bladder and spinal cord injury because of a hyperexcitability of micturation reflexes. It is emerging, that in addition to nNOS, neuropeptides such as substance P, calcitonin gene-related peptide and galanin are involved in inflammation-induced bladder at the primary afferent level as well as the postganglionic efferent level (Callsen-Cencic and Mense, 1999; Zvara et al., 2004).

Male reproductive system

Sexual dysfunction is a significant medical problem that adversely affects health, well-being, quality of life and interpersonal relationships. One of the commonest sexual dysfunctions in males is penile erectile dysfunction, which is due to biochemical and physiological deregulation of erectile tissue trabecular (vascular) smooth muscle function (Lue and Tangho, 1987; Anderson and Wagner, 1995). Studies from both male and female genital tissues indicate that the NO-cGMP pathway is an important regulator of blood flow and engorgement during sexual arousal (Feldman et al., 1994). The discovery of the anti-impotence drug, Viagra (sildenafil, PDE type 5 blocker) inhibits the breakdown of cGMP (Luke and Gerald, 2004; Sussman, 2004), thus facilitating prolonged vasodilatation and penile erection (Castro et al., 2004). NO donor treatment also increases seminal vesicle secretion (Machtens et al., 2003) suggesting that NO has an important role in sexual arousal. Immunoreactivity for nNOS and eNOS, sGC-cGMP, calmodulin, cGK type II and glutamate in the lamina propria of human seminiferous tubules all indicate that the contractility of myofibroblasts could facilitate spermeation (the process of release of mature sperms from the epithelium lining the semeniferous tubules).

Female reproductive system

The NO-cGMP signalling regulates the functions of female reproductive organs by modulating the hormones during different stages of sexual development, oestrous cycle, ovulation, fertilization, implantation, and during pregnancy followed by initiation of uterine muscle contraction during delivery. Of the three well-known isoforms of NOS, nNOS and eNOS appear to play a

major role in mediating functions of female reproductive system. However, in porcine ovary, all three isoforms of NOS are expressed. Immunohistochemical studies have demonstrated the presence of nNOS and eNOS in the surface epithelium, stroma, oocytes, thecal cells, and endothelial cells of blood vessels. The granulosa cells of secondary and tertiary/mature follicles express nNOS and iNOS. In addition, iNOS was detected in the surface epithelium, oocytes, and theca of multilaminar and antral follicles (Kim et al., 2005). Since eNOS regulates the blood flow to the reproductive organs, prolonged inhibition of NO synthesis in pregnant rats reduces the overall foetal growth and litter size (Fernandez Celadilla et al., 2005). Several mechanisms have been proposed to explain what is known as 'maternal tolerance to the foetus', which is now broadly attributed to the placental source of NO. There are many data showing that NO affects the CD95/CD95-L and the balance between TH1/TH2 (Gonzalez et al., 2004). In women with pre-eclampsia, the relaxation of vascular smooth muscles might be attenuated because of the reduced action of endothelial NO or cGMP rather than the decrease in the production of NO (Suzuki et al., 2000; Yamamoto et al., 2005). Overall these observations suggest that NO-cGMP play an important role in mediating the functions of female reproductive organs.

In conclusion, NO being a highly reactive gas, it is capable of regulating various body functions either directly or via cGMP-mediated mechanism. NO produced under physiological concentration is beneficial, however, excessive NO production under pathological conditions could be destructive. Application of appropriate pharmacological inhibitors/NO-donors and/or recombinant viral vectors targeting the gene of interest of NO-cGMP pathway can be utilised for therapeutic purposes.

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