Nitric Oxide Synthase Inhibitors: Future Therapies for CNS Disorders?

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Summary

Nitric oxide (NO) is a messenger that is involved in many physiological events including host defence, vascular tone and neurotransmission. It plays a role in many nervous system functions including synaptogenesis during brain development, synaptic plasticity associated with learning and memory, and regulation of cerebral blood flow. NO is produced from at least 3 different NO synthase (NOS) isoforms: neuronal NOS (type-1; nNOS), immunological NOS (type-2; iNOS) and endothelial NOS (type-3; eNOS).

Under conditions of excessive formation, NO may elicit neuropathological changes. For example, excess NO from nNOS has been implicated in neuronal damage associated with stroke, excitotoxins, mitochondrial toxins and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Activation of iNOS may play a role in the pathogenesis of multiple sclerosis and severe AIDS dementia, while derangements in NO formation from eNOS may play a role in the pathogenesis of migraine headaches. Thus, the development of specific NO inhibitors has become a major challenge. Selective inhibitors are beginning to be developed for the various isoforms of NOS, which raises the possibility that medicinal chemists will be able to develop well tolerated and selective NOS inhibitors that may be used for the treatment of CNS disorders that are due to derangements of NO.

Research in different fields has led to the identification of nitric oxide (NO) as the smallest known biological messenger. NO plays a role in a wide array of important physiological events in the vascular, central, peripheral and immune systems (see fig. 1).[1,2] NO is a new type of transmitter,[3] which acts independently of vesicular release and receptor interactions and instead relies on local synthesis and diffusibility. Therefore, the regulation of NO synthesis is the key to controlling or interfering with the activity of the messenger. Since NO is involved in many apparently independent events, such as host defence, vascular tone and neurotransmission, it has quickly become an important pharmacological target for the treatment of a variety of disorders, including vascular, neurodegenerative, infectious and immunological diseases. The diversity of NO-mediated processes makes it difficult to use changes in global NO levels as a therapeutic tool.[4] Therefore, the future challenge will be to develop compounds that interfere with NO production in a tissue-specific fashion.

1. Synthesis and Regulation of Nitric Oxide (NO)

NO is formed by the conversion of L-arginine to L-citrulline by NO synthase (NOS). This reaction
Fig. 1. Physiological roles of nitric oxide (NO	extsuperscript{•}). Abbreviations:
cGMP = cyclic guanosine monophosphate; LDP = long term depression; LTP = long term potentiation.

leads to an overall 5-electron oxidation of the guanidinium nitrogen of L-arginine.\textsuperscript{[5,6]} NOS is found in a wide variety of cells and tissues,\textsuperscript{[7]} and molecular cloning and biochemical studies have identified at least 3 different NOS isoforms:\textsuperscript{[8]}

- neuronal NOS or type-I NOS (nNOS)
- immunological NOS or type-2 NOS (iNOS)
- endothelial NOS or type-3 NOS (eNOS).

All the known NOS isoforms possess a haem group, require flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin as cofactors, and utilise L-arginine, oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) for their activity.\textsuperscript{[5,6]} Although all the NOS subtypes use the same cofactors and substrates, there are significant differences in their regulatory mechanisms and biophysical properties.

Localisation studies indicate that nNOS is not only expressed in neurons, but also in skeletal muscle, neutrophils, pancreatic islets, endometrium, and respiratory and gastrointestinal epithelia. eNOS is primarily localised to endothelial cells, but it has also been detected in neurons.\textsuperscript{[8]} eNOS is found primarily in the particulate fraction, indicating that it is mainly membrane-bound, whereas nNOS has a more cytoplasmic localisation.\textsuperscript{[9]}

Calcium ions (Ca\textsuperscript{++}) regulate both nNOS and eNOS. The ions form a complex with calmodulin, and the binding of the Ca\textsuperscript{++}-calmodulin complex to eNOS or nNOS is necessary for the shuttling of electrons from NADPH to the haem group of the enzyme.\textsuperscript{[10]} The molecular sequence of the eNOS and nNOS isoforms shows consensus sites for phosphorylation.\textsuperscript{[6]} Biochemical characterisation of the effects induced by phosphorylation on nNOS activity indicates that nNOS is inhibited by Ca\textsuperscript{++}-calmodulin–dependent kinase, cyclic adenosine monophosphate (cAMP)– and cyclic guanosine monophosphate (cGMP)–dependent protein kinases and protein kinase C.\textsuperscript{[11,12]} Moreover, calcineurin, a protein phosphatase, is able to dephosphorylate nNOS and subsequently increase its catalytic activity.\textsuperscript{[13]} eNOS activity is also regulated by phosphorylation. The phosphorylated form is mainly found in the cytoplasm. It is not clear whether phosphorylation mediates translocation of eNOS from the membrane to the cytosol or whether it is a secondary event due to translocation.\textsuperscript{[14]} Protein kinase C modulates eNOS activity by attenuating NO formation.\textsuperscript{[15,16]}

Modulation of the phosphorylation state of NOS may be a potential target for NO synthesis regulation. For instance, tacrolimus (FK 506), which inhibits calcineurin, maintains nNOS in a catalytically inactive phosphorylated state.

iNOS was first characterised in macrophages, but is also expressed in a variety of other cells. In the CNS, it has been identified in vascular smooth muscle cells, astrocytes, and microglia and endothelial cells.\textsuperscript{[17]} Unlike eNOS or nNOS, the regulation of iNOS seems to be dependent on de novo synthesis of the enzyme in response to a variety of cytokines, such as interferon-\(\gamma\), and lipopolysaccharide (LPS). iNOS is insensitive to an increase in intracellular Ca\textsuperscript{++} level.\textsuperscript{[11,5]} Interestingly, iNOS is tightly bound to calmodulin irrespective of Ca\textsuperscript{++} levels; therefore, calmodulin is considered a subunit of the enzyme.\textsuperscript{[18]} Thus, the binding of calmodulin to iNOS provides a continuous flow of electrons, which accounts for the high levels of NO produced by iNOS. The cloning of the iNOS promoter showed that a substantial number of transcription factors are involved in its regulation. Activation of the enzyme by LPS is dependent on the formation of nuclear factor \(\kappa B\) heterodimers, while the synergistic activation of iNOS by interferon-\(\gamma\) utilises interferon-\(\gamma\) response factor-1.\textsuperscript{[119-22]}