Secretin inhibits gastric acid secretion via a vagal afferent pathway in rats

P. Li, T. M. Chang, and W. Y. Chey

Konar Center for Digestive and Liver Diseases, University of Rochester Medical Center, Rochester, New York 14626

SECRETIN IS AN ENTEROGASTRONE THAT INHIBITS BOTH GASTRIC MOTILITY AND ACID SECRETION IN SEVERAL SPECIES, INCLUDING RATS (5, 13, 23), CATS (26), DOGS (4, 12, 26, 29), AND HUMANS (30). A NUMBER OF STUDIES HAVE DEMONSTRATED THAT ENDOGENOUS SECRETIN RELEASED BY DUODENAL ACIDIFICATION SUPPRESSED PENTAGASTRIN-STIMULATED ACID SECRETION IN RESPONSE TO A MEAL (4, 12, 27). IT HAS BEEN SHOWN THAT THE INHIBITION BY DUODENAL ACIDIFICATION ON GASTRIC ACID SECRETION IS MEDIANED BY NEURAL AND HORMONAL MECHANISMS (2, 7, 19). HOWEVER, FEW STUDIES HAVE BEEN CARRIED OUT TO INVESTIGATE THE INTERACTION BETWEEN THE NEURAL PATHWAY AND THE ACTION OF SECRETIN IN THE REGULATION OF GASTRIC ACID SECRETION.

A previous study by Esplugues et al. (8) indicated that afferent sensory neurons located both in the gastric mucosa and celiac ganglion inhibited acid secretion in response to gastric distension. Saperas et al. (24) reported that enterogastric inhibition of gastric acid secretion with duodenal acidification was mediated by extrinsic reflexes involving both the vagal and splanchnic afferent sensory pathways. However, these investigators did not further test whether or not the sensory neural pathway also mediates suppression of acid secretion by endogenous secretin. Recently, Lu and Owyang (18) have observed that secretin at a physiologically dose inhibited gastric motility, which was abolished by pretreatment with a sensory afferent neurotoxin, capsaicin, suggesting that secretin acted on the vagal afferent pathway to suppress gastric emptying. However, the specific pathway involving the inhibition by secretin of gastric acid secretion remains to be fully characterized. A recent study by Kwon et al. (14) showed that vagal nerves modulated secretin binding sites in the rat forestomach. Li et al. (16) reported that calcitonin gene-releasing peptide, a neuropeptide released from afferent sensory neuron endings, inhibited gastric acid secretion in the isolated perfused rat stomach. We have also found that pancreatic exocrine secretion in response to secretin is mediated by a vagal capsaisin-sensitive afferent pathway in rats (15). From this information, we hypothesize that the afferent sensory pathway is involved in the inhibitory action of secretin on gastric acid secretion.

METHODS

Animals

Male Sprague-Dawley rats weighing 260–310 g were used in the experiments with conscious rats prepared with gastric fistulas, and rats weighing 230–280 g were used in the experiments with anesthetized rats with pylorus ligation. Animals were housed under controlled temperature (23°C) and were given free access to rat chow and tap water. Each conscious rat was conditioned in a Bollman cage for 2 h before surgery.
Surgical Preparation

Conscious rats with chronic gastric fistula. The surgical procedure performed in the present study has been described previously (25). In brief, rats were fasted for at least 18 h with free access to water. The animals were anesthetized with intraperitoneal administration of pentobarbital sodium at 30 mg/kg. Through a midline abdominal incision, a stainless-steel cannula (6 mm ID) was inserted into the forestomach via a small incision in the anterior wall near the greater curvature and fixed using a purse-string suture. The gastric cannula was then exteriorized through a stab wound just to the left of the midline fitting with a steel screw cap. Both jugular veins were cannulated with PE-50 polyethylene tubes (0.58 mm ID) that were filled with 100 µl of heparin and fixed behind the ears. Five days were allowed for recovery from the surgery.

Anesthetized rats with ligation of the pylorus. After rats were deprived of food for 24–36 h except for free access to water, they were anesthetized by intraperitoneal and subcutaneous injection of 25% urethan at 0.35 ml/100 g body wt, respectively. The abdomen was opened through a midline incision. A polyvinyl tube (1.4 mm ID) was placed in the proximal stomach 0.5 cm aborad from the esophagogastric junction for infusion of water, and another polyvinyl tube was inserted into the forestomach via a small incision at the greater curvature for drainage of gastric luminal fluid. The pylorus was ligated. For duodenal infusion of acid, a duodenal cannula (1.4 mm ID) was inserted into the proximal duodenum 0.5 cm distal to the pylorus.

Bilateral subdiaphragmatic vagotomy. To investigate the vagal pathway, the stomach in some conscious rats was gently pulled down below the diaphragm to expose the esophagus. The subdiaphragmatic vagal trunks were separated, and both anterior and posterior trunks were transected. In the rats with sham operation, the abdominal vagal trunks were exposed but remained intact (control). Experiments were performed 5 days after surgery.

Capsaicin applications on perivagal region and perceliac ganglia. To study the role of sensory afferent innervation on the secretin-induced inhibition of gastric acid secretion, capsaicin was applied to perivagal trunks and perceliac ganglia 12–14 days before experiments. Briefly, for perivagal capsaicin application, the subdiaphragmatic vagi were exposed and freed in lengths of 0.5 cm from the esophagus. A piece of Parafilm was placed around the nerves to protect the other organs from capsaicin. Both anterior and posterior vagal trunks were surrounded by a 1 × 0.5-cm² wiper soaked in capsaicin solution (10 mg/ml) or vehicle (Tween 80-ethanol-saline, 1:1:8, vol/vol/vol) for 30 min. During that period, 0.1 ml of capsaicin solution was dropped on both nerves every 10 min. The area was then thoroughly rinsed with isotonic saline before the abdomen was closed. For perceliac ganglionic application of capsaicin, the stomach and the spleen were deflected to the right side, and then the celiac-superior mesenteric ganglion complex was exposed through a small incision in the surrounding connective tissue. The capsaicin solution or vehicle was applied to the ganglion complex for 30 min as was done for perivagal capsaicin treatment, and then the area was rinsed with isotonic saline, which was removed with gauze before closing the abdomen.

Collection and Measurement of Gastric Acid Secretion

In conscious rats placed in Bollman cages, the gastric fistula was kept open, and the stomach was gently flushed with warm water until gastric juice became clear. Thirty minutes after flushing, the gastric juice was collected via the fistula at 10-min intervals. In anesthetized rats with pylorus ligation, gastric secretion was collected by a flushing technique. Both gastric cannulas were opened, and the stomach was slowly washed with warm water (~15 ml) followed by a bolus injection of 2 ml of air to completely empty the stomach; next, the stomach was filled with 2 ml of deionized water (pH 7.4), and the two gastric cannulas were temporarily blocked. Gastric secretion was collected by reopening the cannula and injecting 2 ml of air to completely remove fluid out of the stomach at 20-min intervals. The acidity of each sample in both conscious and anesthetized rats was determined by titrating 0.01 N NaOH to a pH of 7.4 using an autotitrator (Fisher Scientific, Pittsburgh, PA).

Experimental Design

Basal gastric acid secretion was determined during 50 min in conscious rats and 60 min in anesthetized rats after experiments began.

To study the influence of the vagal pathway in the inhibitory effect of secretin on acid secretion, pentagastrin at 0.6 µg·kg⁻¹·h⁻¹ was infused intravenously for 100 min. Secretin at 20 pmol·kg⁻¹·h⁻¹ was infused intravenously for 50 min starting 50 min after the initiation of pentagastrin infusion in conscious rats with vagotomy or sham operation.

To investigate the role of the capsaicin-sensitive afferent pathway in regulation of the secretin-induced inhibitory action on acid secretion, similar treatment with pentagastrin and secretin was performed in the conscious rats with perivagal and perceliac ganglion capsaicin treatment. To confirm a complete vagotomy and to rule out possible damage of the vagal efferent pathway by capsaicin, 2-deoxy-D-glucose (2-DG, 120 mg/kg iv) was injected in bolus in both conscious and anesthetized rats with vagotomy or perivagal treatment of capsaicin. To test the inhibitory effect of endogenous secretin on acid secretion in anesthetized rats, 0.03 N HCl was infused intraduodenally at 4.32 ml/h for 60 min starting 1 h after pentagastrin infusion began. In some of these rats, 0.1 ml of a rabbit secretin antiserum (titer = 1:1,000,000) was injected intravenously in a bolus 60 min before HCl was administered.

Statistical Analysis

Gastric acid secretion was expressed as acid output during the 10- to 20-min collection periods. Percentage increase over basal values was calculated by comparing acid output in the last 30 or 40 min of each treatment period with those in the same length of the basal acid secretion period in conscious or anesthetized rats, respectively. All data were designated as means ± SE. Statistical differences were analyzed by a one-way analysis of variance. Tukey's test was used for multiple comparisons. P values <0.05 were considered statistically significant.

RESULTS

Basal Acid Secretion and Stimulated Acid Secretion

Basal acid secretion in conscious rats (4.6 ± 0.5 µeq/10 min) was two times as high as that in anesthetized rats (4.2 ± 0.3 µeq/20 min; Fig. 1). Similarly, both 2-DG- and pentagastrin-stimulated acid outputs in conscious rats were doubled compared with those in anesthetized rats, but the magnitudes of increase after stimulation were similar between them (see Figs. 1, 2, and 6). In the conscious rats, both basal and pentagastrin-stimulated acid secretion were significantly higher in the sham-operated rats compared with those in the...
rats with vagotomy (Fig. 2). In both conscious and anesthetized rats, neither perivagal nor periceliac ganglionic capsaicin treatment exerted any significant influence in the levels of basal acid output when compared with those presented in the vehicle-treated rats (see Figs. 3 and 6). There was no significant difference in 2-DG-stimulated acid secretion between perivagal capsaicin- and vehicle-treated rats (Fig. 1), suggesting that capsaicin treatment did not damage the vagal efferent pathway.

Effect of Vagotomy on Inhibition by Secretin of Pentagastrin-Stimulated Acid Secretion

In conscious rats, 2-DG (120 mg/kg iv) significantly increased acid output in sham-operated rats but did not influence acid secretion in vagotomized rats (Fig. 1), confirming a complete vagotomy in these rats. Intravenous infusion of pentagastrin at 0.6 µg·kg⁻¹·h⁻¹ produced a marked increase in acid output by 123.0 ± 23.6 and 144.8 ± 24.2% in sham-operated and vagotomized rats.

Fig. 1. Effect of 2-deoxy-D-glucose (2-DG) on gastric acid secretion in anesthetized (A) and conscious (B) rats with perivagal treatment of capsaicin or vagotomy. ○, 2-DG (120 mg/kg iv) alone; ●, perivagal application of capsaicin (10 mg/ml); ▲, vagotomy. Capsaicin treatment did not influence basal and 2-DG-stimulated acid secretion. Vagotomy completely blocked 2-DG-stimulated acid secretion. Each point represents mean ± SE of 6 rats in each group.

Fig. 2. Effect of vagotomy on pentagastrin-stimulated acid secretion in response to secretin in conscious rats. A: acid output in response to pentagastrin and secretin. ○, Sham operation (SM); ●, vagotomy (VT). B: percentage over basal acid secretion after administration of secretin and/or pentagastrin. Open bars, pentagastrin (PG, 0.6 µg·kg⁻¹·h⁻¹); hatched bars, pentagastrin + secretin (Sec, 20 pmol·kg⁻¹·h⁻¹). Vagotomy reversed inhibition by secretin of pentagastrin-stimulated acid secretion. Each value expresses mean ± SE; n = 6 in each group. *P < 0.05 vs. vagotomized rats. **P < 0.01 vs. pentagastrin treatment.
rats, respectively (Fig. 2). Secretin (20 pmol·kg⁻¹·h⁻¹ iv) significantly inhibited pentagastrin-stimulated acid secretion by 63.1% in sham-operated rats but not in rats with vagotomy (Fig. 2), suggesting that secretin inhibits pentagastrin-stimulated acid secretion via a vagal pathway.

Effect of Perivagal or Periceliac Ganglionic Application of Capsaicin on the Inhibition by Secretin of Pentagastrin-Stimulated Acid Secretion

Exogenous secretin at 20 pmol·kg⁻¹·h⁻¹ significantly suppressed the pentagastrin-stimulated acid output by 62.8% in the conscious rats with perivagal vehicle treatment; however, the inhibition was completely abolished in the rats with perivagal capsaicin application (Fig. 3). In contrast, inhibition by the same dose of secretin on pentagastrin-stimulated acid secretion was not influenced by periceliac ganglionic application of capsaicin (Fig. 4). It indicated that the capsaicin-sensitive vagal afferent pathway, but not capsaicin-sensitive splanchnic afferent nerves, mediates the inhibitory action of secretin on the pentagastrin-stimulated acid secretion.

In anesthetized rats with ligation of the pylorus, intraduodenal infusion of 0.03 N HCl significantly attenuated pentagastrin-stimulated acid secretion, which was reversed by a rabbit antisecretin serum (Fig. 5). This confirmed that endogenous secretin mediates inhibition of acid secretion by duodenal acidification. Figure 6 shows that pentagastrin-stimulated acid secretion was suppressed by 59.4% (10.8 ± 2.1 vs. 16.0 ± 2.8 μeq/40 min) during intraduodenal administration of acid, but the suppression was abolished by perivagal capsaicin treatment. Similar to the observation in conscious rats, periceliac ganglionic application of capsaicin did not influence the inhibition by duodenal acidification in anesthetized rats (Fig. 7).

**DISCUSSION**

Although studies have revealed that secretin suppresses gastric motility via a capsaicin-sensitive vagal afferent sensory pathway (18, 20), neural action on
secretin-inhibited gastric acid secretion has not been clarified. In the present study, we clearly showed that the inhibitory effect of exogenous and endogenous secretin on pentagastrin-stimulated gastric acid secretion is mediated by a vagal but not a splanchnic capsaicin-sensitive afferent sensory pathway in both awake and anesthetized rats. Thus we demonstrated for the first time that the inhibition by secretin of acid secretion is mediated by a vagal pathway.

To investigate the inhibitory action of endogenous secretin released by duodenal acidification on acid secretion, we designed experiments using anesthetized rats with ligation of the pylorus. Although ligation of the pylorus was reported to enhance gastric acid secretion due to gastric distension, which was regulated by vagovagal reflexes (1, 10), and the absence of the
duodenal inhibitory mechanism, the acid secretion in our model was stable and comparable with that in anesthetized rats with the gastric cannula (data not shown). Moreover, the pattern of acid secretion was similar to that in conscious rats without pylorus ligation after administration of pentagastrin or treatment of capsaicin.

In this study, we found that the acid secretion stimulated by pentagastrin was significantly suppressed after intravenous administration of secretin in sham-operated rats, and this inhibition was completely abolished in vagotomized rats, demonstrating an important role of the vagal pathway on the secretin-induced inhibitory mechanism on the acid secretion. It is possible that the vagal pathway may modulate the secretin receptor binding site in the stomach (14). In addition, vagal tone may play a permissive role on the regulation of the stimulation by secretin of D cells for local release of somatostatin (3, 6). In the present study, we were unable to test whether the vagal pathway influences its inhibitory action on gastrin release from G cells because the acid secretion was stimulated by pentagastrin.

To evaluate the importance of the vagal afferent sensory pathway in the modulation of secretin-inhibited acid secretion, perivagal application of an afferent neurotoxin, capsaicin, was performed to functionally ablate the vagal afferent innervation. We found that ablation of primary afferent neurons did not modify basal and pentagastrin-stimulated acid secretion, which is in agreement with the observations by Esplugues et al. (8) and Raybould and Taché (22). However, the inhibition by intravenous secretin or duodenal acidification on acid secretion was completely reversed by topical treatment of capsaicin in both awake and anesthetized rats. The observation indicates that the inhibition of acid secretion by secretin is entirely dependent on the vagal afferent innervation. These results confirmed and extended the observation of Saperas et al. (24) that duodenal exposure of acid suppressed gastric acid secretion, which was blocked by systemic capsaicin. We believe that afferent sensory nerves also played an important role in regulation of inhibition by secretin of acid secretion in their study, since secretin is the major gut hormone released after duodenal acidification. We provided further evidence that the inhibitory effect of endogenous secretin was blocked by injection of antisecretin serum. In addition, the inhibitory effect of duodenal acidification on acid secretion is, at least in part, due to suppression of endogenous secretin release in these capsaiacinized rats, since capsaicin-sensitive afferent nerves are responsible for the release of secretin and secretin-releasing peptide by 70% after duodenal acidification (15). These observations suggest that secretin may directly signal the afferent sensory nerve to influence secretory function of the stomach. Recently, Wang et al. (28) reported that perineural application of capsaicin on the cervical vagi markedly reduced the binding of 125I-secretin on vagal nerves in rats. This suggests that the secretin receptors are present on the vagal afferent fibers. These findings (28) are in agreement with our in vivo observations that the inhibition by secretin of acid secretions could be suppressed by vagal afferent denervation in rats. It is likely that the secretin receptors in the vagal afferent fibers may serve as target sites of secretin action on both gastric secretion and motility. Further studies are necessary to locate the secretin receptor on the vagal nerve system. We have previously reported that the secretin-induced inhibition of acid secretion is mediated by local release of somatostatin and prostaglandin E2 in the isolated rat stomach and the rat antral mucosa (6, 16). It is not known whether or not capsaicin-sensitive sensory nerve fibers are involved in the local release of somatostatin and prostaglandin E2 in rat gastric mucosa.

In the present study, ablation of the splanchnic afferent sensory nerve by topical capsaicin treatment of the celiac-superior mesenteric ganglia failed to influence the suppression of acid secretion by either exogenous or endogenous secretin in both awake and anesthetized rats. It suggests that the spinal afferent sensory pathway plays a different role in regulation of gastric function compared with vagal afferent innervation. Thus the stomach is innervated by a dual extrinsic afferent neural system from the nodose and spinal sensory ganglia, which have their own receptors to monitor different signals and modulate various functions (9). For example, selective blockade of the spinal afferent fibers supplying the stomach significantly attenuated the increase in gastric mucosa blood flow in response to back diffusion of acid (21). It also suppressed the gastric motility induced by duodenal distension (11). In contrast, ablation of the spinal afferent pathway did not alter the gastric acid inhibitory response to administration of intestinal lipid (17) and acid (24). It appears that only the vagal afferent sensory nerve fibers have specific receptors for secretin, which receives a signal from the hormone and regulates gastric acid secretion.

In conclusion, we have demonstrated that secretin-inhibited gastric acid secretion in response to pentagastrin is mediated by a capsaicin-sensitive vagal afferent sensory pathway. These findings imply that vagal afferent sensory nerves can receive and deliver hormonal signals to modulate gastric function in rats.

We thank Norene Buhner for technical assistance and Pat Faiello for manuscript preparation.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-25962.

Address for reprint requests: W. Y. Chey, GI Unit, PO Box 646, Univ. of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14626.

Received 19 September 1997; accepted in final form 31 March 1998.

REFERENCES

