Concurrent elevation of fundic somatostatin prevents gastrin stimulation by GRP

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Zavros, Yana, William R. Fleming, and Arthur Shulkes. Concurrent elevation of fundic somatostatin prevents gastrin stimulation by GRP, Am. J. Physiol. 276 (Gastro-intest. Liver Physiol. 39): G21–G27, 1999.—Gastrin-releasing peptide (GRP) can stimulate both gastrin and somatostatin (SOM) secretion, but, as gastrin increases SOM and SOM in turn inhibits gastrin, the overall endpoint in terms of gastrin output is variable. To examine the mechanisms involved, we compared the effects of GRP on gastrin secretion in normal sheep and sheep chronically immunized against SOM. In the normal animal, GRP had no effect on either plasma gastrin or SOM. However, in sheep immunized against SOM, GRP stimulated gastrin secretion, suggesting that the concurrent stimulation of SOM prevents the increase in gastrin secretion. To determine the local source of SOM, GRP was then infused into nonimmunized sheep with cannulas draining blood from the fundus and antrum. GRP stimulated fundic SOM output but inhibited antral SOM and gastrin secretion, demonstrating that the fundus was the source of the SOM. Because cholinergic interactions have a major influence on the effects of GRP, a cholinergic stimulus was administered, and we found that the responses were different: SOM output was inhibited in both the antrum and fundus, and antral gastrin secretion was increased. The present study demonstrates two further instances of the differential regulation of SOM from the antrum and fundus. GRP fails to stimulate gastrin because of an increase in fundic SOM, whereas gastrin levels increase following a cholinergic stimulus because of inhibition of both antral and fundic SOM secretion.

Gastrin-releasing peptide (GRP) is distributed throughout the mammalian gastrointestinal tract but is predominantly localized in the fundic and antral mucosal nerve fibers of the stomach (2). GRP, although named for its stimulatory effect on gastrin, is also a strong stimulant of the inhibitory peptide somatostatin (SOM) (6, 19, 24). The relative potency of GRP on gastrin and SOM release is variable and depends on the species and whether the studies are in the whole animal, isolated organ, or tissue sections (2, 6, 10, 12, 25). The concurrent increased secretion of SOM may be the reason for the failure in some circumstances for GRP to increase gastrin or gastric acidity (2, 23). SOM may originate from both the antrum and the fundus but is released via regionally distinct mechanisms (2, 36).

Administration of SOM antiserum potentiates GRP-stimulated gastrin secretion (6, 25), supporting a modulating role for SOM. However, this conclusion is from in vitro studies that use the acute administration of antiserum, which can cause short-term changes that may not be indicative of the steady-state regulatory role of SOM. We have therefore used a chronic model of sheep immunized against SOM (32).

The relative roles and mechanisms of release of SOM from the antrum and fundus in response to GRP have not been resolved. It has been claimed, using a number of in vitro models, that GRP-stimulated SOM secretion from the antrum is mediated by gastrin, whereas fundic SOM secretion is mediated by a neural mechanism (9, 24). However, these indirect mechanisms have not been confirmed when isolated D cells are used (23). To examine the relative contribution of the antral and fundic D cells to GRP-stimulated SOM release in a parallel fashion, we used sheep in which venous drainages of the antrum and fundus are accessed separately (36).

Cholinergic interactions have a major influence on the release and effects of GRP. Cholinomimetics or vagal stimulation releases GRP in vitro (6, 13). The effect of atropine on GRP-stimulated SOM and gastrin secretion is controversial, with no change (6), abolition (9, 19), or augmentation (24) demonstrated. The explanation of this latter finding is based on GRP activation of cholinergic neurons, which would act to inhibit SOM secretion (17, 19, 21, 33). These apparent differences may arise from variations in cholinergic regulation of SOM release between the antrum and fundus (12). We have therefore measured the effects of cholinergic stimulation on antral and fundic SOM secretion. This has been related to changes in antral gastrin output, as gastrin secretion is also under cholinergic control (31, 34). In vitro cholinergic agonists stimulate antral gastrin secretion, whereas they inhibit SOM release (21, 30), implying that the cholinergic inhibition of SOM contributes to the increase in gastrin release. However, assessment of this interaction requires the simultaneous measurement of antral and fundic SOM and antral gastrin secretion, which to our knowledge has not been reported.

Accordingly, the first objective of the study was to investigate the effect of GRP on SOM and gastrin secretion in normal sheep and gastrin secretion in sheep immunized against SOM. Second, the effect of GRP and a cholinergic stimulus (bethanechol) on antral and fundic SOM and antral gastrin secretion was determined in nonimmunized sheep by sampling blood from veins draining the antrum and fundus.
MATERIALS AND METHODS

Materials

Porcine GRP [GRP(1-27); Bachem] was dissolved in 0.02 M CH₃COOH-0.5% BSA to a final stock concentration of 50 nmol/ml. GRP was then diluted in 0.9% saline-0.1% BSA to obtain the administered peptide doses. Bethanexochol (Urecholine; Merck, Sharpe & Dohme, Sydney, New South Wales, Australia) was prepared in 0.9% saline-0.1% BSA to obtain a dose of 0.125 mg·kg⁻¹·h⁻¹. The proton pump inhibitor omeprazole (Astra Hassle) was also diluted in 0.9% saline-0.1% BSA to obtain the administered dose (bolus, 0.65 mg/kg; infusion, 0.50 mg·kg⁻¹·h⁻¹).

Animal Preparation

Chronic studies. UNTREATED SHEEP. The studies were performed in Merino-Corriedale crossbred sheep weighing 35-45 kg. Jugular vein cannulas were inserted as described in a previous study (35). After a local anesthetic (2 ml lignocaine, 1%) was administered, a cannula was inserted into the jugular vein directed toward the head (up), and the same procedure was followed for the contralateral jugular vein except the cannula was directed toward the heart (down). Agonists and antagonists were infused through the cannula directed downward, and blood was collected by the cannula directed upward. A 24-h recovery period was allowed, and animals were housed in metabolism cages for the duration of the experiments.

SHEEP IMMUNIZED AGAINST SOM. Ten Merino-Border Leicester crossbred sheep were immunized at regular intervals with SOM-14 conjugated to BSA with carbodiimide. The antigen used for immunization was a complex of SOM (Novabiochem, South Yarra, Victoria, Australia) and BSA (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) prepared as described previously (15). Sheep immunized with SOM were given subcutaneous injections of 1 ml of antigen solution (0.5 mg SOM/ml), emulsified in an equal volume of Freund’s complete adjuvant (Sigma Chemical, St. Louis, MO) and distributed between two sites in the neck, on three occasions separated by 21 days. After a period of ~6 mo, sheep were injected subcutaneously in the medial thighs, alternating left to right, on a further three occasions separated by 21 days with doses of 1 ml SOM antigen in saline emulsified in an equal volume of Freund’s incomplete adjuvant (Sigma Chemical). At the time of the experiment, titers (defined as dilution to give a bound-to-free ratio of 1 for ¹²⁵I-labeled SOM-14) ranged from 1:10² to 1:1.8 × 10⁶, with a median of 1:3.2 × 10⁵. Affinities ranged from 1.8 × 10¹⁰ to 2.8 × 10¹¹ l/mol. Control studies were performed on four sheep immunized against BSA and four noninjected sheep.

Acute studies. FUNDIC AND ANTRAL CANNULATIONS. Merino-Corriedale crossbred sheep had food withheld for 24 h before surgery. Anesthesia was induced by Pentothal injected into the jugular vein and maintained throughout the experiment with a 1-2% fluothane-oxygen mixture (ICI Australia Operations, Heidelberg, Victoria). The abdomen was opened by a midline incision with bilateral subcostal incisions. Cannulas (0.76 mm ID, 1.65 mm OD; Dow Corning Medical Materials) were inserted into the gastric vein draining blood from the fundus and the gastroepiploic vein draining blood from the antrum. The vein connecting the antrum to the fundus was ligated to prevent blood from flowing from the fundic to the antral vein and contaminating the samples. The stomach was cannulated by the insertion of a catheter (polyvinyl chloride, 10 mm ID × 15 mm OD) through a purse-string suture into the antral region of the abomasum. The cannula was secured by tightening a purse-string suture. Gastric juice was collected by first clearing the cannula contents and then aspirating ~5 ml of gastric secretion; the pH was then measured (36). Cannulas were also inserted into the jugular veins as described in Chronic studies. Basal samples were not collected until 30 min after the surgery was completed.

Experimental Protocols

Chronic studies. PROTOCOL 1: EFFECT OF GRP IN CONTROL SHEEP AND SHEEP IMMUNIZED AGAINST SOM. In preliminary studies, GRP was infused at doses ranging from 50 to 450 pmol·kg⁻¹·h⁻¹ in control sheep. No effect on plasma SOM or gastrin occurred within this dose range; therefore, all subsequent studies in the immunized and control sheep were performed at the intermediate dose of 300 pmol·kg⁻¹·h⁻¹. The infusion lasted for 60 min, and blood was collected every 15 min during the basal, peptide infusion, and postinfusion periods.

Acute studies. PROTOCOL 2: EFFECT OF GRP ON FUNDIC AND ANTRAL SOM AND GASTRIN SECRETION. Protocol 2 experiments were performed in nonimmunized sheep with cannulas in the gastric and gastroepiploic veins. The objective was to determine whether GRP has a differential effect on SOM secretion in the fundus and antrum and to determine whether there was a temporal relationship between fundic or antral SOM release and antral gastrin secretion. After a 30-min basal period, GRP (300 pmol·kg⁻¹·h⁻¹) was infused through the jugular vein cannula (down) at 20 ml/h for 60 min. Blood was collected from the jugular, fundic, and antral veins every 15 min during the basal, peptide infusion, and 30-min postinfusion periods. Gastric secretions were collected every 15 min, and pH was measured.

PROTOCOL 3: EFFECT OF BETHANECHOL ON FUNDIC AND ANTRAL SOM AND GASTRIN SECRETION. As in protocol 2, protocol 3 experiments were performed in nonimmunized sheep with cannulas in the gastric and gastroepiploic veins. To investigate the cholinergic regulation of fundic and antral SOM secretion and antral gastrin release, bethanexochol (0.125 mg·kg⁻¹·h⁻¹) was infused for 60 min at a rate of 20 ml/h through the jugular vein (down). Blood and gastric samples were collected as described for protocol 2.

Laboratory Methods

Radioimmunoassays. SOM. Plasma SOM was measured in ethanol-extracted plasma, as described previously (36). 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, the extracted plasma from the acute studies was reconstituted in 1 ml of assay buffer and 50-100 µl of reconstituted sample were assayed against a buffer standard curve. Only peripheral plasma was collected from the chronic experiments, and this was extracted and assayed using a charcoal-stripped, ovine-extracted plasma (CSOEP) standard curve. Antiserum 8402, which detects SOM-14 and SOM-28, was used at a dilution of 1:30,000, and antiserum 1296. Antiserum 1296 recognizes all carboxy-
terminal fragments larger than the pentapeptide and measures G-17s and G-17ns identically. The ID$_{50}$ was 1 pmol/ml, and the intra- and interassay coefficients of variation were less than 2 and 11%, respectively.

Statistical Methods

Results are expressed as means ± SE. Statistical comparison between more than two groups was made by one-way ANOVA followed by the Student-Newman-Keuls method. Statistical comparison of means between two groups was made using an unpaired t-test. P < 0.05 was considered significantly different. The integrated gastrin and SOM outputs were calculated as the area under the plasma peptide concentration time curve with respect to the average of the basal concentrations preceding the infusion (35).

RESULTS

Chronic Studies

Protocol 1: Effect of GRP in sheep immunized against SOM. Basal plasma gastrin levels in the control and immunized sheep were not significantly different (34 ± 6 and 26 ± 3 pmol/l, respectively). GRP infused at a dose of 300 pmol·kg$^{-1}$·h$^{-1}$ did not affect plasma gastrin in control sheep, but, in the immunized animals, plasma gastrin increased from 26 ± 5 pmol/l to a peak of 42 ± 3 pmol/l at 30 min (P < 0.05) (Fig. 1A). The integrated gastrin output during the 60-min GRP infusion was not changed from the basal level in the control sheep (0.06 ± 0.22 pmol·min$^{-1}$·ml$^{-1}$) (Fig. 1B) but was significantly elevated in the immunized sheep (0.53 ± 0.23 pmol·min$^{-1}$·ml$^{-1}$; P < 0.05) (Fig. 1B). In the control sheep, the withdrawal of the GRP infusion increased plasma gastrin from 39 ± 8 to 50 ± 10 pmol/l, whereas there was no further change in the immunized animals.

Acute Studies

Protocol 2: Effect of GRP on fundic and antral SOM and gastrin secretion. During the infusion of GRP to normal sheep, there was no significant increase in peripheral, antral, or fundic plasma SOM for any of the individual time points. This was probably due to the variance in the degree and timing of the response between the animals (Fig. 2). However, when the integrated responses were calculated, which measure the net rather than the absolute changes, GRP caused a small but significant increase in peripheral (0.42 ± 0.06 pmol·min$^{-1}$·ml$^{-1}$; P < 0.05), a large increase in fundic (3.76 ± 2.12 pmol·min$^{-1}$·ml$^{-1}$; P < 0.05), and a decrease in antral SOM output (−1.04 ± 0.87 pmol·min$^{-1}$·ml$^{-1}$; P < 0.05) (Fig. 3).

As expected, basal gastrin levels in the antral vein (129 ± 21 pmol/l) were significantly higher than those in the fundic (24 ± 6 pmol/l) and peripheral (38 ± 7 pmol/l) circulations (P < 0.05) (Fig. 2). Plasma gastrin remained close to basal concentrations in response to GRP in the antral, fundic, and peripheral circulations (Fig. 2B). In terms of integrated gastrin output, there were small decreases in gastrin output in the peripheral circulation (−0.36 ± 0.04 pmol·min$^{-1}$·ml$^{-1}$; P < 0.05) but no significant changes in the fundic and antral vein concentrations (Fig. 3). GRP caused an increase in gastric pH from a basal value of 3.94 ± 0.26 to 4.41 ± 0.52, but the increase was not significant (P > 0.05).

Protocol 3: Effect of bethanechol on fundic and antral SOM and gastrin secretion. Bethanechol significantly decreased antral vein plasma SOM concentrations (386 ± 138 to 128 ± 45 pmol/l), but the reduction was much greater in the fundic vein (2,397 ± 1,229 to 112 ± 51 pmol/l), and this was reflected in the fall in peripheral SOM concentration (150 ± 61 to 27 ± 10 pmol/l) (Fig. 4).

In contrast to the decrease in SOM concentrations, gastrin levels significantly increased in response to bethanechol in peripheral (16 ± 3 to 91 ± 20 pmol/l), antral (228 ± 64 to 1,355 ± 554 pmol/l), and fundic (13 ± 3 to 59 ± 18 pmol/l) plasma (P < 0.05) (Fig. 4, A–C, respectively).

Figure 5 presents results from Fig. 4 given as the integrated SOM or gastrin outputs. The SOM output was significantly suppressed by bethanechol in the peripheral (−5.20 ± 2.50 pmol·min$^{-1}$·ml$^{-1}$), antral...
28.50 ± 6.50 pmol·min⁻¹·ml⁻¹), and fundic (55.7 ± 19.8 pmol·min⁻¹·ml⁻¹) veins (P < 0.05) (Fig. 5), and this was associated with reciprocal increases in plasma gastrin output.

Figure 6 demonstrates the inverse relationship between peripheral and antral gastrin and peripheral, antral, and fundic SOM concentrations during the bethanechol infusions. There were significant inverse relationships between peripheral and antral gastrin and all three measures of SOM concentration.

Bethanechol caused a significant increase in gastric pH, from a basal value of 3.16 ± 0.12 to 6.07 ± 0.53 (P < 0.05).

DISCUSSION

A number of studies have established that GRP has the potential to stimulate both gastrin and SOM secretion [reviewed by Bunnell (2)], but, as gastrin increases SOM (5, 27) and SOM in turn inhibits gastrin secretion (4, 25), it is not surprising that the overall endpoint in terms of gastrin output can be quite variable. In vitro studies have provided evidence for potential mechanisms such as a tonic inhibitory role for SOM (25), different responses between antrum and fundus (12), and a participatory role for cholinergic innervation (14). However, the results are contradictory even in the one species and seem to depend on the type of model used. Furthermore, these in vitro studies do not determine whether the potential mechanisms are indeed influential in the intact organism. In the present work,
we used sheep chronically immunized against SOM (32) and sheep with fundic and antral vein cannulas (36) to show that the absence of a stimulatory effect of GRP on gastrin secretion is due to a concurrent increase in fundic but not antral SOM. Cholinergic stimulation had a different pattern of response, with a decrease in both antral and fundic SOM and an increase in gastrin.

GRP infusions did not alter peripheral SOM or gastrin concentrations in sheep. However, after the sheep were immunized against SOM, GRP significantly stimulated gastrin secretion, suggesting that the local release of gastric SOM is attenuating the secretion of gastrin. The postinfusion increase in plasma gastrin (Fig. 1) in the nonimmunized sheep is further indication that SOM is performing an active inhibitory role. Previous studies in the isolated perfused rat stomach support this proposal, as the addition of SOM antiserum augmented bombesin-stimulated gastrin secretion (6, 25). The present study demonstrates that this is a functional and relevant mechanism in vivo. Furthermore, the use of sheep chronically immunized against SOM allows the steady-state regulatory role of SOM to be determined rather than the perturbations in gastrin and gastric acidity associated with the acute administration of exogenous SOM antiserum. The usefulness of a chronic model was reinforced by the recent report that the SOM receptor subtype 2 knockout mice (a model that would chronically block most of the effects of SOM on gastrin and gastric acidity) had a normal plasma gastrin and gastric pH, although they were unresponsive to the inhibitory effects of SOM (20). As previously reported, the SOM-immunized sheep have high-titer, high-affinity antisera that can inhibit the biological effects of endogenous SOM (32).

To confirm that GRP stimulated the local release of SOM and to determine whether the antrum or the fundus was the source, blood was sampled from the antral and fundic veins of control sheep. GRP increased fundic but decreased antral SOM secretion. Despite the fall in antral SOM, gastrin secretion also decreased, suggesting that fundic SOM was having the more...

Fig. 5. Peripheral, antral, and fundic integrated outputs for SOM and gastrin in response to bethanechol. Outputs were determined for the 60-min peptide infusion with respect to basal. Results are expressed as means \( \pm \) SE (n = 4). *P < 0.05 vs. basal.

Fig. 6. A: relationship between peripheral gastrin and peripheral SOM concentrations during the infusion of bethanechol. B: relationship between antral gastrin and antral SOM concentrations during the infusion of bethanechol. C: relationship between antral gastrin and fundic SOM concentrations during the infusion of bethanechol. Individual concentrations for each sample taken during the 60-min infusion are plotted.
important influence on gastrin output. As recently summarized (23), the GRP effect on SOM is model and species dependent. In the rat, GRP increases both antral and fundic SOM (24, 25), but the initial claim that this is mediated via gastrin in the antrum and a neural mechanism in the fundus (24) has not been supported, at least for the fundus (23). GRP has no effect on isolated canine fundic D cells (4) and human antral D cells (1), which probably explains why GRP stimulates both gastrin and gastric acid secretion in these species (2). In theperfused pig stomach, GRP stimulates antral SOM (13) and inhibits fundic SOM (14), the opposite of our findings in the sheep. However, the pig studies were performed in separate in vitro preparations of the antral and nonantral parts of the stomach.

The decrease in antral gastrin secretion in response to GRP contrasts with in vitro findings that show a stimulation of gastrin from antral rat segments (24) and canine gastrin cells (8). An explanation of the absence of such an increase in gastrin in the sheep is the concurrent increase in fundic SOM, an influence that would not be observed in vitro in isolated G cells or in cases in which the antrum and fundus are examined separately. A previous study by Holst et al. (13) with the isolated pig antrum showed that intra-arterial GRP caused a dose-dependent increase in antral SOM, resulting in the net stimulation of gastrin at a low dose and an inhibition at higher doses of GRP consistent with a paracrine inhibitory role for SOM. The present work suggests that it is fundic SOM controlling antral gastrin, implying an endocrine mechanism. This is supported by the increase in SOM secretion measured in the peripheral circulation during the infusion of GRP to the antral and fundic vein-cannulated animals. However, basal plasma SOM is higher in the anesthetized animal (Fig. 2), and this peripheral increase in SOM during GRP infusion was not observed in nonanesthetized animals (Fig. 1). Alternatively, there may be a local fundic-antral portal connection as has previously been postulated (29). Similar to the rat, GRP increased gastric pH. In dogs and humans, acid secretion plateaus at higher doses of GRP despite further increases in gastrin (2). These results suggest that the concurrent release of SOM (from the fundus in the case of the sheep) is inhibiting acid secretion.

Vagal influence on gastrin, SOM, and acid secretion is mediated by cholinergic and noncholinergic mechanisms (14). The noncholinergic mediator of gastrin and SOM appears to be GRP (2), although GRP also stimulates the release of ACh (16). It was therefore of interest to compare the effects of cholinergic and GRP stimulation on gastrin and SOM secretion. The cholinergic agonist bethanechol significantly increased antral gastrin release and virtually abolished both fundic and antral SOM secretion. The response was quite different from the effect of GRP, which left antral gastrin secretion unchanged and produced a decrease in antral SOM and an increase in fundic SOM output. This suggests that the effects of GRP are not mediated by an increase in cholinergic activity.

During the cholinergic stimulation, there was a significant inverse relationship between gastrin and SOM secretion, but it is not known whether the increase in gastrin is the result of SOM withdrawal or a direct stimulatory effect on the G cell. Cholinergic agonists have a direct stimulatory effect on gastrin cells prepared from dog, rat, and rabbit antra (31, 34, 37) but have no effect on human G cells (17). The gastrin and SOM response to cholinergic (although not to GRP) stimuli in sheep resembles that found in the rat. In the isolated rat stomach, cholinergic stimulation resulted in the stimulation of gastrin release with the concurrent inhibition of SOM secretion (19, 21). Both antral and fundic SOM were inhibited when isolated segments were examined (26). In contrast, cholinomimetics stimulate antral SOM in human, dog, and pig but inhibit fundic SOM in the dog and pig, with no results reported for the human fundus [reviewed by Koop et al. (18)]. The bethanechol infusion was associated with an increase in gastric pH. A decrease might have been expected, since antral and fundic SOM secretion was inhibited, gastrin secretion was increased, and ACh is a direct stimulant of gastric acidity in many species including sheep (11, 28). However, ACh also increases emptying of rumen contents (pH around 6) into the abomasum, and this would neutralize the increase in gastric acidity (11).

Our study confirms previous reports that SOM regulation in the fundus and antrum is not uniform (12, 36, 22). Appreciation of the differential regulation and local release of SOM is relevant to explaining the absence of a gastrin-stimulating effect for GRP and the potent stimulation of gastrin by cholinergic stimuli. Understanding the mechanisms of these interactions is of general significance, as the gastrin and gastric acidity response to GRP has been proposed as a test for defining the deficiency in gastric regulatory physiology associated with peptic ulcer disease (3, 7).

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