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Physiological and Pathophysiological Functions of Nitric Oxide

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Abstract

Nitric oxide is a ubiquitous mediator with diverse functions. It is generated from L-arginine by nitric oxide synthase, a remarkable regulatory molecule and an enzyme that occurs in endothelial, neuronal and inducible isoforms. In this review, we concentrate on general aspects of NO, especially recent evidence that it can act as a circulating as well as a local mediator, plays an important role in various physiological and pathophysiological functions and conclude with a brief consideration of the therapeutic potential of nitric oxide.
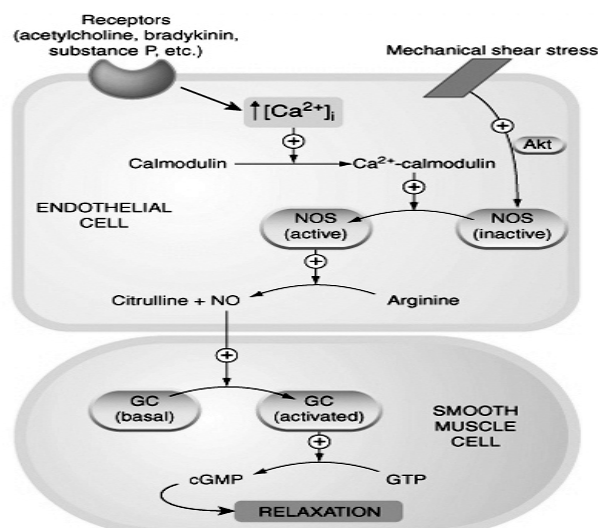
Key words: Nitric oxide, Nitric oxide synthase, L-arginine, Physiology, Pathophysiology
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INTRODUCTION

Nitric oxide (NO) is gaseous signaling molecule that readily diffuses across cell membranes and regulates a wide range of physiologic and pathophysiologic processes including cardiovascular, inflammation, immune and neuronal function. NO is synthesized from L-arginine in endothelial cells in response to various physiological stimuli.[1-2] Nitric oxide is biosynthesized by a family of enzymes that are collectively called nitric oxide synthase (NOS). The enzyme NOS is found in three isoforms neuronal NOS or nNOS or NOS-I, inducible NOS or iNOS or NOS-II and endothelial NOS or eNOS or NOS-III. These isoforms are heme-containing dimeric flavoproteins employing L-argenine as a substrate and requiring NADPH, flavin adenine dinucleotide and tetrahydrobiopterin as cofactors.[3] NO is inactivated by combination with the haem of haemoglobin or by oxidation to nitrite and nitrate, which are excreted in urine. NO is unstable but can react reversibly with cysteine residues (e.g. in globin or albumin) to form stable nitrosothiols; as a result, red cells can act as an O2-regulated source of NO. NO released in this way escapes inactivation by haem by being exported via cysteine residues in the anion exchange

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protein in red cell membranes.[4] Nitric oxide (NO) commonly acts by combining with haem in guanylate cyclase, activating the enzyme, increasing cGMP and thereby lowering [Ca2+], combining with haem groups in other proteins (e.g. cytochrome *c* oxidase), combining with superoxide anion to yield the cytotoxic peroxynitrite anion and by nitrosation of proteins, lipids and nucleic acids.[5-6]

Figure -1 Biosynthetic pathway of NO

Effects of NO include vasodilatation, inhibition of platelet and monocyte adhesion and aggregation, inhibition of smooth muscle proliferation, protection against atheroma, synaptic effects in the peripheral and central nervous system, host defence and cytotoxic effects on pathogens and cytoprotection. Either reduced or increased NO production can contribute to disease. Underproduction of neuronal NO is reported in babies with hypertrophic pyloric stenosis. Endothelial NO production is reduced in patients with hypercholesterolaemia and some other risk factors for atherosclerosis, and this may contribute to atherogenesis. Elevated levels of NO produced within the central nervous system (CNS) are associated with the pathogenesis of neuro-inflammatory and neuro-degenerative human diseases such as multiple sclerosis, HIV, dementia, brain ischemia, trauma, Parkinson's disease, and Alzheimer's disease.[4-6]

Role of nitric oxide in cerebro-cardiovascular system:
Endothelium dependant relaxing activity of NO
Since the determination that EDRF is NO,[7-8] NO has been reported as both a second messenger and neurotransmitter and has been implicated in an extraordinarily diverse range of physiological functions.[5-6, 9] Studied nature of the "blood vessels relaxing factor" derived from endothelium that was identified as nitric oxide, caused intensive scientific research on nitric oxide regarding some aspects of its impact on human physiological and pathological processes. The most important vasodilator and the main substance produced by the endothelium is nitric oxide hence endothelium plays a key role in vasodilation. This led to the proposal of the existence of endothelium-derived relaxing factor, or EDRF.[10] Subsequent pioneering work led to the identification of EDRF as nitric oxide.[11-14] Pharmacologic inhibitors of NOS such as L-

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nitro-arginine (L-NA) and L-*N*-arginine-methyl-ester (L-NAME) also abrogate vasodilation to acetylcholine, and in their presence, acetylcholine actually causes a slight increase in vascular tone.

Blood Pressure

Pressor responses induced by systemic inhibition of NO synthesis may be attributed primarily to the vasoconstriction of peripheral resistance arteries and compensated suppression of sympathetic outflow through the baroreceptor reflex. Central administration of a NOS inhibitor acted at the central nervous system to augment sympathetic outflow, leading to an increase in blood pressure. NO inhibition increases blood pressure but decreases heart rate, muscle sympathetic nerve activity, and plasma norepinephrine levels in humans.[15,17] Habler *et al*.[16] also reported that intravenous injection of L-NAME increases arterial pressure and decreases postganglionic sympathetic nerve activity in anesthetized rats. There are several interacting homeostatic regulators of blood pressure, including the renin–angiotensin system, the autonomic nervous system, and local mediators such as EDRF. L-NA and other NOS inhibitors cause a rise in blood pressure in many species, including rats, guinea pigs, rabbits, dogs and mice.[18] This effect is consistent with the predicted role for basal NO production in the regulation of blood pressure.

Vascular damage and atherosclerosis

Clinical studies have reported that atherosclerosis, hypertension, and hypoxia are related to the NO system[19,20] and that NOS inhibitors may play a role in some cardiovascular regulation. Atherosclerosis is driven by biochemical, cellular, and hemodynamic forces in the vessel wall that cause vascular injury, ultimately leading to endothelial dysfunction, cellular proliferation, recruitment of inflammatory cells, and accumulation of oxidized LDL.[21] The response of blood vessels to injury is formation of neointima. Vascular smooth muscle cells proliferate in the medial layer and migrate across the internal elastic lamina to form the neointima. NO suppresses smooth muscle proliferation in response to vessel injury,[22] suggesting that it normally serves a protective role. In association with other effects such as inhibition of platelet aggregation and adhesion and inhibition of leukocyte activation and adhesion,[23-24] NO normally suppresses the processes that lead to the development of atherosclerotic plaques. NO is critical to the pathophysiology of vascular disease and the concept of endothelial dysfunction. Endothelial dysfunction is defined as impairment of physiologic endothelium-dependent relaxation. It occurs in atherosclerosis, hypertension, diabetes, hypercholesterolemia, and normal aging.[25-27] Impairment of endothelial function occurs before structural changes such as intimal hyperplasia or lipid deposition. This is therefore an early event in the pathophysiology of atherosclerosis. Clinically, endothelial function can be tested by using ultrasound to determine the forearm blood flow response to reflow hyperemia.

Neuronal damage

The neuronal damage that accompanies cerebral ischaemia involves an excessive release of glutamate and a subsequent activation of N-methyl-d-aspartate (NMDA) receptors that, if maintained for a sufficient period of time, induces a massive influx of Ca2+ into the postsynaptic neuron which, in turn, triggers the activation of nNOS and overproduction of NO. In contrast, NO produced by activation of eNOS,[28-29] and even NMDA receptors,[30-31], plays a protective role in brain ischaemia by maintaining regional cerebral blood flow. The first

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indications that NO could mediate neurotoxic effects came with the discovery that inhibition of NOS attenuates glutamate toxicity in primary neuronal cultures from the rat cerebral cortex[33] and induces neuroprotection in animal models of stroke.[32]

Cannabinoid regulation
Activation of cannabinoid receptors by the endocannabinoid, anandamide, in the median eminence led to NO production, which could be curtailed by inhibition of NOS activity by L-NAME. NO signaling has been shown to be involved in cannabinoid-mediated hypothermia and catalepsy.[34] Furthermore, co-treatment of rats with L-NAME and WIN 55,212-2 (a potent cannabinoid receptor agonist) enhanced hypothermia through a synergistic effect.[35] In chronic treatment experiments, repeated dosing of L-NAME inhibited the development of tolerance to the hypothermic and cataleptic effects of WIN 552,12-2.[36] Cannabinoids have been shown to protect against NMDA receptor-mediated neurotoxicity of retinal[38] and cerebrocortical neurons.[37]

Cerebreal learning
Recent studies have shown that multiple internal models are acquired in the cerebellum, and that these can be switched under a given context of behavior,[40] so that, for example, we can walk on stairs or on escalators without losing balance. Contextual information, which consists of various modalities of afferent signals from the entire body and efferent signals from the cerebrum, is transmitted to the cerebellar cortex via dentate granule cell axons also called mossy fibers (MFs) and parallel fibers (PFs).[41-43] Contexts may also be processed in the upstream cerebral regions, such as the superior parietal lobe, the occipital lobe, and the middle temporal lobe. The combination of contexts and tasks is thought to enable the cerebellum to acquire and switch between multiple internal models in a context-dependent manner.[39,42-43] Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry and nNOS immunohistochemistry have been used to detect NOS neurons in rat brain. Results demonstrated that expression of NOS neurons in dentate gyrus and frontal cortex was significantly increased in earlier and later memory stage, after a water-rewarded spatial alternation task when compared with that after sham training and the expression location and cell counts of NOS neurons in dentate gyrus and frontal cortex with NADPH-d staining or nNOS immunoreactivity resembled each other, but the cell counts of NADPH-d positive neurons were a little more than those of nNOS immunoreactive neurons.[44] In an investigation of the effects of L-NAME, on the performance of rats in a radial arm maze and in habituation tasks, and on monoamine metabolism in the brain, results suggest that, NO may play an important role in performance during the acquisition, but not retention, of the radial arm maze task, and that endogenous NO may be involved in the regulation of monoamine metabolism.[45]

Glial Cells
At low concentrations, NO plays a role in neurotransmission and vasodilation, while at higher concentrations, it is implicated in having a role in the pathogenesis of stroke, demyelination, and other neurodegenerative diseases.[46-47] The iNOS, expressed in various cell types, including astroglia and microglia of the CNS, in response to wide variety of stimuli, is, however, regulated mainly at the transcriptional level and does not require calcium for its activity.[48] Astroglia and microglia in the healthy brain do not express iNOS but following ischemic, traumatic, neurotoxic, or inflammatory damage, reactive astroglia and microglia express iNOS in mouse,

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rat, and human.[48] NO derived from activated glial cells is assumed to contribute to oligodendrocyte degeneration in demyelinating diseases (e.g., multiple sclerosis, experimental allergic encephalopathy, and X-adrenoleukodystrophy) and neuronal death during ischemia, trauma, and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, HIV-associated dementia, Huntington's disease, and amyotrophic lateral sclerosis).[49-50]

Astrocytic P2Y Receptors
Astrocytes actively participate in dynamic control of synaptic transmission. Glutamatergic and purinergic receptor-mediated Ca2+ signaling plays the key role in crosstalk between neurons and astrocytes.[51] These neurotransmitters are sensed by astrocytic receptors capable of generating and propagating Ca2+ waves. Several studies have demonstrated the modulatory role of ATP on synaptic transmission. Endogenously released ATP caused homo and heterosynaptic suppression in cultured hippocampal neurons.[52] Synaptic inhibition by exogenous or endogenously released ATP has also been shown in mouse hippocampal slices. It has been shown that ATP modulates neurotransmission by facilitating IPSCs in interneurons. Another study has suggested that ATP can act presynaptically to facilitate or inhibit glutamate release from hippocampal neurons.[53] The mechanisms suggested so far consider direct action of ATP on neuronal P2 receptors and do not fully explain the dynamic regulation of synaptic transmission by astrocytes. Hippocampal neurons in culture form synaptically connected network and exhibit spontaneous synaptic activity. The synchronized Ca2+ oscillations are the faithful indicator of synaptic events and they have been used to study the synaptic modulation. The physiological concentrations of ATP induce a Ca2+-dependent production of nitric oxide. In an investigation of the roles of nitric oxide in astrocytic Ca2+ signaling by exogenous application of a nitric oxide donor, it was found that nitric oxide induced an influx of external Ca2+. This suggest that transmitters that induce Ca2+ signaling in astrocytes lead to the Ca2+-dependent synthesis of nitric oxide. This in turn stimulates a Ca2+ influx pathway that is, in part, responsible for the refilling of internal Ca2+ stores.[54] In an investigation of the mechanism of synaptic suppression by P2Y receptors in mixed hippocampal cultures wherein networked neurons exhibit synchronized Ca2+ oscillations (SCO) due to spontaneous glutamatergic synaptic transmission, pharmacological studies suggested that SCO suppression was mediated by P2Y2/P2Y4 receptors. And it was concluded that under physiological conditions astrocytes use NO as a messenger molecule to modulate the synaptic strength in the networked neurons.[55]

Memory
A new role of the NO-cGMP pathway, namely, stimulation of the cAMP pathway is to induce long-term memory (LTM). The signaling cascade underlying injection of inhibitors of the enzyme forming NO, cGMP, or cAMP into the hemolymph prior to multiple-trial conditioning blocked LTM, whereas injection of an NO donor, cGMP analog, or cAMP analog prior to single-trial conditioning induced LTM. Induction of LTM by injection of an NO donor or cGMP analog paired with single-trial conditioning was blocked by inhibitors of the cAMP pathway, but induction of LTM by a cAMP analog was unaffected by inhibitors of the NO-cGMP pathway. Inhibitors of cyclic nucleotide-gated channel (CNG channel) or calmodulin-blocked induction of LTM by cGMP analog paired with single-trial conditioning, but they did not affect induction of LTM by cAMP analog. The roles of the cAMP pathway in the formation of LTM are often supplemented by other signaling pathways, most notably by the NO-cGMP signaling pathway. [56-57] NO is a membrane-permeable molecule that functions in intercellular signaling in the

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brain.[58] In mice, NO contributes to late-phase long-term potentiation of synaptic transmission by stimulating soluble guanylate cyclase in target cells, and the resulting increase in cGMP concentration stimulates cGMP-dependent protein kinase (PKG).

Role of nitric oxide in gastrointestinal tract:
NO, which is the most important non-adrenergic non-cholinergic (NANC) inhibitory neurotransmitter in the gut,[59] relaxes smooth muscle by activating soluble guanylate cyclase thereby stimulating the formation of cGMP. The major target of cGMP is protein kinase G, which modulates smooth muscle tone by interacting with myosin light chain kinase. In the gastrointestinal tract, cGMP-independent relaxations to NO have been observed in the lower oesophageal sphincter[60-62] stomach[63-64] and duodenum.[65-66] In the earlier studies, the lack of availability of selective inhibitors of guanylate cyclase may be responsible for an incomplete blockade of nitrergic relaxations. Methylene blue and cystamine, for instance, have been extensively used as modulators of NO-stimulated guanylate cyclase, but these compounds lack the specificity of the recently developed guanylate cyclase blockers such as ODQ (1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one).[67] Dysfunction of the inhibitory NANC nerves in the lower oesophageal sphincter results in the motility disorder, achalasia,[68] and is probably involved in oesophageal spasms and related primary motor disorders in the oesophageal body.[69] Gastric NANC-mediated relaxation following vagal stimulation, food intake or distension of the antrum or duodenum was among the first NANC effects described.[70] Indeed, pyloric hypertrophy and gastric distension are the most prominent abnormalities in nNOS knock out mice.[71] The importance of such inhibitory NANC neurones in human gastric function is illustrated by the frequent complaint by vagotomized patients of epigastric bloating and discomfort after meals, which has been related to an abnormal gastric receptive relaxation.[72-73] Infantile hypertrophic pyloric stenosis has been attributed to a lack of NOS-containing nerves at the pylorus[74] while diabetic gastropathy has been linked to a loss of nNOS that can be treated with insulin and the phosphodiesterase-5 inhibitor sildenafil.[75]

Role of nitric oxide in androgen and erectile dysfunction:
The NOS/cGMP pathway has been deemed critical for erectile function.[79] NO mediates relaxation of the vascular smooth muscle of the resistance arteries of the corpus cavernosum and the trabeculae to facilitate penile erection. In castrated animals, testosterone or 5α-dihydrotestosterone (DHT) administration restored the erectile response and NOS expression in the penis.[76-78,80-85] Phosphodiesterase type-5 (PDE5) hydrolyzes cGMP in vascular and trabecular smooth muscle into GMP. Activation of PDE5 terminates NO-induced, cGMP-mediated smooth muscle relaxation, resulting in restoration of basal smooth muscle contractility and penile flaccidity. In penile tissue, the balance between the intracellular levels of cGMP and GMP is primarily regulated by the activities of NOS and PDE5. Thus, it is likely that any disruption in the expression or activity of these enzymes will lead to pathophysiology. Castration has been shown to reduce the expression and activity of PDE5 in rabbits and rats,[78,87,88] and androgen supplementation has been shown to upregulate the expression and activity of PDE5.[78,86-88] Further, administration of PDE inhibitor alone to medically or surgically castrated animals has little effect on the intracavernosal pressure in response to pelvic nerve stimulation,[87,89] suggesting that androgens are critical not only for regulating NOS activity, but also in modulating PDE5 activity.

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Role of nitric oxide in viral infections:

NO frequently is an important mediator in intracellular inhibition of viral replication, which results in lower viral yields and more efficient host clearance of the infection, hence recovery. There are many pathogens which are not inhibited by NO, however, NO may also contribute to tissue damage, especially if substantial numbers of macrophages are activated, producing large quantities of NO, as in Borna disease[91-92] or HSV-1 pneumonia.[90] Since there are many enzyme inhibitors available, those diseases in which iNOS activity is detrimental may benefit from enzyme antagonism. Host organ tropism also does not predict the selectivity of this response. However, in the case of viral encephalitis due to infection with picornaviruses, rhabdoviruses, HSV-1, or JEV, for instance, activation of nNOS may be lifesaving.

Role of nitric oxide in immunity and inflammation:

Nitric oxide induced by $\gamma\delta$T cells and derived from dietary nitrate may limit microorganism growth.[93] Nitric oxide (NO) is synthesised by many cell types involved in immunity and inflammation. The principal enzyme involved is iNOS which produces high-level sustained NO synthesis. NO is important as a toxic defense molecule against infectious organisms. It also regulates the functional activity, growth and death of many immune and inflammatory cell types including macrophages, T lymphocytes, antigen-presenting cells, mast cells, neutrophils and natural killer cells. However, the role of NO in nonspecific and specific immunity in vivo and in immunologically mediated diseases and inflammation is poorly understood. NO does not act through a receptor-its target cell specificity depends on its concentration, its chemical reactivity, the vicinity of target cells and the way that target cells are programmed to respond. At high concentrations as generated by iNOS, NO is rapidly oxidised to reactive nitrogen oxide species (RNOS) that mediate most of the immunological effects of iNOS-derived NO. RNOS can S-nitrosate thiols to modify key signalling molecules such as kinases and transcription factors. Several key enzymes in mitochondrial respiration are also inhibited by RNOS and this leads to a depletion of ATP and cellular energy. A combination of these interactions may explain the multiple actions of NO in the regulation of immune and inflammatory cells.[94]

Role of nitric oxide in skeletal muscles:

In the past five years, skeletal muscle has emerged as a paradigm of NO function and redox-related signaling in biology. All major NOS isoforms, including a muscle-specific splice variant nNOS, are expressed in skeletal muscles of all mammals. nNOS in particular may show a fast-twitch muscle predominance. Muscle NOS localization and activity are regulated by a number of protein-protein interactions and co- and/or posttranslational modifications. Subcellular compartmentalization of the NOSs enables distinct functions that are mediated by increases in cGMP and by *S*-nitrosylation of proteins such as the ryanodine receptor-calcium release channel[95] and also increases in contraction that are dependent on reactive oxygen intermediates and which are thought to occur through reactions with regulatory thiols on the sarcoplasmic reticulum.[96] Skeletal muscle functions regulated by NO or related molecules include force production (excitation-contraction coupling), autoregulation of blood flow, myocyte differentiation, respiration, and glucose homeostasis.[95] When skeletal muscle is stretched or injured, myogenic satellite cells are activated to enter the cell cycle, divide, differentiate and fuse with muscle fibers to repair damaged regions and to enhance hypertrophy of muscle fibers. This process depends on NO production, metalloproteinase (MMP) activation and release of hepatocyte growth factor (HGF) from the extracellular matrix. Generation of a

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fibrotic scar tissue, with partial loss of function, can also occur, and seems to be dependent, at least in part, on local TGF-β expression, which can be downregulated by NO. Hence, regeneration the muscle depends on the type and severity of the injury, the appropriate inflammatory response and on the balance of the processes of remodeling and fibrosis. It appears that in all these phases NO exerts a significant role.[97]

Role of nitric oxide in respiratory system:
In the respiratory system, this molecule is responsible for maintaining pulmonary vascular integrity.[98] nNOS and eNOS and other NO-adduct molecules (nitrosothiols) have been shown to be modulators of bronchomotor tone. The concentration of this molecule in exhaled air is abnormal in activated states of different inflammatory airway diseases, and its monitoring is potentially a major advance in the management of, e.g., asthma. Finally, the production of NO under oxidative stress conditions secondarily generates strong oxidizing agents (reactive nitrogen species) that may modulate the development of chronic inflammatory airway diseases and/or amplify the inflammatory response.[99] NO improves arterial oxygenation, which may be associated with its action on the distribution of blood flow in the lungs. This property is the basis for inhaled nitric oxide (INO) being employed in the treatment of high altitude pulmonary edema (HAPE), acute respiratory distress syndrome and persistent pulmonary hypertension of the newborn. The combined use of NO and oxygen has cumulative effect on the pulmonary haemodynamics and gas exchange.[98]
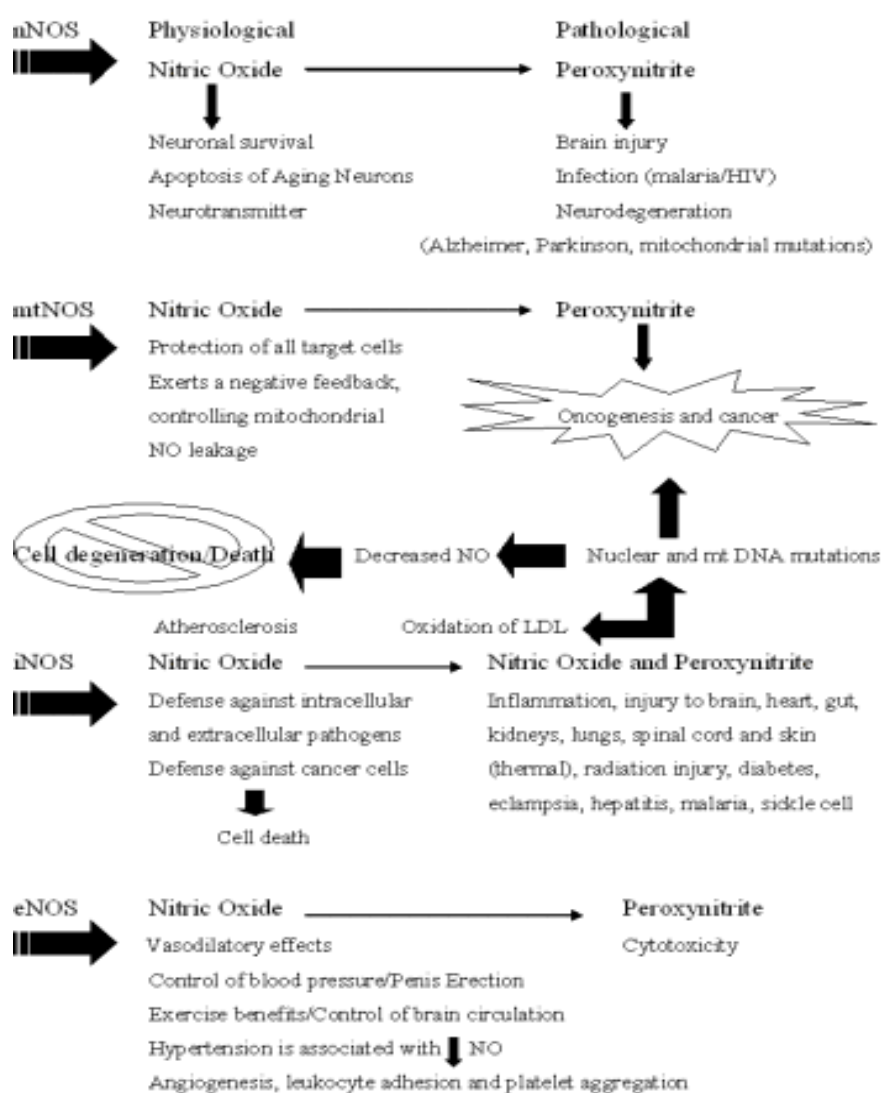
Role of nitric oxide in urinary system:
NO plays a key role in vascular and renal pathway. The activation of the renal NO system is an important mechanism whereby the remnant kidney regulates sodium and water balance, contributing to control the arterial blood pressure in the renal mass reduction and saline load model.[100] Experimental studies in laboratory animals suggest NO might be involved in the pathogenesis of glomerular hyperfiltration in normal as well as type 2 diabetic individuals.[101] NO is also involved in auto-relaxation of urinary bladder.[102]

Current trends
NO is involved in almost every body system, and many potential pulmonary and extrapulmonary applications for NO fall outside the current US Food and Drug Administration (FDA) approval. For instance, NO at doses of less than 80 ppm produces an anti-inflammatory effect by reducing neutrophil adhesion, platelets, and pro-inflammatory cytokines in the circulating blood,[103] also stimulation of apoptosis partly via reduction of NO in HCT -116 cells and NO has been associated with analgesic and anti-inflammatory activities.[104] Research combining NO with new modes of mechanical ventilation may result in protective strategies that could have an impact on diseases such as bronchopulmonary dysplasia and the adult respiratory distress syndrome.[105] Similar paracrine mechanisms for NO are being explored for beneficial effects on sickle-cell crisis, neurological dysfunction during cardiopulmonary bypass, and ischemia-reperfusion injuries.[106-108] The role of NO in the immune system as an endogenous antimicrobial agent is also being explored. Macrophages naturally produce NO as a host-defence mechanism against microbes, but these NO supplies are often overcome and depleted during infection.[109] Exogenous NO may sustain (and even enhance) the ability to defeat invading bacteria and viruses, as well as cancer cells. Potential research is in the way in evaluating the potential of NO to reduce and prevent ventilator-acquired pneumonias.[110, 111] NO also plays a major role in

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the wound-healing process.[112] Varying the level of NO affects collagen and collagenase levels at the wound site. It seems possible that the combination of antimicrobial attributes and vasodilatory effects with the ability to manipulate the pace of collagen formation at a wound site would be effective in promoting healing. Should research prove this to be true, burn patients may greatly benefit from the topical administration of exogenous gaseous NO. Much work still needs to be done to explore the role of NO for myriad potential indications in addition to its reversible vasodilatory effects.

Figure -2: Conceptual framework for physiological and pathological roles of nitric oxide synthase isoforms, nitric oxide and peroxynitrite

Future trends

Riding on the crest of enthusiasm generated after the dramatic but fascinating findings in late 80's, the research on this 'signaling gas' has entered into almost all areas of biology and medicine. As this explosion in research continues, the molecular targets of NO will be more

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precisely defined, and perhaps novel methods by which it regulates enzymes, such as amino acid nitrosylation, will be discovered and newer approaches will be investigated to develop new drugs based on the L-arginine / NO pathway. [113, 114] NO which acts by flipping cellular redox switches rather than acting on specific receptors is first of its kind to have an impact on clinical medicine. The duality of its nature, beneficial, protective and mediating effects in low concentrations while its degenerative and toxic effects in high concentrations has led to this molecule being christened as "Double edged sword" [Table-1]. In diseases characterized by hypofunctioning of L-arginine /NO system which are commonly observed in hypertension and vasospastic diseases, drug design and therapeutic strategies aimed at preserving endothelial integrity and boosting a failing L-arginine/ NO pathway has to be developed. They would include treatment with precursors of L-arginine or NO donors and preventing endothelial damage by pretreatment with antioxidants. Traditional exogenous NO donors such as nitroglycerin will be replaced by newer NO generators having vasodilating effect without the propensity for tolerance development. On the other hand in diseases associated with hyperactive L-arginine/ NO system as in inflammation, diabetes, epilepsy, neurodegenerative diseases like Alzheimers, novel therapies may include the use of NO antagonists or NOS inhibitors. However the potential for NO as a novel therapeutic target or strategy depends heavily on the search for selective NOS inhibitors and NO donors sans tolerance. With such a varied and fundamental nitrergic biology/pharmacology, the hope for novel therapeutic advances in this field continues.

Table -1: Mediatory and toxic functions of NO

System	Mediatory functions	Toxic functions
Cardio-vascular	EDRF, antithrombotic, ischemic protection, anti-atherosclerotic.	Atherosclerosis, septicshock, inflammation, reperfusion injury, myocardial stunning.
Respiratory	Ventilation-perfusion matching, mucus secretion, bronchociliar motility, immune defence	Immune complex induced alveolitis, asthma
Renal	Tubuloglomerular feedback, perfusion, renin-secretion	Acute kidney failure, glomerular glomerulo-nephritis.
CNS	Synaptogenesis, syna- ptic plasticity, memory formation, cerebral blood flow and ischemia, neuroendocrine secretion, visual transduction, olfaction.	Neurotoxic, proconvulsive, migraine
Gastro-intestinal	Blood flow, peristalsis, exocrine secretion, mucosal protection, antimicrobial action, pancreatic insulin secretion.	Mutagenesis, mucosal damage, pancreatic f3 cell destruction,
Immune system	Antimicrobial, antitumor	Transplantation rejection, inflammation, septic shock, tissue damaoe.

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CONCLUSION

Nitric oxide is a simple molecule with many physiologic roles in the cardiovascular, neurologic, and immune systems. Although the general principles of nitric oxide synthesis are known, further research is necessary to determine what role it plays in causing disease. As this explosion in research continues, the molecular targets of nitric oxide will be more precisely defined, and perhaps novel methods by which it regulates enzymes, such as amino acid nitrosylation, will be discovered. Research into the regulation of NOS should lead to a better understanding of its role in the pathogenesis of various diseases. The development of NOS inhibitors to block specific isoforms of nitric oxide as well as stable compounds that release it is also likely. Finally, the study of nitric oxide may lead to an understanding of how other small molecules, such as carbon monoxide, serve as biological messengers.

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