Intraluminal acid induces oesophageal shortening via capsaicin-sensitive neurokinin neurons

William G Paterson, David V Miller, Neil Dilworth, Joseph B Assini, Sandra Lourenssen, Michael G Blennerhassett

Gut 2007;56:1347–1352. doi: 10.1136/gut.2006.115881

See end of article for authors' affiliations

Correspondence to: Dr William G Paterson, GI Division, Hotel Dieu Hospital, 166 Brock Street, Kingston, Ontario, Canada, K7L-5G2; patersow@hdh.kari.net

Revised 1 May 2007
Accepted 4 May 2007
Published Online First
24 May 2007

Objective: Intraluminal acid evokes reflex contraction of oesophageal longitudinal smooth muscle (LSM) and consequent oesophageal shortening. This reflex may play a role in the pathophysiology of oesophageal pain syndromes and hiatus hernia formation. The aim of the current study was to elucidate further the mechanisms of acid-induced oesophageal shortening.

Design: Intraluminal acid perfusion of the intact opossum smooth muscle oesophagus was performed *in vitro* in the presence and absence of neural blockade and pharmacological antagonism of the neurokinin 2 receptor, while continuously recording changes in oesophageal axial length. In addition, the effect of these antagonists on the contractile response of LSM strips to the mast cell degranulating agent 48/80 was determined. Finally, immunohistochemistry was performed to look for evidence of LSM innervation by substance P/calcitonin gene-related peptide (CGRP)-containing axons.

Results: Intraluminal acid perfusion induced longitudinal axis shortening that was completely abolished by capsaicin desensitization, substance P desensitization, or the application of the neurokinin 2 receptor antagonist MEN10376. Compound 48/80 induced sustained contraction of LSM strips in a concentration-dependent fashion and this was associated with evidence of mast cell degranulation. The 48/80-induced LSM contraction was antagonized by capsaicin desensitization, substance P desensitization and MEN10376, but not tetrodotoxin. Immunohistochemistry revealed numerous substance P/CGRP-containing neurons innervating the LSM and within the mucosa.

Conclusions: This study suggests that luminal acid activates a reflex pathway involving mast cell degranulation, activation of capsaicin-sensitive afferent neurons and the release of substance P or a related neurokinin, which evokes sustained contraction of the oesophageal LSM. This pathway may be a target for treatment of oesophageal pain syndromes.

We have previously reported in both the opossum model^{1–3} and humans⁴ that intraluminal acid perfusion results in sustained contraction of the oesophageal longitudinal smooth muscle (LSM) and oesophageal shortening, leading to speculation that acid-reflux-induced oesophageal shortening may actually contribute to the genesis of a hiatus hernia. This acid-induced contraction of the LSM has also been identified using intraluminal ultrasound in humans.⁵ Furthermore, either acid-induced or spontaneous sustained contraction of oesophageal LSM may be a key pathogenic factor in non-cardiac chest pain.⁶

The mechanism whereby intraluminal acid perfusion induces LSM contraction remains incompletely understood. In humans, acid-induced sustained oesophageal contraction is not inhibited by atropine.⁷ In the opossum model *in vivo*, acid-induced oesophageal shortening was unaffected by vagotomy or atropine,¹ but was markedly attenuated by pretreatment of the animals with mast cell stabilizers.² Whether mast cell mediators induce sustained oesophageal shortening directly or indirectly (e.g. via activation of nerves) remains to be determined. Although some mast cells are present in the muscularis propria and throughout the submucosa, most are localized in the lamina propria, just below the epithelium.^{8,9} This suggests that mast cell mediators are more likely to evoke LSM contraction indirectly (i.e. through a neural reflex), but in previous preliminary experiments we found that acid-induced oesophageal shortening was not affected by tetrodotoxin. Action potentials in some capsaicin-sensitive afferent nerves have, however, been shown to be tetrodotoxin resistant.^{10,11} Substance P nerves are present in oesophageal LSM of several

species including humans,^{12,13} and in the opossum it has been reported that certain electrical stimulus parameters evoke sustained LSM strip contraction that is abolished by substance P desensitization,^{14,15} capsaicin desensitization¹⁵ or blockade of the neurokinin 2 receptor.¹⁵ Given this background, the proposed experiments were designed to determine whether capsaicin-sensitive neurokinin nerves are involved in acid-induced sustained LSM contraction and oesophageal shortening.

MATERIALS AND METHODS

Animal preparation

The protocol was approved by the Queen's University Animal Care Committee in accordance with guidelines established by the Canadian Council on Animal Care. Adult opossums (*Didelphis virginiana*) of either sex and weighing between 1.6 and 5.2 kg, were fasted for 8–12 hours before each experiment, but were allowed free access to water. Anesthesia was induced via tail vein injection of pentobarbital sodium (40 mg/kg). Following this, the chest and abdominal cavities were opened and the entire thoracic oesophagus and proximal stomach removed en bloc and transferred to a tissue bath for further dissection. The bath contained oxygenated (95% O₂–CO₂) Krebs solution consisting of 118 mM NaCl, 4.75 mM KCl, 1 mM NaH₂PO₄, 25 mM NaHCO₃, 1.2 mM MgSO₄, 2.5 mM CaCl₂ and 11 mM glucose.

Abbreviations: CGRP, Calcitonin gene-related peptide; CSM, circular smooth muscle; EFS, electrical field stimulation; LSM, longitudinal smooth muscle; PBS, phosphate-buffered saline

Figure 1 Schematic representation of experimental protocol. The distal 8–9 cm segment of opossum smooth muscle oesophagus was excised and divided into two 4 cm segments. Paired experiments were then performed in which acid was perfused in the oral end and effluent collected from the aboral end. A specially designed strain gauge transducer was fixed to the aboral end so as to record movement of the oesophagus in the longitudinal axis.

Oesophageal perfusion experiments

An 8–9 cm segment of the distal smooth muscle oesophagus was divided at its midpoint in order to create two intact segments for intraluminal perfusion studies. These segments were placed in a tissue bath and connected to catheters at the caudal and rostral ends (see fig 1). At the rostral end a specially designed strain gauge transducer was attached as described previously.¹⁶ This transducer measured axial lengthening and shortening of the oesophageal segment. The protocol consisted of a baseline period when saline was perfused through the oesophageal segment at 2 ml/min for 20 minutes. The perfusate was then switched over to 0.1 N HCl for an additional 45 minutes. For each experiment, one segment served as the control and the other as the experimental segment. This was alternated between proximal and distal segments on different days. No difference was noted between the responses of proximal versus distal segments, therefore the results were pooled. For the control segment, the vehicle of the different test agents was added to the tissue bath, whereas in the experimental segment the active agent was added. The effect of the following agents on the acid-induced oesophageal shortening were tested: (1) Capsaicin desensitization. This was achieved by applying 30 μ M capsaicin to the bath and waiting until all induced contractile activity had dissipated; (2) Substance P desensitization. As with capsaicin desensitization,

this was achieved by applying 100 μ M substance P to the bath and then waiting until all evoked contractile activity had dissipated; and (3) The selective neurokinin-2 receptor antagonist MEN 10376 at a concentration of 3 μ M. We have previously demonstrated that this concentration of MEN 10376 causes the maximal inhibition of substance P-induced contraction of oesophageal LSM strips.¹⁷ Although substance P and capsaicin induced transient phasic shortening of the oesophagus, this rapidly dissipated and neither these drugs nor MEN 10376 caused a significant change in resting oesophageal length before the onset of acid perfusion.

Muscle strip experiments

After removal, the oesophagus was opened longitudinally and pinned out, mucosal side up, in a bath containing oxygenated Krebs solution. The mucosa was removed from the underlying submucosa and smooth muscle layers by sharp dissection. Several longitudinally oriented muscle strips (10 mm $\times$ 5 mm) were cut and then hung in 10 ml double-chambered organ baths that contained oxygenated Krebs solution maintained at 35°C. Using silk ligatures, one end of the muscle strip was secured to a hook at the bottom of the organ bath, whereas the other end was attached to a force transducer to record isometric tension. Outputs from the transducers were displayed on an IBM PC compatible computer using Windaq/200 data acquisition software (Data Q instruments). The force transducers were slowly moved away from the chamber until the muscle strips and sutures became straight. A small preload tension ($\sim$ 500 mg) was then placed on each strip. This was followed by an equilibration period of 1 hour, during which the Krebs solution was replaced every 15 minutes. Concentration–response curves for the mast cell degranulating agent, compound 48/80 were established. The lowest concentration that consistently induced maximal contraction was then used in subsequent experiments to determine the effects of tetrodotoxin, desensitization to capsaicin and substance P, and MEN 10376. Desensitization to substance P and capsaicin was achieved as described above. As with the intact oesophagus experiments, substance P and capsaicin induced transient phasic shortening of LSM strips, but this rapidly dissipated and neither of these drugs nor MEN 10376 caused a significant change in resting tone of the strips before application of compound 48/80. The magnitude of LSM strip contractions were expressed as a percentage of the maximal contraction induced by 60 mM KCl (administered at end of protocol). To confirm that compound 48/80 was inducing mast cell degranulation, tissue bath fluid was removed after the administration of 48/80 and assayed for histamine as described previously.¹⁸ In addition, at the end of the experiment tissues

Figure 2 Histological evidence of mast cell activation caused by compound 48/80. (A) control tissue given the vehicle. (B) Tissue exposed to compound 48/80, showing evidence of mast cell degranulation (arrows). Sections are from the deep submucosa. (C) Quantification of mast cell degranulation in control tissue versus tissue given 100 μ g/ml compound 48/80. The mean number of degranulated mast cells per high-powered field was significantly higher in the treated tissue ($p < 0.001$, paired t -test; $n = 5$ per group). Open bars represent the number of intact mast cells whereas black bars represent the number of mast cells with evidence of degranulation (magnification $\times 40$).

were fixed in Carnoy's solution and subsequently processed for histological examination of mast cells using toluidine blue staining. Tissue mast cells were then scored as either being intact or showing evidence of degranulation by a blinded observer and quantified as the total number of cells per high powered field. The mean cell numbers per animal were obtained by counting five adjacent high-powered fields (40 $\times$ magnification). Degranulated mast cells were readily identifiable by the finding of toluidine blue stained particles immediately adjacent to the mast cell membrane (see fig 2).

Immunohistochemical demonstration of substance P/calcitonin gene-related peptide neurons

A 1 cm $\times$ 1 cm full-thickness piece of oesophageal tissue was excised from the distal oesophagus of five animals, pinned flat in a sylgard dish and fixed with 4% neutral buffered formalin, pH 7.1 (Sigma, Oakville, Ontario, Canada) for 30 minutes. Three strips were then cut alternatively in transverse and longitudinal sections and placed into separate, plastic tubes with neutral buffered formalin and gently rocked for 1.5 hours. Tissues were cryoprotected in 30% sucrose in phosphate buffered saline (PBS) overnight, frozen and sectioned at 12 μ m thickness. Slides were then immersed in 1% goat serum (Sigma) in PBS for 30 minutes, followed by the addition of 1 : 50 anti-calcitonin gene-related peptide (CGRP; Chemicon, Temecula, California, USA) and 1 : 250 anti-substance P (Chemicon) in antibody-diluting fluid (Dako, Mississauga, Ontario, Canada), followed by overnight incubation at room temperature. Secondary antibodies were then added for 2 hours, the sections washed in PBS, and then immersed for 3 minutes in the nuclear stain Hoechst H33342 (1 μ g/ml; Sigma).

Tissue was examined using an Olympus BX-51 microscope (Carsen Group Inc., Markham, Ontario, Canada), with independent examination of the LSM, circular smooth muscle (CSM) and mucosa. For both LSM and CSM, photographs of six non-adjacent fields were taken using a CoolSnap digital camera (Roper Scientific, Trenton, New Jersey, USA) and ImagePro Plus Version 4.5 software (Media Cybernetics, Silver Springs, Maryland, USA). On each image, the area of the smooth muscle was measured, and the number of axons, smooth muscle nuclei and axons that co-localized CGRP and substance P were determined. Qualitative observations were also carried out to examine the organization, orientation and presence of varicosities on the substance P/CGRP-positive axons.

Figure 3 Effect of pharmacological antagonists on acid-induced oesophageal shortening. Paired experiments were performed examining the effect of the neurokinin 2 receptor antagonist MEN10376 (n = 5), substance P desensitization (n = 6), and capsaicin desensitization (n = 4). A negative number indicates shortening of the oesophagus. Note that MEN10376, substance P desensitization or capsaicin desensitization markedly attenuated the acid-induced oesophageal shortening. (*p < 0.05 **p = 0.01 versus vehicle-treated control strips)

Figure 4 Results of paired longitudinal smooth muscle strip experiments examining the effects of tetrodotoxin (TTX; n = 10), capsaicin desensitization (CAP; n = 5), neurokinin 2 receptor antagonist MEN 10376 (n = 10), or substance P desensitization (n = 5) on the prolonged contraction induced by the application of compound 48/80. Black bars represent control responses to compound 48/80, whereas open bars represent responses in the presence of pharmacological antagonists. Capsaicin desensitization, substance P desensitization and MEN10376 significantly attenuated the compound 48/80-induced contraction, but tetrodotoxin was without affect. *p < 0.05 versus vehicle-treated control strips.

Data analysis

Data are presented as mean $\pm$ SE, with n referring to the number of animals or cells as indicated. Comparisons were made using either analysis of variance followed by the Neuman-Keuls test or the paired Student's *t*-test.

RESULTS

Oesophageal perfusion experiments

Acid perfusion of the 4 cm oesophageal segments resulted in a prompt (within 10 minutes) and sustained oesophageal shortening that averaged approximately 0.4 cm. As can be seen from figure 3, substance P desensitization, capsaicin desensitization or pretreatment with the neurokinin 2 receptor antagonist MEN 10376 markedly attenuated the acid-induced oesophageal shortening.

Muscle strip experiments

Compound 48/80 caused sustained contraction of LSM strips in a concentration-dependent fashion, with a peak contraction of $35.5 \pm 4.2\%$ KCl response occurring at a bath concentration of 100 μ g/ml. This response persisted for prolonged periods after repeated washing of the tissue bath solution. In strips that eventually returned to baseline tension after repeated washing, subsequent application of compound 48/80 was without effect. In view of this, experiments were performed with paired muscle strips. One strip received either MEN 10376 (3 μ M), tetrodotoxin (1 μ M), Substance P desensitization or capsaicin desensitization, whereas the other strip received the appropriate vehicle as control. After this, the maximally effective concentration of compound 48/80 (100 μ g/ml) was added to the tissue bath. As can be seen from figure 4, the compound 48/80-induced LSM contraction was significantly inhibited by MEN 10376 and capsaicin or substance P desensitization, but not by tetrodotoxin.

Compound 48/80 caused a highly significant increase in bath histamine, which also peaked at a bath concentration of 100 μ g/ml (basal bath histamine levels 4.92 ± 0.95 ng/ml versus 30.18 ± 13.94 ng/ml after the application of 48/80; p < 0.05). Furthermore, compound 48/80 caused significant mast cell degranulation (see fig 2).

Substance P/CGRP immunoreactive innervation of the oesophageal muscularis propria and mucosa

Numerous substance P/CGRP immunoreactive axons were detected within the LSM, exclusively running parallel to the muscle fibres (fig 5 A–C). Furthermore, these fibres had

numerous nerve varicosities in close proximity to LSM membranes. Substance P/CGRP immunoreactive axons were also detected in the CSM, but in smaller numbers (average number of co-localized axons was 2.00 ± 0.18 axons/smooth muscle cell in the LSM versus 0.75 ± 0.21 in the CSM; $p < 0.001$). Furthermore, substance P/CGRP immunoreactive axons were not organized parallel to individual CSM cells. Rather, their orientation appeared to lack specific organization, and nerve varicosities in contact with muscle membranes were rarely observed. Substance P/CGRP immunoreactive axons were also abundant within the submucosa, but were not noted within the epithelial layer (fig 5 D–F).

DISCUSSION

This study suggests that luminal acid induces contraction of the oesophageal LSM and consequent oesophageal shortening through stimulation of capsaicin-sensitive neurons and activation of neurokinin 2 receptors. Furthermore, the degranulation of mast cells by compound 48/80 induces oesophageal LSM contraction by similar neural pathways.

Several years ago Shirazi *et al.*¹⁹ observed that the manometric location of the lower oesophageal sphincter migrated proximally in the opossum model when severe oesophagitis was induced by prolonged acid perfusion. Subsequently, our laboratory began characterizing this shortening response, and suggested that it could play a role in the etiology of hiatus hernia. We found that acid-induced oesophageal shortening occurred independent of vagal or muscarinic mechanisms,¹ which are the predominant contractile mechanisms of swallow-induced LSM contraction.¹⁶ Furthermore, luminal acid was

able to induce mast cell degranulation,⁸ and mast cells stabilizers markedly attenuated this acid-induced oesophageal shortening.² Subsequently, we demonstrated that this response was sustained, in that shortening persisted for at least 24 hours after the last exposure to acid,³ an observation that has recently been confirmed in the cat model.²⁰ Furthermore, this sustained response was associated with the hyperresponsiveness of LSM strips,³ but not single LSM cells,²¹ to the agonist carbachol.

With luminal acid perfusion, oesophageal shortening begins within 10 minutes, which probably represents the time course of acid permeation through the oesophageal epithelium. Although epithelial sloughing was apparent on gross inspection of the oesophageal mucosal surface after 45 minutes of acid perfusion, the onset of shortening occurs at a time when there is no macroscopic evidence of mucosal injury (unpublished observations). This fairly rapid onset suggested a possible role of neural reflexes. Our previous studies had, however, shown that vagotomy and atropine had no effect on the response,¹ and subsequent preliminary experiments found that the neurotoxin tetrodotoxin also did not attenuate the response. Certain capsaicin-sensitive afferent neurons innervating the gastrointestinal tract are, however, known to be tetrodotoxin insensitive,^{10,11} and gastrointestinal sensory-motor reflexes involving these neurons have been well demonstrated.²² Furthermore, Crist *et al.*¹⁴ showed that electrical field stimulation (EFS) of LSM strips caused prolonged contraction that was blocked by substance P desensitization, suggesting that substance P or a related neurokinin may be involved in neurogenic LSM contraction. We have also recently found that the non-cholinergic LSM contraction induced by EFS is antagonized

Figure 5 Immunohistochemistry demonstrating innervation of longitudinal smooth muscle (LSM) cells (top panels, A to C) and submucosa (bottom panels, D to F) by substance P/calcitonin gene-related peptide (CGRP) immunoreactive nerve profiles. In the LSM, axons run parallel to the individual LSM cells, whereas in the submucosa they surround acini of epithelial glands. (A and D) Substance P immunoreactive nerve profiles; (B and E) CGRP immunoreactive nerve profiles; (C and F) Merged image showing substance P/CGRP co-localization. Scale bar 20 μm .

Figure 6 Proposed model for the mechanisms of acid-induced oesophageal shortening: luminal acid permeates the squamous epithelium and causes degranulation of subepithelial mast cells. Mast cell mediators in turn activate capsaicin-sensitive, substance P (SP)/calcitonin gene-related peptide (CGRP) afferents, which then result in the release of substance P or a related neurokinin onto longitudinal smooth muscle (LSM). The reported experimental observations suggest that this is an axo-axonal reflex involving the extrinsic substance P/CGRP afferents. The involvement of intrinsic neurokinin motor neurons cannot be fully excluded. Ach, acetylcholine; CSM, circular smooth muscle; MP, myenteric plexus; NKA, neurokinin A.

by capsaicin desensitization or blockade of the neurokinin 2 receptor.¹⁵ Given this background, we decided to pursue the possibility that capsaicin-sensitive neurons and substance P (or a related neurokinin) are involved in acid-induced oesophageal shortening. In previous preliminary experiments we showed that exogenous substance P caused sustained contraction of oesophageal LSM strips through activation of the neurokinin 2 receptor.¹⁷ In the current experiments either a neurokinin 2 receptor antagonist, capsaicin desensitization or substance P desensitization markedly attenuated acid-induced oesophageal shortening. These results strongly suggest that luminal acid causes LSM contraction via activation of capsaicin-sensitive neurons and the subsequent release of substance P or a related neurokinin.

When we first performed the in-vitro acid perfusion experiments, we used a high concentration of capsaicin (640 μ M) to desensitize the preparation, and this completely prevented acid-induced oesophageal shortening. This concentration had previously been found to induce significant blood flow changes in the rat stomach²³ and opossum oesophagus.²⁴ Furthermore, in preliminary experiments, we noted that in some preparations low amplitude phasic contractile activity persisted as we added progressively higher concentrations of capsaicin up to 480 μ M (adding in increments of 160 μ M). This was not the case in the muscle strip experiments; as progressively increased concentrations of capsaicin were added to the bath, recurrent contractile activity did not occur beyond a

concentration of 30 μ M. Subsequently, it was reported that high concentrations of capsaicin inhibit voltage gated Ca^{2+} channels,²⁵ which would result in the inhibition of smooth muscle contraction, independent of an effect on capsaicin-sensitive neurons. We therefore conducted muscle strip experiments (data not shown) to determine the effects of varying concentrations of capsaicin on the LSM contraction evoked by EFS, using stimulus parameters known primarily to activate cholinergic neurons.¹⁶ This revealed that capsaicin concentrations of 500 μ M inhibited the EFS-induced contractions by approximately 50%, raising the possibility that the effect of 640 μ M capsaicin on the acid-induced oesophageal shortening was non-specific. However, 30 μ M capsaicin caused an insignificant ($\sim 15\%$) inhibition of the EFS-induced contraction. We therefore repeated the acid perfusion experiments using this lower concentration of capsaicin and found that it also completely reversed acid-induced oesophageal shortening (fig 3). It is therefore very unlikely that the effect of capsaicin was solely the result of a non-specific effect on smooth muscle contraction.

Because mast cell stabilizing agents also attenuate acid-induced oesophageal shortening,² we performed additional experiments to establish how mast cell mediators might interact with the capsaicin-sensitive neurons/neurokinin pathway. As expected, compound 48/80, an agent that degranulates connective tissue mast cells,²⁶ caused sustained LSM contraction in muscle strip studies, and was associated with histamine release and histological evidence of mast cell degranulation. This LSM contraction induced by compound 48/80 was tetrodotoxin insensitive, but was blocked by the neurokinin 2 receptor antagonist MEN 10376, or by capsaicin or substance P desensitization. These observations suggest that mast cell degranulation induced by acid results in the release of mediators that in turn activate capsaicin-sensitive neurons and the release of substance P.

Although these studies provide significant new information on the mechanisms of acid-induced LSM contraction and oesophageal shortening, a number of mechanistic questions remain unanswered, including how luminal acid leads to mast cell degranulation, and which of the mast cell mediators is primarily responsible for activation of this reflex pathway. It is also uncertain whether this response represents an axo-axonal reflex whereby activation of the sensory terminal leads to the release of substance P or other neurokinins within the muscle layer. It is also possible that capsaicin-sensitive afferents synapse on intrinsic neurokinin motor neurons, as described by Bartho and colleagues²⁷ in the small bowel. Our immunohistochemical studies demonstrated that the LSM layer of the opossum oesophagus is innervated by numerous substance P-positive neurons that co-localize with CGRP, favouring this being an axo-axonal reflex, involving extrinsic sensory neurons (see fig 6).

In conclusion, this study provides evidence that luminal acid activates a reflex pathway involving mast cell degranulation, activation of capsaicin-sensitive afferent neurons and the release of substance P or a related neurokinin, which in turn causes sustained contraction of the oesophageal LSM. Although these results have been obtained in an animal model, we believe they have relevance to human pathophysiology. Not only has oesophageal shortening in response to luminal acid been demonstrated in humans,⁴ but studies using intraluminal ultrasound suggest that luminal acid causes so-called sustained oesophageal contractions, which appear to be prolonged contractions of the LSM layer.^{5,6} These sustained oesophageal contractions, which can occur in response to acid as well as spontaneously, often correlate temporally with chest pain episodes in patients with non-cardiac chest pain, and are

insensitive to muscarinic blockade.⁷ This acid-induced reflex pathway to the LSM may thus be an important target for pharmacological therapy of non-cardiac chest pain.

Authors' affiliations

William G Paterson, David V Miller, Neil Dilworth, Joseph B Assini, Sandra Lourenssen, Michael G Blennerhassett, Gastrointestinal Diseases Research Unit and Departments of Medicine, Biology and Physiology, Queen's University, Kingston, Ontario, Canada

Funding: Canadian Institutes of Health Research grant MOP-9978

Conflict of interest: None declared.

REFERENCES

- 1 **Paterson WG**, Kolyn DM. Oesophageal shortening induced by short-term intraluminal acid perfusion in opossum: a cause for hiatus hernia? *Gastroenterology* 1994;**107**:1736–40.
- 2 **Paterson WG**. Role of mast cell-derived mediators in acid-induced shortening of the oesophagus. *Am J Physiol (Gastrointest Liver Physiol)* 1998;**274**:G385–8.
- 3 **White RJ**, Zhang Y, Morris GP, et al. Acid-induced oesophageal shortening in opossum is associated with longitudinal muscle hyperresponsiveness. *Am J Physiol (Gastrointest Liver Physiol)* 2001;**280**:G463–9.
- 4 **Dunne DP**, Paterson WG. Acid-induced oesophageal shortening in humans: a cause for hiatus hernia? *Can J Gastroenterol* 2000;**14**:847–50.
- 5 **Pehlivanov N**, Liu J, Mittal RK. Sustained oesophageal contraction: a motor correlate of heartburn symptom. *Am J Physiol (Gastrointest Liver Physiol)* 2001;**281**:G743–51.
- 6 **Balaban DH**, Yamamoto Y, Liu J, et al. Sustained oesophageal contraction: a marker of oesophageal chest pain identified by intraluminal ultrasonography. *Gastroenterology* 1999;**116**:29–37.
- 7 **Mittal RK**, Gui A, Lui J. The sustained oesophageal contraction associated with heartburn of a positive Bernstein test is mediated through a non-cholinergic pathway. *Gastroenterology* 2001;**120**:A421.
- 8 **Barclay RL**, Dinda PK, Morris GP, et al. Morphological evidence of mast cell degranulation in an animal model of acid-induced oesophageal mucosal injury. *Dig Dis Sci* 1995;**269**:G219–24.
- 9 **Ying LTJ**, Hurlbut DJ, Paterson WG. Human mucosal mast cell number and relationship to vasculature in acid-induced inflammatory conditions of the oesophagus. *Can J Gastroenterol* 1996;**10**(Suppl A):51A.
- 10 **Campbell DT**. Large and small vertebrate sensory neurons express different Na and K channel subtypes. *Proc Natl Acad Sci U S A* 1992;**89**:9569–73.
- 11 **Rang HP**, Bevan S, Dray A. In.; Wall PD, Melzack R, eds. *Textbook of pain*. 3rd ed. New York: Churchill Livingstone, 1994:57–78.
- 12 **Singaram C**, Sengupta A, Sugarbaker DJ, et al. Peptidergic innervation of the human oesophageal smooth muscle *Gastroenterology* 1991;**101**:1256–63.
- 13 **Uddman R**, Alumets J, Hakanson R. Peptidergic (enkephalin) innervation of the mammalian oesophagus. *Gastroenterology* 1980;**78**:732–7.
- 14 **Crist J**, Gidda J, Goyal RK. Role of substance P nerves in longitudinal smooth muscle contractions of the oesophagus. *Am J Physiol (Gastrointest Liver Physiol)* 1986;**250**:G336–43.
- 15 **Daya F**, Miller DV, Paterson WG. Studies on the neural mechanisms underlying opossum oesophageal longitudinal muscle contraction. *Neurogastroenterol Mot* 2004;**16**:674.
- 16 **Paterson WG**. Studies on opossum oesophageal longitudinal muscle function. *Can J Physiol Pharmacol* 1997;**75**:65–73.
- 17 **Zhang Y**, Miller DV, Basford T, et al. Mechanism of oesophageal longitudinal smooth muscle contraction induced by sodium nitroprusside (SNP) and substance P (SP). *Gastroenterology* 2000;**118**:A387.
- 18 **Feldman MJ**, Morris GP, Dinda PK, et al. Mast cells mediate acid-induced augmentation of opossum oesophageal blood flow through a histamine- and nitric oxide-dependent mechanism. *Gastroenterology* 1996;**110**:121–8.
- 19 **Shirazi S**, Schulze-Delrieu K, Custer-Hagen T. Motility changes in opossum oesophagus from experimental oesophagitis. *Dig Dis Sci* 1989;**34**:1668–76.
- 20 **Zhang X**, Geboes K, Depoorte I, et al. Effect of repeated cycles of acute oesophagitis and healing on oesophageal peristalsis, tone, and length. *Am J Physiol (Gastrointest Liver Physiol)* 2005;**288**:G1339–46.
- 21 **Wells RW**, Morris GP, Blennerhassett MG, et al. Effects of acid-induced oesophagitis on oesophageal smooth muscle. *Can J Physiol Pharm* 2003;**81**:451–8.
- 22 **Patton D**, O'Reilly M, Vanner S. Sensory peptide neurotransmitters mediating mucosal and distention evoked neural vasodilator reflexes in guinea pig ileum. *Am J Physiol (Gastrointest Liver Physiol)* 2005;**289**:G785–90.
- 23 **Wallace J**, McKnight GW, Befus AD. Capsaicin-induced hyperemia in the stomach: possible contribution of mast cells. *Am J Physiol (Gastrointest Liver Physiol)* 1992;**263**:G209–14.
- 24 **Feldman MJ**, Morris GP, Paterson WG. Role of substance P and calcitonin gene-related peptide in acid-induced augmentation of opossum oesophageal blood flow. *Dig Dis Sci* 2001;**46**:1194–9.
- 25 **Sim JH**, Kim YC, Kim SJ, et al. Capsaicin inhibits the voltage-operated calcium channels intracellularly in the antral circular myocytes of guinea-pig stomach. *Life Sciences* 2001;**68**:2347–60.
- 26 **Koibuchi Y**, Ichikawa A, Nakagawa M. Histamine release induced from mast cells by active components of compound 48/80. *Eur J Pharmacol* 1985;**115**:163–70.
- 27 **Barthó L**, Lénárd L Jr, Patachini R, et al. Tachykinin receptors are involved in the "local efferent" motor response to capsaicin in the guinea-pig small intestine and oesophagus. *Neuroscience* 1999;**90**:221–8.

EDITOR'S QUIZ: GI SNAPSHOT

Answer

From question on page 1336

Multiple high signal cystic lesions in both lobes of the liver, which did not enhance with gadolinium or connect with the intra-hepatic biliary tree, could be seen on the T2-weighted MRI scan. The lesions had low attenuation on the CT scan and low signal on the T1-weighted MR scan. These findings are most consistent with biliary hamartomas.

Bile-duct hamartomas form during embryological development from insufficient resorption of the ductal plate, necessary for bile-duct formation. Also known as von Meyenburg complex, they are characteristically multiple, uniform in size and surrounded by variable amounts of fibrous stroma.

Biliary hamartomas have an estimated incidence of 1%–3%, are commonly asymptomatic, discovered incidentally, and require no treatment. Successful transplantation of an affected donor liver has been reported. Vague abdominal pain and malignancies have been documented. Differential diagnoses include metastases and micro-abscesses. MRI excludes these by demonstrating homogenous cyst size and signal intensity, with sharply demarcated lesions.

doi: 10.1136/gut.2006.104893a