Nitric Oxide Plays a Key Role in Myelination in the Developing Brain

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Abstract

Inhaled nitric oxide (iNO) is one of the most promising therapies used in neonates, but there is little information available about its effect on the developing brain. We explored the effects of both iNO and endogenous NO on developing white matter in rodents. Rat or mouse pups and their mothers were placed in a chamber containing 5 to 20 ppm of NO for 7 days after birth. Neonatal exposure to iNO was associated with a transient increase in central nervous system myelination in rats and C57BL/6 mice without any deleterious effects at low doses (5 ppm) or behavioral consequences in adulthood. Exposure to iNO was associated with a proliferative effect on immature oligodendrocytes and a subsequent promaturational effect. The role of endogenous NO in myelination was investigated in animals treated with the nitric oxides synthase inhibitor L-NAME in the neonatal period; this led to protracted myelination defects and subsequent behavioral deficits in adulthood. These effects were reversed by rescuing L-NAME–treated animals with iNO. Thus, we demonstrate considerable effect of both exogenous and endogenous NO on myelination in rodents. These data point to potential new avenues for neuroprotection in human perinatal brain damage.

Key Words: Brain damage, Development, Myelination, Nitric oxide, Prematurity, Repair.

INTRODUCTION

Brain injury in the premature infant is a problem of major importance, as recently emphasized by EPICure and EPIPAGE population studies (1, 2). Because of major advances in neonatal intensive care, nearly 85% to 90% of these infants now survive. Approximately 10% of the survivors later exhibit cerebral palsy, especially spastic diplegia, and an additional 25% to 50% exhibit cognitive, attentional, and/or behavioral deficits that result in failure to succeed in school. This has a major effect on the ethics of resuscitation of the most immature babies and on the economic and subsequent medical needs of handicapped infants and adults. The neurologic disabilities observed relate in considerable part to cerebral white matter injury, and preventing this injury requires an understanding of its pathogenesis. A combination of hypoxia-ischemia and maternal-fetal infection/inflammation is considered to play an important role in these processes (3). Nitric oxide (NO), which modulates vascular tone, reperfusion, inflammation, and oxidative stress, is a key component of these risk factors (4, 5).

Despite considerable advances in our understanding of the pathophysiology of brain damage during development, therapeutic options are still extremely limited. Developing new drugs for very preterm infants is currently a time-consuming and speculative strategy, with many regulatory constraints. Investigating the neurological effects of drugs that are already in common use may be a faster and more pragmatic option.

Although its use is controversial, inhaled NO (iNO) is one of the most commonly used therapies in neonatal intensive care units. Nitric oxide is thought to have only a local effect that is limited to pulmonary vascular tone and has been proposed for treatment of pulmonary hypertension-related hypoxemia and for preventing chronic lung disease. However, considerable clinical evidence suggests that iNO could also affect the developing central nervous system (CNS) (6, 7). In this study, we explored the neurological effects of iNO on the immature brain and demonstrate the major effects of exogenous (inhaled) and endogenous NO on myelination in rodents.

MATERIALS AND METHODS

Animals and Treatment Procedures

All experiments were carried out in compliance with the ethical rules of INSERM. Mother rats (Sprague-Dawley;
Janvier, Le Genest-St-Isle, France) or C57BL/6 mice (8) and their pups were placed in a normoxic, normocapnic gas chamber containing either 5 or 20 ppm of NO for postnatal days (P) 0 to 7. The concentrations of NO and NO₂ (lower than 1 ppm) were monitored using the iNOvent system (INOTHERapeutics, Clinton, NJ). To determine the specific effects of iNO on rat pups, the mothers were changed daily from exposed to unexposed experimental groups. Control and iNO-exposed animals were treated similarly in 2 identical Plexiglas chambers in the same room. After the exposure periods, rat pups were maintained in the same room under strictly similar conditions.

To investigate the effect of either endogenous NO production or exogenous iNO on the developing brain, we analyzed myelination in neonatal rats treated with the inhibitor of NO synthases (NOSs), N⁵(G)-nitro-L-arginine methyl ester (L-NAME; 15 mg/kg twice a day given intraperitoneally from P0 to P7).

Immunohistochemistry

We studied 6 to 10 pups in each experimental group in 3 separate experiments. Immunolabeling with the primary antibodies (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A141) was visualized using the streptavidin-biotin-peroxidase method as previously described (9). Antibodies NG2 and O4 (the latter was a generous gift from Dr P.A. Rosenberg, Boston, MA) were used as markers of immature and proliferating oligodendrocytes. Antibodies to adenosomatous polyposis coli (APC) and myelin basic protein (MBP) were used as mature and myelinating oligodendrocytes markers. Olig2 was used as a marker of all oligodendrocytes. To assess the specificity of Olig2 for oligodendrocyte lineage cells, double labeling using anti-glial fibrillary acidic protein (GFAP) and Olig2 was performed (Figure, Supplemental Digital Content 2, http://links.lww.com/NEN/A142). Cells positive for GFAP did not express Olig2, consistent with a recent report (10). For NG2 and O4 immunolabeling, rat pups were perfused transcardially with 4% paraformaldehyde in phosphate buffer (0.12 mol/L, pH 7.4). The brains were processed as previously described (9).

Quantitation of Immunoreactive Cells and Fibers

Immunoreactive cells were counted in subcortical white matter (+2.16 to −0.36 mm from the bregma) in animals killed on P3, P7, and P14. Cells were counted within a 0.25-mm² grid in at least 4 sections per animal (n = 6 or more per group).

The optical density (OD) of MBP+ fibers was measured in the cingulum in coronal sections because of the white matter thickness in this area. At least 4 sections each from 6 to 10 animals per group that were killed on either P10 or P14 were examined. Optical densities were measured at 100× magnification using a computerized image analysis system (ImageJ; NIH, MA, http://rsb.info.nih.gov/ij), as previously described (11). Nonspecific background density was measured at each brain level in an area devoid of MBP immunostaining and was subtracted from the values from the cingulum.

Western Blot

Membrane proteins were extracted from the forebrain cortex, including the white matter, from P14 rat pups. Extraction was achieved by homogenization in HEPES buffer containing protease inhibitors (Sigma, St Louis, MO), according to the manufacturer’s instructions. Protein samples (50 μg) were incubated overnight with antibodies either MBP (Santa Cruz Biotechnology, Santa Cruz, CA), platelet-derived growth factor-α (PDGFα; Santa Cruz Biotechnology), semaphorins 3A (Sema3A) and 3F (Sema3F; Abcam, Inc, Cambridge, MA) diluted 1:5000, or an anti-α-actin antibody (Santa Cruz Biotechnology) diluted 1:10,000. Western blot experiments were run in triplicate.

TUNEL Staining

In P7 and P21 animals, dying cells in the white matter were detected using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, as previously described (9). Stained cells were counted in 4 sections each from a single hemisphere of at least 6 animals per treatment group.

Ultrastructural Morphology of Cingulate White Matter

For electron microscopy, 100-μm-thick sagittal brain sections were cut on a vibratome, postfixed with 1% osmium tetroxide, dehydrated, and incubated in araldite. Silver-stained ultrathin sections of the cingulate white matter were made using an ultramicrotome (LKB, Bromma, Sweden), after cutting a semithin section to verify the level. Ultrathin sections were collected on copper grids. They were counterstained and examined using an electron microscope (LEO 912; Carl Zeiss, Le Pecq, France).

Quantitative Real-time Polymerase Chain Reaction

DNA-free total RNA from the brain cortex including white matter was obtained at P3, P7, P10, and P14 using a protocol adopted from Chomczynski and Sacchi (12). To standardize gene expression across samples, we first compared the expression levels of 4 housekeeping genes within the samples. For reverse transcription (RT), we used 600 ng of total RNA and the Iscript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Real-time polymerase chain reaction (PCR) was set up with Supermix (Bio-Rad) containing SYBR green dye in a final volume of 20 μL. The samples were run in triplicate. The applied primers for real-time PCR are listed in Table, Supplemental Digital Content 3, http://links.lww.com/NEN/A143.

Behavioral Assessments

Open Field and Habituation

An open field consisted of a 63 × 63-cm² arena divided into 49 equal-sized squares (9 × 9 cm²) constructed of opaque Plexiglas with 40-cm-high walls and a clear Plexiglas lid. On day 1, each rat was placed in the field facing a corner for 5 minutes. The behavior of the rat was recorded using an overhead digital camera. Behavioral measures extracted from the digital video recordings illustrated the number of times the rats crossed the square sections. To minimize the stress incurred by the presence of an object during subsequent object exploration tests, on day 2, each rat was returned to the same arena with a single object for 3 minutes.
Nonspatial and Spatial Object Recognition Tests

On day 3, each rat was returned to the same arena for a 3-minute exploration period, with 2 objects located at diagonally opposite corners. One hour later, each rat was returned to the arena for a 3-minute exploration period with a familiar and a novel object located in the same corners as during the initial exploration period. On day 4, each rat was returned to the same arena for a 3-minute exploration period with 2 objects located at diagonally opposite corners. One hour later, each rat was returned to the arena for a 3-minute exploration period with the same objects but with the position of 1 object changed to another corner. During each exploration period, the time spent exploring each object was measured. Each behavioral assessment test was run with a least 18 animals per group.

Statistical Analysis

All data were reported as mean ± SEM. Either 1- or 2-way analysis of variance was performed with age and groups as the factors, and the Newman-Keuls post hoc test was used. Statistical tests were run on GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA).

RESULTS

Inhaled NO Promotes Myelination in Neonatal Rodents

We first investigated the effect of iNO on serum concentrations of nitrates and nitrites, 2 of the main metabolites of NO. Inhaled NO induced a dose-dependent increase in serum concentrations of nitrates and nitrites (Figure, Supplemental Digital Content 4, http://links.lww.com/NEN/A144). We next determined whether iNO affected myelination under basal conditions. Exposure to both 5 and 20 ppm of iNO was associated with up to 40% increase in myelin formation in the developing white matter (Fig. 1A, B). Increases were observed at both P7 and P14, suggesting that the effect of iNO on myelination in normal rat pups continued after the treatment was stopped. It seemed to be transient, however, because no further differences were observed at day 21 (Fig. 1B). Upregulation of both MBP protein and gene transcription was detected just after iNO exposure at P14 (Figs. 1C, D). Inhaled NO was also associated with a transient increase in the density of mature (APC+) oligodendrocytes at P14 in the white matter (Figs. 1E, F). These results were not modified when mothers were changed daily from exposed to unexposed experimental groups, indicating that these results were directly related to effects of iNO on the rat pups. The ultrastructural features of white matter myelin were similar in iNO-exposed and controls (Figure, Supplemental Digital Content 5, http://links.lww.com/NEN/A145). We did not observe any differences within the cortical plate between iNO-exposed and control pups (n = 6 per group) using the neuronal markers RT-97 for axons and γ-aminobutyric acid (GABA) or calcium-binding protein for GABAergic neurons and interneurons (Figure, Supplemental Digital Content 6, http://links.lww.com/NEN/A146). Thus, in contrast to its effects on developing oligodendrocytes, iNO did not seem to induce changes in neuronal maturation.

To determine whether the effects on myelination were observed in other rodents, we also examined C57BL/6 mice at similar stages of brain development. The effects of iNO in a myelin proteolipid protein–green fluorescence protein mouse at P10 were comparable to those in P14 rat pups, that is, both proteolipid protein and MBP densities were increased in the lateral corpus callosum of the striatum in iNO-exposed mouse pups (Figs. 2A, B). The myelin fiber ODs were also increased in the white matter and basal ganglia (Fig. 2C).

These findings were sex-independent and were not related to the exposure of the dam to iNO. Exposure to iNO was not associated with any changes of the performances observed in 3 separate behavioral tests, including open field and object exploration tests performed in adult rats (data not shown).

Because 5 ppm of iNO was associated with myelination enhancement in both rat and mouse pups, all further experiments were performed using this low concentration, which is comparable to that used clinically in human neonates.

Inhaled NO Increases the Density of Proliferating Immature Oligodendrocytes in Neonatal Rats

To determine whether iNO acts through preoligodendrocyte proliferation or maturation, we first explored the effects of iNO on proliferating cells in white matter. Exposure to iNO was associated with a significant increase versus controls in the proportion of proliferating (Ki67+) cells (p < 0.05; Fig. 3A). Inhaled NO also induced a small but significant increase in the total number of Olig2+ oligodendrocytes (Fig. 3B). The density of immature oligodendrocytes was assessed using NG2 and O4 markers; it induced a significant increase of both NG2+ and O4+ oligodendrocytes (Figs. 3C, D). The distribution of O4+ and NG2+ cells seemed to be similar in treated and controls. The density of TUNEL+ Olig2+ double-positive cells in rat pups was also similar to that in controls throughout the hemispheric white matter (Fig. 3E). Similarly, TUNEL+ Olig2+ cell densities were not different in iNO-exposed and control groups at P21 (Figure, Supplemental Digital Content 7, http://links.lww.com/NEN/A147). These data strongly suggest that iNO acts first as an enhancer of oligodendrogial proliferation and that this is likely responsible, at least in part, for its effects on myelin content and APC+ cell density, that is, the newly formed oligodendrocytes induced by iNO did not seem to die.

Transcriptional Effects of iNO on Factors Involved in Oligodendrogial Lineage and Myelination

By quantitative RT-PCR analysis, iNO was associated with increased PDGFRα expression at P3 and P10 (Fig. 4A). Quantitative analysis of PDGFRα protein expression in rat pups using Western blot also demonstrated a significant increase in iNO-exposed compared with controls (Figure, Supplemental Digital Content 8, http://links.lww.com/NEN/A148). In contrast, Sox10 expression was not affected by iNO (Fig. 4B), and other transcription factors (i.e. Nkx2.2, CREB, c-jun) were not altered at any time point in animals exposed to iNO (data not shown).

Semaphorins 3A and 3F act as axonal guidance cues and chemotactic factors for oligodendroglial cells in the developing CNS. Semaphorin 3A usually exerts a repulsive
FIGURE 1. Inhaled NO enhances myelination in the developing rat brain. (A) Myelin basic protein+ oligodendrocytes in the lateral corpus callosum in coronal sections from control (Ctl) and rat pups exposed to 5 and 20 ppm of NO (iNO5 and iNO20) at P7. Boxes delineate the regions in which ODs were measured. Scale bar = 100 μm. (B) Optical density quantification of MBP staining in the lateral corpus callosum of iNO-exposed rat pups (normalized to the respective controls) at P7, P14, and P21. *, p < 0.05; **, p < 0.01; ***, p < 0.001; iNO-treated groups versus controls. (C) Western blot shows a dramatic increase of MBP synthesis in iNO-exposed rats at P14. (D) Reverse transcription–PCR shows modulation of MBP transcript levels relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene levels after iNO exposure at P14. *, p < 0.05; iNO-treated groups versus controls. (E) Photomicrographs showing mature APC+ oligodendrocytes in the lateral corpus callosum of control (Ctl) and NO-exposed rat pups (iNO5) at P14. Bar = 100 μm. (F) Quantification of APC+ oligodendrocytes in the lateral corpus callosum of control (Ctl) and NO-exposed rat pups (iNO5 and iNO20) at P7, P14, and P21. *, p < 0.05; ***, p < 0.001; iNO-treated groups versus controls.
FIGURE 2. Inhaled NO enhances myelination in the developing brain of C57BL/6 mice. (A) Optical densities of myelin proteolipid protein (PLP) green fluorescence protein (GFP) in the lateral corpus callosum and striatum of control and iNO-exposed C57BL/6 pups at P10. *, p < 0.05; iNO-treated groups versus controls. (B) Optical density of MBP in the lateral corpus callosum and striatum of control and iNO-exposed C57BL/6 pups at P10. *, p < 0.05; iNO-treated group versus controls. (C) Proteolipid protein (green) and MBP (red) double-immunoreactive oligodendrocytes in the lateral corpus callosum and striatum of control (Ctl) and iNO-exposed C57BL/6 mice (iNO) at P10. Bar = 100 μm.
effect, whereas Sema3F exerts an attractive effect on oligodendrocyte precursors during migration (8, 13). Exposure to iNO did not alter Sema3A transcription on either P7 or P14, whereas Sema3F transcription was upregulated (Figs. 4C, D). Western blot analyses confirmed that iNO exposure induced no change in Sema3A protein level, whereas it was associated with increased Sema3F protein content on P7 (Figure, Supplemental Digital Content 8, part B, http://links.lww.com/NEN/A148).

**FIGURE 3.** Inhaled NO promotes oligodendroglial cell proliferation in vivo. (A) Quantification of Ki67+ cells in the lateral corpus callosum of control (Ctl) and iNO-exposed rat pups at P7 and P14. **, p < 0.01. (B) Quantification of Olig2+ oligodendrocytes in the lateral corpus callosum of control and iNO-exposed rat pups at P7 and P14. *, p < 0.05. (C) NG2+ and O4+ oligodendrocytes in white matter from control and iNO-exposed rat pups at P7. Bar = 50 μm. (D) Quantification of NG2+ and O4+ cells in the lateral corpus callosum of control and iNO-exposed rat pups at P7. ***, p < 0.001. (E) Quantification of TUNEL+ Olig2+ oligodendrocytes in the hemispheric white matter from control and NO-exposed rat pups at P7.

**Inhaled NO Reverses the Myelination Impairment and Behavioral Deficits Induced by NOS Inhibition**

To determine whether endogenous NO has a role in normal brain myelination, we analyzed myelination in neonatal rats treated with the NOS inhibitor L-NAME. The concentration of nitrites and nitrates markedly decreased after L-NAME administration and was restored to normal levels by treatment with 5 ppm of iNO (Fig. 5A). L-NAME administration did not delay the initiation of myelin production in the lateral corpus callosum but significantly reduced myelin density at P14 in both the corpus callosum and striatum (Figs. 5B, C). In contrast, iNO administered to L-NAME-treated rat pups was associated with a complete restoration of normal myelination throughout the brain. The myelin impairment induced by the lack of endogenous NO production was transient. Adult rats subjected to L-NAME during the neonatal period had similar myelin content to that in controls (data not shown). However, behavioral assessments in adulthood demonstrated a dramatic effect of neonatal L-NAME treatment, that is, there was a significant deficit in reactions to novelty, either in the form of object replacement or in a new spatial location for a familiar
object (Fig. 5D). Conversely, L-NAME-treated rat pups rescued with iNO did not show any difference in performance in adult behavioral tasks in comparison to controls. Open field sessions did not reveal any significant differences between experimental groups. In contrast to its effect on myelination, L-NAME treatment did not alter calcium-binding protein+ interneuron density in the cortex (Figure, Supplemental Digital Content 9, part A, http://links.lww.com/NEN/A149). Similarly, GABA+ cells density was not altered by L-NAME in the cortex (data not shown). However, L-NAME did induce a small but significant decrease in GFAP+ astrocyte density in the developing white matter that was reversed by iNO (Figure, Supplemental Digital Content 9, part B, http://links.lww.com/NEN/A149).

**DISCUSSION**

We provide several lines of evidence that both exogenous (inhaled) NO and endogenous NO are involved in myelination of the developing rodent brain white matter. This effect, even if transient, seems to be affect cognitive behavior in adulthood. Our data show that NO may act as a regulator of some genes involved in the maturation of the oligodendroglial lineage and in CNS myelination.

It is well established that NO is a physiological mediator in the CNS. Within the brain parenchyma, NO is produced from arginine and molecular oxygen by NOS expressed by glial and endothelial cells. Despite some degree of specificity, each cell type is capable of expressing all NOS isoforms, indicating the crucial role of NO during brain development. Although the role of NO in the developing brain remains poorly understood, it seems to be involved in the regulation of cerebral blood flow and in memory acquisition. After brain injury, NOS activity may be upregulated, and NO may be produced in excess, such as in excessive stimulation of glutamate receptors or the transcriptional effect of cytokine release (14). In cases of ischemia-reperfusion, there is a marked increase in NO production during the phase of reperfusion and reoxygenation.
that is correlated with the intensity of the ischemic and hypoxic insult (15). Nitric oxide accumulation depends on the brain region, that is, it is particularly noticeable in the cortex, hippocampus, hypothalamus, amygdala, and substantia nigra (16). Lesions of the hippocampus induced by medial cerebral artery occlusion in the rat correlate with tissue NO concentrations

FIGURE 5. Endogenous NO production is needed for normal myelination and behavior in rats. (A) Quantification of nitrite and nitrate serum concentration in rat pups treated with L-NAME alone or with iNO exposure compared with control (Ctl) animals at P1, P7, and P14. *, p < 0.05; **, p < 0.01; ***, p < 0.001. (B) Optical density quantification of MBP immunoreactivity in the lateral corpus callosum and striatum of control and L-NAME-treated (alone or with iNO) rat pups at P7 and P14. *, p < 0.05; **, p < 0.01; ***, p < 0.001. (C) Myelin basic protein+ oligodendrocytes in the lateral corpus callosum and striatum in coronal sections from control and L-NAME-treated (alone or with iNO) rat pups at P14. Boxes delineate the areas in which OD was measured (B). Bar = 100 μm. (D) Quantification of 3 behavioral tasks (i.e., open field, exploration of new object, and exploration of moved object) in adult rats subjected to L-NAME, iNO, or L-NAME + iNO during the first week of life compared with controls (n = 18–24 for each group). When time spent in exploring either a new or a moved object is 50% or less, this indicates a total failure to recognize change in the object. **, p < 0.01; ***, p < 0.001.

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Nitric Oxide Promotes Myelination

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Numerous studies have demonstrated the deleterious effects of NO accumulation in brain ischemia-reperfusion (19, 20). Hypoxia-ischemia results in inflammation, especially in the developing white matter, and high NO concentrations and peroxynitrite produced locally by activated microglia may become toxic to neurons and immature oligodendrocytes in vitro (21). In contrast, NO also induces beneficial effects, probably as a function of the intracellular redox state (22). For example, an increase in brain infarct volume has been reported in sheep and rats when NO production is decreased by NOS inhibitors (23). The extent of the lesions after medial cerebral artery occlusion is reduced in endothelial cell NOS-deficient mice (24), whereas secondary neuronal damage induced by prolonged ischemia is lessened in inducible NOS knockout mice (25). The protection conferred on the ischemic brain by NO seems to be linked to vasodilation, which improves cerebral blood flow and hinders the formation of microthrombi in capillaries (26). Moreover, the nitration or nitrosylation by NO of iron- or thiol-containing enzymes may alter their biological actions by minimizing the generation of reactive oxygen species and associated oxidative stress (27–29). Several studies have demonstrated beneficial effects of NO in iron-related brain injury in vitro and in vivo (30). In addition, the less reactive NO radical scavenges highly reactive oxygen species and converts them into nonradicals such as nitrates and nitriles. Inhaled NO can scavenge thyl radicals, converting them into biologically active S-nitrosothiols, which have a longer half-life in vivo. Among them, the endogenous S-nitrosylated reduced glutathione (GSNO) is approximately a 100-times more potent than glutathione itself (31). Finally, NO inhibits brain lipid peroxidation both in vivo and in vitro (29, 30) through the scavenging of lipid peroxyl radicals and thiol radicals. Moreover, GSNO may be an endogenous NO reservoir that can release NO when it reacts with either Cu+ or thioredoxin (32–35). Freshly prepared GSNO not only produces NO-like biological effects but also protects against oxidative stress in the endothelium, myocardium, and brain tissue (30, 31, 36, 37). S-nitrosylated reduced glutathione is also capable of detoxifying peroxynitrite in vitro, which explains why peroxynitrite is a weak pro-oxidative agent in the brain in vivo and in vitro.

How iNO affects myelination and behavior in adulthood remains unclear. We found here that transient myelin changes observed with L-NAME were associated with long-term behavioral alterations. These results suggest that a transient delay in myelination during the early postnatal period could lead to long-term behavioral and cognitive consequences in adult. Because we did not identify abnormal neuronal differentiation in young and adult rats subjected to NOS inhibitors, we speculate that dysmyelination could be responsible for the behavioral impairment observed in treated animals. Indeed, it is remarkable that iNO was protective of myelination during the first 2 weeks of life associated with and subsequently improved cognitive tasks. However, there is little evidence in the literature to correlate behavioral assays to changes in myelination, and conclusions should therefore be made with caution, but animals with myelin dysregulation have been investigated using the same behavioral assays as used in the present study (38, 39). In these reports, reduction in myelination and dysmyelination led to marked deficiencies in memory and locomotor activity.

In addition to its effect on oligodendroglial cell proliferation and maturation, we demonstrated that expression of semaphorins might also play a role in this process. We found an imbalance between Sema3A and Sema3F (mostly due to an increase in Sema3F expression) associated with the transiently increased white matter myelin content in the iNO-exposed rat pups. Recently, Sema3F has been recognized to be not only an axonal guidance molecule but also an attractant for oligodendroglial precursors and subsequent myelinating oligodendrocytes (8). Moreover, oligodendroglial precursors expressed class 3 semaphorins receptors, that is, neuropilins (NRP1 and NRP2) and Plexin B3 (40–42). Involvement of neuropilins and semaphorins in orchestrating the migration patterns of developing oligodendrocytes may explain the recruitment of NG2/O4+ oligodendrocytes in the developing white matter we observed in response to iNO exposure. Although the distribution of these developing oligodendrocytes throughout the developing white matter seemed to be similar in iNO-exposed pups and controls, the question of the distribution of younger oligodendroglial progenitors could be altered in iNO-exposed pups remains unanswered. In addition to the Sema3F transcriptional regulation induced by iNO, a direct effect of the NO/cyclic guanosine monophosphate (cGMP) signaling pathway on class 3 semaphorins properties cannot be ruled out. Indeed, NO synthesis and the subsequent activation of guanylyl cyclase to produce cGMP induce the conversion of Sema3A repulsion to attraction (43).

Inhaled NO is currently a therapy used worldwide in sick full-term and preterm neonates. In addition to the well-known vasodilatory properties of NO, several recent experimental studies have suggested a possible role for the vascular endothelial growth factor/NO pathway in restoring pulmonary angiogenesis and enhancing distal lung growth (6). In contrast, the effect of iNO on CNS development remains controversial. For many years (because NO was known to increase bleeding time and inhibit platelet aggregation), it was feared that iNO would increase the incidence of intracranial hemorrhage in critically ill preterm neonates (44). Later, clinical studies demonstrated that there was no significant increase in intracranial bleeding in preterm neonates (45). More intriguing and exciting was the fact that iNO seemed to decrease the risk of cognitive impairment by 47% in exposed preterm infants observed at 2 years (7).

Recently, the pathogenesis of cerebral white matter injury in premature infants has been revisited (3). Cerebral white matter injury is characterized by the loss of premyelinating oligodendrocytes and is associated with a high risk of neurodevelopmental impairment. Here, we showed that iNO induces an increased proliferation of oligodendroglial progenitors, suggesting that iNO could be a candidate for preventing white matter injury. The unique cerebrovascular anatomy and physiology of the premature baby underlies the exquisite sensitivity of the white matter to the abnormal milieu of preterm extrauterine life and especially to ischemia-reperfusion promoted by impaired cerebral autoregulation and inflammation.
generation of reactive oxygen species may trigger excitotoxicity and the attack of premyelinating oligodendrocytes by free radicals because of the immaturity of antioxidant enzyme systems and iron accumulation. Again, our study raises the possibility that intracerebral NO concentration might be a mechanism of defense against oxidative stress injury in immature oligodendrocytes (22).

In conclusion, both endogenous and exogenous NO play a crucial role as an effector molecule in the myelination of the developing CNS, and iNO may have effects on the CNS as well as on the lungs in exposed patients. Our data also suggest that iNO could be a potential candidate for neuroprotection in perinatal white matter lesions.

REFERENCES