

Nitric Oxide Synthases

Key References

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Overview

Nitric oxide is a reactive free radical gas that can act as an intracellular or extracellular messenger. It may act locally as an autacoid, paracrine substance or neurotransmitter, and at a distant target if it is carried and delivered as a protected complex, or prodrug. It is therefore, a very unique signaling molecule. It is formed from L-arginine by a family of isoforms of nitric oxide synthases (NOS 1-3). These enzymes are separate gene products encoded on three different chromosomes. The three isoforms have about 50-60% homology and each isoform has considerable homology between species (about 90%). A variety of co-translational and post-translational modifications of the different isoforms can take place, including phosphorylation, myristoylation and palmitoylation, each of which may influence their subcellular location and/or activity. This family of enzymes has considerable homology with cytochrome P450 and has both oxidase and reductase domains with complex cosubstrate and cofactor requirements that include heme, O₂, NADPH, FAD, FMN, tetrahydrobiopterin and calmodulin. The enzyme isoforms are active as homodimers and catalyze the oxidation of the guanidino nitrogen of L-arginine to nitric oxide. The other product of the reaction is citrulline.

Most cell types and tissues possess one or more isoforms of NOS. The regulation and roles of each NOS isoform in various tissues and biological processes is an active area of investigation as is the development of selective and specific inhibitors of the NOS isoforms. Nitric oxide, formed by NOS-1 (nNOS) in central or peripheral neurons, may function as a neurotransmitter, particularly in NANC (nonadrenergic and noncholinergic) neurons. It is thought that NOS-2, or inducible NOS (iNOS), is probably

not present in cells and tissues unless its formation has been induced with endotoxin and/or proinflammatory cytokines such as IL-1, interferon- γ or TNF- α . Formation of nitric oxide by this isoform may participate in antimicrobial activity, cytotoxicity and/or inflammatory responses with or without the formation of peroxynitrite. Nitric oxide formation by NOS-3 (eNOS) in endothelial cells explains the effects of endothelium-dependent vasodilators on vascular relaxation and decreased platelet adhesion and aggregation.

These and many other effects of nitric oxide are mediated through increased cyclic GMP formation due to soluble guanylyl cyclase activation. Thus, nitric oxide via cyclic GMP can regulate protein kinase G activity, protein phosphorylation and numerous biological processes. However, some effects of nitric oxide such as its antimicrobial, cytotoxic and inflammatory effects are independent of cyclic GMP and may result from nitric oxide's interactions with transitional metals, thiol groups and other free radicals such as superoxide anion. These complexes may alter the structure or function of the macromolecule. Some complexes may act as nitric oxide reservoirs or "prodrugs" for nitric oxide release under appropriate conditions.

The participation of nitric oxide and cyclic GMP in cell signaling has been one of the most rapidly developing areas in biology with about 70,000 publications since the first biological effects of nitric oxide were described in 1977. While the field has grown exponentially, many important questions regarding the formation, function and metabolism of these important messengers and signaling molecules remain to be answered. Fortunately, the availability

of numerous compounds that alter their formation, metabolism and function has markedly stimulated research in the field.

Nitric Oxide Synthases

CURRENTLY ACCEPTED NAME	NOS-1 (N3033)	NOS-2 (N1783, N2783)	NOS-3 (N1533)
OTHER NAMES	nNOS Neuronal NOS NOSII	iNOS Inducible NOS NOSII	eNOS Endothelial NOS NOSIII
HUMAN MONOMER SIZE	~160 kDa	~131 kDa	~133 kDa
COFACTORS	NADPH (N7505) FMN (F1392) FAD (F6625) H4 bioppterin (T4425) Calmodulin (P2277) Heme	NADPH (N7505) FMN (F1392) FAD (F6625) H4 bioppterin (T4425) Calmodulin (P2277) Heme	NADPH (N7505) FMN (F1392) FAD (F6625) H4 bioppterin (T4425) Calmodulin (P2277) Heme
SUBCELLULAR LOCALIZATION	Predominantly cytosolic	Both cytosolic and particulate	Predominantly particulate with caveolae
SOME INHIBITORS ^a	N-Methyl-L-arginine (M7033) N-Nitro-L-arginine (N5501) 7-Nitroindazole (N7778) 1-(2-Trifluoromethylphenyl)imidazole (T7313) L-Thiocitrulline (T1090) S-Methyl-L-thiocitrulline (M5171)	Aminoguanidine (A8835, A7009) S-Benzylisothiourea (B9138) 1-(2-Trifluoromethylphenyl)imidazole (T7313) L-N6-(1-Iminoethyl)lysine (I8021) 1400W (W4262)	N-Methyl-L-arginine (M7033) N-Nitro-L-arginine (N5501) N-Iminoethyl-L-ornithine (I8768) 7-Nitroindazole (N7778)
TISSUE EXPRESSION	Ubiquitous	Macrophages, liver, retina, bone cells, epithelial cells	Platelets, placenta, blood vessels, liver, kidney
PHYSIOLOGICAL FUNCTION	Produces NO; displays properties of a neurotransmitter in brain and peripheral nervous system; neurotransmission, renal tubular glomerular interactions, intestinal motility	Produces NO; mediates tumoricidal and bacterial actions in macrophages; antimicrobial, cytotoxic, inflammation, septic shock	Produces NO; mediates VEGF-induced angiogenesis in coronary vessels; inhibits blood clotting through decreased platelet activation; vascular relaxation, decreased platelet adhesion, angiogenesis, aggregation
DISEASE RELEVANCE	Erectile dysfunction, atherosclerosis, endothelial dysfunction	Inflammation	Hypertension

Abbreviations

FAD: Flavin adenine dinucleotide

FMN: Flavin adenine mononucleotide

NADPH: β-Nicotinamide adenine dinucleotide phosphate

1400W: N-(3-(Aminomethyl)benzyl)acetamide

FOOTNOTES

^a While numerous inhibitors are available, some compounds display some partial selectivity.