Nitric Oxide in Brain Function and Dysfunction

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Nitric Oxide Signalling: Nitric oxide (NO) is the signalling molecule originally identified as endothelium-derived relaxing factor (EDRF) mediating relaxation of blood vessels.[1] It is a small, highly diffusible and reactive molecule with a short life-time, generated from arginine by the cytoplasmic enzyme nitric oxide synthase (NOS). Three NOS genes with distinct tissue localisation and properties are known, namely: endothel, inducible and neuronal NOS (eNOS, iNOS & nNOS, respectively). Activation of eNOS and nNOS are classically Ca^{2+}-dependent, with nNOS being closely coupled to Ca^{2+}-permeable NMDA receptor (NMDAR), both of which are linked to postsynaptic densities (PSD-95) of the CNS through their mutual PDZ binding motifs.[2] eNOS and nNOS generate low nanomolar concentrations of NO, whereas iNOS can produce micromolar levels. Such high concentrations affect down-stream signalling mechanisms, with low concentrations being neuroprotective and mediating physiological signalling (e.g. neurotransmission or vasodilatation) whereas higher concentrations are neurotoxic. Excessive activation of iNOS has been linked to several neurodegenerative disorders (see below).

The major physiologically relevant receptor for NO is soluble guanylyl cyclase (sGC) which mediates the production of cGMP from GTP. Downstream transduction can be via cyclic nucleotide-gated ion channels, activation of protein kinase G and protein phosphorylation, or direct actions on proteins via S-nitrosylation and nitrotyrosination (Figure 1). Metabolism of cGMP by phosphodiesterases (PDE) suppresses NO/sGC signalling. There are 11 PDE genes with specific differential expression in nervous tissue. Signalling activity will then reflect the equilibrium between cGMP synthesis and degradation; for instance sildenafil/Viagra is an antagonist of PDE5, reducing degradation so that lower activity of sGC can achieve sufficient signalling to relax corpora cavernosa muscle and achieve erection.

There are several well characterised competitive antagonists for nNOS and sGC, and some allosteric modulators allowing pharmacological intervention. But physiological actions of NO are achieved at very low concentrations, so proof of endogenous

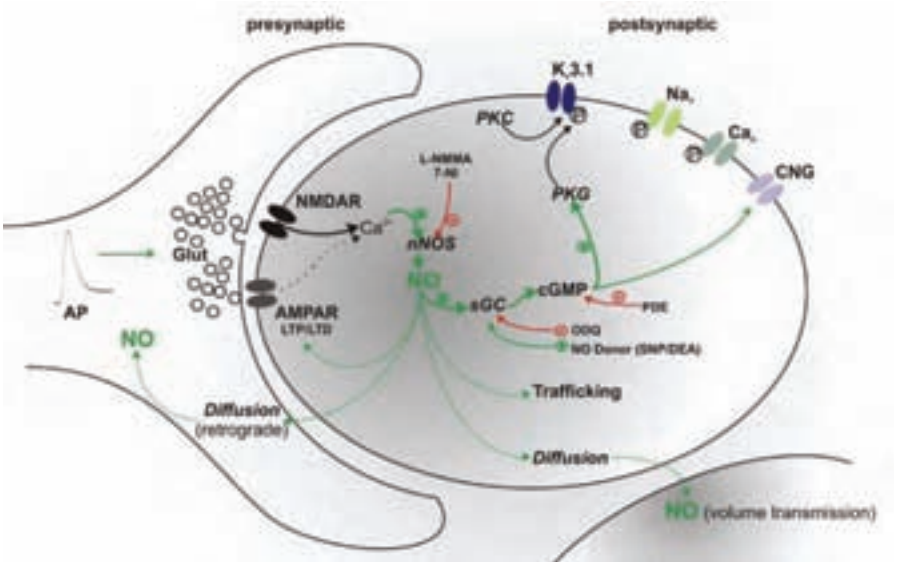

Figure 1: The NO signalling pathways
Nitric oxide, produced from the amino acid arginine by nNOS, has various physiological effects. Synaptic glutamate release activates postsynaptic AMPA and NMDA receptors (AMPAR, NMDAR) leading to Ca^{2+}-induced nNOS activation. This NO will diffuse and subsequently activate sGC to produce cGMP (from GTP) which has several signalling roles, including activation of PKG or cyclic nucleotide-gated ion channels. NO will act locally at the source of production and in neighbouring neurons through a process of volume transmission to affect postsynaptic neuronal excitability or presynaptic neurotransmitter release. Pharmacological studies use 7-NI and L-NMMA as competitive NOS antagonists or ODQ as a sGC inhibitor (red arrows) while there are many different NO donors (e.g. SNP or DEA-NONOate) which generate NO independent to NOS and thereby activate sGC. Other powerful modulation is achieved by PDEs, mediating breakdown of cGMP and reduce NO/sGC signalling. Several ion channel targets for nitrergic signalling are indicated (AP – action potential, Cav - voltage gated calcium channel, CNG - cyclic nucleotide-gated ion channels, Kv3.1 – voltage gated potassium channel, L-NMMA - NG-Methyl-L-arginine, LTD – long term depression, LTP – long term potentiation, Nav – sodium channel, ODQ - 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one, PDE – phosphodiesterase, SNP - Sodium nitroprusside, 7-NI - 7-Nitroindazole).

NO generation by physiological stimuli is difficult. nNOS is widely distributed across the brain, but it is normally expressed in a subpopulation of neurons within a given region. Its mobility, unconstrained by cell membranes, allows action across a broad volume (hence the term 'volume transmitter') limited by inactivation (e.g. scavenging or degradation). It has long been postulated that NO could also act as a retrograde messenger, mediating transmission from target neurons back onto the synapse and regulating synaptic plasticity (for example in the hippocampus and cerebellum).

Nitric oxide signalling in the brain can modulate a range of processes such as various forms of plasticity (long term potentiation and depression, LTP and LTD) regulating rhythmic activity, including gut motility, respiratory rhythm, circadian rhythms, locomotor and thalamocortical oscillation. There is strong evidence for involvement in learning and memory mechanisms through mediation of specific forms of LTP in the cerebellum,[3] hippocampus[4] and neocortex[5] and LTD in the cerebellum. The cellular and molecular targets of nitrergic signalling pathways are also diverse and as yet incompletely resolved; there is evidence for modulation of presynaptic transmitter release at excitatory glutamatergic and inhibitory GABAergic synapses, postsynaptic AMPAR phosphorylation and trafficking, calcium channels, potassium channels and interactions with other signalling pathways (such as mGluR, endocannabinoid and catecholamine). Our recent work in the auditory brainstem has highlighted the role of NO in regulating postsynaptic excitability via Kv3 voltage-gated potassium channels in activity-dependent auditory processing. Enhanced synaptic transmission at the calyx of Held synapse onto principal cells of the medial nucleus of the trapezoid body (MNTB) causes NMDAR-mediated and calcium-dependent activation of postsynaptic nNOS. The NO acts in the target neuron and surrounding neurons to suppress voltage-gated potassium channels (particularly Kv3) through a slow time-course (15-30 minutes) phosphorylation mechanism which has a homeostatic-like function in matching postsynaptic excitability to the synaptic traffic.[6] The broad expression of Kv3 channels in fast-spiking interneurons throughout the brain suggests this modulation might be a general mechanism by which NO influences synaptic processing at a postsynaptic rather than a presynaptic site.

An important consideration from the perspective of disease is the extent to which NO mediates signalling between the vasculature, neurones and glial cells, and involvement of microglia and the immune system in nitrergic signalling of the brain. Given the ease of NO diffusion, a key future challenge is to understand the extent to which over-production of NO in one system (endothelium, immune) can 'spill-over' into triggering brain dysfunction and neurodegeneration.

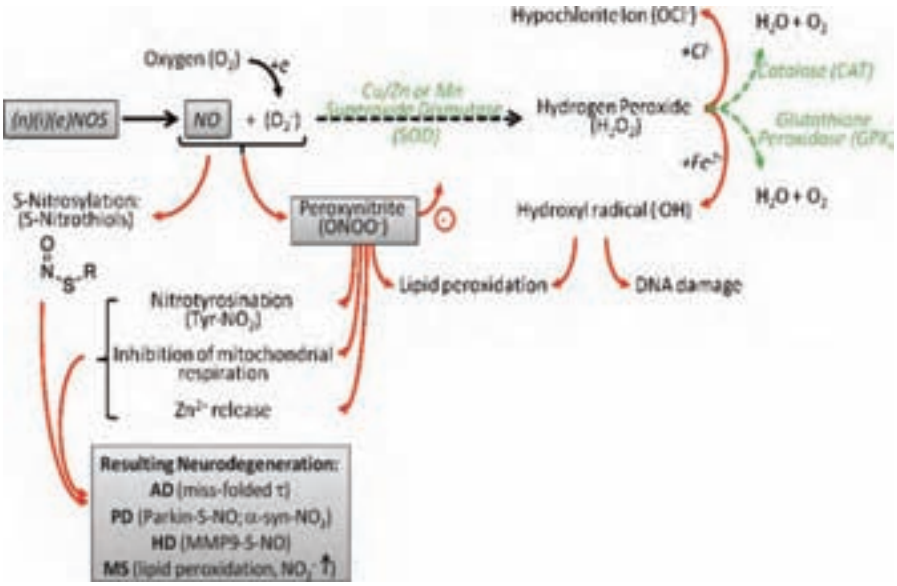

Figure 2: Oxidative and nitrosative stress: pathological consequences of NO generation.
NO will react with superoxide anions (O_2^-) to form the highly reactive peroxynitrite ion ($ONOO^-$). $ONOO^-$ is responsible for protein nitrotyrosination and inhibits mitochondrial respiration. NO itself nitrosylates protein residues leading to the formation of S-nitrothiols. All of the above effects of NO have been implicated in Alzheimer's, Parkinson's and Huntington's disease as well as in multiple sclerosis. There are several cellular antioxidant enzymes (green) which break down reactive oxygen species, namely: Cu/Zn or Mn superoxide dismutase which converts O_2^- into hydrogen peroxide (H_2O_2). H_2O_2 itself can form hypochloride ions or the highly reactive hydroxyl radical ('OH) responsible for lipid peroxidation and DNA damage. Further antioxidant actions (indicated by the dotted arrows) of either Glutathione Peroxidase or Catalase lead to the formation of H_2O from H_2O_2.

So what are the processes whereby NO signalling might contribute to disease?

Production of Reactive Nitrogen Species (RNS): The term nitrosative stress describes this ability of NO and its derivatives (RNS) to damage proteins and DNA. A primary reaction is reaction of NO and O_2- to form peroxynitrite ($ONOO^-$, Figure 2) decreasing the bioavailability of NO.[7] Nitrosylation and nitrotyrosination of proteins are important for the physiological and pathological roles of NO. Nitrosylation is the reaction of NO with cysteine to form nitrosothiols[8] and nitrotyrosination is the reaction of tyrosine with $ONOO^-$ to form 3-nitrotyrosine.

NOS can also directly contribute to O_2^- production since cells with deficient cofactor tetrahydrobiopterin (BH4) or substrate (arginine), cannot catalyze the five-electron oxidation of L-arginine into L-citrulline (thereby generating NO), but can still receive electrons from NADPH and donate them to O_2, reducing it to form O_2^-[9] so further enhancing peroxynitrite production. It is interesting to note that both Alzheimer's (AD) and Parkinson's disease (PD) are associated with a BH4 deficiency.[10,11]

Mitochondria and oxidative stress: Generation of reactive oxygen species (ROS) occurs in every eukaryotic cell; electron 'leakage' from the mitochondrial electron transfer chain reacts with molecular oxygen to make superoxide (O_2^-, Figure 2). Normally this is metabolized by superoxide dismutase (SOD) to H_2O_2, which is further degraded by the antioxidant enzymes, catalase or glutathione peroxidase. Thus mitochondria are also a potential source of RNS. NO and $ONOO^-$ both inhibit the mitochondrial respiratory chain, reducing ATP production[12,13] so that susceptibility to neurodegeneration shows complex dependence on local metabolic rates, oxygen availability, antioxidant activity (reduced glutathione) and cell stress resistance signalling.[14] Other effects of NO/$ONOO^-$ include release of Zn^{2+} from internal stores (such as metallothionein) with concomitant formation of S-nitrosothiol and neurotoxicity.[15,16] Free Zn^{2+} induces respiratory block, opening of the mitochondrial permeability transition pore (mPTP), cytochrome c release, generation of ROS, and p38 MAP kinase activation leading to caspase-independent K^+ efflux with cell volume loss and apoptotic-like death.[17]

Further metabolic compromise may result from mitochondrial fragmentation. This is fast, occurring within minutes after NMDAR activation or NO exposure, and is considered a prelude to neurodegeneration and cell death.[18] Increased mitochondrial fission in response to NO has been reported in AD, PD, amyotrophic lateral sclerosis (ALS) and

NO affects the balance between healthy signalling and neurodegeneration

Huntington's disease (HD).[19] Fragmentation of other organelles, such as the Golgi apparatus is known to occur during apoptosis in several neurodegenerative disorders. NO-mediated Golgi fragmentation is downstream of NMDAR activation and precedes neuronal cell death.[20]

Role in neurodegenerative disease: NO generation in the brain is mediated by NMDAR activation, so excitotoxicity–related neuronal injury could have a nitrergic component. Endogenous levels of oxidizing agents, NO and Zn^{2+} inhibit excessive excitation of NMDAR and limit excessive influx of Ca^{2+} via the NMDAR. Such feedback could ameliorate NMDAR-mediated neurotoxicity. High-affinity Zn^{2+} inhibition, redox modulation or S-nitrosylation of the receptor are mediated with the involvement of at least seven cysteine residues on NMDAR subunits.[21]

NO signalling contributes to several neurodegenerative diseases[22] through production of ROS/RNS and subsequent oxidative/nitrosive stress. Excessive NO production from inflammation is a significant factor in AD, PD, ALS, multiple sclerosis (MS) and HD, and also in the brain damage following ischaemia and reperfusion. Enhanced nitrotyrosine immunoreactivity and oxidative protein damage are evident in brains from AD patients,[23,24] while inhibition of mitochondrial cytochrome c oxidase and enhanced H_2O_2 production in amyloid β (Aβ) mutant mice suggest mitochondrial involvement in ROS generation.[25] The cerebral cortex of patients with AD has high protein nitrotyrosination[26] and nitrated proteins are associated with Aβ deposition[23] along with nitrotyrosination of Tau protein[27] and synaptophysin; consistent with a dysfunction in cholinergic synaptic transmission.[28] Most recently S-nitrosylation of Drp1 has been shown to mediate mitochondrial fission and neuronal damage caused by Aβ.[29]

Exposure of experimental inflammation models to NO cause axonal degeneration, especially when accompanied by propagating electrical activity.[30] Several potential pathogenic mechanisms have been suggested. In PD, S-nitrosylation of Parkin[31,32] initially increases but later decreases Parkin activity. Alpha-synuclein (α-syn) a protein associated with synaptic terminals and synaptic transmission, is heavily nitrated at 4 tyrosine residues and this contributes to aggregation.[33] Nitrated α-syn is more resistant to proteolysis and has reduced lipid binding and solubility.[34] Other contributing mechanisms could include metabolic compromise by RNS (via block of mitochondrial complex I) in substantia nigra[35] since MPTP-induced neuronal loss in this PD model was slowed by competitive nNOS antagonists[36] and nNOS inhibition blocked MPTP-mediated decrease in striatal dopamine levels in mice.[37]

We conclude that nitric oxide has broad physiological actions across many organ systems, in the brain this includes modulation of synaptic transmission as a 'retrograde messenger', but it is also a 'volume transmitter', mediating activity-dependent changes in postsynaptic excitability (Figure 1). Generation of RNS, involvement in oxidative stress and the propensity for 'spill-over' between endothelium and immune signalling into the neuronal environment suggest that we might expect dysfunctional nitrergic signalling to have broad involvement in neurodegenerative disease and these possibilities are under increasing investigation. ◆

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