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REVIEW ARTICLE

Nitric Oxide as a Unique Bioactive Signaling Messenger in Physiology and Pathophysiology

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Dedicated to late Professor Padmanabhan Subbarao Krishnan (1914–1994)
Founder Head of Biochemistry Department, Lucknow University

Nitric oxide (NO) is an intra- and extracellular messenger that mediates diverse signaling pathways in target cells and is known to play an important role in many physiological processes including neuronal signaling, immune response, inflammatory response, modulation of ion channels, phagocytic defense mechanism, penile erection, and cardiovascular homeostasis and its decompensation in atherogenesis. Recent studies have also revealed a role for NO as signaling molecule in plant, as it activates various defense genes and acts as developmental regulator. In plants, NO can also be produced by nitrate reductase. NO can operate through posttranslational modification of proteins (nitrosylation). NO is also a causative agent in various pathophysiological abnormalities. One of the very important systems, the cardiovascular system, is affected by NO production, as this bioactive molecule is involved in the regulation of cardiovascular motor tone, modulation of myocardial contractivity, control of cell proliferation, and inhibition of platelet activation, aggregation, and adhesion. The prime source of NO in the cardiovascular system is endothelial NO synthase, which is tightly regulated with respect to activity and localization. The inhibition of chronic NO synthesis leads to neurogenic and arterial hypertensions, which later contribute to development of myocardial fibrosis. Overall, the modulation of NO synthesis is associated with hypertension. This review briefly describes the physiology of NO, its synthesis, catabolism, and targeting, the mechanism of NO action, and the pharmacological role of NO with special reference to its essential role in hypertension.

INTRODUCTION

Nitric oxide (NO), first characterized as an endothelium-derived relaxation factor, has now emerged as a ubiquitous signaling messenger molecule involved in diverse pathophysiologic processes such as neurotransmission, inflammatory and immune responses, and vascular homeostasis [1, 2, 3, 4]. It is a highly diffusible inorganic radical gas and has been known for many years as a noxious pollutant in car exhaust fumes, fossil fumes, and cigarette smoke. It is also known to bind cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain. However, the relevance of this action only became apparent after the discovery in the 1980s that NO is a biological mediator, and the demonstration in the 1990s that it inhibits respiration in mammalian cells. The roles of NO in the regulation of cell bioenergetics, cell death, and controlling oxygen supply and demand for cell integrity were also suggested [4, 5]. In this context, NO coordinates the respiratory cycle to acquire and deliver oxygen to target tissues by regulating hemoglobin (Hb) function and vascular smooth muscle contractility [4].

NO is synthesized from the amino acid L-arginine by a family of enzymes termed NO synthases (NOS). NO, synthesized in neurons of the central nervous system, acts as a neuromediator with many physiological functions, including the formation of memory, coordination between neuronal activity and blood flow, and modulation of pain [6]. In peripheral nervous system, NO is released by a widespread network of nerves, which mediate some forms of neurologic vasodilatation and regulate certain gastrointestinal, respiratory, and genitourinary functions [7]. NO is also generated in large quantities during host defense and immunological reactions, where it contributes to

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their cytotoxicity against tumor cells, bacteria, viruses, and other invading microorganisms [8, 9]. NO is a biatomic free radical (uncharged molecule) containing an unpaired electron that may undergo several reactions acting either as a weak oxidant or as a reductant compound [10]. Three biological and interrelated active redox forms of NO have been described, namely, NO, nitrosonium (NO^+), and nitroxyl anion (NO^-) [11]. NO can react with oxygen free radicals to form powerful oxidant peroxynitrite ($ONOO^-$), which is involved in protein oxidation reactions under physiological conditions [12]. NO or NO^+ ion is also able to form S-nitrosothiols (RSNO) by reacting with sulphydryl groups of protein, which are potent platelet aggregation inhibitors and vasorelaxant compounds.

Recent studies have also revealed a role of NO as a signaling molecule in plants. As a developmental regulator, NO promotes germination, leaf extension, and root growth, and delays leaf senescence and fruit maturation. Moreover, NO acts as a key signal in plant resistance to incompatible pathogens by triggering resistance-associated hypersensitive cell death. In addition, NO activates the expression of several defense genes (eg, pathogenesis-related genes, *phenylalanine ammonia-lyase*, *chalcone synthase*) and could play a role in pathways leading to systemic acquired resistance [13]. A novel role for NO in the regulation of lateral root development in tomato has been reported, which probably operates in the auxin signaling transduction pathway [14]. NO has been associated with plant defense responses during microbial attack, and with induction and/or regulation of programmed cell death [15].

Studies have indicated that chronic administration of N omega-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, produces marked hypertension. Although the mechanism of this form of hypertension is not well understood, several studies have demonstrated that sympathetic nerve activity is at least acutely elevated after L-NAME administration [16]. Now it is known that hypertensions are linked to the alteration in NO concentration. In this review, we describe the interaction of NO with superoxide anion, the physiology of NO, its synthesis/catabolism/targeting, the mechanism of NO action, and the pharmacological role of NO with special emphasis on its essential role in hypertension.

INTERACTION OF NITRIC OXIDE WITH SUPEROXIDE ANION AND OTHER RADICALS

Besides the role of NO in hypertension and cardiovascular disease, the free radical (eg, superoxide anion) has been reported to play a role in endothelial dysfunction. Free radicals are chemical species (molecules or atoms) possessing an unpaired electron in their outermost orbital. Due to the presence of one or more unpaired electrons, these species are paramagnetic, which makes them highly reactive. A free radical can be formed in a molecule, by gaining an additional electron, for example, reduction of molecular oxygen (O_2) to the superoxide anion radical ($O_2^{-\cdot}$) [17]:

$$O_2 + e^- \longrightarrow O_2^{-\cdot}. \tag{1}$$

Some oxides of nitrogen ($NO^\cdot$, $NO_2^\cdot$) are also free radicals. In the absence of L-arginine, nNOS has been shown to generate superoxide, $O_2^{-\cdot}$. Superoxide, either directly or through its self-dismutation to hydrogen peroxide H_2O_2, is likewise believed to be a cell-signaling agent. It has been estimated that a typical human cell metabolizes about 10^{12} molecules of O_2 per day and generates 3×10^9 molecules of H_2O_2 per hour. In oxidative stress condition $O_2^{-\cdot}$ is an unusual species, where it can act as a reducing agent by donating its extra electron to NO to form peroxynitrite ($ONOO^-$), or it can act as an oxidizing agent; in this case it gets reduced to H_2O_2. Under normal circumstances, the relatively high abundance of superoxide dismutase (SOD) ensures that the latter reaction occurs preferentially. However, when NO is produced in large quantities, a significant amount of O_2 reacts with NO to produce $ONOO^-$:

$$\begin{aligned} O_2^{-\cdot} + NO &\longrightarrow ONOO^-, \\ O_2^{-\cdot} + 2H &\longrightarrow H_2O_2 + 3O_2. \end{aligned} \tag{2}$$

The $NO^\cdot$ radical can react with peroxyl radical ($RO_2^\cdot$), hydroxyl radical ($OH^\cdot$), or NO^-, (a reactive and short-lived species) to produce alkyl peroxinitrite (ROONO), nitrous acid (HNO_2), or nitrous oxide (N_2O), respectively:

$$\begin{aligned} NO^\cdot + RO_2^\cdot &\longrightarrow ROONO, \\ NO^\cdot + OH^\cdot &\longrightarrow HNO_2, \\ NO^\cdot + NO^- &\longrightarrow ONNO^{-\cdot}, \\ NO^\cdot + ONNO^{-\cdot} &\longrightarrow NO_2^- + N_2O, \\ ONNO^{-\cdot} + H^+ &\longrightarrow OH^\cdot + N_2O. \end{aligned} \tag{3}$$

On exposure to air, NO reacts with O_2 to form the brown gas nitrogen dioxide ($NO_2^\cdot$), which is far more reactive than $NO^\cdot$. The overall reaction is

$$2NO^\cdot + O_2 \longrightarrow 2NO_2^\cdot. \tag{4}$$

The oxidation of $NO^\cdot$ dissolved in aqueous solutions produces mainly nitrite ion (NO_2^-); the overall equation is

$$4NO^\cdot + O_2 + 2H_2O \longrightarrow 4H^+ + 4NO_2^-. \tag{5}$$

The above reaction may be the sum of the equations

$$\begin{aligned} 2NO^\cdot + O_2 &\longrightarrow 2NO_2^\cdot, \\ 2NO_2^\cdot + 2NO^\cdot &\longrightarrow N_2O_3 \text{ (addition of 2 radicals)}, \\ N_2O_3 + 2OH^- &\longrightarrow H_2O + 2NO_2^-. \end{aligned} \tag{6}$$

The role of NO, H_2O_2, and $O_2^{-\cdot}$ in regulation of spontaneous tone in aorta of deoxycorticosterone acetate (DOCA)-salt hypertensive rats has been reported [18]. The $NO_2^\cdot$, ONOOH, N_2O_3, and HNO_2 can produce nitration, nitrosation, and deamination of DNA bases, which can make the DNA unstable.

PHYSIOLOGY OF NITRIC OXIDE

The interest in NO arose because it plays many important physiological roles in the nervous system, the vascular system, and other systems (eg, penile erection, bladder control, lung vasodilation, and peristalsis). The physiological stimuli for generation of NO are not fully understood, but pulsatile flow and shear stress may be the main determinants [19]. Griffith and Edwards [20] demonstrated that the activity of endogenous NO was much more in large arterioles in which hydraulic resistance and sheer stress were also highest. The NO was known to be released from endothelial cells in culture in a flow-dependent manner [21]. The role of other factors such as sympathetic or parasympathetic involvement in the generation of NO or whether some endothelial cells releases vasoactive substances, which in turn induce the release of NO, has not been yet established. NO is not stored and diffuses freely to its site of action where it binds covalently to its effectors. In biological systems, it has an estimated half-life of only 3–5 seconds. In coronary artery disease, the basal level of NO as well as stimulated release of NO were reduced [21]. The Hb-mediated inhibition of NO may play a role in the vasospasm that follows subarachnoid hemorrhage, which has long been suspected to be mediated by some product of lysed red blood cells [22]. Since NO is also the inhibitor of platelet activation, any alteration of its formation in the vessel wall will not only predispose to vasoconstriction but will also favor platelet adhesion and aggregation. In this context, platelet products released during aggregation in vivo may cause contraction of de-endothelialized canine coronary artery rings, whereas they induce relaxation in presence of endothelium [23]. Interestingly, isosorbide dinitrate, which releases NO, and prostaglandin E acted synergistically to reduce platelet deposition and to increase their survival time in patients with peripheral vascular disease [24].

The modulation of ion channels, including Ca^{2+} channels and pores, is now emerging as a general mechanism by which NO exerts biological signaling. Due to the fact that the bioactivity of NO involves several second-messenger systems and is profoundly influenced by multiple environmental stimuli, many seemingly divergent observations may be found in the literature [25]. Accordingly, there is an ongoing controversy regarding the impact of NO on Ca^{2+} channels. An important theme for NO signaling, which may partly settle the ongoing controversies regarding it, is the spatial confinement of NOSs with effector molecules, including ion channels. Perhaps the most important environmental stimulus that influences the bioactivity of NO is redox milieu, which in turn is influenced by the formation of reactive oxygen species and oxygen tension. Recently Khan and Hare [25] have reviewed the current knowledge regarding the NOS modulation of Ca^{2+} channels, emphasizing the cardiovascular, musculoskeletal, and central nervous systems. Ruiz-Stewart et al [26] have recently demonstrated that soluble guanylyl cyclase (sGC) is a nucleotide sensor whose responsiveness to NO is regulated by ATP.

NITRIC OXIDE SYNTHESIS

NO is mainly synthesized in living organisms by the action of a group of enzymes called NOSs which convert the amino acid L-arginine into NO and another amino acid, L-citrulline. Oxygen is required and the NOSs contain four cofactors: FAD, FMN, tetrahydrobiopterin, and haem; the haem center has spectral properties resembling those of cytochrome P450. There are three types of NOSs. Two are constitutive (named cNOS) and one is inducible by cytokines and endotoxins (named iNOS). There are two subtypes of cNOS: one was initially detected in the vascular endothelium and named eNOS and the other is present in the central and peripheral nervous systems and named nNOS [5]. The nNOS and iNOS are predominantly soluble enzymes whereas eNOS is more than 90% particulate [27]. The nNOS and eNOS are Ca^{2+}/calmodulin-dependent enzymes [28].

The iNOS releases NO in large quantities (micromolar range) during inflammatory or immunological defense reactions and is involved in host tissue damage. The overexpression of *iNOS* gene might account for NO overproduction, as it is reported in rats with portal hypertension [29]. The iNOSs bind calmodulin tightly and their activity is essentially Ca^{2+}-independent. Miljkovic and Trajkovic [30] have shown that iNOS is activated by interleukin-17 (IL-17), which is a proinflammatory T cell cytokine. They have also proposed the biological consequences of IL-17-mediated NO release that could be relevant to the mechanisms or therapy of autoimmune and inflammatory disorders [30].

The eNOS is expressed constitutively in endothelial cells and synthesizes the NO needed for regulation of blood pressure. The activation of eNOS is induced by increase in intracellular Ca^{2+} resulting from activation of diverse G-protein-coupled receptors (GPCR) or from mobilization of intracellular Ca^{2+} stores. Studies have shown that thapsigargin, a selective inhibitor of Ca-ATPase of endoplasmic reticulum and sarcoplasmic reticulum, activates NO release from pulmonary artery endothelial cells [31]. The actual intracellular Ca^{2+} that is required to activate this enzyme may be significantly different from that released from subcellular compartments as well as the average Ca^{2+} concentration [32]. Recently, Liu et al [33] have demonstrated that Endothelin-1 activates eNOS via heterotrimeric G-protein beta-gamma subunit signaling to protein kinase B/Akt. An increase in the association of heat shock protein 90 (HSP90) with eNOS is well recognized for increasing NO ($NO^{\cdot}$) production. Despite the progress in this field, the mechanisms by which HSP90 modulates eNOS remain unclear. It has recently been suggested by Ou et al [34] that the tyrosine kinase and HSP90-dependent signaling pathways act in concert to suppress uncoupled eNOS activity.

The nNOS is found in a variety of neurons in both the central and peripheral nervous systems and is a constitutionally expressed enzyme, though it can also be induced in neurons by certain treatments. Lately, Raines et al [35] examined the activation of cellular signal transduction pathways in nNOS-transfected cells grown in the presence or absence of L-arginine. Their results indicated that nNOS can differentially regulate the ERK (extracellular signal-regulated kinase) signal transduction pathway in a manner dependent on the presence of L-arginine and the production of NO.

NITRIC OXIDE CATABOLISM

A strict regulation of bioactivity of NO is essential for maintaining vasculature tone and inhibiting platelet activation. Several mechanisms appear to operate for the degradation of NO. Liu et al [36] have shown that erythrocyte sequestration of oxyhemoglobin and flow properties of erythrocytes decreased the reaction of NO with hemoglobin. Uric acid, the product of free radical generating enzyme, xanthine oxidase, has been shown to be the sink for NO activity [32]. In hypertensive state, superoxide anion reacts with NO and forms $ONOO^-$, which accounts for the removal of NO. There are also reports to show the existence of some enzymatic scavengers of NO. For example, Abu-Soud and Hazen [37] observed that NO serves as the substrate for multiple members of mammalian peroxidase family. Another possible enzymatic mechanism of NO catabolism is via catalytic consumption by prostaglandin H synthase [38].

NITRIC OXIDE TARGETING

The smooth muscles are the target for NO produced from endothelial cells, but the route and chemical form of NO have been controversial. The assumption that diffusion alone moves NO is no more feasible. Pawloski et al [39] have suggested that the movement of NO through erythrocytes occurs by a precisely coordinated series of events. Pawloski et al [39] have shown that the RBCs do not consume NO irreversibly by combining with Hb. NO reemerges from RBCs as biologically active S-nitrosothiol, which is protected from reacting with Hb. Some of the NO captured by Fe^{2+} in Hb can be shuttled intramolecularly to a conserved thiol group, producing S-nitrosohemoglobin. This NO may be transferred to other thiol-containing molecule, thus enabling NO activity to leave RBCs. This has been shown to occur preferentially in oxygen-poor tissues where Hb changes conformation.

MECHANISM OF NITRIC OXIDE ACTION

A variety of stimuli such as 5-hydroxytryptamine, acetylcholine, thrombin, calcium ionophore A23187, and arachidonic acid cause changes in arterial pressure and electrical stimulation and are able to release NO from endothelial cells [40]. The stability of NO is lower than other powerful endothelial vasodilators such as prostacyclin, which together with NO causes platelet antiaggregation and vasorelaxation effects by different mechanism [41]. NO is known to readily bind certain transition metal ions and many of its physiological effects are exerted as a result of its initial binding to Fe^{2+} haem groups in the enzyme guanylate cyclase. As a result, the enzyme becomes active and catalyses the production of more cGMP from GTP. This cGMP lowers the intracellular Ca^{2+} and relaxes the muscles, dilating the vessel and lowering blood pressure. Endothelial-derived NO can also diffuse into the lumen of the vessel, where it can prevent platelet aggregation and adhesion to the endothelium by a cGMP-dependent mechanism. NO and its receptor, sGC, are emerging as key mediators coordinating ATP supply and demand. The mechanisms coupling this pathway with metabolic and energetic signaling remain undefined. Ruiz-Stewart et al [26] have demonstrated that sGC is a nucleotide sensor whose responsiveness to NO is regulated by ATP. It has been observed that NO stimulates ADP ribosylation of many soluble and membrane-bound proteins including G-proteins in vascular smooth muscles. This leads to the activation of adenylate cyclase activity and inhibition of phospholipase-C activity leading to vasodilation [42]. They hypothesized that in hypertensive state, the chronically decreased levels of NO lead to decreased ribosylation of G-proteins leading to vasoconstriction.

Recent insights into molecular mechanisms regulating NO generation and the rich diversity of mechanisms by which it propagates signals reveal the role of this simple gas as a principle mediator of systems integration of oxygen balance. NO reversibly regulates protein function by posttranslational modification of cysteine thiols by S-nitrosylation in a fashion analogous to protein phosphorylation. S-nitrosylation can be mediated by NO derivatives including nitrosylated transition metals, NO carriers such as nitrosothiols, or by direct interaction with NO in the presence of electron acceptors [43]. Like phosphorylation, S-nitrosylation is precisely targeted to select cysteine residues in the context of specific structural motifs within proteins. Target cysteines located between acidic and basic amino acids that support the general acid/base chemistry underlying S-nitrosylation confer a guanine nucleotide exchange factor-like effect to p21Ras and activate CNG channels in the brain [4]. Moreover, S-nitrosylation creates opportunities for novel protein interactions that could impact the composition of intracellular signaling networks [4].

In endothelial cells, NO production activated by specific receptor agonists, shear stress, or hypoxia regulates local hemodynamics by inducing vascular smooth muscle relaxation [44]. Cotranslational modification by myristoylation and palmitoylation compartmentalizes eNOS in caveolae, specialized plasma membrane domains enriched in caveolins, which organize molecules to control the temporal and spatial attributes of signaling [44, 45]. In the

resting state, association with caveolins inactivates eNOS, in part, by preventing calmodulin association. The importance of controlling the spatial and temporal characteristics of NO signaling by subcellular compartmentalization with select coregulatory molecules is highlighted by the ability of NO to activate opposing molecular mechanisms in the same cell [4]. Thus, cardiomyocyte ionotropy reflects the integration of eNOS signaling in caveolae, which reduces contractility by preventing calcium entry mediated by colocalized adrenergic receptors and L-type calcium channels, and nNOS signaling in sarcoplasmic reticulum, which promotes contractility by S-nitrosylation of ryanodine receptors, promoting calcium release [4, 45].

PHARMACOLOGICAL ROLE OF NITRIC OXIDE

Many pharmacological roles of NO have been reported. We are describing only a few important roles. The generation of NO in cardiovascular system is dependent on a constant active vasodilation. The vasodilation action of glyceryl trinitrate (GTN) was earlier believed to be due to its conversion in the circulation to NO_2, which, in contrast to NO_3, has some vasodilator action. However, in 1940, Kranz et al [46] demonstrated that an immediate and total conversion in the bloodstream of an effective vasodilator dose of GTN would not yield sufficient NO_2 to explain the observed vasodilation. It is known that all the nitrovasodilators and NO activated the sGC, which increases the cGMP levels in smooth muscle, which in turn induces a sequence of protein phosphorylation associated with smooth muscle relaxation. Feelisch et al [47] have shown that nitrovasodilators also generate NO in a nonenzymatic reaction with cysteine. Prostacyclin and NO act synergistically to inhibit aggregation of platelets [48], suggesting that the release of NO and prostacyclin by the vascular endothelium play a role in its thromboresistance.

NO is implicated to play a role in penile erection. The neurogenic NO is considered the most important factor for relaxation of penile vessels and corpus cavernosum. In erectile dysfunction, NO donors have a direct action on penile tissue facilitating penile smooth muscle relaxation [49]. The defective eNOS-driven NO synthesis causes insulin resistance, arterial hypertension, and dyslipidemia in mice, and characterizes insulin-resistant humans. On the other hand, stimulation of iNOS and NO overproduction in mice may also cause metabolic insulin resistance, suggesting the effect of NO in the regulation of glucose homeostasis. Cook and Scherrer [50] reviewed the evidence for this novel concept and provided the conceptual framework for the use of NO-delivery drugs and pharmacological agents that modulate the bioavailability of endogenously produced NO for the treatment of insulin resistance. A drug, Resveratrol (trans-3,4′,5-trihydroxystilbene), a recently described grape-derived polyphenolic antioxidant, has been found to protect the heart from ischemic reperfusion injury. Hattori et al [51] have demonstrated that Resveratrol can pharmacologically precondition the heart in a NO-dependent manner.

The role of NO in neuronal degeneration of glaucoma is well established, and drugs to inhibit NO production have been introduced in preclinical studies. Giuffrida et al [52] have investigated the pharmacological efficacy of a topical formulation of the nonselective NOS inhibitor, nitro-L-arginine methyl ester (L-NAME), in an experimental model of glaucoma in rabbits. They have provided the first evidence that topical L-NAME significantly reduces the IOP in a model of ocular hypertension. Pearse et al [53] have indicated that acute inhibition of iNOS is beneficial in reducing several patho-physiological processes after spinal cord injury. Furthermore, they demonstrated that the antisense inhibition of iNOS is more efficacious than currently available pharmacological agents. NO is also known to mediate many pharmacological actions of ethanol. Boyadjieva et al [54] have determined the role of NO in ethanol regulation of beta-endorphin (beta-EP) release from primary cultures of rat fetal mediobasal hypothalamic cells. Alcohol treatments blocked sodium-nitroprusside-induced increases in the level of cellular cyclic guanidine monophosphate. The nonspecific NO blocker NG-L-NAME, but not the inactive isomer N-nitro-d-arginine-methyl-ester (d-NAME), inhibited ethanol inhibitory actions on beta-EP release. These results suggested that the cyclic guanidine monophosphate/NO pathway is involved in ethanol alteration of hypothalamic beta-EP release.

NO modulates many behavioral and neuroendocrine responses. Genetic or pharmacological inhibition of the synthetic enzyme that produces NO in neurons evokes elevated and sustained aggression in male mice. NO appears to play an important role in normal brain 5-hydroxytryptamine (5-HT, serotonin) function and may have significant implications for the treatment of psychiatric disorders characterized by aggressive and impulsive behavior [55]. Shortly after the invention of nitroglycerine (NTG), it was noticed that this substance is capable of inducing a violent headache. Only recently, it became known that this is due to the release of NO by NTG. As the molecular mechanism of migraine pain remains to be determined, NTG, being prodrug of NO, has been used to study the etiology and pathophysiology of migraine. Such studies on NTG- and also histamine-induced headaches have led to proposals that NO may be the causative molecule in migraine pain. The importance of NO as a potential initiator of the migraine attack opens new directions for the pharmacological treatment of migraine and other vascular headaches [56].

Mechanisms involved in the protective action of NO in insulin producing cells are a matter of debate. It was previously shown that pharmacological inhibition of c-Src cancels the antiapoptotic action of low and sustained concentrations of exogenous NO. Recently, Tejedo et al [57] have shown that NO triggers the PI3K/Akt survival pathway in insulin producing RINm5F cells by

arousing Src to activate IRS-1. Schizophrenia-like symptoms can be induced in humans by phencyclidine (PCP), a drug with marked psychotomimetic properties. PCP disrupts prepulse inhibition of acoustic startle in rodents, a measure which has also been shown to be disrupted in schizophrenic patients. This effect is blocked by NOS inhibitors, suggesting that NO plays an important role in this effect of PCP. Methylene blue, a guanylate cyclase and NOS inhibitor, has shown therapeutic value as an adjuvant to conventional antipsychotics in the therapy of schizophrenia. Recently, Klamer et al [58] have suggested that the NOS/guanylate cyclase pathway is involved in pharmacological and behavioral effects of PCP. Since PCP as well exerts psychotomimetic characteristics, agents that interfere with the NOS/guanylate cyclase pathway may be of therapeutic value also in the treatment of schizophrenia [58].

NO, xenon, and cyclopropane are anesthetic gases that have a distinct pharmacological profile. Whereas the molecular basis for their anesthetic actions remains unclear, they behave very differently to most other general anesthetics in that they have little or no effect on GABA(A) receptors, yet strongly inhibit the N-methyl-d-aspartate subtype of glutamate receptors. It is shown that certain members of the two-pore domain K^+ channel superfamily may represent an important new target for these gaseous anesthetics [59].

Angiotensin converting enzyme (ACE) inhibitors inhibit the degradation of bradykinin and contribute to accumulation of bradykinin and NO, both of which may be beneficial for diseased hearts. Since ACE produces angiotensin II in the heart, ACE inhibitors (ACEIs) may prevent coronary vasoconstriction and increase coronary blood flow. On the other hand, since ACEIs also inhibit kininase II, which results in reduced degradation of bradykinin, ACEIs may increase cardiac NO levels via stimulation of bradykinin receptors and the accumulation of bradykinin in the ischemic myocardium [60]. Kitakaze et al [61] also suggested that ACEIs attenuate both reversible and irreversible myocardial cellular injury via bradykinin/NO-dependent mechanisms.

Renin-angiotensin-aldosterone systems play a critical role in the development and progression of cardiovascular diseases, and ACEIs have proven effective for the treatment of these diseases. Since angiotensin II receptor antagonists can inhibit the effects of angiotensin II via ACE-independent pathways, for example, chymase, they were considered to be more effective than ACEIs. On the other hand, ACEIs can increase bradykinin, and thus, NO, which may cause potent cardioprotection, inhibition of smooth muscle proliferation, and attenuation of inflammation mechanisms. It appears that angiotensin II receptor antagonists and ACEIs may mediate cardioprotection in different ways [62]. ACEIs have been used extensively in heart failure, where they induce systemic vasodilatation and they have also been shown to reduce ischemic events after myocardial infarction, although their mechanisms of action on the coronary circulation are less well understood. The ACEI, enalaprilat, improves transmural myocardial perfusion at rest and after chronotropic stress, and restores impaired subendocardial coronary flow and vasodilator reserve in dilated cardiomyopathy (DCM). The effects of enalaprilat were bradykinin mediated and NO dependent and were not recapitulated by AT1 antagonist losartan. These data suggested beneficial effects of ACEIs on the coronary circulation in DCM that are not shared by AT1 receptor antagonists [63].

ROLE OF NITRIC OXIDE IN HYPERTENSION

There are several challenges in the study of the role of NO pathway in hypertension. Hypertensive patients have been shown by some, but not all, to have a reduced vasodilatory response to various stimuli of NO release. In hypertension, morphological vascular alteration affecting the endothelium, the intima, and vascular smooth muscle cells is produced. Thus, changes in the size and shape of endothelial cells, as well as in endothelial replication, have been noted. Abnormalities of endothelial cells are considered the main factors responsible for the enhancement of total systemic vascular resistance, leading to an increase in arterial blood pressure [64]. Genetic alteration can produce a predominant formation of endothelium-derived contracting factors and mitogen, which enhance the contraction elicited by vasoconstrictors and decrease the lumen area. Among the contracting substances produced by the endothelium are $O_2^{-\cdot}$, thromboxane A_2, and peptides named endothelin [65]. Endothelin-1 is a potent vasoconstrictor agent formed from a less active intermediate, proendothelin [65]. A significant increase in endothelin-1 plasma concentration is observed in patients with primary hypertension compared with normal subjects. A definite correlation has been established between the activities of free radical generating enzymes and the levels of NO. Mitogenetic activation described in hypertension is induced by (1) an increase in sympathetic activity [66] and (2) the release of vasoactive agents such as endothelins, angiotensin II, prostaglandin, thromboxane, transforming growth factor β, and platelet-derived growth factor, that may additionally modulate vascular growth [67].

In different animal models of hypertension as well as in hypertensive patients, a reduction of endothelium-dependent relaxation has been reported [68]. The impairment of endothelium-dependent relaxation seems to be secondary to sustained hypertension, the intensity and the duration of hypertension being the determining factors for the degree of impairment [69]. Luscher [70] has indicated that endothelial dysfunction is a consequence, and not a cause, of hypertension, though some studies describe an endothelial alteration at an early age. In isolated vessels and in vivo studies, the basal release of NO has been measured by analyzing the contractile responses induced by NOS inhibitors. Their result suggested that

the basal formation of NO is decreased in hypertension [71]. It has been described that the incidence of hypertension as well as isolated systolic hypertension is enhanced in elderly persons. The older hypertensives have a twofold greater risk of suffering from cardiovascular dysfunction than do younger hypertensives. Changes in viscoelastic properties together with augmented vascular stiffness contribute to the increase in both systolic arterial pressure and blood pressure with age [71]. The association of hypertension with aging increases the risk of lacerations in the endothelial modulation of vascular tone, which suggests that aging is an additional risk factor for endothelial dysfunction [69].

The effect of sodium nitroprusside (an NO) and NG-monomethyl-L-arginine (L-NMMA), a specific NOS inhibitor, on platelet was studied, and it was observed that in platelets from hypertensive patients, there is a markedly reduced sensitivity to L-NMMA, which could be explained by a reduction in NO synthesis [72]. The effect of L-arginine administration in patients with essential and secondary hypertension was investigated by measuring hemodynamic parameters, neuroendocrine hormones, and indicators of NO release. During administration, mean arterial pressure decreased, heart rate increased, cardiac output increased, and total peripheral resistance decreased [73]. Essential hypertension also has a genetic basis. Accumulated evidences, including finding of elevation of arterial blood pressure in mice lacking the *eNOS* gene, strongly suggest that alteration in NO metabolism is implicated in hypertension. The Glu 298 Asp mis-sense variant was significantly associated with essential hypertension suggesting that it is a genetic susceptibility factor for essential hypertension [74].

Recently, Das [75] has beautifully summarized the overall role of NO and $O_2^{-\cdot}$ in hypertension. In general, it is reported that patients with uncontrolled essential hypertension have elevated concentrations of superoxide anion ($O_2^{-\cdot}$), H_2O_2, lipid peroxides, endothelin, and transforming growth factor-beta (TGF-beta) with a simultaneous decrease in endothelial NO (eNO), SOD, vitamin E, and long-chain polyunsaturated fatty acids (LCPUFAs). Physiological concentrations of angiotensin II activate NAD(P)H oxidase and trigger free radical generation (especially that of $O_2^{-\cdot}$). Normally, angiotensin II-induced oxidative stress is abrogated by adequate production and release of eNO, which quenches $O_2^{-\cdot}$ to restore normotension. Angiotensin II also stimulates the production of endothelin and TGF-beta. TGF-beta enhances NO generation, which in turn suppresses TGF-beta production. Thus, NO has a regulatory role on TGF-beta production and is also a physiological antagonist of endothelin. Antihypertensive drugs suppress the production of $O_2^{-\cdot}$ and TGF-beta and enhance eNO synthesis to bring about its beneficial actions. LCPUFAs suppress ACE activity, reduce angiotensin II formation, enhance eNO generation, and suppress TGF-beta expression. Prenatal supplementation of LCPUFAs decreases insulin resistance and prevents the development of hypertension in adult life, whereas deficiency of LCPUFAs in the prenatal period results in raised blood pressure later in life. Patients with essential hypertension have low concentrations of various LCPUFAs in their plasma phospholipid fraction. Based on this, it is proposed that LCPUFAs serve as endogenous regulators of ACE activity, $O_2^{-\cdot}$ and eNO generation, and TGF-beta expression. It is proposed that availability of adequate amounts of LCPUFAs during the critical periods of growth prevents the development of hypertension in adulthood [75].

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary vasculature leading to vasoconstriction and remodeling of the pulmonary arteries. The resulting increase in the right ventricular after load leads to right ventricular failure and death [76]. NO administration restored the stroke volume with a decrease in PAH and an improvement of the pulmonary vascular resistance to systemic vascular resistance ratio. Systemic blood pressure and coronary perfusion remained unaffected. This selective effect on the pulmonary circulation should be considered a major advantage of NO inhalation in the treatment of right ventricular dysfunction in acute pulmonary hypertension [76]. Severe pulmonary hypertension and right-sided circulatory failure (RSCF) represent an increasing cause of morbidity and mortality in patients undergoing high-risk cardiac surgery [77]. Increased pulmonary vascular resistance in the setting of cardiopulmonary bypass (CPB) may further lead to decrease in blood flow across the pulmonary vascular bed; thereby decreasing left ventricular filling and cardiac output. Current management techniques for RSCF include both nonspecific vasodilator and inotropic agents (often limited by systemic hypotension) and the placement of right ventricular assist devices (associated with increased perioperative morbidity). Inhaled NO (NOi) represents a novel, specific pulmonary vasodilator that has been proven efficacious in these clinical settings [77].

The role of the NO pathway in both the healthy and the diseased pulmonary circulation is described by Michelakis [78]. The treatment options are limited, expensive, and associated with significant side effects. The NO pathway in the pulmonary circulation provides several targets for the development of new therapies for this disease. However, the NO pathway is modulated at multiple levels including transcription and expression of the *NOS* gene, regulation of the NOS activity, regulation of the production of cGMP by phosphodiesterases, postsynthetic oxidation of NO, and so forth. This makes the study of the role of the NO pathway very difficult, unless one uses multiple complementary techniques. Furthermore, there are significant differences between the pulmonary and the systemic circulation, which make extrapolation of data from one circulation to the other very difficult. In addition, the role of NO in the development of pulmonary hypertension varies among different models of the disease [78]. Recently, Huang et al [79] have suggested that

chronic NO inhibition ameliorates portal-systemic shunting and improves the collateral vascular responsiveness to arginine vasopressin in portal hypertensive rats.

CONCLUSIONS AND PERSPECTIVES

On the basis of the survey presented in this article, we can say that NO is a "universal messenger molecule" so far as its spectrum of actions is concerned. This molecule plays a vital role in a wide variety of pathophysiological and biochemical reactions. In summary, NO is involved in regulation of blood flow in different parts of the body, regulation of blood pressure, prevention of aggregation and adhesion of platelets, assisting the immune system to kill a wide variety of pathogens and block viral replication, promotion of certain types of cancer, promotion of penile erection and spermatogenesis, and facilitating childbirth. Common vascular disease states including hypertension, atherosclerosis, and diabetes are associated with endothelial dysfunction, characterized by reduced bioactivity of NO. Loss of the vasculoprotective effects of NO contributes to disease progression, but the mechanisms underlying endothelial dysfunction remain unclear. Increased superoxide production in animal models of vascular disease contributes to reduced NO bioavailability, endothelial dysfunction, and oxidative stress. In human blood vessels, the NAD(P)H oxidase system is the principal source of superoxide, and is functionally related to clinical risk factors and systemic endothelial dysfunction.

NO is an important mediator of both physiological and pathological responses. Over the past decade, evidence has accumulated, indicating that NO plays a key role in the regulation of metabolic and cardiovascular homeostasis. Pharmacological compounds that release NO have been useful tools for evaluating the broad role of NO in physiology and therapeutics. NO deficiency has been implicated in the genesis and evolution of several disease states. Both medical needs and commercial opportunities have fostered attempts to modulate NO in the human body for therapeutic gain. Strategies for NO modulation encompass anti-inflammatory, sexual dysfunction, and cardiovascular indications. Recent insights suggest that the original model, in which NO production reflects cell-specific isoenzymes with distinct functional properties, does not adequately capture the complexity of NOS regulation. Rather, all three NOS isoforms are constitutively coexpressed in a variety of cells and reside in multiple subcellular compartments [4]. Indeed, the expression of NOS isoforms is transcriptionally and translationally controlled, and they undergo co- and posttranslational modification, resulting in their expression in specific cellular and subcellular compartments, in the context of co-expressed regulatory partners, that precisely controls the spatial and temporal production of and signaling by NO [4]. Interestingly, in plants, nitrate reductase is also responsible for NO production [80]. Various genetic polymorphisms of the *eNOS* gene have been reported as susceptibility genes in a number of cardiovascular diseases. The functional significance of these polymorphisms has not yet been demonstrated.

Although much is known about different aspects of NO, there are many aspects yet not fully understood; further exploration of certain aspects might help in alleviating many pathophysiological disorders. (i) Role of NO within the myocardium is not fully known and is being pursued intensively. It is anticipated that a greater understanding of this action will help to explain some of the processes involved in heart diseases. (ii) So far it seems that increased ingestion of arginine reverses the changes in vascular reactivity and induces internal thickness in atherosclerosis and may also reduce blood pressure and excessive proliferation of smooth muscle cells in hypertension. There may be other ways to increase active concentrations of endogenous NO such as by prolonging its half-life or duration of its actions. Moreover, NO donating compounds can be used as replacement therapy to treat its impaired production. (iii) Selective inhibition of generation of NO may be a useful therapy as in case of brain ischemia and chronic degenerative diseases of the nervous system. (iv) Levels of L-Arginine-NO pathway products in biological fluids may be used as clinical markers for monitoring certain pathological conditions and progress of their treatment. (v) Immune regulatory action of NO appears to target Th1/Th2 balance of immune responses. NO can induce expression of Th2 associated cytokines IL4 whereas IFNγ and IL2 are suppressed. It has also been shown that apoptosis promoting activity of NO also primarily affects Th1 cells. These observations can be exploited to modulate the immune system to favorable conditions by selective activation/inhibition of NOS. (vi) There is enhanced expression of NOS in a variety of human cancers. Regulation of NOS activity in such cases may have therapeutic potential. This aspect needs to be seriously explored to pave treatment of certain carcinomas.

We have superficially scanned a number of pathophysiological/biochemical areas where NO plays a direct or indirect role. The simplicity of this molecule, contrasted with its ubiquitous presence and complexity of its actions, is astonishing. Further knowledge regarding regulation of its production and signal transduction along with its therapeutic potentials would be of much interest. Insights into mechanisms regulating NO production and signaling, and their integration into responses mediating homeostasis place into specific relief the role of those processes in pathophysiology. Moreover, this central role in pathophysiology identifies NO signaling as a key target for novel therapeutic interventions to minimize irreversible tissue damage associated with ischemic cardiovascular disease. NO therapy has been used experimentally to successfully treat idiopathic pulmonary hypertension and pulmonary hypertension associated with cardiac and respiratory diseases. However, the long-term benefits have yet to be studied. They are far from ideal because of the associated side effects mainly due to the catabolism of NO in toxic NO_2. A safer technology to regulate in vivo synthesis of NO by genetic manipulation would be a welcome move.

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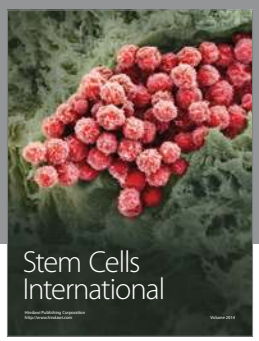

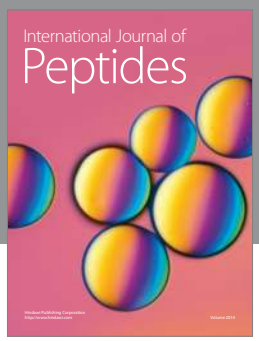

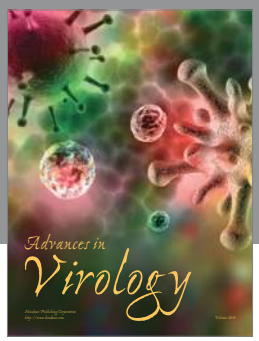

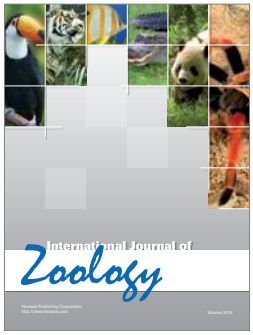

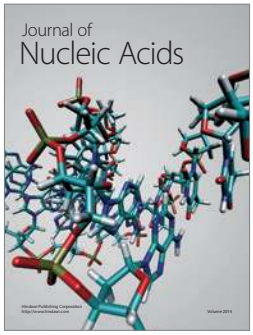

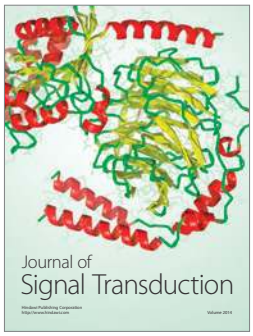

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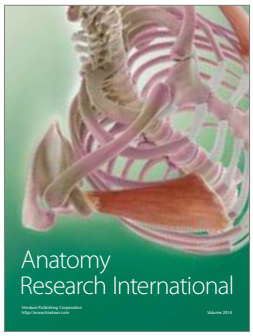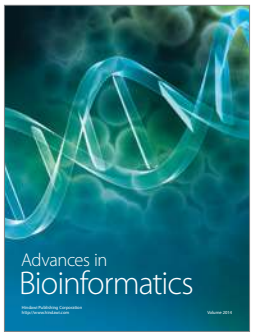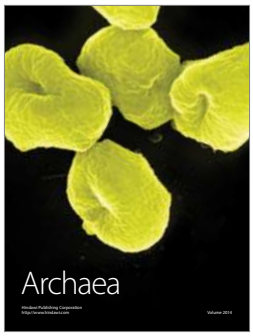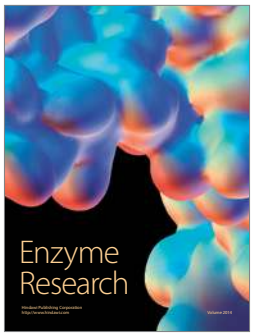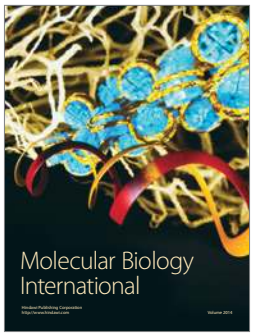
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