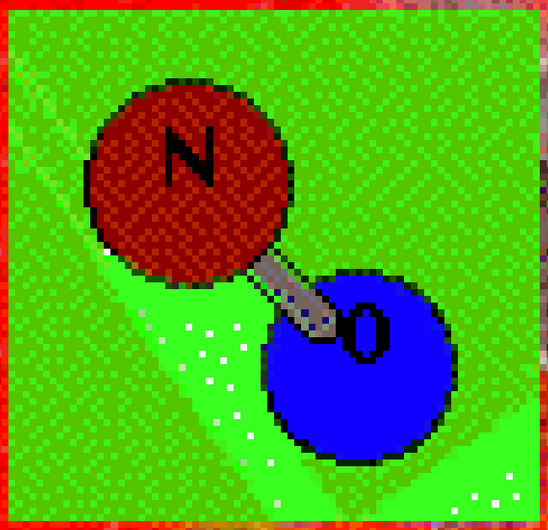

Nitric Oxide Review Online

Editor Weiming Xu

Nitric Oxide: From a mysterious labile factor, to the molecule of the Nobel Prize.

Recent progress on nitric oxide research**.**

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Abstract

NO is now known to be an important messenger molecule in biology. It regulates a variety of functions within cells and tissues including vasodilation, neurotransmission and immnuological process. This review will focus on the nitric oxide synthase gene family and recent progress on molecular genetic analysis of NOS1, NOS2 and NOS3 genes.

Key words: Nitric oixde, NOS, gene family, genetic study

Introduction: Nitric Oxide and Nobel Prize

Scientific breakthrough often comes from unexpected observations, and imaginative adventurous research. In the early 80's at the New York Laboratory of Suny health centre, Dr. Robert Furchgott was presented contridcting results by his two technicians. One technician always found the acetycholine relaxed the blood vessel. Where as the other, found it always caused contraction. Furchgott noticed that one technician handled the vessels roughly, and inadvertently rubbed off the thin layer of endothelium from the surface of the vessel, whilist the other was careful, and kept the endothelium intact. He then realized that an intact endothelium is a prerequisite for the relaxant effect of acetycholine(1). By separating the endothelium from the smooth muscle, he showed that a labile factor is released from the endothelium, he named it the "endothelium-dependent relaxing factor"(EDRF).

Since then the race to hunt down this mysterious factor has been fiercely contested. The three winners of this race have been awarded the Nobel Prize for physiology/medicine this year. They are pharmacologists Robert Furchgott, of the state University of New York; Louis Ignarro(2), of the University of California, Los Angeles; and Ferid Murad, of University of Texas Medical School in Houston(3). Although many

scientists feel that a fourth name should also been recognized-that of Salvador Moncada, currently director of the Wolfson Institute for biomedical Research at University College London for his contribution to conclude that EDRF, and nitric oxide was identical(4)(5).

NO regulates vasodilation, neurotransmission and immnuological process

In late 80's these pioneer scientists, discovered a gas, Nitric Oxide(NO), previously considered to be merely atmospheric pollutant, is a critical signaling molecule for endothelium-dependent, relaxing occurs in response to a wide variety of stimuli. Including acetylcholine, bradykinin, substance P, thrombin, adenine nucleotide, calcium ionophore A23187, and increases in the blood flow through arteries, microvessles and some veins. It is now clear that the endothelium-dependent relaxation, described by Furchgott and the others, is just one of the myriad mediator functions of NO. NO also carries important information in the nervous system:

In the brain, NO have been shown to be a neurotransmitter, and play an important role in learning and memory(6). In male, it is message that translates sexual excitement into an erect penis. Pfizer’s blockbuster drug Viagra reverses impotence by enhancing an NO-stimulated pathway. At a time when vascular pharmacologists were pursuing the identity of EDRF, immunologists were independently exploring the cytotoxic properties of macrophages that appeared to be dependent on the generation of large amounts of NO in response to infection(7)(8). The immune system uses NO in fighting virus, bacteria, parasites, and tumors. By early 1990's, scientists in distinct disciplines-immunology, cardiovascular physiology, and neuroscience- sudden realized they were studying the same molecule, NO.

Nitric Oxide Gene family

NO is a highly labile gas, and a very small compound that is not stored, but diffuses from its site of formation to its site of action (since it is both water/lipid soluble, it diffuses freely within tissues). Because it contains a unpaired electron, it is extremely active. It binds to the haem moiety of guanylate cyclase, and cause a greater than 400-fold activation of the enzyme. NO is formed directly from the guanidino nitrogen of the L-arginine by nitric oxide synthase(NOS) through a process that consumes five electrons, and results in the formation of L-citrulline(Fig.1.) . NOS is an unusual oxidative enzyme, in that most other enzymes consume one or two electrons for a similar function.

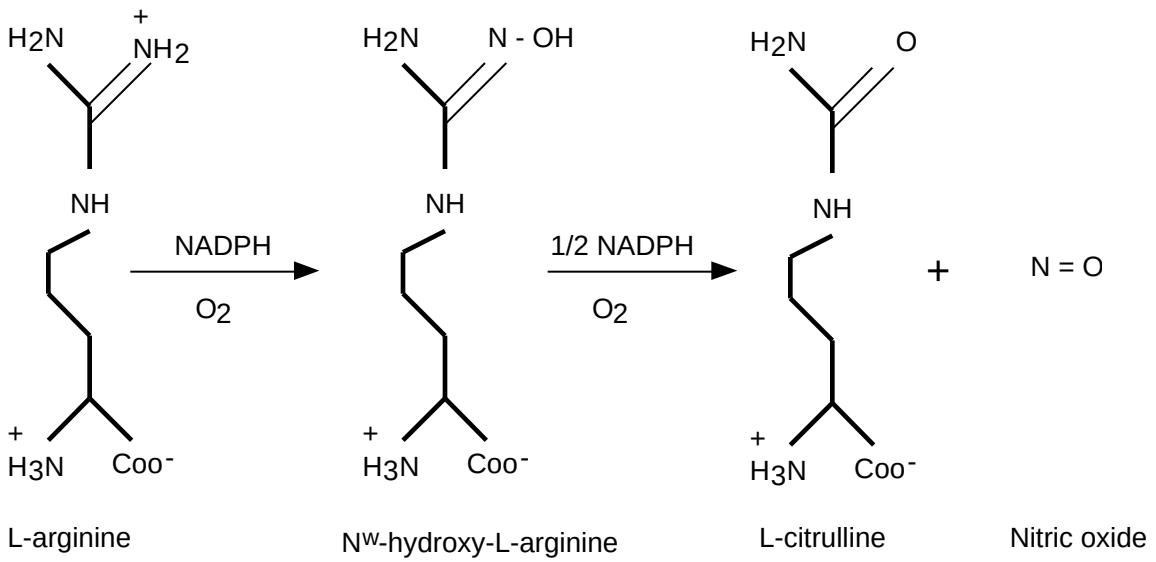

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Fig.1. Biosynthesis of NO. L-arginine is converted to No in two successive steps of which a two-electron oxidation of L-arginine to N-w-hydroxy-L-argninie is the first, then converted

to NO and citrulline, utilizing one and half NADPH and O_2. Both steps require $Ca2^+$ and calmodulin as activators and are accelerated by tetrahydrobiopterin.

NOS's structure, and function have been clarified by molecular cloning of the cDNA from the brain(NOS1)(9), endothelial(NOS3)(10), macrophages, and other type of inducible cells(NOS2)(11) (Fig.2). Three forms of the NO synthase enzyme are known: NOS3 being initially isoalted from the endothelium, NOS1 being initially isolated from brain cerebellum, and NOS2 being intially isolated from murine macrophage and human hepatocytes and chondrocytes. The molecular cloning, functional analysis, and crystal structure study data revealed the NOS gene family shares a similar compositions with each other:

All have two domains : N-terminal half of Heme-oxygenase dome, with tetrahydrobiopterin , heme and arginnine binding sites and C-terminal half of P-450 reductase domain with the positions of recognition sites for NADPH, as well as for flavin mononucleotide(FMN) and flavin adenosine dinucleotide(FAD). Cytochrome P-450 reductase is the only other mammalian enzyme known to contain these recognition sites(Fig.2).

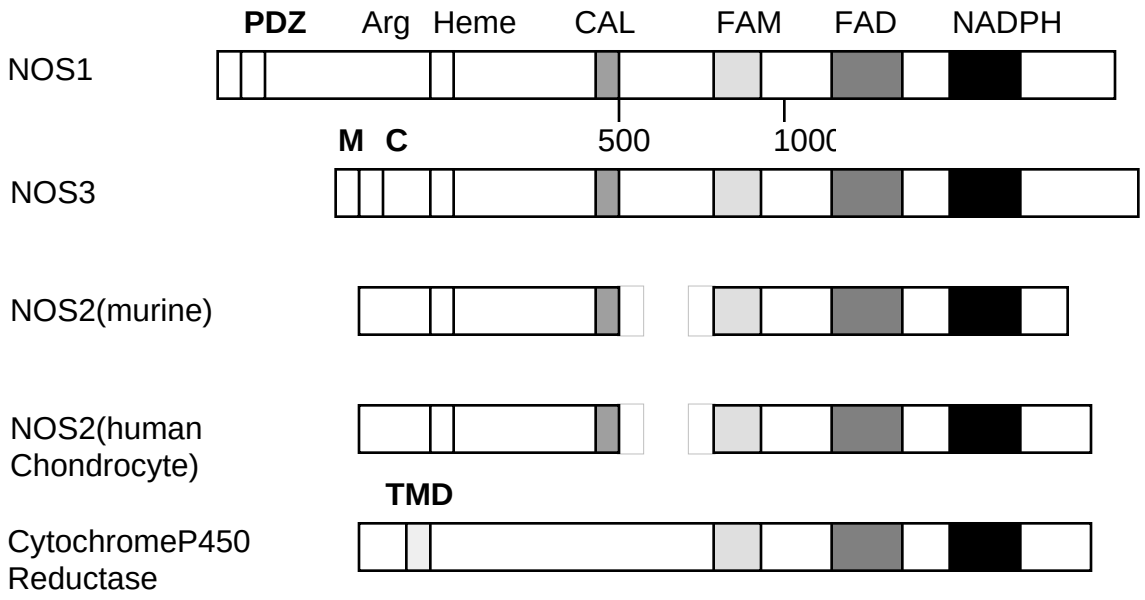

Fig.2. Sequence homologies of molecular isoforms of NOS. All NOS enzymes have consensus binding sites for Arginine(Arg), Heme, FAD, FMN, NADPH and calmodulin(Cal). NOS1 has PDZ binding domain. NOS3 has myristoylation(M) and Caveolin(C) binding sites and Cytochrome P450 reductase has transmembrane domain(TMD).

Both NOS3, and NOS1 enzymes are constitute in the sense that their activation does not required new enzymes protein synthesis. For instance, the NOS1 is activated by glutamate -induced increase in intracellulular $Ca2^{+}$ levels activates NOS via calmodulin. NOS3 is also a constitute, being activated by the increase in intracellular $Ca2^{+}$ level. Agonists such as acetylcholine, bradykinin activating at muscarininc or bradykinin receptor on the endothelial cells active the phosphinosititide cycle to generated $Ca2^{+}$ which then stimulates NOS by biding calmadulin. Constitute NOS enzymes account for the role of NO in mediating rapidly events, such as neurotransmission, and blood vessel

dilatation. In constrast, the inducible nitric oxide , NOS2, which mediated produce large amount of NO, followed by endotoxin, microbacteria, and cytokines induction.

Recent progress on genetic study on NOS gene family

Soon after the first NOS has been cloned in 1991(9), we have mapped this gene on the human chromosome 12q12-q24 in 1992(12). In 1993, the fluorescence -situ hybridization has been used to pinpoint the gene on 12q24.3(13), followed by identifing the highly polymorphic markers for NOS1 gene(14). This was followed by establishing the linkage map of the gene location, which leads to the identification of the human disease association with NOS1 gene. It turns out NOS1 is a susceptibility locus for in infantile pyloric stenosis (PS), one of the most common, and lethal condition in new born babies. In the 27 families studies(15), there was significant overall transmission disequilibrium between PS and NOS1a (P = .006). Consideration of each allele independently revealed a highly significant tendency for allele 7 (210 bp) to be preferentially transmitted to the affected offspring (P = .0006). Coincidentally, the most evident effect of disrupting the NOS1 gene in mice(16), is the development of grossly enlarged stomachs, with hypertrophy of the pyloric sphincter and the circular muscle layer. This phenotype resembles the human disorder infantile pyloric stenosis, in which gastric outlet obstruction is associated with the lack of NADH neurons in the pylorus.

In order to hunt down the other human diseases in which NOS genes may be involved in, we and others also quickly carried out gene mapping studies, and polymorphic marker scans, and disease association studies for the NOS2 and NOS3

genes. The NOS3 have been mapped to 7q35-q36 and NOS2 has been 17q11.2(17). We have discovered the NOS gene family is a dispersed gene family(18). Although NOS1, and NOS3 genes shows only a single chromosomal localization, we have found multiple copies of inducible nitric oxide synthase gene-like sequences in the human genome. They have been called NOS2A, NOS2B and NOS2C (Table 1)(19). The genomic structures of the NOS1, NOS2 and NOS3 gene were also determined by our group and others (Table2)(20).

Tablel 1. Chromosomal localization of NOS1, NOS3, NOS2 and NOS2-like sequences onto different regions of human chromosomes

Table 1 Isoforms of NO Synthase(NOS)

Name	Other Name	Type	Regulated by	Present in	Human chromossome
NOS1	Neuronal NOS nNOS	constitute	Ca^{2+}/ calmodulin	Brain, cerebellum other neuronal tissues	12q24.1-12q24.31
NOS3	Endothelium NOS eNOS	constitute	Ca^{2+}/ calmodulin	Endothelial cells	7q35-7q36
NOS2A	Inducible NOS iNOS	inducible	endotoxin cytokines	Macrophages Neutrophiles Chondrocytes Hepatocytes	17q11.2
NOS2B		Unknown		Unknown	17p11.2-17q11.2
NOS2C		Unknown		Unknown	17p11.2-17q11.2

As expected from physiological role of NOS2 genes, the NOS3 gene knock out mice has been shown to have phenotype of hypertension(21). NOS2 knock out mice has

been shown to be uniformly susceptible to infection by many pathogens, including tuberculosis(22,23). The studies for NOS2 knock mice has shown that it is one of the crucial genes to the host defence against many lethal pathogens. Recently, we have identified a highly polymorphic microsatellite marker in the human NOS2 promoter region(24), and found a strong positive allele association between human NOS2 gene polymorphism and fetal cerebra malaria(25). Infectious diseases are still major health risks in developing countries, future study of the NOS2 gene may help to unlock new methods to combat these diseases.

Alfred Nobel, was prescribed nitroglycerin, one of the key components of dynamite to ease his chest pain when he contracted heart disease. It took 100 years until it was clarified that nitroglycerin acts by releasing nitric oxide gas. However, it also opened floodgate for medical articles detailing the biological activity of nitric oxide (NO) which are now flooding the scientific journals at a pace of 500 per month. There are more than 22,000 medical articles that dealt with nitric oxide, yet nitric oxide was not even a subject 12 years ago. It is impossible for any review to cover such a vast amount of information now. The only hope we have is that this rapid progress of Nitric Oxide Research will bring more benefit for human health.

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Figures and Tables

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Table 2. Comparison of the localization and size of exon/intron among NOS1, NOS2 and NOS3 Genes(ref.20).

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