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Medicinal and Chemical Perspectives of Nitric Oxide: An Overview

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Abstract

Nitric oxide (NO) is a simple, diatomic molecule possessing unique and fascinating chemistry. Indeed, the utility of NO as a biological effector and/or mediator is due to some of its novel chemical properties along with the properties of other nitrogen oxides derived from it. The multiple effects of NO in biological systems have resulted in intense investigation into the mechanisms of NO-mediated events. The chemistry of NO is the primary determinant of its biological properties. However, not all the reactions of NO that can be performed in the test tube are pertinent *in vivo*. Due to the discovery of its endogenous biosynthesis and diversity of its biological actions, nitric oxide (NO) is a molecule of extreme physiological, pharmacological, and pathophysiological interest. Much of the biological utility of NO is due to its unique chemical interactions with biological molecules. Thus, an understanding of the basic chemical properties of NO, and derived species, will provide insight into the intimate mechanisms of its biological activity.

Keywords: Nitric oxide; *In vivo*; Biological activity; Chemistry

Introduction

In the late 1980s and early 1990s it was confirmed by several groups of workers that nitric oxide was a chemical messenger released by the endothelium and other tissues in mammals. It was tentatively identified as being the endothelium-derived relaxing factor (EDRF) discovered earlier in the 1990s and has been linked to a multitude of physiological and pathophysiological states in mammals [1]. It is now known to be involved in the control of blood pressure, neurotransmission and the immune defence system of the body [2,3]. NO in the brain regulates many physiological processes affecting behavior and cognitive function, including synaptic plasticity. In addition, it also controls brain blood flow, promotes angiogenesis, maintains cellular redox state, cell immunity and neuronal survival. Its over-production may lead to neurodegeneration [4]. Excessive production has been linked to atherosclerosis, hypotension, Huntington's disease, Alzheimer's disease and AIDS dementia whilst underproduction has been related to thrombosis, vasospasm and impotence. This diversity of action has resulted in there being a considerable interest in the relationship of NO to both healthy and diseased states [5].

Nitric oxide (NO) released by vascular endothelial cells accounts for the relaxation of strips of vascular tissue [6] and for the inhibition of platelet aggregation [7] and platelet adhesion attributed to endothelium-derived relaxing factor [8]. NO is a colourless paramagnetic gas (B.P. -151.7^{0}C), sparingly soluble in water (2-3 mmol dm^{-3}). It is produced in mammals by the enzyme-catalysed interaction of molecular oxygen and arginine. NO is a key molecule involved in a variety of biological functions throughout the whole body [9]. However, whether it is NO or a derivative of NO, that is ultimately responsible for the observed physiological response to NO generation is still under dispute.

Structure of Nitric Oxide

Nitric Oxide (NO) is a linear molecule. NO is colorless gas at room temperature and pressure. The maximum solubility of ·NO (at 1atm partial pressure) in water at room temperature and pressure is approximately 2mM which is slightly higher than the solubility of dioxygen (O_2) in water. Also, like O_2, ·NO is somewhat lipophilic and possesses 6- to 8- fold higher solubility in nonpolar solvents and lipid membranes compared to water. Thus, the rates of ·NO reactions in hydrophobic environment can be dramatically increased (with O_2, e.g.) over that found in water due to its increased concentration. It becomes immediately evident from a lewis dot depiction that ·NO

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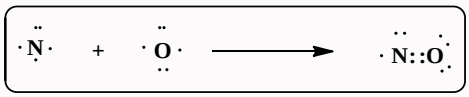

Figure 1: Lewis dot structure of ·NO.

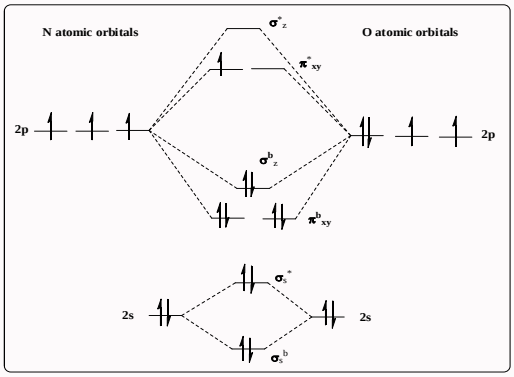

Figure 2: Molecular orbital diagram for ·NO.

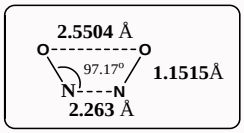

Figure 3: Structure of ·NO dimer.

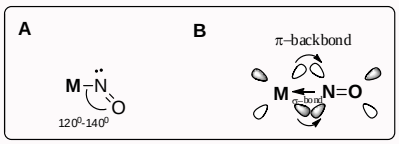

Figure 4: (A) Bent geometry for M-nitrosyl bond. M = metal ion and (B) Linear geometry (arrows depict bonding interactions).

has one unpaired electron and thus is formally a free radical species (Figure 1).

The radical nature of ·NO is evidenced by its ability to react with other species with unpaired electrons such as O_2, superoxides ($O_2^{\cdot -}$) and other radical species. However, unlike many free radicals, ·NO doesn't dimerise appreciably in the gas phase at room temperature and pressure, although it appears to form N_2O_2 (Figure 3) in the liquid state. This may seem curious since dimerization would lead to a structure (ON-NO) whereby all atoms have a full complement of eight valence electrons and, therefore, would satisfy the octet rule. In order to reconcile this apparent anomaly, the molecular orbital of ·NO must be considered. Simple combination of the nitrogen and oxygen atomic orbital's gives the following set of molecular orbital's (Figure 2). In order of increasing energy, the molecular orbital consist of a σ bonding orbital (σ_s^b) and a corresponding σ antibonding orbital (σ_s^*), two degenerate π bonding orbitals (π^b_{xy}), one σ bonding orbital (σ^b_z, only slightly higher in energy than the π bonding orbitals), two degenerate antibonding π orbitals (π^*_{xy}), and a high energy σ antibonding orbital (σ^*_z). Since the total number of valence electrons in the ·NO molecule is 11 (five from nitrogen and six from oxygen), the low-lying σ bonding and σ antibonding orbitals along with the bonding π and σ orbitals are all filled. However, the last electron must reside in a π^* antibonding orbital. In molecular orbital terminology, the electronic structure of ·NO exists as a monomer at room temperature and pressure. The above rationale for the observation that ·NO exists primarily as a monomeric species at room temperature and pressure is purely qualitative. Based on the argument above, ·NO is considered a free radical in spite of the fact that it does not have the tendency to dimerise at room temperature and pressure. Nitric oxide will, however, react with other species with unpaired electrons. One of the most biologically relevant reactions of this type is the reaction of ·NO with O_2. As a ground state triplet, O_2 has two unpaired electrons [electronic configuration for O_2 is KK $(\sigma_s^b)^2(\sigma_s^*)^2(\pi^b_{xy})^4(\sigma^b_z)^2(\pi^*_{xy})^2$] and therefore possesses some chemical characteristics of a biradical. As such, O_2 is known to react with other radical species. Since O_2 has two unpaired electrons, the product of O_2 and ·NO (and other radicals) still has one unpaired electron which can react further. Thus, the reaction of ·NO with O_2 consumes two equivalents of ·NO to give two equivalents of nitrogen dioxide ($\cdot NO_2$) possibly via the reactions (1), (2), and (3).

$$\cdot NO + O_2 \rightarrow \cdot OONO \quad (1)$$

$$\cdot OONO + \cdot NO \rightarrow ONOONO \quad (2)$$

$$ONOONO \rightarrow 2 \cdot NO_2 \quad (3)$$

The simple Molecular orbital (MO) picture for ·NO shows that the unpaired electron is in an antibonding MO (is a type of molecular orbital that if occupied by electrons, weakens the bond between two atoms and helps to raise the energy of the molecule relative to the separated atoms). Consequently, this electron should be readily lost, resulting in the formation of the nitrosonium ion (NO^+). This is in agreement with NO's low ionisation potential of 9.25 eV compared to a value of 15.56eV for nitrogen gas.

Chemical Properties of NO

The chemistry of ·NO is quite varied. It can be oxidised, form salts, act as an electrophile, oxidising agent and free radical and form complexes [10]. It gives rise to a series of salts and complexes with metals. Many of the chemical species identified in laboratory reactions have also been found in biological systems. It is believed that many of these species could be formed in biological systems by reactions similar to those found in the *in vitro* laboratory experiments, although at present few of these reactions have been detected *in vivo*. In biological systems, ·NO often appears to be closely associated with many other simple nitrogen-oxygen containing species such as nitrogen dioxide, nitrogen trioxide, nitrogen tetroxide, nitrate, nitrite and peroxynitrite. Nitrogen dioxide and peroxynitrite are believed to cause tissue damage [11].

Reaction of ·NO with metals

Complexes containing ·NO ligands have been prepared by the direct reaction of ·NO with the metal ion of complexes that have unused coordination sites, the displacement of an existing ligand by ·NO and the reaction of inorganic or organic nitrites [12]. The nitric oxide ligand may be bonded to the metal in three distinct ways (Figure 4), namely:

1. Complexes in which the M-NO bond is linear or almost linear, usually lying between 160⁰ and 180⁰. In these complexes the ·NO donates its odd electron to the metal atom.

2. Complexes in which the M-NO bond angle is bent and lies between 120⁰ and 140⁰. In these structures the ·NO is considered to be a 1 electron donor to the metal atom.

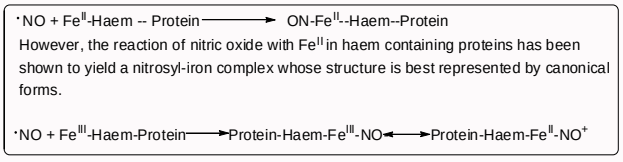

Figure 5: Canonical forms of nitrosyl-iron complex.

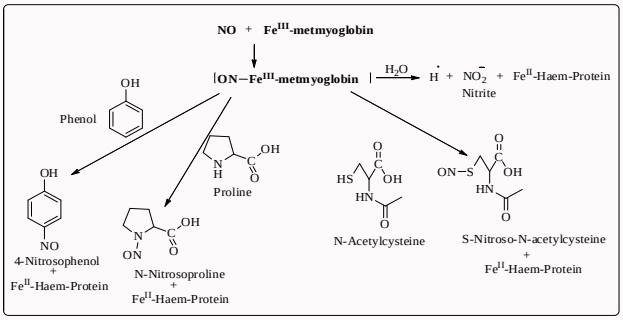

Figure 6: Examples of the reactions of nucleophiles with metmyoglobin-nitric oxide mixtures.

3. Complexes in which the ·NO acts as a bridge. More than 1 bridge may link the metal atom in these complexes.

·NO complexes with Irons

Iron is widely distributed in mammalian cells, both as free ions and complexed with a wide variety of proteins. ·NO readily forms complexes with both ferrous and ferric ions in the presence of other suitable ligands as well as reacting with the Fe^{II} and Fe^{III} centres of iron containing naturally occurring molecules [reactions (4) and (5)]. The coordination state of iron in the resulting complexes is often four, the ·NO occupying any vacant coordination sites [13].

$$Fe^{II}\text{-Hb-}O_2 + \cdot NO \rightarrow Fe^{III}\text{-Hb} + ONOO^- \quad (4)$$

$$ONOO^- \rightarrow NO_3^- \quad (5)$$

Electron paramagnetic resonance (EPR) spectroscopy showed that ·NO reacts with cysteine, histidine and other amino acids in the presence of ferrous ions to form 4-coordinate NO-iron-amino acid complexes. Spectroscopy also showed that ·NO reacted with proteins in the presence of ferrous ions to form complexes that were associated either with thiol groups or imidazole groups. Proteins whose structures contained a high proportion of thiol groups formed complexes involving the thiol groups in preference to imidazole groups.

Electron spin resonance (ESR) has also shown that proteins whose structures didn't contain iron bound to haem but included thiol groups formed complexes in the presence of free iron where one iron was complexed to two thiol groups and two ·NO molecules [reaction (6)]. These structures are probably similar to that proposed for the NO-iron-cysteine complex. Complexes of this type are called dinitrosyl-iron-dithiol complexes.

$$(NO)_2Fe(RS)_2 + 2R'SH \rightarrow (NO)_2Fe(R'S)_2 + 2RSH \quad (6)$$

The interaction of ·NO with cellular iron and proteins usually results in a loss of enzyme activity. However, it is not clear in the case of the proteins with thiol groups if the action of ·NO is the direct cause of the loss of enzyme activity. This is because the dinitrosyl-iron-dithiol protein complexes could lose their activity by exchanging their

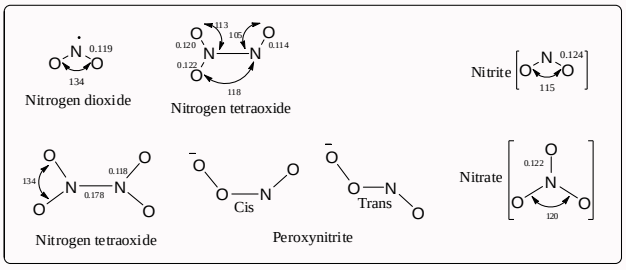

Figure 7: Structure of compounds related to nitric oxide having bond lengths in nanometers.

original protein-thiol ligands (RSH) with other different protein-thiol ligands (R$^{/}$SH). A further complication is that nitrogen dioxide can also react in the same manner as nitric oxide to form dinitrosyl-iron-dithiols. Consequently, it could be that nitrogen dioxide produced from ·NO is the *in vivo* source of dinitrosyl-iron-dithiols, in which case it would be nitrogen dioxide that is responsible for the loss of enzyme activity.

Experimental work has also shown that ·NO reacts with both the Fe^{II} and Fe^{III} oxidation states of iron bound to haem in protein molecules. The reaction of ·NO with Fe^{II} in haem containing proteins has been shown to form stable ON- Fe^{II}-haem-protein complexes [14]. However, the reaction of NO with Fe^{III} in haem containing proteins has been shown to yield a nitrosyl-iron complex whose structure is best represented by canonical forms (Figure 5). The electron deficient nature of the nitrogen explains why these complexes act as electrophiles and react with many nucleophiles, reducing the Fe^{II} and Fe^{III} in the process. e.g., Castro and Wade have shown that ·NO reacts with metmyoglobin to form a NO- Fe^{III}-haem complex that nitrosates a wide variety of nucleophiles.

Chemical Properties of Nitric Oxide Complexes

Nitric oxide (·NO) is a simple, diatomic molecule possessing unique and fascinating chemistry. Indeed, the utility of ·NO as a biological effector and/or mediator is due to some of its novel chemical properties along with the properties of other nitrogen oxides derived from it.

Oxidation exchange and Displacement reaction

Nitric oxide ligands of nitric complexes exhibit a wide variety of different types of reaction that include action as an electrophile or nucleophile, oxidation exchange and displacement reactions [15]. The difference in the attachment of nitric oxide ligand to the metal ion in the structures of linear, bent and bridge nitric oxide complexes, accounts for some of the differences in their reactivity. E.g., some linear M-NO complexes act as electrophiles because the nitrogen atom of the nitric oxide has donated 3 electrons to the metal. This leaves the nitrogen deficient in electrons and so open to attack by nucleophiles such as OH^-, RO^-, RS^- and RNH_2.

Protein-Haem-Fe^{III}-NO = RS-N=O (*S*-Nitrosothiol) + Protein-Haem-Fe^{II} (7)

NO reacts rapidly with free sulphydryl groups to form *S*-nitrosothiols [16]. *S*-Nitrosothiols slowly decompose, releasing nitric oxide, and so are of potential use as nitric oxide donors. Furthermore, *S*-nitrosothiols are one of the agents that act on soluble guanylyl cyclase (is a lyase enzyme, Guanylyl cyclase is often part of

Figure 8: Oxidation of nitric oxide by superoxide.

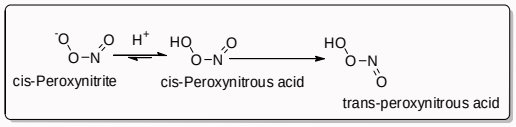

Figure 9: Peroxynitrous acid formation.

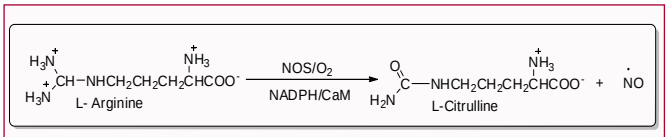

Figure 10: A schematic representation of the formation of nitric oxide.

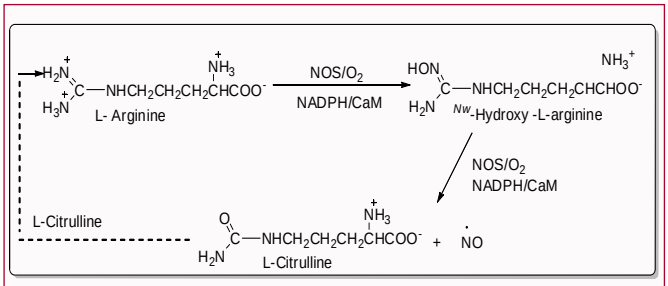

Figure 11: Recycling pathway for *L*-citrulline to *L*-arginine.

the G protein signaling cascade that is activated by low intracellular calcium levels and inhibited by high intracellular calcium levels). The nitric oxide ligands of both bent and bridge nitric oxide complexes act as nucleophiles, reacting with H^+ and other electrophiles. This is because the nitrogen of the nitric oxide has donated one electron to the metal atom and so it has a lone pair of electrons that can react with electrophiles. It has also been reported that the nitric oxide ligand in some nitric oxide complexes is oxidised to nitrogen dioxide. These nitrogen dioxide ligands have been shown to be involved in oxygen transfer reactions to alkenes, disulfides and other organic species, which means that nitric oxide could react through this route in biological systems.

Nitric oxide ligands also undergo exchange reactions in which the nitric oxide is exchanged with a ligand in another complex and displacement reactions in which nitric oxide displaces other ligands from their complexes. The latter type of reaction is believed to be responsible for activation of the enzyme guanylyl cyclase by nitric oxide in cells. The binding of nitric oxide to the iron atom of the haem nucleus of this enzyme releases a histidine residue. It has been suggested that this histidine residue acts as either a catalyst or a nucleophile, which increases the activity of the guanylyl cyclase. Other ligands that bind to the iron of haem do not liberate a histidine residue or activate guanylyl cyclase. Furthermore, removal of the nitric oxide deactivates the enzyme.

Nitric oxide has been reported to react with some metal complexes to form a range of products including cyclic nitrogen-oxygen compounds, nitrous oxide and nitrate. For e.g., nitric oxide rapidly reacts with oxymyoglobin (O_2-My-Fe^{II}) to form nitrate and metmyoglobin (Figure 6); the oxidised form of the oxygen-carrying hemeprotein myoglobin (My-Fe^{III}). Furthermore, oxyhaemoglobin has been found to react in a similar fashion to produce methaemoglobin which is a form of the oxygen-carrying metalloprotein haemoglobin, in which the iron in the heme group is in the Fe^{3+} (ferric) state, not the Fe^{2+} (ferrous) of normal haemoglobin.

Oxidation

Nitric oxide is readily oxidised by oxygen in both the gaseous state and under aqueous aerobic conditions to nitrogen dioxide, which readily dimerized to nitrogen tetroxide, which reacts with water to form a mixture of nitrite and nitrate ions (Figure 7).

Practical evidence suggests that this reaction is not a major metabolite route for nitric oxide. However, nitrogen dioxide is a strong oxidising agent (adds nitroso, NO, groups to a structure) and nitrating agent (adds nitro, NO_2). Nitric oxide is oxidised by superoxide $^{\cdot}O_2$ to form peroxynitrite (Figure 8). The reaction is very fast and is probably a major route for the metabolism of nitric oxide.

Peroxynitrite is a stable anion at alkaline pH (pK_a = 6.8 at 37°C). It has been suggested that the stability of the peroxynitrite ion is due to its structure being held in a *cis* conformation by internal forces of attraction [17]. Under acidic conditions the *cis* isomer is protonated to the *cis*-peroxynitrous acid, which isomerises to the more stable *trans* isomer. At neutral pH peroxynitrite is rapidly protonated to form the unstable peroxynitrous acid (Figure 9), which rapidly decomposes to nitrogen dioxide, hydroxyl radicals in about 20-30% yield and nitrate ions by two separate routes.

The stability of peroxynitrite allows it to diffuse considerable distances through biological systems as well as to cross membranes before it reacts. This reactivity is believed to describe by three main routes:

1. At physiological pH in the presence of hydrogen ions, peroxynitrite can result in the formation of an intermediate with hydroxyl free radical-like activity.

2. Peroxynitrite can react with metal ions and the metal centres of SOD to form a nitrating agent with similar reactivity to the nitronium ion ($^+NO_2$). This nitrating agent readily nitrates phenolic residues, such as the tyrosine residues of lysozyme and histone.

3. Peroxynitrite reacts with sulfhydryl groups of proteins and other naturally occurring molecules.

Salt formation

Both ^+NO and ^-NO ions are known. Their existence may be explained by the low ionisation (9.5 eV) and reduction potentials (0.39eV) of nitric oxide. Nitrosonium salts (^+NO) are well known but are readily hydrolysed in water. For example, nitrosonium sulphate rapidly hydrolyses to nitrous and sulphuric acids [18]. The nitroxyl ion (NO^-) is a less well characterised species. However, it is believed to be formed in the reduction of NO by cuprous (Cu^+) superoxide dismutase (Cu^+ SOD). Nitroxyl ions have been shown to react rapidly with molecular oxygen to from peroxynitrite, which would suggest that the nitroxyl ion would have a very short life in oxygenated tissue.

Reaction as an electrophile

Nitric oxide can act as an electrophile because its electronic configuration is one electron short of a stable octet. It readily reacts with thiols, amines and other nucleophiles. For example, nitric oxide reacts in this manner with primary and secondary amines to form

Table 1: Some characteristic properties of cNOS and iNOS.

Characteristics	cNOS		
	nNOS	eNOS	iNOS
Cellular location	Cytosolic (aqueous medium)	Particulate (membrane bound)	Cytosolic (aqueous medium)
Ca^{2+} dependent	Yes	Yes	No

adducts known as NO-NO-ates. The NO-NO-ate adduct is unstable in aqueous solutions, yielding nitric oxide. The rate at which the nitric oxide is produced has been shown to depend on the pH, temperature and structure of the amine. Consequently, NO-NO-ate adducts may have possible use as drugs to treat cases where the production of endogenous nitric oxide is impaired. Nitric oxide reacts with proteins containing thiol groups, under physiological conditions, to form *S*-nitrosothiol derivatives. *In vitro* evidence suggests that nitric oxide is transported in the plasma in the form of stable *S*-nitrosothiols, about 80% of which are *S*-nitroso-serum albumins. These *S*-nitrosothiols are believed to act as a depot for nitric oxide maintaining vascular tone. Furthermore, it is thought that *S*-nitrosothiols could be intermediates in the cellular action of nitric oxide.

Reaction as an oxidizing agent

Both nitric oxide and nitrogen dioxide have been reported to oxidise thiols under basic conditions.

Nitric Oxide Synthases Isoforms/Iso-Enzymes and Mechanism of NOS Mediated Nitric Oxide Biosynthesis

Nitric oxide is produced *in vivo* by the catalytic oxidation of *L*-arginine by a family of enzymes known as NOS (nitric oxide synthases) [19]. The reaction requires nicotinamide adenosine diphosphate as a cofactor (NADPH) and produces nitric oxide and *L*-citrulline in a 1:1 molar ratio (Figure 10). As a result, the concentration of citrulline is often used as an estimate of the *in vivo* concentration of nitric oxide [20].

NOS have been broadly classified (by chromosomal mapping of the genes encoding NOS) as constitutive NOS (cNOS) and inducible NOS (iNOS) enzymes. The cNOS enzymes are present in endothelial and neural tissue and are usually referred to as eNOS and nNOS enzymes, respectively. These enzymes are not identical but have similar properties. They appear to be present at an approximately constant level in the host cell but only produce nitric oxide when activated by the Ca^{2+} ion binding protein calmodulin (CaM). Conversely, iNOS enzymes are not present in the cell but are produced in response to stimulants of host and bacterial origin. Activation of cNOS results in the production of a short burst of nitric oxide at a low concentration whilst activation of iNOS results in the continuous production of nitric oxide at a high concentration.

It has been shown that the endothelial and neural tissue production of nitric oxide is initiated by agonists such as Ach, ADP, bradykinin and glutamate. The binding of these agonists to appropriate receptors causes an increase in cellular Ca^{2+} ions. These Ca^{2+} ions bind to and activate CaM, which in turn activates the cNOS present in the cell to produce nitric oxide [21].

Experimental work has shown that the first step in the formation of nitric oxide is the synthesis of N^{ω}-hydroxy-*L*-arginine as an enzyme-bound intermediate by a 2-electron oxidation involving molecular oxygen, NADPH and CaM. This intermediate is converted to citrulline with the liberation of nitric oxide by an overall 3-electron oxidation that also involves molecular oxygen, NADPH and CaM. This concentration of cellular *L*-arginine is maintained by the recycling of the *L*-citrulline to *L*-arginine (Figure 11). However, it has been shown that a low concentration of cellular *L*-arginine results in impairment of endothelium (is a type of epithelium that lines the interior surface of blood vessels and lymphatic vessels) dependent relaxation.

iNOS is found in a wide variety of cells such as mast cells derived from the myeloid stem cell. It is a part of the immune system and contains many granules rich in histamine and heparin, macrophages are a type of white blood cell that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the types of proteins specific to the surface of healthy body cells on its surface in a process called phagocytosis. Kupffer cells are specialized macrophages located in the liver lining the walls of the sinusoids that form part of the reticuloendothelial system (RES) and neutrophils. Unlike cNOS it is not calcium dependent. However, it has been found that in macrophages *i*NOS calmodulin is tightly bound to the inducible enzyme and so probably plays a part in its action but possibly by a different mechanism to that found in other NOS enzymes. iNOS enzymes are activated by the presence of substances such as bacterial toxins, gamma interferon and interleukin 1-β. Activation of iNOS results in the continuous production of a high concentration of nitric oxide.

These general forms of NO reflect the two distinct general modes of action of NO. With cNOS the enzyme produces bursts of NO that transmit a message to the target cells without damaging those cells. With iNOS the enzyme is responsible for the continuous production of NO in sufficient production to damage and kill cells that may or may not be benefit to the organism. For example, activated immune cells produce amounts of NO that are lethal to harmful target cells such as those found in cancers and invasive parasites but over production of nitric oxide has been linked to the death of pancreatic β cells in insulin-dependent diabetes mellitus.

Cytotoxic Roles of Nitric Oxide

Nitric oxide generated by the immune system through the *i*NOS pathway acts as a killer molecule. The high concentration of nitric oxide (nanomoles) produced by this process causes lethal oxidative injuries to the target cells, such as cancer and parasite cells. Little is known about this process but it is now thought that the nitric oxide reacts in conjunction with superoxide, which is also produced by activated immune system cells. The immune cells increase their surface area and fold around their target cells or microorganisms. Once in position they release nitric oxide, which attacks the copper and iron complexed proteins in the target cell, liberating copper and iron ions from these proteins. This is accompanied by the formation of hydroxyl free radicals and molecular oxygen that cause massive oxidative injury to the target cell.

Nitric Oxide Synthesis through Cnos Route

This is believed to act by binding to the iron in the haem unit that constitutes the active site of soluble guanylyl cyclase (GC). This alters the conformation of the enzyme, which activates it to act as a catalyst. However, some workers believe that the nitric oxide is converted to an *S*-nitrosothiol and it is a compound, that nitrosates the soluble GC. Activation of the soluble GC enzyme, by either of these routes, results in the conversion of GTP (guanosine triphophate) to cGMP in the target cell. For e.g., increase in cGMP concentration has been

shown to inhibit Na^+ channels of the kidney and to decrease the Ca^{2+} in smooth muscle and platelets. Dissociation of the nitric oxide from the active ON-Fe^{II}-haem-enzyme complex deactivates the GC. The nitric oxide released by the cNOS route that doesn't bind to a haem target area may take part in nitrosation reaction or reacts with thiols to from nitrosothiols, which decompose to release nitric oxide. It has been suggested that nitrosothiols, such as nitrosocysteine may act as a depot for nitric oxide, thereby prolonging its action. Furthermore, it has also been suggested that nitric oxide binds to the thiol groups of mammalian albumin and as such is transported in the plasma.

Therapeutic Significance of NOS Inhibitors and Nitric Oxide

Compounds that reduce nitric oxide generation

A wide range of pathophysiological states have been associated with the overproduction of nitric oxide. E.g., overproduction of nitric oxide by iNOS has been associated with osteoporosis, inflammation, rheumatoid arthritis and morphine dependence while overproduction by nNOS is associated with Alzheimer's disease, strokes and schizophrenia. Consequently, these NOS enzymes offer a potential target for drugs to treat these conditions.

NOS inhibitors

Reduction of nitric oxide production can be achieved by inhibiting the action of NOS or its activating processes, such as calcium ingress. Based on knowledge of the process for the nitric oxide production an obvious line of investigation was to develop NOS inhibitors by synthesising analogues of *L*-arginine. A number of these analogues have been found to inhibit the formation of nitric oxide by acting as NOS blocking agents. These inhibitors have been extensively used to investigate the action of nitric oxide. N^{ω}-Monomethyl-*L*-arginine (*L*-NMMA) has been found to increase Blood pressure in man and other species. However, its selectivity is low. N^5-(1-Iminomethyl)-*L*-ornithine (*L*-NIO) is an irreversible inhibitor of NOS in activated macrophages. N^{ω}-Nitro-*L*-arginine methyl ester (*L*-NAME) has been reported to exhibit a selectivity of 300:1 for nNOS as against iNOS, while *S*-ethyl-*L*-thiocitrolline has been reported to exhibit a 50:1 preference for nNOS against eNOS. Several simple guanidino compounds have also been to inhibit nitric oxide synthesis. It is believed that these compounds inhibit nitric oxide synthesis by preventing the 2^{nd} stage of the oxidation of arginine (Table 1).

Amino guanidine has been shown to be a selective inhibitor of iNOS in animal models. It has a minimal effect on the cNOS that is required to maintain blood pressure. The selectivity of amino guanidine is believed to be due to the presence of the hydrazine residue, since replacement of this moiety by a methyl group, which has similar overall shape and size, resulted in the loss of selectivity and a considerable loss of activity. Many other classes of compound have been examined as sources of selective NOS inhibitors. For e.g., 2-iminoazaheterocycles and imidazole analogues show a preference for iNOS against nNOS. Dipeptides containing an N^{ω}-nitroarginine residue have also shown a range of different selectiveness towards NOS isoenzymes.

Compounds that supply nitric oxide

Sodium nitroprusside and organic nitrates and nitrites have been used from 100 years to treat angina. Glyceryl trinitrate has been used to relieve impotence. It has been demonstrated that sodium nitroprusside and organic nitrates and nitrites act by forming either nitric oxide or a nitric oxide adduct during their metabolism. The metabolic pathways of these drugs appear to be catalysed by enzymes that are specific for each drug. This specific nature would account for the wide diversity of pharmacological action of each of these drugs. Knowledge of the chemistry and biochemical pathway of nitric oxide has resulted in several groups of compounds being investigated as leads to new drugs. E.g., the suggestion that EDRF is an *S*-nitrosothiol has resulted in the investigation of a number of these compounds as potential drugs. *S*-nitrosocaptopril, *S*-nitroso-*N*-acetylcysteine and *S*-nitroso-*N*-acetylpenicillamine have all been shown to have vasodilator properties in animals and may have some use in humans.

NO-NO-ates are also being investigated as potential sources of drugs. These compounds are prepared by the direct action of nitric oxide on a nucleophile. They are stable solids that spontaneously decompose in water. The rate of decomposition depends on the temperature, pH and the nature of the nucleophilic residue X. Since, the rate of release of nitric oxide depends on the nature of X; NONO-ates could be useful as slow-release drugs. Furthermore, the spontaneous nature of the generation of nitric oxide means that the release of nitric oxide *in vivo* would depend on the chemical nature of the NONO-ate rather than the intervention of another biological process such as redox system.

Exception

Sydnomines are a group of compounds used to treat angina. Molsidomine is metabolised in the liver to 3-morpholino-sydnomine (SIN-1), which spontaneously releases nitric oxide under aerobic conditions [22]. SIN-1 doesn't produce nitric oxide under anaerobic conditions, which suggests that the intervention of a redox system is necessary for nitric oxide release. Example: Sildenafil (Viagra, a PDE 5 (phosphodiesterase type 5) inhibitor) and a number of similar nitric oxide releasing compounds are used to treat erectile dysfunction in men.

The Genetic Approach

Each of the different isoforms of NOS is produced by a different gene. Consequently, control of the relevant gene would influence the production of the relevant NOS isoform and the subsequent generation of nitric oxide produced by that isoform. This would enable medicinal chemists to design specific NOS inhibitors and stimulants. A number of NOS enzymes have been cloned from a variety of sources but no compounds have yet been developed for clinical use.

Conclusion

The chemistry of ·NO and ·NO-derived species consists of numerous interrelated and interdependent processes. Nitric oxide is a unique biological messenger molecule. It mediates, in part, the immune functions of macrophages; it is produced by endothelial cells to mediate blood vessel relaxation; and it also serves as a neurotransmitter in the central and peripheral nervous system. Endothelial nitric oxide synthase and neuronal nitric oxide synthase are thought to be primarily constitutive, with activation induced by calcium entry into cells in the absence of protein synthesis. The molecular targets of NO are increasing, as are its pathophysiological and physiological roles in the nervous system. Under conditions of excessive formation, NO is emerging as an important neurotoxin in a variety of nervous system disorders as well as other physiological disorders.

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