Nitric Oxide Production in Rat Dorsal Root Ganglia and Spinal Cord After Sciatic Nerve Lesion

María F. Coronel, Lic
María C. Defagot, Lic, PhD
Patricia L. Musolino, MD
Marcelo J. Villar, MD, PhD

ABSTRACT. Recent studies have analyzed the role of nitric oxide (NO) in pain modulation in several models of sciatic nerve injury. In the present study we have investigated NO production in lumbar dorsal root ganglia (DRG) and spinal cord (SC) over time after sciatic nerve cut. Neuronal nitric oxide synthase (nNOS)-like immunoreactivity (LI) was also determined, since the expression and activity of the enzyme do not always correlate. Nerve section induced a progressive increase in NO production in the ipsilateral L4-5 DRGs and the corresponding levels in the SC in a pattern that correlated with nNOS-LI; this increase was gradual after 7 days of survival time, more pronounced after 14 days, with the highest values detected 28 days after axotomy. This peak was followed by a progressive decrease, reaching control values 42 days after the lesion. The present study shows that nNOS upregulation is related to an increased NO production and release. The temporal pattern of NO production parallels the one observed for the expression of the enzyme, suggesting that the induction of nNOS synthesis yields a protein that is functional and highly active.

KEYWORDS. Primary afferent neurons, neuropathic pain, neuronal nitric oxide synthase, peripheral axotomy, sciatic nerve injury, enzyme activity, enzyme expression

María F. Coronel, Lic (E-mail: fcoronel@cas.austral.edu.ar); María C. Defagot, Lic, PhD (E-mail: cdefagot@cas.austral.edu.ar); Patricia L. Musolino, MD (E-mail: pmusloino@cas.austral.edu.ar); and Marcelo J. Villar, MD, PhD (E-mail: mvillar@cas.austral.edu.ar) are all affiliated with the Facultad de Ciencias Biomédicas, Universidad Austral, Av Presidente Perón 1500, B1629AHJ, Derqui, Buenos Aires, Argentina.

Address correspondence to: Dr. Marcelo J. Villar, Facultad de Ciencias Biomédicas-Universidad Austral, Av Presidente Perón 1500, B1629AHJ, Derqui, Buenos Aires, Argentina (E-mail: mvillar@cas.austral.edu.ar).

Supported by: Facultad de Ciencias Biomédicas-Universidad Austral.

Journal of Neuropathic Pain & Symptom Palliation, Vol. 1(2) 2005
Available online at http://www.haworthpress.com/web/JNPSP

doi:10.1300/J426v01n02_02

INTRODUCTION

Nitric oxide (NO) is a potent biological mediator that acts as an intercellular messenger in the nervous system (Dawson et al. 1992; Dawson et al. 1995) and plays a role in pain modulation (Meller et al. 1990; Meller et al. 1992). NO is synthesized from L-arginine by a family of enzymes called nitric oxide synthases (NOS) giving citrulline as a byproduct (Bredt et al. 1990b). There are three major classes of NOS: neuronal (nNOS), endothelial (eNOS) (both constitutive) and inducible (iNOS) (Alderton et al. 2001). These three isoforms are present, among other sites, in the central and peripheral nervous system (Bredt et al. 1990a; Egberongbe et al. 1994). Being a small reactive molecule, NO has a short half-life (in the order of milliseconds to seconds) and is rapidly oxidized to the inactive and stable end products nitrite (NO_2^-) and nitrate (NO_3^-) (Ignarro et al. 1993). *Ex vivo* concentrations of these nitrogen oxides (NOx) correlate with NOS activity and are used as a quantitative index of NO production (Salter et al. 1996).

Because NO is membrane permeable, it cannot be stored in synaptic vesicles and is produced on demand (Gally et al. 1990). NO may act in the neuron where it is produced, or diffuse from its site of production to act on adjacent glia and/or other neurons, as a "classical neurotransmitter" (Gally et al. 1990; Morris et al. 1992). In addition, it has been suggested to behave as a retrograde transmitter mediating synaptic plasticity and long-term potentiation (Baringa 1991).

Recent studies have analyzed the participation of NO in pain modulation in various models of sciatic nerve injury. Lesion induced NO production has been attributed to have a protective effect on damaged neurons (Thippeswamy et al. 1997a; Thippeswamy et al. 2001b) and might be associated with neuronal regeneration (Cristino et al. 2000) and anti-apoptotic pathways (Chung et al. 2001). However, it is also possibly involved in nNOS positive cell death (Zochodne et al. 1997) and pro-apoptotic mechanisms (Heneka et al. 1998). Supporting the role of NO in neuronal survival and nerve regeneration, it has been shown to be involved in path-finding and establishment of appropriate synaptic connections after nerve transection (Van Wangenen et al. 2001).

Previous histochemical studies have demonstrated that lumbar dorsal root ganglia (DRGs) contain only a few NADPH diaphorase/nNOS positive cell bodies, mainly of small and medium size (Aimi et al. 1991; Zhang et al. 1993). In the lumbar spinal cord (SC), major populations of NADPH diaphorase positive neurons have been found in the superficial laminae of the dorsal horn and around the central canal (Valtschanoff et al. 1992a).

After peripheral nerve axotomy, there is a dramatic increase in nNOS-LI (Fiallos Estrada et al. 1993; Zhang et al. 1993; Shi et al. 1998) and its mRNA levels (Verge et al. 1992) in small and medium sized neurons of DRGs as well as in lamina II of the ipsilateral dorsal horn (Zhang et al. 1993). These findings suggest that the expression of the enzyme is highly plastic in response to a nerve lesion. However, it still remains to be demonstrated whether or not the activity of the enzyme and the amount of NO produced bear the same plasticity. nNOS has been shown to be subject to expressional regulation yielding different mRNA transcripts (Brenman et al. 1997) which in turn can be translated into functional or non functional proteins. In addition, nNOS enzymatic activity has been shown to be tightly regulated by multiple factors (Alderton et al. 2001). As a consequence, the expression and activity of the enzyme do not always correlate (Broholm et al. 2003).

Therefore, the purpose of the present study was to investigate the changes over time in NO production in lumbar DRGs and SC in a model of peripheral axotomy and correlate these changes with those of nNOS-LI.

MATERIALS AND METHODS

Surgical procedure: All experimental procedures were performed in accordance with the animal care guidelines of the local Animal Experimentation and Ethics Committee. Young adult male Sprague Dawley rats (200-250 g, Fucal, Buenos Aires, Argentina) (n = 70) were anaesthetized using chloral hydrate (0.7g/kg i.p.) and the right sciatic nerve was exposed and transected at a mid-thigh level. A 5 mm portion

of the nerve was resected and the proximal stump, ligated.

Tissue collection and preparation of homogenates: After different survival times (7, 14, 28, 35, and 42 days) the lesioned (n = 25) and control (n = 10) animals were decapitated and the ipsilateral and contralateral L4-5 DRGs, as well as the corresponding levels of the SC were rapidly dissected out and washed in saline. Each segment of the SC was sectioned into right and left using a S5 Zeiss dissection microscope. The samples were homogenized in 20 mM Tris-ClH (pH 7.4) with 0.32 M sucrose, 2 mM EDTA, 2 mM DTT and 10% protease inhibitors (Protease Inhibitor Cocktail, Sigma). The homogenates were then centrifuged at 10000 × g for 10 min at 4°C and the supernatants were stored at −30°C until use.

Measurement of NOx: NO production was evaluated by measuring its stable degradation products nitrite and nitrate using the NO Analyser 280 (Sievers Instruments Inc.). NOx present in the samples were reduced by vanadium chloride (VCl_3) in hydrochloric acid (HCl) at 90°C to form NO (Braman et al. 1989). The NO product reacted with ozone in a gas phase chemiluminescent reaction with the generation of nitrogen dioxide, whose emissions in the red and near infrared region of the spectrum were detected by a photomultiplier tube. The analyser was calibrated with solutions of sodium nitrate ($NaNO_3$) 1-100 μM.

Expression of data and statistical analysis: Protein concentration was determined using Lowry's method (Lowry et al. 1951) and NOx levels were normalized to the protein content of each sample and expressed as nmol/mg protein. Data were analyzed with GraphPad Prism. ANOVA test and Newman-Keuls Multiple Comparison Test were used. Results are expressed as mean ± SEM. Statistically significant results were differentiated using these symbols: ns $p > 0.05$, * $0.05 > p > 0.01$, ** $0.01 > p > 0.001$, *** $p < 0.001$

Sample dissection for immunohistochemical analysis: Following similar survival times, lesioned (n = 25) and control (n = 10) animals were deeply anaesthetized (chloral hydrate 1.5 g/kg i.p.) and perfused through the heart with 60 ml of warm (37°C) Tyrode´s buffer (pH 7.4), followed by 60 ml of fixative (4% paraformaldehide and 0.2% picric acid in 0.16M phosphate buffer, pH 7.0) (Zamboni et al. 1967) at 37°C and 300 ml of the same fixative at 4°C. The ipsilateral and contralateral L4-5 DRGs and the corresponding levels of the SC were removed and post fixed in the same fixative for 90 min a 4°C. The tissue was then rinsed in 20% sucrose in phosphate buffer (pH 7.2) containing 0.1% sodium azide and stored at 4°C until immunohistochemical processing.

nNOS immunohistochemistry: The tissue was embebed in OCT compound (Tissue Tek, Miles Laboratories, Elkhart, IN, USA) and cut at 14 μm thickness in a cryostat (Microm, Heildeberg, Germany). Sections were mounted onto chrome alume-gelatin coated slides and processed for standard avidin-biotin peroxidase complex (ABC) immunohistochemistry (Hsu et al. 1981). After rinsing them in PBS and dehydration, endogenous peroxidase activity was inactivated with hydrogen peroxide, followed by rehydration and rinses in PBS. Sections were then incubated in a humid chamber at 4°C for 24 hr with rabbit nNOS polyclonal antibody (1:4000, Santa Cruz Biotechnology), rinsed in PBS, incubated at room temperature for 60 min with biotinylated rabbit anti goat secondary antibodies (1:100, Vector Laboratories, Burlingame, CA, USA), rinsed in PBS and incubated in ABC (1:100, Vectastain Elite ABC kit from Vector) for 1 h at room temperature. Peroxidase activity was demonstrated by reaction with hydrogen peroxide using a nickel-intensified diaminobenzidine protocol for enhancement of the immunoreaction product. After dehydration, the sections were mounted in Permount (Fisher Scientific Company, Fair Lawn, New Jersey, USA). All sections were examined under bright field illumination using a Nikon Eclipse E800 photomicroscope and photographed using a Nikon Digital Camera ND100. The number of neurons exhibiting nNOS-LI in L4-5 DRGs was determined by counting immunostained neuronal profiles in randomly, systematically sampled sections: every 10th section, six to ten sections per ganglion. The total number of neurons was also determined in these sections and the results were expressed as the percentage of immunostained neurons.

RESULTS

nNOS-LI in DRGs: Nerve section induced a dramatic increase in the number of nNOS-IR cells and fibers in the ipsilateral DRGs. Control L4-5 DRGs contained only a few (2%) detectable nNOS-IR cells (Figure 1A), as well as contralateral ganglia at all survival times tested. Some of these cells extended nNOS-IR processes. Seven days after peripheral axotomy 23% of all neurons showed nNOS-LI (Figure 1B). A higher number of nNOS-IR neurons was detected at 14 days (49%) (Figure 1C), reaching a peak 28 days (57%) (Figure 1D) after the surgery, followed by a progressive decrease: 51% of labelled neurons at day 35 (Figure 1E) and 9% at day 42 (Figure 1F). In all cases, labelled neurons corresponded to small and medium sized cells.

nNOS-LI in SC: In the dorsal horn of control animals, the highest density of nNOS-IR nerve fibers and terminals was observed in the inner lamina II, where many nNOS-IR small local neurons could also be found (Figure 2B). Some large neurons with long and thick processes and several small neurons with a strong nNOS-LI were located around the central canal (lamina X). After peripheral nerve axotomy, there was an increased nNOS-IR neuropil with nerve fibers and terminals occupying the outer and inner lamina II of the ipsilateral dorsal horn, while the number of small nNOS-IR neurons was reduced (Figure 2A).

NOx levels in DRGs and SC: In control and contralateral ganglia and SC of any survival time tested, low amounts of NOx could be detected. Nerve section induced a progressive increase in NOx levels in the ipsilateral DRGs (Figure 3) and SC (Figure 4) in a pattern that correlated with nNOS-LI. Seven days after peripheral nerve axotomy, no changes in NOx levels could be detected in the ipsilateral dorsal horn (iDH = 1.48; cDH = 1.52 nmol/mg prot). In DRGs, a mild increase with, however, no statistical significance when compared to control or contralateral ganglia, could be observed in the ipsilateral side (iDRG = 2.25; cDRG = 1.70 nmol/mg prot). This increase reached statistical significance both in DRGs and SC after 14 (iDRG = 4.66; cDRG = 2.32 nmol/mg prot), (iDH = 5.53; cDH = 1.86 nmol/mg prot); 28 (iDRG = 11.40; cDRG = 1.77 nmol/mg prot), (iDH = 9.91; cDH = 2.09 nmol/mg prot); and 35 (iDRG = 5.60; cDRG = 1.95 nmol/mg prot), (iDH = 5.83; cDH = 1.81 nmol/mg prot) days of survival. In both cases, the highest concentration of NOx was observed 28 days after the axotomy. Fourty-two days after the lesion, NOx levels had almost returned to basal levels (iDRG = 2.00; cDRG = 1.96 nmol/mg prot),

FIGURE 1. Immunohistochemistry micrographs showing nNOS-LI in L5 DRGs from control (A) and axotomized (B-F) rats sacrificed after different survival times: 7 (B), 14 (C), 28 (D), 35 (E) or 42 (F) days. Arrows indicate small nNOS-IR cells. Arrowheads point to medium sized nNOS-IR cells. Calibration bar 125 μm.

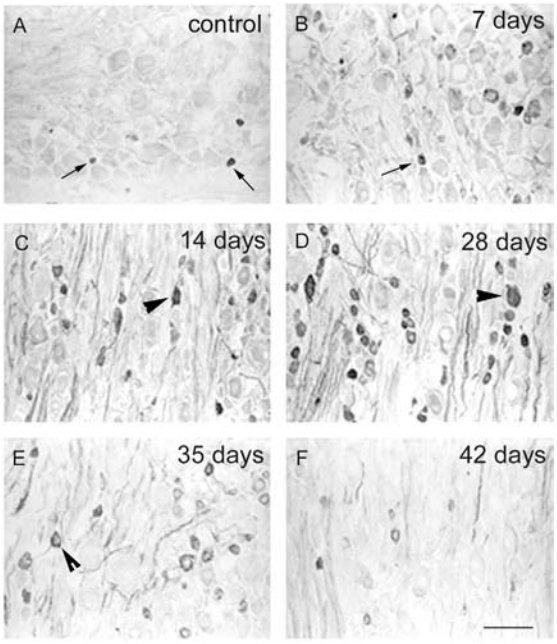


FIGURE 2. Immunohistochemistry micrographs showing nNOS-LI in the dorsal horn of the lumbar SC of a rat subjected to a unilateral peripheral nerve axotomy and sacrificed after 28 days of survival. Arrows indicate the area of increased nNOS-LI. Calibration bar 115 μm.

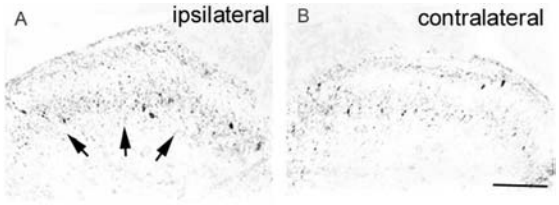


FIGURE 3. NOx levels in L4-5 DRGs from rats subjected to sciatic nerve transection and sacrificed after different survival times. Black bars: ipsilateral (i) DRGs, gray bars: contralateral (c) DRGs, white bar: control (c) animals. ns $p > 0.05$, ** $0.01 > p > 0.001$, *** $p < 0.001$

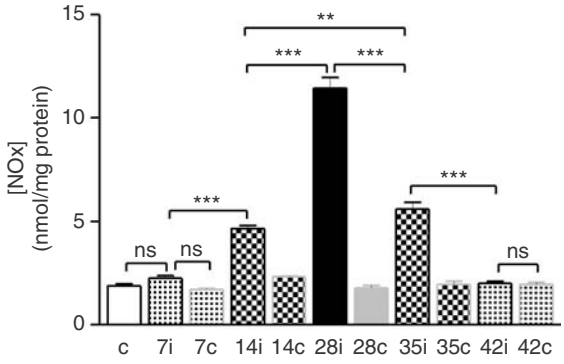
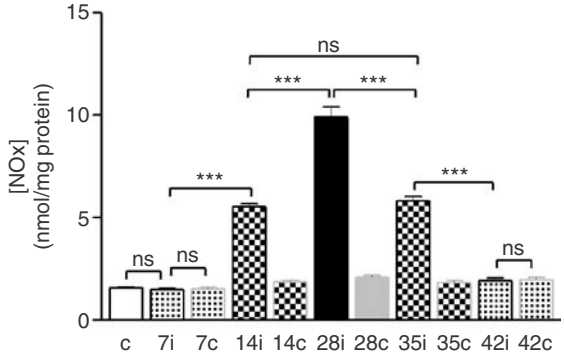


FIGURE 4. NOx levels in the lumbar SC from rats subjected to sciatic nerve transection and sacrificed after different survival times. Black bars: ipsilateral (i) DRGs, gray bars: contralateral (c) DRGs, white bar: control (c) animals. ns $p > 0.05$, *** $p < 0.001$



(iDH = 1.91; cDH = 1.96 nmol/mg prot). No statistically significant difference was detected between control animals (DRG = 1.89 nmol/mg prot; DH = 1.57 nmol/mg prot) and the contralateral ganglia and dorsal horn of the axotomized animals, independently of the survival time tested.

DISCUSSION

The present results confirm that L4-5 DRGs contain only a few nNOS-IR cell bodies, with a progressive increase of labelled neurons after peripheral axotomy. Additionally, in the dorsal horn of the SC the number of nNOS-IR fibers is increased after the lesion. Interestingly, the results also show an increase in the production of NO both in ganglia and SC, thus suggesting a parallel augmentation in the activity of the enzyme after axotomy.

In DRGs, nNOS expression is restricted to small and medium sized neurons, supporting the idea of NO participation in nociception. Immunohistochemical studies have revealed that nNOS is normally transported both to the peripheral (Fiallos Estrada et al. 1993) and central projections (Meller et al. 1993) of these neurons.

In normal circumstances, many nNOS positive fibers, terminals and cells are seen in the dorsal horn. Since the levels of nNOS-LI (present study) and nNOS mRNA (Verge et al. 1992) are very low in control ganglia, only low amounts of the enzyme can be transported through the dorsal roots to the dorsal horn. Thus, it is highly probable that most of the fibers and terminals observed belong to local neurons, as it has been previously suggested (Valtschanoff et al. 1992a). Peripheral axotomy induced a decrease in the number of nNOS-IR local cells of the dorsal horn as well as a marked increase in nNOS-IR nerve fibers in lamina II of the ipsilateral dorsal horn. These findings strongly suggest that after peripheral nerve lesion there is a decrease in the local synthesis of the enzyme, while increased amounts of it are transported from lumbar DRGs into the afferent nerve endings in the superficial dorsal horn.

Additionally, increased nNOS expression in DRG neurons is also probably traduced into increased transport of the enzyme to their peripheral projection. NO produced at the axonal endbulbs may be involved in changes in the microcirculation of the injured peripheral nerve environment and thus influence the likelihood of regeneration of the lesioned nerve (Zochodne 2000).

This increase in nNOS expression in DRGs has been shown to be related to deprivation of retrogradely transported nerve growth factor (NGF) (Thippeswamy et al. 1997a; Thippeswamy et al. 1997b) and possibly others factors, as a consequence of the axotomy. The decrease in the expression of the enzyme observed after the peak of day 28 may be due to local production

of neurotrophic factors by satellite glia cells (Hammarberg et al. 1996; Lee et al. 1998; Zhou et al. 1999) which are activated by the NO produced in the surrounding neurons, resulting in a complex regulatory system.

The present study shows that nNOS upregulation is related to an increased NO production and release, as shown by the high levels of nitric oxides observed after the lesion. The temporal pattern of NO production parallels the one observed for the expression of the enzyme, suggesting that the induction of nNOS synthesis yields a protein that is functional and highly active. The decrease in NOx levels observed at 35 and 42 days of survival time is accompanied by a reduction in nNOS-LI and is therefore probably due to a decrease in the expression of the enzyme and not in its activity.

The physiological/pathological significance of this increase in NO production after peripheral nerve damage has not yet been precisely elucidated. This gaseous agent has been attributed both pronociceptive (Coderre et al. 1994) and antinociceptive roles (Thippeswamy et al. 2001a). These discrepancies may be explained by the fact that NO may be a messenger molecule of different types of cells, including excitatory neurons and inhibitory interneurons (Valtschanoff et al. 1992b; Kawamata et al. 1999). Furthermore, NO has been shown to act on a wide range of downstream targets including ion channels, receptors, intracellular signalling molecules and genes, opening a wide range of possible outcomes in response to this messenger.

More studies should be carried out for further elucidation of the role played by NO in response to peripheral nerve injury and to define whether or not this gaseous messenger is involved in the mechanisms of neuropathic pain.

REFERENCES

Aimi, Y., M. Fujimura, S. R. Vincent and H. Kimura (1991). Localization of NADPH diaphorase-containing neurons in sensory ganglia in the rat. *Journal of Comparative Neurology* 306:382-392.

Alderton, W. K., C. E. Cooper and R. G. Knowles (2001). Nitric oxide synthases: structure, function and inhibition. *Biochemical Journal* 357:593-615.

Baringa, M. (1991). Is nitric oxide the "retrograde messenger"? *Science* 254:1296-1297.

Braman, R. S. and S. A. Hendrix (1989). Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescent detection. *Analytical Chemistry* 61:2715-2718.

Bredt, D. S., P. M. Hwang and S. H. Snyder (1990a). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 347:768-770.

Bredt, D. S. and S. H. Snyder (1990b). Isolation of nitric oxide synthase. A calmodulin requiring enzyme. *Proceedings of the National Academy of Sciences of the USA* 87:682-685.

Brenman, J. E., H. H. Xia, D. S. Chao, S. M. Black and D. S. Bredt (1997). Regulation of neuronal nitric oxide synthase through alternative transcripts. *Developmental Neuroscience* 19:224-231.

Broholm, H., I. Rubin, A. Kruse, O. Braendstrup, K. Schmidt, E. B. Skriver and M. Lauritzen (2003). Nitric oxide synthase expression and enzimatic activity in human brain tumors. *Clinical Neuropathology* 22(6):273-281.

Chung, H. T., H. O. Pae, B. M. Choi, T. R. Billar and Y. M. Kim (2001). Breakthroughs and views of nitric oxide as a bioregulator of apoptosis. *Biochemical and Biophysical Research Communications* 282: 1075-1079.

Coderre, T. J. and K. Yashpal (1994). Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. *European Journal of Neuroscience* 6(8):1328-1334.

Cristino, L., A. Pica, F. Della Corte and M. Bentivoglio (2000). Coinduction of nitric oxide synthase, BCL-2 and growth associated protein 43 in spinal motoneurons during axon regeneration in the lizard tail. *Neuroscience* 101:451-458.

Dawson, T. M. and V. L. Dawson (1995). Physiological and toxicological actions of nitric oxide in the central nervous system. *Advances in Pharmacology* 34: 323-342.

Dawson, T. M., V. L. Dawson and S. H. Snyder (1992). A novel neuronal messenger molecule in brain: the free radical, nitric oxide. *Annals of Neurology* 32: 297-311.

Egberongbe, Y. I., S. M. Gentleman, P. Falkai, B. Bogerts, J. M. Polak and G. Roberts (1994). The distribution of nitric oxide synthase immunoreactivity in the human brain. *Neuroscience* 59:561-578.

Fiallos Estrada, E. C., W. Kummer, B. Mayer, R. Bravo, M. Zimmerman and T. Herdegen (1993). Long lasting increase of in nitric oxide synthase immunoreactivity, NADPH diaphorase reaction and c-JUN coexpression in rat dorsal root ganglion neurons following sciatic nerve transection. *Neuroscience Letters* 150:169-173.

Gally, J. A., P. R. Montague, G. N. Reeke and G. M. Edelman (1990). The NO hypothesis: possible effects of a short-lived, rapid diffusible signal in the development and function of the nervous system. *Proceedings of the National Academy of Sciences of the USA* 87(9):3547-3551.

Hammarberg, H., F. Piehl, S. Cullheim, J. Fjell, T. Hokfelt and K. Fried (1996). GDNF mRNA in Schwann cells and DRG satellite cells after chronic sciatic nerve injury. *Neuroreport* 7:857-860.

Heneka, M. T., P. A. Loschmann, M. Gleichmann, M. Weller, J. B. Schulz, U. Wullner and T. Klockgether (1998). Induction of nitric oxide synthase and nitric oxide mediated apoptosis in neuronal PC12 cells after stimulation with tumor necrosis factor alpha/lipopolysaccharide. *Journal of Neurochemistry* 71:88-94.

Hsu, S., L. Ranid and H. Fanger (1981). Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabelled antibody (PAP) procedures. *The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society* 29:577-580.

Ignarro, L., J. M. Fukuto, J. M. Griscavage, N. E. Rogers and R. E. Byrns (1993). Oxidation of nitic oxide in aqueous solution to nitrite but not nitrate: comparison with enzimatically formed nitric oxide from L-arginine. *Proceedings of the National Academy of Sciences of the USA* 90:8103-8107.

Kawamata, T. and K. Omote (1999). Activation of spinal N-methyl-D-aspartate receptors stimulates a nitric oxide/cyclic-guanosine 3′,5′-monophosphate/glutamate release cascade in nociceptive signalling. *Anesthesiology* 91:1415-1424.

Lee, S. E., H. Shen, G. Taglilatela, J. M. Chung and K. Chunk (1998). Expression of nerve growth factor in the dorsal root ganglion after peripheral nerve injury. *Brain Research* 796:99-106.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193:265-275.

Meller, S. T. and G. F. Gebhart (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain* 52:127-136.

Meller, S. T., S. J. Lewis, J. N. Bates, M. J. Brody and G. F. Gebhart (1990). Is there a role for an endothelium-derived relaxing factor in nociception? *Brain Research* 531(1-2):342-345.

Meller, S. T., P. S. Pechman, G. F. Gebhart and T. J. Maves (1992). Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. *Neuroscience* 50(1):7-10.

Morris, R., E. Southam, D. J. Braid and J. Garthwaite (1992). Nitric oxide may act as a messenger between dorsal root ganglion neurones and their satellite cells. *Neuroscience Letters* 137(1):29-32.

Salter, M., C. Duffy, J. Garthwaite and P. J. Strijbos (1996). Ex vivo measurements of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity in vivo. *Journal of Neurochemistry* 66(4):1683-1690.

Shi, T. J., K. Holmberg, Z. Q. Xu, H. Steinbusch, J. de Vente and T. Hokfelt (1998). Effect of peripheral nerve injury on cGMP and nitric oxide synthase levels in rat dorsal root ganglia: time course and coexistence. *Pain* 78:171-180.

Thippeswamy, T., R. K. Jain, M. Numtaz and R. Morris (2001a). Inhibition of neuronal nitric oxide synthase results in neurodegenerative changes in axotomised dorsal root ganglion neurons: evidence for a neuroprotective role of nitric oxide in vivo. *Neuroscience Research* 44:37-44.

Thippeswamy, T., J. S. McKay and R. Morris (2001b). Bax and caspases are inhibited by endogenous nitric oxide in dorsal root ganglion neurons in vitro. *European Journal of Neuroscience* 14:1229-1236.

Thippeswamy, T. and R. Morris (1997a). Cyclic guanosine 3′,5′-monophosphate mediated neuroprotection by nitric oxide in dissociated cultures of rat dorsal root ganglion neurons. *Brain Research* 774:116-122.

Thippeswamy, T. and R. Morris (1997b). Nerve growth factor inhibits the expression of nitric oxide synthase in neurons in dissociated cultures of rat dorsal root ganglia. *Neuroscience Letters* 230:9-12.

Valtschanoff, J. G., R. J. Weinberg and A. Rustioni (1992a). NADPH diaphorase in the spinal cord of rats. *Journal of Comparative Neurology* 321:209-222.

Valtschanoff, J. G., R. J. Weinberg, A. Rustioni and H. H. Schmidt (1992b). Nitric oxide synthase and GABA colocalize in lamina II of rat spinal cord. *Neuroscience Letters* 148(1-2):6-10.

Van Wangenen, S. and V. Rehder (2001). Regulation of neuronal growth cone filopodia by nitric oxide depends on soluble guanylyl ciclase. *Journal of Neurobiology* 46:206-219.

Verge, V. M., Z. Xu, X. J. Xu, Z. Wiesenfeld Hallin and T. Hokfelt (1992). Marked increase in nitric oxide synthase mRNA in rat dorsal root ganglia after peripheral axotomy: in situ hybridization and functional studies. *Proceedings of the National Academy of Sciences of the U S A* 89:11617-11621.

Zamboni, L. and C. De Martino (1967). Buffered picric acid formaldehyde. A new rapid fixation for elctron microscopy. *Journal of Cellular Biology* 35:148.

Zhang, X., V. Verge, Z. Wiesenfeld Hallin, G. Ju, D. Bredt, S. H. Snyder and T. Hokfelt (1993). Nitric oxide synthase-like immunoreactivity in lumbar dorsal root ganglia and spinal cord of rat and monkey and effect of peripheral axotomy. *Journal of Comparative Neurology* 335:563-575.

Zhou, X. F., Y. S. Debg, E. Chie, Q. Xue, J. H. Zhong, E. M. McLachlan, R. A. Rush and C. J. Xian (1999). Satellite-cell-derived nerve growth factor and neurotrophin-3 are involved in noradrenergic sprouting in the dorsal root ganglia following peripheral nerve injury in the rat. *European Journal of Neuroscience* 11(5):1711-1722.

Zochodne, D. W. (2000). The microenvironment of injured and regenerating peripheral nerves. *Muscle Nerve Supplement* 9:33-38.

Zochodne, D. W., M. Misra, C. Cheng and H. Sun (1997). Inhibition of nitric oxide synthase enhances peripheral nerve regeneration in mice. *Neuroscience Letters* 228:71-74.

Received: December 28, 2004
Accepted: February 20, 2005