Differential relaxation and contractile responses of the human upper esophageal sphincter mediated by interplay of mucosal and deep mechanoreceptor activation

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Szczesniak MM, Fuentealba SE, Burnett A, Cook IJ. Differential relaxation and contractile responses of the human upper esophageal sphincter mediated by interplay of mucosal and deep mechanoreceptor activation. Am J Physiol Gastrointest Liver Physiol 294: G982–G988, 2008. First published February 7, 2008; doi:10.1152/ajpgi.00496.2007.—Background and aims: the neural mechanisms of distension-induced esophagoupper esophageal sphincter (UES) reflexes have not been explored in humans. We investigated the modulation of these reflexes by mucosal anesthesia, acid exposure, and GABA<sub>B</sub> receptor activation. In 55 healthy human subjects, UES responses to rapid esophageal air insufflation and slow balloon distension were examined before and after pretreatment with 15 ml of topical lidocaine, esophageal HCI infusion, and baclofen 40 mg given orally. In response to rapid esophageal distension, UES can variably relax or contract. Following a mucosal blockade by topical lidocaine, the likelihood of a UES relaxation response was reduced by 11% (P < 0.01) and the likelihood of a UES contractile response was increased by 14% (P < 0.001) without alteration in the overall UES response rate. The UES contractile response to rapid esophageal air insufflation was also increased by 8% (P < 0.05) following sensitization by prior mucosal acid exposure. The UES contractile response, elicited by balloon distension, was regionally dependent (P < 0.05) (more frequent and of higher amplitude with proximal esophageal distension), and the response was attenuated by topical lidocaine (P < 0.05). Baclofen (40 mg po) had no effect on these UES reflexes. Abrupt gaseous esophageal distension activates simultaneously both excitatory and inhibitory pathways to the UES. Partial blockade of the mucosal mechanosensitive receptors permits an enhanced UES contractile response mediated by deeper esophageal mechanoreceptors. Activation of acid-sensitive esophageal mucosal chemoreceptors upregulates the UES contractile response, suggestive of a protective mechanism.

esophagus; lidocaine; HCl

Improvements in the neuropharmacology of reflexive responses of the upper esophageal sphincter (UES) and esophagoesophageal reflexes (secondary peristalsis) is relevant to the understanding of the pathophysiology of conditions characterized by impaired esophageal clearance, an abnormal belch reflex, and excessive esophagopharyngeal regurgitation. These conditions are potentially important in the pathogenesis of extraesophageal disorders such as reflux laryngitis, cough, and asthma.

Several reflexive UES responses triggered by stimulation of sensory afferents proximally from the pharynx (14) and distally from the esophagus (1, 5) have been demonstrated. The esophagopharyngeal reflexes are mediated via the brain stem by vagal afferents arising from esophageal mucosa and from muscle layers (11). The efferent targets of these reflexes are the component muscles of the UES and selected muscle groups that exert a distracting force on the hyoid bones (22). Physiological studies have identified a number of esophageal mechanoreceptors that may be responsible for these reflexes and have classified them according to their location in the esophageal wall and specific stimulus-response characteristics (17, 21). Specialized vagal primary afferent nerve endings, interganglionic laminar endings (IGLE), have been identified between the circular and the longitudinal muscle layers of the esophagus (15). A study of receptive fields of vagal afferent fibers in a guinea pig esophagus suggests that the IGLEs are tension-sensitive receptors (28). Various types of nerve endings have also been shown to exist within the esophageal mucosa (26) although it is unknown whether these correspond to the receptive fields of vagal afferent fibers.

The UES responses to esophageal distension are dependent on the nature of the distending stimulus. Both slow and rapid distension can induce secondary peristalsis and/or UES contraction, whereas only rapid esophageal distension induces UES relaxation (9, 11). The UES relaxation response is often accompanied by the esophagoglottal closure reflex and esophageal hyoid distraction (11, 22). This differential response to distension rate suggests that two different mechanoreceptor populations are operative, one slowly adapting and one rapidly adapting. This notion is supported by electrophysiological studies of gastroesophageal vagal afferents in animal models demonstrating slowly adapting mechanoreceptors located in the muscle layers that detect tension and/or stretch while rapidly adapting mucosal mechanoreceptors respond to deformation (20).

It is plausible that esophageal chemostimulation may augment or inhibit the esophag-UES relaxation reflex. The latter scenario would provide a potential “protective response” against retrograde flow, whereas the former scenario could facilitate esophagopharyngeal regurgitation, suggesting that a manipulation of these afferent pathways might be a rational therapeutic target. Secondary peristalsis, which is an important mechanism for the clearance of refluxed acid and retained food boluses, is triggered by esophageal distension. It remains unknown whether chemoreceptor activation or acid sensitization can contribute to the triggering of secondary peristalsis in the human (19).

GABA<sub>B</sub> receptors exist presynaptically at a variety of central sites including vagal afferent terminals in the dorsal me-

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distal to UES midpoint). Subsequently followed by balloon distensions in the proximal esophagus (8 cm through the center core channel of the manometric assembly. Four different experimental conditions, reducible insufflation rates. Driven by compressed air at a fixed pressure giving rapid and reproducible insufflations were performed by a custom-built system that incorporated a plunger-driven pneumatic pump at 0.15 ml/min (Dentsleeve). Sideholes were located proximal to the midsleeve position (3, 4.5, and 6 cm distal to the midsleeve was used for rapid air insufflation of the esophagus. The catheter incorporated two silicone balloons, 8 and 18 cm distal to UES to intraesophageal air insufflation and balloon distension was monitored UES pressure (Dentsleeve, Bowden, South Australia). A center channel (1.2 mm ID) exiting 4.5 cm distal to the midsleeve sensor for several seconds and obtaining the mean pressure. Perfusion of the three manometric sideholes at the proximal margin of the sleeve assembly in randomized order, 8 cm and 18 cm distal to UES midpoint. To minimize irritation to the pharynx by the perfusate, baseline pharyngeal pressure was acquired after every five air insufflations by perfusing the three manometric sideholes at the proximal margin of the sleeve sensor for several seconds and obtaining the mean pressure. UES pressure was monitored throughout the study. Response of the UES to intraesophageal air insufflation and balloon distension was classified as relaxation, when UES pressure dropped to within 3 mmHg of the baseline pharyngeal pressure, or contraction, when the peak pressure during a 10-s interval after the application of the distending stimulus exceeded by at least 5 mmHg the peak basal UES pressure during the 10-s interval before the stimulus. On occasions, rapid air distension triggered both a UES contraction and a relaxation response, generally with the relaxation preceding the contraction. In this circumstance, the response was classified as a relaxation (Fig. 1).

Secondary peristalsis, when triggered in response to rapid air distension, was recorded. Distensions, which were followed immediately by a spontaneous primary swallow, were removed from the analysis of secondary peristaltic responses because the primary swallow made it impossible to distinguish a secondary peristaltic response.

Statistical analysis. Inferences on the proportions of stimuli triggering various UES responses and the influence on these proportions by lidocaine, acid, and baclofen were made by using \( \chi^2 \) analysis. Inferences on the regional differences of stimulus on the frequency of distension-induced UES relaxations, contractions, and secondary peristalsis were made by using \( \chi^2 \) analysis. A Student’s paired \( t \)-test was used to compare the effect of experimental conditions on the amplitude of the UES contractile responses.

RESULTS

We studied a total of 55 subjects in four experimental groups: group I \((n = 16, 8\) men; mean age 30; range 20–50 yr), group II \((n = 15, 5\) male; mean age 30; range 20–50 yrs), group III \((n = 15, 9\) male; mean age 30; range 20–50 yrs), group IV \((n = 9, 3\) male; mean age 30; range 20–50 yrs); four volunteers participated in all four groups.

UES responses to rapid esophageal insufflation and the effect of mucosal blockade with lidocaine (group I). Of a total 800 esophageal air insufflations, 751 were analyzable. Under basal conditions, esophageal air insufflations caused UES relaxation or contraction in 39 and 27%, respectively. After pretreatment with lidocaine, the proportion of relaxation responses decreased by 11% \((P = 0.01, \chi^2)\), whereas the proportion of contractile responses increased by 14% \((P < 0.001, \chi^2)\) (Fig. 2A). The likelihood of a UES relaxation response, but not a contractile response, was volume dependent (Fig. 2B).

UES responses to rapid esophageal insufflation, effect of mucosal acidification (group II). Of the total 750 esophageal air insufflations, 703 were assessable. Under basal conditions, esophageal air insufflations caused UES relaxation or contraction in 55 and 35%, respectively. After acid infusion, the proportion of contractile responses increased by 8% \((P <
0.05), whereas the frequency of relaxation response was not significantly affected (Fig. 3).

UES responses to rapid esophageal insufflation, effect of baclofen (group III). Of a total 750 esophageal air insufflations, 726 were assessable. Under basal conditions esophageal air insufflations caused UES relaxation or contraction in 38 and 33%, respectively. Pretreatment with 40 mg baclofen given orally caused a minor reduction in the frequency of UES relaxations [not significant (NS)] and no change in the frequency of a contractile response (Fig. 4).

UES responses to rapid esophageal insufflation, effect of baclofen (group III). Of a total 750 esophageal air insufflations, 726 were assessable. Under basal conditions esophageal air insufflations caused UES relaxation or contraction in 38 and 33%, respectively. Pretreatment with 40 mg baclofen given orally caused a minor reduction in the frequency of UES relaxations [not significant (NS)] and no change in the frequency of a contractile response (Fig. 4).
UES responses to balloon distension of the esophagus (groups I–IV). Esophageal balloon distension caused either a UES contractile response (80%) or no discernible response in 20%. Occurrence of UES relaxations was negligible (<0.01%). The frequency of UES contractile response to balloon distension was 30% lower in the distal vs. proximal esophagus ($P < 0.001, \chi^2$), whereas the frequency of UES relaxation was unchanged. B: stratification by inflation volume showed the response was not volume dependent.

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Fig. 3. Effect of prior esophageal acid exposure on UES responses to rapid esophageal air insufflation. A: when considering all volumes together, prior esophageal acidification resulted in a significantly greater likelihood of a UES contraction ($P < 0.001, \chi^2$), whereas the frequency of UES relaxation was unchanged. B: stratification by inflation volume showed the response was not volume dependent.

Fig. 4. Effect of baclofen on UES responses to rapid esophageal air insufflations irrespective of inflation volume (A) or stratified by volume (B). Baclofen 40 mg given orally did not influence the likelihood of either UES relaxation or contraction.
to 30 mmHg when the stimulus was changed to the distal esophagus (Fig. 5B). Neither baclofen nor HCl had an effect on the amplitude or frequency of UES contractions induced by esophageal balloon distension.

Secondary peristaltic responses to rapid air insufflation (groups I–III). Under basal conditions, 28% of rapid air insufflations triggered secondary peristalsis. Secondary peristalsis was seen to occur in the context of any or no UES response but was twice as likely to be initiated when the UES response was a contractile compared with UES relaxation or absent UES response \((P < 0.05)\) (Fig. 6). Lidocaine anesthesia increased the proportion of UES contractile responses and decreased the proportion UES relaxation responses (group I) without influencing the likelihood of a secondary peristaltic response. Prior acid exposure significantly decreased the frequency of secondary peristaltic events triggered by rapid air insufflation by 8% \((P < 0.05)\).

**DISCUSSION**

The major findings of this study are that esophageal mucosal anesthesia with lidocaine reduces the likelihood of a UES relaxation response and increases the likelihood of a contractile response to abrupt esophageal distension by air insufflation. Additionally, prior esophageal acid exposure increases the likelihood of this UES contractile response. In contrast, balloon distension almost exclusively triggers UES contraction, and both the magnitude and the likelihood of this response were diminished by esophageal mucosal anesthesia with lidocaine. Baclofen, at least in a dose of 40 mg po, failed to modulate any of the UES reflexes examined.

The presence of esophageal mucosal afferents is well documented in animals (17, 26), and evidence exists for presence of sensory nerve fibers in human esophageal mucosa (13). Our findings indicate that mucosal mechanoreceptors are of primary importance in mediating the esophago-UES relaxation response, whereas deep mechanoreceptors primarily mediate UES contractile responses. Mucosal afferents, which are both lidocaine and acid sensitive, interact at some level with afferent pathways arising from deeper mechanoreceptors and are capable of modulating the likelihood of the UES contractile response.

These findings can be interpreted in relation to centrally projecting vagal afferent endings, which have been found in both mucosal and muscular layers of the esophageal wall (4, 8).
In a ferret, three functional classes of receptors have been identified: 1) mucosal, responsive to light touch (stroking with von Frey hair); 2) muscular tension, responsive to circumferential stretch; and 3) mucosal receptors that respond to both stroking and stretch (17). Rapid distension of the feline esophagus by air insufflation triggers an esophago-UES relaxation reflex, this response being blocked by topical mucosal anesthesia (lidocaine) or by removal of the esophageal mucosa. In contrast, the esophago-UES contractile reflex in the cat, triggered by slow balloon distension, is unaffected by mucosal anesthesia or by stripping off the mucosa (11). This suggests that, in the cat at least, the mechanoreceptors responsible for triggering esophago-UES relaxation are located in the mucosa and receptors responsible for the esophago-UES contraction reflex are located in the muscle layer. Our findings in the present study provide evidence for a similar anatomical distribution of mechanoreceptors responsible for the esophago-UES relaxation reflex in the human esophagus. Although we did not observe complete abolition of the esophago-UES relaxation reflex after topical lidocaine application, this observation is most likely due to subtotal blockade of the total receptive field of mucosal lidocaine-sensitive receptors in the awake human subject with the use of our method of lidocaine delivery.

In contrast to the study of Lang et al. (11) in which the air insufflation parameters were carefully chosen to invariably trigger a UES relaxation in the cat, in the present study [and indeed in earlier studies in the human (9)], we saw a mixed response with 39% UES relaxations and 27% contractions. In the human esophagus, considerably greater amounts of air are required to trigger a response. Because the rapidity of insufflation is limited both by esophageal capacitance and caliber of the insufflation channel within the catheter, in the present study, rapid air insufflations most likely activated both rapidly adapting mucosal mechanoreceptors as well as slowly adapting muscular mechanoreceptors. We propose, in response to abrupt esophageal distension, that activation of mucosal nerves acting via some intermediate neural pathway exerts an inhibitory influence on nerves projecting from the muscularis. These putative afferents are likely to project to the medulla; however, the data that we have is insufficient to specify where those putative interneurons are located. Hence, blockade of input from the mucosal receptors by topical lidocaine would remove this inhibitory influence such that the response to abrupt esophageal distension is mediated by muscular mechanoreceptors predominates, resulting in increased likelihood of a UES contraction.

In contrast to the effects of lidocaine on the UES responses to abrupt esophageal distension by air insufflation, the finding that lidocaine anesthesia reduces the frequency of balloon distension-induced UES contractions could indicate a second population of lidocaine-sensitive mucosal nerves. These nerves may project to central efferent pathways mediating UES contraction where they may act synergistically with those afferents arising in the muscular layer, which are primarily responsible for mediating the UES contractile response. Two types of mucosal receptors have been identified in a ferret esophagus (16), mucosal (responding to light touch) and tension-mucosal receptors (responding to both touch and circumferential stretch). The effect of stimulation of these mucosal receptors on UES tone is unknown. However, if mucosal receptors were to trigger a UES relaxation response and the tension-mucosal receptors triggered the UES contractile response, balloon distension triggering the latter together with activation of the deeper muscular tension mechanoreceptors would provide a dominant input causing the observed UES contractile response. Blockade of these tension-mucosal receptors by lidocaine would only leave the deeper muscular receptors to trigger the UES contractile response and, hence, the observed decreased frequency of UES contractions. These properties of the mucosal receptors is physiologically plausible because it would enable the esophagus to “distinguish” between gaseous reflux (distending stimulus) and liquid or solid reflux (distending plus touch stimuli). In the case of gaseous reflux, UES relaxation should be allowed to vent the gas, whereas during liquid or solid reflux, UES contraction would protect the supra-esophageal structures from acidic refluxate.

There are important clinical implications of the finding that the UES relaxation reflex is mediated via mucosal mechanoreceptors. Our preliminary studies have suggested that this reflex, when compared with healthy controls, is upregulated in patients with laryngitis (23). We had hypothesized that one mechanism for this dysregulation in patients with laryngitis might be prior sensitization of the mucosal afferent mechanoreceptors by acid. However, our findings in the present study, at least in the context of short-term experimental esophageal acid exposure, failed to support the hypothesis that acid-related upregulation of the UES relaxation reflex exists. In fact, we found that experimental acidification enhanced the UES contractile response. This response may protect the esophagus against acid exposure because similar chemostimulatory augmentation of UES pressure has been observed previously during esophageal acid infusion (6) and during spontaneous gastroesophageal acid reflux (25). The upregulation of the UES contractile response by acute esophageal acid infusion observed in healthy controls may not be indicative of UES responses in patients with chronic acid exposure in whom the possibility exists that afferent nerves are desensitized or damaged by prolonged acid exposure.

Ex vivo and in vivo studies in a ferret demonstrated that the GABAB agonist baclofen, acting peripherally on primary vagal afferent receptors, dose dependently attenuates traffic within these afferents in response to esophageal circumferential stretch (16). In a cat model, rapid esophageal distension, acting via lidocaine-sensitive mucosal mechanoreceptors, induces UES relaxation, reflex esophageal (nonperistaltic) contraction, glottic closure, and hyoid bone distraction; all of which are blocked or attenuated by the GABAB agonist baclofen shown to be acting on peripheral vagal afferents (11). The maximal permissible dose of the GABAB agonist baclofen approved in the present study (40 mg po) failed to abolish distension-induced esophago-UES relaxation. No definite conclusions can be made about the lack of response to baclofen in the human as the dose used in the present study [40 mg po (~0.57 mg/kg)] is roughly half that used in the cat (1 mg/kg) (11).

Secondary peristalsis executes a protective function by clearing the esophagus of refluxed gastric contents (7). Our findings have shown that secondary peristalsis occurred more frequently when associated with UES contractile response. This is not unexpected because both responses are initiated by the stimulation of muscular mechanoreceptors (11). Secondary peristalsis, in contrast to the esophago-UES contractile reflex, was unaffected by mucosal anesthesia. This indicates that,
although both responses share a common afferent pathway, the mechanism controlling reflexive UES responses receives input from both mucosal and muscular mechanoreceptors. Because 72% of rapid insufflations did not trigger secondary peristalsis, we explored the hypothesis that UES contraction might act as a protective mechanism when secondary peristalsis failed. However, we observed the UES barrier function was preserved (i.e., active UES contraction or maintenance of basal tone) in only 50% of such instances, which did not support such a hypothesis.

It has been shown that acidic barium initiates secondary peristalsis more frequently than neutral barium in opossum (10) but not in the human (24). On the other hand, it has been shown that patients with gastroesophageal reflux disease with chronic acid exposure exhibit a defect in triggering of secondary peristalsis (18, 27), but it is unclear from these studies whether this defect was contributing to, or was caused by, esophageal inflammation. Our findings in the present study have shown a potential counterprotective response in that a brief (15 min) acid exposure to the normal esophagus reduced the frequency of secondary peristalsis in response to air insufflation. However, this experimental scenario needs to be interpreted with caution as it may not reflect responses in patients with reflux disease.

In conclusion, our findings in humans corroborate the results obtained in animals that mucosal lidocaine-sensitive afferents mediate the distension-induced esophago-UES relaxation reflex and lidocaine-insensitive, presumably muscular, mechanoreceptors mediate the distension-induced esophago-UES contractile reflex. The latter reflex was also upregulated by esophageal acidification indicative of a possible protective mechanism.

GRANTS

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