Chemical Communication Between Vagal Afferent Somata in Nodose Ganglia of the Rat and the Guinea Pig In Vitro

EUN JOO OH AND DANIEL WEINREICH
Department of Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, Baltimore, Maryland 21201-1559

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Oh, Eun Joo and Daniel Weinreich. Chemical communication between vagal afferent somata in nodose ganglia of the rat and the guinea pig in vitro. J Neurophysiol 87: 2801–2807, 2002; 10.1152/jn.00768.2001. The cell bodies of spinal afferents, dorsal root ganglion neurons, are depolarized several millivolts, and their probability of spiking increased when axons of neighboring somata in the same ganglion are electrically stimulated repetitively. This form of neural communication has been designated cross-depolarization (CD) and cross-excitation (CE). The existence of CD and CE between somata of vagal afferents (nodose ganglion neurons, NGNs) of rats and guinea pigs was investigated by electrically stimulating the vagus nerve while recording the electrical activity of NGNs in intact nodose ganglia with sharp intracellular microelectrodes. CD and CE in NGNs were manifested by a membrane depolarization (∼4 mV), the presence of spontaneous action potentials, and a decreased spike threshold. CD was dependent on the frequency and intensity of vagal nerve stimulation. Two distinct types of CD were observed: 1) in NGNs with large input resistances (Rm), CD was dependent on [Ca2+]m, associated with increased membrane conductance, and had an extrapolated reversal potential (Em,n) value of about −25 mV; and 2) in NGNs with low Rm, CD was independent of [Ca2+]m, not accompanied by a membrane conductance change, or a measurable Em,n value. These data reveal the existence of a chemical communication pathway between vagal afferent somata and suggest the possibility that communication between different visceral organs may occur at the level of the primary vagal afferent neuron.

INTRODUCTION

Vagal primary afferent neurons (nodose ganglia neurons, NGNs), like dorsal root ganglion neurons (DRGNs), lack specialized synaptic contacts between their somata (Lieberman 1976) implying that each sensory afferent neuron conveys information from peripheral target tissues to the CNS independently. However, this dogma has been challenged during the last decade. DRG somata are transiently depolarized and become more excitable by repetitive action potential activity in neighboring axons in the same DRG. This form of nonsynaptic communication is measurable in most DRGNs, and it has been designated cross-depolarization (CD) and cross-excitation (CE), respectively (Amir and Devor 1996; Devor and Wall 1990). CD and CE have been shown to be mediated by the release of a diffusible signal molecule(s) whose identity remains unknown (Amir and Devor 1996, 2000). The function of CD and CE under normal physiological conditions is speculative, perhaps supporting the coordinate communication between different dermatomes. Under pathological conditions such as nerve damage, CD and CE have been implicated as mechanisms contributing to the hyperexcitability typical of injured DRGNs (Amir and Devor 1996; however, see Liu et al. 1999).

NGNs innervate a variety of visceral targets, including the heart, airway, and gastrointestinal tract. CD and CE, if present in primary vagal somata, could have important implication for visceral sensory integration. The current work tests whether CD and CE exist among NGNs of the guinea pig and the rat. Here we report that CD and CE are readily detectable among NGNs and they share many of the same mechanisms reported for DRGNs.

METHODS

Animals and tissue preparation

Right nodose ganglia (NG) from adult male rats (n = 17) or guinea pigs (n = 7) with ∼2 cm of the peripheral vagus nerve (VN) attached were dissected with or without the recurrent laryngeal nerve (RLN). Connective tissues surrounding the NG were carefully removed. Tissues were mounted in a recording chamber and perfused with warm (33–35°C) Locke solution containing (in mM) 136 NaCl, 5.6 KCl, 1.2 MgCl2, 2.2 CaCl2, 14.3 NaHCO3, 1.2 NaH2PO4, and 10 dextrose, equilibrated with 95% O2-5% CO2, pH 7.2–7.4. Figure 1 depicts the three different stimulating conditions used in this study.

Electrophysiology

NGNs were impaled with a sharp microelectrode, 40–80 MΩ when filled with 3 M KCl. Electrical membrane properties were recorded in current- and voltage-clamp modes using an Axoclamp II amplifier (Axon Instruments, Union City, CA) as described by Jafri and Weinreich (1998). Silver bipolar electrodes were used to stimulate the VN and the RLN in a separate mineral oil–filled chamber. Criteria for accepting NGNs for study included the following: resting membrane potential less than or equal to −45 mV, input resistance greater than or equal to 10 MΩ, and an action potential overshooting 0 mV. NGNs were classified as either A-type or C-type according to their conduc-

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The initiation of the somal spike elicited by a single suprathreshold stimulus (0.1 ms in duration) applied to the RLN or VN. Rheobase was determined by applying incremental intracellular depolarizing current pulses, 10 ms in duration, until spike threshold was achieved.

To characterize the dependence of CD on stimulus frequency and intensity of nerve trunk stimulation, different frequencies (10–100 Hz) and stimulus intensities (50, 75, or 90% of the stimulus strength necessary to elicit a somal action potential or an absolute stimulus) were applied to the VN or RLN. Absolute stimuli were applied when neuronal axons were not included in the VN, and these intensities (10, 20, or 30 V) were used based on the axonal threshold of other neurons in the preliminary experiments (11.3 ± 1.0 V, mean ± SE; range 5.8–24 V; n = 21). To evaluate the excitability change during CD, subthreshold transmembrane depolarizing current (80% of rheobase) was injected via the intracellular recording electrode. The duration of each stimulus was 10 ms, and 10 stimuli were applied for 5 s (2 Hz). Firing probability was defined as the number of trials that induced action potential firing per 10 trials (Amir and Devor 1996). Reversal potential of CD was estimated by extrapolation, using a ramp voltage protocol (−90 to −50 mV for 500 ms; 0.08 mV/ms) in voltage-clamp mode. Nominal 0 mM [Ca²⁺], Locke solution, balanced with elevated [Mg²⁺], was bath applied for ≥3 min to test whether CD was dependent on influx Ca²⁺ (e.g., on neurotransmitter release). When the presence of CD was ambiguous, we defined CD as a depolarization greater than 0.5 mV and an onset delay less than 1 s. There were only four NGNs (among 111 NGNs) showing CD less than 1 mV (0.8–0.9 mV). Data acquisition and analysis were performed using pClamp 8 software and a Digidata 1200 interface (Axon Instruments).

RESULTS

Prevalence of CD

Several distinct protocols were used to elicit CD. For NGNs whose axons were contained in the vagus (VN, Fig. 1A), CD was evoked by trains of depolarizing stimuli applied to the VN at current intensities that were 10% below that needed to evoke a orthodromic somal action potential. For NGNs whose axons were not contained in the VN (Fig. 1, B and C), an absolute stimulus intensity of 20 V was used.

The data depicted in Fig. 2 illustrate CD and CE recorded in NGNs. Application of a 10 s train (100 Hz, at an intensity 10% below somal spike threshold; see Fig. 1) to the VN evoked a 5 mV membrane depolarization that lasted ~30 s. During CD the excitability of this NGN was enhanced as judged by the dramatic increase in neuronal firing probability (Fig. 2B). In some NGNs CD was sufficient to evoke spontaneous action potential.
activity (Fig. 2C). Because CD averaged ~4 mV, it is likely that CD-induced spontaneous action potential activity originated outside the cell body perhaps in the stem process.

Using a standard stimulating protocol of 50 or 100 Hz for 10 s, 67% of 165 NGNs sampled showed CD with an average amplitude of 3.7 ± 0.28 mV (mean ± SE). Based on the conduction velocity of their axons, NGNs can be classified into two classes; 90% of the NGN are C-fibers having conduction velocities <1.5 ms, the others are A-type fibers with conduction velocities >1.5 m/s (Marsh et al. 1987; Stansfeld and Wallis 1985; Undem et al. 1993). NGNs showing CD were similarly distributed in both classes of NGNs, about 47% of A-type NGNs and 67% of C-type NGNs (Table 1). In 34 NGNs (in the case of Fig. 1B), conduction velocities were not measured. In this population of NGNs, 79% of the cells revealed CD averaging 4.2 ± 0.60 mV.

Several electrical membrane properties were examined to test whether neurons showing CD [CD(+)] could be differentiated from NGNs without CD [CD(−)]. Although the resting membrane potentials were nearly identical in both sets of neurons, CD(+) neurons had significantly higher resting membrane conductances and elevated thresholds for action potential detonation than did CD(−) neurons (Table 2).

**Frequency dependence of CD**

The amplitude of CD was dependent on the frequency of stimuli applied to adjacent NGN axons (Fig. 3A). To compare the efficacy of different frequencies, CD evoked by a given frequency was normalized as a percentage of maximum CD (amplitude produced by 50 or 100 Hz stimulation, Fig. 3B). The amplitude of CD grew with increasing frequency over the range of 10–50 Hz (Fig. 3A). The population results (Fig. 3B) revealed that the amplitude of CD saturated at frequencies above 30 Hz., and that CD evoked by 10 Hz stimulation was significantly smaller in amplitude than at higher frequencies. It is possible that CD can be evoked with frequencies below 10 Hz, but the response amplitude would be about 10% of maximum or a membrane depolarization <1 mV. This response would be difficult to discriminate from baseline noise without averaging.

Because bursting stimuli might more closely mimic physiological activity such as lung inflation and deflation, we tested whether bursting stimuli might also evoke CD. Using bursts of stimuli for 10 s with 30 or 50 Hz intraburst frequency and 1 or 2 Hz interburst frequency, CD was 2.6 ± 0.51 mV in 4/6 NGNs tested with these protocols. Thus it appears that bursting stimuli were as efficacious as continuous stimulation in eliciting CD. There might be an optimal bursting pattern for CD expression, but we did not explore other bursting stimuli in the present study.

**Stimulation intensity dependence of CD**

To examine the relation between the magnitude of CD and the relative number of activated axons, we varied the intensity

![FIG. 3. Frequency dependence of CD. A: different stimulation frequencies induced altered amplitudes of CD. Higher frequencies of stimulation induced larger amplitude CDs. However, increasing stimulation frequency above 30 Hz did not further increase CD amplitude. This NGN had a resting membrane potential of −65 mV, Rm 50 MΩ, and an axonal conduction velocity of 1.1 m/s. Horizontal bar indicates the duration of tetanic stimulation (10 s, 90% of somal spike threshold). B: the frequency dependence of CD for 5 NGNs from 5 different animals was statistically analyzed. The Friedman test revealed that there was a significant difference among the different frequencies tested with 95% confidence (x2 9.240; df 3; P = 0.026). Post hoc comparison with the Newman-Kuels test showed significant differences in 10 Hz vs. 30, 50, and 100 Hz with 95% confidence (indicated by asterisks). Bars indicate SE.](http://jn.physiology.org/)

**TABLE 1. Incidence and size of cross-depolarization**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>A Neurons</th>
<th>C Neurons</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD(+) NGNs, %</td>
<td>67 (111/165)</td>
<td>47 (9/19)</td>
<td>67 (75/112)</td>
<td>27 (13/34)</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>3.7 ± 0.28</td>
<td>3.7 ± 0.57</td>
<td>3.5 ± 0.35</td>
<td>4.2 ± 0.60</td>
</tr>
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Amplitude values represent means ± SE. A neurons and C neurons had conduction velocities of ≥1.5 m/s and <1.5 m/s, respectively. Numerators in parentheses are the number of nodose ganglion neurons (NGNs) showing cross-depolarization (CD), and denominators are the total numbers of NGNs recorded. The percentage of NGNs showing CD [CD(+) and CD(−)] ranged from 29 to 100% in the 31 animals studied; the mean ± SE was 70 ± 3.7%.

**TABLE 2. Electrical membrane properties of CD(+) and CD(−) NGNs**

<table>
<thead>
<tr>
<th></th>
<th>CD(+)</th>
<th>CD(−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>111</td>
<td>54</td>
</tr>
<tr>
<td>Emem, mV</td>
<td>−60 ± 0.7</td>
<td>−60 ± 0.8</td>
</tr>
<tr>
<td>Rm, MΩ</td>
<td>37 ± 2.9</td>
<td>53 ± 5.0</td>
</tr>
<tr>
<td>Rheobase, nA</td>
<td>1.3 ± 0.13</td>
<td>0.7 ± 0.09</td>
</tr>
<tr>
<td>P values</td>
<td>0.773</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of cells. T-test was used for Emem and Mann-Whitney U test for Rm and rheobase. Emem, resting membrane potential; Rm, input resistance. CD(+) and CD(−) signify nodose ganglion neurons (NGNs) with or without CD, respectively. For abbreviations, see Table 1.
of the stimulus applied to the VN. In the case of NGNs whose axons could be directly activated by the stimulating electrodes on the VN (Fig. 1A), the intensity of stimulation was varied as a percentage of the threshold stimulus required to evoke a somal action potential. The maximal intensity used to evoke CD with this paradigm was a stimulus 90% of threshold. As stimulation intensity decreased to 75 and 50% of threshold, the amplitude of CD decreased (Fig. 4A). The amplitude of CD was normalized as a percentage of maximum CD (Fig. 4B).

With this protocol, the amplitude of CD was directly related to the magnitude of applied stimulus, presumably reflecting the number of activated neighboring axons and somata.

In the case of NGNs whose axons were not present in the VN, due to axons exiting proximal to the placement of the stimulation electrode (Fig. 1, B and C), the stimulation intensity was varied in absolute values, 10, 20, or 30 V. The amplitude of CD was normalized to the amplitude produced by 20 V stimulation. A stimulus of 30 V induced a CD 62% larger (29 ± 12.4%; n = 3; P = 0.005) than that produced by a 20 V stimulus.

Calcium dependence and reversal potential of CD

If CD is mediated by neurotransmitter(s) released from NGNs, its amplitude should be reduced by lowering the concentration of Ca2+ in the Locke solution, or application of cadmium (Cd2+), a nonselective blocker of voltage-dependent Ca2+ channels (VDCC). Switching to a Locke solution containing nominally zero mM Ca2+, or one containing 100 μM Cd2+, reversibly blocked CD in 12 of 20 NGNs tested; the remaining NGNs utilized a different mechanism to generate CD (see following text). The data in Fig. 5A illustrate the reversible block of CD produced by switching to a Locke solution containing nominally zero Ca2+ (see also Table 3). These results suggest that CD is produced by the release of a chemical signal molecule in this population of NGNs. Further support for this inference was obtained by estimating a reversal potential (E\text{rev}) value for CD. NGNs were voltage clamped near their resting potential of −60 mV. During the peak inward current evoked by a CD stimulus, voltage-clamp ramps (−90 to −50 mV) were applied, and the E\text{rev} values were estimated by linear extrapolation of the current traces produced by ramp commands before and during the CD responses (Fig. 5B). The slope

![Image](http://jn.physiology.org/content/jn/87/6/2804/F5.large.jpg)
of the current trace recorded during the CD response was always larger than control indicating that CD was associated with an increased membrane conductance. The estimated $E_{\text{rev}}$ in the experiment shown in Fig. 5B was $-36$ mV; in two other cells, $E_{\text{rev}}$ values were $-15$ and $-23$ mV, suggesting that CD was probably due to an opening of ionic channels. Because Cl$^-$ equilibrium potential is near $-30$ mV in these neurons (Galagher et al. 1978), the present data cannot distinguish between CD being produced by an increase conductance to Cl$^-$ or activation of a nonelective cation conductance. Additional work will be required to determine which ionic mechanism underlies this component of CD.

In 8/20 NGNs, lowering the concentration of extracellular Ca$^{2+}$ or the addition of 100 $\mu$M Cd$^{2+}$ did not alter the magnitude of CD (Fig. 6A). When the electrical membrane properties of these NGNs were compared with those with Ca$^{2+}$- and Cd$^{2+}$-sensitive CD, interesting differences emerged: their membrane potentials were 15 mV more hyperpolarized, their resting membrane conductance was more than doubled, and their spike threshold was nearly six times higher (Table 3). The possibility that these cells were glia was ruled out by observing the presence of overshooting action potentials in response to intracellular current injection or following VN stimulation. The extrapolated current traces produced by ramp voltage-clamp commands during a CD stimuli were always parallel to control current traces (Fig. 6B) and never revealed a tendency to cross one another. Thus CDs in these NGNs were not associated with a conductance change nor with a reversal potential value, suggesting that the opening or closing of ionic channels is not critical for CD in this population of NGNs. It is possible that the membranes in this population of CD(+) NGNs are so “leaky” that their membrane potential values closely follow changes in $E_K$ produced by elevated extracellular potassium concentrations associated with the excitation of neighboring neurons. This interpretation is supported by the finding that CD from this population of NGNs had larger amplitudes (179 ± 52.7%) and slower decay rates (70% decay time: 140 ± 9.8%) when nodose ganglia was superfused with 10 $\mu$M ouabain to block the Na$^+$/K$^+$ pump ($n = 5$).

**Effects of $\alpha$-tubocurarine on CD**

In NGNs $\alpha$-tubocurarine (dTC) can block the actions of many endogenous substances that cause a membrane depolarization with an associated increase in membrane conductance. For example, the depolarizing actions of $\gamma$-aminobutyric acid (GABA), acetylcholine (ACh), and 5-hydroxytryptamine (5-HT) are reversibly abolished by low micromolar concentrations of dTC (Higashi et al. 1982). Bath application of 10 $\mu$M dTC reversibly blocked or reduced CD in four of seven NGNs tested (76 ± 16.7% reduction, $n = 4$; range, 30–100%). The resting membrane potential in these NGNs averaged $-58 \pm 1.7$ mV and $R_m$ 75 ± 11.9 $\Omega$. Interestingly, the three NGNs whose CD were unaffected by dTC application had a hyperpolarized resting membrane potential (−68 ± 2.6 mV), low $R_m$ (23 ± 3.3 $\Omega$), and high rheobase (1.4 ± 0.27 nA). These results with dTC further support the hypothesis that there are at least two different mechanisms for CD; one involving a transmitter-mediated opening of ionic channels, and another produced by the activity-evoked accumulation of potassium in the extracellular space.

**DISCUSSION**

The principle finding of this work is that about 70% of vagal sensory somata housed in the nodose ganglion communicate with one another. In more than one-half of these neurons, this coupling is accomplished via an activity-dependent release of a diffusible signal molecule. Because there are no reported synaptic connections between nodose ganglion somata (Liebermann 1976), this unconventional mode of communication must occur via the release of signal molecules directly from somata, stem processes, or intraganglionic axons. Release of neurotransmitter substances from somal compartments of sensory neurons is not unprecedented; this phenomenon has been reported in rabbit NGNs (Palouzier-Paulignan et al. 1992), in acutely dissociated DRG neurons (Huang and Nehér 1996), and in trigeminal neurons in vivo (Neubert et al. 2000; Ulrich-Lai et al. 2001).

The properties of CD and CE (cross-talk) observed among NGNs were qualitatively similar to those previously reported.
for DRG neurons by Amir and Devor (1996). Thus these results suggest that cross-talk is a general physiological property of mammalian primary sensory neurons including both somatosensory and visceral sensory afferents. However, we observed two distinct mechanisms associated with CD: one showing a $E_{\text{rev}}$ value, an increase membrane conductance, a dependency on extracellular Ca$^{2+}$, and blocked by bath applied dTC application, reflecting nonsynaptic chemical communication; the other being devoid of a measurable $E_{\text{rev}}$ value, no change in slope conductance, not dependent on extracellular Ca$^{2+}$, and unaffected by dTC application. This latter mechanism probably reflects an activity-dependent elevation of extracellular K$^+$ concentration ($[K^+]_o$) and subpopulation of CD($\uparrow$) NGNs are susceptible to this ionic change, especially if NGNs have low $R_{\text{in}}$. This hypothesis is supported by the observation by Utzschneider et al. (1992) that $[K^+]_o$ increases with similar time course with CD in rats. Our results differed in one respect from those reported for DRG neurons; namely, NGNs showed an increase in membrane conductance rather than a decrease during CD. We do not know the source of this difference. It may be attributable to methodological differences because we used ramp voltage-clamp commands to estimate slope conductance directly while Amir and Devor (1996) used current-clamp and hyperpolarizing rectangular current steps to estimate changes in membrane input resistance. Alternatively, NGNs might use different signal molecules than those in DRG neurons to support cross-depolarization.

### Possible chemical mediators

Any substance endogenous to NGNs that increases membrane conductance and has a $E_{\text{rev}}$ value near $-25$ mV would be a viable candidate mediator for cross-depolarization. Some candidate substances that meet these criteria include GABA, a viable candidate mediator for cross-depolarization. Some Ebrane conductance and has a

### Functional implication of cross-talk in vagal afferent somata

In the somatosensory system of the rat, intact muscle afferent neurons develop a spontaneous discharge of action potentials when neighboring neurons are axotomized. The mechanism of this phenomenon is speculated to be a paracrine signal produced by damaged neurons (Michaelis et al. 2000). Airway afferent neurons can fire more than 50 Hz when responding to lung inflation in physiological condition (Corderil and Corderil 1984). It is possible that under pathophysiological conditions impulse frequency in vagal afferents might correspond to our experimental conditions. For example, in the presence of airway inflammation, airway afferents can be sensitized by inflammatory mediators and generate action potential synchronously during lung inflation with high frequency. This induction of action potentials could allow neuronal cell body to release neurotransmitter(s) and depolarize neighboring neurons. Although the average amplitude of CD is small, about 4 mV (range 0.8–18.6 mV), some neurons demonstrated spontaneous action potentials on CD. Other neurons revealed an increase in firing probability, suggesting a lowering of rheobase. So, despite the relatively small magnitude of CD, neurons can generate an action potential to subthreshold stimuli. It is possible that the low magnitude of CD may signal that CD is generated at some distance from the somal site of recording, perhaps somewhere in the stem process.

Numerous clinical examples exist whereby pathological disturbances in one organ elicits changes in the function of another organ. A clear relation exists between chronic cough and gastroesophageal reflux disease (Irwin et al. 2000) or between airway hyperresponsiveness and irritable bowel syndrome (White et al. 1991). In animal models, esophageal stimulation by HCl causes airway neurogenic inflammation (release of tachykinin from peripheral vagal afferent nerve endings, Hamamoto et al. 1997). The neural mechanisms for esophageal-bronchial reflex in humans, as in animal models, remain unresolved. Several distinct neural pathways have been implicated that include central sensitization and peripheral mechanisms, i.e., via a local axon reflex mechanism (Canning 1999; Fischer et al. 1998; Hamamoto et al. 1997; Irwin et al. 2000). The existence of CE and CD between neuronal somata in nodose ganglion reported in the current work suggest an additional site for esophageal-bronchial communication; namely, CE between esophageal and airway afferents at the level of the vagal afferent cell body housed in the nodose ganglion. However, before testing this hypothesis we must first demonstrate that cross-talk between nodose somata exists in vivo.

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