**Vagotomy Decreases Excitability in Primary Vagal Afferent Somata**

ERIC LANCASTER, EUN JOO OH, AND DANIEL WEINREICH

1The Neuroscience Program and 2Department of Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, Baltimore, Maryland 21201-1559

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Lancaster, Eric, Eun Joo Oh, and Daniel Weinreich. Vagotomy decreases excitability in primary vagal afferent somata. J Neurophysiol 85: 247–253, 2001. Standard patch-clamp and intracellular recording techniques were used to monitor membrane excitability changes in adult inferior vagal ganglion neurons (nodose ganglion neurons, NGNs) 5 days following section of the vagus nerve (vagotomy). NGNs were maintained in vivo for 5 days following vagotomy, and then in vitro for 2–9 h prior to recording. Vagotomy increased action potential (AP) threshold by over 200% (264 ± 19 pA, mean ± SE, n = 66) compared with control values (81 ± 20 pA, n = 68; P < 0.001). The number of APs evoked by a 3 times threshold 750-ms depolarizing current decreased by >70% (from 8.3 to 2.3 APs, P < 0.001) and the number of APs evoked by a standardized series of (0.1–0.9 nA, 750 ms) depolarizing current steps decreased by over 80% (from 16.9 APs to 2.6 APs, P < 0.001) in vagotomized NGNs. Similar decreases in excitability were observed in vagotomized NGNs in intact ganglia in vitro studied with “sharp” microelectrode techniques. Baseline electrophysiological properties and changes following vagotomy were similar in right and left NGNs. A “sham” vagotomy procedure had no effect on NGN properties at 5 days, indicating that changes were due to severing the vagus nerve itself, not surrounding tissue damage. NGNs isolated after being maintained 17 h in vivo following vagotomy revealed no differences in excitability, suggesting that vagotomy-induced changes occur some time from 1–5 days after injury. Decreased excitability was still observed in NGNs isolated after 20–21 days in vivo following vagotomy. These data indicate that, in contrast to many primary sensory neurons that are thought to become hyperexcitable following section of their axons, NGNs undergo a marked decrease in electrical excitability following vagotomy.

**INTRODUCTION**

The inferior vagal ganglia house the somata of primary vagal afferent neurons (nodose ganglion neurons, NGNs), which provide sensory innervation to the lungs, heart, proximal gastrointestinal tract, aortic baroreceptors, and other thoracic and abdominal viscera. NGNs play an important role in detecting liquid in the upper airway, blood pressure, gastric acidity, hepatic glucose levels, and visceral distension, and they contribute to the febrile and hyperalgesic responses to infection (Mei 1983; Watkins et al. 1994). Vagal afferents may also mediate short-term satiety and satiety-induced analgesia; these functions may contribute to bulimia nervosa (Raymond et al. 1999). In addition, chemical or electrical stimulation of vagal afferents may either facilitate or inhibit nociception, depending on intensity of stimulation (Randich and Gebhart 1992).

Many surgical and pharmacological treatments affect vagal sensory function. Extensive severing of vagal afferents occurs during heart and lung transplants, and may occur during resections of the stomach, esophagus, or intestine. Iatrogenic impairment of vagally mediated airway protection has been reported following the removal of certain neoplasms of the neck, parapharyngeal region, and infratemporal fossa (Leonetti et al. 1996). Carotid endarterectomy (Ballota et al. 1999) and the implantation of electronic vagal stimulators for epilepsy treatment (Amar et al. 1998) have also been reported to damage the vagus nerve. Vagus nerve stimulation with implanted electronic stimulators has been used to treat refractory epilepsy in over 6,000 patients (George et al. 2000), and these devices are currently being studied as a therapy for drug-resistant depression (Rush et al. 2000). The expanding use of these devices increases the importance of understanding vagus nerve injury.

In the rat, bilateral vagotomy induces increased sensitivity to several types of injury and inflammation; this effect has been ascribed to a reduction in the tone of vagally mediated analgesia (Khasar et al. 1998a,b; Miao et al. 1997). Severe vagus nerve bilateral, or just the celiac branches of the vagus nerve, augments inflammatory and nociceptive responses to bradykinin injection, mechanical pinch, and other noxious stimuli (Janig et al. 2000). Either increased pro-nociceptive vagal activity or decreased anti-nociceptive vagal activity could account for these observations.

Despite the importance of the vagus nerve in conveying visceral afferent information, the electrophysiological properties of injured vagal afferents are far less understood than those of injured spinal afferents (i.e., those from dorsal root ganglion neurons, DRGNs) (for review see Devor 1994). Axotomy of DRGNs results from many types of nerve injuries, and chronic pain and allodynia are common results of such injuries. Increased excitability and ectopic spiking noted in axotomized DRGNs may be important in pain syndromes (Devor 1994; Nordin et al. 1984), and blocking ectopic discharge from reaching the CNS may prevent ongoing pain and allodynia (Gracey et al. 1992; Sheen and Chung 1993).

The purpose of the present work was to test whether similar increases in excitability occur in NGNs. We find that within 5 days of a unilateral vagotomy, vagotomized NGNs become profoundly less excitable. Specifically, the current required to...
initiate action potential (AP) firing was increased by over 200%, and the numbers of AP fired in response to standardized strong depolarizing stimuli was reduced by over 80%. Thus this population of visceral afferents responds to injury in a fashion that is radically different from that reported for somatic (dorsal root ganglion, DRG) afferents.

**METHODS**

**Vagotomy**

Vagus nerve injury, vagotomy, was elicited by unilaterally removing a section of the right or left cervical vagus nerve of adult (200–300 g), male Sprague Dawley rats, as approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore. Under ketamine (50 mg/kg ip)/xylazine (10 mg/kg ip) anesthesia, a 5-mm section of the vagus nerve was removed approximatively 1 cm distal to the right or left inferior vagal (nodose) ganglion. This operation severed the afferents processes of approximately 90% of the NGNs on the operated side (with the remainder projecting their afferents via the superior laryngeal nerve, proximal to the nerve injury). We did not observe any gross impairment of respiration or behavior in vagotomized rats, and within 2 days of the operation, rats resumed gaining weight. Vagotomized and control nodose ganglia remained in vivo for some time (usually 5 days) after the operation. Rats were then killed by CO2 inhalation, and nodose ganglia were removed bilaterally. Intact ganglia were used for “sharp” microelectrode recording in vitro, while dissociated NGNs maintained in culture for 2–9 h after plating were used for patch-clamp analysis.

**Dissociation**

NGNs were dissociated enzymatically as described previously (Jafri et al. 1997). Briefly, ganglia were rapidly removed from animals, desheathed, and then incubated in enzyme solution [10 mg collagenase type 1A (Sigma, St. Louis, MO), 10 mg dispase II (Boehringer Mannheim, Mannheim, Germany), and 10 ml Ca2+- and Mg2+-free Hank’s Balanced Salt Solution] for 2 h at 37°C. Neurons were dissociated by trituration, washed by centrifugation (3 times at 700 g for 45 s), suspended in L15 media (GIBCO BRL, Rockville, MD) containing 10% fetal bovine serum (JRH Biosciences, Lenexa, KS), and then transferred onto circular 15-mm glass cover slips (Belco Glass, Vineland, NJ) coated with poly-D-lysine (0.1 mg/ml, Sigma). NGNs adhered to cover slips and were maintained in culture for 2–9 h after plating at 37°C prior to recording.

**Patch-clamp recording**

Whole cell patch-clamp techniques (Hamill et al. 1981) were employed with an Axopatch 200B amplifier and PCLAMP 7 software (Axon Instruments, Foster City, CA). Patch pipettes (1–4 MΩ) were fabricated from glass capillaries (MTW150F-4, World Precision Instruments, Sarasota, FL). Pipettes were filled with a variant of a standard internal solution (composition in mM: 10 NaCl, 30 KCl, 2 Na3HPO4, 1.2 Na2HPO4, 10 HEPES, 0.11 MgCl2, pH 7.3 titrated to pH 7.3 with KOH, 314 mOsm). Except where otherwise specified, all chemicals were from Sigma. Pipette voltage offset was determined from current transients elicited by 5-mV depolarizing steps from a holding potential of −60 mV, delivered using the Membrane Test application of PCLAMP7. Capacitance compensation and 80% Rs compensation were used. Criteria for cell inclusion in the study were as follows: Rs ≤ 10 MΩ, Rm > 100 MΩ, and stable recording with 80% series resistance compensation during the entire experiment. Cover slips were superfused (2–4 ml/min) continuously during recording with room temperature (22–24°C) Locke solution (composition in mM: 10 dextrose, 136 NaCl, 5.6 KCl, 1.2 MgCl2·6 H2O, 2.2 CaCl2·2 H2O, 1.2 NaH2PO4, and 14.3 NaHCO3, equilibrated with 95% O2-5% CO2, pH between 7.3 and 7.5). The recording chamber was grounded via a 3 M KCl agar bridge.

**Sharp electrode recording**

Nodose ganglia were removed from some animals 5 days following vagotomy for study with “sharp” microelectrode recording in vitro. For sharp microelectrode recording, intact ganglia were placed on the floor of the recording chamber, covered with gauge thread, and superfused with Locke solution (3–4 ml/min) at room temperature (22–24°C). Conventional current-clamp recording was performed with an Axoclamp 2A amplifier (Axon Instruments). Sharp microelectrodes filled with 3 M KCl (40–100 MΩ) were inserted into NGNs blindly. Entry into a NGN was confirmed by a resting membrane potential and by the presence of an action potential in response to depolarizing current. Rm was measured from the voltage deflection produced by a 100-pA injection of hyperpolarizing current from a potential of −60 mV, and Em was the membrane potential recorded with zero current injected (corrected for tip potential). Criteria for cell acceptance include the following: an action potential overshooting 0 mV and DC membrane input resistance ≥10 MΩ (typically 20–100 MΩ).

**Statistical methods**

The NGNs associated with a cut vagus (NGNs vagotomized for 5 days) were compared with NGNs from the contralateral (control) ganglia using the statistical software SIGMASTAT (Jandel Scientific, San Rafael, CA). The effects of treatment (vagotomy vs. contralateral control) and intra-animal variation on NGN properties were measured using two-way ANOVA. Vagotomized and contralateral NGNs from animals vagotomized for 17 h were also compared with each other using two-way ANOVA, as were vagotomized and contralateral NGNs from animals vagotomized for 20–21 days, and NGNs from the operated and contralateral ganglia of the “sham” vagotomy animals. To determine whether the properties of contralateral NGNs were altered following vagotomy, vagotomized NGNs, contralateral NGNs, and NGNs from control animals were compared using one-way ANOVA with Dunn’s pairwise comparisons.

The properties of right and left NGNs from control animals were compared using a two-way ANOVA. To determine whether there was a differential effect of right and left vagotomy, a two-way ANOVA examined the effects of vagotomy (vagotomy vs. contralateral control) and side (right vs. left) on NGN properties. P < 0.05 was considered significant for all tests.

**RESULTS**

**Passive and active membrane properties of vagotomized vagal afferents recorded in isolated NGNs**

The vagotomized and contralateral (control) nodose ganglia were removed from 13 animals 5 days after vagotomy and dissociated separately. In eight animals the vagotomy was performed on the right side, and in five animals on the left side. Using the whole cell patch-clamp recording technique, the passive membrane properties, firing properties, and AP characteristics of NGNs from vagotomized ganglia were compared with those of control ganglia. The experimenter was “blind” to cell status (the vagotomized vs. control NGNs). However, the obvious differences in excitability measured in NGNs from
DECREASED EXCITABILITY IN VAGOTOMIZED VAGAL AFFERENTS

TABLE 1. Passive and active membrane properties of isolated control and vagotomized nodose neurons

<table>
<thead>
<tr>
<th>Passive properties</th>
<th>Contralateral Control</th>
<th>Control Animal</th>
<th>Vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{m}$, MΩ</td>
<td>371 ± 21 (67)</td>
<td>432 ± 29</td>
<td>284 ± 20*</td>
</tr>
<tr>
<td>$C_{m}$, pF</td>
<td>34 ± 1.4</td>
<td>32 ± 1.0</td>
<td>46 ± 1.4†</td>
</tr>
<tr>
<td>$E_{m}$, mV</td>
<td>−50 ± 0.8 (67)</td>
<td>−49 ± 1.0 (44)</td>
<td>−53 ± 0.8 (65)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AP characteristics</th>
<th>Contralateral Control</th>
<th>Control Animal</th>
<th>Vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP duration, ms</td>
<td>1.3 ± 0.1 (65)</td>
<td>1.7 ± 0.2</td>
<td>1.2 ± 0.1 (64)</td>
</tr>
<tr>
<td>AP overshoot, mV</td>
<td>49 ± 1.5 (65)</td>
<td>47 ± 1.3</td>
<td>40 ± 2.2 (64)*</td>
</tr>
<tr>
<td>Peak hyperpolarization, mV</td>
<td>−71 ± 0.5 (65)</td>
<td>−71 ± 0.3</td>
<td>−68 ± 0.6 (64)†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Firing properties</th>
<th>Contralateral Control</th>
<th>Control Animal</th>
<th>Vagotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP rheobase, pA</td>
<td>81 ± 20</td>
<td>103 ± 17</td>
<td>264 ± 19†</td>
</tr>
<tr>
<td>Absolute (APs)</td>
<td>16.9 ± 2.3</td>
<td>11.4 ± 1.8</td>
<td>2.6 ± 0.6†</td>
</tr>
<tr>
<td>1 times threshold (APs)</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.1 ± 0.2‡</td>
</tr>
<tr>
<td>2 times threshold (APs)</td>
<td>5.1 ± 0.4</td>
<td>3.7 ± 0.5</td>
<td>1.7 ± 0.4 (65)†</td>
</tr>
<tr>
<td>3 times threshold (APs)</td>
<td>8.3 ± 1.3 (66)</td>
<td>5.6 ± 0.8</td>
<td>2.3 ± 0.4 (65)†</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parentheses are number of neurons for each measurement when different from the total number of neurons in that group. Number of neurons in Contralateral Control is 68, in Control Animal is 46, and in Vagotomy is 66. Passive and active membrane properties of isolated control and vagotomized nodose neurons. $R_{m}$, membrane input resistance; $C_{m}$, membrane capacitance; $E_{m}$, resting membrane potential; AP duration, the width the action potential (AP) measured at 0 mV; AP overshoot, the peak voltage (above 0 mV) recorded during the AP; Peak hyperpolarization, the most negative value of the membrane potential following a single AP; AP rheobase, the minimum amount of current required to evoke a single AP; Absolute firing, the maximum number of APs fired in response to any single 750-ms depolarizing current step of 0.1–0.9 nA magnitude (in 0.1-nA increments); 1, 2, and 3 times threshold, the number of APs fired by an NGN during a 750-ms depolarizing current step of a magnitude 1, 2, or 3 times its rheobase, respectively. Values of vagotomized NGNs were compared to values of control NGNs using 2-way ANOVA (as described in METHODS). *$P < 0.01$, †$P < 0.001$, ‡$P < 0.05$.

Each ganglia allowed the experimenter to guess accurately (13 of 13 experiments) which side was vagotomized.

Passive membrane properties (input resistance and capacitance) were determined in vagotomized and control contralateral NGNs in whole cell voltage clamp at −60 mV using ±2.5-mV voltage steps. The resting membrane potential was estimated in current-clamp mode with zero injected current. The capacitance of vagotomized NGNs (46 ± 1.4 pF, mean ± SE, $n = 66$) was significantly ($P < 0.001$) increased compared with control NGNs (34 ± 1.4 pF, $n = 68$). This indicates that vagotomized NGNs may have decreased sodium current and/or augmented potassium current. There was also a significant decrease in peak hyperpolarization magnitude in vagotomized NGNs (−68 ± 0.6 mV, $n = 64, P < 0.001$) versus control NGNs (−71 ± 0.5, $n = 65$). AP duration was not significantly different in the two groups ($P > 0.45$).

AP firing patterns were also examined in control and vagotomized NGNs. For these experiments, a DC current was applied to adjust baseline membrane potential to between −55 and −60 mV. Subsequently, a series of 750-ms depolarizing current steps were used to evoke APs. For each neuron, a threshold current (rheobase) was determined with incremental (10 pA) 750-ms current steps. Then we applied 750-ms depolarizing current steps to 1, 2, and 3 times threshold and recorded the number of APs evoked by each current step. To determine the responsiveness of NGNs to absolute (as opposed to relative to threshold) depolarizing stimuli, we injected a series of 750-ms incremental current steps (0.1–0.9 nA). As illustrated in Fig. 2, control NGNs typically fired multiple APs in response to maintained suprathreshold stimuli; most neurons accommodated (ceased firing) before the end of the 750-ms current injection for stimuli of any strength. Vagotomized NGNs, by contrast, accommodated much more rapidly, generating a new series of APs when the current was re-applied.
FIG. 2. Action potential firing properties of control and vagotomized nodose neurons. The firing properties of representative control and vagotomized isolated nodose neurons, recorded using patch-clamp methods, are shown. Each neuron was depolarized with 750-ms steps of depolarizing current from a baseline membrane potential between −55 and −60 mV. The control nodose neuron had an AP threshold of 10 pA. At AP threshold, control nodose neurons typically fired a single AP and then accommodated. Increasing the magnitude of injected current to 2 or 3 times threshold elicited multiple APs from most control NGNs. The vagotomized neuron had an AP threshold of 200 pA. Depolarizing currents of 1, 2, or 3 times threshold magnitude only elicited single APs from many vagotomized NGNs.

Passive and active membrane properties of vagotomized vagal afferents recorded in the intact nodose ganglion

To test whether the decrease in excitability observed in isolated vagotomized NGNs may have arisen from neuronal isolation methodology or from the patch-clamp recording methods, we employed sharp microelectrode techniques to record electrical properties from NGNs in the intact nodose ganglia in vitro. Membrane electrical properties from vagotomized (right) and contralateral control ganglia removed 5 days following vagotomy from three rats were compared and are summarized in Table 2. There was a trend (P = 0.28) toward more negative resting membrane potentials in vagotomized NGNs (−56 ± 2 mV, n = 23) versus controls (−51 ± 2 mV, n = 20) and a trend (P = 0.09) toward decreased membrane input resistance in vagotomized NGNs (38 ± 6 MΩ, n = 21) compared with control NGNs (62 ± 10 MΩ, n = 19). Like dissociated vagotomized NGNs, vagotomized NGNs in intact ganglia showed dramatic decreases in action potential discharge in response to supra-threshold depolarizing current injection (Fig. 3). In all NGNs recorded in vagotomized ganglia, only a single action potential could be evoked by 750-ms current steps to 1, 2, or 3 times threshold (in 23 of 23 cells), or by currents steps ranging from 1 to 3 nA (in 15 of 16 cells). In contrast, most (15 of 20) NGNs recorded in contralateral...
(control) ganglia fired multiple (>1) action potentials in response to current steps of 2 times threshold or greater. Vagotomized NGNs fired significantly ($P < 0.01$) fewer APs in response to 2 times threshold stimuli ($1 \pm 0$ vs. $5 \pm 1$ for controls) or 3 times threshold stimuli ($1 \pm 0$ vs. $10 \pm 2$). Significant decreases in the numbers of APs discharged by vagotomized NGNs were also seen in response to 1-, 2-, and 3-nA depolarizing current steps (Table 2). These results from the intact ganglia clearly demonstrate that decreased excitability in vagotomized NGNs was not associated with dissociation procedures, or the whole cell patch-clamp technique.

Excitability in contralateral nodose ganglia

While differences in the excitability of vagotomized and contralateral control NGNs are most likely due to decreased excitability in vagotomized NGNs, it is also possible that the excitability of contralateral NGNs changed. To examine this possibility, we recorded the passive membrane properties, AP characteristics, and firing properties of the right and left NGNs from four control animals. There were no statistically significant differences between the right and left NGNs of control animals for any of the properties measured, so they were tabulated as a single population in Table 1. Further, properties of NGNs from control rats were similar to the properties of NGNs from vagotomized NGNs, so they were tabulated as a single population in Table 1. There were no statistically significant differences between either vagotomized or contralateral NGNs for AP threshold, AP waveform, or the number of APs elicited by the stimulus protocols (2-way ANOVA for each). The only statistically significant difference between the two groups was a modest ($\sim 20\%$) increase in membrane input resistance on the vagotomized side ($626 \pm 60$ MΩ vs. $503 \pm 37$ MΩ for contralateral NGNs). Further, there were no statistically significant differences between either vagotomized or contralateral NGNs from these animals and NGNs from control animals for any of the properties listed in Table 1 (1-way ANOVA).

Effects of right versus left vagotomy on excitability changes in NGNs

The right and left vagus nerves do not innervate visceral tissues in an entirely symmetrical fashion. For example, the liver is innervated predominately by the left vagus (Carobi 1990), the proximal duodenum by the left vagus, and the distal duodenum/jejunum by the right (Berthoud et al. 1997). These differences raise the possibilities that the baseline excitability of right and left NGNs may be different, and that right and left NGNs may respond differently to vagotomy. The data presented in the previous section demonstrate that baseline excitability is similar in right and left NGNs. To test for a differential effect of right and left vagotomy, the effects of treatment (vagotomy vs. contralateral control) and vagotomy side (right or left) on each of the variables in Table 1 were measured using a two-way ANOVA. For each variable, a Student-Newman-Keuls test revealed no significant interaction between the side of vagotomy (right/left) and treatment (vagotomy/control). That is, either right or left vagotomy induced similar decreases in excitability and alterations in membrane and passive properties in NGNs of the vagotomized side.

Sham vagotomy

Although excitability changes are most likely due to severing of the vagus nerve per se, it is possible that they are triggered by damage to tissues surrounding the vagus caused by the vagotomy procedure. To examine this issue, we performed a sham vagotomy surgery on two animals and examined the excitability of their right (sham, $n = 12$) and left (control, $n = 13$) NGNs 5 days later. The sham surgery consisted of exposing the vagus nerve and retracting the surrounding tissues, as during vagotomy, but not touching the nerve itself. The excitability, passive properties, and AP characteristics of the sham and control neurons were similar; no statistically significant differences existed for any of the variables listed in Table 1. Changes in the excitability of NGNs following vagotomy were therefore a result of vagus nerve section, and not damage to surrounding tissues.

Time course

The above data were obtained from NGNs removed 5 days post-vagotomy. To examine the time course of the changes in excitability and passive membrane properties following vagotomy, we repeated similar measurements on NGNs removed 17 h and 20–21 days post-vagotomy.

Properties of vagotomized NGNs ($n = 14$) and contralateral control ($n = 14$) NGNs removed from two animals 17 h after vagotomy were similar to the values for control NGNs shown in Table 1. There were no statistically significant differences between vagotomized and contralateral NGNs for AP threshold, AP waveform, or the number of APs elicited by the stimulus protocols (2-way ANOVA for each). The only statistically significant difference between the two groups was a modest ($\sim 20\%$) increase in membrane input resistance on the vagotomized side ($626 \pm 60$ MΩ vs. $503 \pm 37$ MΩ for contralateral NGNs). Further, there were no statistically significant differences between either vagotomized or contralateral NGNs from these animals and NGNs from control animals for any of the properties listed in Table 1 (1-way ANOVA).

Significantly decreased neuronal excitability was still apparent in vagotomized NGNs removed from two animals 20–21 days following surgery, indicating that the effects of vagotomy were maintained at least this long. Specifically, vagotomized NGNs ($n = 13$) had significantly ($P < 0.05$) higher rheobase ($212 \pm 46$ vs. $56 \pm 1$ pA), and fired significantly fewer APs in response to 3 times threshold stimuli ($6 \pm 1.4$ vs. $34 \pm 48$ APs) and fewer APs in response to the standardized series of current steps ($4.4 \pm 1.2$ vs. $5.5 \pm 1.4$ APs) than their contralateral controls ($n = 15$). Vagotomized NGNs also had decreased AP overshoot ($34 \pm 3.4$ vs. $47 \pm 2.4$ mV for controls) and slightly increased capacitance ($38 \pm 2$ vs. $31 \pm 2$ pF for controls). Interestingly, while the differences between vagotomized NGNs and contralateral control NGNs persisted, there was a trend toward increased excitability in both groups relative to the earlier time points. This observation was not pursued in the current work.

DISCUSSION

Vagotomy dramatically decreased the excitability of NGNs, increasing AP threshold by over 200%, and reducing AP discharge by up to 80% in response to strong depolarizing
stimuli. Despite differences in the target organs of the right and left vagus nerves, baseline excitability and changes induced by vagotomy were similar in right and left NGNs. Sham operations did not duplicate these changes in excitability, suggesting that local inflammatory reactions near the vagus nerve do not trigger changes in the neurons. While the mechanism of vagotomy-induced hypoexcitability in NGNs is unknown, the time course reported here was similar to that of ionic current changes in axotomized DRG neurons that appear to be due to the removal of peripheral trophic factors (Dib-Hajj et al. 1998; Fjell et al. 1999). Vagotomy or the disruption of axoplasmic transport has also been shown to change the expression of mRNA and protein for various neuroepitopes in the nodose ganglia (Zhuo et al. 1994, 1995), with a time course similar to that of the excitability changes we report here.

Approximately 90% of NGNs are C-fiber afferents and the remainder A-afferents (Gallego and Adrover 1990; Gallego and Eyzaguirre 1978; Mei 1983). Previous investigators (Schild et al. 1994) have classified isolated NGNs as presumed A- or C-type afferents based on their electrophysiological characteristics, specifically the presence of a “hump” on the falling phase of the C-cell action potential. Because the AP characteristics of NGNs are changed by vagotomy, we avoided classifying our cells in this regard. Due to the large differences between control and vagotomized NGN populations, we can conclude that most C-type NGNs become less excitable following vagotomy. Additional experiments will be required to definitively establish whether A-afferents are also affected, and whether specific populations of C-afferents (heart afferents, airway afferents, etc.) respond differently to vagotomy.

The concordance of data obtained with patch pipettes in isolated NGNs and sharp microelectrode recording in intact ganglia demonstrated that excitability changes observed were unlikely to have arisen from an artifact of the dissociation process or cell dialysis during patch-clamp recording. The close correspondence of the two data sets (patch and sharp electrode) further validated the use of the isolated soma as a model for studies of excitability of rat nodose ganglion neurons.

The excitability of control NGNs in our study differed from those described previously by Schild et al. (1994), who noted that most neonatal rat NGNs fire only a single AP in response to suprathreshold depolarizing current steps. However, many nodose A- and C-fiber afferents of the adult rabbit and adult cat discharge multiple action potentials in response to suprathreshold depolarizing stimuli (Gallego and Eyzaguirre 1978; Jaffe and Sampson 1976). This suggests that differences between our observations and those of Schild et al. (1994) may reflect a difference between neonatal and adult afferents, although differences in tissue culture conditions, external and internal solutions, and recording protocols may also contribute to the disparate results. Based on the similarity between accommodative AP properties observed in intact ganglia with sharp microelectrodes and in acutely dissociated NGNs with patch pipettes, we believe our results may represent an accurate description of the response of adult rat NGNs to sustained depolarizing stimuli.

The somata and the neuroma of some axotomized DRG neurons have been implicated as regions generating spontaneous AP activity (Wall and Devor 1983). By contrast, the somata of vagotomized NGNs were less excitable than control NGN somata by every measure examined, and less able to generate APs in response to sustained depolarizing current. Our work did not directly study whether vagal neuromas could generate spontaneous AP activity. However, the neuromas were attached to nodose ganglia during our “sharp” microelectrode experiments on vagotomized NGNs, and we did not observe spontaneous APs in any of the NGNs during excitability measurements. It is therefore possible that the vagal neuroma, like the somata of axotomized NGNs, is also electrically hypoexcitable (relative to those of spinal afferents).

Electrical activity in the somata of NGNs, like those of DRGs, may not be necessary for AP conduction to the CNS. Thus, the question arises whether excitability changes recorded in primary sensory somata adequately reflect changes in the electrophysiology of the entire neuron (including central and peripheral axons, growth cone, and central terminals). Although our work reveals that the somata of vagotomized NGNs are hypoexcitable, and conduction velocity has been reported to be decreased in vagotomized vagal C-fiber afferents (Gallego and Adrover 1990), additional work will be required to determine whether AP generation is suppressed throughout the entire vagotomized nodose neuron.

Following vagotomy, rats display enhanced responses to several types of noxious stimuli (Khasar et al. 1998a,b; Miao et al. 1997). Modified pain responses are consistent with the observation that vagal stimulation can either enhance or inhibit nociception, depending on the circumstances (Randich and Gebhart 1992; Ren et al. 1988). If vagal afferents become hyperexcitable following vagotomy, this would suggest continual pro-nociceptive input from the nodose ganglia is enhancing pain responses. However, our data suggest that hyperalgesia in vagotomized rats is more likely a consequence of decreased vagal anti-nociceptive input resulting from a profound decrease in NGN excitability, combined with the removal of normal stimuli.

Profound changes in voltage-gated sodium, potassium, and calcium currents have been reported in axotomized DRG neurons (Baccei and Kocsis 2000; Cummins and Waxman 1997; Everill and Kocsis 1999) and are thought to underlie changes in excitability in these neurons. While changes in passive membrane properties in vagotomized NGNs (3 mV more negative $E_{m}$, 35% increased $C_{m}$, and 23% decreased $R_{m}$) may contribute slightly to increased rheobase, the 200% increase in rheobase and substantial decreases in AP discharge are very likely the results of changes in voltage-dependent ionic currents. The ionic current(s) altered to support decreased excitability in vagotomized NGNs are presently unknown. Based on the data presented here and the roles of sodium and potassium currents in shaping NGN AP discharge (Schild and Kunze 1997; Schild et al. 1994), a reduction in sodium current and/or an augmentation of potassium current is likely. Either change could produce increased AP threshold, decreased AP overshoot, and decreased AP discharge. Additionally, the most likely explanation for more negative resting membrane potentials in vagotomized NGNs is an increased potassium conductance at rest. The relatively homogenous response of NGNs, a population composed mostly (~90%) of C-fiber afferents (Gallego and Adrover 1990; Gallego and Eyzaguirre 1978; Mei 1983), to nerve injury provides a tractable model system for studying the role of ionic currents in shaping AP discharge after injury.
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REFERENCES


Jafari MS, Moore KA, Taylor GE, and Weinreich D. Histamine H1 receptor activation blocks two classes of potassium current, I_K(ramp) and I_AHP to excite ferret vagal afferents. *J Physiol (Lond)* 503: 533–546, 1997.


