Active immunization against somatostatin alters regulation of gastrin in response to gastric acid secretagogues

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Westbrook, Simon L., Graham H. McDowell, Kenneth J. Hardy, and Arthur Shulkes. Active immunization against somatostatin alters regulation of gastrin in response to gastric acid secretagogues. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G751–G756, 1998.—We have examined the coupling between somatostatin, gastrin, and gastric acidity, using sheep chronically immunized against somatostatin. All immunized sheep had high-titer (3.2 × 106 ± 1.1 × 106 M), high-affinity (1.5 × 109 ± 1.2 × 109 M) antibodies. However, basal gastrin and gastric acidity were similar to those in control animals, indicating that an inhibitory somatostatin tone was not required for the maintenance of normal basal gastrin and gastric acidity. Omeprazole (a proton pump inhibitor) increased gastric pH to a similar extent in both the control and immunized groups but resulted in a smaller increase in plasma gastrin in the immunized sheep, thus calling into question the assumption that hypergastrinemia associated with hypochlorhydria is the result of somatostatin withdrawal. Pentagastrin- or histamine-stimulated somatostatin secretion reversed or attenuated the omeprazole-induced hypergastrinemia in control but not immunized sheep, demonstrating a functional role for somatostatin and the biological efficacy of the somatostatin immunization. In a separate series of omeprazole-treated sheep, restoration of an acidic gastric pH with intragastric HCl reversed the hypergastrinemia in both control and immunized animals. We conclude that somatostatin is not essential for the acid-mediated regulation of gastrin. The use of a chronically immunized model as opposed to the acute administration of somatostatin antibodies has important advantages in determining the steady-state regulatory role of somatostatin.

histamine; proton pump; antibody

THE GASTRIC ACID feedback loop whereby increased gastric acidity inhibits gastrin secretion and decreased acidity stimulates gastrin release is the basis of the regulation of gastric acid secretion (1). Somatostatin is one of the more important components of the system, as it inhibits both gastrin and gastric acid secretion and is present in both antrum and fundus (4–6, 19, 20, 22). The precise contribution of somatostatin has been difficult to determine, as somatostatin is not only released into the circulation but also functions as a paracrine agent (10). One approach has been to use acute immunoneutralization with somatostatin antisera both in vitro and in vivo (5, 11, 14, 16, 20). However, administration of somatostatin antisera can cause short-term changes that may not be indicative of the steady-state regulatory role of somatostatin. We have therefore used a chronic model of sheep immunized against somatostatin. These sheep have high-titer, high-affinity antibodies but normal basal levels of gastrin and gastric acidity.

Two of the major stimulants of gastric somatostatin are gastric acid and gastrin (18). In vitro studies utilizing perfused rat, mouse, and pig stomachs indicate that acid is a direct stimulant of somatostatin secretion, which then acts on parietal and G cells to attenuate acid and gastrin secretion (6, 18). However, in vivo studies are less definitive, with changes in gastric acidity having no effect on circulating somatostatin in fasted normal subjects, presumably because the somatostatin is being released and acting locally (12). There is therefore doubt about the contribution of somatostatin to the acid-induced inhibition of parietal cell and G cell function.

The second important stimulant of somatostatin is gastrin, and we and others have shown that gastrin is a direct stimulant of somatostatin, independent of changes in gastric acidity (19, 22, 25). This direct effect of gastrin serves to restore somatostatin secretion and attenuate the gastrin response. However, it is not clear to what extent endogenous somatostatin serves to diminish the changes in gastrin.

The present study was conducted to determine the role of somatostatin in the regulation of gastrin and gastric acidity, using conscious sheep immunized against somatostatin. Changes in circulating gastrin and gastric pH were measured in response to the administration of pentagastrin (a nonimmunoreactive analog of gastrin), histamine (a putative mediator of the effect of gastrin (15)), and intragastric infusion of HCl. To avoid changes in luminal acidification influencing the secretion of gastric hormones, the sheep were pretreated with the gastric proton pump inhibitor, omeprazole.

MATERIALS AND METHODS
Sheep

Twenty-one crossbred sheep (Border Leicester × Merino; 11 ewes and 10 wethers) with live weights ranging from 36–45 kg were used in the experiments. Ten of the animals (5 ewes and 5 wethers) had been immunized against somatostatin previously (see below), whereas the remaining 11 (6 ewes and 5 wethers) served as controls, having received either no treatment (n = 6) or placebo injections (n = 5). The sheep were maintained individually in small pens in an enclosed shed lit artificially or in cages for the experimental procedures. Water was available ad libitum, and sheep consumed 800 g/day of a lucerne chaff and barley mixture.

Somatostatin Antigen and Immunization

The antigen used for immunization was a complex of somatostatin (Novabiochem, South Yarra, Victoria) and BSA
(Commonwealth Serum Laboratories, Parkville, Victoria) prepared as described by Hoskinson et al. (7). On three occasions, each separated by 21 days, sheep immunized with somatostatin were given subcutaneous injections of 1 ml of antigen solution (~0.5 mg somatostatin/ml) emulsified in an equal volume of Freund's complete adjuvant (Sigma, St. Louis, MO) and distributed between two sites in the neck. After a period of approximately 6 mo, on three further occasions separated by 21 days, sheep were injected subcutaneously in the medial thigh region, alternating from the left to right side, with doses of 1 ml somatostatin antigen in saline emulsified in an equal volume of Freund's incomplete adjuvant (Sigma).

Those sheep receiving placebo injections received 1 ml of a saline solution of BSA (0.75%, wt/vol) emulsified in adjuvant at times and in sites corresponding to those for immunized sheep.

**Surgical Preparation**

The surgery was performed 3–4 wk after the final immunization or placebo injection. Food and water were withheld for 24 h before induction with thiopental sodium and subsequent maintenance of anesthesia with halothane-oxygen. An indwelling catheter was inserted in each external jugular vein such that the catheter in the left vein (used subsequently for infusions) was inserted toward the heart, and the catheter in the right vein (used subsequently for the collection of blood) was inserted toward the head. Both catheters were filled with heparinized saline, and the patency of each catheter was maintained by flushing with minimum volumes of heparinized saline.

After the venous catheters were inserted, each sheep was placed in a lateral recumbency, and a right subcostal incision was made for insertion of a catheter (polyvinyl chloride, Dural Plastics; 1.0 mm ID, 1.5 mm OD) into the antral region of the abomasum (22) for the collection of abomasal (gastric) contents and to allow intragastric infusion.

Sheep were allowed 3–7 days to recover from surgery, during which time food intake returned to levels observed before surgery. During the first 3 days after surgery, each sheep received a daily intramuscular injection of 1 ml of tetracycline.

**Experimental Procedures**

The sheep were fasted overnight before each experiment, with at least 4 days between each experiment. Gastric juice and blood samples were obtained during a control period of 0.5 h, an omeprazole infusion period of 2 h, a test period in which omeprazole plus a test substance were infused for 2 h, and a final postinfusion period. All infusions were made up in normal saline and administered via the jugular vein catheter directed toward the heart. Blood was collected into chilled heparinized tubes, and after centrifugation the plasma was stored at −20°C pending analyses for hormones and antibodies.

Aliquots of gastric contents (5 ml) were collected at −1 min, hourly until 4 h, and then 40 min after the cessation of the intravenous test infusion. In the case of intragastric infusions, samples of gastric contents were collected immediately before each bolus infusion. The pH of each aliquot of gastric contents was determined using a pH meter.

The three test procedures were as follows:

**Omeprazole/ histamine.** The protocol followed was as described above for the omeprazole/pentagastrin experiment, except that histamine (John Bull, Melbourne) at the rate of 60 µg·kg⁻¹·h⁻¹ replaced pentagastrin.

**Omeprazole/ HCl.** Omeprazole was infused intravenously for 4 h as described above. A bolus infusion of 50 ml of 0.1 M HCl was administered rapidly at 2 h via the abomasal catheter, and then additional aliquots (50–100 ml) of 0.1 M HCl were administered at intervals of 20 min over the next 2 h to maintain gastric contents at pH ~2. Blockages of the gastric cannulas sometimes restricted sampling and gastric infusions. However, a complete set of results was obtained from 10 sheep (5 immunized and 5 control).

All procedures conducted were approved by the Animal Experimental Ethics Committees of the School of Agriculture, La Trobe University, and the Austin and Repatriation Medical Center.

**Analytical Procedures**

**Gastrin.** The concentrations of plasma gastrin were measured using the radioimmunoassay described by Shulkes and Hardy (21). The antiserum used in the assay recognizes all COOH-terminal fragments of gastrin amide larger than those of pentagastrin (thereby excluding measurement of infused pentagastrin). The sensitivity of the assay was 3.5 pmol/l, and the intra- and interassay coefficients of variation were less than 3% and 7%, respectively.

**Somatostatin.** Concentrations of somatostatin were measured only in plasma of control animals, due to the interference of antibodies to somatostatin with the assay in the immunized sheep. Plasma somatostatin was measured using a radioimmunoassay, as reported previously (22). Somatostatin was measured in ethanol-extracted plasma, using an antiserum that detected somatostatin-14 and somatostatin-28 with similar affinities. The sensitivity of the assay was 12 pmol/l, and the intra- and interassay coefficients of variation were less than 5% and 10%, respectively.

**Antisomatostatin antibodies.** Before the first experiment, somatostatin antibody titers and avidities in plasma were determined using previously published methods (5, 7).

**Statistical Analyses**

The results are expressed as means ± SE. Statistical analysis was by one-way analysis of variance followed by the Student-Newman-Keuls comparison.

**RESULTS**

**Antibodies to Somatostatin**

Somatostatin antibodies were not detected in plasma of control sheep. Plasma from all the immunized animals contained specific antibodies to somatostatin. The mean titer for sheep immunized with somatostatin was 3.2 × 10⁵ ± 1.1 × 10⁴ M, and the mean binding avidity of the antibodies was 1.5 × 10¹¹ ± 1.2 × 10¹⁰ l/mol.

**Response to Omeprazole/ Pentagastrin**

The responses to infusions of omeprazole alone or in combination with pentagastrin are shown in Fig. 1.

**Plasma gastrin.** Before commencement of infusions of omeprazole, plasma concentrations of gastrin for control (~40 pmol/l) and immunized sheep (~30 pmol/l) were not significantly different (P > 0.10). For both groups, gastrin increased significantly during the infusion of omeprazole, with the increase being greater for
control than for immunized animals, such that after 2 h of the omeprazole infusion, plasma concentrations were significantly higher for control than immunized sheep (97 ± 13 vs. 53 ± 9 pmol/l; P < 0.01).

In response to the concurrent infusion of pentagastrin and omeprazole, plasma gastrin decreased rapidly to 60 ± 8 pmol/l by 20 min (P < 0.05) in control sheep and remained at this level for the remainder of the infusion. This reduction in plasma gastrin was presumably because of the pentagastrin-induced increase in somatostatin secretion (Fig. 1B). At the end of the infusion of omeprazole plus pentagastrin in control animals, there was a rebound increase in gastrin, reflecting the withdrawal of the somatostatin inhibition. In contrast, plasma gastrin for immunized animals continued to increase in response to the addition of omeprazole plus pentagastrin, reaching concentrations that were significantly higher after 2 h than at the commencement of the infusion of omeprazole plus pentagastrin (85 ± 23 vs. 53 ± 9 pmol/l; P < 0.05). By 20 min after the cessation of the omeprazole plus pentagastrin infusion, plasma concentrations of gastrin had returned to levels similar to those measured after the 2-h infusion of omeprazole alone.

Plasma Somatostatin

Infusions of omeprazole alone did not affect plasma concentrations of somatostatin in control sheep. Within 20 min of the commencement of pentagastrin infusion (with omeprazole), plasma somatostatin had increased significantly (from 15 ± 3 to 197 ± 73 pmol/l; P < 0.01). Thereafter plasma concentrations decreased but remained significantly higher (P < 0.01) than during the infusion of omeprazole alone. After withdrawal of pentagastrin, plasma somatostatin returned to values that were not significantly different (P > 0.05) from basal levels.

Gastric acidity. At the commencement of infusions, the pH values of the gastric contents were similar for immunized and control sheep (2.1 ± 0.2 vs. 1.9 ± 0.1). For both groups, gastric pH increased gradually throughout the infusion of omeprazole alone, with a trend for gastric pH to plateau during the concomitant infusion of omeprazole plus pentagastrin. Gastric pH remained elevated between 5 and 6 in the postinfusion period. Compared with the immunized animals, the pH of the gastric contents for control animals at any particular time was not significantly different (P > 0.05). However, overall gastric pH was higher for control than immunized animals (P < 0.05) throughout the infusion period.

Response to Omeprazole/ Histamine

Plasma gastrin. As for the pentagastrin study, omeprazole increased plasma gastrin to a greater extent in control (from 36 ± 4 to 94 ± 10 pmol/l) compared with immunized animals (from 30 ± 6 to 58 ± 5 pmol/l) (Fig. 2A). The addition of histamine to the omeprazole infusion blocked any further increase in gastrin in the control animals, while gastrin continued to increase in the immunized animals. Throughout the treatment periods, the concentration of gastrin was significantly higher (P < 0.01) for control than for immunized sheep.

Plasma somatostatin. Plasma somatostatin for control sheep increased significantly (from 21 ± 2 to 43 ± 7 pmol/l; P < 0.05) in response to the addition of histamine, although this increase was much less than that seen with the pentagastrin infusion.

Gastric pH. Basal gastric pH and the increase in gastrin following the omeprazole and the omeprazole plus histamine infusion were similar (P > 0.1) for the control and immunized sheep.

Response to Omeprazole/ Intragastric HCl

Plasma gastrin. Intragastric administration of HCl to the omeprazole-treated sheep reversed the increase in gastrin in both the control and immunized sheep (Fig. 3). With the cessation of the HCl administration, plasma gastrin began to increase again, in parallel with the increase in gastric pH.

Plasma somatostatin. The intragastric infusion of HCl had no significant effect on the concentration of somatostatin for control sheep.

Gastric pH. As with the omeprazole infusions in the previous experiments, gastric pH increased from around 3 to between 5 and 6 at the end of the 2-h infusion period. Intragastric infusion of HCl quickly reduced the pH to between 2 and 3, with an increase in gastric pH.
All immunized sheep produced high-titer, high-affinity antisera against somatostatin. Although antibodies of a high titer are desirable, the effectiveness of immunoneutralization is largely dependent on the binding capacity of the antibody to the antigen [see Westbrook and McDowell (27)]. According to Holst et al. (5), the binding avidity of antisomatostatin antibodies in the order of $10^{11}$ l/mol, equivalent to those reported in the present study, is sufficient to neutralize the inhibitory actions of somatostatin on gastrointestinal function.

Despite the presence of the somatostatin antibodies, plasma gastrin and gastric acidity were similar to those in control animals. In contrast, the administration of exogenous somatostatin antisera results in an acute increase in gastrin and gastric acidity (5, 19, 20). These acute changes have been used as a measure of the tonic inhibitory role of somatostatin, but it is apparent from the present study that a tonic inhibitory effect of somatostatin is not necessary to maintain normal plasma gastrin and gastric acidity. Martinez et al. (14) administered somatostatin monoclonal antibodies to conscious rats and concluded that somatostatin does not play a tonic inhibitory role in basal gastric acid secretion. Holst et al. (6), using polyclonal somatostatin antisera in the isolated perfused pig antrum, suggested that gastrin secretion was not always under local somatostatin control. Other regulatory factors may come into play, but the relative contributions of the

DISCUSSION

Somatostatin is a key component in the regulation of gastric acid secretion. It is synthesized in both the body and antrum of the stomach and reduces acid secretion by direct inhibition of parietal cell function and indirectly through inhibition of gastrin release (1). The complexity of the control systems regulating gastric acid secretion and the multiple modes of action of somatostatin—paracrine, hormonal, and neural—have made it difficult to determine the actual as opposed to the potential roles of somatostatin. To determine quantitatively important endogenous actions of somatostatin, specific inhibitors are required. In the absence of an inhibitor of somatostatin, immunoneutralization has been used. These studies have generally used passive immunoneutralization involving the administration of exogenous antibody in the isolated perfused stomach and in antral and fundic cell cultures. With some exceptions in which monoclonal antibodies were used (11, 14), few studies have been performed in the whole animal because of the need for large amounts of reagent. Active immunoneutralization as performed in the current study allows the steady-state regulatory role of somatostatin to be determined in the conscious animal.
somatostatin-dependent and somatostatin-independent mechanisms remain to be determined.

The influence of somatostatin was clearly revealed after perturbation of the gastrin-gastric acid regulatory loop in four ways: administration of a proton pump inhibitor, a gastrin analog, histamine, and intragastric HCl. The results lead us to suggest that somatostatin has important but variable modulating roles in the dynamic regulation of gastrin and gastric acid secretion.

Omeprazole-induced achlorhydria was a potent stimulant of gastrin release, with plasma gastrin more than doubling within 60 min of commencement of the omeprazole infusion. It has been postulated that the hypochlorhydria stimulates gastrin by reducing somatostatin secretion (6). This is a removal of paracrine influence, since achlorhydria in the control sheep (Figs. 1–3) or in other studies (3, 13, 24) had no effect on basal plasma somatostatin levels. The present results do not support this proposal of a withdrawal of somatostatin tone, since the increase in plasma gastrin was less in the immunized than the control sheep given omeprazole (Fig. 1). There is no simple explanation for the paradoxical blunting in the gastrin increase in the immunized animals, especially as the increase in gastric pH was similar in the control and immunized animals. It is relevant that basal plasma gastrin was normal in the chronically immunized animals despite the absence of the tonic inhibitory effect of somatostatin that is thought to regulate basal gastrin. Because basal gastrin in this instance is being regulated by non-somatostatin-independent mechanisms, the superimposition of achlorhydria would not cause a reduction in somatostatin inhibitory tone. As noted previously, the evidence that the basal concentration of gastrin is determined by an inhibitory paracrine influence of somatostatin is based on the result of acute in vitro administration of somatostatin antisera, causing an increase in plasma gastrin concentration and gastric acidity (5, 6, 19, 20). It appears that in the absence of somatostatin, alternative regulatory mechanisms of gastrin secretion exist.

The biological efficacy of somatostatin immunization on a hormonal effect of somatostatin was clearly demonstrated by the administration of pentagastrin to omeprazole-treated sheep. In the nonimmunized animals there was a large increase in circulating somatostatin, which resulted in a significant decrease in circulating gastrin. This decrease in peripheral gastrin was not observed for the somatostatin-immunized sheep, and in fact plasma gastrin levels continued to increase. We conclude that antisomatostatin antibodies neutralized the pentagastrin-stimulated increase of somatostatin, thus removing the inhibitory actions of somatostatin on gastrin secretion. This finding is consistent with the concept of a regulatory pathway linking gastrin to somatostatin and serving to restore somatostatin secretion (22). Both the antrum and fundus are likely sources of the somatostatin, since in the sheep gastrin increases antral and fundic somatostatin secretion (28).

The concomitant infusion of histamine plus omeprazole was a less potent stimulant of somatostatin than pentagastrin plus omeprazole. Nevertheless, an acid-independent increase of somatostatin was demonstrated for histamine. The concentration of somatostatin increased by some 60% in response to histamine, and similar increases have been reported in other species (4). The histamine-induced rise in somatostatin for control sheep prevented any further increase in gastrin, whereas in the somatostatin-immunized sheep plasma gastrin continued to rise. The histamine-induced increase in somatostatin was not as marked as that observed after administration of pentagastrin, probably because only fundic somatostatin is stimulated by histamine; histamine in vitro is an inhibitor of antral somatostatin secretion (26).

Gastric somatostatin secreted by endocrine D cells of the fundic and antral mucosa functions as both a paracrine and an endocrine agent interacting with parietal, G, D, and enterochromaffin-like cells of the stomach (1). Immunization apparently interferes with both the paracrine (omeprazole-induced hypergastrinemia) and hormonal (pentagastrin-induced somatostatinemia) effects of somatostatin. The effectiveness of this immunization protocol in blocking paracrine interactions was previously demonstrated in a study from our laboratory in which gastrin-releasing peptide (GRP) was used as a stimulant of gastrin (23). In control sheep, GRP at doses ranging from 50 to 450 pmol·kg⁻¹·h⁻¹ had no significant effect on peripheral concentrations of gastrin or somatostatin. In contrast, infusions of GRP (300 pmol·kg⁻¹·h⁻¹) in sheep immunized against somatostatin elicited an increase of 60% in the peripheral concentration of gastrin. It was concluded that the immunization blocked the paracrine influence of somatostatin and that the absence of a gastrinstimulating effect of GRP in the sheep was due to a concurrent paracrine inhibitory effect of somatostatin.

Intragastric acid inhibits gastrin secretion, and there is a body of evidence suggesting that this inhibition is mediated by the release of somatostatin. Evidence in favor of this hypothesis may be found in studies with isolated perfused stomachs. For instance, somatostatin antibodies abolished the inhibition of gastrin secretion by acid in the isolated pig antrum (5) and increased basal acid secretion in the perfused mouse stomach (18, 19). However, the present work in the intact animal and data from previous studies in anesthetized humans (3, 8) are consistent with luminal acid reversing the hypergastrinemia independently of changes in somatostatin. Intragastric infusions of HCl reversed the omeprazole-induced achlorhydria and hypergastrinemia for both immunized and control sheep. Furthermore, intragastric infusions of HCl had no significant effect on plasma somatostatin in control sheep. In omeprazole-treated, fasted rats given intragastric acid, gastrin release is rapidly reversed but gastrin mRNA abundance and peptide concentration remain elevated, indicating a dissociation between synthesis and secretion (2). No indexes of somatostatin were measured after administration of acid, but, as noted by Dockray et al. (2), it was not clear how somatostatin release would account for the transient increase in gastrin synthesis. Indeed, somatostatin is an inhibitor of gastrin synthesis (9). In humans, varying gastric pH altered antral gastrin release without affecting antral somatostatin secretion (3, 8). These re-
sults, taken together with the present study, dearly indicate that changes in gastric pH can alter gastrin secretion via pathways that do not necessarily involve somatostatin. Furthermore, caution must be exercised in drawing a conclusion from in vitro studies to explain the situation in vivo, even in the same species. For instance, peripheral bombesin inhibits gastric acid secretion in the rat, which in vivo is somatostatin independent (14) but in vitro appears to be dependent on an increase in somatostatin secretion (17).

The present study demonstrates that the influence of somatostatin on gastric functions can be variable, depending on the dynamic status of the regulatory pathways. Consequently, the use of chronically immunized animals rather than acute administration of somatostatin antibodies (either in vitro or in vivo) has significant advantages in determining the physiological roles of somatostatin.

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