Gastroesophageal Reflux After Combined Lower Esophageal Sphincter and Diaphragmatic Crural Sling Inactivation in the Rat

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This study tests the hypothesis that either selective or combined destruction of the lower esophageal sphincter and the diaphragmatic crural sling should induce reflux in the rat. Pull-through perfusion manometry was performed before and after lower esophageal myectomy, crural myotomy, or both. pH monitoring was used to detect reflux. Unmanipulated rats served as controls. Paired t tests were used for comparison of pre- and postoperative pressure values and contingency tables with Fisher’s tests for examining the association between the interventions and the appearance of reflux. Esophageal myectomy decreased only sphincteric pressure from 25.9 ± 15.5 to 9 ± 6 mm Hg (P < 0.01), whereas crural myotomy decreased only sling pressure from 26.2 ± 13.3 to 7.3 ± 3.9 mm Hg (P < 0.01). Simultaneous performance of both procedures decreased sphincteric and crural pressures from 20.4 ± 7.5 to 7.6 ± 3.9 mm Hg (P < 0.01) and from 45.9 ± 20.6 to 18.2 ± 7.4 mm Hg (P < 0.01), respectively. None of the control, myectomy, or myotomy animals showed reflux upon pH-metry but 5/8 rats in which both procedures were performed had prolonged acid exposure. No esophagitis was seen. In conclusion, normal rats do not have reflux. Selective destruction of either the sphincter or the crural sling does not induce reflux, despite causing flattening of their respective manometric profiles. Conversely, combined inactivation of both components is significantly associated with reflux.

KEY WORDS: gastroesophageal barrier; lower esophageal sphincter; crural sling; manometry; myectomy; myotomy; pH-metry; reflux; rat.

The gastroesophageal barrier against reflux consists of a double sphincteric mechanism formed by the lower esophageal sphincter (LES) and the diaphragmatic crural sling (1). We recently described the anatomic arrangement and the functional features of both components in the rat, in which they are widely separated by a long intraabdominal esophagus that facilitates their independent manometric assessment (2). We showed also that in this animal there is a striking correspondence of the anatomical structure of the U-shaped muscular bundles forming the LES (open toward the left) and the crural sling (open toward the right) with their respective manometric profiles that reflect pressures exerted on the right and left sides of the esophagus respectively (3). We proposed that their simultaneous and complementary actions result in a powerful and effective sphincteric...
mechanism, but since the occurrence of reflux and its possible mechanisms have not been investigated in this animal, convincing demonstration of the efficiency of the barrier was still lacking.

The present study demonstrates that normal rats have no reflux and tests the hypothesis that either individual or combined inactivation of the components of the gastroesophageal barrier—the LES and the diaphragmatic crural sling—will abolish its function and allow free reflux of gastric contents into the esophagus.

MATERIALS AND METHODS

Animals. Adult male Wistar rats \((N = 34)\) weighing 300–500 g (Criffa, Barcelona, Spain) were housed in our animal quarters under controlled temperature and humidity conditions with 12-hr light cycles. Prior to the experiments they received standard rat chow and tap water \textit{ad libitum}. All these conditions were approved by the local institutional research committee and met the requirements established by the current regulations for animal care and research in Europe (EC 86/L609).

Experimental Design. Rats were randomly divided into four groups: Group 1 \((N = 9)\) was used to test pHmetrically the absence of reflux in unmanipulated animals. Group 2 rats \((N = 8)\) underwent pull-through esophageal manometry immediately before and 24 hr after esophagogastriuginal junction myectomy. In group 3 \((N = 8)\), manometric studies were performed before and after crural diaphragm myotomy. Finally, in Group 4 \((N = 9)\), the rats had both esophagogastriuginal junction myectomy and crural diaphragm myotomy. Twenty-four hours after these interventions, all animals underwent esophageal pH monitoring with repeated abdominal compressions to assess whether or not they had gastroesophageal reflux. A fifth group originally planned for \(\text{pH}\) studies after sham operation was discarded after the results of groups 2 and 3 were known. Four rats from each group were killed 30 days after the experiment and their esophagi were histologically investigated for esophagitis.

Operative Procedures. For esophagogastriuginal junction myectomy, the distal esophagus was exposed and isolated through a midline abdominal incision using a clean but not sterile surgical technique and 10 mm of the distal esophageal muscular wall were excised extramucosally. This procedure was accompanied by anterior myotomy of the lower esophagogastriuginal junction performed with microsurgery scissors and knife under surgical microscope (Wild M-650, Herbrugg, Switzerland). For diaphragmatic crural sling myotomy, the crura were exposed on the left side of the greater curvature of the stomach and underneath the spleen. A transverse incision extending through the whole thickness of the crura was carried out until they were completely detached from their insertions in the prevertebral plane. For combined myectomy–myotomy, both procedures were carried out simultaneously in the same animal. After these interventions, the incisions were closed with a two-layer running suture and the animals were allowed to recover for 24 hr. The operative procedures are depicted in Figure 1.

Esophageal Manometry. All measurements were taken in overnight-fasted animals in the supine position and under intraperitoneal anesthesia (6.25 mg/100 g ketamine hydrochloride and 0.5 mg/100 g diazepam) and spontaneous breathing according to previously described techniques (4, 5). Briefly, a tip-occluded single-lumen catheter (1 mm OD, 0.5 mm ID) with a distal side hole \((1.0 \times 0.5 \text{ mm})\) connected to an external transducer (HP 1280; Hewlett Packard, Palo Alto, California) and continuously perfused with bubble-free distilled water \((0.4 \text{ ml/min})\) using a high-pressure, low-compliance pneumohydraulic pump (Müller Scientific, Mississauga, Ontario, Canada) was advanced into the stomach within which the pressure was registered by a monitor (Schiller) with a screen display and on-line printout at a paper speed of 2.5 mm/sec. The atmospheric pressure at the level of the atrium served as the zero reference. The recording orifice of the catheter was oriented towards the anterior wall of the stomach—the plane where both the LES and the crural diaphragm show higher pressure values (3)—and it was subsequently withdrawn into the esophagus.

**Fig 1.** Schematic drawing of the interventions. For lower esophageal sphincter inactivation, a 10-mm circular extramucosal myectomy was associated with an anterior junctional myotomy (A). For diaphragmatic sling inactivation both crura were divided near their prevertebral insertion (B). Both procedures were combined in order to achieve complete inactivation (C).