Sensory-motor responses to mechanical stimulation of the esophagus after sensitization with acid

Asbjørn Mohr Drewes, Hariprasad Reddy, Camilla Staahl, Jan Pedersen, Peter Funch-Jensen, Lars Arendt-Nielsen, Hans Gregersen

INTRODUCTION

Pain arising from the esophagus is very common clinically and in the normal population, but the mechanisms involved are poorly understood. Due to the difficulties in characterizing clinical pain, human experimental models have been developed to investigate the pain pathways in a standardized way in volunteers and patients. These models provide the possibility to control the stimulus parameters and to assess the response quantitatively. Furthermore, the nociceptive system can be sensitized in the laboratory, resulting in allodynia (painful sensations to stimuli that are not normally painful), hyperalgesia (increased sensation to stimuli that are normally painful) and increase in the evoked referred pain area. The sensitization most likely plays an important role in chronic visceral pain disorders. Experimental chemical stimulation with acid has been used to sensitize the esophagus. However, the literature has not been consistent with respect to the evoked mechanical hyperalgesia, probably due to methodological problems related to the stimulus modalities used.

Distension of the gut is a physiologic stimulus, and consequently most researchers have used experimental balloon distension models to investigate basic pain mechanisms in the gastrointestinal (GI) tract. Most previous studies have used volume and pressure as proxies of the mechanical deformation and force applied to the gut wall. However, the mechanical parameters tension, stress and strain are of more value than pressure and volume when studying the esophagus, as these parameters provide more valid information about the mechanical forces and deformation (elastic properties) during distension.

Furthermore, the muscle function is better evaluated when

METHODS: Thirty healthy subjects were included. Distension of the distal esophagus with a balloon was performed before and after perfusion with 0.1 mol/L hydrochloric acid for 30 min. An impedance planimetry system was used to measure cross-sectional area, volume, pressure, and tension during the distensions. A new model allowed evaluation of the phasic contractions by the tension during contractions as a function of the initial muscle length before the contraction (comparable to the Frank-Starling law for the heart). Length-tension diagrams were used to evaluate the muscle tone before and after relaxation of the smooth muscle with butylscopolamine.

RESULTS: The sensitization resulted in allodynia and hyperalgesia to the distension volumes, and the degree of sensitization was related to the infused volume of acid. Furthermore, a nearly 50% increase in the evoked referred pain was seen after sensitization. The mechanical analysis demonstrated hyper-reactivity of the esophagus following acid perfusion, with an increased number and force of the phasic contractions, but the muscle tone did not change.

CONCLUSION: Acid perfusion of the esophagus sensitizes the sensory pathways and facilitates secondary contractions. The new model can be used to study abnormal sensory-motor mechanisms in visceral organs.

Key words: Esophagus; Mechanical; Sensitization; Motility; Reflux; Pain

© 2005 The WJG Press and Elsevier Inc. All rights reserved.
the forces and tensions can be quantitated, rather than measuring the luminal pressure. However, the sensory-motor responses of the organ during a mechanical stimulus cannot be evaluated independently of the mechanical forces and deformation. Thus, phasic contractions and changes in muscle tone can influence the sensory response themselves, and in diseases of the esophagus hyper-reactivity may give major contribution to the symptoms. Methods to estimate and control the mechanical response will thus allow better explanations of the effects on the sensory-motor response during the mechanical stimulations with and without sensitization of the pain system.

Systematic investigation of both the sensory and motor responses to controlled mechanical stimuli following experimental sensitization of the esophagus has to the best of our knowledge never been investigated. The aims of the current study were to (1) investigate the effect on sensitization of the esophagus with acid on the sensory response to controlled mechanical stimulation; (2) calculate the evoked referred pain areas to the mechanical stimulation before and after sensitization as a proxy for the central neuronal changes; and (3) evaluate the motor response to the sensitization by a new in vivo method evaluating the change in tension during contraction (the afterload tension) as function of the initial muscle length before the contraction (the preload radius).

**MATERIALS AND METHODS**

Thirty healthy subjects, 14 males and 16 females, mean age 36.5 ± 12.9 years, were included. The subjects did not suffer from any kind of chronic pain, GI symptoms or disturbances in personality. All subjects gave informed written and verbal consent prior to the study. The protocol was approved by the local ethics committee and performed in accordance with the Helsinki Declaration.

**Mechanical stimulations**

The impedance planimetry system including the principle for measurement of the cross-sectional area (CSA) has been described in detail previously. The 70-cm long probe with a diameter of 4.5 mm had a cylindrical large-sized bag near the tip. The bag was 40 mm in length and was made of 35-µm thick, non-conducting polyester urethane. A side-hole for acid perfusion was placed 2 cm above the bag. The probe had a four-electrode impedance planimetry system with four sets of ring electrodes inside the bag (GMC, Hornslet, Denmark). The volunteers were trained to clearly separate the non-painful and painful range of the sensory ratings.

Methods to obtain repeatable sensory data have previously been shown to be robust, and to discriminate sensations in the esophagus, and the small and large intestine.

After the last distension before butylscopolamine injection (see below) the volunteers were asked about referred pain to the chest or other remote areas evoked by the distensions at moderate pain (VAS = 7). If present the referred pain area was marked with a pen and transferred to a transparent paper. Later the area was digitized (ACECAD D900+ Digitizer, Taiwan) and the size calculated (Sigma-Scan, Jandel Scientific, Canada).

**Protocol**

The subjects fasted for at least 4 h prior to the experiment. Intubation was performed through the mouth. The bag was inserted into the stomach and then retracted to identify the location of the lower esophageal sphincter as a zone of high resting pressure that decreased with swallowing. Then the bag was placed 7 cm proximal to the sphincter and the probe was taped to the cheek. The subjects were asked to lie down with the head tilted by 30° after the placement of the bag. The experiment was performed in that position after 30 min of rest.

Three bag distension stimuli with a constant infusion rate of 25 mL/min were done to precondition the tissue and to obtain repeatable sensory data. The inter-stimulus interval was 60 s for all experiments. When the subjects reported slight pain (6 on the VAS), the bag was deflated using the same flow rate as during the inflation until it was empty. After these stimuli, two more distensions...
were done at the same infusion rate. When moderate pain intensity (7 on the VAS) was reached, the pump was reversed and the bag deflated. Then 20 mg butylscopolamine was given intravenously and after abolishment of the contractile activity the two last distensions were repeated.

After the first series of mechanical stimuli, the participants underwent a modified acid perfusion test. Hence, during a perfusion channel in the catheter 0.1 mol/L hydrochloric acid was infused at a rate of 7 mL/min for 30 min. If the evoked sensations due to the acid stimulation were reported unpleasant (rated ≥5 on the VAS), the perfusion was stopped for 30 s and the subjects were allowed to swallow 10 mL water. In case the perfusion was too unpleasant for the subjects, it was stopped and the amount of infused acid was measured.

After the acid perfusion, bag distensions before and after butylscopolamine were given using the same protocol as described above, before acid perfusion.

During all stimuli autonomic reactions were monitored and displayed on-screen using a Biopac MP100 system (Biopac Systems Inc., Santa Barbara, CA, USA) including sensors and recording system for electrocardiogram, pulse rate and respiration.

**Data analysis**

The circumferential wall tension was calculated according to the law of Laplace for cylindrical structures as

\[ T = \Delta P r \]

where \( T \) is the circumferential wall tension, \( r \) is the balloon radius, and \( \Delta P \) is the transmural pressure. The geometry of the esophagus during distension can be considered circular except at very low pressure levels. Therefore, the radius was determined as

\[ r = \sqrt{\frac{CSA}{\pi}} \]

All subjects stated that they more reliably rated the sensory intensity at the second distension compared to the first. Therefore, only data from the second distension were used in the analysis. After butylscopolamine the first distension was used as the maximal decrease in contractile activity was seen at the first few minutes after the injection.

As criteria for valid contractions before and after acid perfusion a pressure amplitude above 2.5 kPa was used.

In a representative sample of 10 subjects (5 males and 5 females, mean age 36.1±14.3 years) the change in tension during individual distension-induced contractions (afterload tension) was computed and expressed as function of the radius immediately before the contractions (preload radius). The diagrams were made before and after perfusion of the distal esophagus with a mean of 123 mL hydrochloric acid. An example from an individual subject is shown in Figure 1. The data were fitted with a third order polynomial. These diagrams correspond to the well-known heart ventricular function curves in terms of the ventricular stroke working as function of the mean atrial length. Such curves demonstrate the Frank-Starling mechanism of the heart now adapted to the esophagus-see appendix.

The pressure and CSA data obtained between the evoked contractions (without infusion of butylscopolamine) were used to compute the total tonic tension, whereas the tracings during butylscopolamine infusion were used for calculation of the passive tension. The active tonic tension was obtained by subtracting the passive tension from the total tonic tension

**Statistical analysis**

The results are expressed as mean±SD unless otherwise indicated. Continuous data were analyzed using t-tests. For multiple comparisons, two-way analysis of variance (ANOVA) was used with the factors: (1) before and after acid and (2) the different VAS levels. P<0.05 was considered significant. The software package SPSS v. 10.0 was used for the statistical analysis.

**RESULTS**

**Mechanical stimuli before and after acid**

All subjects completed the experiment. After the preconditioning stimuli, the curve characteristics and sensory ratings were reproducible in all subjects. The stimulus-response curves after preconditioning the tissue are shown in Figure 2 for the infused volume, CSA, pressure, and tension. The sensation intensity was approximately linear as functions of all four stimulation variables. The sensory rating increased after acid, when expressed as a function of the volume (\( F = 4.75, P = 0.03 \)), whereas no differences were found for the CSA (\( F = 1.0, P = 0.3 \)), pressure (\( F = 0.7, P = 0.4 \)) and tension (\( F = 1.2, P = 0.3 \)). The curves during butylscopolamine infusion showed the same pattern as described above, before and after acid perfusion (data not shown).

The acid infusion resulted in a more hyper-reactive esophagus as the number of contractions with pressure amplitudes above 2.5 kPa during the distensions increased from 2.9±1.5 to 3.5±1.5 after acid perfusion (\( P = 0.03 \)).

The change in tension during bag-distension-induced contractions (the afterload tension) was plotted as a function of the preload radius for 10 representative subjects (Figure 3). No contractions were observed at radii below 5 mm. Before acid infusion the afterload tension increased until a plateau was reached. This corresponds to the “Frank-Starling mechanism” relating to the less interdigitation of...
muscle filaments when the muscles are overstretched. Painful sensations (VAS >5) were experienced at preload radii higher than 11.5 mm. After the acid infusion higher afterload tensions were observed at both low and high radii as compared to baseline, and there was a tendency to more spreading of the data as some individuals obtained very high afterload tensions. The painful sensations were also only evoked at radii higher than 11.5 mm.

The total, passive and active tonic tensions before and after acid infusion are shown in Figure 4. There was no difference in curve shape between before and after acid, indicating that acid infusion does not change esophageal muscle tone.

**High and low acid responders**

The subjects tolerated a mean of 101±53 mL of acid. To see if the sensory response was related to the amount of acid infused, the subjects were divided into two groups. One group could accept 100-200 mL of acid (n = 17) and the other group, less than 100 mL of acid (n = 13). There was a relation between the evoked sensitization and the acid load as those who accepted more than 100 mL were sensitized to volume (F = 5.3, P = 0.02), pressure (F = 5.5, P = 0.02) and tension (F = 6.0, P = 0.01), but not to CSA (F = 0.9, P = 0.3). The group tolerating less than 100 mL were not sensitized to neither volume (F < 0.01, P = 0.99), pressure (F = 0.6, P = 0.4), tension (F = 0.8, P = 0.4) nor CSA (F < 0.01, P = 0.99).

**Referred pain areas**

All subjects reported referred pain to the stimulations. The referred pain areas to mechanical stimuli at moderate pain are shown in Figure 5. Additionally one male and two females had referred pain in the back. The referred pain areas increased from 27.9±29.3 cm² before acid to 41.4±39.0 cm² after acid (P = 0.047), although the volume was smaller at the distensions after acid.
DISCUSSION

The current experiment used controlled ramp distensions and preconditioning to evoke experimental pain in the esophagus in 30 subjects. The sensory response was assessed before and after sensitization of the lower esophagus by acid perfusion. The sensory rating increased after acid when expressed as a function of the volume, and the degree of sensitization was related to the infused volume of acid. Furthermore, an increase in referred pain to a standardized distension was seen reflecting activation of central facilitatory pain mechanisms. The mechanical analysis demonstrated hyper-reactivity of the esophagus following acid perfusion, with an increased number and force of the phasic contractions, but the muscle tone did not change. This illustrates that acid perfusion not only sensitizes the sensory pathways, but also facilitates motor reflexes.

Sensory response to sensitization with acid

Chronic pain is associated with modifications of the central nervous system such as central sensitization[4]. Animal experiments have demonstrated neuronal changes such as increased spontaneous activity, decreased firing threshold, and expansion of the receptive fields of spinal neurons subjected to activation and/or experimental sensitization of their peripheral afferents[5,26,27]. Sensitization of the human esophagus with acid is a valuable experimental pain model, as the evoked allodynia, hyperalgesia and referred pain patterns reflect sensitization of the nervous system and can be studied systematically[8]. Hence, decreased thresholds to physiologic stimuli seem to contribute to many of the symptoms reported by patients with inflammatory and functional diseases in the gut[28,29]. Thus, a combination of mechanical stimulation and sensitization of the esophagus may mimic the widespread pain and other sensations reported by patients with reflux disease and unexplained chest pain[30,31].

Acid-sensitive fibers have been demonstrated in animal studies, and mucosal afferents are often sensitive to different chemical stimuli[32,33]. Increased responses to mechanical, electrical and thermal stimuli after acid perfusion of the esophagus have also been demonstrated in human beings[6,9,34], although previous studies using latex balloons were not consistent. This can be due to methodological problems using latex balloons, where the distension data must be corrected for the intrinsic mechanical properties of the balloons and for the uncontrollable deformation in longitudinal direction[9,14]. Non-compliant polyester urethane bags overcome these problems. The effect of preconditioning the tissue by several distensions until the stress-strain relationship becomes reproducible has also not been considered in most previous studies[3,13]. Different modifications of the acid perfusion test have been used as a chemogenic stimulus by several authors[6,9,34,35]. When the current material was divided into those who tolerated below and above 100 mL of acid, significant increased sensation to the mechanical stimulus was only seen in the high acid group. Hence, it is recommended to use volumes higher than 100 mL in future studies.

The present study demonstrated increased sensation to the infused bag volume, but not to pressure and tension. The intraluminal pressure and tension are highly dependent on the contractile force state of the esophageal muscles, and hence not as reliable parameters as the deformation[36]. Despite the decrease in volume after acid infusion, the CSA did not decrease significantly. Thus it seems that the bag conforms to a shape where it is shorter after acid infusion. Such a shape change is likely caused by changes in the contractile activity in the acid exposed area and even in regions affected by nerve-mediated reflex responses.
Secondary contractions and muscle hyper-responsiveness can be evoked by acid in the distal esophagus due to reflex loops between mucosal afferents and the motor system\cite{37-40}. After acid perfusion increased force of the secondary contractions was evoked by the distension in the non-painful and painful range. Animal studies have shown that in contrast to the somatic system-afferents encoding both non-painful and painful sensations can sensitize in the viscera\cite{8}. The current observations in the human esophagus are in line with these studies, as the sensitization of afferents encoding conscious sensations to distension seems to change the contractile activity in the muscle via local and/or central reflexes\cite{42,43}. However, the curve form changed mostly in the pain range (to the right of the vertical line in Figure 3) and hence there seems to be a higher effect of sensitization on the painful sensations. The Frank-Starling mechanism predicts a decrease in contractile activity when the muscle is overstretched corresponding to less optimal interdigitation of actin and myosin filaments. In the present experiment the baseline curve form showed no decrease in afterload tension at maximal distension. The fact that the afterload tensions were higher after acid infusion indicates that bag distension itself does not activate the muscle maximally.

Another manifestation of the acid perfusion was the increased referred pain area to the mechanical stimulation, although the bag volume was lower after the perfusion. Enlarged referred pain areas is also a characteristic in clinical gut disorders\cite{44-46}, and are very similar to what is observed in patients suffering from chronic musculoskeletal pain\cite{12,13,15,50}. Previously, we have shown that sensitization of the esophagus results in increased referred pain areas\cite{8}, which was also shown in previous papers using neurophysiological assessment of the spinal and supraspinal pain response after acid perfusion of the esophagus\cite{8,48}.

**Mechanical and motor responses to sensitization with acid**

The sensitization resulted not only in allodynia and hyperalgesia to the distension volumes, but the esophagus also exhibited hyper-reactivity as illustrated by the increased number of contractions after the acid perfusion. Such hyper-reactivity has also been seen in animal studies\cite{37,38}. The contractions were also stronger to a given preload radius. However, the acid infusion did not change the total tonic tension, the passive tension and the active tonic tensions (Figure 4). Hence, the hyper-reactivity only accounts for phasic contractions, not for tone in the esophageal body. Previously, Sifrim et al\cite{10}, showed that acid reflux into the esophagus stimulated tone in the esophageal body. However, simultaneous distension seemed to inhibit the acid induced tone. These issues obviously need further investigations.

The preload radius where contractions were evoked by the painful stimuli (VAS = 5 and higher) did not change after acid (vertical line in Figure 2). This corresponds with the “strain theory”, i.e., that the mechanoreceptors are activated by circumferential stretch independent of the contractile state of the muscles\cite{12,13,15,50}. The receptors encoding distension of the gut are mainly believed to be localized in the muscle and nerve layers, where they are not exposed to acid\cite{33,43}. Hence, the contractions are probably initiated by reflex loops between strain-sensitive mechanosensitive afferents localized in the muscle layers and the smooth muscle cells. Whether such reflexes are local or mediated via central (vagal and/or spinal) afferents cannot be concluded from the current data\cite{40,42,43}. We believe, however, that a central component is important as the contractions were more powerful after perfusion with acid.

The acid perfusion may thus result in sensitization of mucosal afferents as well as central hyperexcitability\cite{8,43,48}. As the enteric nervous system is partly under inhibitory central control\cite{51}, the sensitization may result in dampening of the central control. This mechanism is expedient as such reflexes will tend to move acid from reflux towards the stomach where it is harmless.

**Modeling diseases of the esophagus**

Sensation and pain detection thresholds to distension, electrical and acid stimuli of the esophagus were found to be lower in patients with non-cardiac chest pain compared to healthy subjects\cite{6,7,32,53}. Such hypersensitivity can be mimicked in the current model. Furthermore, the muscles of the esophagus are hyper-reactive in patients with unexplained chest pain\cite{19,54,56}. In the present model the acid perfusion evoked an increased number of contractions, which were characterized by a higher force. Thus diseases characterized by primary and secondary motor disorders can also be mimicked experimentally, and in patients the preload-afterload plots will be valuable for description of the aberrant motor function. The model can therefore be used to study abnormal sensory-motor mechanisms in visceral organs, and may also prove useful in pharmacological studies with drugs targeted to treat patients with unexplained chest pain and motor disorders of the esophagus.

**Appendix**

The preload is considered in this study to be initial muscle length (radius) preceding the contraction during the distension, whereas the afterload is evaluated as the active tension during the contraction. In cardiac physiology the preload is usually considered to be the end-diastolic pressure or radius and the afterload is considered to be the arterial pressure during the systole. The explanation of the Frank-Starling mechanism is that when an extra amount of blood flows into the ventricles, the cardiac muscle itself is stretched to greater length. This in turn causes the muscle to contract with increased force because the actin and myosin filaments then are brought to a more nearly optimal degree of interdigitation for force generation. In cardiac physiology the importance of the concept of preload and afterload is that in many normal functional states of the heart and circulation, the pressure during filling of the ventricle or the arterial pressure against which the ventricle must contract, or both, are severely altered from the normal. The Frank-Starling mechanism has been important in the understanding of drugs with effect on the myocardial function, and transferring this concept to esophageal physiology, the development in the current model will have interest for evaluation of normal esophageal physiology and in the pathophysiology of esophageal disorders.
REFERENCES

1. Bochus HL. Abdominal Pain. In: Berk JE, ed. Gastroenterology. Philadelphia: WB Saunders 1985: 22-47
2. Arendt-Nielsen L. Induction and assessment of experimental pain from human skin, muscle, and viscera. In: Jensen TS, Turner JA, and Wiesenfeld-Hallin Z, eds. Proceedings of the 8th World Congress of Pain, Progress in Pain Research and Management. Seattle: ISAP Press 1997: 393-425
3. Drewes AM, Gregersen H, Arendt-Nielsen L. Experimental pain in gastroenterology: A reappraisal of human studies. Scand J Gastroenterol 2003; 38: 1115-1130
4. Arendt-Nielsen L, Laursen RJ, Drewes AM. Referred pain as an indicator for neural plasticity. Prog Brain Res 2000; 129: 543-356
5. Garrison DW, Chandler MJ, Foreman RD. Viscerosomatic convergence Onto Feline Spinal Neurons from Esophagus, Heart and Somatic Fields - Effects of Inflammation. Pain 1992; 49: 373-382
6. Mehta AJ, De Caestecker JS, Camm AJ, Northfield TC. Sensitization to painful distention and abnormal sensory perception in the esophagus. Gastroenterology 1995; 108: 311-319
7. Sarkar S, Aziz Q, Woolf CJ, Hobson AR, Thompson DG. Contribution of central sensitisation to the development of non-cardiac chest pain. Lancet 2000; 356: 1154-1159
8. Drewes AM, Schipper KP, Dimcevski G, Petersen P, Andersen OK, Gregersen H, Arendt-Nielsen L. Multi-modal induction and assessment of allodynia and hyperalgesia in the human oesophagus. Eur J Pain 2003; 7: 539-549
9. Hu WH, Martin CJ, Talley NJ. Intraoesophageal acid perfusion sensitizes the esophagus to mechanical distension: a barostat study. Am J Gastroenterol 2000; 95: 2189-2194
10. Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. Dig Dis Sci 1997; 42: 223-224
11. Drewes AM, Schipper KP, Dimcevski G, Petersen P, Andersen OK, Gregersen H, Arendt-Nielsen L. Multimodal assessment of pain in the esophagus: a new experimental model. Am J Physiol Gastrointest Liver Physiol 2002; 283: G95-103
12. Drewes AM, Pedersen J, Liu W, Arendt-Nielsen L, Gregersen H. Controlled mechanical distension of the human oesophagus: Sensory and biomechanical findings. Scand J Gastroenterol 2003; 38: 27-35
13. Gao C, Arendt-Nielsen L, Liu W, Petersen P, Drewes AM, Gregersen G. Sensory and biomechanical responses to ramp-controlled distension of the human duodenum. Am J Physiol Gastrointest Liver Physiol 2003; 284: G461-471
14. Pedersen J, Gao C, Egekvist H, Bjerring P, Arendt-Nielsen L, Gregersen H, Drewes AM. Pain and biomechanical responses to distension of the duodenum in patients with systemic sclerosis. Gastroenterol 2003; 124: 1230-1239
15. Petersen P, Gao C, Arendt-Nielsen L, Gregersen H, Drewes AM. Pain intensity and biomechanical responses during ramp-controlled distension of the human rectum. Dig Dis Sci 2003; 48: 1310-1316
16. Gregersen H, Kassab G. Biomechanics of the gastrointestinal tract. Neurogastroenterol Motil 1996; 8: 277-297
17. Gregersen H, Christensen J. Gastrointestinal tone. Neurogastroenterol Motil 2000; 12: 501-508
18. Gregersen H. Biomechanics of the Gastrointestinal Tract. London: Springer Verlag 2002
19. Rao SS, Gregersen H, Hayek B, Summers RW, Christensen J. Unexplained chest pain: the hypersensitive, hyperreactive, and poorly compliant esophagus. Am Intern Med 1996; 124: 950-958
20. Richter JE. Oesophageal motility disorders. Lancet 2001; 358: 823-828
21. Gregersen H, Andersen MB. Impedance measuring system for cross-sectional area in the gastrointestinal tract. Med Biol Eng Comput 1991; 29: 108-110
22. Gregersen H, Giversen IM, Rasmussen LM, Tottrup A. Biomechanical wall properties and collagen content in the partially obstructed opossum esophagus. Gastroenterology 1992; 103: 1547-1551
23. Drewes AM, Schipper KP, Dimcevski G, Petersen P, Gregersen H, Funch-Jensen P, Arendt-Nielsen L. Gut pain and hyperalgesia induced by capsaicin: A human experimental model. Pain 2003; 104: 333-341
24. Drewes AM, Babenko L, Birket-Smith L, Funch-Jensen P, Arendt-Nielsen L. Induction of non-painful and painful intestinal sensations by hypertonic saline: A new human experimental model. Eur J Pain 2003; 7: 81-91
25. Bernstein LM, Baker LA. A clinical test for esophagitis. Gastroenterology 1958; 34: 760-761
26. Coderre TJ, Katz J, Vaccarino AL, Melzack R. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 1993; 52: 259-285
27. Laird JMA, de la Rubia PG, Cervero F. Excitability changes of somatic and viscero-somatic nociceptive reflexes in the decerebrate-spinal rabbit: role of NMDA receptors. J Physiol 1995; 489: 545-555
28. Mayer EA, Munakata J, Mertz H, Lembo T, Bernstein CN. Visceral hyperalgesia and irritable bowel syndrome. In: Gebhart GF, ed. Visceral pain, Progress in Pain Research and Management. Volume 5. Seattle: ISAP Press 1995: 429-468
29. Yaksh TL. Spinal systems and pain processing: development of novel analgesics with mechanistically defined methods. Trends Pharmacol Sci 1999; 20: 329-337
30. Eilick GD, Fass R. Noncardiac chest pain: evaluation and treatment. Gastroenterol Clin N Am 2003; 32: 531-552
31. Clouse RE, Richter JE, Heading RC, Janssens J, Wilson JA. Functional esophageal disorders. Gut 1999; 45: 31-36
32. Ness TJ, Gebhart GF. Visceral pain: a review of experimental studies. Pain 1990; 41: 167-234
33. Sengupta JN, Gebhart GF. Gastrointestinal afferent fibers and sensation. In: Johnson L, ed. Physiology of the Gastrointestinal Tract. Third ed. New York: Raven Press 1994: 484-519
34. Sarkar S, Hobson AR, Hughes A, Growcott J, Woolf CJ, Thompson DG, Aziz Q. The prostaglandin E2 receptor-1 (EP-1) mediates acid-induced visceral pain hypersensitivity in humans. Gastroenterology 2003; 124: 18-25
35. DeVault KR. Acid infusion does not affect intraesophageal balloon distention- induced sensory and pain thresholds. Am J Gastroenterol 1997; 92: 947-949
36. Fass R, Nahloff B, Higa L, Johnson C, Kodner A, Munakata J, Ngo J, Mayer EA. Differential effect of long-term esophageal acid exposure on mecanosensitivity and chemosensitivity in humans. Gastroenterology 1998; 115: 1363-1372
37. Shirazi S, Schudelbeek K, Custerhaven T, Brown CK, Ren JM. Motility changes in opossum esophagus from experimental esophagitis. Dig Dis Sci 1989; 34: 1668-1676
38. White RJ, Zhang Y, Morris GP, Paterson WG. Esophagitis-related esophageal shortening in opossum is associated with longitudinal muscle hyperresponsiveness. Am J Physiol Gastrointest Liver Physiol 2001; 280: G463-469
39. Schoeman MN, Holloway RH. Integrity and Characteristics of Secondary Esophageal Peristalsis in Patients with Gastroesophageal Reflux Disease. Gut 1995; 36: 499-504
40. Lang IM, Medda BK, Shaker R. Mechanisms of reflexes induced by esophageal distension. Am J Physiol Gastrointest Liver Physiol 2001; 281: G1246-1263
41. Gebhart GF. Pathobiology of visceral pain: molecular mechanisms and therapeutic implications IV. Visceral afferent contributions to the pathobiology of visceral pain. Am J Physiol Gastrointest Liver Physiol 2000; 278: G834-838
42. Grundy D. Neuroanatomy of visceral nociception: vagal and splanchnic afferent. Gut 2002; 51: 12-15
43. Szurszewski JH, Ermlow LG, Miller SM. Prevertebral ganglia and intestinofugal afferent neurones. Gut 2002; 51: 16-110
44. Mayer EA, Gebhart GF. Basic and clinical aspects of visceral pain and hyperalgesia. Gastroenterology 1994; 107: 271-293
45. Sanger GJ. Hypersensitivity and hyperreactivity in the irritable bowel syndrome: An opportunity for drug discovery. Dig Dis 1999; 17: 90-99

Drewes AM et al. Sensitization and mechanical stimuli 4373
Mertz H, Fullerton S, Naliboff B, Mayer EA. Symptoms and visceral perception in severe functional and organic dyspepsia. Gut 1998; 42: 814-822

Johansen MK, Graven-Nielsen T, Olesen AS, Arendt-Nielsen L. Generalised muscular hyperalgesia in chronic whiplash syndrome. Pain 2001; 89: 293-295

Sarkar S, Hobson AR, Furlong PL, Woolf CJ, Thompson DG, Aziz Q. Central neural mechanisms mediating human visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 2001; 281: G1196-1202

Sifrim D, Tack J, Lerut T, Janssens J. Transient lower esophageal sphincter relaxations and esophageal body muscular contractile response in reflux esophagitis. Dig Dis Sci 2000; 45: 1293-1300

Barlow JD, Gregersen H, Thompson DG. Identification of biomechanical factors associated with the perception of distension in the human esophagus. Am J Physiol Gastrointest Liver Physiol 2002; 282: G683-689

Christensen J. Motor functions of the pharynx and esophagus.

In: Johnson LR, Christensen J, Jackson MJ, Jacobsen ED, Walsh JH, eds. Physiology of the gastrointestinal tract. Second ed. New York: Raven press 1987: 595-612

Frobert O, Arendt-Nielsen L, Bak P, Funch-Jensen P, Pedersen BJ. Pain perception and brain evoked potentials in patients with angina despite normal coronary angiograms. Heart 1996; 75: 436-441

Smout AJ, Devore MS, Dalton CB, Castell DO. Cerebral Potentials-evoked by esophageal distension in patients with non-cardiac chest pain. Gut 1992; 33: 298-302

Rao SS. Visceral hyperalgesia: the key for unrevealing functional gastrointestinal disorders. Dig Dis 1996; 14: 271-275

Balaban DH, Yamamoto Y, Liu JM, Pehlivanov N, Wisniewski R, DeSilvey D, Mittal RK. Sustained esophageal contraction: a marker of esophageal chest pain identified by intraluminal ultrasonography. Gastroenterology 1999; 116: 29-37

Pehlivanov N, Liu JM, Mittal RK. Sustained esophageal correlate of heartburn contraction: a motor symptom. Am J Physiol Gastrointest Liver Physiol 2001; 281: G743-751

Science Editor Guo SY  Language Editor Elsevier HK