Amylin, Released From the Gastric Fundus, Stimulates Somatostatin and Thus Inhibits Histamine and Acid Secretion in Mice

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Background & Aims: **Amylin, a peptide that displays 50% homology with calcitonin gene-related peptide (CGRP), is colocalized with somatostatin in endocrine cells of the gastric fundus. The present study was designed to determine the mechanism of action of amylin on gastric exocrine and endocrine secretion.** ***Methods:*** **Acid secretion was measured in the isolated mouse stomach by titration. Somatostatin and histamine secretion were measured in rat fundic segments by radioimmunoassay.** ***Results:*** **In isolated mouse stomach, amylin caused a concentration-dependent decrease in acid secretion. In rat fundic segments, amylin and CGRP each caused a concentration-dependent increase in somatostatin and a decrease in histamine secretion. Changes in histamine secretion induced by amylin reflected changes in somatostatin secretion and could be abolished by addition of somatostatin antibody. Both the somatostatin and the histamine responses to amylin were abolished by the selective amylin antagonist AC187 but were unaffected by the CGRP antagonist CGRP8-37. In contrast, the responses to CGRP were abolished by CGRP8-37 but were unaffected by AC187. AC187 alone decreased somatostatin and increased histamine in fundic segments and increased acid secretion in isolated stomach, indicating that endogenous amylin participates in the regulation of gastric endocrine (somatostatin and histamine) and exocrine (acid) secretion.** ***Conclusions:*** **In gastric fundus, release of amylin from somatostatin cells interacts with distinct amylin receptors to enhance somatostatin secretion via an autocrine pathway that leads to inhibition of histamine and acid secretion.**

Amylin, also known as *islet amyloid polypeptide*, was first isolated from the pancreas of patients with non–insulin-dependent diabetes mellitus[1] and insulinoma.[2] Subsequently, it was localized to pancreatic insulin and somatostatin cells by in situ hybridization and immunohistochemistry.[3,4] Amylin, released in response to ingestion of a meal, inhibits glucose-stimulated insulin release,[5] stimulates pancreatic digestive enzyme secretion,[6] and retards gastric emptying.[7] More recently, amylin has been detected in several extrapancreatic sites including brain, lung, and stomach, with highest concentrations in gastric mucosa.[8,9]

Amylin consists of 37 amino acids and is structurally related to the calcitonin family of peptides that includes calcitonin, adrenomedullin, and calcitonin gene-related peptide (CGRP). Amylin displays about 25% sequence homology with calcitonin and adrenomedullin and 50% homology with CGRP.[10] CGRP receptors have been divided into 2 subtypes, $CGRP_1$ and $CGRP_2$, based primarily on the differential antagonist affinities of CGRP8-37, a potent and selective $CGRP_1$ antagonist.[11,12] Recently, molecular cloning techniques have revealed 2 distinct receptors for CGRP: one belonging to the rhodopsin family and the other to the calcitonin receptor-like (CRLR) family.[13] The CRLR, a 7-transmembrane-domain receptor, can function as either a CGRP receptor or an adrenomedullin receptor, depending on which members of a family of single-transmembrane-domain proteins, called *receptor-activity-modifying proteins,* or RAMPs, are expressed. When CRLR and RAMP1 were cotransfected into *Xenopus* oocytes, a CGRP pharmacological response was acquired, whereas cotransfection of CRLR and RAMP2 or RAMP3 resulted in an adrenomedullin receptor.[14–16]

In many tissues, CGRP and amylin activate adenosine 3′,5′-cyclic monophosphate (cAMP)-signaling pathways and produce similar biological effects.[1,10,17–19] The structural and functional similarities suggest that both peptides may interact with the same receptor. Supporting

Abbreviations used in this paper: CGRP, calcitonin gene-related peptide; CRLR, calcitonin receptor-like receptor; ED_{50}, 50% of maximal response; RAMP, receptor-activity-modifying proteins.

0016-5085/02/$35.00
doi:10.1053/gast.2002.34176

this notion, CGRP8-37 inhibits arterial vasodilation induced by either CGRP or amylin in mesenteric and renal blood vessels.[17,20] However, there is also ample evidence that these 2 peptides can mediate their actions through different receptors. In the Chinese hamster ovary cell line CHO-K1, CGRP is ~100 times less potent than amylin in activating adenylate cyclase activity, and the effect of CGRP, but not that of amylin, is blocked by CGRP8-37.[21] Similarly, the inhibition of twitch contractions in rat vas deferens induced by CGRP, but not that induced by amylin, is blocked by CGRP8-37.[22] In rat soleus muscle, the effect of amylin on glycogenolysis is blocked by the selective amylin antagonist AC187 but not by CGRP8-37.[23] Therefore, the current literature suggests that, depending on the tissue and species, amylin can interact either with CGRP receptors or distinct amylin receptors.

In the stomach, CGRP is present in extrinsic sensory neurons[24] and is capable of stimulating somatostatin and inhibiting gastrin and acid secretion.[25–27] Although amylin messenger RNA (mRNA) and immunoreactivity have recently been localized to endocrine cells in the fundus and antrum of rat stomach, the majority of which are somatostatin cells,[8,9] little is known concerning the role of amylin in the regulation of gastric exocrine and endocrine secretion. In conscious rats equipped with gastric fistula, intravenous infusions of amylin and CGRP inhibit acid secretion.[28,29] Because amylin receptors are present in the brain,[30] in vivo preparations cannot discriminate between central and peripheral effects of the peptide.

In the present study, we have used the isolated, luminally perfused mouse stomach to examine the effect of amylin on acid secretion and superfused rat fundic segments to examine the effect of amylin on somatostatin and histamine secretion; both preparations retain intact paracrine pathways but eliminate central nervous system and hormonal (e.g., gastrin) influences.[31] The results indicate that amylin, released from fundic somatostatin cells, enhances somatostatin secretion via an autocrine pathway that leads to inhibition of histamine and acid secretion.

Materials and Methods

Materials

Rat amylin, CGRP, and the CGRP antagonist hCGRP8-37[12] were purchased from Bachem (Torrance, CA). The well-characterized amylin antagonist AC187[23,32,33] was a gift from Dr. Andrew Young (Amylin Pharmaceutical Inc., San Diego, CA). Tetrodotoxin was purchased from Sigma Chemical Company (St. Louis, MO).

Animals

Albino mice, weighing 25–40 g, and Sprague-Dawley rats, weighing 250–400 g, were deprived of food overnight but allowed free access to water containing 10% glucose. The animals were anesthetized with 20% urethane (0.25 mL/50 g body weight), and injected intraperitoneally. The protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Isolation and Luminal Perfusion of Stomach

Mouse stomachs were isolated according to the methods of Bunce and Parsons[34] as detailed previously.[31] The stomach was cannulated at the esophageal and pyloric ends with polyethylene tubes (PE 160) then immersed in 20 mL of serosal solution with the following composition (in mmol/L): NaCl 115.4, $NaHCO_3$ 24.3, KCl 4.5, $MgSO_4$ 2.4, $CaCl_2$ 1.3, and dextrose 31. The lumen was perfused at the rate of 1 mL/min with a solution of the following composition (in mmol/L): NaCl 140, KCl 4.5, $MgSO_4$ 2.4, $CaCl_2$ 1.3, and dextrose 31. The serosal solution was gassed with 95% O_2.5% CO_2 and the luminal solution with 100% O_2. Drugs were added to the serosal solution.

Superfusion of Fundic Segments

The serosa and muscle layers were partly stripped off the proximal portion of rat stomach to improve drug delivery to the mucosa, and a segment, about 1 cm^2, was obtained from the fundic region as previously described.[35] Each piece of tissue (average weight 122 ± 7 mg) was cut into 6–8 segments, washed with saline, and placed on a porous grid separating the 2 halves of a minichamber (Swinnex 25; 1.4 mL volume; Millipore Corp., Bedford, MA). Krebs bicarbonate solution containing 0.2% bovine serum albumin, 4% dextran, and 4.5 mmol/L glucose was perfused into the bottom of the chamber at the rate of 1 mL/min and the effluent collected via a catheter leading from a small aperture at the top of the chamber. The perfusate was gassed with 95% O_2 and 5% CO_2. Drugs were delivered at the rate of 0.1 mL/min via a side arm close to the inlet. The entire preparation was contained within a chamber maintained at 37°C.

Experimental Design

For both mouse stomach and rat fundus, a 30-minute equilibration period was followed by an 80-minute sampling period. For isolated mouse stomach, the sampling period consisted of a 30-minute control basal period; a 20-minute period during which amylin (1 pmol/L to 1 μmol/L), the amylin antagonist AC187 (1 μmol/L), the CGRP antagonist CGRP8-37 (1 μmol/L), or a combination of AC187 (1 μmol/L) and CGRP9-37 (1 μmol/L) was added to the serosal solution; and a final 30-minute control period.

For rat fundic segments, the sampling period consisted of a 30-minute control basal period, a 20-minute period during which amylin or CGRP was superfused at various concentra-

tions (0.1 pmol/L to 0.1 μmol/L), and a final 30-minute control period. In some experiments, the selective amylin antagonist AC187 (10 nmol/L), the selective CGRP antagonist CGRP8-37 (10 nmol/L), or the axonal blocker tetrodotoxin was superfused for 20 minutes before superfusion with either amylin (1 nmol/L) or CGRP (1 nmol/L).

One-milliliter samples of the luminal effluent were obtained at 5-minute intervals from the isolated mouse stomach for immediate measurement of acid concentration by titration to pH 7.4 with 0.01 N NaOH using an automatic titrator (Radiometer, Copenhagen, Denmark). One-milliliter samples of the superfusate were obtained at 5-minute intervals from rat fundic segments and stored in 0.5-mL aliquots at −20°C for subsequent measurement of somatostatin and histamine concentrations by radioimmunoassay.

Radioimmunoassay

Somatostatin concentration was measured in duplicate by radioimmunoassay as described in detail previously.[36] Somatostatin antibody 1001 (final dilution 1:66,000) was a gift from Dr. Tadataka Yamada and Dr. John DelValle, University of Michigan (Ann Arbor, MI). [^{125}I]-Somatostatin was purchased from New England Nuclear (Boston, MA). The limit of detection was 4 pmol/L somatostatin, and the IC_{50} was 58 ± 10 pmol/L of sample (mean ± SD; n = 7 assays). Interassay and intra-assay coefficients of variability were 12% and 8%, respectively.

Histamine concentration was measured in duplicate using a commercial radioimmunoassay kit (Amac, Westbrook, ME) as previously described.[37] The kit includes tubes coated with monoclonal antibody against acylated histamine, acylating agent, and ^{125}I-histamine as tracer. The limit of detection was 0.1 nmol/L histamine, and the IC_{50} was 8 ± 1 nmol/L of sample (mean ± SD; n = 8 assays). Interassay and intra-assay coefficients of variability were 11% and 5%, respectively.

Data Analysis

Acid, somatostatin, and histamine secretions were expressed as the mean increase or decrease, in moles per minute or as percentage change, from the preceding basal level during the 5 minutes immediately preceding the experimental period. Changes in secretion were tested for significance using Student *t* test for unpaired values. All values are given as means ± SE of n experiments on different animals. Concentrations eliciting 50% of maximal response (ED_{50}) were calculated using linear regression analysis.

Results

Basal Acid, Somatostatin, and Histamine Secretion

Mean basal acid secretion in the isolated mouse stomach and mean basal somatostatin and histamine secretion in rat fundic segments were reproducible between animals and reverted to initial control levels at the end of the experimental period (isolated stomach: acid secretion, 81 ± 3 and 77 ± 3 nmol/min; fundic segments: somatostatin, 78.0 ± 6 and 85 ± 6 fmol/min; histamine, 427 ± 45 and 531 ± 55 nmol/min).

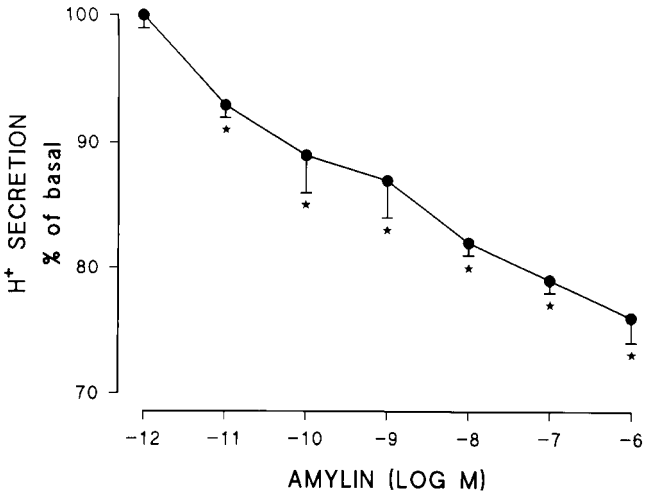

Figure 1. Effect of amylin (1 pmol/L–1 μmol/L) on basal acid secretion in isolated mouse stomach. Data are means ± SE of 6–8 experiments each. *Asterisks* denote significant difference from basal levels at $P < 0.01$.

Effect of Amylin, Amylin Antagonist, and CGRP Antagonist on Acid Secretion From Isolated Mouse Stomach

Addition of amylin, in the range of 1 pmol/L to 1 μmol/L, to the serosal solution of the isolated mouse stomach caused a prompt, reversible, and concentration-dependent decrease in acid secretion (Figure 1). The EC_{50} value was 9×10^{-11}. Maximal inhibition of acid secretion, expressed as the integrated 20-minute response, was obtained at a concentration of 1 μmol/L (24% ± 3% below basal level, $P < 0.001$, n = 6). Addition of the amylin antagonist AC187 (1 μmol/L) had an opposite effect and caused a prompt and reversible increase in acid secretion (9% ± 1% above basal level, $P < 0.001$, n = 5; Figure 2). The effect of the antagonist implies that endogenous amylin inhibits acid secretion.

The CGRP antagonist CGRP8-37 (1 μmol/L) also caused a prompt and reversible increase in acid secretion (6% ± 2% above basal level, $P < 0.05$, n = 5; Figure 2), implying that endogenous CGRP also exerts a tonic restraint on acid secretion. A combination of AC187 and CGRP8-37 increased acid secretion by 14% ± 1% ($P < 0.001$; n = 5), which was significantly greater than that achieved with each antagonist individually ($P < 0.01$ for the difference between the

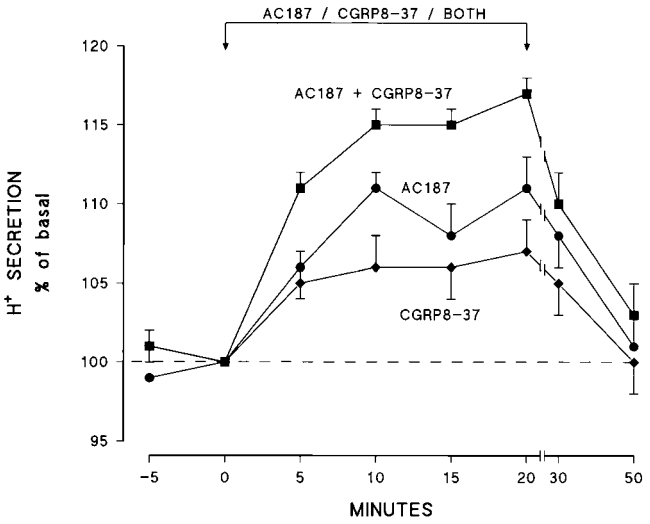

Figure 2. Time course for the effect of the amylin antagonist AC187 (1 μmol/L; ●), the CGRP antagonist CGRP8-37 (1 μmol/L; ◆), or a combination of AC187 and CGRP (1 μmol/L each; ■) on basal acid secretion in isolated mouse stomach. Data are means ± SE of 5 experiments each.

combination and either AC187 or CGRP8-37 alone; (Figure 2). The additive effects of the antagonists are consistent with the existence of distinct amylin and CGRP receptors that regulate acid secretion.

Effect of Amylin on Somatostatin and Histamine Secretion From Rat Fundic Segments

Superfusion of rat fundic segments for 20 minutes with amylin, in the range of 0.1 pmol/L to 0.1 μmol/L, caused a prompt, reversible, and concentration-dependent increase in somatostatin and decrease in histamine secretion (Figure 3). The EC_{50} value for stimulation of somatostatin secretion was 4×10^{-11} and for inhibition of histamine secretion was 5×10^{-11}. Maximal stimulation of somatostatin secretion (79% ± 9% above basal level, $P < 0.001$, n = 6) and inhibition of histamine secretion (32% ± 6% below basal level, $P < 0.01$, n = 6), expressed as the integrated 20-minute response, was obtained at a concentration of 10 nmol/L. The responses were not significantly affected by the axonal blocker tetrodotoxin (5 μmol/L; n = 5).

To determine whether the effect of amylin on histamine secretion was mediated by changes in somatostatin secretion, experiments were performed under conditions in which the effect of somatostatin was precluded. Superfusion with somatostatin antibody alone (final dilution 1:200) for 30 minutes caused an increase in histamine secretion (23% ± 2% above basal level, $P < 0.001$, n = 6; Figure 4), confirming previous studies showing that endogenous somatostatin exerts an inhibitory paracrine influence on histamine secretion.[38] In the presence of somatostatin antibody, the decrease in histamine secretion induced by amylin was abolished, implying that the effects were mediated by changes in somatostatin (Figure 4). Thirty minutes after cessation of superfusion with somatostatin antibody, histamine secretion remained slightly above basal control levels (Figure 4). This most likely represents a residual effect of the antibody as has been observed in prior studies.[39]

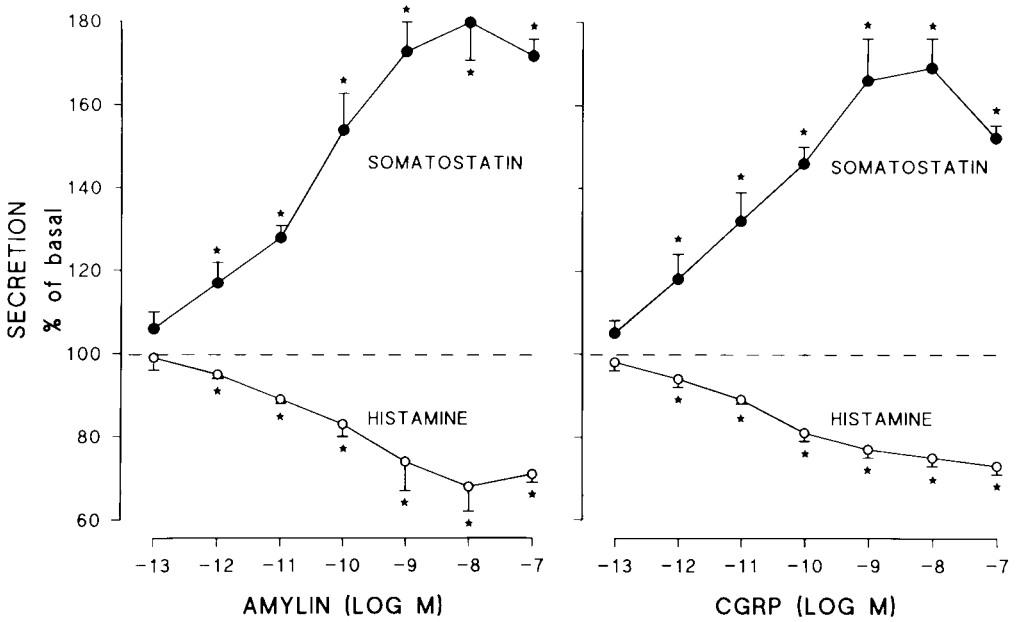

Figure 3. Effect of superfusion with amylin (0.1 pmol/L–0.1 μmol/L; *left*) or CGRP (0.1 pmol/L–0.1 μmol/L; *right*) on somatostatin (●) and histamine (○) secretion from rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6–8 experiments each. *Asterisks* denote significant difference from basal levels at $P < 0.05$.

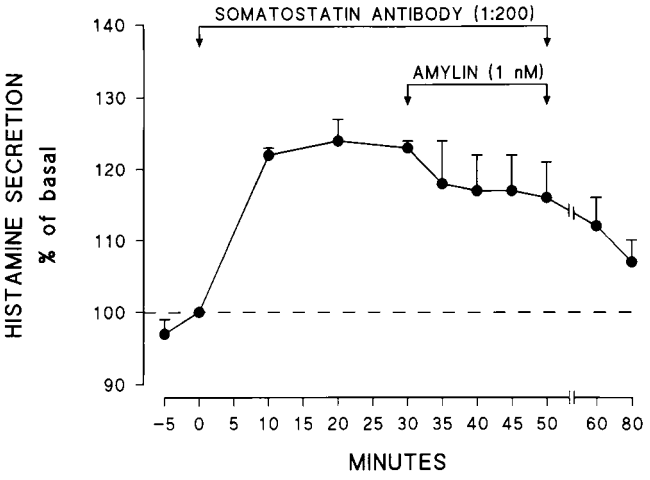

Figure 4. Time course for the effect of amylin (1 nmol/L) on histamine secretion induced by superfusion with somatostatin antibody (final dilution 1:200) in rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6 experiments each.

Effect of CGRP on Somatostatin and Histamine Secretion From Rat Fundic Segments

Superfusion of rat fundic mucosal segments for 20 minutes with CGRP, in the range of 0.1 pmol/L to 0.1 μmol/L, also caused a prompt, reversible, and concentration-dependent increase in somatostatin and decrease in histamine secretion (Figure 3). The EC_{50} value for stimulation of somatostatin secretion was 1×10^{-11} and for inhibition of histamine secretion was 2×10^{-11}. Maximal stimulation of somatostatin secretion (69% ± 7% above basal level, $P < 0.001$, n = 5) and inhibition of histamine secretion (25% ± 2% below basal level, $P < 0.001$, n = 5) was obtained at a concentration of 10 nmol/L.

Effect of Amylin Antagonist on Somatostatin and Histamine Secretion From Rat Fundic Segments

Superfusion with the amylin antagonist AC187 (10 nmol/L) for 20 minutes caused a decrease in somatostatin secretion and, thus, an increase in histamine secretion, implying that endogenous amylin exerts a regulatory influence on somatostatin and histamine secretion (Figure 5). The mean decrease in somatostatin secretion for the 20-minute period was 21% ± 2% below basal level ($P < 0.001$, n = 12), and the mean increase in histamine secretion was 21% ± 5% above basal ($P < 0.01$, n = 12; Figures 5 and 6).

AC187 (10 nmol/L) abolished the somatostatin and histamine responses to amylin (1 nmol/L) but had no significant effect on the responses to CGRP (1 nmol/L; Figures 5 and 6). The somatostatin and histamine responses to CGRP alone and in combination with AC187, respectively, were as follows: somatostatin, 67% ± 9% vs. 87% ± 12% above basal level (NS for the difference between the 2 responses, n = 6 each); histamine, 23% ± 3% vs. 30% ± 4% below basal level (NS for the difference between the 2 responses, n = 6 each; Figure 6). The

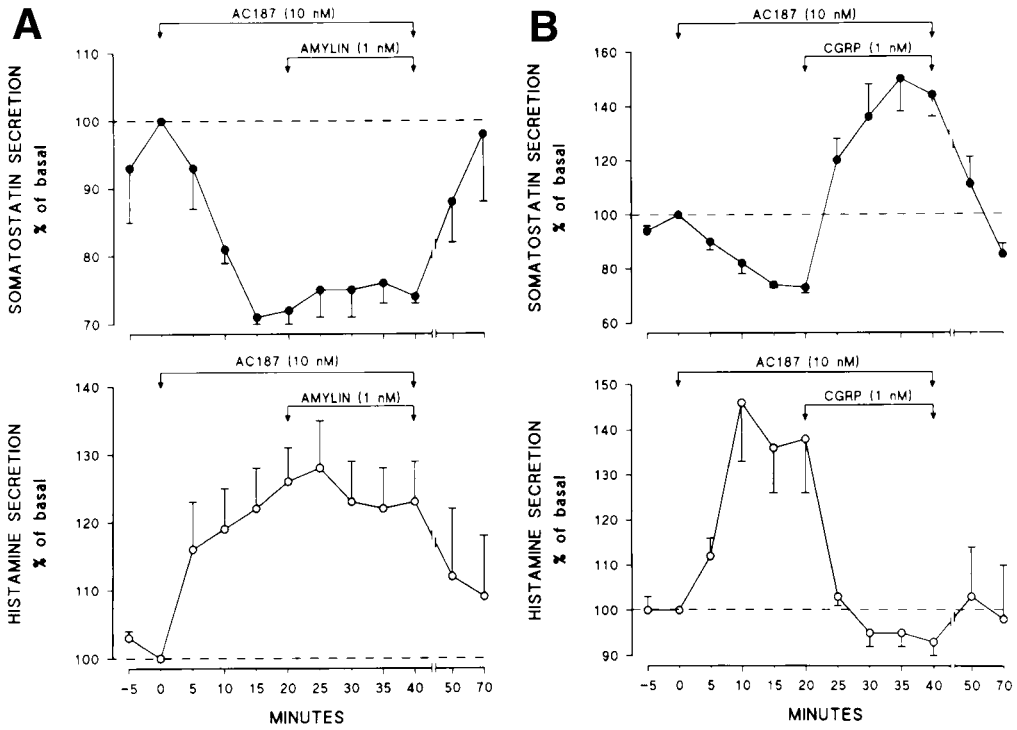

Figure 5. (*A*) Time course for the effect of superfusion with the amylin antagonist AC187 (10 nmol/L), alone and in combination with amylin (1 nmol/L) on somatostatin (●) and histamine (○) secretion from rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6 experiments each. (*B*) Time course for the effect of superfusion with the amylin antagonist AC187 (10 nmol/L), alone and in combination with CGRP (1 nmol/L) on somatostatin (●) and histamine (○) secretion from rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6 experiments each.

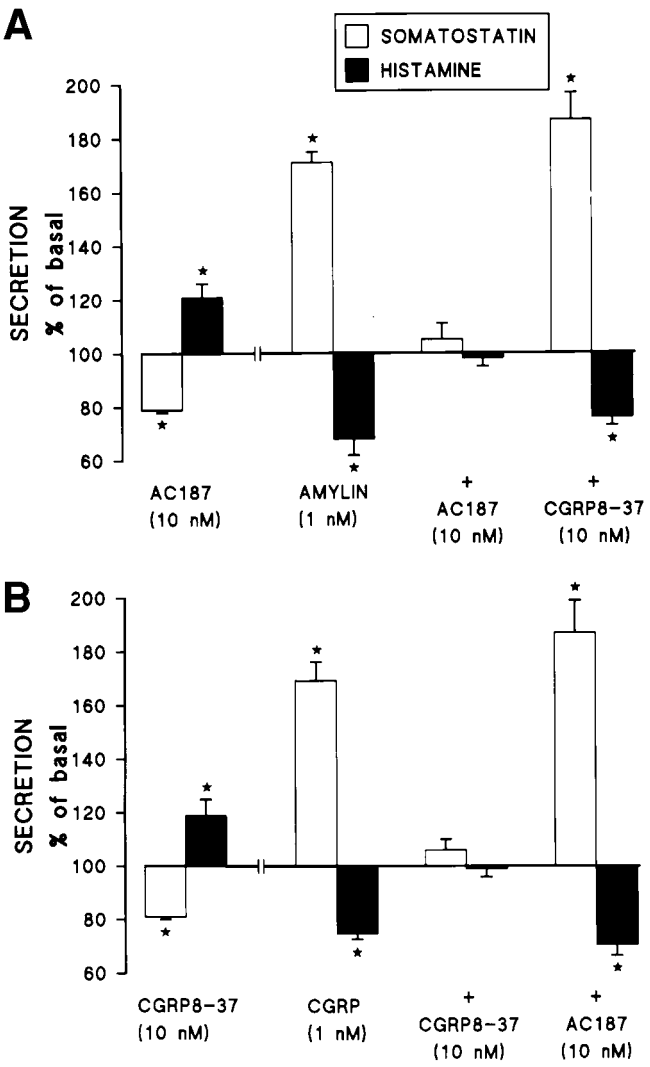

Figure 6. (*A*) Mean somatostatin and histamine responses during 20-minute period of superfusion of rat fundic segments with the amylin antagonist AC187, or with amylin alone and in combination with AC187 or the CGRP antagonist CGRP8-37. *Asterisks* denote significant difference basal levels at $P < 0.01$. (*B*) Mean somatostatin and histamine responses during 20-minute period of superfusion of rat fundic segments with the CGRP antagonist CGRP8-37, or with CGRP alone and in combination with CGRP8-37 or the amylin antagonist AC187. *Asterisks* denote significant difference from basal levels at $P < 0.01$.

divergent effects of the amylin antagonist on amylin- and CGRP-stimulated somatostatin secretion imply that the peptides interact with separate receptors.

Effect of CGRP Antagonist on Somatostatin and Histamine Secretion From Rat Fundic Segments

Superfusion with the CGRP antagonist CGRP8-37 (10 nmol/L) for 20 minutes also caused a decrease in somatostatin and increase in histamine secretion, implying that endogenous CGRP, like endogenous amylin, exerts a regulatory influence on somatostatin and histamine secretion (Figures 6 and 7). The mean decrease in somatostatin secretion for the 20-minute period was 18% ± 2% below basal level ($P < 0.001$, n = 12) and the mean increase in histamine secretion was 17% ± 5% above basal ($P < 0.01$, n = 12; Figure 7).

CGRP8-37 (10 nmol/L) abolished the somatostatin and histamine responses to CGRP (1 nmol/L) but had no significant effect on the responses to amylin (1 nmol/L; Figures 6 and 7). The somatostatin and histamine responses to amylin alone and in combination with CGRP8-37, respectively, were as follows: somatostatin, 71% ± 4% vs. 86% ± 10% above basal level (NS for the difference between the 2 responses, n = 6 each); histamine, 29% ± 7% vs. 24% ± 3% below basal level (NS for the difference between the 2 responses, n = 6 each). The fact that the CGRP antagonist blocked the effect of CGRP but not that of amylin further implies that the effects of the peptides are mediated via separate receptors.

Discussion

The present study examined the role of amylin, which is present in gastric endocrine (mainly somatostatin) cells in the regulation of somatostatin, histamine, and acid secretion in the fundus of the stomach. The results show, for the first time, a physiological role for fundic amylin in the regulation of gastric endocrine and exocrine secretion: endogenous amylin, acting via specific amylin receptors, stimulates somatostatin secretion via an autocrine pathway that acts to attenuate histamine and acid secretion. The evidence on which this conclusion is based can be summarized as follows.

First, in isolated mouse stomach, a preparation that retains intact paracrine pathways but eliminates the influence of gastrin, the amylin antagonist AC187 stimulated acid secretion, implying that endogenous amylin inhibits acid secretion. Consistent with this notion, amylin caused a concentration-dependent decrease in acid secretion.

Second, in segments obtained from rat gastric fundus, amylin and CGRP each caused a concentration-dependent increase in somatostatin and a decrease in histamine secretion. The changes in histamine secretion during addition of amylin reflected changes in somatostatin secretion. This was evident in experiments in which the influence of somatostatin was eliminated by addition of somatostatin antibody. Under these conditions, amylin did not alter histamine secretion. Somatostatin antibody by itself increased histamine secretion, consistent with previous studies showing that endogenous somatostatin

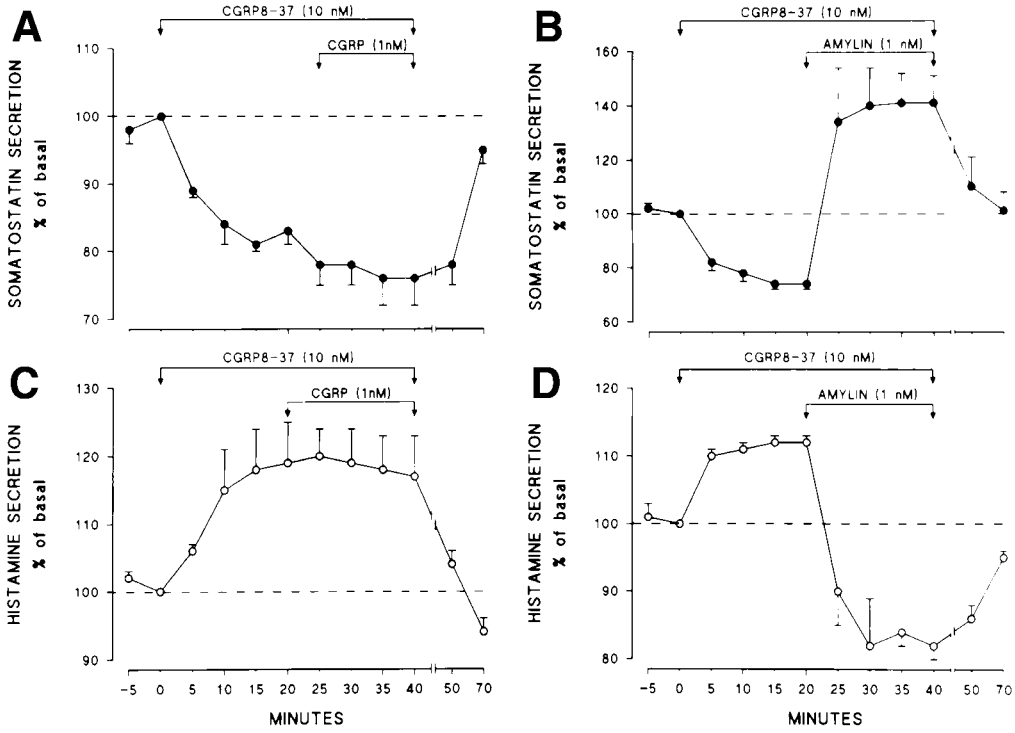

Figure 7. (*A*) Time course for the effect of superfusion with the CGRP antagonist CGRP8-37 (10 nmol/L), alone and in combination with CGRP (1 nmol/L) on somatostatin (●) and histamine (○) secretion from rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6 experiments each. (*B*) Time course for the effect of superfusion with the CGRP antagonist CGRP8-37 (10 nmol/L), alone and in combination with amylin (1 nmol/L) on somatostatin (●) and histamine (○) secretion from rat fundic segments. *Dashed line* indicates level of basal secretion. Data are means ± SE of 6 experiments each.

exerts a tonic inhibitory influence on the secretion of histamine.[38,40,41]

Third, the somatostatin and histamine responses to amylin in fundic segments were abolished by addition of the selective amylin antagonist AC187 but unaffected by addition of the CGRP antagonist CGRP8-37. In contrast, the responses to CGRP were abolished by CGRP8-37 but unaffected by AC187. The results imply that amylin and CGRP interact with distinct receptors to stimulate fundic somatostatin secretion. Consistent with this notion, AC187 and CGRP8-37 each stimulated acid secretion in isolated mouse stomach, and their effects were additive.

Fourth, the amylin antagonist AC187 alone decreased somatostatin and increased histamine secretion in fundic segments, implying that endogenous amylin exerts a tonic stimulatory influence on somatostatin secretion that acts to attenuate histamine secretion.

Although amylin and CGRP share similar structures and, in many tissues, similar actions,[1,10,17–20] in the stomach, their localization is quite different. CGRP is present exclusively in extrinsic sensory neurons that project into cell bodies in the dorsal root ganglia of the spinal cord and into the gastric mucosa.[24,42] Studies in rat, dog, and human indicate that CGRP stimulates somatostatin and inhibits acid secretion.[43–45] In those studies, the precise source of somatostatin could not be distinguished because somatostatin cells are present in both fundic and antral mucosa, and changes in somatostatin secretion detected in peripheral blood reflect net secretion from both regions. Using rat antral segments, Ren et al.[46,47] report that the CGRP antagonist CGRP8-37 inhibits somatostatin and stimulates gastrin secretion, implying that endogenous CGRP exerts a tonic stimulatory influence on antral somatostatin secretion. Stimulation of antral somatostatin and thus inhibition of gastrin secretion may represent one mechanism by which antral CGRP inhibits acid secretion. The present study suggests that CGRP, released from the fundus of the stomach, inhibits acid secretion by stimulating fundic somatostatin secretion. This is supported by the finding that the CGRP receptor antagonist CGRP8-37 alone inhibited somatostatin and stimulated histamine secretion, whereas exogenous CGRP had the opposite effect. The effect of the CGRP antagonist implies that endogenous CGRP contributes to ambient somatostatin secretion in the fundus of the stomach.

In the stomach, amylin is present in endocrine cells.[8] In mouse and rat fundus, the amylin-containing cells are located in the basal half of the mucosal glands, have cytoplasmic processes, and display somatostatin-like immunoreactivity; such colocalization suggests that amylin may function in an autocrine fashion. In support of this notion, (1) the amylin antagonist AC187 inhibited fundic somatostatin and thus stimulated histamine and acid secretion, and (2) the stimulatory effect of amylin on somatostatin secretion was unaffected by the axonal blocker tetrodotoxin.

The inhibitory effects of amylin on acid secretion and the stimulatory effects on somatostatin secretion are consistent with and extend beyond those obtained by Rossowski et al.[28,29] in conscious rats equipped with gastric fistula. These investigators showed that (1) intravenous infusions of amylin inhibited basal, pentagastrin-, and 2-deoxy-D-glucose-stimulated acid secretion, and (2) the effect of amylin on pentagastrin-stimulated acid secretion was inhibited by infusion of a selective somatostatin subtype 2 receptor antagonist. Studies in conscious animals, in which agonists and antagonists are administered systemically in vivo, cannot distinguish between peripheral and central actions of amylin and somatostatin[48] and do not necessarily reflect the action of amylin in the stomach. The present study indicates that endogenous amylin, released from the fundus of the stomach, acts in an autocrine fashion to stimulate fundic somatostatin and thus inhibit histamine and acid secretion.

There had been some debate as to whether amylin and CGRP act via a common CGRP receptor or whether some tissues have an additional high-affinity amylin receptor. Although the greater potency of amylin in some systems suggests the existence of separate amylin receptors, definitive proof awaited the development of the selective amylin antagonist AC187.[32] There is now abundant evidence, derived both from physiologic and radioligand binding studies using selective CGRP and amylin antagonists and, more recently, from molecular biological studies in which rabbit aortic endothelial cells containing a calcitonin receptor were cotransfected with RAMP1 and RAMP3 that, in some tissues, amylin and CGRP interact with distinct receptors.[21–23,49,50] In the present study, although amylin and CGRP both stimulated somatostatin and thus inhibited histamine secretion with similar efficacy and potency, the responses to amylin were abolished by AC187 but unaffected by CGRP8-37, whereas the responses to CGRP were abolished by CGRP8-37 but unaffected by AC187. Furthermore, the effects of the antagonists on acid secretion were additive. Thus, the results strongly indicate that amylin and CGRP interact with distinct receptors to stimulate somatostatin secretion from the fundus of the stomach.

In conclusion, the present study shows, for the first time, a physiologic role for amylin in the regulation of gastric endocrine and exocrine secretion. The results show that release of endogenous amylin from somatostatin cells in the fundus of the stomach enhances somatostatin secretion via an autocrine pathway that leads to inhibition of histamine and acid secretion. Stimulation of somatostatin secretion occurs via high-affinity amylin receptors that are distinct from CGRP receptors.

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Received January 18, 2002. Accepted April 8, 2002.

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Supported by grants from the Veterans Administration Medical Research Fund and Glaxo Wellcome Institute for Digestive Health (to L.V.).