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Inflammatory Neurodegeneration Mediated by Nitric Oxide, Glutamate, and Mitochondria

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Abstract

In inflammatory, infectious, ischemic, and neurodegenerative pathologies of the central nervous system (CNS) glia become "activated" by inflammatory mediators, and express new proteins such as the inducible isoform of nitric oxide synthase (iNOS). Although these activated glia have beneficial roles, in vitro they potently kill cocultured neurons, and there is increasing evidence that they contribute to pathology in vivo. Nitric oxide (NO) from iNOS appears to be a key mediator of such glial-induced neuronal death. The high sensitivity of neurons to NO is partly due to NO causing inhibition of respiration, rapid glutamate release from both astrocytes and neurons, and subsequent excitotoxic death of the neurons. NO is a potent inhibitor of mitochondrial respiration, due to reversible binding of NO to cytochrome oxidase in competition with oxygen, resulting in inhibition of energy production and sensitization to hypoxia. Activated astrocytes or microglia cause a potent inhibition of respiration in cocultured neurons due to glial NO inhibiting cytochrome oxidase within the neurons, resulting in ATP depletion and glutamate release. In some conditions, glutamate-induced neuronal death can itself be mediated by *N*-methyl-D-aspartate (NMDA)-receptor activation of the neuronal isoform of NO synthase (nNOS) causing mitochondrial damage. In addition NO can be converted to a number of reactive derivatives such as peroxynitrite, NO_2, N_2O_3, and S-nitrosothiols that can kill cells in part by inhibiting mitochondrial respiration or activation of mitochondrial permeability transition, triggering neuronal apoptosis or necrosis.

Index Entries: Inflammation; neurons; microglia; astrocytes; nitric oxide; brain; excitotoxicity; cell death; Alzheimer's disease.

The Concept of Inflammatory Neurodegeneration

Astrocytes and/or microglia become "activated" by inflammatory mediators in a wide range of central nervous system (CNS)

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pathologies, including brain trauma and stroke, brain infections such as AIDS dementia and bacterial meningitis, neurodegenerative diseases such as Alzheimer's and Multiple Sclerosis (MS), and during normal aging *(1–3).* Glial activation involves changes in cell phenotype (often described as "reactive") and gene expression, including the *de novo* expression of MHC class I and II antigens, cell adhesion molecules, cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), and the inducible isoform of nitric oxide synthase (iNOS) *(4–6).* Activation is caused by mediators including the pro-inflammatory cytokines interferon-γ, TNF-α, and IL-1β, bacterial and viral components, prions and peptides such as β-amyloid (Fig. 1). Glial activation is thought to be protective via destruction of pathogens, removal of debris and promotion of tissue repair, however, excess activation can be deleterious *(7–9).* Activated glia can kill neurons in coculture *(10–13),* and this may occur in vivo during brain trauma, inflammation, postischemia and infection, and in neurodegenerative diseases *(14–16).* For example in Alzheimer's disease brains, inflammatory-activated microglia are found within, and activated astrocytes around, the β-amyloid-containing neuritic plaques containing dead and dystrophic neurites *(17–22).* β-amyloid can activate glia to kill cocultured neurons via inflammatory mediators *(11,23–25),* and anti-inflammatory drugs reduce the prevalence of Alzheimer's disease *(26–28).* Thus we have the concept of "inflammatory neurodegeneration" i.e., *death or degeneration of neurons caused by inflammation,* and the more general idea that in many CNS pathologies inflammatory activation of nonneuronal cells causes neuronal cell death which contributes to the pathology.

Inflammation is unlikely to initiate any CNS pathology, because something else must first cause the inflammation, but it may—if excessive and long lasting—contribute to pathology and cell death, turning a relatively benign pathology into a dangerous one. This does not mean that all inflammation in the CNS is harmful: the ostensive purpose of inflammation is to kill, limit-the-growth-of and remove pathogens

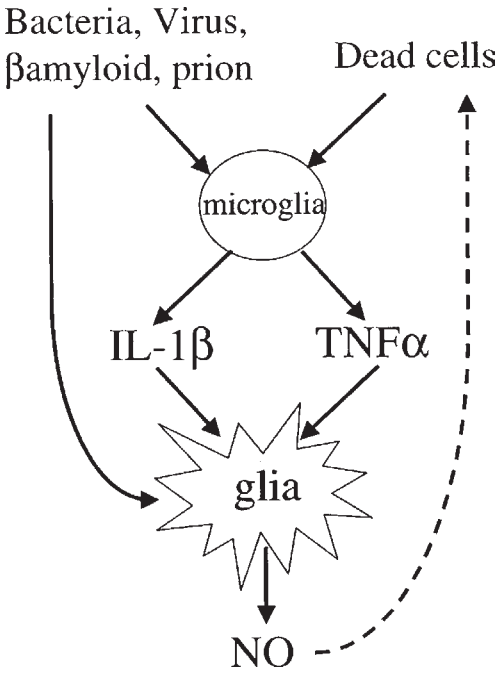

Fig. 1. Mechanisms of glial activation. Macrophages and microglia can be activated by various pathogens and peptides, and then produce cytokines IL-1β and TNF-α, which together with the pathogens further activate microglia and astrocytes (glia) to induce iNOS and produce NO, which in turn may kill neurons, resulting in further inflammation. Not depicted is the potential for phagocytosed antigens to be presented to T-cells, resulting in interferon-γ production by T-cells, which synergistically activates glia.

or virally-infected, cancerous, or dead/dying host cells. But inflammation may in particular circumstances be excessive, or it may be triggered by a benign stimulus, or it may be self-perpetuating if it produces more damage than it resolves. Different CNS pathologies involve different disease mechanisms in different brain structures (with variable susceptibility of different neuronal cell type to inflammatory neurodegeneration) with a variety of time courses, but some form and degree of inflammation occurs in virtually all of them. The extent to which inflammation is positive or negative in a particular pathology will vary and may vary at

different stages of a disease. Understanding the mechanisms by which inflammation damages host cells should help us design strategies to prevent such damage.

A large amount of recent work has revealed the mechanisms by which activated glia kill neurons, and the main part of this review discusses these mechanisms, in particular the roles of nitric oxide, glutamate, and mitochondria. We briefly outline the evidence that inflammatory neurodegeneration contributes to various CNS pathologies.

Inflammation in the CNS

Inflammation occurs both in the CNS and nonCNS tissues in response to stimuli such as pathogens or tissue damage. However, inflammation within the CNS differs from that outside in that it occurs largely behind the wall of the blood–brain barrier. Usually there is little-or-no vascular response to CNS inflammation, and therefore no recruitment of neutrophils; only local, resident microglia show a rapid response becoming "activated," but nonlocal microglia and other macrophages do not migrate into the inflamed area until several days after inflammation begins *(29).* An inflammatory response more like that in the rest of the body can occur if the blood–brain barrier is damaged, as in traumatic brain injury or brain ischemia, where neutrophils and monocytes are recruited into the damaged tissue. In the absence of damage to the blood–brain, the inflammatory response within the CNS is mainly mediated by microglia (regarded as resident macrophages of the brain) and astrocytes. Both these glial cell types can act as antigen-presenting cells, at least in vitro *(30),* and thereby activate T-cells, which can apparently cross the blood–brain barrier *(31).* Activated microglia, astrocytes, and T-cells then produce a variety of cytokines that regulate inflammation.

Inflammation in the CNS is in general induced by cytokines and/or bacterial/viral components, although other inducers may be involved in particular cases. Pro-inflammatory cytokines, in particular IL-1β and TNF-α, are elevated in most CNS pathologies, and there is evidence that they contribute to pathology, although in some conditions they may be protective *(32).* Injection of pro-inflammatory cytokines into the brain or addition to cell cultures can cause neuronal cell death *(32).* Such cytokines can have direct effects on neurons in culture, but these effects are mostly protective. The main target of cytokines are glia, and glia appear to mediate the toxic effects of cytokines on neurons by causing inflammatory activation of glia, which then release factors toxic to neurons (*32;* Fig. 1).

The mechanisms by which activated glia kill neurons in culture have been suggested to include the release of nitric oxide *(9–11,14),* superoxide and/or hydrogen peroxide *(33–35),* glutamate *(36–38),* TNF-α and/or IL-1β *(39–40),* and mitochondrial damage (*41;* Fig. 9). However, it is now emerging that these seemly disparate processes may be part of a single causal chain resulting in cell death: the cytokines induce iNOS in the glia which then release high levels of NO *(10,42,43),* the NO causes glutamate release from astrocytes and neurons resulting in excitotoxicity (*44–47;* Fig. 9), excitotoxicity or activated microglia produce superoxide reacting with NO to give peroxynitrite *(48–50),* which in turn causes mitochondrial damage and cell death *(51–53).* We outline below the interconnected roles of NO, glutamate, and mitochondria in inflammatory neurodegeneration.

Nitric Oxide in Inflammatory CNS Pathology

Nitric oxide (NO) appears to play two radically different roles in the brain: (i) as an intercellular signaling molecule regulating a variety of physiological processes, such as synaptic plasticity, blood flow, and development *(54),* and (ii) as a cytotoxic molecule killing both pathogenic cells and benign host cells in disease *(43,50).* NO is produced by three isoforms of NO synthase: (a) neuronal nitric oxide synthase

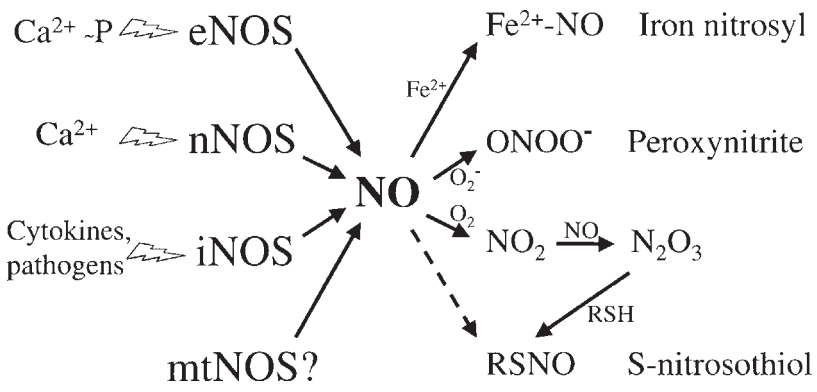

Fig. 2. Sources and sinks of NO. NO is produced by: eNOS, constituitively expressed in endothelial cells and some astrocytes, regulated by calcium and phosphorylation; nNOS expressed in CNS neurons, regulated by calcium; iNOS not normally expressed but induced in glia by pathogens and cytokines; and/or mtNOS (mitochondrial NOS), the existence and origin of which are controvercial. At low levels, NO mainly reacts with ferrous iron in haemproteins. Higher levels may react with superoxide or oxygen. S-nitrosothiols derive from N_2O_3 or other ill-defined means.

(nNOS), which is constituitively expressed in a proportion of brain neurons, and is activated by calcium/calmodulin, particularly after stimulation of NMDA-type glutamate receptors; (b) endothelial nitric oxide synthase (eNOS), which is constituitively expressed in brain endothelial cells and some astrocytes, and is regulated by calcium/calmodulin and phosphorylation/dephosphorylation; and (c) inducible nitric oxide synthase (iNOS), which is not normally expressed in the "healthy" brain but is induced in glia and endothelial cells by pro-inflammatory cytokines, bacterial/viral components and/or hypoxia, and once expressed continuously produces high levels of NO (Fig. 2).

iNOS expression is one of the characteristic changes of the "activated" state of microglia and astrocytes induced by inflammation. NO has been implicated in most neurodegenerative diseases, including multiple sclerosis, AIDS dementia, prion, Parkinson's, Huntingdon's, Alzheimer's, and motor neuron diseases *(43)* (Table 1). iNOS is rarely seen in neurons, but there may be limited expression in some neuronal types induced by cytokines in culture *(55).* There is still some uncertainty as to the relative expression of iNOS in astrocytes and microglia. In cultures of rodent glia it has been reported that iNOS is only expressed in microglia not astrocytes *(56),* however there are many other papers reporting that iNOS can occur in both cell types *(43,57,58).* In contrast to rodent macrophages, iNOS is difficult to induce in human macrophages, apparently due to a very different promoter of human iNOS compared to the rodent iNOS gene *(59).* Similarly iNOS is apparently difficult to induce in human microglia, but by contrast is easy to induce in human astrocytes to high levels of expression *(35,60).* iNOS can also be induced in rodent oligodendrocytes *(61),* brain endothelial cells *(62),* and fibroblasts *(63)* by inflammatory mediators.

NO itself at physiological concentrations (unclear, but probably 0.1–100 n*M*) is relatively unreactive, and most of its physiological actions are mediated by NO binding to Fe^{2+} in the haem of soluble guanylate cyclase, causing activation and guanosine 3′,5′-cyclic monophosphate (cGMP) production. However, NO may be converted to a number of more reactive derivatives, known collectively as reactive nitrogen species (RNS) (Fig. 2). At high concentrations (or within membranes) NO reacts directly with oxygen to produce NO_2, which rapidly reacts with a further NO to give N_2O_3. NO_2 may oxidize or

Table 1
Evidence that NO and/or Inflammation Contribute to Various CNS Pathologies

CNS pathology	Increased iNOS expression in glia	Nitrotyrosine or nitrite presence	Evidence that NO or inflammation is detrimental
Parkinson's disease	*(198,1990)*	*(200)*	NOS inhibition or knockout protects in animal model *(16,201,202,204,265)*
Alzheimer's disease	*(21,22,191)*	*(192,193)*	Anti-inflammatory drugs protect in humans or animals *(26–28)*
Amyotrophic lateral sclerosis	*(208,209)* nNOS upregulation *(207,209)*	*(255)*	
Multiple sclerosis	*(215–218)*	*(215,217,218)*	iNOS inhibitor protects against EAE (animal model of MS) *(220,221)*
Cerebral ischemia	*(229,230)* nNOS upregulation *(225,226,230)*	*(229,230)*	iNOS inhibitors or antisense reduce cell death *(231,232)* nNOS knockouts or inhibitor protect in animals *(227–266)*
Viral infection	*(248,249,251,257)*	*(252,253)*	NOS inhibitors or knockout prevent neuronal cell death *(250,267,257)*
Epilepsy	increased nNos *(241)*		NOS inhibitor blocks induced seizures in animals *(241,242)*
Bacterial infection: meningitis	*(258,259)*	*(258,266)*	NOS inhibitor or peroxynitrite scavenger protects in animal *(189,258,259,266)*
Trauma	*(235–238)*	*(268)*	iNOS inhibitors protect in animal model *(272,366)*
Aging	*(243,244,246,247)*	*(245,246)*	

For each pathology (first column) evidence is referenced for increased iNOS expression in glia (second column), increased NO production measured as nitrotyrosine or nitrite (third column), and/or a detrimental role in the pathology (fourth column).

nitrate (add an NO_2^+ group to) a variety of molecules, (e.g., tyrosine can be nitrated to 3-nitrotyrosine), while N_2O_3 can nitrosate/nitrosylate (add an NO^+ group to) amines or thiols (e.g., cysteine can be nitrosated to S-nitroso-cysteine). NO reacts at the diffusion-limited rate with O_2^- to produce peroxynitrite ($ONOO^-$), which can oxidize or nitrate other molecules, or can decay producing other damaging species (possibly the hydroxyl radical $OH^\bullet$ and NO_2). NO may indirectly (possibly via N_2O_3) nitrosate thiols (e.g,. in proteins or glutathione) to give S-nitrosothiols (RSNO, e.g., S-nitroso-glutathione and S-nitroso-albumin) (Fig. 2). The NO^+ group is directly transferable between different S-nitrosothiols, a process known as transnitrozation or transnitrosylation. S-Nitrosated or tyrosine-nitrated proteins may have altered function. S-Nitrosothiols breakdown and release NO in the presence of either light, reduced thiols or metal ions, such as Cu^+ *(64).*

Mitochondria as Targets for NO

Mitochondria are central to neuronal energy metabolism and neuronal cell death *(65).* It has been suggested that damage to the mitochondrial respiratory chain is an important factor in the pathogenesis of many neu-

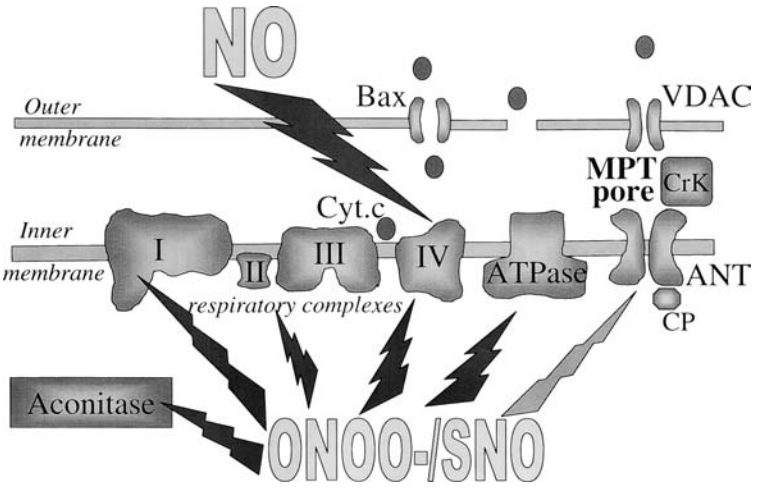

Fig. 3. Mitochondrial targets of NO and reactive nitrogen species (RNS). NO selectively and reversibly inhibits complex IV (cytochrome oxidase). Peroxynitrite ($ONOO^-$) inhibits all the respiratory complexes (numbered I, II, III, and IV), the ATP synthase and aconitase, and activates mitochondrial permeability transition (MPT). S-nitrosothiols (SNO) inhibit complex I and activate MPT. The MPT pore is thought to consist of cyclophilin D (CP) and the adenine nucleotide translocator (ANT) plus possibly mitochondrial creatine kinase (CrK) and the voltage-dependent anion carrier (VDAC). Cytochrome c may be released from the intermembrane space (triggering apoptosis) either by: (a) Bax (or homologous proteins) pores; (b) rupture of the outer membrane (e.g., by MPT); or (c) possibly VDAC in an altered conformation.

rodegenerative diseases *(66).* NO has also been implicated in such diseases *(41),* and NO can inhibit components of the mitochondrial respiratory chain, compromising cellular energy metabolism *(67–72)* (Fig. 3).

Reversible NO Inhibition of Cytochrome Oxidase

NO can potently, acutely, and reversibly inhibit mitochondrial respiration at cytochrome oxidase *(70,73–75).* The NO inhibition is competitive with oxygen due to NO reversibly binding to the oxygen-binding site of the respiratory complex. For example, NO reversibly inhibited the respiration of isolated nerve terminals (synaptosomes) in competition with oxygen: thus 270 n*M* NO half inhibited respiration at 150 μ*M* O_2, but 60 n*M* NO half inhibited respiration at 30 μ*M* O_2 *(73).* Similar concentrations of NO reversibly inhibit the oxygen consumption of cultured neurons causing ATP depletion *(45,76,77).* Because NO inhibits respiration in competition with oxygen it can dramatically increase the apparent K_m of respiration for oxygen *(69,73).* For example, the apparent K_m of isolated cytochrome oxidase or mitochondria is about 0.1–1.0 μ*M* O_2, but in the presence of 60 n*M* NO (a plausible physiological level of NO) the K_m of cellular respiration would be 30 μ*M* O_2, similar to tissue levels of oxygen measured in the brain *(73).* Thus NO can make cells effectively hypoxic at relatively high oxygen levels, and potentially sensitize tissues to hypoxic damage *(78).* NO from activated astrocytes potently and reversibly inhibits the cellular respiration of those astrocytes *(42)* and of co-incubated neurons *(45).* Similarly, activated microglia reversibly inhibit the respiration of co-incubated neurons *(45,48),* activated macrophages inhibit the respiration of co-incubated fibroblasts *(79),* and activated endothelial cells reversibly inhibit their own respiration as

well as that of aorta *(80)*—all via NO inhibition of cytochrome oxidase.

Irreversible Inhibition of Respiration by NO

Cells exposed to NO (or NO-producing cells) show immediate but reversible inhibition of respiration at cytochrome oxidase. However, after several hours of exposure to NO an irreversible inhibition develops, probably due to conversion of NO to RNS that inhibit respiration at multiple sites *(81–83)*. One of the more rapid effects is an inactivation of complex I *(82,84)*, followed by inhibition of aconitase and complex II/III, possibly due to removal of iron from iron–sulphur centers *(85,86)*. Inactivation of complex I can be induced by NO, peroxynitrite or S-nitrosothiols—possibly due to S-nitrosation of the complex—and can be reversed by reduced thiols such as glutathione or by light *(82,84)*. Addition of peroxynitrite to a neuroblastoma cell-line resulted in relatively selective nitration of tyrosines in complex I and associated apoptosis *(87)*. High concentrations of NO can destroy iron–sulphur centres by binding and displacing the iron; there is EPR evidence for such damage in complex I *(88,89)*. Peroxynitrite can inhibit complex I, complex II, cytochrome oxidase (complex IV), the ATP synthase, aconitase, Mn-superoxide dismutase (SOD), creatine kinase, and probably many other proteins *(69,81,90)*. Peroxynitrite is a strong oxidant and can also cause DNA damage, induce lipid peroxidation, and increase mitochondrial proton (and other ion) permeability (probably by lipid peroxidation or thiol cross-linking) *(91,92)*.

Peroxynitrite has relatively little effect on the V_{max} of cytochrome oxidase when the peroxynitrite is added to mitochondria at levels that inhibit the other respiratory complexes *(81)*. However, it does have various damaging effects on isolated cytochrome oxidase, particularly increasing the K_m for oxygen *(93)*. High concentrations (>1 μ*M*) of NO (possibly via NO_2 or N_2O_3) can also induce an irreversible rise in K_m for oxygen both in isolated cytochrome oxidase or in cells treated with NO *(93,94)*.

NO and Mitochondrial Permeability Transition

RNS, S-nitrosothiols, or reactive oxygen species (ROS) cause mitochondrial permeability transition (MPT) in isolated, calcium-preloaded mitochondria *(95–97)*. MPT is a dramatic increase in permeability of the inner mitochondrial membrane to small (up to 1.5 kDa) molecules due to the assembly of a pore, possibly involving the adenine nucleotide carrier, cyclophilin D, the voltage-dependent anion carrier, and mitochondrial creatine kinase (Fig. 3) (for reviews *see 98,99*). Mitochondrial membrane potential and matrix calcium are known to determine the ability of other compounds to induce MPT, thus inhibition of respiration by NO and the subsequent decrease in membrane potential should favor MPT opening. On the other hand, NO itself can inhibit MPT due to the direct inhibition of respiration, preventing calcium accumulation in mitochondria *(100)*. However, NO at high concentrations can promote MPT probably due to the production of peroxynitrite, nitrosothiols, or NO_2/N_2O_3 that may either (a) directly oxidize the protein thiols that regulate opening of the MPT pore *(101,102)*, or (b) oxidize glutathione that normally keeps these thiols reduced. MPT plays an important role in both necrotic- and apoptotic cell-death. MPT dissipates the protonmotive force, causing uncoupling of oxidative phosphorylation, and reversal of the ATP synthase, potentially hydrolyzing cellular ATP, resulting in necrosis. MPT also causes rapid swelling of the mitochondria, such that the outer membrane can be ruptured releasing intermembrane proteins, such as cytochrome *c* (Fig. 3). Release of cytochrome *c* and matrix components such as NADH inhibits respiration, thus potentially causing necrosis. But release of cytochrome *c* and other apoptogenic intermembrane proteins such as apoptosis-inducing factor (AIF) and SMAC/Diablo potentially triggers apoptosis *(103,104)*. Transient MPT opening may be a physiological process, and usually does not cause cell damage, while longer sustained MPT opening may cause either apoptosis or necrosis

(98,105). The mode of cell death after MPT opening is likely to depend on additional factors such as activation of Bid/Bax/Bad pathway or availability of ATP (ATP depletion probably favoring necrosis) *(98).*

Permeability transition-inhibitor cyclosporin A can protect the brain from damage induced by ischemia *(106).* Minocycline, a tetracycline, has recently been found to block permeability transition, and protects in animal models of cerebral ischemia, traumatic brain injury, Huntington's disease, Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) *(107),* however, it also inhibits iNOS and has other effects.

Superoxide, Peroxynitrite, and Other Oxidants

Superoxide (O_2^-) is an oxidant that can, in particular circumstances, lead to the production of a variety of other stronger oxidants such as hydrogen peroxide (H_2O_2), the hydroxy radical (OH), hypochlorous acid (HOCl), or peroxynitrite (ONOOH) which are cytotoxic. However, superoxide production is usually very limited, and many molecules can scavenge superoxide and peroxynitrite. On the other hand some cells, particularly microglia *(34,48,108),* macrophages, and neutrophils can produce extracellular superoxide at very high rates from a specialized enzyme, NADPH oxidase, when appropriately stimulated. Proinflammatory cytokines can prime microglia to produce superoxide in response to protein kinase C activation *(34,35,108).* As superoxide reacts rapidly with NO to produce peroxynitrite, stimulation of the NADPH oxidase in activated microglia expressing iNOS results in rapid disappearance of all NO and the production of peroxynitrite *(48).* Stimulation of the NADPH oxidase in neutrophils similarly causes very rapid NO breakdown *(109).* Thus the simultaneous production of superoxide by microglia, macrophages, or neutrophils and the production of NO by astrocytes, microglia, macrophages, or endothelial cells could produce extracellular peroxynitrite, but whether this would be more or less toxic than NO alone has not been tested.

Mitochondria are another source of superoxide, although at much lower levels than the NADPH oxidase, and while the NADPH oxidase produces superoxide outside the cell or within phagocytotic vesicles, the mitochondrial respiratory chain produces superoxide within the mitochondria. NO can stimulate this mitochondrial superoxide production, and react with it to produce peroxynitrite within the mitochondria, which might then irreversibly inhibit mitochondrial respiration or induce permeability transition and thus cell death *(83).*

There are a number of other potential sources of superoxide, such as xanthine oxidase. NO synthases also produce superoxide and peroxynitrite when the L-arginine level is below saturation *(110–112),* and it has been suggested that this is a means by which excitoxic death occurs in nNOS-expressing neurons *(50).* Superoxide is normally broken down by SOD, of which there two main types: Mn-SOD located in mitochondria, and Cu,Zn-SOD located in the cytosol and extracellularly. Two molecules of superoxide react with SOD to give one molecule of oxygen and one of H_2O_2. However, H_2O_2 and NO can react with SOD to produce peroxynitrite *(113),* which might be one possible reason that H_2O_2 and NO are apparently synergistic in killing nonneuronal and neuronal cells *(92,114).* NO also acutely inhibits catalase due to reversible binding to the haem *(115),* and acutely stimulates H_2O_2 production from mitochondria *(83,116).* Thus, NO can acutely raise H_2O_2 levels in cells , potentially contributing to cell death *(117).*

Peroxynitrite has been implicated in mediating neuronal death induced by NO, glutamate or activated glia, as in some models where peroxynitrite scavengers or catalysts of peroxynitrite breakdown prevent such death *(118–121).* The mechanisms by which peroxynitrite induces neuronal death are unclear, but have been suggested to include: (a) activation of poly (ADP-ribose) polymerase (PARP) (via DNA damage) *(122),* (b) activation of vesicular

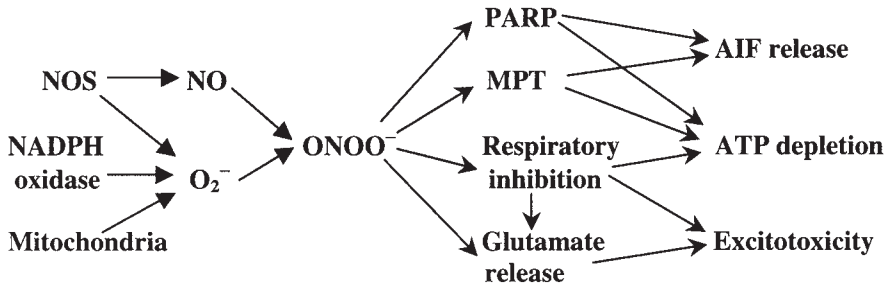

Fig. 4. Peroxynitrite-mediated neuronal death. Potential mechanisms of peroxynitrite ($ONOO^-$) production and actions to cause cell death.

exocytosis of glutamate and NMDA-receptor-mediated excitotoxicity *(123,124)*, or (c) induction of mitochondrial permeability transition which can result in ATP depletion causing necrosis, and/or cytochrome *c* and AIF release causing apoptosis or related forms of cell death *(125)* (Fig. 4). Interestingly addition of peroxynitrite to cultured astrocytes can itself cause activation of the astrocytes to a reactive phenotype expressing iNOS, which is then capable of killing cocultured neurons via iNOS and peroxynitrite-dependent mechanisms *(126)*.

Glutathione can act as an endogenous antioxidant against peroxynitrite and other oxidants, and may be a key determinant of whether neurons live or die in response to NO *(41,68,127,128)*. NO or RNS can cause glutathione depletion *(82)*, and neuronal glutathione may be depleted in a range of brain pathologies, such as PD *(129)*, potentially making neurons more sensitive to NO-induced cell death.

Glutamate, NO, and Excitotoxicity

Excitatory amino acids, in particular glutamate, can at high extracellular levels kill neurons—a process known as excitotoxicity. It has also been proposed that excitatory amino acid neurotoxicity contributes to the pathogenesis of various neurodegenerative diseases *(130)*. Glutamate-induced death of neurons can be mediated by: (a) activation of the NMDA subtype of glutamate receptor, resulting in Ca^{2+} and/or Na^+ overload of the neuron; (b) activation of AMPA receptors, resulting in Ca^{2+} and/or Na^+ overload of the neuron; or (c) glutamate inhibition of cystine uptake, resulting in oxidative stress/death of the neuron *(131–133)* (Fig. 5). A possible mediating event in glutamate neurotoxicity is mitochondrial dysfunction *(65,134)*. Respiratory inhibitors can strongly potentiate NMDA receptor-mediated death by depolarizing the plasma membrane, as depolarization is required together with glutamate for activation of NMDA receptors *(135)*.

Mitochondria and NO in Excitotoxicity

It has also been reported that glutamate neurotoxicity is mediated in part by production of NO by neuronal NO synthase *(136,137)* and NO-induced mitochondrial inhibition or depolarization *(127,138,139)*. In some cases inhibition of nNOS blocks glutamate-induced death of neurons, and cell death has been attributed to NO and calcium-induced mitochondrial depolarization, although mechanisms remain unclear *(127,136–141)*. High concentrations of cyclosporin A can block glutamate-induced mitochondrial depolarization, cytochrome *c* release, and/or cell death suggesting the involvement of MPT *(138,140,142)*. However, cyclosporin A also inhibits calcineurin, which regulates nNOS and the proapoptotic protein Bad—interpretation of these experiments remain ambiguous *(140)*. Keelan et al. *(138)* found that glutamate caused mitochondrial

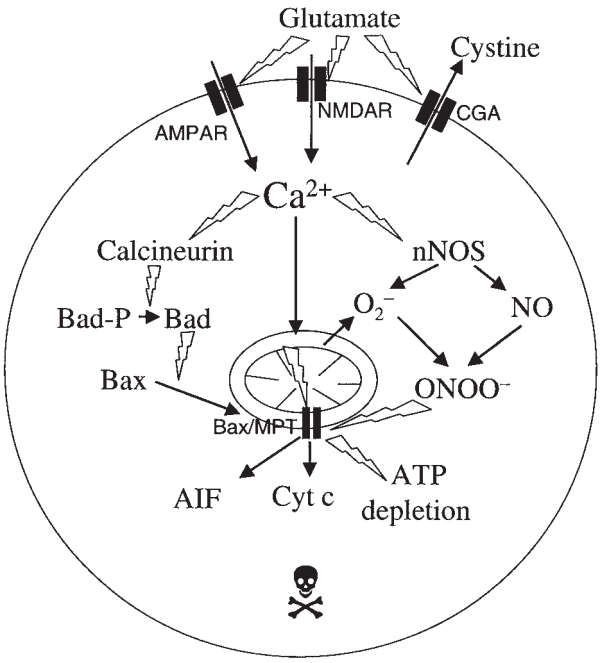

Fig. 5. Possible mechanisms of glutamate-induced neuronal death. Arrows indicate movement of reaction/production; thunderbolts indicate activation. Extracellular glutamate activates either AMPA receptors (AMPAR), NMDA receptors (NMDAR), or the cystine-glutamate antiporter (CGA), the latter resulting in oxidative stress. Calcium elevation may: (a) stimulate calcineurin causing Bad and Bax activation; (b) stimulate mitochondrial oxidant production and MPT; and (c) stimulate nNOS production of NO and oxidants. Activation of Bax pores and/or MPT may result in release of AIF and cytochrome c, and/or cause ATP depletion.

depolarization in hippocampal neurons kept in culture for >11 d, and this required a synergy between high calcium and NO within the neurons, possibly mediated by permeability transition. Interestingly Grima et al. *(143)* found that L-arginine could block NMDA-induced neuronal mitochondrial depolarization and death, apparently by suppressing NOS generated-superoxide and -peroxynitrite. Thus excitotoxicity might be due to calcium activation of nNOS to generate peroxynitrite that activates MPT. Grima et al. *(143)* provided some evidence that astrocytes may suppress excitoxicity by exporting L-arginine to neurons.

Strijbos et al. *(137)* found that brief application of NMDA to cultured striatal neurons caused delayed death preventable by inhibitors of the NMDA receptor, sodium channels, or NOS. They proposed that NMDA receptor-activation caused partial depolarization, which opened sodium channels that led to glutamate release, which in turn completed a vicious circle by activating the NMDA receptors, with the output of this circle continuing stimulation of nNOS that caused neuronal death.

In motor neurons, Urushitani et al. *(144)* distinguished chronic from acute excitotoxicity, the former being mediated by stimulation of nNOS (and therefore blocked by NOS inhibitors), while the latter apparently being mediated by calcium uptake into mitochondria resulting in mitochondrial ROS production (and therefore acute toxicity was blocked by mitochondria uncouplers and ROS scavengers).

Damage to the Mitochondrial Respiratory Chain Caused by NO from Activated Glia

Coculture of neurons with cytokine-stimulated astrocytes results in significant neuronal mitochondrial damage via a NO-dependent mechanism *(71,72).* In particular there is loss of neuronal complex II–III and IV activities, while complex I activity is relatively preserved as long as neuronal glutathione levels are maintained *(128).* However, all of this mitochondrial damage induced by activated astrocytes as well as by NO donors is prevented by the NMDA receptor-antagonist MK-801, indicating that the mitochondrial damage is secondary to NO-induced excitotoxicity *(53).* Furthermore, glutamate exposure alone is known to damage complex II–III and complex IV of the neuronal mitochondrial respiratory chain *(127).* Since the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) prevented this glutamate-induced respiratory chain damage *(127),* the mitochondrial damage may result from peroxynitrite production. Thus,

NO from activated glia causes excitotoxic stimulation of neurons, which in turn cause mitochondrial damage partly mediated by nNOS.

NO-Induced Glutamate Release from Neurons and Glia

NO, at concentrations where it inhibits respiration, causes acute (within seconds) glutamate release from synaptosomes and neurons, *(44–46,145)*, which has been attributed either to inhibition of mitochondrial respiration followed by reversal of glutamate uptake *(44,45,146)*, or to a direct (calcium-independent) activation of vesicular exocytosis *(147)*.

Recently we found that NO also caused acute glutamate release from astrocytes *(47)*. But in contrast to neurons this NO-induced release from astrocytes was prevented by calcium chelators or an inhibitor of vesicular exocytosis, was not inhibited by an inhibitor of glutamate transport, and was not replicated by specific respiratory inhibitors. NO also caused a rapid rise in intracellular calcium released from intracellular stores, and induced the exocytosis, of vesicular ATP. Astrocytes are now known to have a vesicular pool of glutamate (and possibly ATP) that is rapidly exocytosed in response to agonists that raise intracellular calcium. Thus, NO appears to induce glutamate release from astrocytes by calcium-induced exocytosis of vesicular glutamate *(47)*.

NO-induced glutamate release from glia and neurons may also be important for physiological and/or pathological signaling between these cells. We found that activated astrocytes maintained considerably higher levels of extracellular glutamate, which were lowered by an iNOS inhibitor, however the rate of glutamate uptake was unaffected, suggesting that NO from iNOS was causing continuous glutamate release *(47)*. The extracellular glutamate level maintained by these astrocytes was potentially excitotoxic to neurons. Thus, NO from activated glia might kill neurons by three excitotoxicity-related means: (1) NO-induced glutamate release from astrocytes; (2) NO-induced glutamate release from neurons; and (3) potentiation of NMDA-receptor activation via respiratory inhibition-induced neuronal depolarization (*see* below).

Microglial Glutamate Release

Microglia apparently release high levels of glutamate via a specific transporter in exchange for cystine, and this release is increased by inflammatory activation of the microglia *(38,148)*. The reversible sodium-dependent glutamate transporter may also have increased activity in activated microglia and result in glutamate release in conditions where extracellular potassium is raised *(149)*. Supernatants (conditioned media) from microglial cultures can have high glutamate levels, and when added to cultured neurons may kill them by excitotoxic mechanisms *(36,37)*.

Inhibitors of the Mitochondrial Respiratory Chain Potentiate or Cause Excitotoxicity

In cultured neurons, inhibitors of oxidative phosphorylation or the sodium pump allow relatively low levels of NMDA or glutamate to become neurotoxic *(135)*. This is probably due to ATP depletion causing failure of the sodium pump, resulting in plasma-membrane depolarization and removal of the Mg^{2+}-block of the NMDA channel, but ATP-depletion induced changes in calcium transport may also contribute to this synergistic effect *(135,148)*. Inhibitors of the mitochondrial respiratory chain or sodium pump also cause rapid glutamate release from neurons and isolated nerve terminals (synaptosomes), again apparently due to ATP depletion inducing failure of the sodium pump, causing plasma-membrane depolarization resulting in a rapid, transient exocytosis of vesicular glutamate, followed by a sustained reversal of the glutamate uptake carrier *(45,65,149)*.

Mitochondrial respiratory inhibitors also cause neuronal death in vivo, which is generally excitotoxic. Mitochondrial complex II inhibitor, 3-nitropropionic acid causes neuronal death in the striatum that can be attenuated by an

NMDA-receptor antagonist *(150–152).* Another complex II inhibitor, malonate, exacerbates NMDA, AMPA, and glutamate excitotoxicity in vivo *(153).* Complex I inhibitors, rotenone and methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP), cause neuronal death in the substantia nigra, leading to Parkinson's disease symptoms *(154).*

Synergy Between NO and Glutamate in Excitotoxicity

Hewett et al. *(8)* and Kim and Ko *(155)* found that induction of iNOS or addition of NO donors to neuronal-glia cocultures markedly potentiated NMDA-induced excitotoxicity of the neurons. They found that antioxidants blocked this synergy leading them to suggest that NMDA receptor activation-induced superoxide production reacted with NO to cause neuronal death. Another interpretation of the synergy might be that NO inhibition of neuronal mitochondrial respiration would depolarize the plasma membrane, removing the Mg^{2+} block of the NMDA receptor, in which case NMDA toxicity is known to be greatly sensitized *(135,148).*

Capano et al. *(156)* found that glutamate and NO were synergistic in inducing apoptosis in a neuronal cell line, and this was blocked by cyclosporin A or an antisense oligodeoxynucleotide to cyclophilin-A. The latter suggests that calcineurin may be required for apoptosis in this system as it is in excitotoxic death of hippocampal neurons, where the calcium elevation apparently activates calcineurin to dephosphorylate the pro-apoptotic Bcl-2 homologous protein Bad, which then translocates to mitochondria to cause cytochrome *c* release *(157).*

Mechanisms Whereby NO Kills Neurons

There are three different ways in which NO can induce cell death *(67):* (a) energy-depletion induced necrosis (Fig. 6); (b) oxidative apoptosis (Fig. 7); and (c) excitotoxicity (Fig. 8).

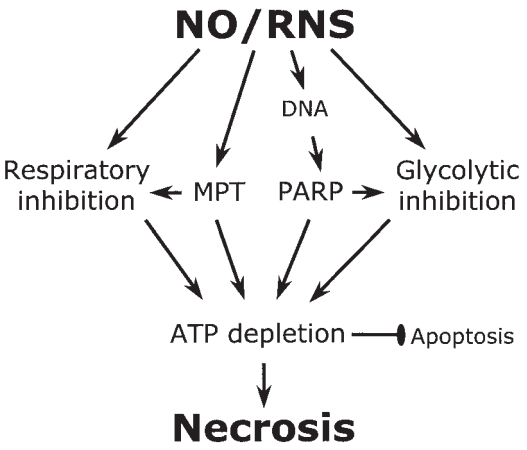

Fig. 6. Potential mechanisms by which NO/RNS causes energy-depletion induced necrosis. NO and RNS directly inhibit mitochondrial respiration. Activation of MPT by RNS can inhibit respiration due to loss of mitochondrial cytochrome c and NADH, and can more directly cause ATP depletion by allowing protons and ATP free access across the inner membrane, resulting in reversal of the ATP synthase. RNS damage to DNA can cause PARP activation, leading to depletion of its substrate NAD,+ which may deplete ATP either because ATP is required to resynthesis NAD^+ or because it is required for glycolysis. RNS directly inhibits (or uncouples) glycolysis at glyceraldehyde-3-phosphate dehydrogenase when glutathione is depleted. ATP depletion inhibits apoptosis, and causes necrosis, usually via failure of the sodium and/or calcium pumps.

The mechanisms of NO neurotoxicity has been proposed to include: (i) activation of PARP followed by NAD^+ and ATP depletion *(122);* (ii) induction of apoptosis by poorly defined mechanisms *(158–160);* (iii) glutamate release *(45,46,145,146)* and excitotoxicity *(8,45,124,158);* and (iv) inhibition of mitochondrial respiration *(41,45).* Direct chemical modification of DNA has been linked to NO or peroxynitrite-induced neurotoxicity *(92).* DNA damage may lead to prolonged activation of the DNA repair enzyme PARP, which then depletes the cell of NAD,+ which may in turn deplete the cell of ATP (causing necrosis) both

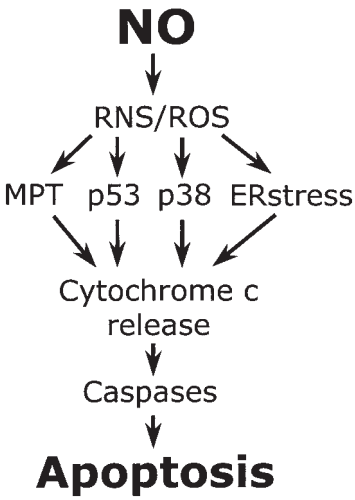

Fig. 7. Potential mechanisms by which NO/RNS causes oxidative apoptosis. NO can induce oxidative and/or nitrosative stress by various means, including increasing superoxide production from mitochondria and inhibiting catalase, RNS inactivation of glutathione peroxidase and Mn-SOD, and RNS reaction with GSH. The resulting stress activates the mitochondrial pathway of apoptosis by several different pathways, including: (a) stimulation of MPT; (b) upregulation of p53; (c) activation of the p53 MAP kinase pathway, and (d) induction of endoplasmic reticulum stress.

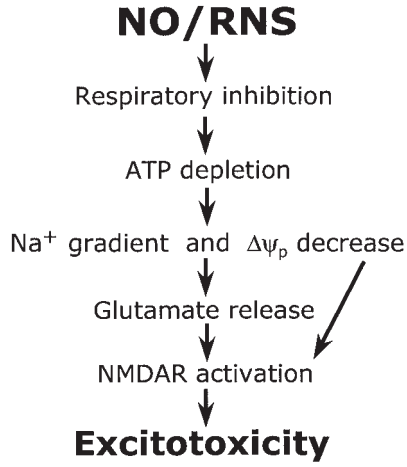

Fig. 8. Potential mechanisms by which NO/RNS causes excitotoxicity. NO/RNS-induced respiratory inhibition causes neuronal ATP depletion, decreasing the plasma membrane sodium gradient and membrane potential, resulting in glutamate release, which together with the decrease in membrane potential activates the NMDA receptor, causing excitotoxicity.

because NAD^+ is required for glycolysis and because it is synthesized from ATP *(122).* PARP appears to mediate some cell death induced by ischemia/reperfusion, glutamate or inflammation, as indicated by protection against these pathologies by PARP inhibitors or in PARP knockout mice *(161).* There is recent evidence that PARP-induced cell death is mediated by AIF release from mitochondria, which translocates to the nucleus to induce DNA fragmentation *(162).* PARP may mediate NO-induced death of some neurons *(122)* but not others *(158).*

Both NO-induced respiratory inhibition and MPT can cause cellular ATP depletion if the cellular glycolytic capacity is low or inhibited. Thus glycolytic capacity is a key determinant of cell survival or the mode of cell death is response to NO *(69,77,165).* Peroxynitrite and S-nitrosothiols can directly inhibit or uncouple glycolysis at glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in condition of glutathione depletion, potentially causing necrosis *(163,164)* (Fig. 6). NO inhibition of GAPDH potentially inhibits glycolysis, however GAPDH is not normally rate-limiting for glycolysis, and NO inhibition of respiration causes an acute stimulation of glycolysis to compensate for the fall in ATP, so that NO often stimulates glycolysis at least initially *(67,77,165).* Furthermore, NO-induced oxidation of GAPDH can result in its uncoupling from ATP production, potentiating ATP depletion *(164).*

NO can induce apoptosis in nonneuronal cells by a variety of means *(67)* including: (a) activation of MPT (probably by peroxynitrite or S-nitrosothiols); (b) activation of the ER stress response (possibly due to ryanodine receptor-mediated calcium release); (c) activation of p53 (probably due to DNA damage); and (d) activation of the p38 MAP kinase pathway (possibly due to oxidants) (Fig. 7). Activation of apoptosis

by NO in neuronal cells may be secondary to excitotoxicity *(124,158,166).* For example, in cerebellar granule neurons NO-induced apoptosis is prevented or delayed by MK-801 (an NMDA-type glutamate receptor antagonist) *(45,124,158).* Death in these cells is eventually necrotic, but is preceeded by nuclear condensation and mitochondrial depolarization with some characteristics of apoptosis. Nuclear condensation and cell death are prevented by caspase inhibitors, at low NO concentrations but not high concentrations *(158),* but are also prevented by calpain inhibitors *(167),* so "apoptosis" may not be an appropriate term to describe cell death in this case.

Excitotoxicity itself generally causes necrosis if high, sustained levels of glutamate or aspartate are involved, or when sensitizers such as glycine or respiratory inhibitors are present *(65).* Such necrosis can result from acute swelling due to sodium influx, or from delayed mitochondrial dysfunction due to calcium influx. Whether a cell dies by necrosis or apoptosis depends in part on whether cellular ATP levels are maintained, as the triggering and execution of apoptosis require ATP, and ATP depletion itself causes necrosis due to the ATP-dependence of sodium and calcium pumps (Fig. 6). Since NO inhibits mitochondrial ATP production and independently activates apoptosis, whether the cell dies by apoptosis or necrosis depends on whether cellular ATP levels are maintained by glycolysis *(165,168).*

NO-induced cell death of cortical neurons and SH-SY5Y neuroblastoma cells was accompanied by caspase activation and Bax translocation to mitochondria, but not blocked by caspase inhibitors *(169).* However, an inhibitor of p38 MAP kinase (SB203580), blocked NO-induced Bax translocation, caspase activation, and cell death; and neurons from Bax knockout mice were resistant to NO-induced apoptosis *(169).* Similarly, in neural progenitor cells the p38 MAP kinase inhibitor prevented NO-induced cytochrome *c* release, caspase activation, and cell death *(170).* This suggests that NO-induced neuronal death in some cases involves p38 MAP kinase activation followed somehow by Bax translocation to mitochondria, which then causes release of cytochome *c* and other apoptogenic proteins from mitochondria *(169).* However, it is unclear whether such death is secondary to excitotoxicity. NO-induced acidification followed by activation of endonucleases by the acidification has also been implicated in NO-induced cell death *(171).*

There are almost certainly a variety of different ways that NO can kill neurons, just as in other cell types *(69).* NO-induced death of cultured neurons is initially prevented by NMDA receptor antagonists, but if the presence of NO is sustained, the later necrosis is not prevented by such antagonists *(45).* Younger neurons (shorter time in culture, lacking NMDA receptors) are just as sensitive to NO but, in contrast to the older neurons, die by non-NMDA receptor-mediated mechanisms *(172).* Other factors that perhaps affect how neuron cells respond to NO are: the concentration of NO, the time course of exposure to NO, the type of NO derivative likely to be present, the cell type, the thiol status of the cell, the glycolytic capacity, and NO and NO-independent protective mechanisms.

NO-Protection of Neurons

NO can be converted into a number of different reactive derivatives, including NO_2, N_2O_3, S-nitrosothiols, and peroxynitrite, collectively known as reactive nitrogen species (RNS). And these species can induce cell death by different means *(97,165,173,174).* S-nitrosating species, particularly S-nitrosothiols, can inhibit apoptosis in nonneuronal cells by S-nitrosating and inhibiting caspases *(175,176).* Also, in neurons S-nitrosating species can inhibit excitotoxicity by S-nitrosating and inhibiting the NMDA receptor *(173,177).* In astrocytes, cGMP (from NO-activated soluble guanylate cyclase) can also apparently protect against cell death by inhibiting MPT (via a protein kinase G-mediated phosphorylation) *(178).*

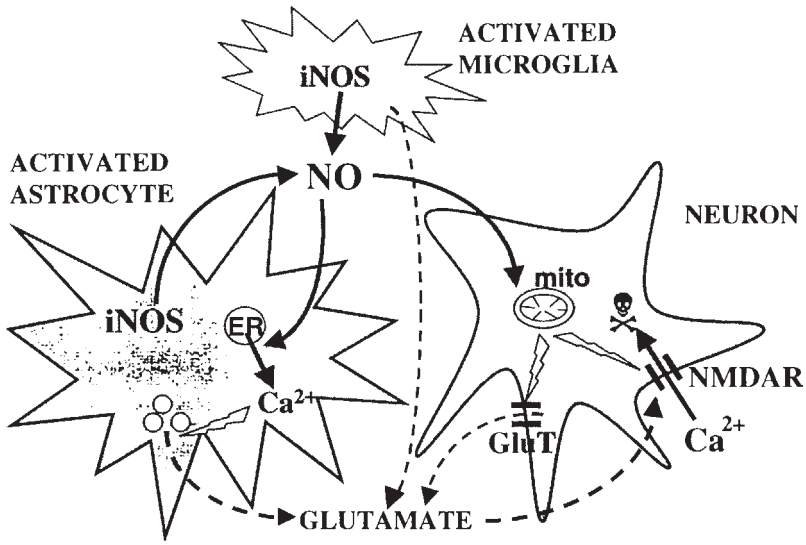

Fig. 9. Mechanisms by which activated glia kill neurons. Activated glia express iNOS and produce NO that: (a) causes calcium mobilization from the endoplasmic reticulum (ER), which activates release of vesicular glutamate from astrocytes; and (b) inhibits mitochondrial (mito) respiration in neurons. This mitochondrial inhibition may depolarize the neuron, causing both release of glutamate via the glutamate transporter (GluT) and sensitization of the NMDA receptor (NMDA), resulting in neuronal death. Activated microglia may also release glutamate that contributes to the activation of the NMDA receptor.

Mechanisms by Which Activated Glia Kill Neurons In Vitro

It has been suggested that the mechanisms by which activated glia kill neurons in culture include the release of nitric oxide *(9–11,219)*, ROS *(33,34)*, glutamate *(12,36–38)*, and/or cytokines *(39,40)*. Potent excitotoxins, particularly glutamate, recovered from medium conditioned by microglia, either untreated or treated with LPS and/or IFN-γ *(36)*, phorbol 12-myristan 13-acetate (PMA) *(37)*, or chromogranin-A *(12)* have been implicated as the principal mediators of microglial toxicity to neurons *(36,37)*, acting via NMDA receptors (thus death is blocked by NMDA-receptor antagonists). However, most of these studies did not test for the involvement of NO, shown in separate studies to be critical for microglial toxicity towards cortical neurons *(180,182)*, cerebellar granule cells *(45,179)*, dopaminergic neurons *(183)*, or cholinergic neurons *(184)* using microglia activated by IFN-γ and LPS *(45,180–182)*, LPS and several cytokines *(179)*, or LPS alone *(183,184)*.

NO is released from activated astrocytes and microglia *(10,14,42,43)*, and neurons are remarkably sensitive to NO-induced cell death *(45,124)*. NO-induced neuronal death is at least initially mediated by glutamate and the NMDA receptor, thus microglial-induced cell death is blocked by both iNOS inhibitors and NMDA-receptor antagonists *(45,172)*. NO from activated microglia or astrocytes has been shown to rapidly inhibit the cellular respiration of co-incubated neurons, resulting in glutamate release and excitotoxic death of neurons *(45)* (Fig. 9).

Human neurons and glia are apparently as susceptible to inflammatory neurodegeneration as rodent cells *(10,35,185)*, although it would appear that it is easier to induce iNOS in human astrocytes than human microglia *(35,60)*, whereas the reverse is true of rodent glia *(56)*. IL-1β and TNF-β (or IFN-γ) synergistically

induced iNOS in astrocytes of human mixed neuronal-glial cultures, and the consequent neuronal death was blocked by a nitric oxide synthase (NOS) inhibitor or a NMDA receptor antagonist, showing that the basic mechanism of cell death is the same in human cells *(10,11,34,186).*

Is Glial Activation In Vivo Sufficient to Induce Neurodegeneration?

In contrast to the large number of published papers showing that inflammatory-activated glia kill neurons in culture, there are relatively few published studies showing that glial inflammation in vivo is sufficient to induce neurodegeneration. Injection of bacterial cell-wall endotoxin (LPS) plus IFN-γ into the rat hippocampus caused iNOS expression 1–7 d later in activated microglia, and nuclear apoptosis of neurons that was prevented by a NOS inhibitor *(187).* Similarly, injection of LPS into the substantia nigra of rats to induce inflammation and iNOS expression caused loss of dopamine neurons that was partially prevented by iNOS inhibitors *(188).* A NOS inhibitor also attenuated the acute inflammation and brain injury induced by *E. coli* in piglet brain *(189).* Others, however, have found that inflammation alone is not sufficient to induce neuronal damage, but inflammation synergizes with excitoxins to induce neuronal death *(32,190).* It is unclear whether the relative poverty of literature in this area is due to lack of effort or due to suppression of inflammation and inflammatory damage in vivo. Clearly the subject merits further research.

CNS Pathologies Where Inflammatory Neurodegeneration is Implicated

Alzheimer's Disease

In Alzheimer's disease brains, activated microglia expressing iNOS are found within, and activated astrocytes around, the β-amyloid-containing neuritic plaques containing dead and dystrophic neurites *(17,18,20–22,191,192)* and there is widespread nitration of proteins *(192,193).* β-amyloid or other Alzheimer's associated proteins (S100β and chromogranin-A) can activate microglia and induce the production of NO *(11,12,23,24).* Anti-inflammatory drugs reduce the prevalence of Alzheimer's disease by about 50% *(27,28)* (Table 1), supporting the idea that activated glia contribute actively to neurodegeneration through inflammatory mechanisms *(194).* Injection or infusion of β-amyloid into animal brains results in a marked increase in NO generation from iNOS and associated neuronal dysfunction *(25,195).* Ischemia is now thought to contribute to the pathogenesis of Alzheimer's, and transgenic mice overexpressing a 751-amino acid isoform of human beta-amyloid precursor protein had increased susceptibility to focal-ischemia-induced brain damage that was prevented by inhibitors of inflammation and iNOS expression *(196).*

Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the loss of dopamine neurons in the substantia nigra, decreased striatal dopamine levels, and consequent extrapyramidal motor dysfunction. There is increasing evidence for the involvement of inflammation in Parkinson's *(197).* There is increased iNOS expression in reactive glia associated with degenerating dopaminergic neurons of the substantia nigra from Parkinson's patients *(198,199)* and increased protein nitration *(200).* Injection of 6-hydroxydopamine into one side of rat stiatum causes loss of dopaminergic neurons and rotational movement of the rat, and this is associated with iNOS induction and inhibited by a NOS inhibitor *(201).* A mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a Parkinson's like syndrome in rodents (and humans) accompanied by gliosis and iNOS expression in the striatum, and both the iNOS expression and

dominergic degeneration are blocked by a synthetic tetracycline, minocycline *(202),* which however also blocks MPT *(107).* A specific defect of Complex I activity was also found in the substantia nigra of patients with Parkinson's disease *(203).* In iNOS knockout mice the dopaminergic neurons of the substantia nigra are protected from MPTP-induced death *(16, 204)* (Table 1). Injection of LPS into the substantia nigra of rats to induce inflammation and iNOS expression caused loss of dopamine neurons that was partially prevented by iNOS inhibitors, suggesting that iNOS could mediate loss of dopamine neurons in Parkinson's disease *(188).* In addition, NO derived from nNOS could contribute to neuronal cell-death as the selective inhibition of nNOS prevents MPTP-induced parkinsonism in experimental animals *(205,206).*

Motor Neuron Diseases

There is growing evidence for the involvement of NO and peroxynitrite in the degeneration of motor neurons in the ventral horn of the spinal cord and motor neurons in the cerebral cortex, characteristic of motor neuron diseases *(33,207).* There is increased expression of iNOS in the degenerating neurons and reactive astrocytes of the spinal cords of amyotrophic lateral sclerosis (ALS) patients *(208,209),* and nNOS may also be upregulated *(207,209).* The activity of nNOS is high even in healthy human skeletal muscle *(257).* Mutations in copper/zinc superoxide dismutase (SOD1) are causal in a proportion of familial ALS patients, and transgenic mice expressing the mutant SOD develop a similar phenotype with degenerating motor neurons *(210).* In these transgenic mice degeneration of the neurons is associated with reactive gliosis of astrocytes and activated microglia, and expression of iNOS in these astrocytes and neurons *(211,212).* The mutant SODs can rapidly produce peroxynitrite and catalyze nitration of tyrosine residues, which are found to be elevated in normal, sporadic ALS *(33,213),* and may contribute to the neurodegeneration *(210).*

Multiple Sclerosis

Activated microglia and macrophages are found in the lesions of multiple sclerosis (MS) in close association with damaged axons *(214).* The expression of iNOS in astrocytes and/or the presence of nitrotyrosine were also found in chronic and acute MS lesions in postmortem tissues of MS patients *(215–218).* NO could play a role in the pathology of demyelination and destruction of the oligodendrocytes as this type of glial cell is also sensitive to NO-induced cell death *(219).* In animal models of autoimmune demyelinating disease iNOS inhibition has ambiguous effects. iNOS knockout mice have more severe and prolonged disease *(220).* Other studies with iNOS inhibitors suggest that NO is protective during induction of disease *(221,222),* but detrimental later at the site of disease *(221).*

Brain Ischemia and Stroke

Brain ischemia appears to cause a very large, transient rise on NO levels at the onset of ischemia and/or at reperfusion, probably due to glutamate activation of nNOS via NMDA receptors and calcium *(141,223,224).* NO produced in penumbra areas may potentially sensitize surrounding areas to hypoxia, due to NO inhibition of cytochrome oxidase in competition with oxygen (*see* above). Ischemia appears to rapidly stimulate nNOS expression as well, which may protect those neurons expressing it but damage others *(225,226).* The nNOS knockouts develop smaller infarcts than the wild-type mice when subjected to MCA occlusion *(227,228).* Death of cultured neurons induced by in vitro ischemia is prevented by nNOS inhibitors or in neurons from nNOS-knockout mice *(141).*

Experimental brain ischemia/reperfusion in rats results in iNOS expression and nitrotyrosine staining in damaged areas of the brain *(229,230)* (Table 1). Antisense oligonucleotide to iNOS *(231),* and iNOS inhibitors *(232)* reduce damage in such a model. nNOS- or iNOS-knockout mice are more resistant to global

brain ischemia/reperfusion-induced damage, whereas eNOS-knockout mice are more sensitive, suggesting that nNOS activation during ischemia and subsequent iNOS induction both contribute to the brain damage, whereas eNOS protects by maintaining blood flow *(228,233,234).*

Traumatic Brain Injury

The increase of proinflammatory cytokine levels in traumatic injury induces iNOS expression that can be detected shortly after injury *(235)* (Table 1). Later on astrocytes surrounding the lesion also express iNOS *(236).* Spinal-cord injury in rats resulted in rapid invasion of the lesion by iNOS-positive macrophages, and a NOS inhibitor reduced the number of associated apoptotic cells *(237).* Stab wounds cause expression of iNOS and other inflammatory proteins in rat brain *(238).* iNOS inhibitors have been shown to reduce injury to the brain induced by fluid percussion *(236).*

Epilepsy

It has been suggested that the electrical activity, resulting in this chronic disorder characterized by seizures, is a consequence of the reactive gliosis from infection or trauma *(239).* Nitric oxide (NO) (especially produced by upregulation of nNOS) could be involved in the mechanism triggering limbic seizures and delayed excitotoxic damage in the hippocampus, since in an experimental model of limbic seizures, systemic administration of a selective nNOS inhibitor prevented motor and electrocortical seizures and abolished neuronal-cell death in the hippocampus *(240).* NOS inhibitors also prevented seizures induced by hyperbaric oxygen *(241)* or pilocarpine *(242).* Since the expression of nNOS protein is significantly increased shortly after induction of seizures, while the expression of eNOS and iNOS remains unchanged for a few days, NO produced by nNOS may play a role in the pathogenesis of epilepsy *(241).*

Aging

Aging is accompanied by increased activation of microglia and astrocytes, and increased expression of inflammatory proteins such as iNOS in rodents and humans, suggesting the "Inflammation Hypothesis of Aging" *(243)* (Table 1). Aging also increases glial responsiveness, so for example, aged rats have increased iNOS expression in response to stab wounds *(238).* iNOS expression and protein nitration increase with age of rats *(244–246).* It has been suggested that iNOS expression contributes to brain (and other organ) aging *(247).*

Viral Infections

Human immunodeficiency virus (HIV) infection can cause acquired immunodeficiency syndrome (AIDS) dementia complex (ADC) or HIV encephalitis. ADC brains show iNOS expression mainly in perivascular macrophages/microglia of the basal ganglia in association with expression of the HIV coat protein gp41 *(248,249)* (Table 1). In culture, gp41 can induce microglia to produce IL-1β and astrocytes to express iNOS, resulting in the death of cocultured neurons via NO-dependent mechanisms *(250).*

HIV-1 encephalitis patients show high levels of iNOS expression in astrocytes in association with virally-infected microglia or macrophages expressing IL-1β and caspase-1 *(251)* and increased nitrite in cerebrospinal fluid (CSF) or serum *(252,253).* Injection of the HIV envelope protein gp120 in rat brains induces iNOS expression *(254).* Hori et al. *(255)* found that HIV-1 infected macrophages could induce iNOS in human astrocytes, and the consequent NO production blocked viral replication, and they speculated that the NO contributes to pathology. Double-stranded RNA can induce iNOS in microglia and macrophages *(256).*

Infection of mice with mosquito-transmitted Venezuelan equine encephalitis virus causes meningoencephalitis, induction of iNOS and TNF-α, and astrogliosis associated with neuronal apoptosis; survival time was increased in iNOS-knockout mice *(257).*

Bacterial Infections and Sepsis

Bacterial meningitis results in expression of iNOS and tyrosine nitration; and iNOS-knockout mice, or those treated with peroxynitrite scavengers, are partially protected from the resulting damage to the blood–brain barrier *(258,259,266).* A NOS inhibitor attenuated the acute inflammatory response and brain injury induced by *E. coli* in piglet brain *(189).* However, inhibition of iNOS with aminoguanidine in a neonatal model of bacterial menigitis resulted in increased bacterial levels, reduced perfusion, and increased seizures *(313),* suggesting that NO may also limit the pathology by killing bacteria. Injection of bacterial cell wall endotoxin (LPS) into the rat brain ventricles caused rapid glial activation and iNOS expression throughout the brain *(260).* Injection of LPS plus IFN-γ into the rat hippocampus caused iNOS expression 1–7 d later in activated microglia, and nuclear apoptosis of neurons that was prevented by a NOS inhibitor *(187).* Similarly injection of LPS into the substantia nigra of rats to induce inflammation and iNOS expression caused loss of dopamine neurons that was partially prevented by iNOS inhibitors *(188).*

Prion Diseases

Prion protein fragments or a homolog of the prion protein (doppel) induce iNOS expression in cultured human microglia *(261,262).* Expression of scrapie or prion homolog proteins in mice causes increased iNOS and nNOS expression in the brain *(261,263)* (Table 1). Microglial activation has been implicated in prion pathology *(264),* but whether NO contributes to the pathology is unknown.

Conclusions

Inflammatory neurodegeneration is the death or degeneration of neurons caused by inflammation. It may contribute to a wide range of CNS pathologies, but the extent to which inflammation contributes positively or negatively to particular diseases remains controversial and requires considerably more research to elucidate. The mechanisms by which inflammatory-activated microglia and astrocytes kill cultured neurons appears to initially involve NO inhibition of mitochondrial respiration, glutamate release from neurons and glia, resulting in excitotoxic death of neurons potentiated by NO inhibition of neuronal respiration. However, other mechanisms may contribute to more chronic damage and degeneration. The extent and mechanisms by which inflammation damages neurons in vivo as opposed to in culture requires further research.

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