Mechanism of Acid-induced Release of Secretin in Rats
Presence of a Secretin-releasing Peptide

P. Li, K. Y. Lee, T.-M. Chang, and W. Y. Chey
The Isaac Gordon Center for Digestive Diseases and Nutrition, The Genesee Hospital, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14607

Abstract
In fasting rats, intraduodenal infusion of dilute hydrochloric acid results in significant increases in both pancreatic exocrine secretion and plasma concentration of secretin. To test the hypothesis that acid-induced release of secretin is mediated by a secretin-releasing factor (S-RF), anesthetized rats were prepared with pyloric ligation, duodenal and jejunal cannulas, and pancreatic duct cannulas. Donor rats were infused intraduodenally with 0.01 N HCl, 0.15 M NaCl, or a combination of 0.01 N HCl and 0.05 N NaHCO3 at 0.3 ml/min for 1.5 h, and the perfusates were collected via jejunal cannulas. The perfusates with pH adjusted to 6.0 were concentrated threefold and infused into the duodena of recipient rats. The concentrate of acid perfusate (CAP) significantly increased both pancreatic volume flow and bicarbonate output and plasma concentration of secretin, whereas concentrates of the saline perfusate (CSP) or the perfusate of a combination of 0.01 N HCl and 0.05 N NaHCO3 (CABP) did not influence pancreatic secretion or plasma concentration of secretin. The increased pancreatic secretion by CAP was attributed to increased circulating secretin because when secretin was immunoneutralized by a rabbit antiserum, CAP-stimulated pancreatic secretion was abolished. The bioactivity of CAP was trypsin-sensitive and heat stable. The active substance in CAP had a molecular weight of < 5,000 and > 1,000, as determined by ultrafiltration and bioassay. In conclusion, dilute HCl releases an S-RF into the upper small intestinal lumen to stimulate release of secretin. This substance, with molecular weight of < 5,000, is heat stable and trypsin sensitive. Thus, the acid-stimulated release of secretin is mediated by a secretin-releasing peptide in the upper small intestinal lumen. (J. Clin. Invest. 1990. 86:1474-1479.) Key words: release of secretin • duodenal acidification • secretin-releasing peptide

Introduction
As early as 1950, Annis and Hollenbeck found in dogs that during postprandial state diversion of pancreatic juice from the upper small intestine produced a greater amount of pancreatic juice than when pancreatic juice was allowed to enter the duodenum (1). They suggested that the increased pancreatic secretion might have resulted from increased release of secretin (1). In fasting rats, Green and Lyman (2) observed that diversion of pancreatic juice from the upper small intestine resulted in increased pancreatic exocrine secretion and infusion of either pancreatic juice or trypsin into the duodenum reversed the increase. They suggested that the increase in pancreatic secretion was attributed to increased release of cholecystokinin (CCK) (2). In recent years, this original observation in rats was confirmed by several groups of investigators (3-5).

It has been found that the increased pancreatic secretion was due to increased release of not only CCK (3-5) but also secretin (6). However, when the increase in pancreatic secretion was reversed by either pancreatic juice or trypsin in the duodenum, the increases in plasma concentration of CCK (3-5) and secretin (6) were also abolished in rats. Recently, in rats, it was learned that the increases in both pancreatic exocrine secretion and plasma concentrations of secretin and CCK in response to intraduodenal administration of sodium oleate was suppressed significantly by either pancreatic juice or a combination of both trypsin and chymotrypsin (7). However, interestingly, in similar experiments in dogs (8), pancreatic juice or trypsin caused significant decreases in both pancreatic secretion and plasma concentration of secretin but no decrease of CCK.

These observations in rats and dogs suggest that there is a trypsin-sensitive releasing factor in the upper small intestine that stimulates the release of secretin. A releasing factor for secretin was searched for in the upper small intestinal perfusate while duodena were infused with a dilute hydrochloric acid in anesthetized rats. Hydrochloric acid is a well-recognized stimulant for the release of secretin in many species (9, 10). In the present investigation, a secretin-releasing peptide was found in the upper small intestinal perfusate in rats.

Materials and Methods
Animal preparation
Male Sprague-Dawley rats weighing between 230 and 300 g were fasted for 24 h with free access to drinking water before surgery. Under anesthesia with 0.35 ml of 25% urethane per 100 g of body weight given intraperitoneally, followed by subcutaneous injection of urethane in the same dose, a midline abdominal incision was made. A polyethylene tube (ID 3.0 mm, OD 4.0 mm) was inserted into the proximal duodenum 5 mm distal to the pylorus via the stomach followed by ligation of the pylorus for intraduodenal infusion of 0.01 N HCl, 0.15 M NaCl or intestinal perfusates (Fig. 1). A polyethylene tube (PE-10, ID 0.28 mm, OD 0.61 mm) was inserted into the pancreatic duct at the junction between the bile-pancreatic duct and the duodenal wall for collection of pancreatic juice. Another PE-10 tube was inserted into the bile duct proximal to the pancreatic duct for diversion of bile to the exterior. An additional cannula was placed into the jejunum 15 cm distal to the ligament of Treitz for collection of perfusates (Fig. 1).
or CAP was boiled for 15 min after surgery each rat received intraduodenal infusion of 0.15 M NaCl at a rate of 0.3 ml·min⁻¹ for 1.5 h to wash the cannulated intestinal segment. Then 0.01 M HCl or 0.15 M NaCl was administered at a rate of 0.3 ml·min⁻¹ for another 1.5 h. Pancreatic juice was collected continuously in 30 min samples. In addition, identical experiments were performed in another group of rats to determine the effect of a combination of 0.01 N HCl and 0.05 N NaHCO₃ on pancreatic secretion and plasma hormone levels. The infusion rate of the testing solution was 0.3 ml/min which consisted of 0.25 ml of 0.01 N HCl and 0.05 ml of 0.05 N NaHCO₃. The pH of this solution was 6.0. The perfusates collected via the jejunal cannulas were kept in ice-chilling beakers, centrifuged at 3,000 g at 4°C for 25 min, and their supernates were lyophiliized. The dried material was reconstituted in H₂O to make a threefold concentrate with pH adjusted to 6.0 with 1 N NaOH before it was reinfused into the duodenum of recipient rats as described below. At the end of the infusion with either one of these solutions for 1.5 h, blood was drawn from the abdominal aorta. Plasma was separated by centrifugation at 1,000 g and 4°C for 15 min, mixed with a cocktail of various protease inhibitors to final concentrations of 100 μg/ml of soybean trypsin inhibitor, 1.5 μg/ml of bovine pancreatic trypsin inhibitor, and 9.9 × 10⁻⁸ M of D-Phe-L-Phe-L-Arg CH₂Cl₂ and stored at −20°C before radioimmunoassay for secretin and CCK (11-13).

Bioassay of concentrated perfusates: To determine the effect of the concentrated perfusate on pancreatic exocrine secretion and the release of secretin and CCK, three groups of seven rats each were prepared with upper small intestinal cannulas as described above (Fig. 1). After initial 1.5 h of perfusion with 0.15 M NaCl, the threefold concentrate of acid perfusate (CAP), saline perfusate (CS), or a combined acid and sodium bicarbonate perfusate (CABP) was infused into the duodenum at 0.3 ml·min⁻¹ for 1.5 h (Fig. 1, groups 1-3). To study the effect of a rabbit antiserum to secretin on the action of CAP, another two groups of five recipient rats each received intravenous injection of either 0.1 ml of a rabbit antiserum to secretin or a normal rabbit serum 15 min before CAP was administered (Fig. 1, groups 4 and 5). To determine the properties of CAP, it was incubated with bovine crystalline trypsin (Worthington Biochemical Co., Freehold, NJ), 100 μg/ml, at 37°C for 1 h and then boiled for 15 min to destroy the enzyme activity, or CAP was boiled for 15 min without incubation with trypsin. Thus, in another two groups of seven rats each, either trypsin-treated CAP or boiled CAP was infused into the duodenum at a rate of 0.3 ml·min⁻¹ as described above (Fig. 1, groups 6 and 7).

Estimation of molecular weight of secretin-releasing factor: To estimate the molecular weight of the active substance in the perfusate, the acid perfusate collected from the upper small intestine was adjusted to pH 6.0, boiled for 15 min and ultrafiltrated through various Amicon membranes (W. R. Grace and Co., Danvers, MA). The materials were first filtered through a PM-10 membrane (molecular weight cut off = 10,000). The material retained by the membrane (mol wt > 10,000) was invariably inactive in stimulating pancreatic secretion in several experiments (data not shown). The active material filtered through the membrane was further fractionated by filtering through a YM-2 membrane (molecular weight cut off = 1,000). The filtrate of YM-2 membrane was further filtered through a YM-5 membrane (molecular weight cut off = 500). The filtrate of YM-5 membrane was concentrated threefold and infused into the upper duodenum at 0.3 ml·min⁻¹ for 1.5 h in three groups of five rats each (Fig. 1, groups 8-10).

Measurement of pancreatic exocrine secretion and release of secretin: Pancreatic juice was collected continuously by a glass micropipette (Drummond Scientific Co., San Francisco, CA) in 30-min samples. The volume of pancreatic flow was measured by calculating the length of pancreatic juice in the micropipette with a capacity of 3.85 ml·cm⁻¹ tube length. The minimal detectable volume change was 0.5 mm, i.e., 0.2 μl. 10 μl of the pancreatic juice was immediately blown into an ice-chilled covered test tube and diluted twice with H₂O for determination of bicarbonate concentration using a chloride/carbon dioxide analyzer (Beckman Instruments, Inc., Fullerton, CA). The minimally detectable amount of bicarbonate was 0.05 μEq. The result was expressed as microequivalents per 30 min. At the end of each experiment blood samples were drawn immediately from the aorta and collected into ice-chilling heparinized glass tubes. Plasma was obtained as described above.

Determination of duodenal pH and trypsin activity: Five conscious rats each weighing ~ 300 g were prepared with a stainless cannula (without pancreatic duct cannulation device) placed in either proximal duodenum (1.0 cm distal to the pylorus) or distal duodenum (5.0 cm distal to the pylorus) as described previously (14). After 24 h fast, the animals were placed in Bollman cage. Duodenal contents were collected continuously through the cannulas in both fasting and postprandial state. To collect duodenal contents during postprandial period, rats were fed with Purina rat chow in an average amount of 1.1 g in 5 min. Duodenal contents were collected continuously for two consecutive 30 min. pH of the liquid portion of the duodenal contents was determined using a pH meter 145 (Corning Medical and Scientific, Meadfield, MA). Trypsin activity of the duodenal contents was determined by the method of Hummel (15).

Effect of exogenous secretin in the duodenal lumen on pancreatic secretion and plasma secretin: To determine if secretin in the duodenal lumen can affect pancreatic exocrine secretion and/or plasma secretin level in 24 h fasted anesthetized rats, porcine secretin in three different concentrations including 2, 5, and 10 nM dissolved in 0.2% BSA saline solution was administered intraduodenally via a polyethylene tube (PE-90). Pancreatic juice was collected for 1.5 h as described above. Blood was collected by aortic puncture at the end of secretin administration. The doses of secretin used in this study were similar to the secretin content found in CAP (2.6 nM).

Statistical analysis All results were expressed graphically as means±SE. The changes in pancreatic volume flow and bicarbonate output were expressed as percentage increase over basal values during the last 30-min period. Student’s t test was used to analyze data. Statistical significance was set at P < 0.05.
Results

Pancreatic secretion and plasma concentrations of secretin and CCK in response to intestinal perfusion in donor rats: Upper intestinal perfusion of 0.01 M HCl resulted in a significant increase in pancreatic exocrine secretion which included both volume flow and bicarbonate output, and plasma concentration of secretin (Fig. 2). In contrast, neither pancreatic secretion nor plasma concentration of secretin was influenced by the perfusion of 0.15 M NaCl or a combined solution of 0.01 M HCl and 0.05 M NaHCO3. Plasma concentration of CCK was not influenced by either one of the three testing solutions.

Effects of intraduodenal perfusion on pancreatic secretion and the release of secretin and CCK in recipient rats: In each group of rats, pancreatic volume flow and bicarbonate output were stable during 1.5-h period with intraduodenal infusion of 0.15 M NaCl. Subsequent intraduodenal administration of CAP significantly increased pancreatic secretion including both volume flow and bicarbonate output by 99.9±16.1% and 183.2±36.3%, respectively, compared with the basal values (P<0.01) (Fig. 3, A and B). However, intraduodenal infusion of CSP or CABB failed to increase significantly pancreatic exocrine secretion. After the infusion of CAP, plasma secretin concentration was 6.2 pm which was higher significantly than plasma secretin concentration after the infusion of CSP or CABB (P<0.05) (Fig. 4). There was no change in plasma concentration of CCK in response to either one of these three perfusates (data not shown).

Effect of a rabbit antisecretin serum on CAP-stimulated pancreatic secretion and plasma secretin concentration: Intravenous injection of the antisecretin serum completely abolished the CAP-stimulated pancreatic volume flow and bicarbonate output (Fig. 5, A and B) and the increase in plasma secretin (Fig. 6 A), whereas a normal rabbit serum (NRS) did not affect either pancreatic secretion or plasma secretin (Fig. 5, A and B, and 6 A).

Partial characterization of secretin releasing factor (S-RF) in CAP: Intraduodenal perfusion of CAP as incubated with trypsin and followed by boiling resulted in a significant sup-

Figure 2. Mean percent increases (above basal value) of pancreatic secretion (volume flow and bicarbonate output) and plasma concentrations of secretin and CCK in response to intraduodenal administration of 0.15 M NaCl (n = 14), 0.01 M HCl (n = 22) and a combination of 0.01 M HCl and 0.05 M NaHCO3 (n = 15) in 51 anesthetized rats. Each bar represents a mean±SE. P values represent comparison between the values produced by 0.15 M NaCl and those by 0.01 M HCl or combination of HCl and NaHCO3.

Figure 3. Pancreatic secretion including volume flow (A) and bicarbonate (B) in response to intraduodenal infusion of CAP, CSP, and CABB in recipient rats. Each value represents a mean±1 SE of seven rats. Bars represent mean percentage increases during last 30 min of perfusion over basal values, *P<0.05; **P<0.01 (compared with values at 0 time); (c) P<0.01 (compared with percent change of CABB group).

pression of both plasma secretin levels (Fig. 6 B) and pancreatic secretion which included volume flow and bicarbonate output (Fig. 7, A and B) in seven rats. In contrast, CAP boiled for 15 min did not change the stimulatory action of CAP on the release of secretin (Fig. 6 B) or pancreatic exocrine secretion (Fig. 7, A and B).

The molecular weight of S-RF in CAP was estimated by ultrafiltration through various Amicon membranes as described in Methods. As shown in Fig. 8, only the material with mol wt 1,000–5,000 increased significantly pancreatic volume flow and bicarbonate output by 81.0±20.2% and 166.0±54.2% (P<0.05), respectively. Plasma secretin concentration was also increased to 4.8±0.6 pm. The materials with mol wt
with bovine serum; (o) of secretin.

CAP in of secretin concentration 6.

In five conscious rats, pH of the luminal contents of proximal duodenum is 7.1. During postprandial state, the mean pH in the proximal duodenum decreased to 2.4, whereas in the distal duodenum it was 7.1 or greater. The trypsin activity paralleled the pH values: in fasting state with duodenal pH 7.0, trypsin activity was high, whereas it was very low when luminal pH was low in the proximal duodenum during postprandial period. At the distal duodenum, trypsin activity was high as pH was 7.0 or higher.

**Effect of intraduodenal secretin on pancreatic secretion and release of secretin:** As depicted in Table 2, secretin given intraduodenally in concentrations up to 10 nM did not increase plasma concentration of secretin, whereas CAP increased secretin to the level of 6.6 pM. The volume flow and bicarbonate output paralleled the increase in the secretin level.

**Discussion**

Although secretin has been known to exist in the upper small intestinal mucosa and to stimulate pancreatic exocrine secretion since the turn of this century (9), only in the past decade has it been firmly established that secretin is a circulating hormone that increases significantly in the circulation in response to ingestion of a meal (16–18). During the postprandial period, the major factor that stimulates the release of secretin is hydrochloric acid entering the duodenum (16–18) and it was shown...
that the circulating secretin was indeed responsible for pancreatic bicarbonate secretion (11). However, the release mechanism of secretin by hydrochloric acid has not been well understood.

The present study indicates that an S-RF is released into the upper small intestine of the rat when a dilute hydrochloric acid was infused into the duodenum, whereas the perfusate collected during an isotonic saline perfusion failed to show any evidence of the presence of S-RF. CAP stimulated not only the release of secretin but also pancreatic secretion of bicarbonate. The bicarbonate secretion was attributable to increased circulating secretin because immunoneutralization of secretin with the antisecretin serum resulted in a complete suppression of pancreatic secretion. However, CAP did not increase plasma concentration of CCK. The S-RF is heat stable and its bioactivity is completely abolished by trypsin. Thus, it is a peptide, probably secreted from the upper intestinal mucosa. The molecular weight of this peptide appears to be < 5,000 and > 1,000.

The secretin-releasing peptide (S-RP) could not be found in the perfusate when 0.01 N HCl was neutralized with 0.05 N NaHCO₃ before the solution was perfused into the upper small intestine. This observation suggests that acid in the upper

### Table I. pH and Trypsin Activity of Duodenal Contents after Meal in Five Conscious Rats

<table>
<thead>
<tr>
<th></th>
<th>pH*</th>
<th>Trypsin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>U·ml⁻¹</td>
<td>U·ml⁻¹</td>
<td>U·ml⁻¹</td>
</tr>
<tr>
<td>Proximal (n = 5)</td>
<td>2.4 + 0.4</td>
<td>2.4 + 0.3</td>
</tr>
<tr>
<td>Distal (n = 5)</td>
<td>7.1 + 0.3</td>
<td>7.5 + 0.2</td>
</tr>
</tbody>
</table>

*pH and trypsin activity of duodenal contents in fasting state was 7.1±0.5 and 26.2±4.5 U·ml⁻¹, respectively.

### Table II. Effect of Luminal Secretin (ID), Concentrated Acid Perfusate (CAP), or Concentrated Saline Perfusate (CSP) on Pancreatic Secretion and Secretin Concentration in Plasma

<table>
<thead>
<tr>
<th>Volume</th>
<th>HCO₃ output</th>
<th>Plasma secretin</th>
</tr>
</thead>
<tbody>
<tr>
<td>% increase</td>
<td>% increase</td>
<td>pm</td>
</tr>
<tr>
<td>Secretin (ID)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 nM (n = 5)</td>
<td>6.7±6.7</td>
<td>17.8±7.3</td>
</tr>
<tr>
<td>5 nM (n = 5)</td>
<td>26.8±11.6</td>
<td>24.0±8.7</td>
</tr>
<tr>
<td>10 nM (n = 5)</td>
<td>19.4±10.0</td>
<td>38.2±22.1</td>
</tr>
<tr>
<td>CAP (ID)</td>
<td>99.9±16.1</td>
<td>183.2±36.3</td>
</tr>
<tr>
<td>CSP (ID)</td>
<td>14.5±5.1</td>
<td>17.2±10.2</td>
</tr>
</tbody>
</table>

Luminal secretin concentration was 2.5±1.7 nM (n = 5) during 0.01 N HCl infusion.

Small intestine triggers the release of an S-RP which in turn releases secretin. It is possible that during normal postprandial period, because acid delivered from the stomach is the major stimulant for the release of secretin, S-RP is released into the duodenal lumen by acid to stimulate secretin cells for the release of secretin. Indeed, the postprandial pH of the proximal duodenal contents in rats was found to range from 1.5 to 4.0 in the present study. It is quite possible, therefore, that S-RP released by HCl can remain intact in the acid medium of the duodenal lumen without being destroyed by pancreatic proteases. The trypsin activity in the duodenal content was very low when its pH was below 3.0. Another possibility is that S-RP may escape from the pancreatic proteases in the upper gut lumen because they are probably bound to food particles, particularly protein. Thus, S-RP may well remain bioactive in the upper intestinal lumen during the postprandial period to signal secretin cells for the release of secretin. However, the present study does not determine whether or not acid also stimulates the secretin cells directly to release secretin. Such a study will be possible only when a specific antibody to the releasing peptide will become available for immunoneutralization or when its specific receptor antagonist will be available.

Secretin which is both immunoreactive and bioactive has been found in the luminal fluid of the duodenum in rats (in the present study) and dogs (19). However, exogenous secretin infused into the duodenum failed to show any significant effect on either pancreatic secretion or plasma secretin level. There are many gut peptides or hormones that have been found in the luminal fluid of the gastrointestinal tract. These include gastrin (20), cholecystokinin (21), substance P (22), somatostatin (23), and a cholecystokinin releasing peptide (24, 25). When administered intraluminally, in experimental animals, some of these peptides exert certain biological actions. Substance P regulates blood flow in feline jejunum (26), and intrajeunal infusion of gastrin enhances intestinal absorption of carbohydrates (27, 28), and exerts a trophic action on mucosal cells (29). Cholecystokinin was shown to stimulate motility of the small intestine in the rabbit (30).

It is apparent that at least two peptides, including secretin and CCK-releasing peptide in the upper small intestinal fluid, release two classic intestinal hormones (secretin and cholecystokinin) into the circulation. A cholecystokinin releasing peptide has been found recently in the upper intestinal washing in rats by Lu et al. (24) and Miyasaka et al. (25). Like S-RP the
pancreatic secretion. pancreatic terology. 98:1642-1648.

Effect of pancreatic juice diverted into the upper small intestinal lumen. Because it was observed previously that diversion of pancreatic juice from the upper small intestine during interdigestive state resulted in significant increases in both plasma secretin concentration and pancreatic exocrine secretion (6), spontaneous release of S-RP is likely. A study is in progress to clarify this possibility. Nevertheless, secretin appears to be another intestinal hormone that has a significant importance on enteropancreatic feedback regulation of exocrine pancreas, specifically secretion of bicarbonate. Moreover, one of the factors responsible for the release of secretin by duodenal acidification may be an S-RP.

Acknowledgments

The authors wish to thank Mrs. Noreen Naud for preparation of this manuscript.

This study was supported by the National Institutes of Health grant No. 25692.

References


Secretin-releasing Factor 1479