Regulation of fundic and antral somatostatin secretion by CCK and gastrin

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The objectives of the present study were to determine the separate effects of CCK and gastrin on fundic and antral SOM secretion and to assess the type of receptor involved, using CCK-A (L-364,718) and CCK-B/gastrin (L-365,260) receptor antagonists. Changes in SOM were measured in plasma collected from cannulas draining blood from the fundus (gastric vein) and antrum (gastroepiploic vein) in anesthetized sheep. Both CCK and gastrin significantly stimulated SOM from the fundus and antrum. Sulfated CCK-8 (CCK-8S) increased SOM secretion from the fundus and antrum through interaction with both type A and B receptors. In contrast to CCK-8S, sulfated gastrin-17 (G-17S) stimulated SOM from the fundus via the type B receptor alone, whereas in the antrum G-17S stimulated SOM secretion independent of the A and B receptors. Histamine mediated, at least in part, the SOM-stimulatory effects; an H2-receptor antagonist blocked CCK-stimulated SOM secretion in both the fundus and antrum and reduced gastrin-stimulated SOM secretion in the fundus. The present study demonstrates regionally distinct regulatory mechanisms for gastric SOM secretion by CCK and gastrin.

GASTRIN, secreted by antral G cells, is the principal stimulant of gastric acid secretion (36). The duodenal peptide CCK is structurally related to gastrin and is a major hormonal regulator of gallbladder contraction (11). Both CCK and gastrin stimulate somatostatin (SOM), but the potency and the receptors involved are not identical. CCK appears to be more potent than gastrin (31, 38) and stimulates SOM secretion by binding to both the CCK-A (type A) and CCK-B/gastrin (type B) receptors, whereas gastrin acts through the CCK-B/gastrin receptor alone (38).

Although the stomach appears to be the source of SOM, there are no in vivo data comparing the regulation of antral and fundic SOM by CCK and gastrin. Differences in regulation are likely, as the D cells in the fundus and antrum vary in the response to acidity and food (8). Furthermore, gastrin is present in high concentrations in the antrum, whereas CCK is confined to the duodenum (11, 36). The distribution of the type A and B receptors is not uniform. Ligand binding demonstrates that both type A and type B receptors are present on isolated canine fundic D cells, whereas only type A receptors are detected on isolated antral D cells (3, 19). However, immunohistochemistry in a number of species has shown that the CCK-B/gastrin receptor is present on D cells of the antrum as well as the fundus (7, 32). Immunohistochemistry of canine and human fundic mucosa demonstrated both receptors in the fundus, although the receptors were not localized to the D cell (17, 24). The CCK-B/gastrin receptor present on parietal cells, enterochromaffin-like (ECL) cells, and D cells has an equal affinity for gastrin and CCK, whether sulfated or not (29). The CCK-A receptor has a greater affinity for sulfated CCK than sulfated or nonsulfated gastrin (29).

The role of the CCK-A and CCK-B/gastrin receptors in SOM secretion is often investigated by receptor-specific antagonists. The benzodiazepine derivative L-364,718 (MK-329, devazepide) is highly selective for the CCK-A receptor and antagonizes CCK-regulated gastrointestinal functions including SOM release (15), pancreatic secretion, and gallbladder contraction (12). A related compound, L-365,260, is selective for the CCK-B/gastrin receptor and has been used to study the role of the type B receptor in acid secretion (34).

We and others (8, 25, 38) have suggested that the mechanisms regulating SOM synthesis and secretion differ between the fundus and antrum. The different morphology of the SOM-secreting D cells in these regions supports this proposal. Antral D cells are open cells with apical processes in contact with the lumen and are sensitive to changes in gastric acidity. Fundic D cells are closed cells and are responsive to peptide regulation (8, 26).

Gastrin can stimulate SOM secretion by acting directly on the D cell (19, 31). Histamine may be a mediator of this response, because gastrin stimulates histamine release (22). It is not clear whether the effect of histamine on SOM secretion is a direct action on the D cell (30) or the result of an increase in gastric acid secretion, as studies with H2-receptor antagonists have been contradictory (5, 16, 30). The effect of H2-receptor antagonists on CCK-stimulated SOM secretion has not been reported. There are many more ECL cells in the fundus than in the antrum (6); thus it would be expected that histamine has a more important role in mediating gastrin-stimulated SOM secretion from this region than from the antrum. In addition, ECL cells purified from rat fundus are sensitive to gastrin as well as CCK (21, 22). However, the relative roles of histamine in CCK- and gastrin-stimulated SOM secretion from the fundus and antrum have not been defined.

The objectives of this study were to investigate functional differences in the regulation of fundic and

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antral SOM secretion. This was achieved by measuring changes in SOM and gastrin secretion in plasma collected from fundic and antral veins of the sheep stomach (abomasum) in response to gastrin and CCK stimulation. The nature of the receptors involved was then determined by repeating the study using the CCK-A and CCK-B/gastrin receptor antagonists. The role of histamine as a mediator of CCK- and gastrin-stimulated SOM secretion was determined by measuring changes in fundic and antral SOM release in response to CCK and gastrin plus the \( H_2 \)-receptor antagonist ranitidine.

**MATERIALS AND METHODS**

**Materials**

Sulfated forms of CCK-8 (CCK-8S) and G-17 (G-17S) were purchased from Bachem (Torrance, CA). Peptides were dissolved in 0.05 M \( \text{NH}_4\text{HCO}_3 \)-0.5% BSA to a final stock concentration of 50 nmol/ml. Peptide stock concentrations were then diluted in 0.9% saline and 0.1% BSA to obtain the administered peptide dose (150 pmol·kg\(^{-1}\)·h\(^{-1}\)). The CCK-A and CCK-B/gastrin receptor antagonists L-364,718 (1 mg/kg) and L-365,260 (3 mg/kg) were supplied by Merck Research Laboratories (Rahway, NJ). Antagonists were dissolved in DMSO-polyethylene glycol-400 (PEG-400) (4:5:0.5, vol/vol) and administered as a 5-ml bolus. The \( H_2 \)-receptor antagonist ranitidine (Glaxo Australia) was diluted in 0.9% saline and 0.1% BSA to obtain the administered dose (bolus 0.5 mg/kg; infusion 1.5 mg·kg\(^{-1}\)·h\(^{-1}\)).

**Animal Preparation**

Merino × Corriedale sheep, ranging in weight from 35 to 45 kg, had food withheld for 24 h before surgery. Anesthesia was induced by intravenous Pentothal sodium (1 g) and maintained throughout the experiment with a 1–2% Fluothane-oxygen mixture (ICI Australia). The abdomen was opened by a midline incision. A cannula (0.76 mm ID, 1.65 mm OD; Dow Corning) was inserted into the gastric vein draining blood from the fundus. The gastroepiploic vein, draining blood from the antrum, was also cannulated. Both the gastric and gastroepiploic veins were ligated on each side of the cannula, allowing drainage of blood from the main branch of the vein and preventing blood from flowing from the fundus to the antrum (Fig. 1). To insert the gastric cannula, a right subcostal incision was made for insertion of the catheter (polyvinyl chloride, 10 mm ID × 15 mm OD) through a purse-string suture into the antral regions of the abomasum. The cannula was secured by tightening the purse string (Fig. 1). Cannulas were also inserted into the jugular veins. One cannula was directed toward the heart (down) for infusion, and the contralateral cannula was directed toward the head (up) for blood sampling (38). After surgery 0.5 h was allowed before the experiment began. All animal experiments were approved by the Austin and Repatriation Medical Center Animal Ethics Committee (95/030).

**Experimental Design**

Three different experimental protocols were used: CCK and gastrin infusions, CCK and gastrin infusions preceded by administration of either the CCK-A or CCK-B/gastrin receptor antagonists, and CCK and gastrin infusions preceded by administration of the \( H_2 \)-receptor antagonist. A 10-ml blood sample was collected from the jugular vein immediately before anesthesia and after the animal preparation to measure the effect of the anesthetic on basal SOM and gastrin concentrations.

**Control experiments. Effect of CCK-8S and G-17S infusion on SOM secretion.** The first series of experiments was performed to determine the stimulatory effects of CCK-8S and G-17S alone in the fundus and antrum. After a 60-min basal period, CCK-8S (150 pmol·kg\(^{-1}\)·h\(^{-1}\)) was infused for 30 min at 20 ml/h via the jugular vein cannula (down) using a syringe infusion pump (Sage Instruments). A 45-min post-peptide-infusion period was allowed before G-17S (150 pmol·kg\(^{-1}\)·h\(^{-1}\)) was infused for a further 30 min, followed by a 30-min postinfusion period. Blood samples were collected simultaneously from the jugular, fundic, and antral veins every 15 min during the basal and postpeptide infusion periods, and every 10 min during the peptide infusions. Gastric secretions were collected every 30 min during the experiment, and pH was measured.

**Antagonist experiments. Effect of L-364,718 or L-365,260 on CCK-8S- and G-17S-stimulated SOM secretion.** The second series of experiments was performed to determine the nature of the receptors that mediate SOM secretion from the antrum and fundus in response to gastrin and CCK. These experiments were similar to the control experiments except that the CCK-A (L-364,718) (1 mg/kg) and CCK-B/gastrin (L-365,260) (3 mg/kg) receptor antagonists were administered on separate occasions, 30 min before the infusion of CCK-8S. Blood samples were collected from the jugular vein immediately before anesthesia and after the preparation period and the effect of the anesthetic on basal SOM and gastrin concentrations was measured.
and gastric juice samples were collected as previously described.

**Effect of Ranitidine on CCK-8S- and G-17S-Stimulated SOM Secretion.** The effect of ranitidine (H₂-receptor antagonist) on fundic and antral SOM secretion was studied to determine the role of histamine as a mediator of gastrin- and CCK-stimulated SOM release. After a 60-min basal period ranitidine was administered as a bolus (0.5 mg/kg) followed immediately by an infusion of 1.5 mg·kg⁻¹·h⁻¹ for 60 min at a rate of 20 ml/h. This dose of ranitidine inhibits ~90% of meal-stimulated gastric acid secretion in dogs (5) and basal and histamine-stimulated gastric acid secretion in sheep. The ranitidine infusion was followed by a 30-min infusion of ranitidine (1.5 mg·kg⁻¹·h⁻¹) plus CCK-8S (150 pmol·kg⁻¹·h⁻¹). A 45-min postinfusion period was allowed before ranitidine plus G-17S was infused for a further 30 min, followed by a 30-min postinfusion period. Blood and gastric juice samples were collected as previously described.

**Laboratory Methods**

Reverse-phase HPLC: predominant SOM molecular form. To determine the proportions of SOM-14 and SOM-28 that were released from the fundus and antrum in response to G-17S and CCK-8S, plasma samples obtained during the infusion of either gastrin or CCK were pooled and concentrated using Sep-Pak C₁₈ cartridges (Millipore). SOM-14 and SOM-28 standards (1 pmol) or Sep-Pak concentrated plasma samples were loaded onto a C₁₈ Bondapak column (Waters) and separated using a 22-min gradient from 28% to 32% acetonitrile and 0.05% trifluoroacetic acid. The flow rate was set at 3 ml/min, and 44 0.5-min fractions were collected. Two hundred microliters of each fraction were dried down under air. Standards and samples were assayed using a buffer standard curve and under normal SOM radioimmunoassay conditions.

Radioimmunoassay. SOM. Plasma SOM was measured in ethanol-extracted plasma as described previously (23). One milliliter of plasma was mixed with 2 ml of absolute ethanol, and the coagulated protein was removed by centrifugation. The ethanol extracts were then divided into 1-ml aliquots, evaporated to dryness, and stored at −20°C until assay. Before assay, extracted plasma was reconstituted in 1 ml assay buffer, and 50–200 µl of reconstituted sample were assayed against a buffer standard curve. Antiserum 8402, which detects SOM-14 and SOM-28 to an equal extent, was used. ¹²⁵I-Tyr-SOM-14 was prepared using the chloramine T method and purified by reverse-phase HPLC. SOM-14 was used for generating the buffer standard curve. The ID₅₀ was 8 fmol/ml, and the inter- and intra-assay coefficients of variation were less than 5% and 12%, respectively.

GAstrin. Plasma gastrin was assayed directly, using 50 µl of plasma. The plasma samples were incubated at 4°C in duplicate with the ¹²⁵I-Met¹⁵ human nonsulfated G-17 (G-17NS) label and antiserum 1296. Antiserum 1296 recognizes all COOH-terminal fragments larger than the pentapeptide and measures G-17S and G-17NS identically. G-17NS was used for generating the standard curve. The ID₅₀ was 1 fmol/ml, and inter- and intra-assay coefficients of variation were less than 2% and 11%, respectively.

**Statistical Analysis**

Results are means ± SE. Statistical comparison between more than two groups was made by one-way analysis of variance followed by the Student-Newman-Keuls method. Data that were not normally distributed were analyzed by the Mann-Whitney test. Statistical comparisons of means between two groups were made using an unpaired t-test. P < 0.05 was considered significantly different. The integrated SOM response was calculated as the area under the plasma SOM concentration time curve with respect to the average of the two basal concentrations preceding the infusion (38).

**RESULTS**

**Control Experiments**

Effect of anesthesia on basal SOM and gastrin secretion. A peripheral blood sample was collected before anesthesia and then immediately after completion of the surgery (−90 min after administration of the Fluothane-oxygen mixture) to measure the effect of the anesthetic on basal SOM and gastrin concentrations. Basal plasma SOM concentrations significantly increased from 24 ± 4 to 130 ± 28 pmol/l (P < 0.05). Basal plasma SOM decreased over the subsequent 60-min basal period to a value of 60 ± 8 pmol/l, measured at 0 min (P < 0.05). Basal SOM was highest in the fundic vein (602 ± 97 pmol/l), followed by the antral vein (153 ± 46 pmol/l) and peripheral circulation (60 ± 8 pmol/l).

Peripheral plasma gastrin concentrations significantly decreased from 20 ± 2 pmol/l just before anesthesia to 15 ± 1 pmol/l at the end of surgery (P < 0.05) and then increased over the following 60-min basal period to 37 ± 7 pmol/l (P < 0.05). As expected, basal gastrin concentration was highest in the antral vein (218 ± 83 pmol/l); values in the fundic vein (28 ± 5 pmol/l) and peripheral circulation (37 ± 7 pmol/l) were similar.

**Effect of CCK-8S and G-17S infusion on SOM secretion**

The first series of experiments was performed to determine the stimulatory effects of CCK and gastrin on fundic and antral SOM secretion before the receptor antagonist study. Both CCK-8S and G-17S increased plasma SOM measured in the peripheral circulation. CCK-8S caused a significant increase in plasma SOM from a basal value of 60 ± 8 pmol/l to a peak response of 82 ± 15 pmol/l (P < 0.05) (Fig. 2A). G-17S also significantly increased plasma SOM from 50 ± 11 to 84 ± 17 pmol/l (P < 0.05) (Fig. 2A).

Antral plasma SOM significantly increased in response to CCK-8S, from 153 ± 46 to 267 ± 51 pmol/l (P < 0.05) (Fig. 2B). Antral SOM remained elevated in the postinfusion period, but G-17S then increased plasma SOM from 248 ± 56 to 349 ± 84 pmol/l; this change was not significant (P > 0.05) (Fig. 2B).

Fundic plasma SOM significantly increased in response to CCK-8S, from 602 ± 97 to 930 ± 163 pmol/l (P < 0.05) (Fig. 2C). Fundic SOM secretion also increased in response to G-17S, from 655 ± 219 to 1,282 ± 323 pmol/l (P < 0.05) (Fig. 2C).

The results are also represented as the integrated SOM output (Fig. 3). The integrated SOM output in response to CCK-8S and G-17S in the peripheral circulation (0.47 ± 0.13 and 0.50 ± 0.11 pmol·min⁻¹·ml⁻¹, respectively), antrum (1.93 ± 0.80 and 1.83 ± 0.78 pmol·min⁻¹·ml⁻¹, respectively), and fundus (3.76 ± 1.60 and 12.9 ± 3.81 pmol·min⁻¹·ml⁻¹, respectively)
was significantly greater than basal levels (P < 0.05) (Fig. 3). The SOM output in the periphery and antrum in response to CCK-8S was similar to that of G-17S. The response to G-17S in the fundus was significantly greater than the response to CCK-8S (P < 0.05) (Fig. 3).

CCK-8S had no effect on gastrin concentration in plasma from the periphery, antrum, and fundus. The net increase in plasma gastrin during the G-17S infusion was similar in the periphery (from 41 ± 8 to 300 ± 34 pmol/l), antrum (from 172 ± 53 to 465 ± 85 pmol/l), and fundus (from 26 ± 4 to 256 ± 46 pmol/l), with peak responses measured at 105 min (P < 0.05).

CCK-8S caused a significant increase in gastric pH, from a basal value of 3.31 ± 0.25 to 4.01 ± 0.42 (P < 0.05). During the infusion of G-17S the gastric pH did not vary significantly from basal (3.52 ± 0.26).

Antagonist Experiments

Effect of L-364,718 or L-365,260 on CCK-8S- and G-17S-stimulated SOM secretion. The CCK-A (L-364,718) and CCK-B/gastrin (L-365,260) receptor antagonists were used to determine the receptors involved in CCK- and gastrin-stimulated SOM secretion.
decreased from 3.76 ± 1.60 to −3.70 ± 0.32 pmol·min⁻¹·ml⁻¹ (P < 0.05) (Fig. 4). The inhibitory effect of L-364,718 on CCK-8S-stimulated antral and fundic SOM output resulted in a decrease in peripheral plasma SOM output from 0.47 ± 0.13 to −0.20 ± 0.22 pmol·min⁻¹·ml⁻¹ (P < 0.05) (Fig. 4).

Similar to the CCK-A receptor antagonist, the CCK-B/gastrin receptor antagonist L-365,260 significantly suppressed CCK-8S-stimulated SOM secretion in both the antrum (−2.21 ± 1.15 pmol·min⁻¹·ml⁻¹) and fundus (−3.79 ± 1.18 pmol·min⁻¹·ml⁻¹) (P < 0.05) (Fig. 4). The decreases were reflected in the peripheral circulation, where SOM output also decreased to −0.39 ± 0.24 pmol·min⁻¹·ml⁻¹ (P < 0.05) (Fig. 4).

During the infusion of CCK-8S plus either L-364,718 or L-365,260 the pH did not vary significantly from basal (3.80 ± 0.35).

G-17S-STIMULATED SOM SECRETION. L-364,718 and L-365,260 had different effects on G-17S-stimulated SOM secretion in the fundus and antrum. L-364,718 had no effect on either antral, fundic, or peripheral SOM secretion stimulated by G-17S (Fig. 5). The CCK-B/gastrin receptor antagonist L-365,260 significantly suppressed G-17S-stimulated SOM secretion in the fundus, from 12.9 ± 3.81 to 0.87 ± 0.67 pmol·min⁻¹·ml⁻¹ (P < 0.05) (Fig. 5) but had no effect on antral secretion (Fig. 5). SOM output in the periphery also decreased from 0.50 ± 0.11 to −0.43 ± 0.34 pmol·min⁻¹·ml⁻¹ (P < 0.05) (Fig. 5), reflecting the decrease in the fundus.

The gastric pH did not significantly change from basal during the infusion of G-17S plus L-364,718 (4.03 ± 0.25). However, during the infusion of G-17S plus L-365,260 the pH increased from 4.11 ± 0.18 to 4.67 ± 0.22 (P < 0.05).

Effect of ranitidine on CCK-8S- and G-17S-stimulated SOM secretion. To determine the role of histamine as a mediator in the regulation of SOM secretion, ranitidine was infused with CCK or gastrin. Ranitidine alone decreased SOM concentration in the periphery (from 175 ± 44 to 87 ± 23 pmol/l) and fundus (from 2,645 ± 578 to 1,361 ± 236 pmol/l) (P < 0.05) but had no effect on antral plasma SOM concentrations. Ranitidine blocked the SOM-stimulatory effect of CCK-8S, and this was observed in the antral, fundic, and peripheral circulations (Fig. 6). In contrast, the coadministration of ranitidine plus G-17S did not prevent the release of G-17S-stimulated SOM from the antrum (Fig. 7). In the fundus G-17S-stimulated SOM output was partly suppressed by ranitidine from 12.9 ± 3.81 to 3.63 ± 3.18 pmol·min⁻¹·ml⁻¹ (Fig. 7). The SOM output in response to G-17S plus ranitidine remained greater than the basal output (P < 0.05) (Fig. 7).

Ranitidine alone increased basal gastrin levels in the periphery (from 21 ± 3 to 42 ± 7 pmol/l), fundus (from 20 ± 3 to 37 ± 8 pmol/l), and antrum (from 166 ± 44 to 252 ± 36 pmol/l). Ranitidine with CCK-8S infusion did not change gastrin measured in plasma collected from the periphery, antrum, and fundus. The net increase in plasma gastrin during the G-17S infusion was similar in the periphery (from 38 ± 7 to 288 ± 32 pmol/l), antrum (from 197 ± 51 to 434 ± 53 pmol/l), and fundus (from 25 ± 4 to 190 ± 19 pmol/l).

Ranitidine alone caused a significant increase in gastric pH from 4.00 ± 0.60 to 4.62 ± 0.61 (P < 0.05). The gastric pH did not change from basal during the infusion of CCK-8S plus ranitidine. However, during the infusion of G-17S plus ranitidine the gastric pH significantly increased, from 4.53 ± 0.50 to 5.11 ± 0.54 (P < 0.05).
HPLC: Predominant SOM Molecular Form Released After CCK-8S and G-17S Stimulation

In the peripheral circulation CCK-8S-stimulated SOM secretion was composed of two peaks of immunoreactivity: 95% coeluted with SOM-14 and 5% coeluted with SOM-28 (data not shown). After CCK stimulation, SOM immunoreactivity in the plasma from the antrum consisted of 99% SOM-14 and 1% SOM-28 and plasma from the fundus consisted of 97% SOM-14 and 3% SOM-28. The predominant SOM molecular form secreted in response to G-17S was also SOM-14, with small amounts of SOM-28 measured in the peripheral (7%) and antral (3%) plasma but none detected in plasma from the fundus.

DISCUSSION

In vitro and in vivo studies have demonstrated that both CCK and gastrin stimulate gastric SOM secretion (1, 5, 15, 31, 38). Through separate sampling of blood from veins draining the fundus and antrum, we have shown differences between the SOM regulatory mechanisms in these regions. CCK-8S stimulates SOM from the fundus and antrum through the interaction with both the type A and B receptors. In contrast to CCK-8S, G-17S stimulates SOM from the fundus via the type B receptor alone, whereas in the antrum G-17S stimulates SOM secretion independent of the type A and B receptors. Histamine mediates, at least in part, the SOM-stimulatory effects; an $H_2$-receptor antagonist blocked CCK-stimulated SOM secretion in both the fundus and antrum and reduced gastrin-stimulated SOM secretion in the fundus.

In agreement with studies of other species, SOM concentration was higher in blood samples from the fundic than the antral veins (10). Conversely, in sheep and humans (20, 23), but not rats (33), the concentration of SOM in the antral mucosa exceeds the fundic tissue concentrations, supporting the concept of a difference in the secretory regulation of SOM secretion from these two regions. As expected, antral veins contained a higher concentration of gastrin, with the fundic vein having a concentration similar to the peripheral levels. This agrees with the distribution of gastrin; >90% of gastrin is found in the antrum (36).

Although we and others (5, 15, 38) have established that gastrin and CCK stimulate SOM secretion, it was not clear in vivo whether the SOM originated from the stomach. The present study shows that both gastrin and CCK stimulate SOM secretion from the fundus and the antrum and that these increases were also detected in the peripheral circulation. These results confirm findings in conscious sheep, in which we have reported significant increases in peripheral SOM secretion in response to gastrin and CCK (38). Based on blood levels achieved, CCK is a more potent stimulant, as it is cleared more rapidly, reaching an equilibrium plasma concentration of 35 pmol/l, compared with more than 300 pmol/l for the same dose of gastrin (38). It is important to note that the SOM secretory response in the anesthetized sheep was about one-half the response observed in the conscious animal. Administration of the anesthetic (Fluothane-oxygen mixture) produced a significant increase in basal plasma SOM concentrations, whereas basal gastrin decreased, presumably as a result of the higher SOM. Other studies have reported an increase in SOM synthesis and secretion in rats anesthetized with urethane (13, 37). An increased production of SOM may be a phenomenon of anesthesia rather than the result of a specific anesthetic.

In a previous study in conscious sheep, in which changes in SOM were measured only in the peripheral circulation, we reported that CCK-stimulated SOM secretion was mediated by both the CCK-A and CCK-B gastrin receptors (38). The present study extends these findings by demonstrating that SOM is released in response to CCK from the fundus and the antrum and that both type A and B receptors mediate these responses. Our results agree with ligand binding evidence and immunohistochemistry demonstrating the presence of both of these receptor types on fundic (3, 19) and antral D cells (7, 32).

G-17S-stimulated fundic SOM secretion was not inhibited by the CCK-A receptor antagonist, confirming that the CCK-A receptor is selective for CCK peptides (3, 29). The CCK-B/gastrin receptor antagonist inhibited G-17S-stimulated SOM secretion in the fundus but not the antrum, indicating that gastrin stimulates SOM secretion in the fundus by activating the CCK-B/gastrin receptor. The mechanism in the antrum was different, since antral SOM output in response to G-17S was not inhibited by either the CCK-A or CCK-B/gastrin receptor antagonists. The absence of an effect of the CCK-B receptor antagonist on gastrin-stimulated antral SOM secretion is surprising since the receptors are present in the antrum (7, 32). However, the level of expression (mRNA) of the B receptor is ~20–50 times...
less in the sheep and rat antrum compared with the fundus (Kolivas, Dow, and Shulkes, unpublished observations; and Ref. 18). The low levels of CCK-B gastrin receptor coupled with the very high concentration of endogenous gastrin in the antrum would mean that most receptors would be occupied, thus making it difficult to block the SOM response to gastrin with L-365,260. In contrast to the antrum, the fundus has a large population of the CCK-B/gastrin receptor, low circulating gastrin concentrations, and a greater SOM-stimulatory response to gastrin.

There was a direct relationship between changes in fundic and antral SOM secretion and peripheral concentrations. In the past it has only been postulated through measurement of peripheral SOM immunoreactivity and HPLC analysis that circulating SOM stimulated by gastrin or CCK originates from the stomach (5, 38). The present study demonstrates that increases in fundic or antral SOM secretion are reflected by an increase in peripheral blood levels. Our results also suggest that peripheral plasma SOM concentrations probably better reflect fundic secretion. During the infusion of G-17S and the CCK-B/gastrin receptor antagonist a decrease in fundic SOM output and an increase in antral output resulted in an overall decrease in circulating SOM (Fig. 5).

SOM-14 was the predominant molecular form in the peripheral, antral, and fundic circulations after CCK or gastrin stimulation, consistent with the fact that >90% of the stored gastric SOM is SOM-14 (20, 23). The proportion of SOM-28 in the peripheral circulation was higher (7%) than that in the antral (3%) and fundic (0%) secretions, suggesting that some SOM originates from the small intestine, which has a higher proportion (50%) of SOM-28 (4). However, the longer half-life of SOM-28 (27) could explain the higher proportion of SOM-28 in the peripheral circulation.

Ranitidine decreased the basal peripheral and fundic plasma SOM concentrations, increased gastrin levels, and increased gastric pH. These results are consistent with histamine regulating SOM secretion either via acid secretion or by acting directly on the D cell (5, 16, 30). In addition to reducing basal SOM concentration, ranitidine abolished CCK-stimulated SOM secretion in both antrum and fundus and reduced gastrin-stimulated SOM in the fundus. However, gastrin-stimulated antral SOM secretion was not affected by ranitidine. Therefore, in the fundus, gastrin- and CCK-stimulated SOM secretion is mediated in part by the release of histamine, presumably from ECL cells. Both gastrin and CCK equipotently stimulate histamine release from rat fundic ECL cells via the CCK-B/gastrin receptor (21, 22), although the effect of the CCK-A receptor antagonist on CCK-8S-stimulated histamine release has not been reported. Histamine in turn stimulates SOM release, either directly (Zavros and Shulkes, unpublished data; and Ref. 30) or by increasing acid secretion (5, 16).

Antral SOM secretion in response to G-17S was not mediated by the release of histamine. This may be explained by the regional distribution of ECL cells, which are concentrated in the oxyntic mucosa (6). In the antrum, the histamine-containing cells are mast cell-like, and these cells do not secrete histamine in response to gastrin (35). In contrast to gastrin, CCK-stimulated antral SOM secretion was mediated by histamine. The mechanisms of the differential effect of ranitidine on CCK- and gastrin-stimulated antral SOM secretion are not apparent, but studies on the relative sensitivities of antral mast cells to gastrin and CCK would help clarify the issues.

The 30-min infusion of CCK-8S increased gastric pH from approximately pH 3 to pH 4 and this increase is probably related to the associated increase in SOM secretion. In agreement with this proposal, CCK-8S infused after the administration of either the CCK-A or CCK-B/gastrin receptor antagonists inhibited SOM secretion, and the gastric pH was unchanged from basal. Although SOM secretion increased during the infusion of G-17S alone or together with the type A receptor antagonist, the pH did not increase. This is consistent with the countervailing effect of gastrin stimulating gastric acid secretion (36). Longer infusions of gastrin produce a decrease in gastric pH (28). G-17S infused with the CCK-B/gastrin receptor antagonist resulted in an increase in gastric pH, although fundic secretion of SOM was inhibited. The increase in gastric pH during G-17S plus the type B antagonist is consistent with studies in conscious sheep (38), in which it was postulated that this increase in pH is related to the direct inhibition of the CCK-B/gastrin receptor on the parietal cell. However, CCK-B/gastrin receptors are also present on the ECL cell (22).

Our study has shown that SOM regulation in the fundus and antrum is not uniform. In the antrum and fundus CCK-8S stimulates SOM secretion by both the CCK-A and CCK-B/gastrin receptors, and these effects can be blocked by a histamine H₂ antagonist. G-17S-stimulated SOM secretion in the fundus is mediated by the CCK-B/gastrin receptor, either directly or with histamine as an intermediary, while in the antrum G-17S stimulates SOM secretion independent of either the type A or type B receptors. The combined effects of gastrin acting via a histamine-dependent and a histamine-independent pathway on SOM secretion may explain the greater potency of G-17S in the fundus compared with CCK-8S. Assessing the physiological significance of the present work is somewhat problematic, as the peptides infused singly cannot reproduce the postprandial milieu. However, this study provides important information on potential regulatory mechanisms among CCK, gastrin, and SOM in the fundus and antrum and supports the concept that postprandial gastrin and gastric acid secretion are regulated at least in part by the release of CCK and the subsequent stimulation of SOM (9, 14). It has been proposed that the excess gastrin and gastric acid secretion in patients with Helicobacter pylori infection and duodenal ulcer disease are related to a deficiency in SOM secretion (2, 9). Our finding that SOM regulation differs between

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fundus and antrum, taken together with reports that the gastric distribution of Helicobacter pylori can vary (2), suggests that both the antrum and fundus should be considered when determining the deficiency in gastric regulatory physiology in peptic ulcer disease.

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