Intraesophageal chemicals enhance responsiveness of upper thoracic spinal neurons to mechanical stimulation of esophagus in rats

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Qin C, Farber JP, Foreman RD. Intraesophageal chemicals enhance responsiveness of upper thoracic spinal neurons to mechanical stimulation of esophagus in rats. Am J Physiol Gastrointest Liver Physiol 294: G708–G716, 2008. First published January 10, 2008; doi:10.1152/ajpgi.00477.2007.—Esophageal hypersensitivity is one of the most common causes of noncardiac chest pain in patients. In this study, we investigated whether exposure of the esophagus to acid and other chemical irritants affected activity of thoracic spinal neurons responding to esophageal distension (ED) in rats. Extracellular potentials of single thoracic (T3) spinal neurons were recorded in pentobarbital sodium-anesthetized, -paralyzed, and -ventilated male rats. ED (0.2 or 0.4 ml, 20 s) was produced by water inflation of a latex balloon placed orally into the middle thoracic region of the esophagus. The chemicals were administered via a tube that was passed through the stomach and placed in the thoracic esophagus. To irritate the esophagus, 0.2 ml of HCl (0.01 N), bradykinin (10 μg/ml), or capsaicin (10 μg/ml) were injected for 1–2 min. Only neurons excited by ED were included in this study. Results showed that intraesophageal instillation of HCl, bradykinin, and capsaicin increased activity in 3/20 (15%), 7/25 (28%), and 9/20 (45%) neurons but enhanced excitatory responses to ED in 9/17 (53%), 8/15 (53%), and 7/11 (64%) of the remaining spinal neurons, respectively. Furthermore, intraesophageal chemicals were more likely to enhance the responsiveness of low-threshold neurons than high-threshold neurons to the esophageal mechanical stimulus. Normal saline (pH 7.4, 0.2 ml) or vehicle instilled in the esophagus did not significantly affect activity or ED responses of neurons. We conclude that enhanced responses of thoracic spinal neurons to ED by the chemically challenged esophagus may provide a possible pathophysiological basis for visceral hypersensitivity in patients with gastroesophageal reflux and/or esophagitis.

ESOPHAGEAL PAIN AND/OR HYPERSENSITIVITY is one of the most common causes of noncardiac chest pain (NCCP). About 60% of such patients have gastroesophageal reflux disease (GERD). The typical symptoms of GERD are heartburn and/or acid regurgitation arising from the reflux of gastric acid. Surveys of the U.S. population suggest that 7–10% of adults experience these symptoms daily (21, 23, 24, 31). Therefore, ~19 million people per year have to live with GERD, and as a result they experience a reduced quality of life. Among the various gastrointestinal diseases in the U.S. population, GERD creates the highest financial burden with the highest annual direct costs ($9.3 billion U.S. dollars) (44). Some patients with GERD have chronic complications such as various laryngeal and pulmonary disorders (22) and may develop esophagitis, Barrett’s esophagus (precancerous lesion), or esophageal adenocarcinoma (5, 55). Despite the prevalence and clinical significance, the pathophysiological mechanisms underlying esophageal pain and/or hypersensitivity are incompletely understood (50, 51).

Investigations of visceral pain have increased extensively during the last 10 years (15). The majority of human studies elucidating visceral pain and/or hypersensitivity in patients with NCCP have used balloon distension in the esophagus and evaluated esophageal pain by scoring pain perception and/or by imaging cortical brain activity in response to different esophageal stimuli. Repeated esophageal balloon distensions and acid exposures decrease the pain threshold in response to the mechanical stimulus of esophagus in patients with NCCP (10, 12, 20, 29, 45–47). However, one study contradicted this result (8). Esophageal hypersensitivity has been attributed to effects on primary afferent mecanoreceptors and chemoreceptors in the esophageal muscular and mucosal layers (41, 49). There is also dysfunction of the central nervous system, because patients with NCCP have abnormalities in cerebrally evoked potentials in response to symptomatic esophageal distension (17, 18). Since acid infused into the lower esophagus reduces the pain threshold within the upper non-acid-exposed esophagus and an area of the overlying skin in both healthy volunteers and patients with NCCP, the investigators suggested that abnormal spinal processing of esophageal sensory information might be involved (45). It has been proposed from a human study that the mechanism underlying esophageal hyperalgesia may be due to an increase of spinal visceroreceptive neuronal excitability (spinal sensitization), which is dependent on the activation of the N-methyl-D-aspartate receptor in spinal dorsal horn neurons receiving primary afferent inputs (17). However, there is very limited electrophysiological support for the idea of enhanced nociceptive processing by the spinal cord and the sympathetic afferent fibers in animals (Refs. 11, 14; see reviews, Refs. 17, 26, 32, 51).

It is well known that the esophagus is innervated by both the vagal and spinal visceral afferent systems with primary sensory neurons (pseudounipolar cell bodies) located in the nodose and dorsal root ganglia, respectively. Generally, it is believed that vagal afferents are involved in physiological esophageal reflexes related to digestive and homeostatic regulation functions, whereas esophageal nociception is carried exclusively by the spinal visceral nerves to the spinal cord (25, 26, 50, 51). The esophageal spinal afferent input travels in cervical and thoracic dorsal roots, as well as the splanchnic nerve, that project to the spinal cord. Spinal cord innervation of esophagus spans from upper cervical (C1) to upper lumbar (L2) segments.
in dogs, cats, and rats, with peak distributions in cervical and thoracic spinal cord (see reviews, Refs. 26, 51). We and others have characterized upper thoracic and cervical spinal neurons with responses to esophageal distension (ED) and determined afferent pathways in cats and rats (11, 14, 37, 38). Although we and others have experimentally examined the innervation as well as the afferent pathways and spinal processing of mechanical stimulation of the esophagus in animal models, very little is known about spinal (sympathetic) afferent and spinal neuronal response characteristics to esophageal distension when specific chemical stimuli are administered directly into the esophagus during the distension period (see reviews, Refs. 26, 51). Only one study has been done to demonstrate that intraaortic injections of bradykinin can produce responses from distension-sensitive sympathetic afferent fibers, but esophageal distension was not induced during the chemical stimulus period (49). It also should be noted that no studies have been done to determine the response characteristics of dorsal horn neurons to the intraesophageal administration of HCl or capsaicin during esophageal distension. In a previous study from our laboratory, it was shown that turpentine-induced inflammation can decrease the threshold of thoracic spinal neuronal responses to esophageal distension, but other response characteristics of the neurons were not examined (14). Furthermore, specific chemical stimuli that contributed to the inflammatory effects of turpentine were not examined. Thus no experimental studies have characterized the responses of upper thoracic spinal neurons to relevant esophageal chemical stimuli such as HCl, and no studies have been done to determine whether neuronal responses to esophageal distension were sensitized by chemical stimulation of the esophagus. The present study in rats was designed to examine whether 1) upper thoracic neurons respond to intraesophageal injections of chemicals (HCl, bradykinin, and capsaicin); 2) intraesophageal chemicals alter the responsiveness of spinal neurons to esophageal distension; and 3) the chemical stimuli differentially affect the responses of spinal neurons that are classified as low threshold or high threshold to esophageal distension. We used HCl as a relevant chemical stimulus, whereas capsaicin and bradykinin are expected to activate nociceptive nerve endings through specific receptors. A preliminary report of this work has been published as an abstract (39).

**METHODS**

Experiments were performed on 23 male Sprague-Dawley rats (Charles River) weighing between 330 and 460 g. The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center and followed the guidelines for animal experiments of the International Association for the Study of Pain. After initial anesthesia with pentobarbital sodium (60 mg/kg ip), catheters were inserted into the right carotid artery to monitor arterial blood pressure and into the left jugular vein for infusion of pentobarbital sodium (15–25 mg·kg⁻¹·h⁻¹) to maintain a constant level of anesthesia throughout the experiment. A tracheotomy was performed for artificial ventilation using a constant-volume pump (55–60 strokes/min, 3.0- to 5.0-ml stroke volume). Animals were paralyzed with pancuronium bromide (0.4 mg/kg ip), and a supplemental dose (0.2 mg/kg ip) was administered hourly to maintain muscle relaxation during the experiment. A thermostatically controlled heating pad and overhead infrared lamps were used to keep rectal temperature between 36.7 and 37.3°C.

ED was produced by infusing warm water into a small latex balloon (1.0 cm long) at the end of PE-240 tubing (Becton Dickinson, Sparks, MD). The balloon was inserted perorally 6–8 cm from the front incisors into the middle thoracic region of the esophagus (37). ED was produced by injection of warm water (0.2 or 0.4 ml at a rate of 0.05–0.1 ml/s and held for 20 s) in an esophageal balloon. These volumes have been employed as natural stimuli in behavioral and neuronal processing studies of esophageal reflexes in rats (37). The low volume of ED (0.2 ml) (low threshold) is considered to be an innocuous distension such as occurs when solid or liquid boluses of foods transit in the esophagus, whereas ED ≥ 0.4 ml (high threshold) is likely to be noxious or painful (9, 11, 37). Only neurons excited by ED were included in this study, although inhibitory and biphasic response patterns of spinal neurons during esophageal input also were found in this and previous investigations (37). This study was designed to focus on the effects of intraesophageal chemicals on intraspinal nociceptive processing for mechanical afferent information from esophagus, but not on the characteristics of the spinal neuronal pool receiving esophageal input.

Intraesophageal chemicals were administered via a polyethylene (PE) tube (2–3 cm) that was passed through the cardiac wall of the stomach into the lower thoracic esophagus. The PE tube was attached to silicone tubing (8–10 cm) connected to a syringe (1 ml). To chemically stimulate the esophagus, 0.2 ml of HCl (0.01 N), bradykinin (10 μg/ml in normal saline), or capsaicin (10 μg/ml in a vehicle solution of 1% ethanol and 1% Tween 80 in normal saline) was injected and maintained for 1–2 min. Spinal neuronal responses to ED were evaluated before, during, and after recovery from intraesophageal chemicals. As control manipulations, 0.2 ml of normal saline (n = 2) or vehicle solution (n = 2) was instilled in the esophagus. These manipulations were combined for analysis. Compared with previous studies (8, 28), a low concentration of HCl (0.01 N) instead of a high concentration (0.1 N) was employed (7, 16). Our purpose for using the lower concentration of HCl was to sensitize esophageal receptors as well as spinal neurons and to examine whether hyperresponsiveness occurs during ED. We did not aim to provide a maximal stimulus for these receptors. Since we examined characteristics of spinal neurons responding to as many as three specific chemicals using repeated ED, we were particularly concerned that the higher concentration of HCl might increase the chances for long-term changes of excitability of esophageal receptors or might even damage the esophagus. The study procedure for evaluating a spinal neuron was as follows. 1) ED with 0.4 ml was used as search stimulus to find a spinal neuron with an excitatory response to ED. 2) Two to three consistent responses to ED (0.2 and 0.4 ml) were documented as control responses before an intraesophageal chemical was instilled. 3) After an injection of a chemical was made into the esophagus (>30 s), ED was repeated again during the exposure of chemicals in the esophagus to observe the effects of intraesophageal chemicals on the excitatory responses to ED. 4) After intraesophageal chemicals were removed and rinsed away (2–3 flushes with 0.2 ml of warm saline), ED was repeated every 3–5 min until ED responses recovered to >80% of control. This typically occurred in <15 min. Therefore, to avoid desensitization of spinal neurons, at least 3 min elapsed between each ED and 20 min between the chemical injections in the esophagus during experiments. In addition, to avoid chemical damage of the esophagus, we conducted three to five trials in one to two cells for each animal. Thoracic laminectomies were performed to expose the T3 spinal segment for recording neuronal activity. After rats were mounted in a stereotactic head holder, the dura mater of the T3 segment was carefully removed and the spinal cord was covered 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. All recordings were made 0.5–2 mm laterally from the midline and at depths between 0 and 1.2 mm from the dorsal surface of the spinal cord. Neuronal activity was recorded online with the Spike 3 data acquisition system (Cambridge
Electronic Design, Cambridge, UK). To quantify neuronal responses, raw traces of neuronal activity were stored and evaluated as rate histograms (1 s per bin). Spontaneous activity was determined by counting impulses for 10 s and then dividing by 10 to obtain impulses per second. After mechanical or chemical stimulation, maximum activity was obtained over a 10-s interval and divided by 10 to obtain impulses per second. A neuron with spontaneous activity was classified as responsive to various stimuli if the maximal change in activity was at least 20% compared with control activity. For neurons with a firing rate of <5 Hz, activity needed to increase by 1 Hz to be counted. Changes (total impulses) in neuronal activity during a stimulus also were measured and calculated as the total response to mechanical stimulation of the esophagus in a procedure that examined sensitization to ED after instillation of esophageal chemicals. The duration of the spinal neuronal responses to esophageal stimuli was measured from the onset of the responses to the time when neuronal activity returned to control levels. Data are means ± SE. Statistical comparisons were made using the Fisher exact test and repeated-measures ANOVA followed by Tukey’s comparison. Differences were considered statistically significant at \( P < 0.05 \).

Protocols were performed in the following sequence of steps: 1) obtain background cell activity; 2) perform ED 1 (0.2 and 0.4 ml) and obtain cell activity; 3) allow recovery and obtain background cell activity; 4) introduce esophageal chemical and obtain cell activity; 5) perform ED 2 (0.2 or 0.4 ml) and obtain cell activity; 6) remove esophageal chemical and allow recovery time to obtain background cell activity; and 7) perform ED 3 (0.2 or 0.4 ml) and obtain cell activity. Steps 6 and 7 are repeated until the cell response to ED recovers to control levels. After completion, the protocol may be repeated using another esophageal chemical. Further explanations of time intervals and analyses of cell activity are provided in the preceding paragraphs.

To mark the locations of spinal neurons, electrolytic lesions (50 μA direct current, 20 s) were made at recording sites after neurons with esophageal input had been studied. At the end of experiments, animals were euthanized with an overdose of pentobarbital sodium. The thoracic spinal cord was removed and placed in 10% buffered formalin solution. After at least 3 days, frozen sections (55–60 μm) of the upper thoracic cord were made and lesion sites in the spinal cord were viewed under a microscope. Laminae of the spinal cord gray matter were identified using the cytoarchitectonic scheme in rats (30).

RESULTS

A total of 30 upper thoracic (T3) spinal neurons with excitatory responses to ED were examined for effects of intraesophageal application of HCl, bradykinin, or capsaicin. Based on the excitatory responses to ED, spinal neurons were divided into the following two groups (37): 18 low-threshold (LT) neurons that started to respond to ED of \( \leq0.2 \) ml (Fig. 1, A and B) and 12 high-threshold (HT) neurons that only responded to ED of \( \geq0.4 \) ml (Fig. 1C). Electrolytic lesions of recording sites were verified histologically for 17 of 30 spinal neurons responding to ED and/or intraesophageal chemicals (Fig. 2). Spinal neurons sensitized by intraesophageal chemicals were located in laminae I, II, III, V, and VII, which was similar to the locations of neurons not sensitized by intraesophageal chemicals (Fig. 2).

Responses to intraesophageal HCl were examined for 20 (12 LT, 8 HT) spinal neurons excited by ED. During intraesophageal application of HCl (0.01 N, 0.2 ml, 1 min), activity of 3 (15%) LT neurons significantly increased to 29.4 ± 10.6 impulses/s from a background level (background cell activity 2) of 9.7 ± 4.7 impulses/s (\( P < 0.05 \)). These neurons were not tested further for sensitization of ED responses to intraesophageal HCl. An example of a spinal neuronal response to intraesophageal HCl is shown in Fig. 1D. Of the 17 spinal neurons whose activity was not increased from background levels (background cell activity 2) in response to HCl (7.2 ± 1.6 vs. 7.7 ± 1.6 impulses/s), intraesophageal HCl enhanced excitatory responses of 7 LT neurons to 0.2-ml ED and 2 HT neurons to 0.4-ml ED but did not affect ED responses in 8 neurons (2 LT, 6 HT). Figure 3A shows a spinal neuron whose ED response was enhanced by intraesophageal HCl. For the 2 HT neurons whose ED response was increased during intraesophageal HCl, mean total responses to ED (0.4 ml) were 78.8, 436.8, and 107.3 impulses before intraesophageal HCl (ED 1), during intraesophageal HCl (ED 2), and after recovery from intraesophageal HCl (ED 3), respectively. For the 7 LT neurons whose discharge was increased during intraesophageal HCl, mean total responses to ED (0.2 ml) were 156.0 ± 31.0 and 421.9 ± 132.2 impulses before intraesophageal HCl (ED 1) and during intraesophageal HCl (ED 2), respectively. After 6.6 ± 0.6 min (range 4.7–9.3 min) of recovery, the mean total response to ED (ED 3) of these neurons was 170.1 ± 40.9 impulses. Response to ED 2 was greater than that to ED 1 and ED 3 (\( P < 0.05 \)). Also, background activity from the 7 LT neurons was not statistically different among conditions: background cell activity 1 (before the initial ED) was 5.3 ± 1.5 impulses/s, background cell activity 2 (before the application of HCl) was 7.1 ± 2.0 impulses/s, and background cell activity 3 (after recovery from HCl) was 7.4 ± 2.6 impulses/s.

Responses to intraesophageal bradykinin were examined for 25 (14 LT, 11 HT) spinal neurons excited by ED. During intraesophageal application of bradykinin (0.2 ml, 1 min), activity of 7 (28%) neurons (5 LT, 2 HT) significantly increased to 26.3 ± 6.3 impulses/s from a background level (background cell activity 2) of 8.3 ± 3.2 impulses/s (\( P < 0.01 \)). These neurons were not tested further for sensitization of ED responses to intraesophageal bradykinin. An example of a spinal neuronal response to intraesophageal bradykinin is shown in Fig. 1E. Of the 15 spinal neurons whose activity was not increased from background levels (background cell activity 2) in response to bradykinin (4.1 ± 1.7 vs. 4.0 ± 1.6 impulses/s), intraesophageal bradykinin enhanced excitatory responses of 7 LT neurons to 0.2-ml ED and 1 HT neurons to 0.4-ml ED but did not affect ED responses in 7 neurons (2 LT, 5 HT). Figure 3B shows a spinal neuron whose ED response was enhanced by intraesophageal bradykinin. For 1 HT neuron whose ED response was increased during intraesophageal bradykinin, mean total responses to ED (0.4 ml) were 86.9, 311.6, and 89.0 impulses before intraesophageal bradykinin (ED 1), during intraesophageal bradykinin (ED 2), and after recovery from intraesophageal bradykinin (ED 3), respectively. For the 7 LT neurons whose discharge was increased during intraesophageal bradykinin, mean total responses to ED (0.2 ml) were 127.0 ± 25.7 and 395.5 ± 94.5 impulses before intraesophageal bradykinin (ED 1) and during intraesophageal bradykinin (ED 2), respectively. After 8.0 ± 1.6 min (range 3.5–14.7 min) of recovery, the mean total response to ED (ED 3) of these neurons was 106.8 ± 20.1 impulses. Response to ED 2 was greater than that to ED 1 and ED 3 (\( P < 0.05 \)). Also, background activity from the 7 LT neurons was not statistically different among conditions: background cell activity 1 (before the initial ED) was 4.6 ± 1.7 impulses/s, background cell activity 2 (before the application of bradykinin) was 4.5 ± 1.6 impulses/s, and background cell activity 3 (after recovery from bradykinin) was 3.9 ± 1.4 impulses/s.
pulses/s. In this series, three neurons without response to bradykinin could not further tested for sensitization.

Responses to intraesophageal capsaicin were examined for 20 (13 LT, 7 HT) spinal neurons excited by ED. During intraesophageal application of capsaicin (0.2 ml, 1 min), activity of 9 (45%) neurons (6 LT, 3 HT) significantly increased to 28.2 ± 3.9 impulses/s from a background level (background cell activity 2) of 7.2 ± 2.6 impulses/s (P < 0.01). These neurons were not tested further for sensitization of ED responses to intraesophageal capsaicin. An example of a spinal neuronal response to intraesophageal capsaicin is shown in Fig. 1F. Of the 11 spinal neurons whose activity was not increased from background levels (background cell activity 2) in response to capsaicin (6.9 ± 3.1 vs. 7.8 ± 3.5 impulses/s), intraesophageal capsaicin enhanced excitatory responses of 6 LT neurons to 0.2-ml ED and 1 HT neuron to 0.4-ml ED but did not affect ED responses in 4 neurons (1 LT, 3 HT). Figure 3C shows a spinal neuron whose ED response was enhanced by intraesophageal HCl (0.01 M, 0.2 ml). E: a neuron excited by intraesophageal bradykinin (BK; 10 μg/ml, 0.2 ml). F: a neuron excited by intraesophageal capsaicin (Cap: 10 μg/ml, 0.2 ml).
26.6 and 328.2 ± 56.0 impulses before intraesophageal capsaicin (ED 1) and during intraesophageal capsaicin (ED 2), respectively. After 5.5 ± 0.6 min (range 3.0–7.2 min) of recovery, the mean total response to ED (ED 3) of these neurons was 189.6 ± 47.4 impulses. Response to ED 2 was greater than that to ED 1 (P < 0.05). Also, background activity from the 7 LT neurons was not statistically different among conditions: *background cell activity 1* (before the initial ED) was 7.7 ± 3.2 impulses/s, *background cell activity 2* (before the application of capsaicin) was 8.7 ± 3.7 impulses/s, and *background cell activity 3* (after recovery from capsaicin) was 7.2 ± 3.9 impulses/s.

Fig. 2. Recording sites of upper thoracic (T3) spinal neurons responding to mechanical and/or chemical stimulation of esophagus. A: solid squares represent spinal neurons excited by ED and also sensitized by intraesophageal chemicals. B: solid circles represent spinal neurons excited by ED but not sensitized by intraesophageal chemicals. C: schematic drawing of the T3 spinal segment (30). I–X, laminae; Liss, Liss’s tract; LSN, lateral spinal nucleus; Pyr, pyramidal tract; IM, intermediomedial nucleus; IL, intermediolateral nucleus; CC, column of Clarke.

Fig. 3. Intraesophageal chemicals enhanced excitatory responses of spinal neurons to ED (0.2 ml, 20 s). A: intraesophageal HCl (0.01 M, 0.2 ml, 2 min) enhanced excitatory response of a spinal neuron to ED. B: intraesophageal BK (10 μg/ml, 0.2 ml, 2 min) enhanced excitatory response of a spinal neuron to ED. C: intraesophageal Cap (10 μg/ml, 0.2 ml, 2 min) enhanced excitatory response of a spinal neuron to ED. D: intraesophageal normal saline (0.2 ml, 2 min) did not affect excitatory response of this spinal neuron to ED.
A summary of responses to ED (0.2 ml) for LT neurons that were sensitized by intraesophageal HCl, bradykinin, or capsaicin is shown in Fig. 4. In addition, intraesophageal saline or vehicle did not affect the total responses to ED (0.2 ml) before, during, or after intraesophageal chemicals (146.3 ± 26.2, 174.0 ± 43.2, or 165.8 ± 45.2 impulses, n = 4) (Fig. 4). An example of the effect of intraesophageal saline on a neuronal response to ED is shown in Fig. 3D.

When spinal neuronal responses to all three chemical stimuli were combined to obtain more trials, 20/25 sensitized responses were found for HT neurons, whereas 4/18 sensitized responses were found for HT neurons. LT neurons were more likely to be sensitized than HT neurons (P < 0.001). Furthermore, for 10 spinal neurons responding to ED and also examined for response to all of three chemicals, 5 neurons responded to capsaicin, 1 neuron responded to both capsaicin and bradykinin, 1 neuron responded to bradykinin alone, 3 neurons responded to all of the chemicals, and no neuron responded to HCl alone. Capsaicin was more likely to cause a response than HCl (P < 0.02).

**DISCUSSION**

The results of this study show that instillations of esophageal chemicals (HCl, bradykinin, and capsaicin) alter background activity and enhance responses of some spinal neurons with mechanical esophageal afferent input. Intraesophageal HCl increased activity of 15% of the spinal neurons and enhanced the excitatory responses of 53% (9/17) of the remaining spinal neurons with esophageal mechanical input. Intraesophageal bradykinin increased activity of 28% of the remaining spinal neurons and heightened responses of 53% (8/15) of the remaining spinal neurons with esophageal mechanical input. Intraesophageal capsaicin altered activity of 45% and increased the responses of 64% (7/11) of the remaining neurons to ED. It was also observed that low-threshold neurons are more often sensitized by chemicals than neurons categorized as high threshold. Hyperexcitability and hyperresponsiveness of mechanical afferent reception of spinal neurons associated with esophageal chemicals observed in the present study may be involved in the development of esophageal pain and/or hypersensitivity.

**Effects of HCl.** Although no studies have been done to examine the effects of sensitizing the esophagus with acid on visceral afferent fibers or spinal neurons, a few studies have investigated the effects of acid on vagal esophageal afferents and neurons of the nucleus tractus solitarii (28, 48). With single afferent unit recordings, 10% (3/28) of the dog vagal esophageal fibers responding to ED are specifically stimulated by HCl/pepsin in the esophagus (48). Even more fibers are stimulated in cats, in which infusion of acid/pepsin into the esophagus produces an increase in firing rate of 31% (5/16) of vagal afferent tension-sensitive fibers (28). Furthermore, extracellular recordings from the nucleus of the solitary tract shows that 57% (12/21) of the neurons excited by ED in an intensity-dependent manner exhibits a decrease in threshold for responses to ED and an increase in firing after acid/pepsin exposure in cat esophagus (28). Sensitization of these central neurons to ED after acid/pepsin infusion is not attenuated by cervical spinal transection at C1–C2 segments (28). Therefore, data suggest that spinal pathways do not contribute to neuronal sensitization of the nucleus of the solitary tract in medulla. However, the present study showed that administering acid to the esophagus excited 15% of the cells that also were excited by ED and enhanced the ED responses of 53% of the neurons that responded only to ED. These results showed that esophageal neuronal processing of esophageal information also contributed to sensitization for mechanical esophageal input to spinal neurons by intraesophageal chemicals.

How acid acts on the primary sensory neurons is not completely known. Esophageal spinal afferents include a mixture of small-diameter (Aδ and C) fibers that transmit stimuli from the muscular, serosal, and interepithelial layers of the esophagus to spinal cord (50, 51). Afferent Aδ-fibers respond to mechanical stimuli and mediate esophageal pain that is generally perceived as abrupt and sharp, whereas C-fibers are polymodal receptors that respond to a wide variety of tissue damaging stimuli such as noxious mechanical inputs, heat, acid, and chemicals (25, 26, 50, 51). The acid-sensing ion channel (ASIC) family is a strong candidate as mediator for HCl transducers on sensory neurons. Of these, ASIC3 has been suggested as the main acid receptor for esophageal noceception (27, 33). Furthermore, the terminals of small spinal visceral afferent fibers also contain a high density of transient receptor potential vanilloid receptor-1 (TRPV1), which is a cation channel activated by capsaicin, heat, acidity, and ethanol. Histological and functional evidence has shown that the esophagus is innervated by TRPV1-expressing sympathetic afferent fibers that are critical for triggering heartburn or pain in esophagitis and modulating esophageal blood flow (27, 51, 54). Activation of these channels by acid leads to Na+ and Ca2+ influx and excitation of the sensory neurons (a primary function), which lead to the release of neuropeptides including glutamate, substance P, and calcitonin gene-related peptide (a secondary function). These neurotransmitters may cause a neuroplastic change in the primary sensory neurons and, ultimately, in central neurons (49). Therefore, ASIC3 and TRPV1 channels may play a critical role in spinal neuronal hyperresponsiveness of mechanical reception by acid exposure in the esophagus as observed in the present study.

**Effects of bradykinin.** Bradykinin is a powerful algesic agent released from ischemic and inflamed tissues. Bradykinin has been called a vasoneuroactive agent because of its action of vasodilation, increased capillary permeability, and excitation of nociceptors. Bradykinin stimulates the nerve endings that primarily encode nociceptive impulses transmitted from somatic and visceral (vagal, sympathetic) fine afferents, such as spinal visceral afferents originating from the esophagus, heart, stomach, duodenum, gallbladder, colon, and testis (see re-
views, Refs. 3, 13, 19). Bradykinin does not stimulate only nociceptors but also excites afferents that most likely are not involved in nociception. For example, vagal afferents from the gastrointestinal tract are traditionally believed to play an important role in conveying information about autonomic regulatory function (e.g., absorption, secretion, storage, emptying) and conscious sensations (e.g., satiety, hunger, nausea), whereas nociceptive signals travel via spinal visceral afferents. However, bradykinin stimulates small vagal afferent fibers from the esophagus, stomach, and duodenum (35, 36, 50, 51). It has been presumed that nociceptive input through vagus nerves may play a role in the emotional-affective and autonomic response component of visceral pain, whereas spinal visceral afferent pathways may be related to the sensory-discriminative and somatomotor response aspects of visceral pain (15, 40, 57).

Differential sensitivity to bradykinin of ED-sensitive mechanoreceptors in vagal and sympathetic afferents of the opossum has been examined. It has been observed that 66% of the vagal low-threshold afferent fibers with mechanical esophageal input respond to systemically administered bradykinin (49). In contrast, all wide-dynamic-range and high-threshold mechanoreceptive fibers in T6–T8 sympathetic chain and splanchnic nerve are activated by bradykinin. However, there are no available data to show the effects of bradykinin on activity of the vagal and spinal central neurons receiving esophageal input. The present study showed that intraesophageal bradykinin increased activity of 28% (7/25) of T3 spinal neurons excited by ED and also enhanced excitatory responses of 53% (8/15) of tested neurons to mechanical esophageal input. Regarding the mechanism of action on the sensory neurons, it has been shown in opossum that bradykinin can excite sympathetic wide-dynamic-range and high-threshold afferent fibers in the esophagus, mainly through B2 receptors, with or without a contractile effect on the esophageal muscle (49). However, other subtypes of bradykinin receptors may be involved (B1, B3–5) (42, 43). Recently, it has been suggested that bradykinin-induced excitation and sensitization of nociceptors also occurs through sensitization and/or activation of TRPV1 in the primary afferent neurons via an intracellular secondary messenger pathway (52, 53).

Effects of capsaicin. Capsaicin is the pungent ingredient in a wide variety of red peppers of genus Capsicum. It can stimulate the afferent neurons through activation of a specific receptor, which was called the capsaicin receptor (4) and, more recently, the TRPV1 (6). Spinal visceral afferent Aδ- and C-fibers and their cell bodies in dorsal root ganglia contain TRPV1 and respond to noxious mechanical stimuli, heat, acid, and chemicals. TRPV1 activation in primary afferent neurons evokes the sensation of burning pain and neurogenic inflammation. Therefore, it may play an important role in the development of esophageal pain and/or hypersensitivity (26, 27, 50, 51). Increased TRPV1 expression in the inflamed human esophagus may mediate the heartburn in esophagitis, and TRPV1 blockers are presumed to provide novel treatment for esophageal hypersensitivity and hyperactivity (27). A few studies have been performed to examine the effects of capsaicin on vagal afferent or efferent fibers originating from the esophagus. In the guinea pig, administration of capsaicin on the outside of esophagus excites 17% of A-fibers and 94% of C-fibers that have mechanosensitive properties and originate from the nodose (inferior) vagal ganglion (57). In dogs, only 14% of the slowly and rapidly adapting mechanoreceptors in the cervical vagal nerve respond to topically applied capsaicin on the esophagus (48). In ferrets, 20% (2/10) of the tension mechanoreceptors in the esophagus respond to intraluminal application of capsaicin (2); intraesophageal capsaicin reflexly elicits responses in 88% (7/8) of vagal efferent fibers that also respond to mechanical esophageal input (36). Furthermore, application of capsaicin on the outside of guinea pig esophagus evokes action potential discharge in all of the tested jugular A- and C-fibers originating from jugular (superior) vagal ganglia (57). The jugular neurons are derived from the embryonic neural crest, as are the spinal sensory neurons (1). However, no study has been done to examine the effects of extra- or intraesophageal capsaicin on activity of spinal neurons in any animal species. The present study is the first to characterize the spinal neuronal responses to intraesophageal capsaicin and show the enhancement of excitatory ED responses of spinal neurons during capsaicin exposure in esophagus. The results show that intraesophageal capsaicin increased the background activity of 45% of the T3 spinal neurons responding to ED, including six LT and three HT neurons; capsaicin exposure in esophagus also heightened the responsiveness of 64% of the spinal neurons with mechanical esophageal input. It is suggested that capsaicin activating TRPV1 may play an important role in peripheral receptors and/or spinal central neuronal hypersensitivity of chemical and mechanical perception in the esophagus such as esophagitis (27).

Considering all chemical stimuli with respect to sensitization. The relatively moderate HCl stimulus to the esophagus had the least ability to increase spontaneous discharge of spinal neurons, whereas capsaicin was most effective on the basis of responses observed in 10 spinal neurons tested with all 3 stimuli. Nonetheless, a similar percentage of ED responses were augmented in spinal neurons whose spontaneous firing rates were unaffected by HCl, bradykinin, or capsaicin. Of the studied neurons, although we did not observe significant changes in background activity for the different groups, responses to ED were enhanced by intraesophageal chemicals. The reason for this observation was not clear. It was presumed that subthreshold chemical stimulation of ASIC, TRPV1, and/or bradykinin receptors in the esophageal nerve endings might involve an increase of the currents generated by intraesophageal HCl, bradykinin, or capsaicin, but the increased current was insufficient to generate action potentials in the afferent fibers. Alternatively, the number of additional action potentials/time traveling in afferent fibers was insufficient to increase the firing rate of spinal neurons. When the esophagus was distended, the previous subthreshold activation by intraesophageal chemicals interacted with the mechanical stimulus to increase the number of action potentials traveling in afferent fibers and further enhanced an excitatory response of spinal neurons to ED. Therefore, sensitization of spinal neurons to mechanical esophageal stimulus did not require or was not necessarily associated with the increase in background activity of different groups of spinal neurons.

Summary. The present study shows that some thoracic spinal neurons responding to esophageal distension are also excited by intraesophageal chemicals, including a gastric fluid component (HCl), a food ingredient (capsaicin), and an inflammatory mediator (bradykinin). These intraesophageal irritants also en-
hanced the excitatory responses of >50% of tested spinal neurons with mechanical esophageal input. In conclusion, intraesophageal chemicals sensitize the activity of upper thoracic spinal neurons responding to esophageal distension. Mechanisms underlying spinal neuronal hyperresponsiveness or hyperresponsiveness may involve both peripheral esophageal receptors and a central visceroreceptive network. The heightened responsiveness of spinal neurons receiving esophageal input observed in the present study may contribute to the primary and secondary hyperresponsiveness in the patients with heartburn, esophagitis, gastroesophageal reflux, and noncardiac chest pain.

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