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Pharmacological strategies for the regulation of inducible nitric oxide synthase: Neurodegenerative versus neuroprotective mechanisms

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Abstract

Inducible nitric oxide synthase (iNOS) is one of three NOS isoforms generating nitric oxide (NO) by the conversion of L-arginine to L-citrulline. iNOS has been found to be a major contributor to initiation/exacerbation of the central nervous system (CNS) inflammatory/degenerative conditions through the production of excessive NO which generates reactive nitrogen species (RNSs). Activation of iNOS and NO generation has come to be accepted as a marker and therapeutic target in neuroinflammatory conditions such as those observed in ischemia, multiple sclerosis (MS), spinal cord injury (SCI), Alzheimer's disease (AD), and inherited peroxisomal (e.g. X-linked adrenoleukodystrophy; X-ALD) and lysosomal disorders (e.g. Krabbe's disease). However, with the emergence of reports on the neuroprotective facets of NO, the prior dogma about NO being solely detrimental has had to be modified. While RNSs such as peroxynitrite ($ONOO^-$) have been linked to lipid peroxidation, neuronal/oligodendrocyte loss, and demyelination in neurodegenerative diseases, limited NO generation by GSNO has been found to promote vasodilation and attenuate vascular injury under the same ischemic conditions. NO generated from GSNO acts as second messenger molecular which through *S*-nitrosylation has been shown to control important cellular processes by regulation of expression/activity of certain proteins such as NF-κB. It is now believed that the environment and the context in which NO is produced largely determines the actions (good or bad) of this molecule. These multi-faceted aspects of NO make therapeutic interference with iNOS activity even more complicated since complete ablation of iNOS activity has been found to be rather more detrimental than protective in most neurodegenerative conditions.

Investigators in search of iNOS modulating pharmacological agents have realized the need of a delicate balance so as to allow the production of physiologically relevant amounts of NO (such as those required for host defence/neutotransmission/vasodilation, etc.) but at the same time block the generation of RNSs through repressing excessive NO levels (such as those causing neuronal/tissue damage and demyelination, etc.). The past years have seen a noteworthy increase in novel agents that might prove useful in achieving the aim of harnessing the good and blocking the undesirable actions of NO. It is the aim of this review to provide basic insights into the NOS family of enzymes with special emphasis of the role of iNOS in the CNS, in the first part. In the second part of the review, we will strive to provide an exhaustive compilation of the prevalent strategies being tested for the therapeutic modulation of iNOS and NO production.

Keywords: CNS; iNOS; Inflammation; NF-κB; Nitrosylation; Cellular redox; Isoprenoids; Lactosylceramide; PPAR-γ; AMPK

1. General aspects

Nitric oxide (NO) is a unique informational molecule first identified as the endothelium-derived relaxation factor (ERDF) (Furchgott and Zawadzki, 1980). It is considered to be a ubiquitous endogenous system which is involved in opposite actions, on one hand maintenance of homeostasis and on the other mediation of pathological processes. Since its identification in 1980 (Furchgott and Zawadzki, 1980; Murad, 1998), the study of NO signaling has been an area of aggressive investigation and is one of the fastest growing fields in biomedical research. The significance of this molecule has been recognized by the award of the 1998 Nobel Prize in Physiology or Medicine to Furchgott, Ignarro and Murad (the pioneers in the field). NO is a free-radical

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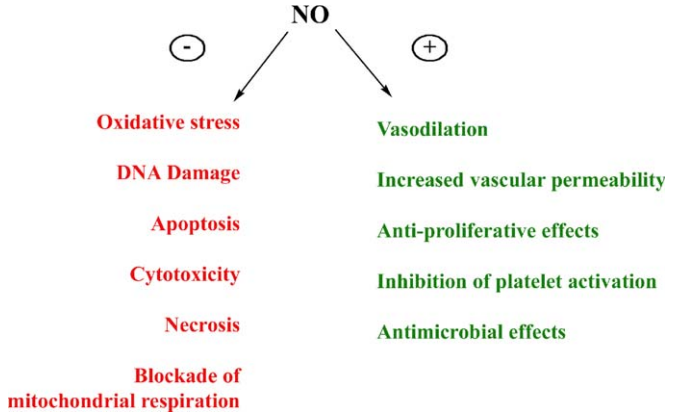

Fig. 1. The various physiological and pathological effects of NO.

gas with an unshared electron that is freely membrane diffusible. As summarized in Fig. 1, depending on the environment in which NO is generated, it can mediate regulatory physiological functions such as vasodilation and neurotransmission (Moncada and Higgs, 1993) or react with superoxide radicals to generate destructive reactive nitrogen species (RNSs) such as dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$) as in neurodegenerative conditions (Bolanos et al., 1997).

1.1. Nitric oxide synthase (NOS)-mediated NO production

NO is synthesized by NOS through sequential oxidation that converts the amino acid L-arginine to L-citrulline as depicted in Fig. 2 (Stuehr, 1999). Three different forms of NO synthase (types I–III) have been identified representing distinct gene products. Out of these, two isoforms are constitutively expressed. These include the neuronal NOS (nNOS or NOS I), which is predominantly expressed in neuronal tissue and the endothelial NOS (eNOS or NOS III) which is predominantly expressed in vascular endothelial cells (Popp et al., 1998; Ignarro et al., 1999). The synthesis of physiologically vital amounts of NO from these constitutive isoforms is Ca^{2+}/calmodulin-dependent (Bredt and Snyder, 1990). Due to their Ca^{2+} dependence, a number of agonists that affect intracellular Ca^{2+} levels interfere with NO synthesis too. The third is an inducible isoform of NOS (iNOS or NOS II). In contrast to the constitutively active forms, iNOS functions in a Ca^{2+}-independent manner. However, calmodulin is non-covalently bound to the iNOS complex and constitutes an essential subunit of this isoform (Cho et al., 1992). iNOS produces NO in response to a wide range of stimuli, most prominently endotoxin and endogenous proinflammatory mediators (Nathan, 1997). Once induced, iNOS generates copious amounts of NO for prolonged periods (until substrate depletion) that have been linked to pathological manifestations observed in neuroinflammatory conditions (Hickey et al., 2001).

All NOS isoforms are homodimeric enzymes that depend on the substrate L-arginine as well as on the cofactors/coenzymes nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (BH_4), flavin adenine dinucleotide (FAD), flavine mononucleotide (FMN), oxygen (O_2) and protoporphyrin IX (Knowles and Moncada, 1994; Stuehr, 1999). The presence of heme, BH_4 and L-arginine promotes dimer formation and stabilization (Alderton et al., 2001). NOS dimerisation is crucial for catalysis because each reductase domain transfers NADPH-derived electrons to the heme located in the adjacent subunit, whereas, electron transfer between reductase and oxygenase domains on the same subunit does not occur (Hemmens and Mayer, 1998). Each NOS peptide consists of an N-terminal oxygenase domain and a C-terminal reductase domain, with a recognizing sequence for Ca^{2+}/CAM located between the two domains. As per the crystal structure, the NOS oxygenase domain shows a core region that binds heme, L-arginine and forms the active site of the enzyme (Hemmens and Mayer, 1998). FMN, FAD, and NADPH are bound by the reductase domain. During NO synthesis the reductase flavins acquire electrons from NADPH and transfer them to heme iron, which activates O_2 and catalyses NO synthesis (Hemmens and Mayer, 1998). In the constitutive NOSs (eNOS and nNOS), the electron transfer is triggered by CAM binding, in this way Ca^{2+} may regulate the activity of these constitutive NOSs. On the other hand, since iNOS is Ca^{2+} independent, the binding of CAM to iNOS is irreversible, which explains why iNOS remains active once assembled (Cho et al., 1992).

1.2. Nitric oxide biosynthesis in the brain

All three NOSs can affect brain function and have been characterized in various brain cells. Neurons produce NO mostly by activation of nNOS, which is constitutively expressed in these cells (Knowles and Moncada, 1994). Glutamate binding to NMDA receptors increases the levels of intracellular Ca^{2+} which activates nNOS via calmodulin for the mediation of rapid events such as neurotransmission (Dawson et al., 1994). nNOS is found in the cerebellum, cerebral cortex and hypothalamus, and also in various ganglion cells of the autonomic nervous system (Bredt and Snyder, 1990).

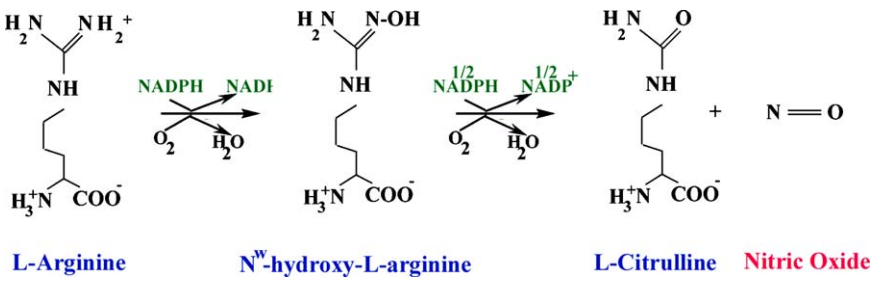

Fig. 2. Biosynthesis of NO from L-arginine.