AN INTERCELLULAR MESSENGER : NITRIC OXIDE

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ABSTRACT

Nitric oxide (NO), a potentially toxic molecule, plays a role as an important second messenger. It has been implicated in a wide range of biological functions, such as blood pressure regulation, blood clotting, and neurotransmission. The regulation, signal transduction and cytotoxicity of NO is strictly dependent upon its chemical reactivity with oxygen and metals rather than specific structural interactions with physiological targets. The properties, biosynthesis, physiological roles, involvement in pathophysiology and therapeutic potential of NO are reviewed.

Key words: Nitric oxide, NO, neurotransmitter, second messenger

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INTRODUCTION

Recently, the intense interest of nitric oxide (NO) research has occurred, because of the significant therapeutic potential. NO plays a role as an important biological second messenger in human physiological processes, which is involved in neurotransmission, blood clotting and blood pressure control. In addition, NO has been shown to serve as part of the immune system against cancer cells and intracellular parasites and microbes. Due to the molecule's reactivity, very small size and diffusibility, the actions of NO depend on its chemical reactivity with oxygen and metals rather than its molecular shape or specific structure interactions with physiological targets as any other biological messengers.

PROPERTIES AND BIOSYNTHESIS OF NO

NO is an inorganic gas produced by many mammalian cells. It is an uncharged molecule containing one unpaired electron and thus is both a paramagnetic compound and a free radical. Nitric oxide diffuses freely in a surprisingly large distance, in all directions from its site of origin[1] and acts as a dissolved nonelectrolyte in all its biological activities, with the exception of the lung in the presence of gaseous phase[2]. It is much more soluble in apolar solvents such as *n*-hexane and dissolve selectively in the membrane and lipid phase of cells.

The chemical reactions of NO with oxygen and metal in biological system are characterized as stabilization of the unpaired electron. The reaction of NO with oxygen species, such as O_2, superoxide anion ($O_2^{\cdot-}$) or peroxy radicals, results in the formation of a stabilized diamagnetic species. The most important reaction of NO with metal is its reaction with oxyferrohemoglobin. The reaction is a transfer of oxygen and electron to NO, forming nitrate anion and oxidized methemoglobin[2].

The reaction of NO with O_2

$$2\,NO + O_2 \longrightarrow 2\,NO_2 \text{ (nitrogen dioxide)}$$
$$NO_2 + NO_2 \longrightarrow N_2O_4 \longrightarrow NO^+$$
$$NO_2 + NO \longrightarrow N_2O_3 \longrightarrow NO^+$$

The transferation of nitrosonium ion : Transnitrosation

$$[NO^+] + OH^- \longrightarrow HNO_2 + H^+ + NO_2 \text{ (nitrite)}$$
$$[NO^+][NO_2^-] + H_2O \longrightarrow 2\,NO_2^- + 2\,H^+$$
$$[NO^+][NO_3^-] + H_2O \longrightarrow NO_2^- + NO_3^- + 2\,H^+ \text{ (nitrate)}$$

Nitrosation of biological nucleophilic targets

$$[NO^+] + R_2NH \longrightarrow R_2NNO + H^+ \text{ (nitrosamine)}$$
$$[NO^+] + RSH \longrightarrow RSNO + H^+ \text{ (s-nitrosothiol)}$$

Nitrosamine : potent mutagen and carcinogen
s-Nitrosothiol : source of NO

The reaction of NO with superoxide $O_2^{\cdot-}$

$$NO + O_2^{\cdot-} \longrightarrow \underset{\text{(peroxynitrite)}}{ONOO^-} \xrightarrow{H^+} ONOOH \longrightarrow NO_3^- + H^+$$

The reaction of NO with oxyferrohemoglobin

$$Hb(Fe^{2+})O_2 + NO \longrightarrow Hb(Fe^{3+}) + NO_3^-$$

Nitric oxide is produced from the substrates arginine, O_2 and reduced nicotinamide adenine dinucleotide phosphate (NADPH) by a Ca^{2+}-dependent mixed function oxidase, nitric oxide synthase (NOS). NOS is a homodimeric protein of 125 to 160 KD subunits. Each NOS subunits contains one flavin adenine dinucleotide (FAD), one flavin mononucleotide (FMN), one tetrahydrobiopterin (H_4B) and one Fe(III)-heme (iron protoporphyrin IX) as its prosthetic groups (Figure 1 and 2) or cofactors.

The synthesis of NO (Figure 3) is a two-step mechanism, the oxidation of arginine (Arg) to L-hydroxyarginine (NOHArg) at the heme site of NOS and the conversion of NOHArg to NO. The N in nitric oxide comes from the guanidino group of arginine, the electron from NADPH and the oxygens in both NO and citrulline from molecular O_2[3].

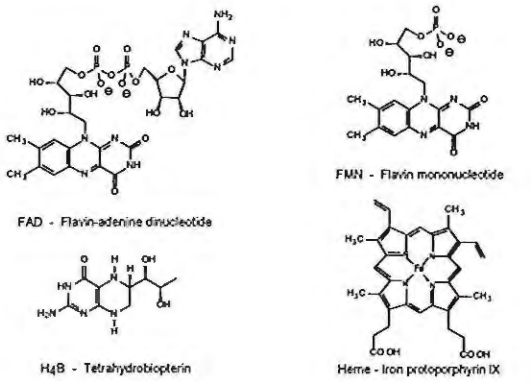

Figure 1 Nitric oxide synthase prosthetic groups.

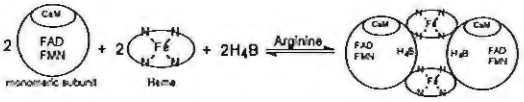

Figure 2 A model shows NOS associate with its prosthetic groups and Ca-calmodulin (CaM) to form an active form of NOS in catalysing the synthesis of nitric oxide from arginine.

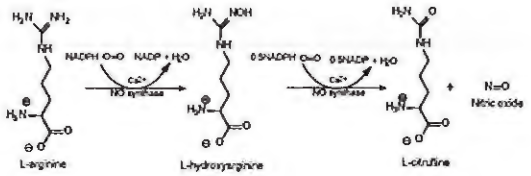

Figure 3 Biosynthesis of Nitric oxide (NO).

THE PHYSIOLOGICAL ROLE OF NO

The effects of NO have been many and varied due to its interplay between different possible reaction pathways. Several researches including the areas of cardiovascular pharmacology, immunology and neurobiology had been done to study the actions of NO in the biological system.

NO IN BLOOD PRESSURE REGULATION

The vascular endothelial cell is now regarded as an endocrine gland that releases several substances including NO[2] which is produced by the activation of NOS in vascular endothelial cells, eNOS. The activation of eNOS occurs through its interaction with Ca^{2+}-calmodulin complex which formed after a rise in intracellular Ca^{2+} causes by influx of Ca^{2+} to the cell. The activated eNOS then catalyses the synthesis of NO which rapidly diffuses from the endothelial cell to the adjacent vascular smooth muscle cells and interacts with its physiological target, the soluble guanylate cyclase (sGC)[4]. Soluble guanylate cyclase (sGC) is a cytosolic enzyme containing a heme as well as a copper ion[2]. The sGC catalyses the production of guanosine 3',5'-cyclic monophosphate (cGMP), an intracellular second messenger, that mediates smooth muscle relaxation through its stimulation of cGMP-dependent protein kinase or protein kinase G to phosphorylate the myosin light chain[1]. NO activates sGC by binding with very high affinity to iron in the heme of sGC displacing the heme iron from the plane of porphyrin ring, eliciting a conformational change and this change enhances the enzyme's catalytic activity[4]. Various nitrates have been used as vasodilators and antianginal agents. The relaxing properties of these agents were once thought to depend on the formation or the release of NO and this idea is now shown to be true. Some of these agents spontaneously decompose into NO and some of them, such as nitroglycerine and amyl nitrite, interact with the thiol groups on protein or enzymes and form an intermediate S-nitrosothiol (RS-NO) before releasing NO[5] (Figure 4).

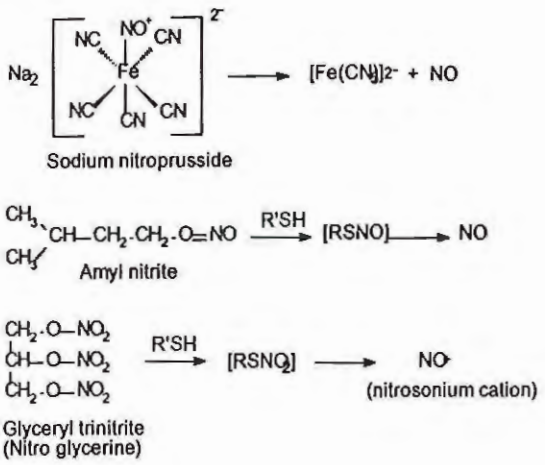

Figure 4 Examples of nitric oxide releasing agents

In addition to the effects on vascular smooth muscle, NO also inhibits platelet aggregation via sGC and cGMP dependent pathway. Together with prostacyclin, NO increases the platelet affinity for both the vascular endothelial surface (platelet adhesion) and for each other (platelet aggregation)[2].

NO AS NEUROTRANSMITTER

The second messenger role for NO in the central nervous system (CNS) and peripheral nervous system (PNS) followed studies in the cardiovascular and immune system. In 1988, John Garthwaite and colleages showed the relationship of NO and N-methyl-D-aspartate (NMDA) receptor, important receptor in the transmission of nerve impulse from cell to cell. They found that the slices from cerebellar portion of rat brain release a labile substance upon the activation of NMDA receptor.(6) This substance is called the endothelial relaxing factor (EDRF) which has been identified as NO or a close derivative that release NO[4].

The exact effector. pathways of NO (Figure 5) is activation of its physiological target, the enzyme soluble guanylate cyclase (sGC). Binding of NO to sGC caused a 100-fold increase in the production of cGMP which leads to the ultimate cellular response. NO does not fit the concept of classical neurotransmitter or neuromodulator. It is synthesized in the neurons when glutamate released from a stimulated neuron binds to and activates NMDA receptor on the adjacent neuron, causing an ion channel in the receptor to open, admitting influx of Ca^{2+} to the neuron. In the neuron, Ca^{2+} binds to calmodulin to form Ca^{2+}/calmodulin complex that associates with NOS and upon the presence of oxygen and nicotinamide-adenine dinucleotide phosphate (NADPH), it converts L-arginine to L-citrulline and NO. NO is released from the neuron through diffusion not the exocytosis[1]. Since it is synthesized on demand, there are no storage or formal uptake mechanism for NO and because of its highly reactivity, NO is rapidly inactivated *in situ*.

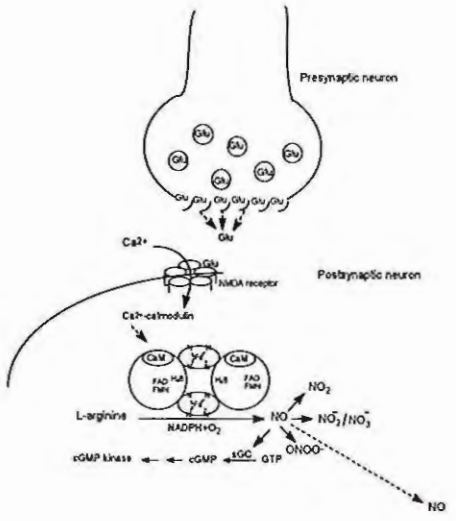

Figure 5 Overview of the biochemical pathway for nitric oxide synthesis and suggested targets for nitric oxide

NO potentiates transmitter release via direct stimulation of synaptic vesicle release. There are evidences that NO acts as a retrograde messenger,[7] that is, it diffuses back to the presynaptic neuron and binds to the iron of the heme cofactor of sGC leading to the increase of synthesis of cGMP which affects the amount of transmitter released. It has been found that because of the action of NO as retrograde messenger, a neuron or group of neurons in the hippocampus and the Purkinje cells of the cerebellum is repeatedly stimulated which leads to the occurrence of long term potentiation (LTP) and long term depression (LTD)[7,8]. LTP and LTD are identified as two processes that link with memory formation.

There are also several lines of evidence that suggest a role for NO in neurotoxicity of the brain[2,3]. During a stroke, delivery of oxygen and nutrients to affect regions of the brain is compromised and neurons in those areas become unable to exclude Ca^{2+}. When blood flow is restored to these calcium rich neurons, overstimulation of NMDA receptor occurs which in turn would activate the synthesis of large amounts of NO. The elevated level of NO has been linked to the inflammation associated tissue damage and the formation of NO active species such as peroxynitrite, a strong oxidant, capable of oxidizing thiols and DNA bases[9]. Mechanisms proposed for NO neurotoxicity are the same as its action in the immune system.

NO IN THE IMMUNE SYSTEM

The role of NO in the immune system is quite different from its function in either neurons or blood vessels. The activation of macrophages and neutrophils by cytokines and/or endotoxins, results in the synthesis of NOS, called inducible NOS[10] or iNOS. In contrast to NOS found in brain (bNOS) and vascular endothelial cells (eNOS), iNOS is not Ca^{2+}-dependent and always contains tightly bound calmodulin that allows the enzyme to be fully active at basal level of Ca^{2+}. The activity of iNOS lasts for many hours following the stimulation of its synthesis. The activated macrophages and neutrophils produce much more large quantities of NO reactive species, such as the potent oxidant peroxynitrite ($OONO^-$), than vascular endothelial cells or neurons do[3].

The reactive species of NO diffuse to tumor cells nearby and interfere with several cellular processes by interact with the iron-sulfur center of several important macromolecules including *cis*-acotinase, an enzyme involved in tricylic AMP cycle and the complex I and complex II in the mitochondrial electron transport chain by forming complexes of general formula $(RS)_2Fe(NO_2)^2$. These interaction diminish the cell's ability to produce and use NAPDH, leading to a decrease in ATP synthesis. NO is produced by the activated macrophages in enough amount to inhibit ribonucleotide reductase, an enzyme catalyses the forming of deoxynucleotides, which is a precursor in DNA synthesis. The interaction of NO to the active sites on this enzyme, a tyrosyl radical, a nonheme iron and thiol groups, causes depletion of deoxyribonucleotides and ultimately leads to inhibition of cell growth[3].

NO has also been reported for its neurotoxicity as well as tumoricidal and bactericidal action by inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) through S-nitrosylation of the active site cysteine[2]. NO also enhances ADP-ribosylation of poly (ADP-ribose) synthase, (PARS), a nuclear enzymes-activated by DNA strand breaks.

The increase of ribosylation causes cell death through depletion of ATP and the source of ADP-ribose, β–nicotinamide adenine dinucleotide[11].

NO IN NANC TRANSMISSION

NO-sensitive neurons that do not response to the neurotransmitter acetylcholine or norepinephrine can be found in several peripheral tissues including the cardiovascular, urogenital, respiratory and digestive system. Therefore NO has also been implicated as one mediator of nonadrenergic noncholinergic (NANC) neurotransmission.

NOS can be found in the myenteric plexus of neurons in all regions of the gastrointestinal (GI) tract[12]. These neurons mediate the physiologic relaxation of the part of GI tract that participates in the normal peristaltic activities of digestion. The gastric mucosa can become more susceptible to lesion and ulceration, when NO synthesis in GI system is impaired[2]. These evidences suggest the involvement of NO in the muscle relaxation and the cytoprotection of the gastric lining. The abnormality of enlarged stomach was found in mutant mice lacking a form of NO-synthesizing enzyme called bNOS which can be found in brain and peripheral nerve cells. Lacking of bNOS leads to decrease production of NO which controls the sphincter that must open to allow food to pass from stomuch to intestine[2]. NO was also found to mediate penile erection by relaxing smooth muscle in the corpus cavernorsum, the major erectile tissue of this organ[13]. Relaxation of the tissue allows increased blood flow into the penis, causing erection.

FUTURE DIRECTIONS

As several studies have been done on NO as an important compound play roles in many different physiological processes, an understanding of the involvement of NO in pathophysiology is evolving. The physiology and biochemistry of NO-mediated processes have been translated into therapeutic benefits. There are several researches on NO leading to a potential drugs, either research on NO-releasing agents or the supplement to the old drugs with agents that will make them more effective to be used in vascular disorders, pulmonary hypertension[9] or erectile dysfunction or impotence and the apoptotic cell death induced by treatment with tumor necrosis factor α (TNFα) plus actinomycin D[14]. These NO donors may also play a beneficial role in the tissue transplant or autoimmune disease such as rheumatoid arthritis. Selective inhibitors of individual forms of NOS which offered the therapeutic benefit with lower toxicity can be used in cerebral ischemia or postischemic damage in stroke to prevent the neurotoxicity from NO. The inhibition of NO synthesis was also found to have a significant beneficient effect on disease states such as schizophrenia, migraine headache, Alzeimer's diseases, developement of colitis[15]. NOS inhibitors were also found to prevent tolerance to morphine and the destruction photoreceptors in the retina[9,15].

Although several studies have been done in NO research and results from these trials are promising, much remains to be accomplished and efforts will undoubtedly increase to use this knowledge to discover new NO-related therapies.

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