Secretin, a Stimulus for Duodenal and Pancreatic "Gastrin" Release: Possible Pathogenetic Significance in Zollinger–Ellison (ZE) Syndrome


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Ex vivo hemoperfused pancreaticoduodenal preparations from dogs have been used to study intraluminal and circulatory patterns of release of immunoreactive gastrin under basal conditions and after secretin stimulation. Bidirectional release of immunoreactive gastrin was maximal at 3 U/min secretin infusion, and release into pancreatic and duodenal juice exceeded that into portal venous blood. Molecular sieving chromatography of peptides with gastrin-like immunoreactivity recovered from duodenal and pancreatic juice indicated a single species of a molecular size equivalent to CCK* and smaller than minigastrin (G-14). The exact identity has not been defined. This study demonstrates that secretin stimulates release of gastrin-like peptides into blood and lumen of extra-antral gastrin-producing tissues in the dog. Unidirectional gastrin release patterns from gastrinoma tissue may explain the paradoxical increase in plasma gastrin levels in response to secretin in patients with gastrinomas (Zollinger–Ellison syndrome).

INTRODUCTION

Seventy-five years have elapsed since Edkins first reported that extracts of antral mucosa stimulated gastric acid secretion [6]. Gregory and Tracy are credited with the isolation and physicochemical characterization of gastrin in pure form [9]. For many years gastrin was viewed as a hormone originating primarily from the gastric antrum, but precise localization of the site(s) of release has not been demonstrated.

Recent observations in man and dog suggest that gastrin may be elaborated by extrantral tissues [22, 33]. The greatest proportion of mucosal gastrin in both species is localized to the gastric antrum, with diminishing tissue concentrations in the small intestine from duodenum to ileum [33]. Although little is known about the ontogeny of gastrin-producing cells, they have been identified prenatally in man and rat [17, 18, 21]. Gastrin release into both the gut lumen and the portal circulation has been documented, but to date there is equivocal evidence of "true" gastrin-producing cells in adult pancreas or of gastrin release into pancreatic juice.

Gastrin release is mediated through complex neuroendocrine and paracrine servomechanisms. Several hormones, including secretin and somatostatin, are effective in suppressing gastrin release [7, 10, 12]. Acidification of both the antrum and the duodenum has been shown to suppress the release of gastrin from the gastric antrum, presumably via different mechanisms [2, 8, 11, 20]. Secretin has been implicated as the major mediator of the latter servomechanism, and appears to act both by suppressing the release of antral gastrin.

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and by blocking the action of gastrin on oxyntic or parietal cells [12, 16]. The administration of exogenous secretin normally decreases fasting levels of immunoreactive gastrin in dogs and in humans with "usual" forms of duodenal ulcer disease [28]. The increase in fasting serum gastrin levels observed following the administration of secretin to patients with Zollinger–Ellison syndrome remains a paradox [16, 28]. Although the precise mechanism underlying this response is unknown, a direct stimulating effect of secretin upon gastrinoma cells has been postulated [16].

Recent data suggest that immunoreactive gastrin is released into the antral and duodenal lumens of cats following electrical vagal stimulation or topical application of acetylcholine [30]. In vitro studies have further shown that secretin may inhibit luminal release of immunoreactive gastrin from the antrum of the dog [7].

The present studies were designed to test the following hypotheses: first, that immunoreactive gastrin in the dog may be released from extra-antral tissues, notably pancreas and duodenum; second, that secretin may stimulate the release of gastrin from extra-antral tissues in the dog; and third, that bidirectional release into the portal circulation and lumens of pancreas and duodenum following secretin infusion may be a normal phenomenon.

MATERIALS AND METHODS
Specimen Preparation and Perfusion Technique

Pancreaticoduodenal preparations were obtained from six mongrel dogs (20–25 kg body weight) using the technique of Ritchie and Hardcastle [25]. The preparation was maintained at normothermia and perfused through the superior mesenteric artery and one branch of the splenic artery. Venous effluent was collected from the portal vein. An 8- to 10-cm juxtapancreatic segment of duodenum was isolated, with preservation of the inferior and superior pancreatic-duodenal veins. The ampulla of Vater was identified through a 3-cm duodenotomy, and the pancreatic duct cannulated with a fine metal probe. The extraduodenal portion of the pancreatic duct was isolated, then

![Diagram](image-url)
The entire perfusion system with oxygenator/reservoir, in-line dialysis unit, and pulsatile perfusion pump.

cannulated with 18.5-G medical-grade polyethylene tubing, and the proximal portion was ligated. Accessory pancreatic ducts were suture ligated when identified. Flexible collection tubes were secured at the proximal and distal ends of the duodenum to permit perfusion of the isolated bowel segment and collection of specimens. The final preparation is illustrated in Fig. 1.

A closed-circuit perfusion apparatus (Fig. 2), similar to that of Vaysse and colleagues [31], was modified by the addition of an in-line dialysis unit (Technicon heated dialyzer, Model 1). Both the perfusion chamber and the dialysis unit were primed with an albumin-containing perfusate solution, and the final hematocrit in the reservoir was adjusted to 25–30% by adding autologous red blood cells. Blood perfusate and stock perfusate were withdrawn from the system at equal rates (28–32 ml/30 min through a mixing coil into the dialysis chamber and filtered through a Gelman 1.2-μm metrical membrane at a rate approximating the intake flow. Damaged red blood cells and free hemoglobin were removed from circulation and half the blood perfusate volume was replaced with fresh stock solution with each passage through the dialyzer. A rotating disk oxygenator

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2 Perfusate composition: Na⁺, 127 meq/liter; Cl⁻, 105 meq/liter; HCO₃⁻, 40 meq/liter; Ca²⁺, 5 meq/liter; K⁺, 3.5 meq/liter; Mg²⁺, 2 meq/liter; PO₄, 2 meq/liter; SO₄, 1 meq/liter; bovine serum albumin (BSA), 4 g%; glucose, 150 mg%; and mannitol to adjust the final osmotic pressure to 315 mosmoles/liter. One thousand units of aqueous heparin was added per liter of perfusate solution.
supplied with a 95% O₂-5% CO₂ gas mixture, served as the blood reservoir. The preparation was perfused at a constant blood flow (CBF) of 20–25 ml/min using a pulsatile infusion pump (Harvard Products, Model 1405). Arterial and portal pressures were monitored continuously and recorded (Hewlett-Packard multichannel recorder, Model 7754A). The pH, pO₂, and pCO₂ were analyzed (pH blood gas analyzer, Radiometer, Copenhagen) and corrected at hourly intervals as needed.

**Experimental Procedure**

The duodenal segment was perfused continuously at 2 ml/min with lactated Ringers solution containing phenol red indicator, 0.6%. Addition of the dye indicator permitted rapid assessment of the luminal pH; change from the acid orange range signified contamination by alkaline pancreatic secretions. Duodenal effluent was collected on ice every 10 min, the volume recorded, and a sample obtained for subsequent gastrin assay. Each sample was boiled for 10 min, quick-frozen in a dry ice-alcohol bath, and stored at -20°C. Samples of dialysate were collected and processed in a similar fashion. Following a 30-min equilibration period, basal samples were obtained from the portal vein (PV), pancreatic duct (PD), and duodenum (D). Tranexol (Aprotinin, Mobay Chemicals, New York) was added to pancreatic juice at a volume ratio of 1:5 and the samples were quick frozen.

Boots secretin (Boots Co. Ltd., Nottingham, England) was infused at 1 U/min. Samples for immunoreactive gastrin assay were collected from portal vein, pancreatic duct, and duodenum at intervals of 1, 3, 5, 10, 20, and 30 min. After 30 min of infusion, the dose of secretin was increased to 3 U/min, and after another 30 min, to 5 U/min. Samples were collected at the same intervals as above during each secretin infusion period and were processed rapidly and quick-frozen for assay at a later date. At the conclusion of the experiment 0.5- to 1.0-g biopsy samples of pancreas and duodenum were obtained. These specimens were boiled in distilled water for 10 min, and after removing the tissue plug, the extract was quick-frozen.

In order to calculate the amount of immunoreactive gastrin lost from the system by dialysis, an additional experiment was performed. Thirty nanograms of synthetic heptadecapeptide gastrin (G-17) was added to a perfusion system not containing an organ. The final concentration of gastrin in the system was 100 pg/ml. Blood flow and reservoir volume remained constant throughout the procedure. Average percentage cumulative losses of immunoreactive gastrin were calculated by dividing the amount of gastrin measured in the dialysate per 30-min collection period by the total gastrin measured in the system (amount lost and that remaining) and multiplying by 100.

**Immunoreactive Gastrin Assay and Validation**

Immunoreactive gastrin was measured by a radioimmunoassay previously described [32]. Antiserum raised against synthetic human gastrin (SHG 17-I) demonstrated 1% cross-reactivity with CCK® (courtesy of Dr. M. Ondetti, Squibb Pharmaceuticals), 0.003% with tetragastrin, and 0.003% with cerulean using ¹²⁵I-gastrin 17-I as a tracer.

Samples of juice from the duodenum and pancreatic duct were chromatographed on Sephadex G-50 superfine columns (pH 8.0; flow rate, 4.0 ml/hr; column, 100 x 1.0 cm). Affinity chromatography using gastrin antisera G₂ conjugated to Sepharose 4B was performed on samples of pancreatic and duodenal juice. The bound fractions were desorbed with 0.1 M acetic acid at pH 2.5. These samples were lyophilized, reconstituted in assay buffer, and assayed for immunoreactive gastrin as described previously. In a control experiment, ¹²⁵I-gastrin 17-I was applied to the column and eluted in the same manner.
Statistical Analysis

Results are expressed as the mean ± standard error. The standard t test was used to analyze the data for significant differences between the means. Differences with a P value of less than 0.05 were considered significant.

RESULTS

Evidence of Immunoreactive Gastrin Release into Duodenal and Pancreatic Juice

Duodenum. The infusion rate of lactated Ringers-phenol red indicator solution into the duodenal segment was manipulated during basal and stimulated periods to maintain a minimum effluent volume of 1–2 ml/min. The average cumulative 30-min basal volume (67 ± 11 ml) was not significantly different from the volumes collected at 1, 3, and 5 U/min secretin infusion (69 ± 11, 59 ± 10, and 79 ± 10 ml, respectively; P NS). The cumulative basal release of immunoreactive gastrin into duodenal juice was 2225 ± 334 pg/30 min. Stimulation with 1 U/min secretin increased this value to 3622 ± 902 pg. Infusion of 3 U/min secretin significantly (P < 0.05) increased the secretion to 4949 ± 1072 pg, and 5 U/min secretin infusion caused no further increase (4175 ± 583 pg). Gastrin release at 5 U/min secretin infusion was, however, significantly (P < 0.02) different from basal (Fig. 3).

Pancreas. Spontaneous flow from the pancreatic duct was not observed in the basal state. In response to secretin infusion, the average cumulative flow rose from 2.7 ± 0.7 ml at 1 U/min of secretin to 4.6 ± 1.2 ml at 3 U/min and fell to 3.7 ± 0.8 ml at 5 U/min. These values were not significantly different. In each instance there was a delay lasting about 10 min before the secretory response to secretin was observed (Fig. 4). Patterns of gastrin release were similar to those of juice flow (Fig. 5). There was no basal secretion. During infusion of 1 U/min secretin the average cumulative release of immunoreactive gastrin was 128 ± 40 pg. With secretin infusion rates of 3 and 5 U/min, 202 ± 48 and 138 ± 9.0 pg were released, respectively, values which were not significantly different from that with the 1 U/min infusion. Regression analysis of the rate of release of immunoreactive gastrin into pancreatic juice (pg/min) versus the...
rate of pancreatic juice flow (ml/min) yielded a correlation coefficient of 0.83 ($P < 0.001$), signifying that the rate of pancreatic gastrin release was flow dependent (Fig. 6).

By extraction, immunoreactive gastrin was recovered from tissue samples of duodenum and pancreas. In a limited number of experiments, the mean immunoreactive gastrin recovered from samples of duodenum ($N = 3$) was $1690 \pm 235$ pg/g and that from pancreas ($N = 2$) was $706 \pm 214$ pg/g.

**Evidence for Immunoreactive Gastrin Release into the Portal Vein in Response to Secretin Infusion**

By calculation of dialysate gastrin losses, there was an average of 7.8% total gastrin in the system lost per 30-min collection period. This necessitated correction of each portal vein gastrin concentration by a factor of 0.03%. The average basal gastrin concentration was $49.8 \pm 4.8$ pg/ml. In response to 1, 3, and 5 U/min secretin infusion, these values rose to $52.7 \pm 3.5$, $65.7 \pm 2.9$, and $77.1 \pm 3.2$ pg/ml (Fig. 7). The average gastrin concentrations at 3 and 5 U/min secretin infusion were significantly ($P < 0.01$) different from basal and the values with the infusion of 1 U/min. In addition, the gastrin values at all secretin infusion rates were significantly ($P < 0.01$) different from each other. Because gastrin accumulated in a closed system without active removal, portal vein values alone reflect neither the total mass secreted nor changes due to increasing secretin doses. Hence, the average net increment in portal vein immunoreactive gastrin content over and above the preceding level was calculated. The incremental changes in portal venous gastrin release are compared with those measured in duodenal and pancreatic juice, and are depicted in Table 1. Similar release patterns over time were observed in all six experiments.

**Isolation and Characterization of Immunoreactive Gastrin Released from Different Tissues**

When pancreatic juice containing 540 pg of immunoreactive gastrin was applied to an affinity column containing an antiserum covalently bound to Sepharose, 44% of that gastrin was bound and eluted with 0.1 M acetic acid. On rechromatography of this material, 100% was bound. Fifty-six percent of the gastrin in duodenal juice was bound by the column. The column capacity was estimated to be 250 pg and the unbound fraction represented an over-
Secretin infusion significantly increased the average portal vein immunoreactive gastrin concentrations (Table 1).

Table 1: Average Net Changes in Luminal and Portal Venous Release of Immunoreactive Gastrin (pg/30 min)

<table>
<thead>
<tr>
<th>Secretion Rate</th>
<th>Portal Vein</th>
<th>Duodenum</th>
<th>Pancreatic Duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 U/min secretion</td>
<td>870 (+60%)</td>
<td>3622 (+63%)</td>
<td>128*</td>
</tr>
<tr>
<td>3 U/min secretion</td>
<td>3900 (+25%)</td>
<td>4949 (+37%)</td>
<td>202 (+58%)</td>
</tr>
<tr>
<td>5 U/min secretion</td>
<td>3420 (-17%)</td>
<td>4175 (-16%)</td>
<td>138 (-32%)</td>
</tr>
</tbody>
</table>

* No basal output recorded.

Fig. 7. The effect of secretin infusion upon the average portal vein immunoreactive gastrin concentrations.

load of the capacity of the column to bind gastrin (Fig. 8).

Immunoreactive gastrin in both pancreatic and duodenal juice was detected using a C-terminal reacting antiserum and duodenal juice eluted on Sephadex G-50 columns as a peptide equivalent in molecular weight to CCK. This peptide did not react with a NH₂-directed gastrin antiserum, suggesting that it contained the C-terminal portion of gastrin or CCK. Rechromatography of the gastrin in pancreatic juice obtained by acetic acid elution of that bound to the affinity column resulted in a single peak again equivalent in molecular weight to CCK (Fig. 9).

Portal vein IG eluted on Sephadex G-50 predominantly as G34, with a lesser fraction corresponding to G17 and smaller peptides (Fig. 10).

DISCUSSION

The use of an ex vivo pancreaticoduodenal perfusion system facilitates study of the mechanism(s) of circulatory and luminal immunoreactive gastrin (IG) release from extra-antral tissues, and avoids many of the problems of in vivo systems. The placement of separate collection cannulae into the duodenal and pancreatic duct lumens allows observation of the dynamics of gastrin release from each endocrine organ in response to the administration of exogenous secretin.

The gastrin concentration in the dog duodenal mucosa was approximately 0.0017 μg/g mucosa, a value somewhat lower than that observed by Watson et al. [33]. Similar values of duodenal gastrin content, however, have been observed by Nilsson...

Fig. 8. The results of affinity chromatography of immunoreactive gastrin recovered from pancreatic and duodenal juice.
Gastrin content in the dog pancreas was approximately 0.0008 μg/g tissue, 50% lower than that in duodenal tissue. Immunoreactive gastrin was recovered in duodenal juice during basal and secretin-stimulated periods. The lack of spontaneous juice flow (basal state) from the pancreatic duct precludes any comparative statements, but gastrin immunoreactivity was present in samples collected during secretin-stimulated periods. The elution patterns on gel filtration of pancreatic and duodenal juice samples indicated an immunoreactive gastrin-like peptide smaller than Rehfeld’s component IV (G-14 or minigastrin [23, 24] and nearly equivalent in molecular size to CCK. Uvnäs-Wallensten et al. similarly observed gastrin release into the duodenal lumen of cats [29], but the peptide was not further characterized.

The concept of hormone release into the gut lumen has been viewed with skepticism, but several investigators have demonstrated gastrin-like peptides in gastric juice of man, dog, and cat [1, 8, 13–15, 30, 32]. In the cat, gastrin from gastric juice has been characterized by gel filtration as mainly G-17. The recovery from dog duodenal and pancreatic juice of a smaller gastrin-like peptide containing approximately eight of the carboxy-terminal amino acids of gastrin or CCK has not been reported. It is not surprising that gastrin-like peptides are recoverable from pancreatic juice. Lom-

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**Fig. 9.** Sephadex G50 superfine chromatography of immunoreactive gastrin in canine duodenal and pancreatic juice.

**Fig. 10.** Sephadex G50 superfine chromatography of immunoreactive gastrin in canine portal vein plasma.
sky et al. [21] demonstrated in the human pancreas cells with gastrin-like immuno-reactivity. This observation, however, has been disputed by others [3, 17]. True gastrin cells have been shown to occur in the pancreas and duodenum prenatally in human [17] and rat [18] species, but rarely are identifiable for longer than 2–3 weeks following birth. In addition, gastrin-like immunoreactivity to specific C terminal-reacting gastrin antisera, although present in gastrinomas and prenatal pancreas, has not been demonstrated in adult pancreas [17]. These dissimilar observations, however, may be related to fixation problems, as pancreatic gastrin-producing D or A, cells have histological and histochemical properties which differ from those of antral gastrin-producing G cells and gastrinoma cells.

The administration of secretin to dogs and patients with “usual” forms of duodenal ulcer disease results in a fall or no change in fasting levels of circulating immunoreactive gastrin [28]. Circulating gastrin peptides in fasting man and dog have been characterized mainly as component II (G34) along with minute quantities of component IV (G-14) [4, 5, 22, 24, 32]. The infusion of 1, 3, and 5 U/min Boots secretin in our ex vivo preparation resulted in prompt increases in immunoreactive gastrin release into duodenal and pancreatic juice and, to a lesser degree, into portal venous blood as well. The quantities of gastrin released into duodenal juice were at least equal to, if not greater than, those released into portal venous blood (Table 1). The lesser rise in gastrin release at 5 U/min secretin into gut and pancreatic lumens as well as into portal venous blood suggests either a fatigue phenomenon or inhibition at the higher dose. In all experiments a 10- to 15-min lag period preceded the spike increase in luminal gastrin release at each secretin infusion rate. A similar lag period preceding the fall in circulating gastrin levels in humans following secretin infusion has been observed by Korman et al. [16].

The increased levels of portal vein gastrin observed in the present study cannot be attributed solely to pH changes of the duodenal perfusate. Becker et al. [2] observed increases in regional venous gastrin release in response to perfusing the proximal duodenum of dogs with neutral or alkaline saline solutions. The constant acid orange color of duodenal effluent in all of the present experiments with dye indicator suggests that duodenal juice alkalinity was not a contributing factor.

The reason that others have not found that secretin is a stimulant for gastrin release may be due to measurements taken in the whole animal. Thompson et al. have shown that endogenous gastrin undergoes rapid extraction and degradation (35–37% per transit) in the small bowel of the dog [27]. Previous experiments (see Materials and Methods) showed that less than 8% of the immunoreactive gastrin in the total system is lost through dialysis per 30-min collection period, a cumulative loss of less than 25% for the entire study period. The lack in our system of an efficient mechanism for extraction and degradation of circulating gastrin may serve to explain the rise in portal vein gastrin levels following the infusion of secretin.

The concept of bidirectional release of hormone peptides into the gut lumen and regional venous circulation is not new. Fiddian-Green, Knight, and Vinik have demonstrated luminal gastrin release in man [8, 15, 32], and Uvnäs-Wallensten et al. described bidirectional immunoreactive gastrin release from the antrum of anesthetized cats following vagal stimulation [30]. Solcia and colleagues have demonstrated two types of endocrine cells in gastric mucosa: Closed-type cells are limited to the gastric corpus and lack direct luminal communications; open-type cells, on the other hand, are found in the antrum and possess microvilli which project into the gastric lumen [26]. Ultrastructural studies of open-type cells indicates that their organization is appropriate not only for bidirectional hormone release but also
for response to luminaly active stimuli, including other hormones [19].

Our findings suggest further that bidirectional gastrin release may occur from extra-antral tissues. In the dog, secretin stimulation inhibits luminal release of antral gastrin and stimulates release from duodenum and pancreas in excess of that into portal venous blood. This phenomenon may also occur in the human but must be confirmed with further investigation. Ultrastructural studies of gastrinomas from patients with Zollinger–Ellison syndrome have shown the presence of nonuniform-appearing secretory cells which contain granules typical of antral G cells [3]. In addition, ductular structures have been frequently observed in histologic sections of gastrinomas [3].

Gastrinoma cells are immunohistochemically similar to antral G cells and react equivalently with carboxy terminal-directed gastric antisera [17]. The responses of gastrinoma cells and antral G cells to exogenous secretin, however, appear to differ markedly, suggesting that the two cell types are qualitatively dissimilar. However, following the administration of secretin in the dog, immunoreactive gastrin is released bidirectionally into pancreatic and duodenal juice as well as into portal venous blood. This observation implies that gastrin-producing G or D cells in the dog are functionally similar to the "open-type" gastric mucosal endocrine cells described by Solcia. If a similar pattern of gastrin release occurs in normal human subjects following secretin stimulation, one might anticipate negligible changes in circulating levels of gastrin because most of the gastrin appears in the intestinal lumen. Gastrinoma cells, on the other hand, may be morphologically distinct (closed versus open cell types) and differ in their communication with ductal structures.

Hence, the administration of exogenous secretin to patients with gastrinomas (Zollinger–Ellison syndrome) may result in a unidirectional release of immunoreactive gastrin into the portal circulation, the magnitude of which depends on the mass of endocrinologically active tumor.

These observations may serve to explain the paradoxical gastrin response to secretin in patients with gastrinomas.

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