Role of mast cell-derived mediators in acid-induced shortening of the esophagus

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Paterson, W. G. Role of mast cell-derived mediators in acid-induced shortening of the esophagus. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G385–G388, 1998.—It has recently been demonstrated that acid-induced esophageal mucosal injury leads to esophageal shortening, raising the possibility that reflex esophagitis per se may contribute to the development of hiatal hernia. The aim of the present study was to determine whether mast cell-derived mediators are involved in this acid-induced esophageal shortening. Changes in esophageal length were continuously monitored in anesthetized opossums while the esophageal lumen was perfused with 100 mmol/l HCl or normal saline. Changes in esophageal length were compared between animals perfused with acid, with or without pretreatment with the mast cell stabilizers doxantrazole or disodium cromoglycate (DSCG), and animals perfused with normal saline, with or without pretreatment with DSCG. In separate in vitro studies the effect of the mast cell stabilizers on electrical field stimulation-induced esophageal longitudinal muscle contraction was determined. Gradual esophageal lengthening occurred during saline perfusion, irrespective of whether animals were pretreated with DSCG. In contrast, acid perfusion induced esophageal shortening, which was abolished by pretreatment with either doxantrazole or DSCG in doses sufficient to attenuate the acid-induced mucosal histamine release. In vitro, the mast cell stabilizers had no effect on electrical field stimulation-induced esophageal shortening. This study suggests that esophageal shortening associated with acute acid-induced esophageal mucosal injury in the opossum is dependent on mast cell-derived mediators.

reflux esophagitis; mast cell stabilizers; longitudinal muscle; hiatal hernia

ALTHOUGH LOWER ESOPHAGEAL SPHINCTER (LES) hypotension and inappropriate LES relaxation are considered to be primary factors contributing to gastroesophageal reflux events (5, 6), it appears that a hiatal hernia can contribute to the pathogenesis of gastroesophageal reflux disease (GERD) by acting as a reservoir for gastric juice that has ready access to the gastroesophageal junction (11, 15). The etiology of hiatal hernia remains unclear. It has been assumed that because intra-abdominal pressure is greater than intrathoracic pressure, the proximal stomach has a tendency to migrate into the chest as the anatomic factors that normally resist this weaken with age (9). However, for migration of the stomach into the chest to occur, there must be either structural or physiological shortening of the esophagus. In 1989, Shirazi et. al. (14) reported that the manometric location of the LES in opossums with experimental acid-induced esophagitis was displaced orally. We subsequently demonstrated this directly and found that the longitudinal shortening was not mediated by the vagus nerves or cholinergic mechanisms (13). In separate studies, Barclay et al. (1) and Feldman et al. (8) have found that acid-induced mucosal injury in the opossum is associated with mast cell degranulation and histamine release. Furthermore, anatomic studies in humans have indicated that the number of lamina propria mast cells is comparable to that seen in the opossum model (10, 19). Many mast cell-derived mediators are known to induce gut smooth muscle contraction (16), thus raising the possibility that mast cell-derived mediators are involved in acid-induced shortening. The aim of the current experiments was therefore to determine whether mast cell-derived mediators are involved in acid-induced esophageal shortening in the opossum.

MATERIALS AND METHODS

The protocol was approved by the Queen's University Animal Care Committee. Experiments were performed in 28 adult opossums (Didelphis virginiana) of either sex, weighing between 2.1 and 4.9 kg. Animals were fasted for 12–16 h before the experiment. Anesthesia was induced with pentobarbital sodium (Somnotol; MTC Pharmaceuticals, Cambridge, ON, Canada), at a dose of 35–40 mg/kg body wt given by tail vein injection, followed by 20 mg/kg iv a-chloralose (BDH Chemicals, Poole, UK). The animals were strapped supine to a specially designed animal board so that they were in a 30° head-up position. Body temperature was maintained at 35°C with a heating pad. An endotracheal tube was placed, and the cuff was inflated to minimize the chance of aspiration. A brachial or femoral artery was cannulated for constant blood pressure recording, and a corresponding vein was cannulated for administration of intravenous fluids and drugs.

An esophageal catheter was assembled consisting of two polyvinyl tubes (0.8 mm ID, 1.16 mm OD) that were glued together with tetrahydrofuran. In one of these tubes, a 1-mm side-hole opening was made, and the distal portion was sealed. This tube was used to locate the LES by a perfused manometry system. The end of the second tube was left open and used for luminal perfusion. The catheter was passed through the mouth of the animal and positioned initially in the stomach. It was then withdrawn so that the perfusion catheter was positioned ~3 cm proximal to the manometrically defined LES.

Longitudinal axis shortening was measured using a specially designed force transducer as previously described (13). This consisted of a flexible copper metal arm that terminated in an inverted U-type configuration so that it could fit over the distal esophagus like a saddle (Fig. 1). A pin was then passed through a hole in each limb of the inverted U as well as through the muscularis propria of the esophagus, to fix the transducer arm at the region of the gastroesophageal junction. In this way, longitudinal axis shortening or lengthening of the esophagus caused deflection of the metal arm in proportion to the magnitude of movement. The transducer was calibrated in terms of millimeters of deflection of the distal tip of the metal arm. Output from the transducer was amplified and recorded on a physiograph (Gould RS 3800; Gould, Cleveland, OH). Recordings were made in the DC mode with a high-frequency filter set at the maximal setting.
The protocol consisted of a 45-min stabilization period, during which the esophagus was continuously perfused with normal saline at a rate of 1 ml/min. This was followed by a 30-min normal saline baseline period and then a 90-min period during which either normal saline (controls, n = 4) or 100 mmol/l HCl were perfused at a rate of 1 ml/min. Three groups of acid-perfused animals were studied (n = 4 in each). One group was pretreated (30 min before the start of the perfusion period) with the mast cell stabilizer doxantrazole (a gift from Burroughs-Wellcome, Research Park, NC) at a dose of 60 mg/kg iv, the second with the mast cell stabilizer disodium cromoglycate (DSCG; Sigma, St. Louis, MO) at a dose of 80 mg/kg iv, and the third with an equal volume of saline (placebo). An additional control group (n = 4) was pretreated with intravenous DSCG and luminaally perfused with normal saline throughout the experiment.

A separate series of in vitro experiments (n = 3) were performed to exclude nonspecific inhibitory effects of the mast cell stabilizers on opossum longitudinal muscle contraction. After anesthesia, a midline laparotomy and sternotomy were performed and an endotracheal tube was positioned for ventilation of the animal. The entire thoracic esophagus with a cuff of proximal stomach was then excised en bloc and placed in a Plexiglas tissue bath containing modified Krebs solution and continuously bubbled with 95% O2-5% CO2. The Krebs solution consisted of (in mM) 118 NaCl, 4.75 KCl, 1.0 NaH2PO4, 25 NaHCO3, 1.2 MgSO4, 2.5 CaCl, and 11 glucose. After baseline observations the EFS response was repeated with varying concentrations of the mast cell stabilizer in the perfusate. A separate series of in vivo experiments (n = 5 in each) was pretreated with DSCG (n = 4) or doxantrazole (n = 4) or saline. Changes in esophageal length during in vivo perfusion studies in the different groups are depicted in Fig. 2. During the 30-min baseline period (saline perfusion), esophageal lengthening occurred (0.21 ± 0.06 cm; n = 20). Intraluminal acid perfusion then caused gradual, sustained esophageal shortening, which differed significantly from both baseline and control groups by 15 min (P < 0.01). After 90 min of acid perfusion, shortening averaged 0.54 ± 0.20 cm (range 0.20–1.05 cm; n = 4). In contrast, in control animals perfused with normal saline throughout the experiment, there was further esophageal shortening, which averaged 0.39 ± 0.04 cm (range 0.27–0.45 cm; n = 4) at the end of the experiment. Pretreatment with DSCG and doxantrazole abolished the acid-induced esophageal shortening. These doses were chosen on the basis of our previous experiments, which showed significant attenuation of the acid-induced luminal appearance of histamine, as well as reversal of the acid-induced augmentation of esophageal blood flow, by these mast cell stabilizers (8). The change in esophageal length in the acid-perfused animals that had been pretreated with mast cell stabilizers was not significantly different from control animals perfused with normal saline or normal saline-perfused animals that were pretreated with DSCG.

Data are presented as means ± SE. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test for in vivo experiments and ANOVA with repeated measures for the in vitro experiments. A P value of < 0.05 was considered significant.

RESULTS

In vivo experiments. Changes in esophageal length during in vivo perfusion studies in the different groups are depicted in Fig. 2. During the 30-min baseline period (saline perfusion), esophageal lengthening occurred (0.21 ± 0.06 cm; n = 20). Intraluminal acid perfusion then caused gradual, sustained esophageal shortening, which differed significantly from both baseline and control groups by 15 min (P < 0.01). After 90 min of acid perfusion, shortening averaged 0.54 ± 0.20 cm (range 0.20–1.05 cm; n = 4). In contrast, in control animals perfused with normal saline throughout the experiment, there was further esophageal shortening, which averaged 0.39 ± 0.04 cm (range 0.27–0.45 cm; n = 4) at the end of the experiment. Pretreatment of the animals with either DSCG (n = 4) or doxantrazole (n = 4) abolished the acid-induced esophageal shortening. These doses were chosen on the basis of our previous experiments, which showed significant attenuation of the acid-induced luminal appearance of histamine, as well as reversal of the acid-induced augmentation of esophageal blood flow, by these mast cell stabilizers (8). The change in esophageal length in the acid-perfused animals that had been pretreated with mast cell stabilizers was not significantly different from control animals perfused with normal saline or normal saline-perfused animals that were pretreated with DSCG.

Fig. 2. Effect of mast cell stabilizers on acid-induced esophageal shortening. Time 0 represents when baseline (saline perfusion) period ended and perfusion of test solution began. With acid perfusion, esophageal shortening occurred that was significantly different from baseline and all other groups by 15 min (P < 0.01). In contrast, neither change in esophageal shortening occurred in acid-perfused animals pretreated with disodium cromoglycate (DSCG) or doxantrazole, which was not significantly different from the lengthening seen in control animals perfused with saline, with or without pretreatment with DSCG. •, Acid perfused; ■, acid perfused + pretreated with doxantrazole; ◊, acid perfused + pretreated with DSCG; –×–, saline perfused; +, saline perfused + pretreated with DSCG.
In vitro experiments. EFS caused a frequency-dependent phasic shortening of the esophagus that was unaffected by either DSCG or doxantrazole in concentrations of $10^{-6}$ to $10^{-3}$ M (Fig. 3). This suggests that these drugs do not have a nonspecific inhibitory effect on esophageal longitudinal muscle contraction.

DISCUSSION

This study demonstrates that mast cells are involved in acid-induced esophageal shortening and provides further indirect support for the concept that esophagitis per se may cause esophageal shortening and may contribute to the development of a hiatal hernia. It is highly unlikely that results were related to nonspecific effects of the mast cell stabilizers; not only was the same effect seen with two different mast cell stabilizers, but high concentrations of these agents did not affect EFS-induced longitudinal muscle contraction in vitro.

Previous studies in the opossum model have demonstrated that longitudinal muscle contraction during peristalsis is primarily mediated by the vagus nerve through release of acetylcholine (4, 12). In our previous study we found that neither vagotomy nor atropine could attenuate the acid-induced esophageal shortening, suggesting that some other mediator must be involved (13). Furthermore, Barday et al. (1) and Feldman et al. (8) have recently demonstrated in the opossum model that acute acid injury is associated with mast cell degranulation and release of histamine into the lumen. The released histamine appears to play a role in the pathogenesis of the injury, in that it contributes to the reactive increase in mucosal blood flow via a nitric oxide-dependent mechanism (8). On the basis of the current studies, it appears that a mast cell-derived mediator released in the course of acid-induced injury also caused longitudinal muscle contraction, resulting in esophageal shortening.

Given the high number of mast cells in the lamina propria and their proximity to the muscularis mucosa, it is possible that this muscle layer, which is oriented longitudinally and has physiological and pharmacological properties similar to the longitudinal muscle of the muscularis propria (7), contributes to esophageal shortening. However, it is unclear whether the muscularis mucosa can actually shorten the entire esophagus as opposed to causing axial movement of the mucosa only. Furthermore, we attempted to fix our transducer to the esophagus by pinning it through the muscularis propria to minimize any contribution from the muscularis mucosa. Nevertheless, it is impossible to exclude a contribution from this muscle layer in the acid-induced esophageal shortening.

It is unclear whether mast cell mediators induce contraction of longitudinal muscle directly or via nerve pathways. We have previously shown, however, that atropine and vagotomy have no effect on acid-induced esophageal shortening (13). Furthermore, our previous morphological studies show evidence of acid-induced mast cell degranulation not only in the lamina propria but also in the deep submucosa and the muscularis propria (1). Therefore, mediators from these more deeply located mast cells may well be able to diffuse to directly stimulate the longitudinal muscle of the muscularis propria.

The mast cell mediator(s) that might be causing the acid-induced esophageal shortening is also unknown. Preliminary studies suggest that it is not histamine, as histamine causes fairly weak and unsustained contraction of esophageal longitudinal smooth muscle in vitro (personal observations). Numerous other mast cell-derived inflammatory mediators are known to induce contraction of gut smooth muscle (16). For instance, platelet-activating factor is a potent contractor of intestinal smooth muscle (17), as is 5-hydroxytryptamine (18) and the leukotrienes $C_4$ and $D_4$ (2). Therefore, identification of the mediator(s) and cellular mechanisms that produce this response, as well as a possible intermediate role for neurons, will require further detailed study.
The degree of shortening measured in response to acid perfusion was relatively small, but this does not necessarily mean that this is a pathophysiological response without clinical significance. Not only is the opossum esophagus much shorter than the human esophagus, but in the current experiments the use of the flexible metal arm to measure the change in length as it occurred provided some resistance to free axial movement of the esophagus. It would therefore significantly underestimate the degree of esophageal shortening that would occur in the unencumbered esophagus. This is supported by the previous experiments by Shirazi et al. (14), in which the LES migrated -2 cm oral after induction of acid-induced esophagitis.

It remains to be established whether the findings in the opossum model are applicable to humans. Certainly, the duration of acid exposure required before significant shortening was detected is comparable to that which occurs spontaneously in patients with GERD (3). Ying et al. (19) and Marinell et al. (10) have also found that the number of subepithelial mast cells in humans is substantial and is comparable to that seen in the opossum model. Furthermore, esophageal mast cells appear to be increased in patients with GERD (10, 19). Nevertheless, the current studies describe the acute response to intraluminal acid. GERD is a condition in which there is repeated exposure of the esophageal mucosa to gastric juice over a prolonged period. Whether such chronic exposure also causes mast cell mediator release and esophageal shortening remains to be established.

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