ELSEVIER

Brain Research 878 (2000) 240–246

BRAIN RESEARCH INTERACTIVE

www.elsevier.com/locate/bres

Interactive report

Axonal transport of NADPH-diaphorase and [^{3}H]nitro-L-arginine binding, but not [^{3}H]cGMP binding, by the rat vagus nerve[1]

Angelina Y. Fong[a], William T. Talman[b], Andrew J. Lawrence[a,*]

[a]*Department of Pharmacology, Monash University, Wellington Road, Clayton, VIC 3168, Australia*
[b]*Department of Neurology, University of Iowa and Veterans Affairs Medical Center, Iowa City, IA 52242, USA*

Accepted 8 August 2000

Abstract

Previous studies have shown that the NO$^{\cdot}$-cGMP pathway may be functionally relevant in the nodose ganglion and at afferent terminations of the vagus nerve. The technique of unilateral vagal ligations, using double ligatures, was combined with the techniques of NADPH-diaphorase histochemistry, as an index of nitric oxide synthase (NOS) activity, and autoradiography using the radioligands [^{3}H]nitro-L-arginine and [^{3}H]cGMP, to examine axonal transport of NOS and cGMP-dependent effectors by the rat vagus nerve. A population of perikarya in the nodose ganglia was NADPH-diaphorase positive, and binding of both [^{3}H]nitro-L-arginine and [^{3}H]cGMP was found on the nodose ganglia. Following vagal ligation, NADPH-diaphorase reactivity accumulated proximal to the proximal ligature and distal to the distal ligature. Vagus nerve transection beyond the distal ligature eliminated NADPH-diaphorase reactivity at the distal ligature. Similarly, [^{3}H]nitro-L-arginine binding was found over the nodose ganglion; and after vagal ligation, an accumulation of [^{3}H]nitro-L-arginine binding was seen adjacent to the proximal ligature, though little binding was found adjacent to the distal ligature. No accumulation of [^{3}H]cGMP binding was found adjacent to either the proximal or the distal ligatures. These findings suggest that the rat vagus nerve bidirectionally transports NOS, the enzyme involved in biosynthesis of NO$^{\cdot}$ by nitroxidergic nerves. As anticipated, [^{3}H]nitro-L-arginine, a competitive inhibitor of the amino acid precursor for NO$^{\cdot}$, binds only to a centrifugally transported moiety that we conjecture is NOS, while cGMP apparently is not subject to transport. These data further support the use of NO$^{\cdot}$ in transmission at vagal afferent terminals.

Theme: Sensory systems

Topic: Somatic and visceral afferents

Keywords: Nodose ganglion; NOS; NADPH-diaphorase; Vagus nerve; Vagal ligation; Axonal transport

1. Introduction

The vagus nerve contains afferent axons including those from arterial baro- and chemoreceptors, cardiopulmonary receptors and gastrointestinal receptors. These fibers originate from cell bodies in the nodose (inferior vagal) ganglion and project centrally to terminate in the nucleus tractus solitarii (NTS) [11]. Vagal afferent terminals distally [8,33,34], as well as those in the NTS [21], are immunoreactive for nitric oxide synthase (NOS), the enzyme that is critical to the biosynthesis of nitric oxide (NO$^{\cdot}$). Demonstration of NOS mRNA expression in the rat nodose perikarya [18] confirms vagal afferent neurones contain NOS. Furthermore, NO$^{\cdot}$ is functionally active in nodose ganglion neurones where it is synthesized [18]. NO$^{\cdot}$ released by vagus nerve terminals may participate in transmission both centrally at the level of the NTS [20] and peripherally at arterial baroreceptors [25], in the heart [5], and in the gut [1].

In fact, NO$^{\cdot}$ is widely distributed and is believed to act as a transmitter in a number of different pathways [31]. Its best described means of signal transduction is through stimulation of soluble guanylate cyclase with resulting accumulation of cyclic GMP (cGMP) within the target cell [9]. Indeed, a number of studies both centrally [20,23] and peripherally [18] have shown the actions of NO$^{\cdot}$ to be

[1]Published on the World Wide Web on 18 August 2000.

*Corresponding author. Tel.: +61-3-9905-4855; fax: +61-3-9905-5851.

E-mail address: andrew.lawrence@med.monash.edu.au (A.J. Lawrence).

0006-8993/00/$ – see front matter
PII: S0006-8993(00)02789-X

sensitive to antagonism by inhibitors of soluble guanylate cyclase, and a previous study [18] has shown evidence to suggest that the NO·-cGMP pathway is functionally relevant within the nodose ganglion. In addition, not only has NO· been demonstrated to have a physiological role within the NTS [19,20,23] but NO· has also been shown to be capable of modulating the release of L-glutamate [16], a neurotransmitter at baroreceptor, chemoreceptor and cardiopulmonary afferent terminals [17,32]. Together, these data suggest that NO·-cGMP may be involved both in central and peripheral neurotransmission by the vagus nerve.

We hypothesized that NOS is transported bidirectionally from nodose ganglion cells but that the enzyme transported from the periphery to the neurone; if it is retrogradely transported back to the cell body for metabolic reprocessing, may not bind, or bind with reduced affinity, to [^{3}H]nitro-L-arginine, a competitive inhibitor of L-arginine, the amino acid precursor for NO· synthesis [7]. Further, since NO· is functionally active in nodose ganglion neurones [18], we hypothesized that cGMP-dependent effectors, that could be labelled with [^{3}H]cGMP [2], may be present within the ganglion. Central transport of NOS to the NTS has been implied by earlier studies that demonstrated a reduction of NOS in the NTS after removal of the nodose ganglion or vagotomy [15,21,28]. However, as the vagus nerve rostral to the nodose ganglion is surrounded by bone and is relatively inaccessible, the current study focussed on the nerve distal to the ganglion. We would anticipate that characteristics of transport demonstrated in these peripheral vagal fibers would also apply to centrally projecting axons of the pseudounipolar nodose ganglion neurones.

In the present study, the techniques of unilateral vagal ligation and/or vagotomy together with NADPH-diaphorase histochemistry and [^{3}H]nitro-L-arginine and [^{3}H]cGMP autoradiography were employed to examine possible axonal transport of NOS and its second messenger system, cGMP along the rat vagus nerve.

2. Materials and methods

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986 and under the guidelines of the Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. The studies also adhered to the *Guide for Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996). Efforts were made to minimize animal suffering and the number of animals used.

2.1. NADPH-diaphorase histochemistry

2.1.1. Surgical procedure

Five male Wistar–Kyoto rats (335–390 g) were anaesthetized with sodium methohexitone (60 mg/kg, i.p.). A ventral midline incision was made in the neck and the left vagus nerve was exposed. Two ligatures (3/0 silk), ~5 mm apart, were secured around the vagus nerve immediately distal to the nodose ganglion. In three cases, the vagus nerve, distal to the ligatures was transected to examine retrograde transport. The right vagus nerve was also exposed and loose ligatures were placed around the nerve peripheral to the nodose ganglion for sham controls. The wounds were sutured, infiltrated with lidocaine (1% w/v), and dusted with Cicatrin® antibiotic powder. Rats were housed individually and allowed to recover for 2 days. At the end of the recovery period, the rats were again anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and transcardially perfused with 100 ml of phosphate buffered saline (PBS 0.1 M, pH7.4) followed by 500 ml fixative (4% paraformaldehyde in 0.1 M PBS, pH 7.4). Both nodose ganglia and vagus nerves were isolated, removed, postfixed, and cryoprotected for 2 h at 4°C in fixative (4% paraformaldehyde in 0.1 M PBS, pH 7.4) containing 10% sucrose before being frozen in cryoprotectant tissue embedding medium over supercooled isopentane. Longitudinal sections (10 μm) were cut on a cryostat and thaw-mounted onto gelatin/chrom-alum coated glass microscope slides and stored at −80°C until used.

2.1.2. NADPH-diaphorase histochemistry

The slide-mounted tissue slices were allowed to return to room temperature before being pre-incubated in 0.1 M Tris–HCl (pH 8.0) for 5 min. The sections were then transferred to the reaction solution containing 0.5% β-NADPH, 0.125% nitroblue tetrazolium, 0.1% Triton X-100 in 0.1 M Tris–HCl (pH 8.0) for 30 min at 37°C, as adapted from previously published protocol [38]. Following the reaction, the slides were air-dried, dehydrated in serial alcohol, cleared and coverslipped prior to analysis and photography under normal light field microscopy conditions (Olympus BH2 microscope).

2.2. Autoradiographic experiments

2.2.1. Surgical procedure

Five male Wistar–Kyoto rats (335–390 g) were anaesthetized with sodium methohexitone (60 mg/kg, i.p.) and a left unilateral vagal ligation and corresponding sham operations were performed as described above. At the end of the 2 day recovery period, the rats were anaesthetised with sodium methohexitone (60 mg/kg, i.p.), the left and right nodose ganglia and vagus nerves were isolated, removed and frozen in cryoprotectant (OCT tissue embedding compound) over supercooled isopentane. The rats were then euthanazed by decapitation. Longitudinal sections (10 μm) of the nerves and ganglia were cut with a cryostat and sections were thaw-mounted onto gelatin/chrome-alum coated glass microscope slides that were then stored at −80°C until used.

2.2.2. [^{3}H]Nitro-L-arginine autoradiography

[^{3}H]Nitro-L-arginine autoradiographic experiments were conducted using a modified version of a previously published protocol [12]. In brief, tissue sections were allowed to return to room temperature prior to pre-incubation for 30 min in 50 mM Tris–HCl, 3 mM $CaCl_2$ buffer (pH 7.4) containing 0.025% Triton X-100 at room temperature. The sections were then incubated for 60 min in [^{3}H]nitro-L-arginine (40 nM) in 50 mM Tris–HCl, 3 mM $CaCl_2$ buffer at 4°C. Non-specific binding was assessed in adjacent sections incubated in the presence of the radioligand plus L-arginine hydrochloride (1 mM). The slide-mounted sections were then washed in ice-cold buffer (3×1 min) followed by a rinse in ice-cold distilled water. Sections were then dried in a cool stream of air and desiccated overnight. The next day, the slides were tightly apposed to Hyperfilm-[^{3}H] and exposed in the dark at room temperature for 12 months.

2.2.3. [^{3}H]cGMP autoradiography

The protocol for [^{3}H]cGMP autoradiography was adapted from a previously published protocol [2]. Sections were allowed to return to room temperature prior to 30 min incubation in the radioligand, [^{3}H]cGMP (20 nM), in Krebs buffer with 25 mM Hepes (pH 7.4) in the presence of IBMX (1 mM), a potent phosphodiesterase inhibitor, at room temperature. Non-specific binding was determined on adjacent sections incubated in the presence of the radioligand, IBMX (1 mM) plus a cGMP analogue, 8-Bromo-cGMP (1 mM). The slide-mounted sections were then washed in ice-cold buffer (2×1 min) followed by a rinse in ice-cold distilled water. The sections were dried in a cool stream of air, desiccated overnight and tightly apposed to Hyperfilm-[^{3}H] and exposed in the dark at room temperature for 8 weeks.

2.2.4. Materials

N^G-Nitro-L-[2,3,4,5-^{3}H]arginine hydrochloride (specific activity 4.0 Ci/mmol) and Hyperfilm-[^{3}H] were purchased from Amersham Life Science, USA; Guanosine 3′,5′-cyclic monophosphate [8,5′-^{3}H] (specific activity 34.1 Ci/mmol) was purchased from NEN Life Science Products, USA. L-Arginine hydrochloride, β-NADPH and nitroblue tetrazolium were obtained from Sigma Chemicals Co., Australia; 3-isobutyl-1-methylxanthine (IBMX) and 8-Bromoguanosine-3′,5′-cyclophosphate from Research Biochemicals Inc., USA. Triton X-100 was purchased from Ajax and OCT tissue embedding compound from Miles Diagnostics. Sodium methohexitone, sodium pentobarbitone were both purchased from Lilly Laboratories; lidocaine hydrochloride (1% w/v) from Delta West; and Cicatrin® from Wellcome. All other reagents were of either laboratory or analytical grade from various suppliers.

3. Results

3.1. NADPH-diaphorase staining

Examination of the nodose ganglia revealed a population of neurones that stained for NADPH diaphorase (Fig. 1A). In intact sections, NADPH diaphorase staining was barely detectable along the entire length of the vagus nerve. On the other hand, analysis of ligated vagus nerves revealed marked accumulation of NADPH-diaphorase staining adjacent to both the distal and the proximal ligatures (Fig. 1A, B). Distal vagotomy resulted in a lack of NADPH-diaphorase reactivity adjacent to the distal ligature (Fig. 1C, D).

3.2. [^{3}H]Nitro-L-arginine autoradiography

In intact vagus nerve sections, binding of [^{3}H]nitro-L-arginine was restricted to the nodose ganglion, and no binding of radioligand was seen along the vagus nerve. In contrast, following unilateral vagal ligation (Fig. 2B), in addition to the binding over the nodose ganglion, [^{3}H]nitro-L-arginine binding was apparent adjacent to the proximal ligature in 4 out of 5 rats examined. Binding of [^{3}H]nitro-L-arginine adjacent to the proximal ligature was greater than that adjacent to the distal ligature. Non-specific binding in the presence of L-arginine (1 mM) was too faint to be detected on the autoradiograms.

3.3. [^{3}H]cGMP autoradiography

In ligated vagus nerve sections, [^{3}H]cGMP binding was essentially restricted to the nodose ganglion (Fig. 2C). No accumulation of [^{3}H]cGMP binding was seen adjacent to ligatures, though ligature sites were clearly discernible (Fig. 2C). Similarly, in vagus nerves subjected to sham ligation, [^{3}H]cGMP binding was almost entirely restricted to the nodose ganglion (Fig. 2D) although faint binding could be detected over the entire length of the vagus nerve. Densitometric analysis of [^{3}H]cGMP binding indicated ~50% specific binding, as defined by 8-Bromo-cGMP (1 mM, Fig. 2D).

4. Discussion

This study makes three contributions. Firstly, these data represent direct evidence of bidirectional axonal transport of NADPH-diaphorase activity along the rat vagus nerve. Second, they show that demonstrable [^{3}H]nitro-L-arginine binding apparently only occurs on a centrifugally transported moiety or moieties. Third, the study provides evidence of binding of [^{3}H]cGMP in the rat nodose ganglion and suggests that [^{3}H]cGMP-sensitive binding is not transported axonally along the rat vagus nerve.

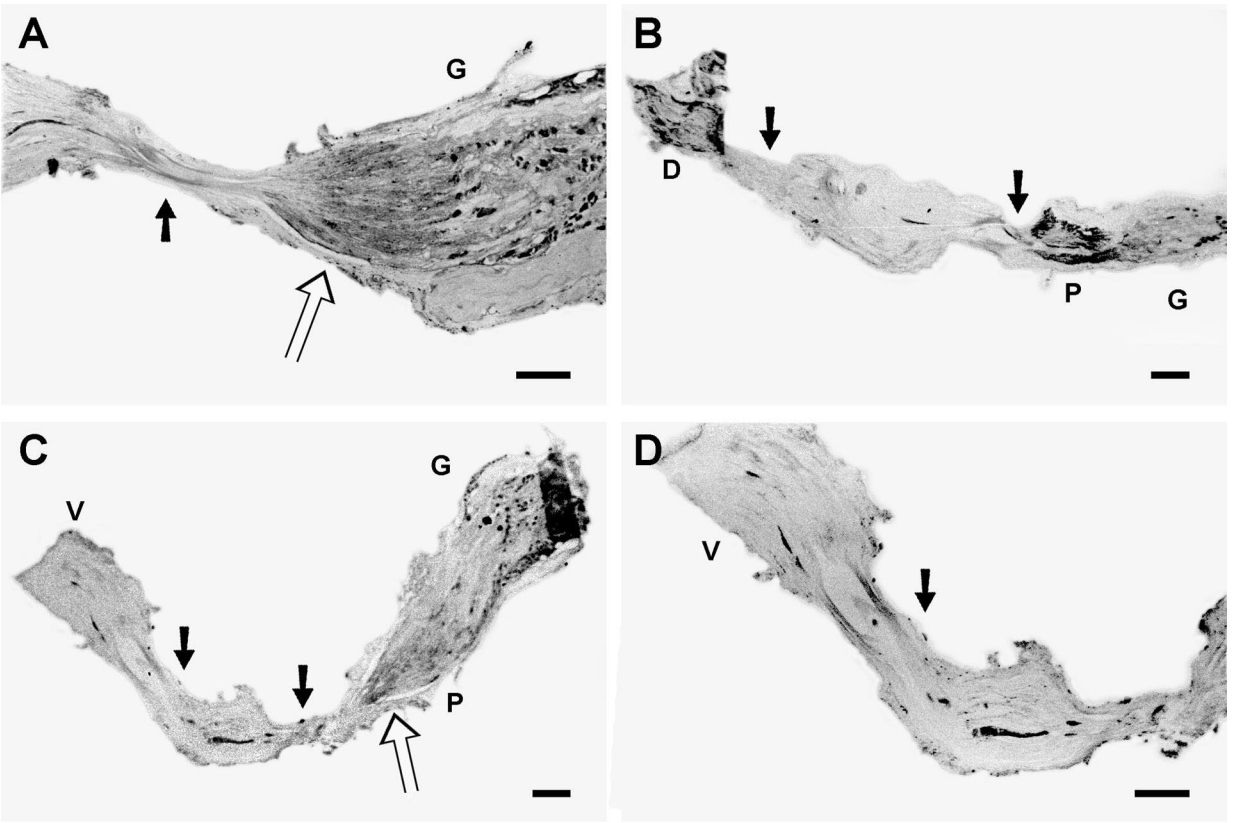

Fig. 1. Photomicrographs of NADPH-diaphorase activity on rat nodose ganglion-vagus nerve sections, closed arrows indicating the site of ligation. (A) NADPH-diaphorase reactivity can be clearly seen in a number of neurons of the nodose ganglion (G), in addition, an accumulation of NADPH-diaphorase reactivity (open arrow) is seen proximal to the ligation site (closed arrow). (B) Accumulation of NADPH-diaphorase reactivity is clearly apparent adjacent to both proximal (P) and distal (D) ligatures, indicated by arrows, suggesting bi-directional transport of NADPH-diaphorase. (C) Accumulation of NADPH-diaphorase reactivity adjacent to the distal ligature is abolished following distal vagotomy (V) whereas accumulation still occurs adjacent to the proximal (P) ligature (open arrow). (D) Magnification of distal ligation site in (C) demonstrating that retrograde transport of NADPH-diaphorase activity is abolished by vagotomy (V). Scale bars=250 μm.

The accumulation of NADPH-diaphorase reactivity and [^{3}H]nitro-L-arginine binding adjacent to proximal ligature suggests that the vagus nerve transports NADPH-diaphorase/[^{3}H]nitro-L-arginine binding (and we conjecture NOS) anterogradely from the cell body, where the mRNA encoding for NOS is present [18], to the peripheral vagal terminals. NADPH-diaphorase accumulation adjacent to the distal ligature also suggests that the enzyme is then apparently transported retrogradely from the peripheral terminals towards the cell body for possible reprocessing or recycling. The elimination of NADPH-diaphorase reactivity adjacent to the distal ligature by vagotomy further supports retrograde transport of NAPDH-diaphorase, or NOS, along the vagus nerve. Agreement between [^{3}H]nitro-L-arginine binding and NADPH-diaphorase reactivity in this study supports the use of NADPH-diaphorase as an index of NOS transport. Despite the specificity of [^{3}H]nitro-L-arginine for NOS [4,27], the low specific activity of the ligand (4.0 Ci/mmol), and the subsequent long exposure time to film, limits the usefulness of this ligand alone for the study of NOS. On the other hand, NADPH-diaphorase staining is a straightforward technique for the localization of NOS [36] and is thus, preferable to [^{3}H]nitro-L-arginine, particularly if quantification is not a pre-requisite. The higher level of [^{3}H]nitro-L-arginine binding adjacent to the proximal ligature compared to the distal ligature may be due in part to greater transport of the enzyme NOS anterogradely. However, the lower [^{3}H]nitro-L-arginine binding adjacent to the distal ligature may be caused by a conformational change in the enzyme due to metabolic processing, and a corresponding change in affinity, as reflected in the lower level of ligand binding. This issue is further complicated by the low specific activity of the ligand, resulting in difficulty in quantification. Therefore, further analysis of this phenomenon is needed to allow a secure interpretation. Use of a radiolabeled ligand with high specific activity would be beneficial, but such a ligand is currently not available.

Previous studies have shown a correlation between the distribution of NADPH-diaphorase activity, NOS mRNA and NOS immunoreactivity [3] and suggested that NADPH-diaphorase staining reflects the presence of NOS.

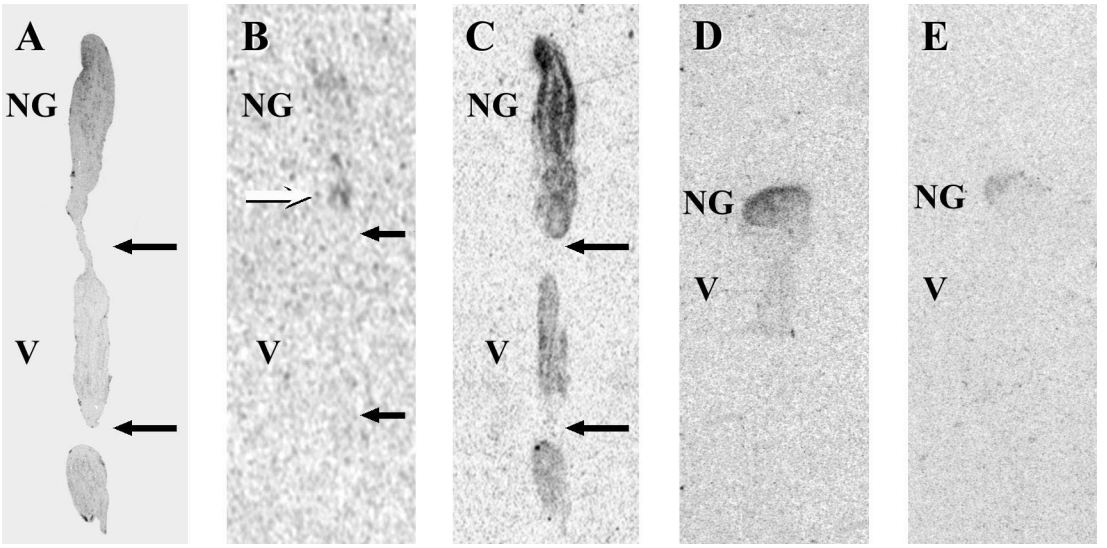

Fig. 2. (A) Photomicrograph of rat nodose ganglion (NG) with distally ligated vagal trunk (V), stained with 0.1% thionin, black arrows indicating the site of ligatures in the distal vagus nerve. (B, C) Autoradiograms of total [^{3}H]nitro-L-arginine binding (B) and [^{3}H]cGMP (C) binding in rat nodose ganglia and ligated vagus nerve sections, arrows indicating site of ligatures in the distal vagal trunk. Accumulation of [^{3}H]nitro-L-arginine binding, indicated by white arrow, is apparent adjacent to the proximal ligature (B), suggesting centrifugal transport of the NOS enzyme, while no visible accumulation of [^{3}H]nitro-L-arginine was seen adjacent to the distal ligature. In addition, no accumulation of [^{3}H]cGMP was apparent adjacent to either the proximal or distal ligatures (C). Autoradiogram of total binding of [^{3}H]cGMP in intact rat nodose ganglion-vagus nerve section (D) and non-specific binding of [^{3}H]cGMP (E), as determined by the cGMP analogue, 8-Bromo-cGMP (1 mM).

Some studies have even extended the interpretation and demonstrated that NADPH-diaphorase is, in fact, neuronal NOS [10]. In contrast, additional studies showed that NOS and NADPH-diaphorase are distributed differentially, and that colocalization of NOS and NADPH-diaphorase is not exclusive [37]. However, a number of other studies support the use of NADPH-diaphorase as a biochemical marker of NOS in this study. Firstly, the reactivity of NADPH-diaphorase in the current study agrees with previous studies that showed NADPH-diaphorase staining [26] and NOS mRNA [18] within the nodose ganglion. Secondly, similar axonal transport of NADPH-diaphorase activity has been demonstrated along autonomic nerves [22]. Finally, in the present study, the binding of [^{3}H]nitro-L-arginine, a specific NOS inhibitor, correlates with staining of NADPH-diaphorase in the nodose ganglion and anterogradely transported NADPH-diaphorase.

The localization of NOS within the nodose ganglion has been examined previously using a number of techniques, including immunohistochemistry [22], in situ hybridization [18] and NADPH-diaphorase [22,26]; however, this is the first study where localization of NOS in the rat nodose ganglion and vagus nerve has been studied with the radioligand [^{3}H]nitro-L-arginine. On the other hand, previous studies have utilized [^{3}H]nitro-L-arginine for the localization of NOS within the rat brain [4,12,27].

Though the transport of NADPH-diaphorase reactivity and NOS along the ligated vagus nerve has been investigated previously, the earlier study only placed a single ligature placed on the nerve [22]. Double ligatures that allow more accurate assessment of bidirectional transport have not been used. In addition, a previous study has shown that an increase in the level of inducible NOS (iNOS) in rat ganglia followed nerve crush injury [24]. Thus, the use of double ligatures and distal transection of the vagus nerve in the present study was important and clearly indicates that accumulation of the enzyme NOS (NAPDH-diaphorase reactivity) is due to bidirectional axonal transport, rather than accumulation of iNOS at a site of neuronal damage.

While NOS (NADPH-diaphorase) appears to be transported by vagus nerve axons, binding of [^{3}H]cGMP did not reveal axonal transport of [^{3}H]cGMP. This finding suggests that cGMP may be involved in NO-mediated signalling within the nodose ganglion [18]. In the rat brain, [^{3}H]cGMP may bind to cGMP dependent protein-kinase [2], but some now propose that cGMP, and cAMP, may elicit some of their actions through activation of a novel class of Ca^{2+} permeable ion channel, termed cyclic-nucleotide-gated channels [14,29,35]. In fact, mRNA encoding for cyclic-nucleotide gated channels seems to be widespread throughout rat brain [13]. We cannot discount the possibility that the [^{3}H]cGMP binding we demonstrated is to cyclic-nucleotide-gated channels as well as cGMP protein-dependent kinase. Binding of [^{3}H]nitro-L-arginine within the nodose ganglion further supports the premise that the $NO^{\cdot}$-cGMP pathway may be active in intercellular signalling and autoregulation within the nodose ganglion. Indeed, studies in cultured nodose ganglion neurones [30] and isolated nodose ganglion preparations

[18] have shown NO to be functionally active within the nodose ganglion, acting via the guanylate cyclase effector [18].

In conclusion, the present study has demonstrated that NOS binding and NADPH-diaphorase reactivity is subject to bidirectional transport along the vagus nerve while the binding of [^{3}H]cGMP is not. Although the current study focussed on transport distal to the ganglion, we suggest that the findings apply to transport of NOS to afferent terminals in the NTS as well. Because the number of microtubules, which are critical to axoplasmic transport, is greater rostral than it is caudal to the nodose ganglion [6], central transport of NOS by the vagus nerve may be even more prominent than we have shown in the peripheral vagus nerve.

Acknowledgements

AJL is an RD Wright Fellow of the NH&MRC (Australia). WTT is the recipient of a Cottman Fellowship from Monash University. WTT is supported in part by NIH R01 HL59593.

References

[1] L. Bartho, G. Koczan, G. Petho, C.A. Maggi, Blockade of nitric oxide synthase inhibits nerve-mediated contraction in the rat small intestine, Neurosci. Lett. 145 (1992) 43–46.
[2] C. Bladen, D. Loewen, S.R. Vincent, Autoradiographic localization of [^{3}H]-cyclic GMP binding sites in the rat brain, J. Chem. Neuroanat. 10 (1996) 287–293.
[3] D.S. Bredt, C.E. Glatt, P.M. Hwang, M. Fotuhi, T.M. Dawson, S.H. Snyder, Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase, Neuron 7 (1991) 615–624.
[4] T.C. Burazin, A.L. Gundlach, Localization of NO synthase in rat brain by [^{3}H]L-N^G-nitro-arginine autoradiography, Neuroreport 6 (1995) 1842–1844.
[5] K. Conlon, C. Kidd, Neuronal nitric oxide facilitates vagal chronotropic and dromotropic actions on the heart, J. Auton. Nerv. Syst. 75 (1999) 136–146.
[6] J.A. Donoso, Microtubule stability along mammalian peripheral nerves, J. Neurobiol. 17 (1986) 383–403.
[7] P.N. Dubbin, M. Zambetis, G.J. Dusting, Inhibition of endothelial nitric oxide biosynthesis by *N*-nitro-L-arginine, Clin. Exp. Pharmacol. Physiol. 17 (1990) 281–286.
[8] E.R. Forster, E. Southam, The intrinsic and vagal extrinsic innervation of the rat stomach contains nitric oxide synthase, NeuroReport 4 (1993) 275–278.
[9] J. Garthwaite, S.L. Charles, R. Chess-Williams, Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain, Nature 336 (1988) 385–388.
[10] B.T. Hope, G.J. Michael, K.M. Knigge, S.R. Vincent, Neuronal NADPH diaphorase is a nitric oxide synthase, Proc. Nat. Acad. Sci. (USA) 88 (1991) 2811–2814.
[11] M. Kalia, J.M. Sullivan, Brainstem projections of sensory and motor components of the vagus nerve in the rat, J. Comp. Neurol. 211 (1982) 248–265.
[12] E.J. Kidd, A.D. Michel, P.P. Humphrey, Autoradiographic distribution of [^{3}H]L-N^G-nitro-arginine binding in rat brain, Neuropharmacology 34 (1995) 63–73.
[13] P.A. Kingston, F. Zufall, C.J. Barnstable, Widespread expression of olfactory cyclic nucleotide-gated channel genes in rat brain: implications for neuronal signalling, Synapse 32 (1999) 1–12.
[14] S. Kusaka, I. Dabin, C.J. Barnstable, D.G. Puro, cGMP-mediated effects on the physiology of bovine and human retinal Muller (glial) cells, J. Physiol. (Lond.) 497 (1996) 813–824.
[15] A.J. Lawrence, M. Castillo-Melendez, K.J. McLean, B. Jarrott, The distribution of nitric oxide synthase-, adenosine deaminase- and neuropeptide Y-immunoreactivity through the entire rat nucleus tractus solitarius: Effect of unilateral nodose ganglionectomy, J. Chem. Neuroanat. 15 (1998) 27–40.
[16] A.J. Lawrence, B. Jarrott, Nitric oxide increases interstitial excitatory amino acid release in the rat dorsomedial medulla oblongata, Neurosci. Lett. 151 (1993) 126–129.
[17] A.J. Lawrence, B. Jarrott, Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius, Prog. Neurobiol. 48 (1996) 21–53.
[18] A.J. Lawrence, E. Krstew, B. Jarrott, Actions of nitric oxide and expression of the mRNA encoding nitric oxide synthase in rat vagal afferent neurons, Eur. J. Pharmacol. 315 (1996) 127–133.
[19] S.J. Lewis, B.H. Machado, H. Ohta, W.T. Talman, Processing of cardiopulmonary afferent input within the nucleus tractus solitarii involves activation of soluble guanylate cyclase, Eur. J. Pharmacol. 203 (1991) 327–328.
[20] S.J. Lewis, H. Ohta, B. Machado, J.N. Bates, W.T. Talman, Microinjection of *S*-nitrosocysteine into the nucleus tractus solitarii decreases arterial pressure and heart rate via activation of soluble guanylate cyclase, Eur. J. Pharmacol. 202 (1991) 135–136.
[21] L.H. Lin, M.D. Cassell, A. Sandra, W.T. Talman, Direct evidence for nitric oxide synthase in vagal afferents to the nucleus tractus solitarii, Neuroscience 84 (1998) 549–558.
[22] A. Lumme, S. Vanhatalo, S. Soinila, Axonal transport of nitric oxide synthase in autonomic nerves, J. Auton. Nerv. Syst. 56 (1996) 207–214.
[23] B.H. Machado, L.G. Bonagamba, Microinjection of *S*-nitrosocysteine into the nucleus tractus solitarii of conscious rats decreases arterial pressure but L-glutamate does not, Eur. J. Pharmacol. 221 (1992) 179–182.
[24] S. Magnusson, P. Alm, M. Kanje, Inducible nitric oxide synthase increases in regenerating rat ganglia, NeuroReport 7 (1996) 2046–2050.
[25] T. Matsuda, J.N. Bates, S.J. Lewis, F.M. Abboud, M.W. Chapleau, Modulation of baroreceptor activity by nitric oxide and *S*-nitrosocysteine, Circ. Res. 76 (1995) 426–433.
[26] R. Morris, E. Southam, S.R. Gittins, J. Garthwaite, NADPH-diaphorase staining in autonomic and somatic cranial ganglia of the rat, NeuroReport 4 (1993) 62–64.
[27] V.L. Rao, R.F. Butterworth, Regional distribution of binding sites for the nitric oxide synthase inhibitor L-[^{3}H]nitroarginine in rat brain, Neurochem. Res. 21 (1996) 355–359.
[28] D.A. Ruggiero, E.P. Mtui, K. Otake, M. Anwar, Central and primary visceral afferents to nucleus tractus solitarii may generate nitric oxide as a membrane-permeant neuronal messenger, J. Comp. Neurol. 364 (1996) 51–67.
[29] M.L. Ruiz, J.W. Karpen, Single cyclic nucleotide-gated channels locked in different ligand-bound states [see comments], Nature 389 (1997) 389–392.
[30] M. Sato, M. Kawatani, Nitric oxide raises cytosolic concentrations of Ca^{2+} in cultured nodose ganglion neurons from rabbits, Neurosci. Lett. 206 (1996) 69–72.
[31] E. Southam, J. Garthwaite, The nitric oxide-cyclic GMP signalling pathway in rat brain, Neuropharmacology 32 (1993) 1267–1277.
[32] W.T. Talman, M.H. Perrone, D.J. Reis, Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers, Science 209 (1980) 813–815.

[33] K. Tanaka, T. Chiba, Nitric oxide synthase containing neurons in the carotid body and sinus of the guinea pig, Microsci. Res. Tech. 29 (1994) 90–93.
[34] K. Tanaka, T. Chiba, The vagal origin of preganglionic fibers containing nitric oxide synthase in the guinea-pig heart, Neurosci. Lett. 252 (1998) 135–138.
[35] G.R. Tibbs, E.H. Goulding, S.A. Siegelbaum, Allosteric activation and tuning of ligand efficacy in cyclic-nucleotide-gated channels, Nature 386 (1997) 612–615.
[36] S.R. Vincent, H. Kimura, Histochemical mapping of nitric oxide synthase in the rat brain, Neuroscience 46 (1992) 755–784.
[37] M.A. Vizzard, S.L. Erdman, J.R. Roppolo, U. Forstermann, W.C. de Groat, Differential localization of neuronal nitric oxide synthase immunoreactivity and NADPH-diaphorase activity in the cat spinal cord, Cell Tissue Res. 278 (1994) 299–309.
[38] B.S. Zhu, W.P. Gai, Y.H. Yu, I.L. Gibbins, W.W. Blessing, Preganglionic parasympathetic salivatory neurons in the brainstem contain markers for nitric oxide synthesis in the rabbit, Neurosci. Lett. 204 (1996) 128–132.