Duodenal acid-induced gastric relaxation is mediated by multiple pathways

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Lu, Yuan-Xu, and Chung Owyang. Duodenal acid-induced gastric relaxation is mediated by multiple pathways. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1501–G1506, 1999.—In this study, we used an in vivo anesthetized rat model to investigate the mechanisms responsible for duodenal acid-induced inhibition of gastric motility. Intradaodenal infusion of HCl produced a rate-dependent decrease in intragastric pressure. Infusion of HCl at 2 ml/h produced a physiological plasma secretin level and elicited a decrease in intragastric pressure of 3.0 ± 0.2 cmH₂O. Infusion of rabbit secretin antiserum reduced the acid-induced inhibition of gastric motility by 85 ± 5%, suggesting mediation mainly by endogenous secretin. Administration of the cholecystokinin (CCK)-A antagonist MK-329 caused only a modest 10 ± 3% reduction in gastric relaxation, whereas the serotonin antagonist ICS-205930 had no effect. In contrast, immunoneutralization with the serotonin antibody caused only a 15% reduction in the relaxation evoked by a higher rate of HCl infusion (3 ml/h), whereas MK-329 and ICS-205930 caused a 20 ± 4% reduction and no reduction, respectively. Bilateral truncal vagotomy or perivagal application of capsaicin completely abolished gastric relaxation in response to low rates (1–2 ml/h) of 0.1 N HCl infusion but only partially affected gastric relaxation in response to a higher infusion rate (3 ml/h). These observations indicate that multiple pathways mediate the duodenal acid-induced inhibition of gastric motility. At low rates of HCl infusion, gastric relaxation is mediated primarily by endogenous secretin, which acts through vagal afferent pathways. At higher rates of HCl infusion, gastric relaxation is mediated by endogenous secretin, CCK, and possibly by the direct action of HCl on vagal afferent pathways or yet unidentified neuropathways.

vagal afferent; serotonin; secretin; cholecystokinin; duodenal acidification

Duodenal acidification plays a major role in the regulation of gastric emptying (14). Gastric emptying of test meals is rapid when the meal pH is greater than seven and progressively slower as the pH of the test meal declines (18). It has been shown that the greater the concentration of acid in a test solution, the greater the inhibition of gastric emptying (15). This action may be important in protecting the duodenum from excessive amounts of acid.

Currently, the mediators responsible for the feedback inhibition of gastric motility by duodenal acidification remain undefined. A potential candidate is the hormone secretin, which is localized in the S cells of the duodenum and upper jejunum (26). Many studies have demonstrated that duodenal acidification plays a central role in the release of secretin. Simultaneous measurements of intraduodenal pH and plasma secretin concentration in both the fasted and fed states have demonstrated that rapid falls in duodenal pH were followed by transient increases in plasma secretin concentration (29, 23). The threshold pH in the duodenum for the release of secretin is 4.5, although the magnitude of this response is related to duodenal acid load (5). In humans, a single intravenous bolus of secretin inhibits both spontaneous and stimulated motor activity of the antrum (8). It has also been shown that intravenous secretin delays gastric emptying of distilled water and normal saline in a dose-dependent manner (4, 32). Hence, it is probable that secretin mediates delayed gastric emptying induced by duodenal acidification.

Raybould and Holzer (25) reported that duodenal acidification inhibited gastric motility through vagal and spinal sensory pathways. This inhibition appeared to be mediated partially by cholecystokinin (CCK), but not by secretin, because administration of a secretin polyclonal antibody had no effect on the acid-induced inhibition of gastric emptying. It should be noted that a high acid load was used in this study, and, therefore, the physiological significance of this observation remains to be determined.

In this study, we propose to test the hypothesis that a physiological load of acid in the duodenum inhibits gastric motility through the release of secretin acting through the vagal afferent pathway, whereas a supra-physiological acid load affects gastric motility through non-secretin-dependent pathways, such as CCK and direct vagal stimulation. To test this hypothesis, HCl was infused in the rat duodenum at different rates, and gastric motility was measured in the presence or absence of in vivo immunoneutralization with secretin antibody, CCK antagonists, and vagotomy. In addition, we compared the neural pathways used by a physiological load of acid to mediate gastric relaxation to the neural pathways used by secretin.

METHODS

Materials. The following materials were purchased: capsaicin and atropine sulfate from Sigma Chemical (St. Louis, MO) and secretin from Peninsula Laboratories (Belmont, CA). The CCK-A receptor antagonist MK-329 was a generous gift from Merck Sharp and Dohme. ICS-205930, a serotonin (5-HT₃) antagonist, was obtained from Research Biochemicals International (Natick, MA). The polyclonal antibody to secretin was a generous gift from Dr. William Y. Chey (University of Rochester). The drugs were dissolved in physiological saline and were stored at −20°C.
Animal preparation. Male Sprague-Dawley rats weighing between 250 and 300 g were fasted for 24 h with water available ad libitum. The animals were anesthetized with urethane (1.5 g/kg ip). A tracheotomy was performed, and a tracheal tube was inserted through which the animals breathed room air spontaneously. Through a midline incision, a catheter (PE-100, 0.86 mm ID) with an attached rubber balloon was inserted in the body of the stomach through an incision in the duodenum immediately distal to the pylorus and was secured at the pylorus. The jugular veins were cannulated with polyethylene tubing.

Measurement of intragastric pressure. Intragastric pressure was measured using a rubber balloon inserted in the body of the stomach as described previously (19). Pressure was recorded by a Gould Statham P231D pressure transducer and pen recorder. The balloon was filled with water at 37°C (1.6–2.0 ml). This volume was determined to be the level required to induce an intragastric pressure of 10 cmH2O. The exact location of the balloon was verified after each experiment.

Intraduodenal HCl administration. For duodenal infusion of HCl, a duodenal canula (0.5 mm ID) was inserted in the proximal duodenum immediately distal to the balloon catheter. After a 50-min equilibrium period, normal saline or 0.1 N HCl was perfused intraduodenally at 1, 2, and 3 ml/h, during which time intragastric pressure was recorded continuously. Blood samples for the measurement of secretin by RIA (26) were obtained 15 min after the initiation of HCl administration.

Pretreatment with secretin antiserum. To test if HCl-induced gastric relaxation is mediated by endogenous secretin, we examined the effects of secretin antiserum on gastric motility. Rabbit secretin antiserum with a titer of 1:2,000,000 to bind 50% of 125I-labeled secretin at 1 pmol was provided by Dr. William Y. Chey (27). After a 60-min equilibrium period, 0.5 ml of rabbit secretin antiserum or normal rabbit serum was administered through the jugular vein, and intraduodenal perfusion of 0.1 N HCl at 1–3 ml/h was begun 15 min later. Similar immunoneutralization studies have been used successfully to study the role of secretin in oleic acid-induced inhibition of gastric acid secretion in rats (27). Intragastric pressure was monitored before and after HCl infusion, with and without rabbit secretin antiserum.

Pretreatment with CCK and 5-HT antagonists. To determine if endogenous CCK is involved in mediating acid-induced gastric relaxation, we examined the effect of the CCK-A receptor antagonist MK-329 during HCl infusion. After a 60-min equilibrium period, MK-329 (1 mg/kg) was administered through the jugular vein, and intraduodenal 0.1 N HCl perfusion at 1–3 ml/h was begun 15 min later. Similar pressure was monitored before and after HCl infusion. Similar studies were performed with the 5-HT3 antagonist ICS-205930 (0.2 mg/kg iv), which has been shown to prevent 5-HT-induced vagal responses (22).

Bilateral subdiaphragmatic vagotomy. To investigate if low- or high-dose HCl infusion acts through stimulation of the vagal pathways, acute bilateral subdiaphragmatic vagotomy was performed. Through a midline incision of the abdominal wall, the stomach was carefully manipulated to expose the esophagus. The subdiaphragmatic vagal trunks were exposed halfway between the diaphragm and the gastric cardia. The anterior and posterior trunks of the vagal nerves were transected (21). For the control experiments, the abdominal vagal nerves were exposed but not cut. HCl infusion studies were performed as described previously. To demonstrate the completeness of vagotomy, gastric response to electrical stimulation of the vagus nerve was tested as described below.

Nerve stimulation. Through a midline incision on the anterior surface of the neck, the right cervical vagus nerve was dissected free, and the distal end was placed on a nerve electrode and covered with liquid paraffin. The nerve was stimulated with a Grass stimulator (10 V, 5 Hz, 1 ms for 30 s) at 30 min before and 10 min after intraduodenal infusion of 0.1 N HCl (2 ml/h).

Perivagal application of capsaicin. To investigate the role of the vagal afferent pathway in mediating the action of intraduodenal HCl, we examined the effects of perivagal application of capsaicin. After anesthesia, the abdominal vagal trunks were exposed. A small piece of gauze soaked in 1% capsaicin solution (0.1 ml/rat) was left on the vagal trunks for 30 min (21). The capsaicin solution was prepared by sonicating 10 mg of capsaicin in 0.1 ml of Tween 80 for 10 min; 0.9 ml of olive oil was added, and the mixture was sonicated for another 10 min. The vehicle solution (a mixture of Tween 80 and olive oil) alone was applied to the control rats.

Intraduodenal HCl studies as described previously were performed 5 days after surgery in both groups of rats.

Statistical analysis. Results were expressed as means ± SE. The multivariate ANOVA method was used to evaluate the effect of the repeated measurements over time, the effect of treatment, and the interaction between them. Significance was accepted at the 5% level.

RESULTS

HCl dose-response studies. The mean basal secretin level was 1.6 ± 0.5 pmol/L. The plasma secretin levels during intraduodenal perfusion of 0.1 N HCl at 1, 2, and 3 ml/h were 3.3 ± 1.0 pmol, 5.5 ± 0.6 pmol, and 14 ± 2 pmol, respectively (n = 6 for each infusion rate of HCl). Previous studies have shown that the postprandial increase in plasma secretin is in the range of 5–6 pmol/L (27). Therefore, it appears that intraduodenal infusion of 0.1 N HCl at 1–2 ml/h produces physiological plasma secretin levels, whereas perfusion of 0.1 N HCl at 3 ml/h produces a supraphysiological level.

The intragastric pressure was set at 10 cmH2O with balloon distension and was stable for at least 60 min before the infusion of secretin. Intraduodenal infusion of 0.1 N HCl at 1–3 ml/h produced a dose-dependent decrease in intragastric pressure (Fig. 1). HCl infused at 2 ml/h produced a physiological plasma secretin level in the rat and caused intragastric pressure to fall by 3.0 ± 0.2 cmH2O (n = 6).

Effect of secretin antiserum pretreatment on acid-induced gastric relaxation. In a separate study, 0.5 ml of a rabbit antisecretin serum (AB R11 7.1, titer 1:2,000,000) was administered through a jugular vein 15 min before intraduodenal administration of 0.1 N HCl. Similar immunoneutralization studies have been used successfully to investigate the role of secretin in the mediation of oleic acid-induced inhibition of gastric acid secretion (27). Administration of antisecretin serum did not affect basal gastric pressure, but it abolished intragastric relaxation evoked by infusion of 0.1 N HCl at 1 and 2 ml/h and reduced by <15% relaxation caused by 0.1 N HCl infusion at a higher rate of 3 ml/h (Fig. 2).

Effects of CCK-A and 5-HT3 antagonists on acid-induced gastric relaxation. Administration of the CCK antagonist MK-329 (1 mg/kg) caused a 10 ± 3% (n = 6)
and 21 ± 4% (n = 6) reduction in gastric relaxation when 0.1 N HCl was infused at 2 and 3 ml/h, respectively. Therefore, 60–70% of the gastric relaxation in response to 0.1 N HCl infusion at 3 ml/h was not affected by secretin antiserum or by the CCK antagonist, suggesting that duodenal acid directly stimulates vagal fibers. To investigate if duodenal acid-induced inhibition is mediated by 5-HT3 receptors, we examined the effect of intravenous infusion of the 5-HT3 antagonist ICS-205930 (0.2 mg/kg; see Ref. 22). As shown in Fig. 3, ICS-205930 did not affect gastric relaxation induced by the infusion of 0.1 N HCl at 2 ml/h. Similar results were observed when HCl was infused at 3 ml/h (n = 6; data not shown).

Effect of vagotomy. Immediately after truncal vagotomy, there was a reduction in intragastric pressure by 21 ± 9%, but it returned to its original level within 30 min. At this time, we began the intraduodenal HCl infusion experiment. Subdiaphragmatic vagotomy marked reduced the gastric relaxation evoked by intraduodenal infusion of 0.1 N HCl at 3 ml/h but only reduced ~35% of the relaxation evoked by infusion at 3 ml/h (Fig. 4).

Effect of perivagal application of capsaicin. Similar to truncal vagotomy, perivagal application of capsaicin markedly reduced gastric relaxation in response to low rates of 0.1 N HCl infusion (1 and 2 ml/h) but only decreased the relaxation induced by a higher infusion rate (3 ml/h) by 48% (Fig. 5). In contrast, perivagal application of the vehicle solution did not affect gastric relaxation induced by 0.1 N HCl (1–3 ml/h).

Effect of HCl on gastric contraction evoked by electrical vagal stimulation and carbachol. Electrical vagal stimulation (10 V, 5 Hz, 1 ms for 30 s) evoked a significant increase in gastric relaxation. This contraction was completely abolished by atropine (100...
tion did not affect the gastric contraction evoked by carbachol (1.5 M) produced a 3 ± 0.5 cmH₂O increase in gastric pressure over basal. Intraduodenal infusion of 0.1 N HCl (2 ml/h) failed to inhibit the increase in gastric pressure over basal. Intraduodenal administration of 0.1 N HCl at 1 and 2 ml/h increased plasma secretin to 3–5 pmol, which was within the physiological range (27), and it induced a dose-dependent decrease in intragastric pressure. This action was markedly antagonized by the administration of a secretin antibody (AB R11 7.1), indicating the importance of endogenous secretin in duodenal acid-induced inhibition of gastric motility. In contrast, intraduodenal infusion of 0.1 N HCl at 3 ml/h caused an elevation of plasma secretin to supraphysiological levels (>14 pmol), and gastric relaxation was minimally affected by the administration of a secretin antibody. This suggests that duodenal acid-induced inhibition of gastric motility is mediated by more than one mechanism. Gastric relaxation induced by low doses of acid is mediated mainly by endogenous secretin, whereas inhibition of gastric motility by supraphysiological doses of acid is mediated by mechanisms largely independent of secretin. This may explain the discrepancies between our observations and those reported by Raybould and Holzer (25) where 0.1 or 0.2 N HCl infused at 3 ml/h produced nonphysiological conditions.

In addition to secretin, acid in the duodenum may release CCK (8), which, in turn, may induce gastric relaxation. In our study, the contribution of CCK to gastric relaxation is small; the blockade of the CCK-A receptor reduced only ~10% of the inhibition induced by low doses of acid. When HCl was infused at a higher rate (3 ml/h), we observed a reduction in gastric relaxation of ~20%. This finding is in agreement with that of Raybould and Holzer (25). In contrast, Forster et al. (10) and Green and colleagues (12) observed that the CCK receptor antagonist had no effect on the inhibition of gastric emptying induced by acid in the stomach. These differences may be attributed to the experimental design and the dosage of CCK antagonists administered.

It is interesting to note that, when HCl was infused at 3 ml/h, administration of the CCK antagonist and the secretin antibody only partially reduced acid-induced gastric relaxation, suggesting the involvement of other mediators. It is conceivable that acid in the duodenum may directly, or indirectly through the release of other hormones such as 5-HT, stimulate vagal or splanchnic afferent pathways to induce gastric relax-
Gastric relaxation evoked by duodenal acid

secretin-independent pathways. Also, because va-
Raybould and Holzer (25), indicating participation of
affected by immunoneutralization of endogenous secre-
by high doses of HCl (3 ml/h) was only minimally
scious rats may also be mediated by endogenous secre-
relaxation evoked by duodenal acidification in con-
Similar findings were observed with inhibition of gas-
tors are not involved in duodenal acid-induced inhibi-
tion of gastric motility.
Our studies clearly demonstrated that duodenal acidi-
this appears to be mediated mainly by endog-
ous secretin, because immunoneutralization with a
secretin antibody markedly reduced gastric inhibition
induced by duodenal acid. This is further supported by
the observation that vagotomy or capsaicin abolished
or reduced the action of duodenal acid and that of
secretin (21, 26). Similar to secretin, infusion of 0.1 N
HCl at 1 and 2 ml/h also failed to inhibit gastric con-
traction evoked by electrical field stimulation or
carbocbol. This suggests that duodenal acid does not
have a direct inhibitory effect on the vagal release of
ACh, nor does it directly affect contraction of gastric
smooth muscle cells. Taken together, these observa-
tions support the proposal that low doses of acid in
the duodenum release secretin, which, in turn, acts through
a vagal afferent pathway.
All of our studies were performed in anesthetized
rats; therefore, it is not known if our findings are
applicable to conscious rats. However, recently, Li and
colleagues (20) reported that secretin significantly inhib-
ited pentagastrin-stimulated acid secretion, which was
abolished by both vagotomy and peripheral capsaicin
treatment in both anesthetized and conscious rats.
Similar findings were observed with inhibition of gas-
tric acid secretion by duodenal acidification, which
could be prevented by the administration of a rabbit
antiserum to secretin (20). Hence, it is likely that gastric
relaxation evoked by duodenal acidification in con-
scious rats may also be mediated by endogenous secre-
atin, which acts via a vagal afferent pathway.
It is interesting to note that gastric relaxation evoked
by high doses of HCl (3 ml/h) was only minimally
affected by immunoneutralization of endogenous secre-
This observation agrees with that reported by
Raybould and Holzer (25), indicating participation of
secretin-independent pathways. Also, because va-
gotomy inhibited ~60% of the gastric relaxation in-
duced by high doses of acid in the duodenum, the
participation of secretin- and vagal-independent path-
ways is suggested. It is possible that HCl induces the
release of paracrine or endocrine substances, which act
either directly or indirectly through neuropsychways, or
that HCl directly activates splanchnic or enterogastro
neuropsychways. Therefore, depending on the acid load
in the duodenum, the induction of gastric relaxation by
duodenal acidification may be mediated by the release of
secretin, CCK, or other paracrine mediators, by the
direct action of acid on the vagal afferent pathway, or by
other yet unidentified neuropsychways. However, under
physiological conditions, acid in the duodenum causes
gastric relaxation mainly through the release of endog-
ous secretin, which acts through vagal afferent path-
ways.

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