Esophagitis-related esophageal shortening in opossum is associated with longitudinal muscle hyperresponsiveness

ROBERT J. WHITE,1,2 YONG ZHANG,1 GERALD P. MORRIS,1,2 AND WILLIAM G. PATERSON1–4
1Gastrointestinal Diseases Research Unit and Departments of 2Biology, 3Physiology, and 4Medicine, Queen’s University, Kingston, Ontario, Canada K7L 3N6

Received 2 August 2000; accepted in final form 5 October 2000

White, Robert J., Yong Zhang, Gerald P. Morris, and William G. Paterson. Esophagitis-related esophageal shortening in opossum is associated with longitudinal muscle hyperresponsiveness. Am J Physiol Gastrointest Liver Physiol 280: G463–G469, 2001.—Acute intraluminal acid perfusion induces esophageal shortening in humans and opossums. Lower esophageal sphincter (LES) hypotension and peristaltic dysfunction occur in patients and animal models of reflux esophagitis. This study examined whether similar shortening and motor dysfunction occur in anesthetized opossums after repeated esophageal acid exposure and whether this is associated with longitudinal muscle (LM) hyperresponsiveness. Manometry used before and after 3 consecutive days of 45-min perfusion with 100 mmol/l HCl or normal saline measured esophageal length and motor responses to induced swallows. LM electrical and mechanical responses were assessed using standard isometric tension and intracellular recording techniques. Compared with controls, repeated acid perfusion induced erosive esophagitis and significant esophageal shortening, associated with enhanced LM responses to carbachol, a significantly depolarized resting membrane potential, and abnormal spike patterns. LES resting pressure and swallow-induced peristalsis were unaffected. In this model of reflux esophagitis, marked persistent esophageal shortening and associated LM hyperresponsiveness occur before significant LES or peristaltic dysfunction, suggesting that esophageal shortening is the earliest motor disorder induced by acid injury.

Hiatal hernia; peristalsis; lower esophageal sphincter; reflux esophagitis

Transient relaxation of the lower esophageal sphincter (LES) and LES hypotension are thought to be the primary factors contributing to gastroesophageal reflux events (6). However, there is now considerable evidence that a large hiatal hernia can adversely affect the gastroesophageal anti-reflux barrier and exacerbate reflux (11, 19, 28). Not only is the external sphincter mechanism of the diaphragmatic crura lost in patients with hiatal hernia (11, 28), but the hernia sac may act as an acid reservoir with ready access to the LES whenever it relaxes (19, 27). A recent study (13) also reported that the threshold for induction of transient LES relaxations was lower in gastroesophageal reflux disease (GERD) patients with hiatal hernia than in nonhernia GERD patients. However, a control group with hiatal hernia, but no reflux disease, was not included in this study. Therefore, the observed results could also be explained by an increased likelihood of hiatal hernia formation in patients with preexistent low threshold for transient LES relaxation.

The etiology of hiatal hernia remains unclear. It has long been assumed that since the intra-abdominal pressure exceeds the intrathoracic pressure, the proximal stomach will tend to migrate into the chest as the anatomic factors that usually resist such movement weaken with age (15). For this to occur, however, there must be either structural or physiological shortening of the esophagus.

In 1989, Shirazi et al. (26) observed an oral displacement of the manometric location of the LES in opossums after prolonged intraluminal acid perfusion, raising the possibility that reflux esophagitis per se may contribute to the development of hiatal hernia. We (21, 23) subsequently demonstrated this in the opossum, using techniques to directly measure longitudinal esophageal shortening, and found that the shortening was not affected by vagotomy or atropine but was markedly attenuated by pretreatment with mast cell stabilizers. Similarly, we (7) recently observed a proximal migration of the manometrically located LES after short-term intraluminal acid perfusion in humans. Kahrilas et al. (14) noted that the esophageal shortening normally observed during peristalsis was inhibited in patients with hiatal hernia, which would be expected if the esophagus was already in a shortened state.

Esophageal peristaltic dysfunction has also been implicated in the pathogenesis of GERD. In humans with reflux esophagitis, incomplete or failed peristalsis (12), decreased amplitudes (12, 30, 31) and durations (30, 31) of peristaltic contractions in the smooth muscle esophagus, and decreased resting LES pressure (12, 31) have all been observed. However, such disturbances of esophageal motility are most pronounced in

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patients with more severe cases of esophagitis (12, 30) and are often not demonstrated in lower-grade cases (1, 10, 29, 30). In opossums, frequent failure of primary peristalsis and occurrence of spontaneous esophageal body contractions have been reported (26) in experimentally induced esophagitis. In addition, a significant decline in LES pressure has been observed after intraluminal acid exposure in the opossum (23) and the cat (3, 8).

The present study was designed to test the hypothesis that esophageal shortening similar to that described after acute acid exposure also occurs in an opossum model of esophagitis in which repeated intraluminal acid perfusion was performed. The possibility that this shortening is related to hyperresponsiveness of esophageal longitudinal muscle was also examined. In addition, the effects of acid-induced esophagitis on LES and esophageal body motor function were assessed.

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. All experiments were 4 days in length and took place at approximately the same time each day. Adult opossums (Didelphis virginiana) of either sex, weighing between 2.7 and 5.9 kg, were fasted for 8–12 h before each day of experimentation but allowed free access to water. Anesthesia was induced at the start of each day via tail vein injection with ketamine hydrochloride (25 mg/kg) and valium (1 mg/kg; Hoffman-La Roche, Mississauga, ON, Canada). Buprenorphine (0.01 mg/kg; Reckitt & Colman Pharma, Richmond, VA) was given intravenously to maintain analgesia. Animals were secured supine, at a 30° head-up angle, to a heated surgery table. This angle served to minimize reflux of the esophageal perfusate. Body temperature was maintained at 35°C with a heating pad. An endotrachial tube was placed, and the cuff was inflated to minimize the chance of aspiration. At the end of days 1–3, lactated Ringer solution (100 ml) was administered subcutaneously to maintain fluid and electrolyte balance, and atropine sulfate (0.05 mg/kg; Abbott Laboratories, Montreal, PQ, Canada) was given intravenously to prevent vomiting and excessive secretion.

Manometry

Esophageal manometry was accomplished using a custom-designed opossum LES sleeve assembly catheter system with an overall diameter of 3.5 mm (Dentsleeve, Bowden, SA, Australia). The assembly consisted of eight recording tubes (0.65 mm ID), each containing a 1-mm diameter side hole recording port. The tubing immediately distal to each recording port was sealed, and bubble-free water was perfused through each port at a rate of 0.3 ml/min with the use of a capillary tube. A sleeve attached to the catheter covered the area between the recording ports 1 and 3.5 cm from the distal tip, and a single recording port within this sleeve served to record the highest pressure within this area. Pressure transducers (Transpack II; Sorenson Research, Abbott, Salt Lake City, UT) connected to the recording tubes displayed their outputs on an IBM PC-compatible computer using WinDaq/200 data acquisition software (DataQ Instruments, Akron, OH).

A second catheter, used to luminally perfuse the esophagus, was fixed to the manometric catheter so that its end was positioned 7 cm above the proximal margin of the sleeve. These catheters were passed orally until all distal pressure-recording ports were in the stomach. The assembly was then withdrawn slowly in increments of 0.25 cm until both the sleeve and the recording port at the proximal sleeve margin (3.5 cm from the distal tip) were recording the high-pressure zone of the LES. The catheter was then withdrawn as slowly as possible while continuously monitoring the pressure tracing. Withdrawal was stopped immediately when the pressure recorded from the side hole at the proximal sleeve margin abruptly dropped to a stable intraesophageal pressure. This point was taken as the proximal extent of the LES. The recording port within the sleeve continued to measure LES pressure while additional recording ports allowed for the measurement of esophageal body contractions at sites 1, 3, and 5 cm proximal to the LES.

Experimental Protocol

Assessment of changes in esophageal length. On each day of experimentation, animals were allowed a 30-min stabilization period after anesthesia and instrumentation. Baseline LES position was then determined manometrically as described above, and a mark was made on the catheter relative to a fixed point in the animal’s mouth. Animals were then perfused intraluminally for 45 min on each of 3 consecutive days with either 0.9% saline (normal saline, NS) (control, n = 10) or 100 mmol/l hydrochloric acid (HCl) (n = 12), which was made isosmolar with NS. Perfused solutions were prewarmed to 37°C and administered at a rate of 2 ml/min using a peristaltic cassette pump (Manostat, New York, NY).

Twenty-four hours later (day 4), the position of the LES was again determined manometrically by a blinded observer who was unaware of the nature of the perfused solution in each experiment. A second mark was made on the catheter, relative to the same fixed point in the animal’s mouth that was used at baseline. The distance between these two marks was then measured, and any difference was taken as a change in LES position. Such a change was then interpreted as a change in the overall length of the esophagus, with a proximal movement of the LES indicating an overall shortening of the esophagus and a distal movement of the LES representing an overall lengthening of the esophagus. Changes in esophageal length measured in this way were found to be of a similar magnitude to changes observed previously in our laboratory (21, 23) after direct measurement of longitudinal axis shortening using a strain-gauge device.

Assessment of peristaltic function and LES responses. In some animals (n = 9 for each group), a bipolar silver wire electrode was taped to the surface of the mylohyoid muscle on days 1 and 4. The electromyogram activity of the mylohyoid muscle was then recorded as described previously (24). This, along with direct observation of the animal, allowed for monitoring of the deglutition reflex. At least five separate series of swallow stimulations were induced at baseline (on day 1 before perfusion) and on day 4. Swallows were induced by firmly stroking the animal’s posterior pharynx with a cotton-tipped swab. Such mechanical stimulation has been used previously to reliably evoke swallows (22).

Swallow-induced esophageal body contraction amplitudes were measured at 1, 3, and 5 cm proximal to the LES. Resting LES pressure, measured just before an induced swallow, and
the percent LES relaxation, measured as [(resting pressure – lowest pressure after a swallow)/resting pressure] × 100, was also recorded. Changes in mean amplitude at each site, mean resting LES pressure, and mean percent LES relaxation from baseline to day 4 were calculated for each animal. The elapsed time between swallow induction and the onset of contractions at each site was also measured. This allowed for characterization of the response to swallowing as either peristaltic or nonperistaltic. Responses were classified as nonperistaltic when simultaneous contractions occurred at two or more sites, when a contraction at a distal site occurred before that at a more proximal site, or when no contractions at one or more sites were observed after an induced swallow.

Measurement of Myeloperoxidase Activity and Epithelial Injury

The distal esophagus was excised on day 4 from all animals, which were then killed with an overdose of pentobarbital sodium (Somnotol; MTC Pharmaceuticals, Cambridge, ON, Canada). Transverse sections of tissue (0- to 1-cm and 4- to 5-cm proximal to the LES) were cut, blotted dry, weighed, immediately frozen in liquid nitrogen, and stored at −70°C. They were subsequently assayed for myeloperoxidase (MPO) activity, an index of inflammation, using the method of Krawisz et al. (16), with modifications described by Schierwagen et al. (25). Other tissue sections were removed, immersion fixed in Carnoy’s fixative, and processed for routine histological examination of epithelial damage. The percent epithelial denudation (complete absence of epithelium) was calculated as %denudation = (total length of all denuded portions/total length of section) × 100.

Longitudinal Muscle Mechanical and Electrical Recordings

A section of the distal esophagus was excised on day 4 and pinned out, mucosal side up, in a bath containing oxygenated (95% O₂-5% CO₂) Krebs solution. The Krebs solution consisted of (in mM) 118 NaCl, 4.75 KCl, 1 NaH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, 2.5 CaCl₂, and 11 glucose. The mucosa and submucosa were removed from the underlying smooth muscle layers by blunt dissection. A sheet of longitudinal muscle (5 mm × 10 mm) was excised for intracellular recordings. Conventional intracellular microelectrode recordings were then employed as described previously (33). In addition, several longitudinally oriented muscle strips (10 mm × 5 mm) were cut and then hung in 10-ml double-chambered organ baths that contained oxygenated Krebs solution maintained at 35°C. With silk ligatures, the muscle strips were secured in the baths at one end to a hook while the other end was attached to force transducers used to record isometric tension. Outputs from the transducers were displayed on an IBM PC-compatible computer using Windaq/200 data acquisition software (DataQ Instruments).

The force transducers were slowly moved away from the chamber until the muscle strips and sutures became straight. A small preload tension (~500 mg) was then placed on each strip. This was followed by an equilibration period of 1 h, during which the Krebs solution was replaced every 15 min. The stretch at this point was defined as 0% stretch or resting length. Strips were stretched, in 10% increments, to a maximal stretch of 100% (2 cm final length). The resulting tension, which represented the passive length-tension relationship, was recorded at each level of stretch. The active length-tension relationship was examined by measuring the response to 1 μmol/l carbachol at each level of stretch. Tissues were rinsed twice between each exposure, and a 15-min equilibration period was allowed before the next application of carbachol.

At the completion of the length-tension experiments, the strips were removed from the organ baths, blotted dry, and weighed. Muscle tension was expressed in grams and normalized for cross-sectional area (CSA) of the strip as described by Barbara et al. (2): CSA (mm²) = tissue wet weight (mg)/(tissue length (mm) × density (mg/mm³)), where the density of smooth muscle bathed in Krebs solution was assumed to be 1.05 mg/mm³.

Statistics

Data are presented as means ± SE. All statistics were performed on an IBM PC-compatible computer using Excel (Microsoft, Redmond, WA) and Instat (Graphpad Software, San Diego, CA) analytical software. Differences in MPO activity and resting membrane potential of longitudinal smooth muscle cells and changes in esophageal length (from baseline to day 4) between NS- and acid-perfused groups of animals were tested for statistical significance using two-tailed unpaired Student’s t-test. Differences in percent epithelial denudation and changes from baseline to day 4 in esophageal body contraction amplitude at any site, LES resting pressure, and percent relaxation were tested between groups using nonparametric Mann-Whitney U-test. Differences in the frequency of peristaltic vs. nonperistaltic waves within groups between baseline and day 4 were tested statistically using Fisher’s exact test. P < 0.05 was considered statistically significant.

RESULTS

MPO Activity and Epithelial Injury

Macroscopically, repeated intraluminal acid perfusion induced mucosal sloughing, reactive hyperemia, necrosis, and ulceration of the esophagus. This was associated with increased inflammation as indicated by significantly higher MPO levels than in NS-perfused animals within the region 0–1 cm above the LES (1.12 ± 0.23 vs. 0.33 ± 0.06 U/mg tissue; P < 0.005) and within the region 4–5 cm above the LES (1.37 ± 0.26 vs. 0.011 ± 0.004 U/mg tissue; P < 0.0001). Repeated acid exposure also caused significantly increased epithelial damage, as the epithelium was found to be 31.7 ± 11.6% denuded after acid but only 5 ± 3.8% denuded after NS perfusion (P < 0.01).

Assessment of Changes in Esophageal Length

In control NS-perfused animals, the esophagus actually lengthened over the course of the experiment (0.51 ± 0.23 cm vs. baseline). The reason for this is unclear. In unperfused animals subjected to all other experimental manipulations, a gradual lengthening of the esophagus was noted after the anesthesia and stabilization period that was similar to the lengthening observed in animals perfused with NS (data not shown). It is possible that the stress of anesthesia and other preexperiment manipulations causes a nonspecific shortening response on day 1 that dissipates by day 4 as the animal becomes acclimatized to the procedures. On the other hand, repeated intraluminal acid perfusion induced significant shortening of the esoph-
Assessment of Peristaltic Function

minal acid perfusion (5 n did swallow at baseline and on animals did not swallow at baseline. In animals that perfused with acid, with no swallows induced in one acid-perfused animal. One of nine NS-perfused animals did not swallow at baseline. Technical difficulties pre-vented data collection of esophageal body contractions in three of nine animals. Technical difficulties pre-

Table 2: Effect of repeated esophageal intraluminal perfusion of saline or acid on peristaltic function

<table>
<thead>
<tr>
<th></th>
<th>Saline Baseline</th>
<th>Acid Day 4</th>
<th>Peristaltic</th>
<th>%Peristaltic</th>
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<tbody>
<tr>
<td></td>
<td>Peristaltic</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>27</td>
<td>26</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Day 4</td>
<td>10</td>
<td>3</td>
<td>11</td>
<td>25</td>
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Values are no. of swallow-induced responses. The frequency of peristaltic swallow-induced responses, compared with nonperistaltic, was not significantly affected by repeated exposure to saline (n = 8) or acid (n = 5).

Fig. 1. Effect of repeated esophageal luminal perfusion of 0.9% saline (NS) or 100 mmol/l HCl on esophageal length. Data represent change in length on day 4 vs. baseline. Proximal migration of the lower esophageal sphincter (LES) indicates esophageal shortening, whereas distal migration indicates esophageal lengthening. Perfusion of acid resulted in significant shortening of the esophagus (mean shortening of 0.68 ± 0.21 cm vs. mean lengthening of 0.51 ± 0.23 cm in NS-perfused controls; group mean difference = 1.19 cm; *P = 0.0006).

and percent LES relaxation from baseline to day 4 in acid animals (n = 6) were not significantly different from those in controls (n = 8) (Table 3).

**Longitudinal Muscle Mechanical and Electrical Recordings**

Active tension generated in response to 1 μmol/l carbachol was significantly greater at each increment of stretch from 0 to 50% in muscle strips from acid-perfused animals (n = 7), compared with strips from NS-perfused controls (n = 8) (P < 0.05 for 0, 40, and 50% stretch, P < 0.01 for 30% stretch, P < 0.005 for 10 and 20% stretch) (Fig. 2A). From 50 to 100% stretch, active tension generated by muscle strips from acid-perfused animals was not significantly different from strips from NS-perfused controls. In both groups, passive tension increased exponentially with increasing stretch (Fig. 2B). Despite a tendency toward increased passive tension at higher increments of stretch in muscle strips from acid-perfused animals vs. controls, this did not reach statistical significance.

Longitudinal smooth muscle cells from the NS-perfused esophagus displayed spontaneous electrical activity in which one to six (4.4 ± 0.4) spike-like action potentials with a mean amplitude of 17.6 ± 0.9 mV were superimposed on a slow wave-like potential with a mean frequency of 2.32 ± 0.27/min and mean resting membrane potential of −53.1 ± 1.6 mV. Compared with controls, cells from acid-perfused animals demonstrated a significantly depolarized resting membrane potential (−47.6 ± 1.1 mV; P < 0.05). In addition, cells from esophagitis animals displayed three distinct patterns of spontaneous action potential spiking (Fig. 3). Of the 31 cells recorded from these animals, 19 displayed a normal pattern, whereas 7 cells were charac-

Table 1: Effect of repeated esophageal intraluminal perfusion of saline or acid on esophageal body contraction amplitudes

<table>
<thead>
<tr>
<th></th>
<th>5 cm Saline</th>
<th>5 cm Acid</th>
<th>3 cm Saline</th>
<th>3 cm Acid</th>
<th>1 cm Saline</th>
<th>1 cm Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (a)</strong></td>
<td>89.2 ± 10.1</td>
<td>86.7 ± 11.9</td>
<td>66.4 ± 9.7</td>
<td>57.9 ± 4.9</td>
<td>45.3 ± 4.1</td>
<td>52.7 ± 10.1</td>
</tr>
<tr>
<td><strong>Day 4 (b)</strong></td>
<td>84.8 ± 12.6</td>
<td>53.8 ± 25.2</td>
<td>73.0 ± 10.4</td>
<td>49.9 ± 8.6</td>
<td>52.2 ± 4.0</td>
<td>57.3 ± 4.0</td>
</tr>
<tr>
<td><strong>Difference (b - a)</strong></td>
<td>−4.5 ± 15.7</td>
<td>−32.9 ± 27.1</td>
<td>6.6 ± 10.4</td>
<td>−7.9 ± 6.8</td>
<td>6.9 ± 3.3</td>
<td>4.6 ± 8.1</td>
</tr>
</tbody>
</table>

Values represent means ± SE for contraction amplitudes (in mmHg) at recording sites 5, 3, and 1 cm proximal to the manometrically located lower esophageal sphincter (LES). Changes in amplitudes between baseline and day 4 for 100 mmol/l HCl (acid)-perfused animals (n = 5) were not significantly different at any site from the changes observed in 0.9% saline (saline)-perfused controls (n = 8).
terized by an abnormal, unstable resting membrane potential, and 5 cells revealed ongoing spike-like action potentials.

**DISCUSSION**

The present study demonstrates that in the opossum, esophagitis induced by repeated intraluminal acid perfusion leads to marked shortening of the esophagus, which is associated with hyperexcitability of the longitudinal muscle layer. Furthermore, this shortening is not a transient event, as the esophagus remained shortened 24 h after the final period of acid challenge. The average length of the opossum esophagus is 15 cm. Thus the shortening observed in this study (1.2 cm vs. control animals) was not significantly different from changes in saline-perfused controls (n = 6).

Acid-induced esophageal shortening has been observed previously in humans and in experimental models of esophagitis. Dunne and Paterson (7) have shown that short-term intraluminal acid perfusion induces a proximal movement of the manometrically located LES in healthy volunteers. Our previous opossum studies in which longitudinal axis shortening was measured directly using a strain-gauge device during 90 (21) and 150 min (23) of acid perfusion have demonstrated esophageal shortening of a similar magnitude to that observed after day 1 of perfusion in the present investigation. However, a partial reversal of this shortening was observed over time (23). Using a more chronic model, Shirazi et al. (26) found a significant proximal migration of the opossum LES after intraluminally perfusing the esophagus with acid for 2 or 4 h and then examining changes in the manometric location of the LES at 24 and 72 h, respectively. Such sustained shortening, up to 3 days after the cessation of perfusion in this model, is consistent with the results of the present study.

This esophageal shortening and the associated marked inflammation occurred in the absence of detectable esophageal peristaltic dysfunction or LES impairment. In contrast, several studies (3, 23, 26) have demonstrated such dysfunction in animal models of esophagitis. Shirazi et al. (26) observed significantly reduced esophageal body contraction amplitudes, frequent failure of primary peristalsis, and frequent occurrence of spontaneous esophageal body contractions in the distal esophagus of opossums after acid perfusion. We (23) also previously observed a reduced resting LES pressure after acute intraluminal perfusion of acid in the opossum. However, in each of these studies, the duration of perfusion, being 2–4 h and 150 min, respectively, was at least three times longer than any day of perfusion in the present study. Using a model similar to the opossum model described in this study, Biancini et al. (3) observed a significantly reduced resting LES pressure in the cat after acid perfusion. It is likely that in these animal models, LES and peristaltic dysfunction occur with more advanced esophagitis than is required to induce longitudinal shortening.

### Table 3. Effect of repeated esophageal intraluminal perfusion of saline or acid on resting LES pressure and %LES relaxation

<table>
<thead>
<tr>
<th></th>
<th>Resting LES Pressure (mmHg)</th>
<th>%LES Relaxation</th>
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<tr>
<td></td>
<td>Saline</td>
<td>Acid</td>
</tr>
<tr>
<td>Baseline (a)</td>
<td>34.0 ± 2.7</td>
<td>36.3 ± 5.8</td>
</tr>
<tr>
<td>Day 4 (b)</td>
<td>37.3 ± 2.6</td>
<td>30.2 ± 5.9</td>
</tr>
<tr>
<td>Difference (b – a)</td>
<td>3.3 ± 2.6</td>
<td>–6.1 ± 6.3</td>
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Values represent means ± SE obtained in each group. Changes in LES resting pressure and %LES relaxation from baseline to day 4 in acid-perfused animals (n = 8) were not significantly different from changes in saline-perfused controls (n = 6).

![Fig. 2. Effect of repeated esophageal luminal perfusion of 0.9% NS or 100 mmol/l HCl on passive and active length-tension relationships for esophageal longitudinal muscle. Longitudinal muscle strips were stretched in 10% increments from 0% (resting length) to 100%. Active tension (induced by 1 μmol/l carbachol) (A) and passive tension (B) were recorded isometrically at each increment of stretch. Strips from acid-perfused animals generated significantly greater active tension at each increment of stretch between 0 and 50%, compared with strips from NS-perfused controls. No significant differences in passive tension generated at any increment of stretch were observed between groups. *P < 0.05; **P < 0.01; and ***P < 0.005.](image-url)
Peristaltic dysfunction and LES hypotension are also commonly observed in patients with GERD. Compared with healthy controls, significantly decreased esophageal contraction amplitudes after swallowing, increased frequency of failed peristaltic sequences, and hypotensive LES pressures have all been noted in individuals with the disease (12, 31). Significantly decreased contraction durations and peristaltic propagation velocity in patients with severe reflux esophagitis have also been observed (31). It is important to note that such abnormalities appear to be related to the severity of the esophagitis in GERD patients (12). Indeed, a study by Timmer et al. (30) suggests that only patients with severe esophagitis (Savary-Miller grade III–IV) exhibit alterations in amplitude and duration of esophageal motor waves, whereas patients with low-grade esophagitis (grade I–II) do not display abnormalities to any significant degree. Twenty-four-hour pH manometry has been used to compare esophageal responses with spontaneous swallows in patients with low-grade esophagitis (grade I–II) and in healthy controls. No differences were found in terms of amplitude or duration of esophageal body contractions in the distal esophagus or in the velocity of peristaltic contractions (1, 10).

Although no significant peristaltic dysfunction or LES impairment was found in the current study, a significant shortening of the esophagus was observed. This suggests that esophageal shortening is an earlier and more sensitive motor response to acid-induced esophagitis. The mechanism by which esophagitis induces esophageal shortening is not completely understood. It is known that, unlike the longitudinal muscle contraction and resulting shortening of the esophagus observed during peristalsis, acid-induced esophageal shortening is not mediated by cholinergic neurons or vagal pathways (5, 20, 23). Furthermore, mast cell-derived mediators are important in the esophageal shortening observed with acute acid exposure (21). Whether this finding is relevant to the more chronic model used in the present investigation is unknown.

It was observed in this study that longitudinal muscle strips from acid-perfused animals generated significantly greater tension in response to carbachol stimulation. This is comparable to recent findings in the inflamed small intestine. Increased longitudinal muscle contractility has been observed in rats infected with *Nippostrongylus brasiliensis*, a response that could be prevented by suppressing the inflammatory response with corticosteroid treatment (9). Jejunal longitudinal muscle from rats infected with *Trichinella spiralis* has been found to be hyperresponsive to carbachol and 5-hydroxytryptamine (32). Similarly, in NIH Swiss mice infected with *T. spiralis*, there is an enhanced longitudinal muscle response to carbachol and KCl (2). An important finding of the latter study is that the change in longitudinal muscle function persisted long after the expulsion of the nematode and the resolution of the inflammatory response. This provides evidence that a persistent, fundamental change can occur in the longitudinal muscle during inflammation. A similar change in the esophageal longitudinal muscle layer may have occurred in this study as a result of the inflammatory response to acid and could be an important factor in the prolonged shortening observed.

Further support for the idea that some alteration of the longitudinal muscle layer is related to esophageal shortening comes from the intracellular recordings in this investigation. The resting membrane potential of cells from animals repeatedly perfused with acid was significantly depolarized relative to control animals, cells from animals repeatedly perfused with acid was significantly depolarized relative to control animals, which is consistent with observations from inflamed intestinal circular smooth muscle cells (4, 18). In addition, spontaneous electrical activity, unstable membrane potentials, and ongoing spike-like action potentials not observed in control cells became apparent after acid challenge. Thus the hyperexcitability of the longitudinal muscle layer may be related to a dramatic change in cellular membrane properties, although it is not clear if an ionic basis underlies the observed alterations in spontaneous electrical activity. Such a change could be due to an alteration of the mediator composition of the extracellular fluid (including mediators released from mast cells) and the way in which these...
mediators interact with the membrane, changes in membrane ion channel expression, or to some other unknown mechanism.

Cause and effect relationships between reflux esophagitis, hiatal hernia, and impaired motility remain speculative. This study, however, provides evidence to support the notion that reflux esophagitis per se causes esophageal shortening, which in turn could contribute to the formation of hiatal hernia. This shortening precedes esophageal peristaltic or LES dysfunction. Once developed, the hernia, acting as an acid reservoir with ready access to the LES during swallowing, may then contribute to a worsening of the esophagitis, possibly leading to the impaired motor and LES function observed in patients with more severe cases of GERD.

We thank D. V. Miller for excellent technical assistance and G. C. Pringle for helping with the挂号.

This study was supported by Medical Research Council of Canada Grant MA-10475 (W. G. Paterson), National Science and Engineering Research Council (NSERC) Grant 138239 (G. P. Morris), and NSERC Postgraduate Scholarship (PGS B) and the School of Graduate Studies at Queen’s University (R. J. White).

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