Current Topics in Pharmacology
Vol. 15, *2*, 2011

Review

Role of nitric oxide in developmental biology in plants, bacteria, and man

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ABSTRACT

Since its discovery, nitric oxide (NO) has been observed to play an important role in the physiology of single-celled organisms as well as high-order vertebrates. In this review, we will discuss the involvement of NO in bacterial, plant and human systems. NO originates from a variety of sources, namely bacterial, plant, and mammalian nitric oxide synthases which oxidize L-arginine. Bacterial NO is involved in toxin synthesis, signaling and biofilm formation. Organisms use NO to mediate oxidative stress incurred during the innate immune response. In plants, large amounts of NO hinder plant growth, while lower concentrations regulate normal development. NO and the associated reactive oxygen species (ROS) are effective antibacterial, anti-parasitic, and antifungal agents. Though NO has therapeutic effects in the immune system, the NO response is biphasic and concentration-dependent. NO promotes tumorigenesis within a concentration range, and induces apoptosis of cancerous cells at other concentrations. The biphasic response to NO is also evident in the regulation of chemokine, interleukins, and NF-κB, which can promote or inhibit inflammation. The physiologic response to NO is concentration dependent. NO, by way of non-adrenergic non-cholinergic (NANC) nerve transmission, propagates a cascade of molecular signaling that facilitates smooth muscle cell relaxation and increased arterial inflow into the corpora, initiating an erectile response. Additional NO is released through NOS activity in the endothelium in response to cholinergic nerve activity and shear stress, which helps to maintain erection.

KEYWORDS: nitric oxide, nitric oxide synthase family, nitric oxide and erectile function, nitric oxide and bacterial cells, nitric oxide and plants

ABBREVIATIONS

NO	: nitric oxide
Nar	: respiratory nitrate reductase
Nap	: periplasmic nitrate reductases
NirK, NirS	: nitrite reductase genes
NOS	: nitric oxide synthase
GTPase	: large family of enzymes that can bind and hydrolyze guanosine triphosphate
ROS	: reactive oxygen species
RNS	: reactive nitrogen species
H-NOX	: heme nitric oxide/oxygen
SNP	: sodium nitroprusside
iNOS	: inducible nitric oxide synthase
L-NMMA	: L-NG-monomethyl Arginine citrate
L-NAME	: L-NG-Nitroarginine methyl ester (hydrochloride)
cGMP	: cyclic guanosine monophosphate
NK cells	: natural killer cells
DNA	: deoxyribonucleic acid

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Research Support: NIH Grant HL 62000 and HL 77421

DNA-PKcs : DNA-dependent protein kinase
IL : interleukins
IFN-γ : interferon-gamma
TNF : tumor necrosis factors
TGF-β : transforming growth factor beta
G-CSF : granulocyte colony-stimulating factor
M-CSF : macrophage colony-stimulating factor
VEGF : vascular endothelial growth factor
CC chemokine : ß-chemokine
NF-κB : nuclear factor kappa-light-chain-enhancer of activated B cells
Th : T helper cell
NANC : Nonadrenergic, noncholinergic
sGC : soluble guanylate cyclase
GTP : guanosine-5'-triphosphate
eNOS : endothelial nitric oxide synthase
PDE-5 : type-5 phosphodiesterase
GDP : guanosine diphosphate
EDRF : endothelium-derived relaxing factor
EFS : electrical field stimulation

History of NO

Discovery and major players

Atmospheric NO (nitrous air) was first observed in 1774 by Joseph Priestly [1]. Amyl Nitrite was first synthesized in 1844 by Antoine Balard. Vasoactive properties were first reported by Frederick Guthrie in 1859 [2, 3]. Brunton in 1867 reported on the use of nitrite of amyl as a treatment for angina pectoris [4]. In 1903 Francois-Franck suggested that amyl nitrate is a vasodilator [5]. The metabolic sources and functions of NO in bacteria, humans, and plants is illustrated in the Figure 1.

Role of NO in bacteria

Bacterial synthesis of NO proceeds from the oxidation of L-arginine via the *N*-ω-hydroxy-L-arginine intermediate. Additionally, denitrification is a facultative pathway shared by many bacteria and archaea whereby nitrate is reduced to nitrite, nitric oxide, nitrous oxide and finally nitrogen gas [6]. The four steps of denitrification are characterized by seven enzymes [7]. Of these enzymes, the reductases Nar or Nap catalyze the reduction of nitrate to nitrite, and the nitrite reductases NirK or NirS reduce soluble NO_2- to NO gas [6].

The identification of bacterial proteins homologous to mammalian NOS have shed light on new roles of NO in microbes [8]. Studies on the biological functions of these bacterial NOSs show that NO is involved in toxin biosynthesis, defense against oxidative stress, and regulation of growth responses after radiation exposure [8].

Toxin synthesis and host defense

In certain *Streptomyces* strains, NOS plays a role in the biosynthesis of thaxtomin, a plant toxin contributing to the virulence in scab-causing pathogens [9, 10]. NO, produced by NOS, is directly linked to the nitration of thaxtomin [11]. *Bacillus subtilis*, a bacteria that exhibits many multicellular traits such as biofilm formation and swarming motility, uses NOS-derived NO defensively and collectively to protect against host oxidative attack [12]. This is employed against a common form of host defense which is driven by the Fenton reaction, and uses ferrous iron and peroxide to generate hydroxyl radicals that have a deleterious effect on cells [13]. Reduction of the ferric iron by cellular reductants, such as cysteine, maintains the continuity of the Fenton reaction by providing ferrous iron. Using NO, bacterial s-nitrosation of cysteine inhibits recycling of this ferrous iron, thereby preventing oxidative damage to the *Bacilli* [8]. NO also blocks the detrimental effects of oxidation of DNA and proteins by activating a *Bacilli*-specific catalase that breaks down peroxide [13]. The same defensive mechanism involving endogenous and exogenous NO also occurs in *Bacillus anthracis* and is activated upon macrophage-induced oxidative stress which helps ensure the survival of the pathogen [14]. During a host-pathogen interaction, the hosts fight infection by causing indiscriminate oxidative damage, but, like their bacterial assailants, the hosts also produces NO in response to pathogen virulence and oxidative stress [8].

Bacterial NO signaling

*Deinococcus radiodurans*is is a highly resilient bacteria able to survive in stringent conditions, including desiccation, exposure to reactive oxygen species,

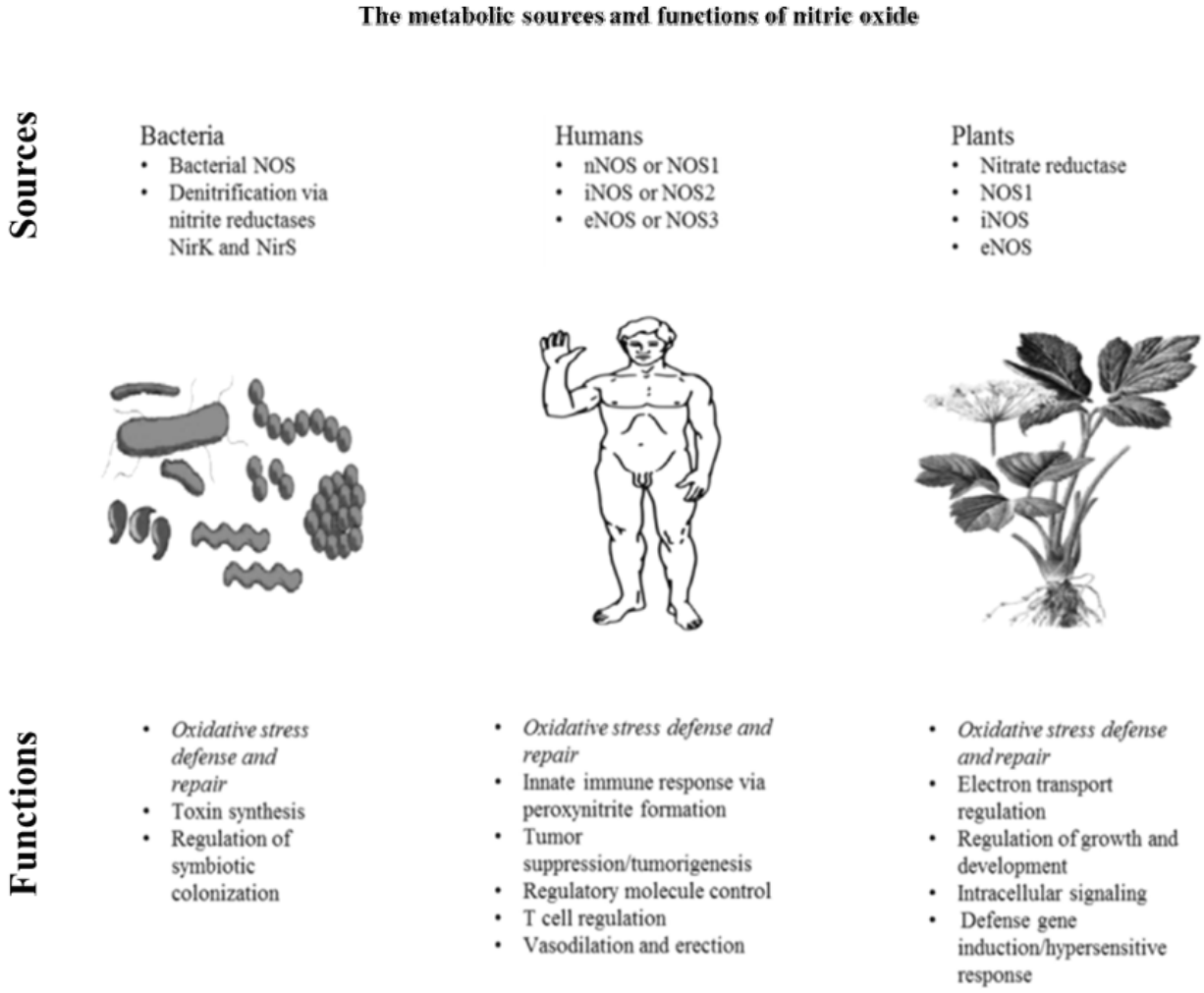

Figure 1. This figure illustrates the role of nitric oxide synthase (NOS) and nitric oxide in bacteria, humans, and plants in regard to oxidative stress, cellular defense and repair, and other cellular processes. There are 3 isoforms of NOS in humans and more are present in plants. It has recently been discovered that NOS and NO play an important role in cardiovascular health and erectile function. The roles of NOS and NO are diverse and important in bacteria, humans, animals, and plants.

and significant radiation exposure [15]. However, Δ*nos,* a strain of *D. radiodurans* in which the *nos* gene has been deleted, displays minimal cell repair after irradiation. The addition of exogenous NO at any stage of damage promotes the growth recovery of the strain [15]. The protective mechanism exhibited by bacterial NOS is further established through the correlation observed between NO generation, levels, and the activation of the obgE gene. The gene codes for GTPases involved in regulation of developmental processes and cell proliferation [16]. *D. radiodurans* exposed to UV light synthesizes NO. This results in the upregulation of obgE gene which induces cell repair signaling [15]. NO also elicits responses involving regulatory proteins in other bacteria [17].

Symbiosis and NO

NO is involved in signaling pathways of endosymbionts, used primarily as a way to avoid cascades of host derived ROS and RNS from attacking their proteins and lipids [18]. In the

squid-vibrio light organ, bacterial symbionts with bacterial heme-containing H-NOX proteins sense host-derived NO and regulate the symbiotic colonization of the light organ [19]. NO is also involved in signaling in plant root nodules containing nitrogen-fixing bacteria. Recent studies indicate that the amount of NO generated by a host's immune response in response to a pathogenic or beneficial microbe is modulated by class 1 hemoglobin genes, which lower concentrations of NO in the presence of nitrogen fixing bacteria [20]. NO also plays a role in signaling involving the symbiotic relationships of a diverse collection of animal hosts [21]. Cellobiose, a cell wall component in plants, also induces production of NO at the host-pathogen interface and the excess of NO in toxin biosynthesis implicate the role of NO in tissue growth [8].

Role of NO in plants

Physiology

NO has been shown to stimulate seed germination in plants. It is also known to play a role in mitochondrial respiration and chloroplast electron transport, where it serves to regulate the terminal transport step and rate of electron transport [22-25]. Treatment of plants exposed to an oxidative stress inducing herbicide along with the NO donor sodium nitroprusside showed a protective effect against ROS [26]. At high doses, NO can retard plant growth, whereas at lower concentrations NO promotes normal growth and development [27]. Application of SNP to roots has shown to stimulate lateral root development whereas applying a NO scavenger to the root has been shown to stimulate primary root growth and hinder lateral root growth [28]. Nitric oxide has also been indicated to be involved with the regulation of fertility. A plant mutant under-producing NO develops faster, whereas plants given NO donors or plants with higher endogenous production of NO exhibit a delayed flowering time [29].

Plants are known to possess many distinct nitric oxide synthases (NOS). The enzymes generally catalyze NO from arginine, similar to what is seen in mammalian cells [30]. Plant iNOS was found to be induced upon virus inoculation and was present in very low numbers in uninfected cells. This enzyme was also shown to be sensitive to inhibitors of animal NOS such as L-NMMA, L-NAME and aminoguanidine [31, 32]. Another NOS, NOS1, has been shown to be required for maximal growth and proper organ development [33]. Distinct NOS enzymes have also been discovered in peroxisomes and apoplasts [34, 35].

Second messengers and defense

Cyclic GMP has been proposed as a common second messenger for NO in plants. Experimentally, NO has been shown to regulate calcium ion channels in stomatal guard cells (cells regulating gas exchange in the leaf through the opening and closing of the stoma) via promotion of calcium release from intracellular stores [36, 37]. The subsequent rise of free calcium in the cytosol was blocked by guanylate cyclase antagonists, implicating a cGMP dependent second messenger system [37].

Defense genes such as pathogenesis-related 1 protein and phenylalanine ammonia lyase have been observed to be induced by the addition of NO donors (SNP). The same genes were observed to be induced by cGMP, further implying a guanylate cyclase pathway [38]. NO plays a key signaling role during the hypersensitive response, a reactive oxygen species generating response resulting in localized cell death limiting nutrient availability to an invading pathogen. NO, in concert with hydrogen peroxide, can induce cell death in this role [39].

Physiologic roles of NO

Immune system

Many cell types express NO within the immune system, such as macrophages, neutrophils, NK cells, mast cells, phagocytic cells and dendritic cells. Other cells involved in immune response also express NO such as endothelial cells, epithelial cells, vascular smooth muscle cells, fibroblasts and many others [40]. The NO response in infection has direct effects on DNA through mutation, the inhibition of DNA repair and synthesis, the S-nitrosylation of proteins, tyrosine nitration, or enzymatic inactivation [40].

The oxidation of proteins and DNA at different sites is often carried out by peroxynitrite and NO_2 [41].

The targeting of this nitrogen-reactive species in bacteria such as *Salmonella typhimurium* shows its efficacy as an antibacterial agent [42]. In viral infections, such as seen in Epstein-Barr virus infections, peroxynitrite formation from NO hinders viral replication and blocks activation of the genome, thereby inhibiting the virus [43]. Anti-parasitic and antifungal activity has also been credited to peroxynitrite synthesis [41].

iNOS

iNOS induction contributes to indirect effects of NO antimicrobial activity [40]. Induced iNOS in macrophages diminishes the growth factor arginine, contributing to growth inhibition or possibly parasitic death [44]. In a similar manner, *N*ω-hydroxy-L-arginine, a stable intermediate in the NO synthesis pathway, blocks arginase activity, which leads to the killing of *Leishmania* [45].

Tumors

The ability of interferon-λ to suppress tumor cell growth in mice established the first known function of NO in the immune system [46]. The sulfated polysaccharide fucoidan exhibits cytotoxic properties against tumor cells, which mechanistically stems from the activation of the NOS gene and the increase of NO synthesis [47]. Studies show that the NOS inhibitor L-NAME blocks NO production as well as the cytocidal effects seen in fucoidan. However, contradictory evidence exists regarding the endogenous expression of iNOS in tumor cells inducing DNA-dependent protein kinase that protect the cells from NO cytotoxicity [40]. NO is still considered a "double-edged sword" in oncology due to its involvement in both inhibition and promotion of tumorigenesis [48]. Within a certain concentration range, NO facilitates the survival of tumors, but beyond this critical NO concentration NO has been observed to sensitize the cancerous cells to apoptosis. Therefore, the NO response in tumor cells is biphasic. The duality of the NO response has been exploited in pre-clinical cancer models to hinder tumor growth as well as improve the effectiveness of chemotherapy and radiotherapy [49].

Tissue damage

Though NO has proven therapeutic effects in the immune system, it can also act in tissue-damaging pathways [40]. NO has been implicated in neurodegeneration through its connection to the neurotransmitter glutamate [50]. Deviation in glutamate signaling transduction and release of NO are factors in ischemic stroke [51]. Microglia are brain immune cells responsible for preservation of the neural environment, including the regulation of immune responses, and increased activity of NOS in microglia following transient brain ischemia leads to the production of cytotoxic NO, which has a deleterious effect [52, 53]. The combination of nitric oxide and superoxide yields peroxynitrite, which causes the cleavage of DNA strands. This leads to the activation of a DNA repair protein and consumption of NAD^+, the root cause of the observed brain damage [52].

Immune response to NO

The physiologic response to NO in the immune system is varied due to the expression of all known isoforms of NOS in the immune system as well as the ability of NO to easily cross membranes, and be transported with various low molecular weight compounds such as S-nitrothiols at sites distal to NO production. NO can bind a variety of targets, many of which are regulatory molecules [40]. Consequently, nitric oxide possesses both an anti-inflammatory and immunosuppressive effect in the body [54]. In rat models, evidence for this effect has been observed in the attenuation of acute inflammation and adjuvant arthritis. L-arginine was shown to enhance these effects [55-63]. In humans, synthesis of NO in the colon is increased in patients with ulcerative colitis and NOS inhibitors have been shown to suppress an experimental model of induced chronic ileitis [64]. There are a wide range of responses mediated by NO, from vasodilation and edema, sensory nerve modulation, leukocyte activity modulation, to tissue cytotoxicity [65-68]. These varied responses support reports showing evidence of tissue protection through the administration of both NO donors and NOS inhibitors [54, 69, 70].

Immune system regulation

Another function of NO within the immune system is cytokine, chemokine, and growth factor regulation. NO induces both pro and anti-inflammatory responses through these mediators. NO produced by macrophages, T cells, endothelial cells and

fibroblasts can cause the up and down regulation of interleukins (e.g. IL-1, IL-6, IL-8, IL-10, IL-12, IL-18), IFN-γ, TNF, growth factors (TGF-β, G-CSF, M-CSF, VEGF), CC chemokines, and macrophage inflammatory proteins. The modulation of these molecules affects signaling cascades, transcription factors, proteins regulating mRNA translation, and cytokine enzymes and precursors [71-78]. Regulation of NF-κB is also determined in a biphasic manner by the concentration of NO present [79].

T cell regulation

NO also modulates T helper cell levels and ratios. Th1 cells are more susceptible to apoptosis than Th2 cells and this apoptotic pathway has been shown to be regulated by NO, likely through its reactions with other ROS within the cell. Th2 ratios in relation to Th1 are increased in the presence of NO through the up regulation of IL-2 in murine lymphocytes and the up regulation of IL-4 in human cells [80-85]. NO also down regulates the expression of P and E selectin, vascular cell adhesion molecule, and intracellular adhesion molecule-1, which inhibits the rolling of leukocytes along the endothelium and prevents migration of helper cells from vessels into tissues. P and E selectins preferentially recruit Th1 cells into inflamed tissues. Suppression of these selectins shows that NO works to inhibit the accumulation of Th1 cells at sites of inflammation via adhesion interference [86-88].

Role of NO in erection

Erection is achieved through an integration of central and peripheral processes which result in physiologic vasodilation, arterial inflow into the paired corpora cavernosa and veno-occlusion in the penis. Nonadrenergic, noncholinergic transmission of NO from the cavernosal nerve terminals initiates a cascade of molecular signaling mediated through the heterodimericheme protein soluble guanylate cyclase. sGC activation results in conversion of intracellular GTP to cGMP. Increased intracellular levels of cGMP activate a cGMP-dependent protein kinase, causing membrane hyperpolarization and uptake of calcium into the endoplasmic reticulum. The decrease in bioavailable intracellular calcium facilitates smooth muscle cell relaxation. As the arterial inflow into the corpora increases, shear stress activates an endothelial nitric oxide synthase, serving as an additional source of NO to facilitate an erection. A cGMP dependent PDE-5 converts cGMP to GDP, blocks membrane hyperpolarization and abolishes the erectile response [89-91].

Early investigations performed with human corporal smooth muscle cell cultures showed that these cells respond identically to vascular smooth muscle cells when cGMP was added to the culture media and suggested a role for EDRF in the process of erection [92, 93]. This notion was further developed with additional experiments in which rat aortas were harvested and hung on a column over a rat cavernosal smooth muscle cell culture. Calcium efflux was measured in the cavernosal culture after human serum had been dripped through the aortas. The results of this study also suggested EDRF was responsible for cavernosal smooth muscle cell relaxation [93]. Dr. Jake Rajfer contributed significantly to these early studies and one of his colleagues at UCLA medical center, Dr. Louis Ignarro, had recently reported that the identity of EDRF released from the vascular smooth muscle was NO [86]. The two investigators began a collaboration to show that NO was the compound responsible for smooth muscle cell relaxation in the corpora cavernosa and erection. Their initial study showed that relaxation of rabbit cavernosal tissue by electrical field stimulation was attenuated by compounds that inhibited NO synthesis [94, 95]. Within the next two years, 3 studies were published showing the role of NO in relaxation of human corporal smooth muscle tissue [93, 96-98]. Rajfer and Ignarro's 1992 article in the New England Journal of Medicine was especially significant because it was the first article to demonstrate that inhibition of phosphodiesterase enhanced the relaxation response of corporal strips to EFS and NO [93, 98]. A study by Dr. Arthur Burnett at Johns Hopkins demonstrated the localization of NO to the penile nerves of the rat using antibodies [89]. Shortly thereafter, the effects of phosphodiesterase inhibition upon erectile responses was reported *in vivo* [99]. These experiments provided the basis for what is now considered to be the gold standard in treatment of erectile dysfunction, the PDE-5 inhibitor.

SUMMARY

Since the time NO (nitrous air) was first described in 1774 a large body of information about the role of this gaseous molecule in physiologic function in bacteria, plants, and humans has been published. There are now more than 112,686 publications on or dealing with NO and more papers are appearing every week. Organisms use NO to mediate oxidative stress, whereas plants use NO to regulate growth and development. In humans NO has a key role in the regulation of numerous physiologic and pathophysiologic processes and drugs that enhance the release of NO have very wide and important uses in modern medicine. The report discusses some of the roles of NO in plants, animals, and bacteria and it is anticipated that knowledge about NO will continue to expand in the future. We believe that the NO-SGC-cGMP pathway will be an important target for drug development for many years in the future.

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