Responses and Afferent Pathways of C1–C2 Spinal Neurons to Cervical and Thoracic Esophageal Stimulation in Rats

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Qin, Chao, Margaret J. Chandler, Chuanchau J. Jou, and Robert D. Foreman. Responses and afferent pathways of C1–C2 spinal neurons to cervical and thoracic esophageal stimulation in rats. J Neurophysiol 91: 2227–2235, 2004. First published December 24, 2003; 10.1152/jn.00971.2003. Because vagal and sympathetic inputs activate upper cervical spinal neurons, we hypothesized that stimulation of the esophagus would activate C1–C2 neurons. This study examined responses of C1–C2 spinal neurons to cervical and thoracic esophageal distension (CED, TED) and afferent pathways for CED and TED inputs to C1–C2 spinal neurons. Extracellular potentials of single C1–C2 spinal neurons were recorded in pentobarbital-anesthetized male rats. Graded CED or TED was produced by water inﬂation (0.1–0.5 ml) of a latex balloon. CED changed activity of 48/219 (22%) neurons; 34 were excited (E), 12 were inhibited (I), and 2 were E/I. CED elicited responses for 18/18 neurons tested after ipsilateral cervical vagotomy, for 12/14 neurons tested after bilateral vagotomy and for 9/11 neurons tested after bilateral vagotomy and C6–C7 spinal cord transection. TED changed activity of 31/190 (16%) neurons (28E, 3I). Ipsilateral cervical vagotomy abolished TED-evoked responses of 5/12 neurons. Bilateral vagotomy eliminated responses of 2/4 neurons tested, and C6–C7 spinal transection plus bilateral vagotomy eliminated responses of 2/2 neurons. Thus inputs from CED to C1–C2 neurons most likely entered upper cervical dorsal roots, whereas inputs from TED were dependent on vagal pathways and/or sympathetic afferent pathways that entered the thoracic dorsal roots. These results supported a concept that C1–C2 spinal neurons play a role in integrating visceral information from cervical and thoracic esophagus.

INTRODUCTION

Esophageal pain is often referred to somatic structures associated with angina pectoris, such as the submucosal region, upper abdomen, face, neck, and upper back (Bernstein and Baker 1958; Kramer and Hollander 1955; Polland and Bloomfield 1931). Therefore pain of esophageal origin is frequently confused with cardiac pain in clinical practice (Bernstein et al. 1962; Davis et al. 1982; Foreman 1999; Kramer and Hollander 1955; Lee et al. 1985; Richter et al. 1989). Previous studies in cats and rats have shown that upper thoracic dorsal horn neurons responsive to thoracic esophageal afferents also receive inputs from the thoracic wall, triceps, axillary regions, and forelimbs (Euchner-Wamser et al. 1993; Garrison et al. 1992; Qin et al. 2003a). This convergence of visceral and somatic inputs provides a neural basis for explaining how esophageal pain is referred to the chest. However, neural mechanisms for referred pain from the cervical esophagus as well as pain originating in the thoracic esophagus and referred to the neck and head are unknown.

Previous studies from this laboratory in monkeys and rats have suggested a neural mechanism for referred pain that originates in the heart but is perceived in the neck and jaw. Chemical or electrical stimulation of cardiac vagal and sympathetic afferent fibers activate C1–C3 spinothalamic tract and dorsal horn neurons that receive somatic inputs from the neck, ears, jaw, and face areas (Chandler et al. 1996, 2000; Foreman 1999; Fu et al. 1992; Qin et al. 2001; Zhang et al. 1997). Furthermore, gastric distension and stimulation of splanchic afferent fibers activate upper cervical neurons (Akeyson and Schramm 1994; Qin et al. 2003c). Afferent information from the esophagus enters the CNS via spinal visceral afferent fibers and via vagal afferent fibers to the nucleus of the solitary tract (Clerc 1983; Collman et al. 1992; Fryscak et al. 1984; Hudson and Cummings 1985; Mei 1983; Neuhuber 1987; Neuhuber and Clerc 1990). Additionally, labeled neurons have been identified in upper cervical dorsal root ganglia after injection of anatomical tracers into the cervical esophagus of rats (Uddman et al. 1995). Based on the preceding results, we hypothesized that stimulation of the esophagus would activate upper cervical spinal neurons and thus provide a neural mechanism underlying esophageal pain referred to the neck and head.

The purposes of this study were to quantitatively compare responses of C1–C2 spinal neurons to cervical and thoracic esophageal distension (CED, TED) and to examine afferent pathways for CED and TED input to C1–C2 segments. Results showed that ~20% of C1–C2 spinal neurons had excitatory and/or inhibitory responses to CED or TED. Most neurons responding to CED or TED received convergent somatic input from head, ears, neck, and shoulder areas. Sensory inputs from CED to C1–C2 spinal neurons primarily entered upper cervical dorsal roots, whereas inputs from TED primarily traveled in vagal pathways to reach the upper cervical spinal cord. A preliminary report of this work has been presented in abstract form (Jou et al. 1998).

METHODS

Experiments were performed in 53 male Sprague-Dawley rats (Charles River) weighing between 330 and 460 g. After animals were initially anesthetized with pentobarbital sodium (60 mg/kg ip), catheters were inserted into the right carotid artery to monitor blood pressure and into the left jugular vein to inject saline and drugs. During the experiment, a continuous intravenous infusion of pentobarbital (5–10 mg · kg⁻¹ · h⁻¹) was used to maintain the level of anesthesia. Animals were paralyzed with pancuronium bromide (0.4 mg/kg ip) and a supplemental dose (0.2 mg · kg⁻¹ · h⁻¹ iv) was necessary.

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infused to maintain muscle relaxation during the experiment. A tracheotomy was performed for artificial ventilation using a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). A thermostatically controlled heating pad and overhead infrared lamps were used to maintain rectal temperature between 36.7 and 37.3°C.

Laminectomies were performed to expose spinal segments C1–C2 for neuronal recordings and C6–C8 segments for spinal cord transections. Rats were mounted in a stereotactic headholder and stabilized with a clamp on T2 vertebra. Dura mater of the cervical spinal cord was carefully removed. Dental impression material was used to make a small well at C1–C2 that was filled with warm agar (3–4% in saline) to improve stability for neuronal recording. Carbon-filament glass microelectrodes were used to record extracellular action potentials of single spinal neurons in the C1–C2 segments. All recordings were made from 0.5 to 3 mm lateral to the midline and at a depth of 0–1.6 mm from the spinal dorsal surface. The left and right cervical vago nerves were separated from the carotid artery, and a suturo was looped around each nerve trunk to permit identification of the vago nerves for transections. If a C1–C2 neuron still responded to esophageal distension after interruption of vago pathways, spinal transection was made at C6–C7 to determine if activation of the neuron occurred through spinal (sympathetic) pathways.

Esophageal distension was produced by water inflation of a small latex balloon (length: 1.0 cm) at the end of PE-240 tubing. Balloons were inserted perorally 5–6 cm or 9–10 cm from the upper front incisors for CED or TED, respectively (Lu and Bieger 1998; Qin et al. 2003a). In some experiments, the esophageal balloon was carefully moved between cervical and thoracic regions to examine responses of a single spinal neuron to CED and TED. Esophageal distension of 0.3–0.4 ml was used as the search stimulus for identifying neurons that responded to stimulation of esophageal afferents. Graded CED or TED was produced with distension volumes of warm water (0.1, 0.2, 0.3, 0.4, 0.5 ml, 20 s), and the injection rate was controlled manually at 0.05–0.1 ml/s. These distension volumes were chosen because they have been employed as a natural stimulus in a study of esophageal reflexes in rats (Wei et al. 1997). Furthermore, a previous study showed that ≥0.3 ml water distension produces changes in the diameter and length of the esophagus that are similar to changes observed with ≥1.0-ml inflation of an air balloon (Qin et al. 2003a). Because ED with air inflation of ≥1.0 ml induces pseudosomatic responses and a passive avoidance behavior paradigm in rats, this volume of air inflation is considered a noxious stimulus (Euchner-Wamser et al. 1993; Gebhart and Sengupta 1996). Therefore water inflation of ≥0.3 ml ED was considered to be a noxious esophageal stimulus, and the volumes of ED used in this study ranged from innocuous to noxious stimulation intensities (Qin et al. 2003a).

Cutaneous receptive fields of spinal neurons were tested for responses to innocuous brushing with a camel-hair brush and noxious pinch of skin with a blunt forceps. Neurons were categorized as follows: wide dynamic range (WDR) neurons were activated by brushing the hair and had a greater response to noxious pinching of the somatic field; high-threshold (HT) neurons responded only to noxious pinching of the somatic field; low-threshold (LT) neurons responded primarily to hair movement. If a cutaneous receptive field was not found, arm or shoulder-joint movement was examined.

To determine locations of spinal neurons with esophageal inputs, an electrolytic lesion (50 μA DC anodal for 20s, cathodal for 20 s) was made at most recording sites after a neuron with esophageal input was studied. At the end of experiments, animals were killed with intravenous euthanasia-5 solution (0.2–0.3 ml) or an overdose of pentobarbital, and the cervical spinal cord was removed and placed in 10% buffered formalin solution. The segmental location of C6–C8 spinal transection was confirmed. After ≥3 day, frozen sections (55–60 μm) of the upper cervical cord were made and viewed with a microscope. Laminae of lesion sites were identified using the cytoarchitectonic scheme reported by Molander et al. (1989).

Spontaneous neuronal activity was determined by counting impulses for 10 s and then dividing by 10 to obtain impulse/s. Neuronal responses to ED were calculated as the change in activity during ED from the spontaneous (control) activity. A response to a stimulus was defined as a change of neuronal activity ≥20% compared with control activity (Qin et al. 2003a). Stimulus-response curves were plotted for some neurons responding to graded ED, and regression analysis was used to obtain an extrapolated threshold for neuron with esophageal input (Euchner-Wamser et al. 1993; Qin et al. 2003a). Latencies of responses were measured from the onset of ED to the onset of a response. Durations of responses were measured from the onset of a response to the time of recovery to control activity. Statistical comparisons were made using Student’s paired or unpaired t-test and χ2 test. P values <0.05 were considered significant. Data are presented as means ± SE.

RESULTS

Cervical and thoracic esophageal distension (0.4 ml) changed the activity of 48/219 (22%) and 31/190 (16%) spinal neurons recorded from C1–C2 segments, respectively. Three patterns of neuronal responses to ED were observed: excitation (E), inhibition (I), and exhibition-inhibition (E-I). E and I responses of spinal neurons were found to either CED or TED, whereas E-I responses were found only to CED. Electrolytic lesions of recording sites of some neurons responding to ED were histologically reconstructed (Fig. 1). No difference was found in the regional distribution of neurons responding to CED and TED. Convergent somatic inputs from head, ears, neck and shoulder area were found for 45/48 (94%) and 30/31 (97%) neurons that responded to CED and TED, respectively. The relationships between somatic field properties and neuronal response patterns to ED are shown in Table 1.

Neuronal responses to CED

Noxious CED (0.4 ml) increased activity of 34 spinal neurons and decreased activity of 12 neurons in C1–C2 segments; responses of two neurons were excitatory-inhibitory. Examples of these neurons are shown in Fig. 2, A–D, and characteristics of neuronal responses to CED are shown in Table 1. Graded CED induced excitatory responses in 27 neurons in an intensity-dependent manner (Fig. 2E). The average threshold volume of these neurons was 29.8 ± 6.5 μl calculated by using a least-squares-linear-regression analysis. The average excitatory response of superficial neurons (depth <0.35 mm, 16.4 ± 3.2 impulse/s, n = 8) to CED was significantly lower than that of neurons in deeper lamina (27.5 ± 3.4 impulse/s, n = 26, P < 0.01). Based on time of recovery to control activity after CED (0.4 ml, 20 s) was removed, spinal neurons excited by CED were subdivided into two groups: neurons with recovery time ≤5 s were classified as short-lasting excitatory (SL-E, n = 15) and neurons with after-discharges >5 s were classified as long-lasting excitatory (LL-E, n = 19). Examples of graded responses to CED in SL-E and LL-E neurons are shown in Fig. 2, A and B, respectively. Average duration of LL-E responses to CED was 38.5 ± 4.0 s compared with 20.9 ± 0.6 s for SL-E responses. Average latency of LL-E responses was significantly longer than for SL-E responses (2.4 ± 0.4 vs. 1.3 ± 0.3 s, P < 0.05). Based on graded responses to CED, spinal neurons were subdivided into two groups: low-threshold excitatory (LT-E, n = 19) and high-threshold excitatory (HT-E, n = 8). The LT-E neurons responded to CED distending volume
HT-E neurons responded only to distending volumes \( \geq 0.3 \) ml of CED. Furthermore, noxious CED (0.4 ml) decreased spontaneous activity from 13.3 ± 2.5 to 2.5 ± 0.9 impulse/s in 12 neurons. An example of an inhibitory response to CED is shown in Fig. 2D, and a summary of the stimulus-response relationship of inhibitory responses to graded CED in nine neurons is shown in Fig. 2F.

**Neuronal responses to TED**

Thoracic esophageal distension (0.4 ml) increased activity of 28 neurons and decreased activity of three neurons in C1–C2 segments of the spinal cord. Characteristics of spontaneous activity and neuronal responses to TED are shown in Table 1. Excitatory responses of 22 neurons to graded TED are summarized in Fig. 2G. The average threshold volume of spinal neurons with excitatory responses to TED was 31.2 ± 7.2 μl.

**Comparison of responses to CED and TED**

The proportions of superficial neurons that were responsive to either CED (10/115, 9%) or TED (5/73, 7%) were signifi-

![Figure 1](image-url) Lesion sites of neurons recorded in C1–C2 spinal cord based on Molander et al. (1989). ●, neurons excited by cervical or thoracic esophageal distension (CED or TED); ○, neurons inhibited by esophageal distension. I-X, laminae. CCN, central cervical nucleus. IBN, internal basilar nucleus. LCN, lateral cervical nucleus. LSN, lateral spinal nucleus. Pyr, pyramidal tract.

### TABLE 1. Comparison of characteristics of C1–C2 neurons responding to cervical and thoracic esophageal distension

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Responses to CED</th>
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<th>Responses to TED</th>
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<tbody>
<tr>
<td></td>
<td>E</td>
<td>I</td>
<td>E-I</td>
<td>E</td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>12</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Spontaneous activity, impulse/s</td>
<td>9.1 ± 1.9</td>
<td>13.2 ± 2.5</td>
<td>14.8 ± 4.2</td>
<td>8.5 ± 2.3</td>
</tr>
<tr>
<td>Latency, s</td>
<td>1.9 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.5 ± 2.4</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>E responses, impulse/s</td>
<td>24.5 ± 3.1</td>
<td>N/A</td>
<td>12.8 ± 8.2</td>
<td>12.1 ± 1.9</td>
</tr>
<tr>
<td>I responses, impulse/s</td>
<td>N/A</td>
<td>10.8 ± 2.1</td>
<td>5.6 ± 6.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of responses, s</td>
<td>30.8 ± 2.8</td>
<td>35.5 ± 6.2</td>
<td>52.5 ± 6.6</td>
<td>28.9 ± 2.5</td>
</tr>
<tr>
<td>Properties of somatic fields WDR/HT/MJ/NR</td>
<td>16/15/2/1</td>
<td>1/9/0/2</td>
<td>0/2/0/0</td>
<td>9/16/2/1</td>
</tr>
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Values are means ± SE. CED and TED, cervical and thoracic esophageal distension; E, excitatory response; I, inhibitory response; WDR, wide dynamic range; HT, high threshold; MJ, moving joints; N/A, not applicable.
cantly less than those of deeper spinal neurons to CED (38/104, 37%) or TED (26/117, 22%, \( P < 0.05 \)). The average excitatory response of deeper neurons to CED (27.5 ± 3.4 impulse/s, \( n = 26 \)) was significantly greater than of superficial neurons to CED (16.4 ± 3.2 impulse/s, \( n = 8 \)) and also of deeper neurons to TED (12.5 ± 1.9 impulse/s, \( n = 24, P < 0.01 \)). Of 67 neurons examined for responses to both CED and TED, 10 (15%) neurons responded to both stimuli and 6 neurons responded to either CED or TED. Of 10 neurons receiving convergent inputs from cervical and thoracic esophagus, 9 neurons exhibited excitatory responses to both CED and TED, and 1 neuron was inhibited by both visceral stimuli. The LT-E neurons responded to CED more frequently than to TED (70 vs. 32%, \( P < 0.01 \)). An example of excitatory responses of a neuron that received convergent inputs from cervical and thoracic esophagus is shown in Fig. 3, A and B.

FIG. 2. Responses of C1–C2 neurons activated by CED and TED. A: a neuron with short-lasting excitatory (SL-E) responses. B: a neuron with long-lasting excitatory (LL-E) responses. C: a neuron with excitatory-inhibitory responses. D: a neuron with inhibitory responses. E and F: a summary for excitatory and inhibitory responses to graded CED. G and H: a summary for excitatory and inhibitory responses to graded TED. Bin width of rate histograms is 1 s in all examples. Imp/s, impulses/s. The duration of CED is indicated (■) at the bottom of each graph in all figures.
Effects of vagotomy and spinal transection

To determine afferent pathways of esophageal inputs to upper cervical spinal neurons, neuronal responses to CED (n = 18) and TED (n = 12) were compared before and after cervical vagus nerves and the lower cervical spinal cord were transected. Of these neurons tested for afferent pathways, seven neurons received convergent inputs from cervical and thoracic esophagus. Examples of effects of vagal and spinal transections while recording from a neuron that was excited by both CED and TED are shown in Fig. 3, A–H. A summary of the effects of vagotomy and spinal transection on CED and TED responses is presented in Fig. 4. Neuronal responses to esophageal stimuli were considered abolished if the original response was reduced by ≥80% after vagotomy or spinal transection. Ipsilateral cervical vagotomy (ICV) did not interrupt CED input to 18/18 C1–C2 neurons (14 E, 4 I). Sequential contralateral vagotomy (CCV) abolished responses of 2/14 E neurons to CED; 4 neurons were lost during the CCV procedure. Transection at C6–C7 spinal segments eliminated responses of two E neurons (Fig. 3G); nine neurons (7 E, 2 I) still responded to CED, and one neuron was lost during spinal transection. Thus excitatory and inhibitory responses to CED of 9/13 (69%) neurons were dependent on spinal visceral pathways that entered the spinal cord above the C6–C7 segments, whereas excitatory responses of 4/13 neurons were evoked either by vagal afferents or by spinal afferents that entered spinal segments below C6–C7. Examples of effects of cervical vagotomy and spinal transection on excitatory and inhibitory responses to CED are shown in Fig. 5, A and B, respectively.

For 12 neurons excited by TED, ICV and CCV eliminated responses to TED in 7 neurons; 3 neurons were lost during the CCV procedure (Fig. 4). Figure 5, C and D, shows two examples of these neurons. For two neurons that still responded to TED input after bilateral vagotomy, sequential spinal transection at C6–C7 eliminated responses to TED. Thus for nine neurons that were not lost during CCV, excitatory responses of 7/9 (78%) neurons to TED were dependent on vagal pathways, whereas responses in two neurons were produced by spinal

<table>
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<th>Transsections</th>
<th>CED neurons</th>
<th>TED neurons</th>
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<tr>
<td>intact</td>
<td>18 (14E, 4I)</td>
<td>12E</td>
</tr>
<tr>
<td>ICV</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>18 (14E, 4I)</td>
<td>7E</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CCV</td>
<td>2E</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>12 (9E, 3I)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4 (3E, 1I)</td>
<td>3</td>
</tr>
<tr>
<td>cut C6–C7</td>
<td>2E</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9 (7E, 2I)</td>
<td>0</td>
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<td></td>
<td>1I</td>
<td>0</td>
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<tr>
<td>abolished</td>
<td>responded</td>
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<td>responded</td>
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<td>lost</td>
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FIG. 3. Responses of a C2 neuron receiving convergent inputs from cervical and thoracic esophagus before and after cervical vagotomy and spinal transection at C6. A: excitatory responses to graded CED. B: responses to graded TED. C: effects of ipsilateral cervical vagotomy (ICV) on neuronal responses to graded CED. D: effects of ICV on neuronal responses to graded TED. E: effect of contralateral cervical vagotomy (CCV) on responses to graded CED. F: ablation of responses to TED by CCV. G: ablation of response to CED by spinal transection at C6 segment. H: no response to TED.

FIG. 4. Effects of ipsilateral and contralateral cervical vagotomy (ICV, CCV) and spinal transection at C6–C7 segments on responses of C1–C2 neurons to esophageal distension.
visceral afferents that entered spinal segments below C6–C7. Figure 3 shows that excitatory responses of a neuron to TED were eliminated by bilateral vagotomy, but responses to CED in this neuron were abolished by spinal transection at C6 segment. In summary, vagal afferents were the major pathway for thoracic esophageal inputs to C1–C2 spinal neurons, whereas CED predominately activated C1–C2 neurons by spinal visceral afferents that entered cervical segments.

**DISCUSSION**

This study revealed several interesting observations about the upper cervical spinal processing of afferent information arising from the esophagus. First C1–C2 spinal neurons that responded to esophageal distension were primarily excited by inputs from both the cervical and thoracic regions of the esophagus. Second, these cells received convergent somatic inputs from overlying structures including the neck and jaw. Finally, most of the afferent input from the cervical esophagus entered the spinal cord via upper cervical dorsal roots while input from the thoracic esophagus was transmitted in vagal afferent fibers and thoracic sympathetic afferent fibers.

**Neuronal responses to ED**

One interesting observation in the present study was that C1–C2 spinal neurons not only responded to mechanical stimulation of the cervical esophagus but also to distension of the thoracic esophagus. Similar to this finding, thoracic spinal neurons also receive convergent inputs from cervical and thoracic regions of the esophagus (Qin et al. 2003a). Thus it might be a universal phenomenon that sensory neurons in many spinal segments are activated by afferent inputs originating in extensive areas of the esophagus. These neurons with convergent inputs, therefore could play a role in spinal sensory
processing and integration of afferent information from different regions of the esophagus.

The majority of C1–C2 spinal neurons with esophageal input were excited by CED or TED, and the remainder were either inhibited or excited and then inhibited. Additionally, SL-E and LL-E responses of spinal neurons to esophageal distension were identified in the present study. Of spinal neurons excited by CED, 56% had LL-E responses, and the remaining neurons were SL-E responsive, whereas 43% of neurons excited by TED had LL-E responses and 57% were SL-E responsive. Rapidly and slow/nonadapting responses of mechanoreceptors in the esophagus have been characterized (Mei 1983). Furthermore, these characteristics were observed in recordings from esophageal vagal and sympathetic afferent fibers in opossums (Sengupta et al. 1989, 1990). It is reasonable to assume that rapidly adapting mechanoreceptors correlate with the SL-E responsive neurons and slow/nonadapting mechanoreceptors correlate with the LL-E neurons recorded in this study. These characteristics imply that there is a differential spinal processing of peripheral afferent information from the esophagus. The present results generally agree with previous studies in rats that examined neuronal responses to esophageal distension in the thoracic spinal cord (Euchner-Wamser et al. 1993; Qin et al. 2003a). However, a study in cats has reported that ~80% of thoracic spinal neurons with excitatory esophageal inputs show slow or nonadaptation to ED, and the remainder rapidly adapt to esophageal distension (Garrison et al. 1992). This disparity could result from species differences, the spinal cord segments used for neural recordings, or the regions of the esophagus distended.

Differential characteristics of neuronal responses in superficial and deeper laminae of spinal cord to various visceral nociceptive inputs have been examined previously (Cervero et al. 1987; Ness and Gebhart 1989; Qin et al. 2003a–c). In the present study, the proportion of deeper neurons responding to CED was significantly larger than for neurons in superficial laminae; excitatory responses of deeper neurons to CED were significantly greater than for superficial neurons. These data agree with previous observations in upper thoracic spinal cord neurons with esophageal and cardiac inputs (Qin et al. 2003a,b) and suggest that deeper spinal neurons are more likely to process visceral information than spinal neurons in superficial laminae.

Viscerosomatic convergence

Esophageal pain is referred primarily to midsternal and epigastric areas, to the back, and sometimes to suprasternal notch, neck, and jaw (Polland and Bloomfield 1931). The somatic location of referred pain from the esophagus correlates with the level of esophageal stimulation in humans. During distension of the upper esophagus, subjects feel pain in the suprasternal notch, throat and neck. Mid-esophageal distension elicits a wide scattering of pain to the body of sternum, sternal manubrium, and upper back, and lower esophageal distension produces pain referred to the suprasternal region, epigastrium, and middle back area (Currie 1979; Kramer and Hollander 1955). In the present study, the majority of C1–C2 neurons that were activated by CED and/or TED also responded to mechanical stimulation of somatic receptive fields located on the face, neck, ears, and shoulder areas. In contrast, somatic receptive fields for upper-thoracic spinal neurons with esophageal input are located on more caudal areas of the body, such as axillary, thoracic, and back regions (Euchner-Wamser et al. 1993; Qin et al. 2003a). Convergence of visceral and somatic afferents on the same neurons is an explanation of the clinical finding of visceral pain referred to somatic structures (Foreman 1999; Ruch 1961). Thus viscerosomatic convergence onto C1–C2 spinal neurons observed in the present study might be a neural basis of referred somatic pain and muscular spasm originating from the cervical and thoracic esophagus. For example, thoracic esophageal distension elicits a viscerosomatic motor reflex that results in paraspinal muscular contraction of the upper back in rats (Jou et al. 2002), whereas oral esophageal distension and chemical (HCl) nociceptive stimulation elicits an increase of electromyographic activity from neck muscles (Hummel et al. 2003).

Another potential correlation with observations in the present study is an etiology for Sandifer syndrome, which consists of abnormal contractions of the neck (torticollis) and gastroesophageal reflux with or without a hiatus hernia (Kinsbourne 1964; Sutcliffe 1969). Wide variability in the expression and degree of severity of this syndrome may cause misdiagnosis in pediatric practices (Mandel et al. 1989; Werlin et al. 1980). The pathophysiology of these abnormalities is not clear, but it is thought that the head and neck posturing is secondary to the gastroesophageal reflux and is a viscerosomatic-motor response to provide relief from the discomfort of the reflux (Deskin 1995; Werlin et al. 1980). The present study found that C1–C2 spinal neurons received viscerosomatic convergent inputs from the thoracic esophagus and the neck, which might provide a central relay for an esophagosomatic-motor reflex in the upper cervical spinal cord to explain symptoms in patients with Sandifer syndrome.

Spinal visceral afferent pathways

Spinal afferent innervation from all regions of the esophagus extends from C2–L1 dorsal root ganglia with peak distributions in the upper cervical and upper thoracic spinal cord in various species (Clerc 1983; Collman et al. 1992; Hudson and Cummings 1985; Khurana and Petras 1991; Neuhuber and Clerc 1990). In the present study, afferent information to 69% of C1–C2 neurons responsive to CED was transmitted to the CNS by visceral spinal afferents that entered above C6–C7 segments. In support of this observation, injection of True Blue into the cervical esophagus in rats results in the appearance of labeled neurons in the C2–C5 dorsal root ganglia (Uddman et al. 1995). In contrast, spinal afferent input to C1–C2 neurons responding to TED entered the spinal cord via thoracic spinal visceral afferent pathways, which might involve the paravertebral sympathetic chain and splanchnic nerves (Sengupta et al. 1990). Most likely, primary afferent fibers from the thoracic esophagus directly and/or indirectly synapse on neurons in thoracic spinal segments that transmit information to C1–C2 neurons via intraspinal ascending projections (Molenaar and Kuypers 1975, 1978). Additionally, because cervical dorsal root ganglia can contain sensory neurons from the lower esophagus (Christensen 1984; Collman et al. 1992), the possibility of direct spinal afferents from the thoracic esophagus to the cervical spinal cord cannot be excluded.

In a previous study from this laboratory, afferent pathways
of upper thoracic spinal neurons with esophageal inputs were examined for responses to CED and TED (Qin et al. 2003a). We found that excitatory responses to TED result from activation of afferent inputs that enter thoracic spinal segments, whereas excitatory responses to CED result from afferent inputs entering cervical or thoracic spinal segments (Qin et al. 2003a). Results of the present study showed a similar afferent pathway of spinal visceral fibers originating from cervical and thoracic regions of esophagus in upper cervical (C1–C2) spinal neurons. However, the present study showed that vagal afferents also were involved in the responses of upper cervical spinal neurons to TED.

Vagal afferent pathway

Another neural pathway of esophageal sensory input to C1–C2 segments is primary afferent fibers that travel in the vagus nerve. The majority of vagal afferent fibers synapse in the nucleus tractus solitarius (NTS) and project to other brain stem nuclei and also to the upper cervical spinal cord (Norgren 1978). Only a small portion of vagal afferents project directly to the upper cervical spinal cord in rats (Kalia and Sullivan 1982; McNeill et al. 1991). It is presumed that esophageal inputs traveling in vagus nerves reach the NTS and afferents from the NTS then transmit information to C1–C2 spinal neurons.

In rats, the cervical portion of the esophagus is innervated predominantly by afferent fibers in the recurrent laryngeal nerves, the middle third is supplied by both the superior laryngeal and vagus nerves, and the abdominal third is innervated by vagal afferent fibers (Fryscak et al. 1984; Mei 1983; Neuhauser 1987). After an injection of True Blue into the cervical esophagus, numerous labeled neurons are found in the dorsal root ganglia (Uddman et al. 1995). Midthoracic esophageal distension induces considerable c-fos expression in the nucleus of the solitary tract (Traub et al. 1994). Quantitative studies document that vagal fibers respond in an intensity-dependent manner to distension of the lower esophagus in opossum (Sengupta et al. 1989, 1990). The present study showed that responses to TED of 78% of C1–C2 neurons were abolished by bilateral cervical vagotomy, whereas only 31% of neurons responding to CED were dependent on vagal input. Thus sensory information from the thoracic esophagus was more likely transmitted in vagal afferent fibers than information from the cervical esophagus.

Relative contribution of vagal and spinal afferents to esophageal nociception

Although both vagal and spinal innervation are responsible for sensory and motor function of the esophagus, the relative contribution of these two nerve routes in esophageal pain is unknown (Loomis et al. 1997; Lynn 1992). The few studies done in this area come to different conclusions. Esophageal vagal afferents are significantly more numerous than esophageal spinal afferents (Collman et al. 1992). Furthermore, the mean response threshold to ED of vagal afferent fibers is significantly lower than that of spinal afferent fibers (Sengupta et al. 1989, 1990). Therefore, it was concluded that vagal afferents are involved in physiologic reflexes and homeostatic mechanisms, whereas esophageal pain sensation is transmitted via spinal afferents (Lynn 1992). However, pseudoaffective or cardiovascular responses to noxious ED are attenuated by unilateral vagotomy and abolished by bilateral vagotomy. Moreover, administration of morphine at the thoracic level of the spinal cord inhibited the cardiovascular responses to a noxious cutaneous pinch but failed to inhibit responses elicited by noxious ED in rats (Hummel et al. 2003; Loomis et al. 1997). Results of the present study showed that input from the cervical esophagus to C1–C2 neurons primarily entered upper cervical spinal roots; however, input from the thoracic esophagus traveled in vagal pathways and/or entered thoracic dorsal roots. Therefore, the relative contributions of vagal and spinal afferents to activation of C1–C2 neurons in rats depended on the region where the esophagus was stimulated. These results support a concept that C1–C2 spinal neurons play a role in integrating visceral information from the cervical and thoracic esophagus. Pain originating in the cervical esophagus and referred to somatic regions innervated from upper cervical segments might be transmitted via spinal afferent fibers that enter cervical segments. However, pain originating from the thoracic esophagus and referred to the same somatic regions might be transmitted primarily via vagal fibers or by spinal visceral afferents that enter thoracic segments.

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