ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006

Forum Review

Nitric Oxide Synthase Expression and Nitric Oxide Toxicity in Oligodendrocytes

ANNE I. BOULLERNE[1] and JOYCE A. BENJAMINS[2]

ABSTRACT

Oligodendrocytes (OLG) have more complex interactions with nitric oxide (NO) than initially suspected. Historically, OLG were seen only as targets of high NO levels released from other cells. Expression of nitric oxide synthase type II (NOS-2) in primary cultures of OLGs stimulated by cytokines led to controversy due to the presence of small numbers of microglia, cells also inducible for NOS-2 expression. The present review summarizes the findings that immature OLG express NOS-2, but that they do not in their most mature stage in culture as membrane sheet-bearing cells. This raises questions about the regulation of NOS-2 expression in OLG. Additionally, novel data are presented on NOS-3 expression in cultured OLG. If confirmed *in vivo*, this finding suggests that constitutive NOS-3 expression may play a key role in OLG injury due to its activation by calcium, in interaction with pathways mediating glutamate toxicity. The authors discuss *in vivo* NO levels to place *in vitro* findings in context, and compare OLG sensitivity to NO with that of other brain cells. Lastly, the multiple interactions of NO are considered with regard to glutamate cytotoxicity, the antioxidant glutathione, mitochondrial function, and myelin architecture. *Antioxid. Redox Signal.* 8, 967–980.

EXPRESSION OF NITRIC OXIDE SYNTHASES

Constitutive nitric oxide synthases

Expression in brain. Nitric oxide synthase (NOS) type I, also named neuronal NOS (nNOS or NOS-1), and NOS type III, also named endothelial NOS (eNOS or NOS-3), are constitutively expressed in endothelial cells, and in some neurons and astrocytes in certain brain areas. Unlike inducible NOS (iNOS or NOS-2), the activity of both of these enzymes is regulated by changes in calcium concentrations. NOS-1 and NOS-3 have seldom been investigated in oligodendrocytes (OLG) since initially they were not found in OLG in guinea pig optic nerve either by NADPH-diaphorase activity (NADPH-d), common to all NOS, or by immunodetection (85). That same year, NADPH-d was reported in trout OLG (83). This difference was ascribed to species differences, but not to the detection method.

The presence of NOS-1 has never been found in OLG. Even though immunostaining for NOS-3 *per se* has never been reported in OLG, the presence of NOS-3 cannot be completely ruled out. Since NOS-3 is mostly bound to the membrane fraction (79), it responds similarly as do other membrane antigens to fixation methods; thus, harsh treatment to remove paraffin from embedded sections disrupts most of the plasma membranes and can lead to loss of membrane-associated antigens. Moreover, NOS-3 and its NADPH-d activity are especially sensitive to aldehyde fixation versus the relative sturdiness of NOS-1 (35). As a result, the two oldest and most used methods, namely paraffin-embedding and 4% paraformaldehyde (PAF) fixation, greatly impair detection of the moderate levels of NOS-3 found in glial cells. Highly variable intensity and frequency of NOS-3 detection in astrocytes illustrate the importance of fixation methods. NOS-3 and NADPH-d activity in astrocytes were first reported in normal rat brain after light fixation with 2% PAF (35). Later, brain sections from primate and rat, when lightly fixed or em-

[1]Department of Anesthesiology, University of Illinois at Chicago, Chicago, Illinois.
[2]Department of Neurology, Wayne State University, Detroit, Michigan.

bedded in OCT compound, showed widespread and robust NOS-3 expression in astrocytes throughout the gray matter and the white matter (52, 98). In contrast, paraffin-embedded and heavily fixed sections displayed NOS-3 expression in astrocytes only in a few discrete areas in normal rodent brain or in brain lesions from multiple sclerosis patients as well as from healthy controls (5, 15, 43).

When taking into account detection methods, circumstantial evidence suggests that NOS-3 is expressed in OLG in normal brain. NADPH-d activity was found in fish OLG after light fixation with 1% PAF, and revealed characteristic staining of rings around axons reminiscent of myelin sheaths (83). Similar ring-like structures were found adjacent to neurons by NOS-3 immunodetection in the primate cortex lightly fixed with 2%–4% PAF (98). As might be predicted in brain strongly fixed with 4% PAF, NADPH-d activity was detectable in rat OLG only by electron microscopy without NOS-3 immunodetection (56). Additional circumstantial evidence for NOS-3 expression in OLG is suggested by the detection of its mRNA by *in situ* hybridization in rodent white matter (29). However, the signal was so low that it was ascribed to background despite the absence of this faint staining in NOS-3 null mice. Furthermore, Schwann cells, the myelinating homologue of OLG in peripheral nervous system, express NOS-3 protein *in situ* as shown by immunohistochemistry (60). Until additional studies are done using OLG markers to detect NOS-3 mRNA or protein under optimal conditions, the question whether OLG express NOS-3 in normal brain remains unanswered.

NOS-3 expression in primary culture. Despite the lack of clear evidence for NOS-3 in OLG in brain, our preliminary studies show that NOS-3 is expressed in primary cultures of OLG (13). Using immunodetection with polyclonal and monoclonal antibodies, we reproducibly found strong expression of NOS-3 in enriched OLG preparations from epilepsy patients that was absent in contaminating astrocytes and microglia (Fig. 1). NOS-3 was present at all stages of maturation from bipolar to membrane-bearing OLG expressing myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG), suggesting it is expressed early in the lineage. To assess the possibility of a pathological expression due to the epileptic source of cells, or a species-specific expression, NOS-3 was investigated in OLG isolated from normal adult rat brain where it was immunodetected in soma and thick processes. Functional evidence for NOS-3 was demonstrated in cultured adult baboon OLG using the general NOS inhibitor *N*-methylamidino-L-ornithine (L-NMMA). After one week incubation with low dose L-NMMA (4 μ*M*, close to the Ki of purified human NOS-3), marked changes in arborization occurred in OLG (Fig. 1). L-NMMA does not inhibit NOS-3 specifically, and inhibits NOS-1 with a similar efficacy. However, NOS-1 was not found in cultured human OLG (unpublished observations), suggesting the arborization change was related to a decrease in NOS-3 activity.

The lack of NOS-3 detection in OLG *in situ* can be reconciled with the strong expression in culture if NOS-3 expression is too low to be detected by classic immunohistochemistry using resin-embedded or strongly fixed sections. This does not rule out a possible increased transient expression of NOS-3 during brain repair, since cultured OLG isolated from adult brain reenact the myelination program and are a model for remyelination. NOS-3 does not appear to play a major role

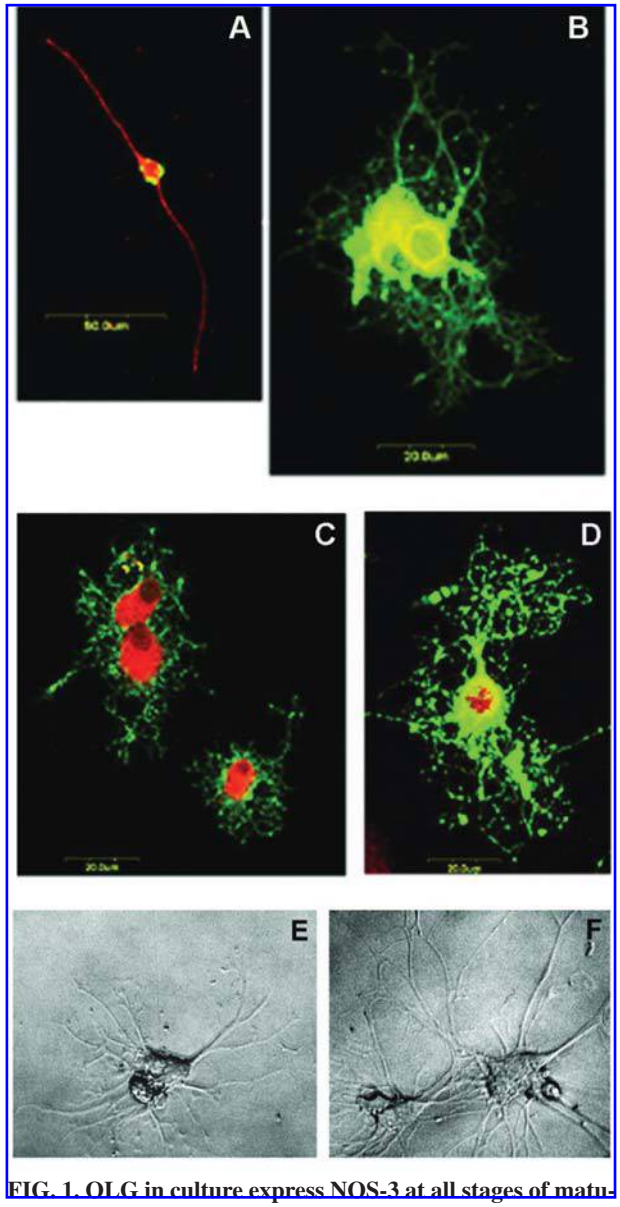

FIG. 1. OLG in culture express NOS-3 at all stages of maturation from bipolar to sheet-bearing OLG (A–D). OLG cultures were prepared from adult brain and maintained in serum-free medium. A human bipolar OLG expresses NOS-3 (*red*) but not MBP (*green*) (**A**). Human mature OLG express both NOS-3 (*red*) and MBP (*green*) with overlay in *yellow.* (**B**). NOS-3 (*red*) expression is not restricted to humans and is present in human (**C**) and rat (**D**) mature OLG expressing MOG (*green*). (**D**) is courtesy of Dr. Dello-Russo (University of Illinois at Chicago). Note that the presence of NOS-3 is restricted to cytoplasm while MBP and MOG are present on the entire cell surface as best illustrated by a single confocal scan (**C**). All other panels (**A**, **B**, and **D**) show merged stacks of confocal imaging scans. NOS-3 function was assessed in maturing baboon OLG bearing ramified processes as shown by phase (**E**). A week after a single addition of the NOS-3 inhibitor L-NMMA, long-lasting changes in arborization were observed, including flattened cell bodies, and thinner, less ramified processes (**F**).

during development as attested by the normal brain of NOS-3 null mice on gross examination (88). Only subtle differences were found in NOS-3 null mice when compared to the wild type, such as modulation of neurotransmitter release and reduced neurogenesis (50, 86). All studies of NOS-3 null mice focused on neuronal functions and none addressed glial functions of brain repair and remyelination. Whether NOS-3 expression *in vitro* is an artifact of culture conditions or represents upregulation that might occur during remyelination deserves further investigation. NOS-3 was present in cultured OLG across species and across maturation stages from bipolar progenitors to mature membrane-bearing cells, and may have, at least *in vitro,* a function in membrane extension.

Inducible nitric oxide synthase

A series of publications has reported expression of inducible NOS (iNOS or NOS-2) in cultured OLG (69, 71, 75), while one report convincingly conveyed negative results (46). The controversy is fueled by the classification of NOS-2 as a hallmark of inflammation, induced by inflammatory cytokines to provide a high germicidal NO output. NOS-2 expression in OLG contradicts the dogma that they are passive targets of inflammation. In addition, NOS-2 activity in a cell highly susceptible to oxidative damage is puzzling, as OLG are likely to suffer greatly from high levels of NO. However, the same can be said of neurons, clearly shown to express NOS-2 under pathological conditions such as Alzheimer's disease (45).

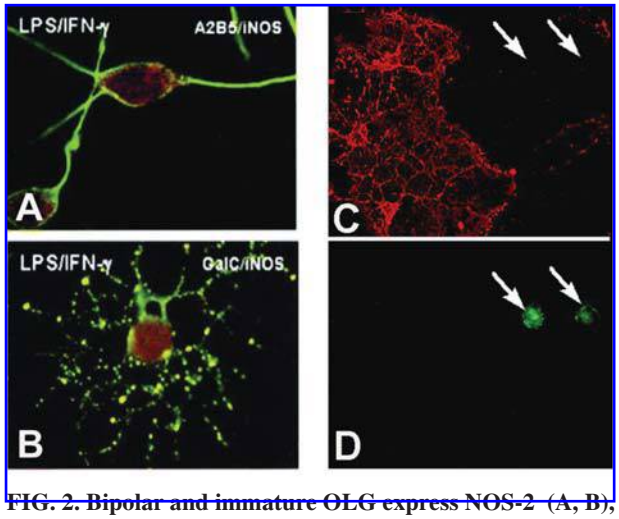

FIG. 2. Bipolar and immature OLG express NOS-2 (A, B), while mature sheet-bearing OLG do not (C, D). OLG cultures were prepared from neonatal rat brain, then treated with LPS plus IFNγ for 24 h and co-labeled with antibodies to NOS-2 (*red*) and A2B5 (*green*) (**A**) or galactocerebroside (*green*) (**B**), from Molina–Holgado *et al.* (75) with permission. The same OLG culture model (control not depleted of microglia) was treated with LPS and IFNγ for 48 h and stained for O1 (galactocerebroside) (**C**) and NOS-2 (**D**). Panels **C** and **D** are from the same microscopic field. *Arrowheads* indicate two O1-negative, NOS-2-positive cells, from Hewett *et al.* (46) with permission. Note the immature multipolar OLG (**B**) when compared to mature sheet-bearing OLG (**C**).

Evidence for and against NOS-2 expression in oligodendrocytes. *In vitro*, the combined inflammatory cytokines tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) elicited a strong NOS-2 expression in the rat OLG progenitor cell line CG4 as revealed by RT–PCR and Western blot analysis (9). NOS-2 was active and produced NO, as measured by colorimetric Griess reaction. A number of reports have shown NOS-2 induction in primary OLG culture with similar stimuli. The first report showed NOS-2 expression after 2 days incubation with lipopolysaccharide (LPS) plus IFNγ or IFNγ plus interleukin-1 beta (IL-1β), based on several methods, including mRNA analysis, immunochemistry, enzymatic assay, and NO production (71). A majority of OLG were found to express NOS-2, and staining for 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) revealed branched processes devoid of large membrane extensions, suggesting that the NOS-2 expressing OLG had not reached full maturation. Another report showed NOS-2 expression after 24 h incubation with the same stimuli of LPS and IFNγ in most OLG progenitors at the A2B5+ bipolar stage, as well as in OLG deemed mature, that is, positive for galactocerebroside (GalC) (75). However, the image illustrating a "mature" OLG showed an immature OLG decorated with GalC that expressed NOS-2 (Fig. 2). NOS-2 expression was also induced by a completely different stimulus, the ionotropic glutamate receptor agonist kainate, after a short 2-day culture period that generated differentiating OLG of bipolar and multipolar phenotypes (69). More recently, NOS-2 expression was induced with the combination of cytokines IFNγ, TNFα, and LPS, or with bilirubin alone, and was detected by RT–PCR, Western blot analysis, and NO production (31, 39). However, these two reports lacked detailed characterization of the OLG cultures and did not show NOS-2 immunostaining nor their stage of maturity. These cultures were enriched with 95% OLG expressing GalC, raising the concern that the 5% remaining cells, primarily microglia and astrocytes, were also induced to express NOS-2 in these conditions. In an elegant study, Hewett *et al.* (46) concluded that NOS-2 expression was due to microglial expression in enriched OLG cultures by selectively killing residual microglia, then subsequently treating with LPS and IFNγ. The cultures depleted of microglia showed fully mature OLG bearing large membrane extensions that were negative for NOS-2 in contrast to nondepleted cultures with microglia that expressed NOS-2 (Fig. 2). However, even after microglia were removed, some residual NO production remained upon stimulation, suggesting that some cells in the culture, possibly OLG progenitors, were still able to express NOS-2.

In situ, NOS-2 has never been reported in OLG in normal adult brain, as might be expected in the absence of high levels of inflammatory cytokines. In the rare pathological situation of pure OLG tumors, NOS-2 was immunodetected in more than half of the oligodendrogliomas (14), consistent with NOS-2 expression in immature OLG. In traumatic brain injury with increased levels of inflammatory cytokines, NOS-2 has occasionally been immunodetected in OLG (36). In multiple sclerosis, NOS-2 has never been detected in OLG, despite an abundance of IFNγ and TNFα in lesions, but it should be noted that multiple sclerosis lesions are characterized by a paucity of premyelinating OLG (22), rendering any NOS-2 detection unlikely.

Conclusion. The fact that all the publications used the same cell culture system from neonatal rat brain facilitates their comparison. Strikingly, a common characteristic in the reports showing NOS-2 expression by immunochemistry was the early stage of maturity, from bipolar progenitors to immature/young OLG devoid of large membrane extensions. In contrast, when OLG were fully mature and harbored membrane sheets, the so-called "mossy type," they failed to express NOS-2 with the same stimuli of LPS and IFNγ. That suggests NOS-2 expression is inducible only in progenitors and maturing OLG, but is inhibited in membrane sheet-bearing OLG. It was clearly documented in two studies that cultures elicited to promote NOS-2 expression were less mature than control cultures (69, 75). It is possible that the inducing conditions for NOS-2 stress cells and hamper their progress into maturation, resulting in a younger phenotype that permits NOS-2 expression. Inhibition of NOS-2 induction in mature OLG is possibly a protective mechanism to prevent the production of large amounts of NO that could otherwise disrupt myelin (see paragraph on mechanisms of NO toxicity). NOS-2 expression, as occurs following cytokine exposure in younger OLG, is not likely to be involved in maturation and the production of myelin. In support of this, NOS-2 null mice have apparently normal brains (67). Interestingly, nonmyelinating Schwann cells also express NOS-2 and produce NO following activation with the same cytokines TNFα and IFNγ (42), providing another analogy between the two myelinating cell types, and further supporting the conclusion that immature OLG can express NOS-2.

NO TOXICITY: DOSES AND MECHANISMS

NO concentration in vivo *versus* in vitro

NO is a gas that diffuses freely across cells, but as a free radical it reacts very quickly with other molecules. NO is labile and therefore relatively hard to quantify. *In vivo*, it can be monitored directly by a microelectrode sensor. However, since calibration to standard concentrations of NO is difficult, measurements are often given as percentage of the control baseline rather than actual concentrations. A rare report of direct NO concentration detected by microsensor in ischemic brain areas indicated an increase from less than 10 nanomolar to the micromolar range within 20 min of artery occlusion that began to decline upon substrate depletion (68). Another method to quantify NO in tissue is to trap the gas in a stable complex with iron that is then detected by electron paramagnetic resonance (EPR) spectroscopy. In normal rat brain, NO was detected in the nanomolar range with 0.9–1.5 nmol/g (28–46 ng/g) tissue by EPR, confirming the microsensor values (89). In a rat model of epilepsy where NOS-1 is activated, NO was again measured at less than 1 nmol/g in normal brain, and increased up to 10–15 nmol/g during tonic convulsions (51, 54). In pathological situations where high NO output NOS-2 is induced, such as septic shock in rat, the NO level in brain was found to be 5 nmol/g (150 ng/g) after 4 h, and 8–17 nmol/g after 6 h (33, 34, 89). During prolonged inflammation in rat brain such as occurs in experimental allergic encephalomyelitis (EAE) and rabies virus infection, NO was found to be 20- to 30-fold higher (20–30 μ*M*) than the 1 μ*M* levels in normal brain and spinal cord (48). However, this study contradicts other NO values in being an order of magnitude higher than the 1 n*M* levels usually found in normal brain by microsensor and EPR.

In cerebrospinal fluid (CSF), devoid of hemoglobin that converts nitrite into nitrate, nitrite is a reliable indicator of NO production. An increase in nitrite level in CSF is considered an indicator of intrathecal NO production and a sign of brain inflammation. Nitrite level in normal human CSF is in the micromolar range, from 0.4 to 2.0 μ*M* (17, 26, 93). In normal rat brain, nitrite and nitrate levels are within the same range as in human CSF and average 2–4.5 μg/g tissue (23, 89). In pathological conditions such as a relapse in multiple sclerosis, nitrite CSF level can reach 9 μ*M* (26). *In vitro*, the method of choice to quantify NO is to measure nitrite and nitrate levels in culture supernatant. Rodent macrophages and microglia are able to synthesize micromolar concentrations of NO over a 24-h period, resulting in nitrite concentrations close to 30 μ*M* (28). Human fetal astrocytes in culture accumulate up to 70 μ*M* nitrite after 7 days (16). Overall nitrite values are an order of magnitude higher than direct NO measurements due to several factors such as other sources (e.g., dietary intake), and accumulation of NO over time, further enhanced *in vitro* by reduced degradation.

In summary, physiological concentrations in brain under normal conditions are around 1 n*M* NO and 1 μ*M* nitrite. During pathological conditions, NO level can reach up to 1 μ*M* while nitrite levels are around 100 μ*M*. Another consideration to take into account is the inactivation of NO by interaction with tissue. For example, in an *ex vivo* study of optic nerves from rats with EAE, analysis of endogenous NO levels, as monitored by cGMP accumulation from guanyl cyclase activation, indicated the ambient NO concentration was only around 1 n*M* (37), considerably lower than the toxic 1 μ*M* levels generated in glial cultures treated with cytokines. Taking into account that NO is inactivated by tissue and that NO concentrations are probably higher at the site of production, it is likely that physiological concentrations reach up to 1 μ*M* NO and 100 μ*M* nitrite. Therefore, studies using NO donors should not go too far above this concentration range (see section on sensitivity to NO).

NO sensitivity of oligodendrocytes compared to other brain cells

In vitro *studies.* Comparisons of sensitivity to NO of various cell types in brain are complicated by uncertainties with accurate measurement of NO in culture or *in situ*, as discussed above. Further, both NO and peroxynitrite can damage cells, so the contribution of each species is often unknown. In culture, the type of NO donor, the specific culture conditions and the presence of other cells in the culture can affect NO toxicity as well. Finally, very few studies have examined simultaneously both glia and neurons in the same culture for their sensitivity to NO under identical conditions.

However, comparisons of results from separate cultures or individual studies show that developing rat OLG and mature mouse OLG are as sensitive to NO as neurons, whereas astroglia are considerably more resistant to NO. Studies using

neuronal cultures point to a high vulnerability to NO. In one study, rat hippocampal neurons and B104 neuroblastoma cells in culture showed 28% and 19% cell death, respectively, over 48 h at a concentration of 10 μ*M* nitrite as measured by the Griess assay, whereas 4 μ*M* nitrite was not toxic (49). In that study, NO was generated by astroglia treated with S100β, in co-cultures with the neurons or the B104 cells. Another study in organotypic hippocampal cultures used the slow releasing NO donor DETA-NO (NOC-18) and found that a steady concentration of 4.5 μ*M* NO released from 3 m*M* DETA-NO caused minimal cell death after 24 and 48 h. However, 3 m*M* NOC-12, a faster releasing NO donor producing a higher steady concentration of 10 μ*M* NO, resulted in ATP depletion and extensive cell death at 24 h (58). The authors measured the NO concentrations using an electrochemical probe (57).

In comparison to neurons, exposure of astroglia in culture to 1 m*M* DETA-NO resulted in about 8% cell death at 24 h, and about 50% at 48 h. NO concentrations were not measured directly, but can be estimated to be around 1 μ*M* (102). Astroglial sensitivity to NO at 48 h was more than for hippocampal neurons in organotypic culture that showed less than 10% cell death with 3 m*M* DETA-NO after 48 h (58), suggesting a protective mechanism in tissue slices during long-term incubation. In another study, using separate neuronal and astrocyte cultures, DETA-NO at 0.5 m*M* killed 95%–100% of neurons, but not astroglia (38), supporting further the greater vulnerability to NO of neurons in culture versus in organotypic slice, and showing without ambiguity that neurons are more sensitive to NO than astroglia. A recent study, using the fluorescent NO indicator diaminofluoroscein diacetate in mouse neocortical slices, demonstrated that acute neuronal death triggers rapid release of high levels of NOS-2-dependent NO from astrocytes (18).

The sensitivity to NO of developing rat OLG is comparable to that of neurons in culture with 50% OLG death at 24 h with 0.3 m*M* DETA-NO (estimated to be 0.3–0.6 μ*M* NO) (6), similar to 95% neuron death at 24 h with 0.5 m*M* DETA-NO. There was no survival of developing OLG with 1 m*M* DETA-NO (6), compared to only 8% death for astroglia at 1 m*M* DETA-NO (102), showing the much greater sensitivity of OLG than astroglia. In contrast, Baud *et al.* (6) found 50% cell death in MBP+ rat OLG at 2 m*M* DETA-NO after 24 h, but 50% death of developing OLGs at 0.3 m*M*, indicating that mature rat OLG are considerably more resistant to NO than developing OLG. Mature mouse OLG expressing GalC and MBP, in the presence of 15%–20% astroglia and less than 5% microglia, were killed by 150 μ*M* NOC-18 (DETA-NO, 0.15–0.3 μ*M* NO) at 64% and by 300 μ*M* NOC-9 at 80% by 18 h (12), suggesting that mature mouse OLG are as sensitive to NO as developing rat OLG. While 150 μ*M* NOC-12 (0.5 μ*M* NO) kills 60%–70% of differentiated murine OLG, 25 μ*M* NOC-12 (concentration estimated to be 80–85 n*M* NO, Ref. 101) kills only 10% of OLG, and in fact is protective against kainate toxicity (unpublished results). Table 1 summarizes these studies. The factors contributing to the differences in sensitivity of differentiated OLG in the rat system, and between rat and mouse for mature OLG are not known, but may be due to differences in the conditions for differentiating the OLG, to species differences (rat vs. mouse) or to the presence of ciliary neurotrophic factor and fewer astroglia (<5%) in the mature OLG cultures that were resistant to NO.

In vivo *studies.* The relative sensitivity of neurons, astroglia and OLG to NO and oxidative injury *in vivo* has been assessed primarily by histologic and immunocytochemical examination in numerous human diseases and animal models. Although a comprehensive summary of these *in vivo* studies is beyond the scope of this review, as in culture, NO and glutamate excitotoxicity have been implicated in death of neurons and OLG. For example, the role of astrocytes in ischemic neuronal death has been extensively examined (reviewed in Ref. 94). Premyelinating oligodendrocytes are extremely sensitive to both nitrosative and oxidative injury in periventricular leukomalacia in newborns (44). Recently, increasing attention has been focused on the roles of astroglia and microglia in

TABLE 1. COMPARISON OF SENSITIVITY TO NITRIC OXIDE BETWEEN NEURONS AND GLIAL CELLS

Cell type	*NO donor*	*NO concentration*	*Half life*	*Cell death*	*Reference*
Neurons, organotypic	3 m*M* DETA-NO	4.5 μ*M* NO	20 h	minimal at 24 h	58
Neurons, organotypic	3 m*M* NOC-12	10 μ*M* NO*	3 h	most at 24 h	58
Neurons	0.5 m*M* DETA-NO	0.9 μ*M***	20 h	95% at 24 h	38
Astroglia	0.5 m*M* DETA-NO	0.9 μ*M***	20 h	none	38
Astroglia	1 m*M* DETA-NO	1–2 μ*M* NO	20 h	8% at 24 h	101
Developing rat OLG	0.3 m*M* DETA-NO	0.3–0.6 μ*M* NO	20 h	50% at 24 h	6
Mature rat OLG	2 m*M* DETA-NO	2–4 μ*M* NO	20 h	50% at 24 h	6
Mature mouse OLG	0.15 m*M* DETA-NO	0.15–0.3 μ*M* NO	20 h	64% at 18 h	12
Mature mouse OLG	0.3 m*M* NOC-9	nd	3 min	81% at 18 h	12
Mature mouse OLG	0.15 m*M* NOC-12	0.5 μ*M* NO	3 h	60% at 18 h	Benjamins (unpub.)

*Measured at 5 min.
**Measured at 30 min, 4 h, and 24 h.
nd, not determined.
DETA-NO (also called NOC-18), NOC-12, and NOC-9 are NO donors of NONOate class.

damage to neurons and OLG in multiple sclerosis and in other inflammatory processes in white matter, as occurs in Alzheimer's disease (reviewed in Ref. 72).

NO toxicity in cell lines and primary cultures of oligodendrocytes

Proliferating rat OLG precursors after 24 h co-culture with microglia in separate permeable chambers showed 40% cytotoxicity in basal conditions with 10 μ*M* nitrite levels. Cell death increased to 70% when microglia were activated to produce up to 35 μ*M* nitrite, and reached 100% upon addition of L-arginine, which increased nitrite levels up to 55 μ*M* (70). Cytotoxicity was largely prevented by L-arginine analogues that inhibited NOS-2. This study launched several cytotoxicity studies using NO donors in OLG cell lines and in primary cultures. A comparative study of the three types of primary glial cultures isolated from the same rat brains showed that OLG were the most vulnerable to NO of all glial cells with 20% cell death at 18 h with 1 m*M* SNAP, but negligible death of astroglia and microglia (73). Doubling of SNAP (2 m*M*) killed 40% OLG, 20% astroglia, and less than 5% microglia. A subsequent study confirmed 48% and 52% cell death of primary rat OLG after 18 h with 1 m*M* SNAP and 2 m*M* SNAP, respectively, with cell death assessed by a different method (74). A study by another group corroborated these findings with half-maximal toxicity reached at 1.4 m*M* SNAP for mature MBP+ rat OLG (87). Under similar conditions, mouse OLG in primary culture achieved 50% cell death with 1.2 m*M* SNAP (200 μ*M* nitrite) and 74% cell death with 2 m*M* SNAP (300 μ*M* nitrite) (12), showing similar sensitivity to NO released from SNAP for mouse and rat OLG. Several mouse OLG cell lines under similar conditions with 1 m*M* SNAP after 18 h showed NO sensitivity with significant differences among them, from 5% to 50% cell death (66). These differences in sensitivity, however, did not correlate with maturation state. N19, the least mature cell line with bipolar morphology akin to progenitors, and N20.1, the most mature cell line, multipolar and expressing MBP mRNA, shared similar low sensitivity to 1 m*M* SNAP (5%–10% cell death). Another cell line, N1, with an intermediate maturity phenotype expressing GalC and CNPase, showed the highest 50% cell death with 1 m*M* SNAP, possibly because of expression of a different set of glutamate receptors not present in N19 and N20.1 cell lines. More recently, the lack of NO sensitivity for the N20.1 cell was confirmed, with 1 m*M* SNAP after an 18 h exposure (11). Half maximal cell death (50%) was reached by 5.5 m*M* SNAP (2.8 m*M* nitrite) for N20.1 cells, while 8 m*M* SNAP (4 m*M* nitrite) led to 60%–80% cell death. This suggests the N20.1 OLG cell line is five times more resistant to NO than OLG in primary culture. Comparison of these studies with others using a different class of NO donors (NONOates including DETA-NO and NOCs) is complicated by the fact that SNAP releases NO dependent on the composition of the medium on a mole to mole basis, while NONOates release NO spontaneously on a 1:2 molar basis with varying half-lives (20 h at 37°C for DETA-NO, compared to 1–6 h at 37°C for SNAP). This negates any direct comparison of SNAP and DETA-NO concentrations unless the actual level of released NO is monitored by a microsensor or by nitrite and nitrate levels.

Mechanisms of NO toxicity in oligodendrocytes

Role in glutamate toxicity. The interplay between glutamate receptor signaling and levels of NO has been well characterized in neurons, but less so in OLG. In neurons, NO can prevent or induce neuronal death depending on its concentration and the redox state of the cell (55, 59, 76). Neuroprotection can occur by *S*-nitrosylation and thus inactivation of NMDA receptors, caspases and p21 ras, or by upregulating protective genes such as Bcl-2 and HSP70. Activation by NO of the protective cGMP pathway via guanyl cyclase can lead to protein kinase G-dependent activation of PIP3-kinase and the antiapoptotic Akt serine/threonine kinase. Conversely, high levels of NO and peroxynitrite may kill neurons by indirect activation of caspases or by caspase-independent disruption of mitochondrial function (see section on mitochondrial toxicity). Like neurons, mature OLG are terminally differentiated cells, and also maintain extensive membrane structures, often at a distance from the cell body. However, several key differences between OLG and neurons indicate that neurotoxic or neuroprotective mechanisms operative for neurons cannot be directly applied to OLG. Thus, mature OLG do not express NOS-1, nor do they express NOS-2 when treated with inflammatory cytokines, at least in culture, in contrast to astroglia and microglia. Whether axonal contact alters expression of NOS-2 in mature OLG exposed to cytokines is not known.

OLG express ionotropic AMPA and kainate glutamate receptors. In one study with kainate receptors, three different NO donors did not modulate binding of kainate to its receptor in human brain tissue, suggesting that the kainate receptor is not inactivated by *S*-nitrosylation (62). This study, however, did not assess whether the kainate receptors were localized in gray or white matter. In sharp contrast with rodent cells, adult human OLG in culture were recently found resistant to excitotoxicity mediated through prolonged activation of AMPA and kainate receptors, probably due to low expression and little calcium permeability (99). If confirmed, this finding will have important implications for our understanding of glutamate cytotoxicity in human OLG. Until recently, OLG were reported to express few or no NMDA receptors. One earlier study found electrophysiological evidence for transient expression of atypical NMDA receptors in rat spinal cord (104). Recent reports have documented the presence of NMDA receptors in OLG at all stages of development, as well as in compact myelin (55a, 71a, 87a). These receptors showed low sensitivity to Mg^{2+} in contrast to neuronal NMDA receptors. NMDA antagonists blocked myelin damage due to oxygen/glucose deprivation or hypoxia/ischemia (71a, 87a). Whether the interaction of these atypical NMDA receptors and NO in OLG is similar to that described above for neurons has not been investigated.

In astrocytes, NO exposure upregulates glutathione synthesis, potentially protective (38), but induces rapid release of glutamate (2), inhibits glutamate uptake (100), and upregulates NOS-2. Conversely, glutamate, kainate, and AMPA all markedly increase cGMP levels in astroglia; this response requires NO synthase activity, and is dependent on extracellular calcium (3). Quiescent astroglia and microglia protect cultured neurons from glutamate toxicity; when these glia are activated with LPS, they produce high levels of NO, but do not kill the neurons unless high levels of glutamate are also

present (82). Whether OLG are more susceptible to glutamate toxicity in the presence of NO is not known.

Whether differentiating and mature OLG respond to NO and glutamate receptor activation in similar ways *in vivo* is not clear. In culture, kainate toxicity in immature OLGs is dependent on NOS-2 activity (69), similar to astroglia, as reviewed by Baltrons and Garcia (4). Elevated levels of cGMP or activation of protein kinase G protects immature and mature OLG and an immortalized OLG line from kainate toxicity, as do low levels of NO (87, 101). These results indicate that low levels of NO stimulate guanyl cyclase to increase cGMP levels. We have recently shown that cGMP and agonists of Group I metabotropic glutamate receptors act via protein kinase G to partially protect mature OLGs against killing by high levels of NO (7, 8). Metabotropic glutamate receptors are expressed in both rodent (64) and adult human OLG progenitors (65). Activation of Group I metabotropic glutamate receptors attenuates excitotoxicity by inhibiting the accumulation of reactive oxygen species and the loss of intracellular glutathione in OLG (30). Takahashi *et al.* (95) demonstrated that glutamate toxicity of cultured OLG initiated by IL-1β was dependent on the presence of both microglia and astroglia, and resulted from inhibition of glutamate uptake by astroglia. The role of NO was not investigated directly, but the inhibition of astroglial uptake of glutamate and the increased death of OLG via ionotropic glutamate receptors implicate NO as a mediator of these effects.

Of interest, white matter OLG express the glutamate transporters EAAT-1 and EAAT-2, and may be the predominant cells for glutamate clearance in white matter, as for astroglia in gray matter (84). Expression of the glutamate transporters were reduced in multiple sclerosis lesions and in cultured OLG exposed to the proinflammatory cytokine TNFα, suggesting that cytokine-induced downregulation of glutamate transport may also be mediated in OLG via NO-related mechanisms, as in astroglia. In summary, as in neurons, NO can act by protective pathways at low concentration, and by damaging pathways at high concentration to mediate the effects of glutamate toxicity in OLGs.

Effects of NO on mitochondria. Most studies of the effects of NO on mitochondria in OLG used exogenous NO from chemicals or from activated glial cells without considering the intrinsic capacity of OLG to synthesize endogenous NO. OLG do not express NOS-1, making it unlikely they express mitochondrial NOS, a splice variant of NOS-1 recognized by antibody to NOS-1 and absent in NOS-1 null mice (41). NOS-2, however, can be induced in immature OLG under various conditions, and could augment the provided exogenous NO. Likewise, OLG NOS-3 expression could contribute to endogenous NO.

There is overwhelming evidence that NO inhibits mitochondrial respiration in OLG. Incubation of 0.5 m*M* SNAP with mature rat OLG had a relatively low 5% cytotoxicity, but led to inhibition of mitochondrial activity, retraction of processes and cell clustering (73). Activity of the ferrosulfur enzyme succinate dehydrogenase, from complex II of the electron transport chain, was decreased by 30% from 12 to 18 h in mature rat OLG and by 45%–60% in several OLG cell lines (66). A higher dose of NO with 2 m*M* SNAP decreased by 20% the activity of the ferrosulfur enzyme after 6 h, and almost completely abolished its activity after 18 h in mature rat OLG (73). In contrast, the nonferrosulfur enzyme isocitrate dehydrogenase was unaffected by NO in mature rat OLG (73), showing that mitochondrial enzymes with a ferrosulfur center are specifically affected by NO. Other known inhibitory mechanisms of the electron transport chain include *S*-nitrosylation of the embedded proteins of complex I (24), and inactivation of cytochrome *c* oxidase by competitive binding of NO on the O_2-binding site (40). Additionally, peroxynitrite can be formed in a diffusion-controlled reaction in the presence of NO and superoxide ion leaking from the inhibited electron transport chain as illustrated in Figure 3. Peroxynitrite nitrosates tyrosine residues in proteins, induces the peroxidation of membrane lipids, releases cytochrome *c* and damages mitochondrial DNA (41). As proof of peroxynitrite formation, mitochondrial DNA became damaged subsequent to cytokine-induced NOS-2 in a pattern virtually identical to that elicited by addition of peroxynitrite to rat OLG cultures (31).

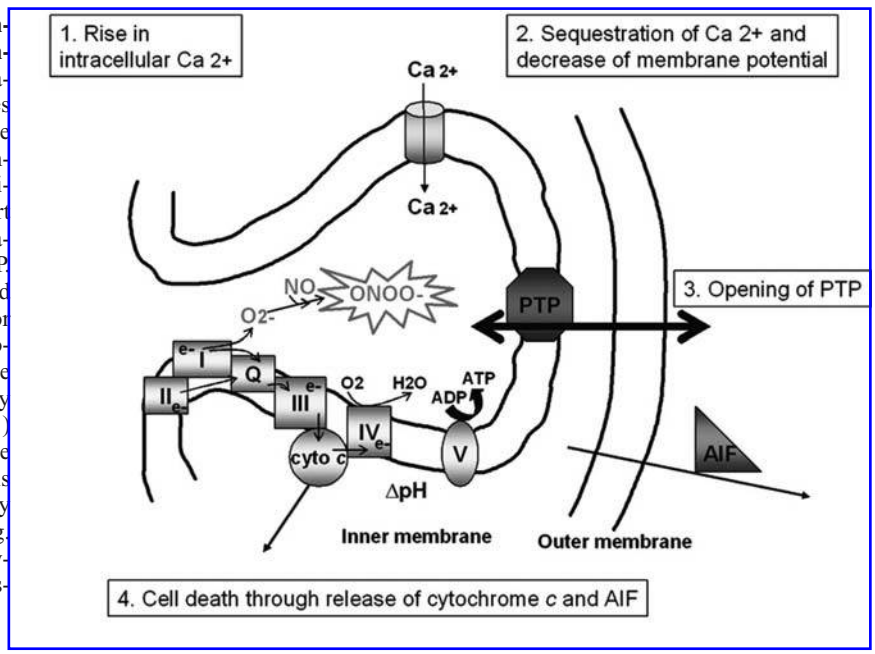

FIG. 3. A model of the possible sequential effects of NO on OLG mitochondria. 1. The first step is a rise in intracellular calcium (Ca^{2+}) within minutes following addition of NO donors. 2. The second step is a decrease in mitochondrial membrane potential (ΔpH) by direct inhibition of the electron transport chain by NO, combined with sequestration of calcium, further depleting ATP. Sequestration of calcium is performed by the mitochondrial uniporter, the major calcium entry pathway into the mitochondrial matrix. 3. The third step is the opening of the PTP further facilitated by formation of peroxynitrite ($ONOO^-$) from superoxide (O_2^-) and NO. 4. The fourth step is cell death by apoptosis with 10%–20% PTP opening, and by necrosis with at least 50% PTP opening. The opening of the PTP releases cytochrome *c* and translocates apoptosis-inducing factor (AIF) to the nucleus.

In contrast to mature OLG, developing rat OLG were 10-fold more sensitive to NO with half-cytotoxicity achieved by 200 μ*M* DETA-NO at 24 h (6). This differential vulnerability resulted from a compromised mitochondrial membrane potential as early as 4 h in developing OLG, accompanied by a decrease of 80% in ATP content after 12 h (6). In contrast, mature rat OLG did not show a decrease in mitochondrial membrane potential under the same conditions despite enduring a similar 80% decrease in ATP content. ATP could be consumed in mature OLG to maintain the mitochondrial membrane potential compromised by the inhibition of the electron transport chain. The transmembrane potential is the driving force to produce ATP and pump in relatively large quantities of calcium. Calcium influx was observed in mature mouse OLG within 2–9 min of SNAP incubation (12) as illustrated in Fig. 4. Calcium influx accounts for a significant part in NO toxicity as shown by a complete protection of calcium chelator EGTA against SNAP and DETA-NO (12). Calcium influx suggests the opening by NO of metabotropic calcium channels yet to be identified.

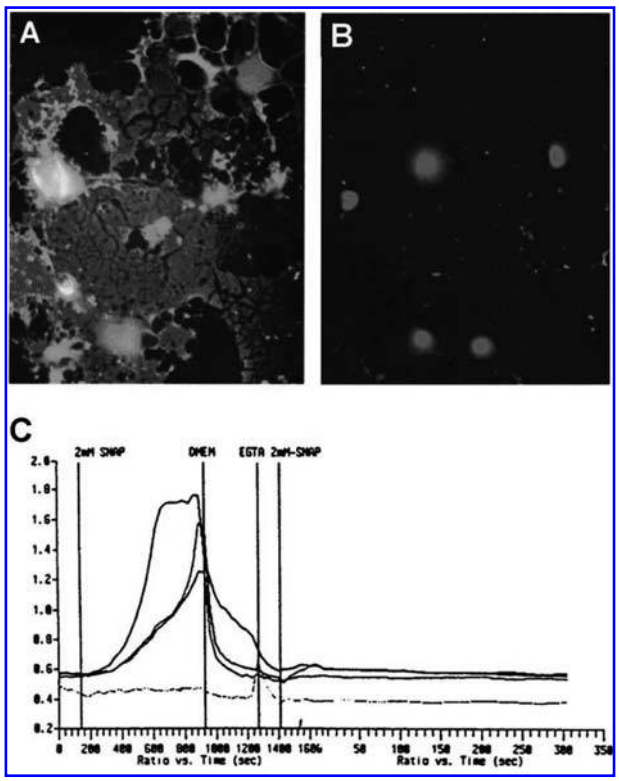

FIG. 4. High levels of exogenous NO initiate a calcium influx in OLG leading to cell death. OLG prepared from neonatal mouse brain show extensive membrane sheets stained by antibody A007 to sulfatide, and their normal nuclei are visualized by propidium iodide staining (**A**). Upon exposure to 2 m*M* SNAP, OLG die within 18 h, harboring shrunken nuclei and destroyed membranes (**B**). Intracellular calcium rise is an early event occurring in the majority of OLG within 2–9 min of SNAP addition, as shown by calcium imaging (**C**). Addition of EGTA to the medium prevented this rise, indicating an influx of intracellular calcium, from Boullerne *et al.* (12) with permission.

Surprisingly, despite an anticipated leakage of superoxide due to an almost certainly compromised electron transport chain, excess superoxide formation was not involved in NO toxicity in developing OLG, as demonstrated by the lack of protection from various superoxide scavengers and catalases, or overexpression of the cytosolic and mitochondrial superoxide dismutases (6). In agreement, neither reactive oxygen species nor peroxynitrite were produced under these conditions.

Complete inhibition of mitochondrial activity by 2 m*M* SNAP leads to death by necrosis for 52% mature rat OLG and 74% mouse OLG after 18 h (12, 74). Similarly, SNAP induced necrosis of the mouse OLG cell line N20.1, as shown by lack of DNA fragmentation and absence of protection by a pan-caspase inhibitor (11). In contrast to mature OLG, NO-induced cell death in developing OLG occurred through apoptosis, as shown by TUNEL staining, pyknotic nuclei and translocation of apoptosis-inducing factor from mitochondria to nuclei (6). Apoptosis in developing OLG is probably caused by the much lower dose of NO to induce cell death, allowing for a smaller degree of opening of permeability transition pores (PTP), as seen in Fig. 3.

The increased NO toxicity of developing O4+ OLG when compared to mature MBP+ OLG does not have an obvious explanation. Developing OLG are more challenged than mature OLG to maintain their mitochondrial transmembrane potential. It cannot be attributed to a difference in levels of the iron-binding proteins transferrin and ferritin, able to release iron leading to cytotoxicity with NO. Both transferrin and ferritin are expressed in O4+ OLG, with transferrin driving MBP expression, and are still present in mature MBP+ OLG (27, 80). Similarly, the basal level of glutathione is not significantly higher in MBP+ OLG compared to O4+ OLG (53) and only A2B5+ progenitors have much lower levels of glutathione (32). It is likely the mitochondrial glutathione pool becomes depleted in O4+ OLG progenitors following NO incubation, because depletion of mitochondrial glutathione leads to the opening of the PTP that precedes translocation of apoptosis-inducing factor from mitochondria to nuclei (21). In agreement with the role of glutathione *in vitro*, a significant decrease in reduced glutathione level paralleled by a significant increase in oxidized glutathione and *S*-nitrosothiol levels were found in CSF of multiple sclerosis patients when compared to controls (20).

In conclusion, the effect of NO to trigger cell death by apoptosis or necrosis in rodent OLG can be divided into four steps. The first step is a rise in intracellular calcium within minutes following incubation with NO donors (12) that is likely to be enhanced by glutamate. The second step is a decrease in mitochondrial membrane potential as early as 4–6 h, caused by direct inhibition of NO binding to the ferrosulfur enzymes of the electron transport chain (6, 66, 73). Inhibition of the electron transport chain by NO combined with calcium pumping by the channel uniporter exhausts the transmembrane potential over time. Moreover, calcium leakage from the mitochondrial matrix occurs through flickering of the PTP between open and closed states, as shown in OLG cultures in resting state and when the cytosolic calcium was raised by agonists (90). Sequestration of calcium by mitochondria to maintain a low calcium concentration in cyto-

plasm can last several hours as shown by the protection during the first 6 h of chelation with calcium EGTA (12). The third step occurs when the transmembrane potential is exhausted and leads to the opening of the PTP, further facilitated by a depleted glutathione pool and by formation of peroxynitrite. The fourth and last step is cell death occurring by apoptosis when the percentage of PTP opening is limited to 10%–20% and by necrosis when it reaches 50%. Opening of the PTP leads to release of cytochrome *c* and translocation of apoptosis-inducing factor from mitochondria to the nucleus.

Effects of NO on myelin. The effects of reactive oxygen and nitrogen species on the myelin membrane have been studied directly in isolated myelin fractions (97), or inferred by analyzing cellular and molecular changes occurring during inflammatory damage to myelin in multiple sclerosis or EAE (91). Increased formation of lipid and protein peroxides and the presence of nitrotyrosine in white matter, in both human and animal inflammatory demyelinating diseases, point to roles for oxidative and nitrative stress as mediators of damage to myelin.

In isolated myelin, peroxynitrite from the donor SIN-1, formed by the reaction of NO with superoxide, resulted in lipid peroxidation, as measured by formation of malondialdehyde after 1–2 h incubation (97). Neither NO nor superoxide alone had this effect. Further, the peroxidation was inhibited by superoxide dismutase but not catalase, confirming superoxide and not hydrogen peroxide as the source of the damaging agent peroxynitrite.

In vivo, malondialdehyde is increased in plaques and CSF of multiple sclerosis patients when compared to normal brain tissue and CSF (19, 77), supporting further the finding on purified myelin. Injection of the peroxynitrite donor SIN-1 into rat corpus callosum (white matter) led to severe myelin alteration as early as 2 days after injection, starting with myelin vacuolization, followed by demyelination along with immunodetection of nitrotyrosine, the footprint of peroxynitrite (96). Accordingly, nitrotyrosine staining has been consistently detected in acute lesions of multiple sclerosis (1, 25, 47, 63, 78).

In addition, NO by itself has an effect on another parameter, that of myelin compaction. Incubation of rat optic nerve with 1 m*M* SNAP for only 2 h, under similar conditions as for purified myelin, led to irreversible decompaction of myelin at the level of the intraperiod line (10). Alteration in myelin architecture could not be assigned to lipid peroxidation, since no malondialdehyde was formed, but to *S*-nitrosylation of proteins including proteolipid protein. Proteolipid protein is responsible for stabilization of the intraperiod line, representing compaction of the extracellular surfaces of myelin lamellae. Accordingly, peripheral myelin, which does not depend on proteolipid protein for stabilization, was not decompacted by NO. This suggests a direct and rapid action of NO on myelin architecture in the CNS.

In vivo and in culture, as discussed above, OLG and especially differentiating OLG are more sensitive than other glia to NO and to oxidative damage, resulting in selective OLG death and demyelination (91). This may be due to reduced capacity for defense against oxidative insults and mitochondrial damage, and the high iron content in OLG. As in isolated myelin, reactive species may lead to peroxidation and directly damage myelin *in vivo*, stimulating phagocytosis by macrophages. Similarly, activation of proteases and lipases may disrupt the stable structure of the myelin sheath. In the EAE model of inflammatory demyelination, several therapeutic strategies have been directed at the prevention of damage from reactive oxygen or nitrogen species. The effects of antioxidant treatment have been more successful than attempts to reduce reactive nitrogen species. In some cases, reduction of reactive nitrogen species has worsened disease, in agreement with the protective effects of low levels of NO under some conditions in glial and neuronal cultures.

FUTURE DIRECTIONS FOR UNDERSTANDING THE ROLE OF NO IN OLIGODENDROCYTES

Endothelial nitric oxide synthase

If confirmed *in vivo*, the expression of NOS-3 offers promising new venues of research in the biology of OLG as a signaling molecule possibly involved in the regulation of cytoskeletal structure. Potential regulation of NOS-3 activity with regards to increases in intracellular calcium levels should be explored. Pathways to excitotoxicity induced by glutamate through increased intracellular calcium may involve NOS-3. Other conditions leading to a rise in intracellular calcium, such as increased NO levels *in vivo* and incubation with NO donors *in vitro* should also be addressed with regards to NOS-3 activity. Pharmacologic regulation of calcium channels and the use of NOS-3 selective inhibitors may provide further information about the mechanisms leading to toxicity, and suggest possible strategies for protection. Models using chemical agents such as cuprizone or ethidium bromide to induce noninflammatory demyelination in wild-type versus NOS-3 null mice could provide important information about the role of NOS-3 in remyelination. Findings about NOS-3 activity in OLG primary cell cultures can be applied to NOS-3 null mice immunized to induce EAE to better understand neuroinflammatory demyelinating diseases such as multiple sclerosis.

Inducible nitric oxide synthase

Since NOS-2 has convincingly been shown to be expressed upon stimulation in premyelinating OLG by several independent laboratories, one must assume bipolar progenitors and young OLG are able to express NOS-2. One question is whether the results found in neonatal rodent cultures are applicable to adult human OLG. As an example of species differences, adult human microglia are notoriously difficult to induce for NOS-2 expression, while rodent microglia are readily induced. Another question concerns the regulation of NOS-2 induction; the mechanisms are largely unknown and appear to involve a variety of different signals, including the ionotropic kainate glutamate receptor and inflammatory cytokines, at the exclusion of LPS (62a), suggesting OLG are missing the receptors for innate immunity. In the rat OLG progenitor cell line CG4, p38 mitogen-activated protein ki-

nase is known to be involved in NOS-2 expression induced by cytokines (9). The problem of unavoidable contamination of OLG primary culture by astrocytes and microglia, also inducible for NOS-2 in similar conditions, needs to be solved, and the use of cell lines would provide a pure population of OLG, albeit at an immature stage. A recent study circumvented the problem of contaminating cells by deriving OLG from rat cortical neurospheres that were induced to differentiate for 48 h until expression of the early mature markers GalC and CNPase, hence achieving virtually pure primary cultures of OLG. NOS-2 expression was induced by TNF-α, and that induction was greatly potentiated by amyloid-β peptide (103). It is likely that after 48 h of differentiation, OLG were of immature/young phenotype with processes but no large membrane extension, as illustrated earlier (61). The signaling pathways involved neutral sphingomyelinase and ceramide. As in the other glial cell types, astrocytes and microglia (81, 92), the transcription factor NF-κB was involved in NOS-2 expression in OLG derived from neurospheres. Finally, an unknown inhibitory mechanism prevents NOS-2 expression in mature OLG with membrane extensions. Evidences for a similar mechanism *in vivo* are lacking due to the paucity of NOS-2 expression in OLG in brain. It would be informative to know this mechanism to help understand the maturation process and the production of myelin.

Glutamate and NO

The interactions between NO and glutamate receptors, both ionotropic and metabotropic, need to be assessed more comprehensively to gain insights into mechanisms of cytotoxicity. Metabotropic receptors have been found only recently in OLG, and their role with regard to NO need to be more fully characterized. Another contentious issue is the question of species difference between rodent and human cells. In sharp contrast to rodent cells, one study reports that adult human OLG weakly express AMPA and kainate receptors and are refractory to excitotoxicity (99), raising questions about the glutamate excitotoxicity model proposed for multiple sclerosis. Results in human cells need to be replicated and extended to the effects of glutamate and NO in the human nervous system.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Douglas Pfeiffer (Ohio State University Medical Center) for constructive discussions on mitochondria metabolism. This work was supported in part by NIH grant NS13143 (JB).

ABBREVIATIONS

A2B5, ganglioside present on OLG progenitor; CNPase, 2′,3′-cyclic nucleotide-3’-phosphodiesterase; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; EPR, electron paramagnetic resonance; GalC, galactocerebroside; IFNγ, interferon gamma; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; L-NMMA, *N*-methylamidino-L-ornithine; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; NADPH-d, NADPH-diaphorase activity; NO, nitric oxide; NOS-1, nitric oxide synthase type I; NOS-2, nitric oxide synthase type II; NOS-3, nitric oxide synthase type III; OLG, oligodendrocytes; PAF, paraformaldehyde; PTP, permeability transition pore; TNFα, tumor necrosis factor alpha.

REFERENCES

1. Bagasra O, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Fu ZF, Tawadros R, and Koprowski H. Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proc Natl Acad Sci USA* 92: 12041–12045, 1995.
2. Bal-Price A, Moneer Z, and Brown GC. Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. *Glia* 40: 312–323, 2002.
3. Baltrons MA and Garcia A. AMPA receptors are coupled to the nitric oxide/cyclic GMP pathway in cerebellar astroglial cells. *Eur J Neurosci* 9: 2497–2501, 1997.
4. Baltrons MA and Garcia A. The nitric oxide/cyclic GMP system in astroglial cells. *Prog Brain Res* 132: 325–337, 2001.
5. Barna M, Komatsu T, and Reiss CS. Activation of type III nitric oxide synthase in astrocytes following a neurotropic viral infection. *Virology* 223: 331–343, 1996.
6. Baud O, Li J, Zhang Y, Neve RL, Volpe JJ, and Rosenberg PA. Nitric oxide-induced cell death in developing oligodendrocytes is associated with mitochondrial dysfunction and apoptosis-inducing factor translocation. *Eur J Neurosci* 20: 1713–1726, 2004.
7. Benjamins JA and Nedelkoska L. Protection of mature oligodendrocytes: cross talk between cyclic GMP and growth factors. *J Neurochem* 85 (Suppl. 1): 37, 2003. (Abstract)
8. Benjamins JA and Nedelkoska N. Metabotropic glutamate receptors 1 and 5 activate protein kinase G to protect against kainate excitotoxicity in oligodendrocyte. *J Neurochem* 94 (Suppl.1): 107, 2005. (Abstract)
9. Bhat NR, Zhang P, and Bhat AN. Cytokine induction of inducible nitric oxide synthase in an oligodendrocyte cell line: role of p38 mitogen-activated protein kinase activation. *J Neurochem* 72: 472–478, 1999.
10. Bizzozero OA, DeJesus G, and Howard TA. Exposure of rat optic nerves to nitric oxide causes protein *S*-nitrosation and myelin decompaction. *Neurochem Res* 29: 1675–1685, 2004.
11. Boullerne AI, Nedelkoska L, and Benjamins JA. Synergism of nitric oxide and iron in killing the transformed murine oligodendrocyte cell line N20.1. *J Neurochem* 72: 1050–1060, 1999.
12. Boullerne AI, Nedelkoska L, and Benjamins JA. Role of calcium in nitric oxide-induced cytotoxicity: EGTA protects mouse oligodendrocytes. *J Neurosci Res* 63: 124–135, 2001.

13. Boullerne AI, Dello-Russo C, Frim D, Arnason BGW, Feinstein D. Adult primate oligodendrocytes in culture express NOS-3 and are functionally different in white matter and gray matter. XXVIIth International Symposium of Neuron–Glia Interactions, June 20–22, 2005. Montreal, Canada. (Abstract)
14. Broholm H, Braendstrup O, and Lauritzen M. Nitric oxide synthase expression of oligodendrogliomas. *Clin Neuropathol* 20: 233–238, 2001.
15. Broholm H, Andersen B, Wanscher B, Frederiksen JL, Rubin I, Pakkenberg B, Larsson HB, and Lauritzen M. Nitric oxide synthase expression and enzymatic activity in multiple sclerosis. *Acta Neurol Scand* 109: 261–269, 2004.
16. Brosnan CF, Battistini L, Raine CS, Dickson DW, Casadevall A, and Lee SC. Reactive nitrogen intermediates in human neuropathology: an overview. *Dev Neurosci* 16: 152–161, 1994.
17. Brundin L, Morcos E, Olsson T, Wiklund NP, and Andersson M. Increased intrathecal nitric oxide formation in multiple sclerosis; cerebrospinal fluid nitrite as activity marker. *Eur J Neurol* 6: 585–590, 1999.
18. Buskila Y, Farkash S, Hershfinkel M, and Amitai Y. Rapid and reactive nitric oxide production by astrocytes in mouse neocortical slices. *Glia* 52: 169–176, 2005.
19. Calabrese V, Raffaele R, Cosentino E, and Rizza V. Changes in cerebrospinal fluid levels of malondialdehyde and glutathione reductase activity in multiple sclerosis. *Int J Clin Pharmacol Res* 14: 119–123, 1994.
20. Calabrese V, Scapagnini G, Ravagna A, Bella R, Foresti R, Bates TE, Giuffrida Stella AM, and Pennisi G. Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and *S*-nitrosothiols and with changes in glutathione levels. *J Neurosci Res* 70: 580–587, 2002.
21. Cande C, Vahsen N, Metivier D, Tourriere H, Chebli K, Garrido C, Tazi J, and Kroemer G. Regulation of cytoplasmic stress granules by apoptosis-inducing factor. *J Cell Sci* 117: 4461–4468, 2004.
22. Chang A, Tourtellotte WW, Rudick R, and Trapp BD. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *N Engl J Med* 346: 165–173, 2002.
23. Clement P, Sarda N, Cespuglio R, and Gharib A. Changes occurring in cortical NO release and brain NO-synthases during a paradoxical sleep deprivation and subsequent recovery in the rat. *J Neurochem* 90: 848–856, 2004.
24. Clementi E, Brown GC, Feelisch M, and Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci USA* 95: 7631–7636, 1998.
25. Cross AH, Manning PT, Keeling RM, Schmidt RE, and Misko TP. Peroxynitrite formation within the central nervous system in active multiple sclerosis. *J Neuroimmunol* 88: 45–56, 1998.
26. Danilov AI, Andersson M, Bavand N, Wiklund NP, Olsson T, and Brundin L. Nitric oxide metabolite determinations reveal continuous inflammation in multiple sclerosis. *J Neuroimmunol* 136: 112–118, 2003.
27. De Los Monteros AE, Zhao PM, and De Vellis J. *In vitro* injury model for oligodendrocytes: development, injury, and recovery. *Microsc Res Tech* 52: 719–730, 2001.
28. Dello Russo C, Boullerne AI, Gavrilyuk V, and Feinstein DL. Inhibition of microglial inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1beta production. *J Neuroinflam* 1: 9, 2004.
29. Demas GE, Kriegsfeld LJ, Blackshaw S, Huang P, Gammie SC, Nelson RJ, and Snyder SH. Elimination of aggressive behavior in male mice lacking endothelial nitric oxide synthase. *J Neurosci* 19: RC30, 1999.
30. Deng W, Wang H, Rosenberg PA, Volpe JJ, and Jensen FE. Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. *Proc Natl Acad Sci USA* 101: 7751–7756, 2004.
31. Druzhyna NM, Musiyenko SI, Wilson GL, and LeDoux SP. Cytokines induce nitric oxide-mediated mtDNA damage and apoptosis in oligodendrocytes. Protective role of targeting 8-oxoguanine glycosylase to mitochondria. *J Biol Chem* 280: 21673–21679, 2005.
32. Fragoso G, Martinez-Bermudez AK, Liu HN, Khorchid A, Chemtob S, Mushynski WE, and Almazan G. Developmental differences in HO-induced oligodendrocyte cell death: role of glutathione, mitogen-activated protein kinases and caspase 3. *J Neurochem* 90: 392–404, 2004.
33. Fujii H, Wan X, Zhong J, Berliner LJ, and Yoshikawa K. In vivo imaging of spin-trapped nitric oxide in rats with septic shock: MRI spin trapping. *Magn Reson Med* 42: 235–239, 1999.
34. Fujii H and Berliner LJ. Ex vivo EPR detection of nitric oxide in brain tissue. *Magn Reson Med* 42: 599–602, 1999.
35. Gabbott PL and Bacon SJ. Localisation of NADPH diaphorase activity and NOS immunoreactivity in astroglia in normal adult rat brain. *Brain Res* 714: 135–144, 1996.
36. Gahm C, Holmin S, and Mathiesen T. Nitric oxide synthase expression after human brain contusion. *Neurosurgery* 50: 1319–1326, 2002.
37. Garthwaite G, Batchelor AM, Goodwin DA, Hewson AK, Leeming K, Ahmed Z, Cuzner ML, and Garthwaite J. Pathological implications of iNOS expression in central white matter: an *ex vivo* study of optic nerves from rats with experimental allergic encephalomyelitis. *Eur J Neurosci* 21: 2127–2135, 2005.
38. Gegg ME, Beltran B, Salas-Pino S, Bolanos JP, Clark JB, Moncada S, and Heales SJ. Differential effect of nitric oxide on glutathione metabolism and mitochondrial function in astrocytes and neurones: implications for neuroprotection/neurodegeneration? *J Neurochem* 86: 228–237, 2003.
39. Genc S, Genc K, Kumral A, Baskin H, and Ozkan H. Bilirubin is cytotoxic to rat oligodendrocytes *in vitro*. *Brain Res* 985: 135–141, 2003.
40. Ghafourifar P, Bringold U, Klein SD, and Richter C. Mitochondrial nitric oxide synthase, oxidative stress and apoptosis. *Biol Signals Recept* 10: 57–65, 2001.
41. Ghafourifar P and Cadenas E. Mitochondrial nitric oxide synthase. *Trends Pharmacol Sci* 26: 190–195, 2005.
42. Gold R, Zielasek J, Kiefer R, Toyka KV, and Hartung HP. Secretion of nitrite by Schwann cells and its effect on T-cell activation *in vitro*. *Cell Immunol* 168: 69–77, 1996.

43. Hashiguchi A, Yano S, Morioka M, Hamada J, Ushio Y, Takeuchi Y, and Fukunaga K. Up-regulation of endothelial nitric oxide synthase via phosphatidylinositol 3-kinase pathway contributes to ischemic tolerance in the CA1 subfield of gerbil hippocampus. *J Cereb Blood Flow Metab* 24: 271–279, 2004.
44. Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, and Kinney HC. Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. *J Neuropathol Exp Neurol* 62: 441–450, 2003.
45. Heneka MT and Feinstein DL. Expression and function of inducible nitric oxide synthase in neurons. *J Neuroimmunol* 114: 8–18, 2001.
46. Hewett JA, Hewett SJ, Winkler S, and Pfeiffer SE. Inducible nitric oxide synthase expression in cultures enriched for mature oligodendrocytes is due to microglia. *J Neurosci Res* 56: 189–198, 1999.
47. Hill KE, Zollinger LV, Watt HE, Carlson NG, and Rose JW. Inducible nitric oxide synthase in chronic active multiple sclerosis plaques: distribution, cellular expression and association with myelin damage. *J Neuroimmunol* 151: 171–179, 2004.
48. Hooper DC, Ohnishi ST, Kean R, Numagami Y, Dietzschold B, and Koprowski H. Local nitric oxide production in viral and autoimmune diseases of the central nervous system. *Proc Natl Acad Sci USA* 92: 5312–5316, 1995.
49. Hu J, Ferreira A, and Van Eldik LJ. S100beta induces neuronal cell death through nitric oxide release from astrocytes. *J Neurochem* 69: 2294–2301, 1997.
50. Huang PL. Neuronal and endothelial nitric oxide synthase gene knockout mice. *Braz J Med Biol Res* 32: 1353–1359, 1999.
51. Itoh K, Watanabe M, Yoshikawa K, Kanaho Y, Berliner LJ, and Fujii H. Magnetic resonance and biochemical studies during pentylenetetrazole-kindling development: the relationship between nitric oxide, neuronal nitric oxide synthase and seizures. *Neuroscience* 129: 757–766, 2004.
52. Iwase K, Miyanaka K, Shimizu A, Nagasaki A, Gotoh T, Mori M, and Takiguchi M. Induction of endothelial nitric-oxide synthase in rat brain astrocytes by systemic lipopolysaccharide treatment. *J Biol Chem* 275: 11929–11933, 2000.
53. Juurlink BH, Thorburne SK, and Hertz L. Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. *Glia* 22: 371–378, 1998.
54. Kaneko K, Itoh K, Berliner LJ, Miyasaka K, and Fujii H. Consequences of nitric oxide generation in epileptic-seizure rodent models as studied by in vivo EPR. *Magn Reson Med* 48: 1051–1056, 2002.
55. Kang YC, Kim PK, Choi BM, Chung HT, Ha KS, Kwon YG, and Kim YM. Regulation of programmed cell death in neuronal cells by nitric oxide. *In Vivo* 18: 367–376, 2004.
55a. Karadottir R, Cavelier P, Bergersen LH, and Attwell D. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature* 438: 1162–1166, 2005.
56. Keilhoff G, Seidel B, and Wolf G. Absence of nitric oxide synthase in rat oligodendrocytes: a light and electron microscopic study. *Acta Histochem* 100: 409–417, 1998.
57. Keynes RG, Griffiths C, and Garthwaite J. Superoxide-dependent consumption of nitric oxide in biological media may confound *in vitro* experiments. *Biochem J* 369: 399–406, 2003.
58. Keynes RG, Duport S, and Garthwaite J. Hippocampal neurons in organotypic slice culture are highly resistant to damage by endogenous and exogenous nitric oxide. *Eur J Neurosci* 19: 1163–1173, 2004.
59. Kim PK, Kwon YG, Chung HT, and Kim YM. Regulation of caspases by nitric oxide. *Ann NY Acad Sci* 962: 42–52, 2002.
60. Lee Y and Shin T. Expression of constitutive endothelial and inducible nitric oxide synthase in the sciatic nerve of Lewis rats with experimental autoimmune neuritis. *J Neuroimmunol* 126: 78–85, 2002.
61. Lee JT, Xu J, Lee JM, Ku G, Han X, Yang DI, Chen S, and Hsu CY. Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol* 164: 123–131, 2004.
62. Lees RD, Slater P, and D'Souza SW. Nitric oxide does not modulate kainate receptor binding in human brain. *Neurosci Lett* 233: 133–136, 1997.
62a. Li J, Band O, Vartanian T, Volpe JJ, and Rosenberg PA. Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc Natl Acad Sci USA* 102: 9936–9941, 2005.
63. Liu JS, Zhao ML, Brosnan CF, and Lee SC. Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. *Am J Pathol* 158: 2057–2066, 2001.
64. Luyt K, Varadi A, and Molnar E. Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. *J Neurochem* 84: 1452–1464, 2003.
65. Luyt K, Varadi A, Halfpenny CA, Scolding NJ, and Molnar E. Metabotropic glutamate receptors are expressed in adult human glial progenitor cells. *Biochem Biophys Res Commun* 319: 120–129, 2004.
66. Mackenzie-Graham AJ, Mitrovic B, Smoll A, and Merrill JE. Differential sensitivity to nitric oxide in immortalized, cloned murine oligodendrocyte cell lines. *Dev Neurosci* 16: 162–171, 1994.
67. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, Stevens K, Xie QW, Sokol K, Hutchinson N, et al. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* 81: 641–650, 1995.
68. Malinski T, Bailey F, Zhang ZG, and Chopp M. Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 13: 355–358, 1993.
69. Martinez–Palma L, Pehar M, Cassina P, Peluffo H, Castellanos R, Anesetti G, Beckman JS, and Barbeito L. Involvement of nitric oxide on kainate-induced toxicity in oligodendrocyte precursors. *Neurotox Res* 5: 399–406, 2003.
70. Merrill JE, Ignarro LJ, Sherman MP, Melinek J, and Lane TE. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *J Immunol* 151: 2132–2141, 1993.
71. Merrill JE, Murphy SP, Mitrovic B, Mackenzie–Graham A, Dopp JC, Ding M, Griscavage J, Ignarro LJ, and

Lowenstein CJ. Inducible nitric oxide synthase and nitric oxide production by oligodendrocytes. *J Neurosci Res* 48: 372–384, 1997.

71a. Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J, Yin X, Trapp BD, McRory JE, Rehak R, Zamponi GW, Wang W, and Stys PK. NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature* 439: 988–992, 2006.

72. Miller G. Neuroscience. The dark side of glia. *Science* 308: 778–781, 2005.

73. Mitrovic B, Ignarro LJ, Montestruque S, Smoll A, and Merrill JE. Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells *in vitro*. *Neuroscience* 61: 575–585, 1994.

74. Mitrovic B, Ignarro LJ, Vinters HV, Akers MA, Schmid I, Uittenbogaart C, and Merrill JE. Nitric oxide induces necrotic but not apoptotic cell death in oligodendrocytes. *Neuroscience* 65: 531–539, 1995.

75. Molina–Holgado E, Vela JM, Arevalo–Martin A, and Guaza C. LPS/IFN-gamma cytotoxicity in oligodendroglial cells: role of nitric oxide and protection by the anti-inflammatory cytokine IL-10. *Eur J Neurosci* 13: 493–502, 2001.

76. Nelson EJ, Connolly J, and McArthur P. Nitric oxide and *S*-nitrosylation: excitotoxic and cell signaling mechanism. *Biol Cell* 95: 3–8, 2003.

77. Newcombe J, Li H, and Cuzner ML. Low density lipoprotein uptake by macrophages in multiple sclerosis plaques: implications for pathogenesis. *Neuropathol Appl Neurobiol* 20: 152–162, 1994.

78. Oleszak EL, Zaczynska E, Bhattacharjee M, Butunoi C, Legido A, and Katsetos CD. Inducible nitric oxide synthase and nitrotyrosine are found in monocytes/macrophages and/or astrocytes in acute, but not in chronic, multiple sclerosis. *Clin Diag Lab Immunol* 5: 438–445, 1998.

79. Ortiz PA and Garvin JL. Trafficking and activation of eNOS in epithelial cells. *Acta Physiol Scand* 179: 107–114, 2003.

80. Paez PM, Garcia CI, Davio C, Campagnoni AT, Soto EF, and Pasquini JM. Apotransferrin promotes the differentiation of two oligodendroglial cell lines. *Glia* 46: 207–217, 2004.

81. Pahan K, Sheikh FG, Namboodiri AM, and Singh I. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. *J Clin Invest* 100: 2671–2679, 1997.

82. Perez–Capote K, Serratosa J, and Sola C. Glial activation modulates glutamate neurotoxicity in cerebellar granule cell cultures. *Glia* 45: 258–268, 2004.

83. Perez SE, Adrio F, Rodriguez MA, Rodriguez–Moldes I, and Anadon R. NADPH-diaphorase histochemistry reveals oligodendrocytes in the rainbow trout (teleosts). *Neurosci Lett* 205: 83–86, 1996.

84. Pitt D, Nagelmeier IE, Wilson HC, and Raine CS. Glutamate uptake by oligodendrocytes: Implications for excitotoxicity in multiple sclerosis. *Neurology* 61: 1113–1120, 2003.

85. Qi X and Guy J. Localization of NADPH diaphorase/nitric oxide synthase in the optic nerve of the normal guinea pig: a light and electron microscopic study. *J Comp Neurol* 370: 396–404, 1996.

86. Reif A, Schmitt A, Fritzen S, Chourbaji S, Bartsch C, Urani A, Wycislo M, Mossner R, Sommer C, Gass P, and Lesch KP. Differential effect of endothelial nitric oxide synthase (NOS-III) on the regulation of adult neurogenesis and behaviour. *Eur J Neurosci* 20: 885–895, 2004.

87. Rosenberg PA, Li Y, Ali S, Altiok N, Back SA, and Volpe JJ. Intracellular redox state determines whether nitric oxide is toxic or protective to rat oligodendrocytes in culture. *J Neurochem* 73: 476–484, 1999.

87a. Salter MG and Fern R. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. *Nature* 438: 1167–1171, 2005.

88. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, and Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 93: 13176–13181, 1996.

89. Sjakste N, Sjakste J, Boucher JL, Baumane L, Sjakste T, Dzintare M, Meirena D, Sharipova J, and Kalvinsh I. Putative role of nitric oxide synthase isoforms in the changes of nitric oxide concentration in rat brain cortex and cerebellum following sevoflurane and isoflurane anaesthesia. *Eur J Pharmacol* 513: 193–205, 2005.

90. Smaili SS and Russell JT. Permeability transition pore regulates both mitochondrial membrane potential and agonist-evoked Ca^{2+} signals in oligodendrocyte progenitors. *Cell Calcium* 26: 121–130, 1999.

91. Smith KJ, Kapoor R, and Felts PA. Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol* 9: 69–92, 1999.

92. Stasiolek M, Gavrilyuk V, Sharp A, Horvath P, Selmaj K, and Feinstein DL. Inhibitory and stimulatory effects of lactacystin on expression of nitric oxide synthase type 2 in brain glial cells. The role of Ikappa B-beta. *J Biol Chem* 275: 24847–24856, 2000.

93. Svenningsson A, Petersson AS, Andersen O, and Hansson GK. Nitric oxide metabolites in CSF of patients with MS are related to clinical disease course. *Neurology* 53: 1880–1882, 1999.

94. Swanson RA, Ying W, and Kauppinen TM. Astrocyte influences on ischemic neuronal death. *Curr Mol Med* 4: 193–205, 2004.

95. Takahashi JL, Giuliani F, Power C, Imai Y, and Yong VW. Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity. *Ann Neurol* 53: 588–595, 2003.

96. Touil T, Deloire–Grassin MS, Vital C, Petry KG, and Brochet B. *In vivo* damage of CNS myelin and axons induced by peroxynitrite. *Neuroreport* 12: 3637–3644, 2001.

97. Van der Veen RC and Roberts LJ. Contrasting roles for nitric oxide and peroxynitrite in the peroxidation of myelin lipids. *J Neuroimmunol* 95: 1–7, 1999.

98. Wiencken AE and Casagrande VA. Endothelial nitric oxide synthetase (eNOS) in astrocytes: another source of nitric oxide in neocortex. *Glia* 26: 280–290, 1999.

99. Wosik K, Ruffini F, Almazan G, Olivier A, Nalbantoglu J, and Antel JP. Resistance of human adult oligodendrocytes to AMPA/kainate receptor-mediated glutamate injury. *Brain* 127: 2636–2648, 2004.

100. Ye ZC and Sontheimer H. Glial glutamate transport as target for nitric oxide: consequences for neurotoxicity. *Prog Brain Res* 118: 241–251, 1998.
101. Yoshioka A, Yamaya Y, Saiki S, Kanemoto M, Hirose G, and Pleasure D. Cyclic GMP/cyclic GMP-dependent protein kinase system prevents excitotoxicity in an immortalized oligodendroglial cell line. *J Neurochem* 74: 633–640, 2000.
102. Yung HW, Bal-Price AK, Brown GC, and Tolkovsky AM. Nitric oxide-induced cell death of cerebrocortical murine astrocytes is mediated through p53- and Bax-dependent pathways. *J Neurochem* 89: 812–821, 2004.
103. Zeng C, Lee JT, Chen H, Chen S, Hsu CY, and Xu J. Amyloid-beta peptide enhances tumor necrosis factor-alpha-induced iNOS through neutral sphingomyelinase/ceramide pathway in oligodendrocytes. *J Neurochem* 94: 703–712, 2005.
104. Ziak D, Chvatal A, and Sykova E. Glutamate-, kainate- and NMDA-evoked membrane currents in identified glial cells in rat spinal cord slice. *Physiol Res* 47: 365–375, 1998.

Address reprint requests to:
Anne I. Boullerne, Ph.D.
Department of Anesthesiology
University of Illinois at Chicago
Jesse Brown VA Medical Center
820 South Damen Avenue, 6th Floor R&D office
Chicago, IL 60612

E-mail: abouller@uic.edu

Date of first submission to ARS Central, October 12, 2005; date of acceptance, December 2, 2005.

This article has been cited by:

1. S.-Y. Yao, C. Natarajan, S. Sriram. 2012. nNOS mediated mitochondrial injury in LPS stimulated oligodendrocytes. *Mitochondrion* . [CrossRef]
2. Subramaniam Sriram. 2011. Role of glial cells in innate immunity and their role in CNS demyelination. *Journal of Neuroimmunology* . [CrossRef]
3. George H. Vries, Anne I. Boullerne. 2010. Glial Cell Lines: An Overview. *Neurochemical Research* **35**:12, 1978-2000. [CrossRef]
4. S. Yao, P. Pandey, A. Ljunggren-Rose, S. Sriram. 2010. LPS mediated injury to oligodendrocytes is mediated by the activation of nNOS: Relevance to human demyelinating disease. *Nitric Oxide* **22**:3, 197-204. [CrossRef]
5. Jean E Merrill. 2009. In Vitro and In Vivo Pharmacological Models to Assess Demyelination and Remyelination. *Neuropsychopharmacology* **34**:1, 55-73. [CrossRef]
6. Takeshi Izawa, Shigeo Takenaka, Hideshi Ihara, Takao Kotani, Jyoji Yamate, Robin J.M. Franklin, Mitsuru Kuwamura. 2008. Cellular responses in the spinal cord during development of hypomyelination in the mv rat. *Brain Research* **1195**, 120-129. [CrossRef]
7. F. Florenzano, M.T. Viscomi, S. Amadio, N. D'Ambrosi, C. Volonté, M. Molinari. 2008. Do ATP and NO interact in the CNS?. *Progress in Neurobiology* **84**:1, 40-56. [CrossRef]
8. Joyce A. Benjamins, Liljana Nedelkoska. 2007. Cyclic GMP-Dependent Pathways Protect Differentiated Oligodendrocytes from Multiple Types of Injury. *Neurochemical Research* **32**:2, 321-329. [CrossRef]
9. Hari Shanker SharmaInteraction between amino acid neurotransmitters and opioid receptors in hyperthermia-induced brain pathology **162**, 295-317. [CrossRef]
10. Narayan R. Bhat , Douglas L. Feinstein . 2006. NO and Glial Cell Biology. *Antioxidants & Redox Signaling* **8**:5-6, 869-872. [Citation] [Full Text PDF] [Full Text PDF with Links]