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Research Article

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Differential Control of Somatostatin Messenger RNA in Rat Gastric Corpus and Antrum

Role of Acid, Food, and Capsaicin-sensitive Afferent Neurons

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Abstract

Somatostatin messenger RNA in the antrum and corpus of rat stomach was quantified by Northern and slot blotting using a probe generated by the polymerase chain reaction. Fasting for 48 h enhanced the abundance of somatostatin mRNA in the pyloric antral region, but not in the acid-secreting region of the stomach. In fasted rats, somatostatin mRNA in antrum, but not corpus, was decreased by inhibition of acid secretion with omeprazole. In contrast, in rats treated with capsaicin to lesion small diameter afferents there was a significant decrease in somatostatin mRNA abundance in the corpus but not antrum. The effects of capsaicin cannot be attributed to nonspecific changes in gastric endocrine cell gene expression, since the abundance of histidine decarboxylase mRNA (which is a functionally regulated marker for a different gastric endocrine cell type) did not change with capsaicin. Gastric capsaicin-sensitive afferents are rich in calcitonin gene-related peptide, and in rats with antibodies to this peptide there was reduced corpus somatostatin mRNA. Moreover, infusion of calcitonin gene-related peptide in control rats produced a significant increase in somatostatin mRNA in the gastric corpus. The results indicate that somatostatin mRNA abundance is controlled by the gastric luminal contents and the extrinsic afferent innervation, but the relative importance of these factors differs in antrum and corpus: luminal contents are relatively more important in antrum and primary afferents using calcitonin gene-related peptide in the corpus. (*J. Clin. Invest.* 1993. 91:244–250.) Key words: somatostatin • omeprazole • food • capsaicin • mRNA • calcitonin gene-related peptide

Introduction

The secretory functions of the gastric mucosa are regulated by the luminal contents of the stomach, and by humoral factors which include neurotransmitters such as acetylcholine, peptide hormones like gastrin, and paracrine regulators such as histamine and somatostatin (1). It is now thought that somatostatin acts locally in the antral mucosa to mediate the well known inhibitory effect of luminal acid on gastrin (G−) cell function (2–4). However, somatostatin is also released by endocrine (D−) cells in the acid-secreting mucosa where it is thought to inhibit acid secretion at least in part by direct action on parietal cells (4–6). The factors controlling gastric somatostatin release have been intensively studied in a variety of different preparations, including the perfused stomach and isolated D cells in short-term culture (3, 7–10). The available data indicate that in addition to luminal acid a number of peptides and neurotransmitters influence somatostatin release, among which is the neuropeptide calcitonin gene-related peptide (CGRP)[1] (11–13). The latter is of interest, because it is localized exclusively to the primary afferent innervation of the stomach. Thus, > 80% of gastric spinal afferents contain CGRP immunoreactivity compared with < 10% of vagal afferents; CGRP is absent from intrinsic gastric neurons in the rat, but is present in the peripheral terminals of afferents found in mucosa, submucosa, muscle layers, and myenteric plexus (14–18).

Recent work suggests that the luminal contents of the stomach determine not only somatostatin release but also the abundance of somatostatin mRNA in the gastric mucosa. Brand and Stone (19) described reciprocal decreases in antral somatostatin and increases in gastrin mRNA abundance in rats treated with the H^+/K^+ ATPase inhibitor omeprazole; Wu et al. (20) also reported that reduction of acid secretion by fundectomy had similar effects. However, the factors controlling somatostatin mRNA abundance in the acid-secreting part of the stomach remain uncertain. We describe here the results of modulating the gastric luminal contents and of lesioning primary afferents on the abundance of somatostatin mRNA in corpus and antrum. The results are interesting in that they suggest differential control of somatostatin mRNA abundance in corpus and antrum, and identify CGRP released by small diameter (capsaicin-sensitive) primary afferent neurons as one of the influences regulating corpus mRNA levels.

Methods

Animals. The present studies were performed on female Wistar rats weighing ~ 250 g. They were housed on a 12-h light-dark cycle. For experiments involving withdrawal of food, animals were kept in individual wire-bottomed cages and allowed access to water ad lib.

Experimental treatments. The relative importance of food and acid in the gastric lumen was determined by studies in experimental rats that were either fed ad lib. or fasted for 48 h. Both groups included rats that were treated with vehicle (0.25% wt/vol, methyl cellulose, 3 ml by gavage, at 0 and 24 h), or with omeprazole (400 μmol/kg in vehicle, at 0 and 24 h). Animals were killed after 48 h and tissues taken as described below.

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1. *Abbreviations used in this paper:* CGRP, calcitonin gene-related peptide; HDC, histidine decarboxylase; PCR, polymerase chain reaction; SSPE, sodium chloride sodium phosphate EDTA buffer.

The role of small diameter primary afferent neurons was examined by studies in rats treated with the selective sensory neurotoxin, capsaicin. Neonatal rats (0–24-h old) were anesthetized with halothane and received capsaicin (50 mg/kg, subcutaneously in Tween:ethanol:water, 10:20:70) or vehicle. They were then reared normally. The success of the capsaicin-treatment was verified by the absence of an eye-wipe response to 0.1% (wt/vol) capsaicin when applied topically to adult animals.

The role of CGRP was examined using (*a*) immunoneutralization to block endogenous CGRP, and (*b*) by intravenous infusion of exogenous peptide. The protocol used to generate neutralizing antibodies has previously been described (21). In brief, experimental animals were induced to produce their own antibody by immunization with rat Tyr^0 $\alpha CGRP_{28-37}$ conjugated to thyroglobulin by glutaraldehyde as described previously (21). Animals received the equivalent of ~ 25 nmol of peptide subcutaneously in CFA when their body weight was 100 g, and two further injections at 4–6-wk intervals of ~ 12 nmol. Control rats were immunized with thyroglobulin alone. Blood samples were taken from a tail vein 7–10 d after the third immunization for monitoring of antibody titres. Thereafter animals were taken for experiments as described below. The presence of circulating CGRP antibodies was verified by incubation of plasma in serial dilutions with ^{125}I-labeled rat Tyr^0 $\alpha CGRP_{28-37}$ as previously described (15, 21, 22).

The action of exogenous CGRP was examined by intravenous infusion in control and capsaicin-treated rats. Animals were anesthetized with pentobarbitone sodium (60 mg/kg) and an indwelling line established in the jugular vein and exteriorized at the back of the neck. The following day, animals were given intravenous infusions of either rat α-CGRP (1 nmol/kg per h) or saline, for 6 h. The animals were then killed and corpus taken for mRNA analysis.

Tissue extraction. Animals were killed by decapitation and trunk blood collected for radioimmunoassay of plasma gastrin which was used to monitor the effects of food deprivation and omeprazole treatment (23). Plasma gastrin was determined using antibody L2 which is specific for the COOH terminus of heptadecapeptide gastrin (24). The antral and corpus regions of the stomach were separately excised leaving behind a generous band of tissue at the antro-corpus border. A modification of the method of Chirgwin et al. (25) was used to extract total RNA. Briefly, tissues were homogenized in 10 vol of 4 M guanidinium isothiocyanate, containing 25 mM sodium acetate, pH 6.0 and 0.84% (vol/vol) β mercaptoethanol. The homogenate was adjusted to 2.3 M CsCl containing 25 mM sodium acetate, pH 6.0, layered on a cushion of 5.7 M CsCl and centrifuged at 135,000 *g* for 20 h. RNA pellets were redissolved in Tris-EDTA containing 0.2% SDS, and reprecipitated with 3 vol ethanol in the presence of 0.3 M sodium acetate pH 6.0. The recovery of RNA per unit weight of antrum (i.e., micrograms total RNA per milligram tissue) in the various experimental groups was not significantly different (analysis of variance) from their respective controls (fasting: 2.2±0.1, fasting and omeprazole: 2.2±0.2; fed: 2.2±0.2, fed and omeprazole 2.4±0.2; capsaicin: 1.5±0.1, vehicle, 2.1±0.3); there were slightly higher recoveries of RNA from the corpus, but the difference between corpus and antrum was not significant and again there were no significant differences between experimental groups and controls (fasting: 4.4±0.6, fasting and omeprazole: 3.5±0.5; fed: 4.2±0.7, fed and omeprazole: 4.5±0.6; capsaicin 2.5±0.2, vehicle 1.8±0.2).

Preparation of probes. The identification of somatostatin mRNA was made using a cDNA probe generated by the polymerase chain reaction (PCR). Oligonucleotide primers corresponding to sequences in the 5′ noncoding (sense) and 3′ noncoding (anti-sense) regions of rat somatostatin cDNA (26) were synthesized on a DNA synthesizer (391; Applied Biosystems, Inc., Foster City, CA) and purified by ion exchange HPLC. PCRs were performed in 1X Taq polymerase buffer (Promega Corp., Madison, WI) containing dATP, dGTP, and dTTP (200 μmol; Pharmacia Fine Chemicals, Piscataway, NJ), oligonucleotide primers (50 pmol each), cDNA template (~ 2 μg), Taq DNA polymerase (2 U; Promega Corp.), and either 200 μmol dCTP or 2 μmol dCTP and [^{32}P]dCTP (100 μCi; ~ 3,000 Ci/mol; Amersham Corp., Arlington Heights, IL). After initial denaturation, 33 cycles of amplification (55°C for 1 min, 72°C for 2 min, 95°C for 1 min) were performed followed by a final extension cycle of 10 min at 72°C. The cDNA template used for these studies was generated from rat antral total RNA using a commercially available kit (Boehringer Mannheim Corp., Indianapolis, IN). Probes were purified on Qiagen-tip 5 mini columns (Diagen, Dusseldorf, Germany). In some experiments, gastrin, histidine decarboxylase (HDC), and β actin mRNA's were studied using radiolabeled PCR products of 362 bp, 1,238 bp, and 644 bp, respectively, generated by inclusion of [^{32}P]dCTP in the reaction mixture as previously described (23, 27). Gel electrophoresis of the somatostatin PCR product and of the internally radiolabeled probe confirmed a size compatible with the predicted one of 464 bp, and digestion with PvuII generated products consistent with the predicted fragments of 289 and 164 bp (Fig. 1).

DNA sequencing. Somatostatin PCR product was generated as described above, purified by Qiagen Tip-5 column, precipitated with isopropanol and the pellet dissolved in TE buffer. The concentration of DNA was estimated using a fluorometer (TKO 100; Hoefer Scientific Instruments, San Francisco, CA) and ~ 1 pmol heated at 90°C for 3 min then snap frozen on dry ice/ethanol. Forward or reverse sequencing primer (i.e., sense or antisense PCR primer, 1 pmol) was added and the mixture annealed at room temperature for 30 min. Sequencing was performed using a T7 DNA sequencing kit (Promega Corp., Southampton, UK) according to standard protocols using [^{32}P]dATP. Sequencing reactions were electrophoresed on 6% polyacrilamide gels, and the dried gels exposed to Kodak RP film for 48 h.

Northern blots and quantification. Samples of total RNA in rat stomach extracts were analyzed by Northern and slot blot. Aqueous samples of total RNA (20 μg) were denatured in 3 vol of formamide:formaldehyde:10 × 3-(4-Morpholino)propane sulfonic acid (MOPS) (4:1.75:1) at 55°C for 15 min and applied to 1% agarose formaldehyde gels. After electrophoresis, RNA was electroblotted onto nylon membranes (Hybond N; Amersham Corp.) and cross-linked by ultraviolet light. For slot blots, total RNA was denatured as above and blotted onto Hybond N membranes using a commercial slot blot apparatus, according to the manufacturers instructions (Bio-Rad Laboratories, Hemel Hempstead, UK). Membranes were prehybridized at 42°C for 6 h in 50% formamide containing 5 × Denhardt's solution, 5 × sodium chloride sodium phosphate EDTA buffer (SSPE), 0.5% SDS and sonicated salmon sperm DNA (200 μg/ml), then hybridized overnight with 2×10^6 cpm/ml of cDNA probe.

Membranes were washed twice with 2 × SSPE containing 0.1% SDS at room temperature for 20 min, once with 0.1 × SSPE/0.1% SDS at 65°C for 20 min, then exposed to Kodak X-AR film at −70°C with an intensifying screen for 24–48 h. Signals were removed from mem-

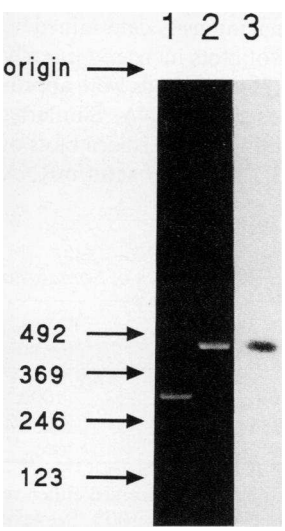

Figure 1. Agarose gel separation of PCR-generated somatostatin DNA. Samples were applied to a 1.5% agarose gel containing 0.1 μg/ml ethidium bromide, and electrophoresed in Tris-borate-EDTA. Markers are 123 bp ladder (Gibco BRL, Paisley, UK). Lane *1*, PvuII digest of PCR-generated DNA; predicted products 289 and 164 bp. Lane *2*, undigested PCR product; predicted size 464 bp. Lane *3*, ^{32}P-labeled somatostatin PCR probe (see Methods) after exposure to Kodak X-AR x ray film.

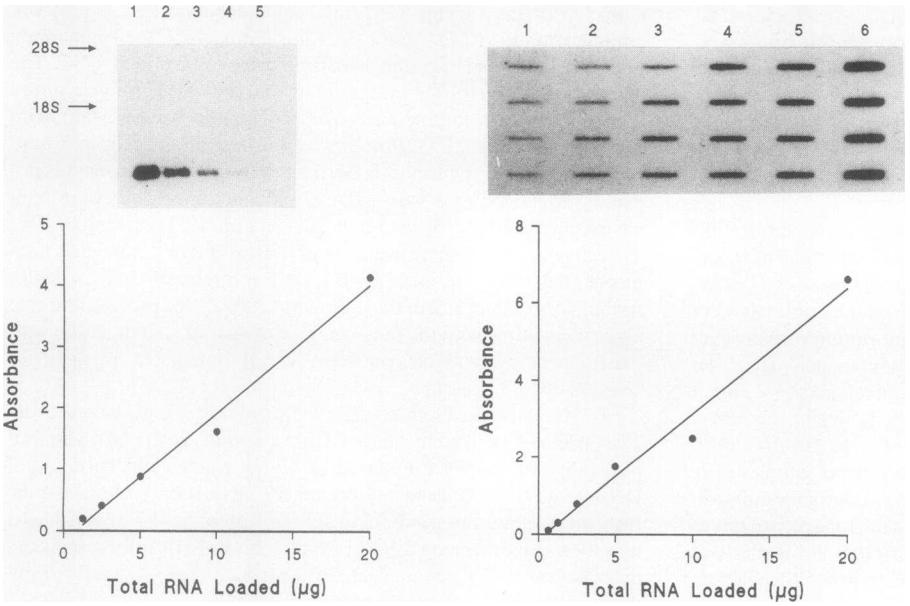

Figure 2. Relationship between the densitometric signal and graded quantities of total RNA. Upper left hand panel, Northern blot of gastric somatostatin mRNA. Samples of 20, 10, 5, 2.5, and 1.25 μg total antral RNA (lanes *1–5*, respectively) were electrophoresed in a 1% agarose formaldehyde gel as described in the text, transferred to Hybond N membrane, and hybridized with the somatostatin PCR probe. Lower left hand panel, relationship between densitometric signal and total RNA loaded. Upper right hand panel, slot blot analysis of somatostatin mRNA. Samples of 0.625, 1.25, 2.5, 5, 10, and 20 μg total antral RNA (*1–6*, respectively) were applied to a Hybond N membrane using a Bio-slot apparatus (Bio-Rad). The membrane was hybridized with the somatostatin PCR probe. Lower right hand panel, relationship between densitometric signal and total RNA slotted onto membrane.

branes using boiling 0.1% SDS and membranes rehybridized with probes to gastrin, HDC, or β actin. Finally, all membranes were hybridized with an end-labeled oligonucleotide probe to the 18s ribosomal subunit to check for equal loading and transfer efficiency. Signals on the x ray films were quantified using video densitometry. Statistical significance between control and treated groups of animals was determined using an unpaired *t* test, and results are presented as mean±SE with reference to control samples expressed as 100%.

Results

General. Withdrawal of food for 48 h produced an ~ 40 g decrease in body weight in both vehicle and omeprazole-treated rats. In fasted rats, plasma gastrin concentrations (13.0±3.1 pmol/liter) were significantly ($P < 0.001$) reduced compared with rats fed ad lib. (51.7±4.4 pmol/liter) and this decrease was reversed by omeprazole (107±24 pmol/liter). In rats fed ad lib. and treated with omeprazole for 48 h, plasma gastrin (217±46 pmol/liter) was ~ fourfold ($P < 0.01$) higher than in vehicle-treated rats fed ad lib.

In Northern blots, a single species of somatostatin mRNA of ~ 0.85 kb was identified in extracts of both corpus and antrum of all groups of rats. The signal intensity determined by laser densitometry of Northern or slot blots increased linearly with RNA loaded (Fig. 2), so that these methods were appropriate for quantification of somatostatin mRNA. Similarly, bands of the expected size were identified in Northern blots of gastrin (0.65 kb), HDC (2.7 and 3.5 kb), or β actin mRNA (~ 2 kb) in all groups of rats. The sequence of the PCR-generated somatostatin cDNA probe was identical to that described by Montminy et al., for the corresponding region of the somatostatin gene (28).

Somatostatin mRNA abundance: effect of food and acid. Withdrawal of food for 48 h produced a 486±94% increase in antral somatostatin mRNA abundance ($P < 0.001$), while gastrin mRNA abundance was reduced to 25.9±2.3% ($P < 0.001$) that in control rats fed ad lib. Both observations are in line with previous findings (23, 29). Unexpectedly, however, we found that in the same rats, somatostatin mRNA abundance in the acid-secreting part of the stomach decreased on withdrawal of food to 63±18% of control. In both antrum and corpus there was a modest fall in β actin (to 81.4±6.9 and 74.2±4.9%, respectively) with fasting. It is known that treatment of rats with omeprazole reverses the fasting-induced reduction of gastrin mRNA (23) and decreases somatostatin mRNA in the antrum (19, 20); the same pattern of responses was observed here (Table I). In contrast, somatostatin mRNA in the acid secreting part of the stomach did not change when fasted rats were treated with omeprazole (Fig. 3, Table I). In rats fed ad lib., omeprazole increased antral gastrin mRNA as expected and decreased antral somatostatin mRNA; in addition, however, omeprazole treatment of fed rats also decreased somatostatin mRNA in the corpus (Table II). The abundance of β actin mRNA was unchanged in either region of the stomach by omeprazole (Tables I and II).

Table I. Influence of Omeprazole on the Abundance of Somatostatin, Gastrin, or β Actin mRNA in Antrum and Corpus of Fasted Rats

	Antral gastrin	Antral somatostatin	Corpus somatostatin	Antral β actin	Corpus β actin
Fasted	100±31.6	100±14	100±14	100±16	100±11.5
Fasted + omeprazole	256±19.7*	54.5±4.1‡	98±12	114.6±18	99.1±16.9

Rats ($n = 6$ in each group) were fasted (48 h), and received either vehicle or omeprazole (400 μmol/kg, per os, 0 and 24 h). The effect of omeprazole is compared to the control group expressed as 100%. See text for further information. *t* test, * $P < 0.001$, ‡ $P < 0.01$.

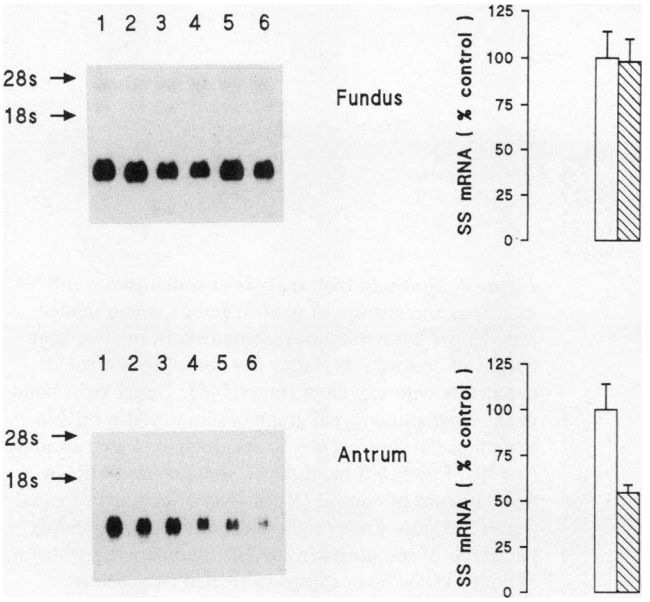

Figure 3. Northern blot analysis of fundic and antral somatostatin mRNA in control and omeprazole-treated rats. 20-μg samples of total RNA were applied to 1% agarose-formaldehyde gels and electrophoresed in MOPS. RNA was transferred to Hybond N membranes which were hybridized with the somatostatin PCR probe. Upper left hand panel, lanes *1–3* RNA from acid-secreting stomach from 48 h fasted (vehicle treated) animals; lanes *4–6* corresponding samples from animals fasted for 48 h and treated with omeprazole (400 μmol/kg at 0 and 24 h). Upper right hand panel, bar graph of fundic somatostatin mRNA abundance in vehicle or omeprazole treated, fasted animals (both $n = 6$). Lower left hand panel, lanes *1–3* antral RNA from 48 h fasted animals, lanes *4–6* antral RNA from omeprazole treated, 48 h fasted animals. Lower right hand panel, bar graph of antral somatostatin mRNA abundance in vehicle or omeprazole treated, 48 h fasted rats (both $n = 6$). □, Fasting; ▨, fasting + omeprazole.

Capsaicin treatment and immunoneutralization. The results described above suggest that while the luminal contents of the stomach regulate somatostatin mRNA abundance, different mechanisms are at work in the corpus and the antrum. Since CGRP stimulates somatostatin release, and is exclusively localized to the primary afferent innervation of the rat stomach, we investigated the role of these nerves in controlling somatostatin mRNA abundance. In rats that had been treated with capsaicin to lesion small diameter afferent neurons, corpus somatostatin mRNA was markedly depressed ($P < 0.01$) compared with vehicle-treated controls (Fig. 4). In contrast, antral somatostatin abundance was unchanged. These changes were not attributable to nonspecific effects on corpus endocrine cell function because HDC mRNA, which is located in a different population of corpus mucosa endocrine cells, was unchanged by capsaicin treatment. Antral gastrin mRNA was also unaltered by capsaicin pretreatment.

The role of CGRP was examined in rats that had been previously immunized to produce CGRP antibodies. The dilution of plasma from these rats required for binding of 50% of 2,000 cpm of radiolabeled peptide in radioimmunoassay buffer (4°C, 24 h) varied from 1:2,700 to 1:13,000. In these rats somatostatin mRNA abundance in the corpus was depressed significantly ($P < 0.025$) (Fig. 5). Again this was not a nonspecific effect because HDC mRNA abundance was unchanged (Fig. 6).

Direct evidence for the idea that CGRP increased somatostatin mRNA abundance was sought by intravenous infusion of the peptide. In control rats, α-CGRP infusion (1 nmol/kg per h) for 6 h produced a significant increase in somatostatin mRNA abundance in the corpus compared with saline-infused rats (saline: 100±7.0%, $n = 4$; CGRP: 158±14.7%, $n = 6$; $P < 0.05$). It was of interest that in parallel experiments in capsaicin-treated rats the infusion of CGRP did not increase somatostatin mRNA abundance (saline: 100±4.8%, $n = 5$; CGRP: 97.8±7.3%, $n = 6$).

Discussion

The main finding of the present study is that separate factors determine the abundance of somatostatin mRNA in the acid-secreting part of the stomach, and in the antrum. The data therefore indicate that D cells of the antrum and corpus are functionally distinct. In fasted rats, the presence of gastric acid appears to be less important in maintaining corpus compared with antral somatostatin mRNA abundance. In contrast, small diameter primary afferent neurons appear to be important in maintaining corpus, but not antrum, somatostatin mRNA levels. These results extend earlier studies that have been directed at understanding the control of antral somatostatin mRNA levels (19, 20, 29), and they draw attention to the primary afferent innervation of the stomach, and in particular to CGRP, as a factor determining gastric endocrine cell function.

Previous work by ourselves and others has shown that CGRP occurs in ~ 80% of spinal afferent neurons projecting to the rat stomach, in < 10% of vagal gastric afferents, and is absent from gastric intrinsic neurons; CGRP is transported towards the peripheral terminals of spinal afferents that are located in the mucosa, around submucosal blood vessels, in circular smooth muscle and myenteric plexus (14–18). In rats pretreated with the sensory neurotoxin capsaicin there is complete loss of gastric CGRP and of a high proportion of gastric

Table II. The Influence of Omeprazole on the Abundance of Somatostatin, Gastrin, or β Actin mRNA in Antrum and Corpus of Rats Fed Ad Lib

	Antral gastrin	Antral somatostatin	Corpus somatostatin	Antral β actin	Corpus β actin
Fed	100±32.4	100±8.6	100±9.7	100±8.9	100±15.1
Fed + omeprazole	307.3±62.9*	29.8±2.7‡	76.2±3.3§	101.3±11.8	104.7±14.6

Rats ($n = 6$ in each group) were fed ad lib., and received either vehicle or omeprazole (400 μmol/kg^{-1}, per os, 0 and 24 h). The effect of omeprazole is compared to the control group expressed as 100%. See text for further information. *t* test, * $P < 0.05$; § $P < 0.01$, ‡ $P < 0.001$.

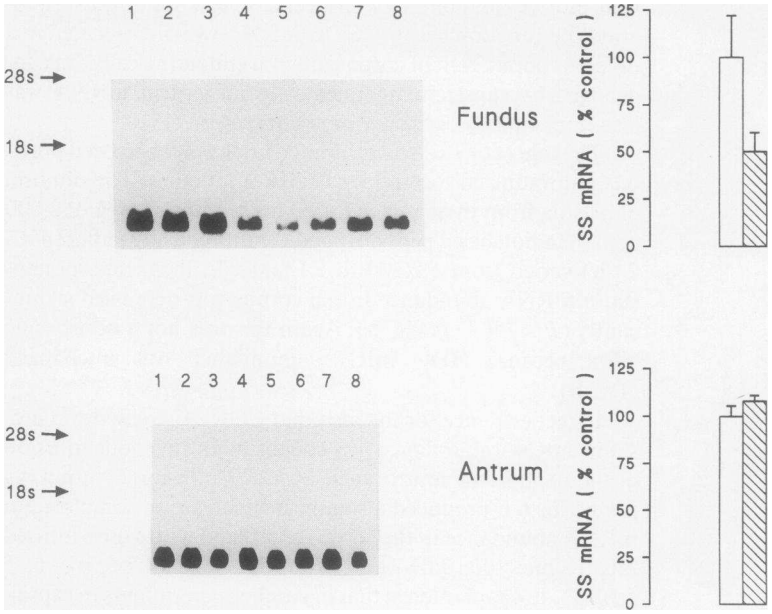

Figure 4. Northern blot analysis of somatostatin mRNA in corpus and antrum of control and capsaicin treated rats. Upper left hand panel, somatostatin mRNA from corpus of control rats (lanes *1–4*) or animals treated neonatally with capsaicin (lanes *5–8*). Upper right hand panel, corresponding bar graph of somatostatin mRNA abundance in control (n = 5) or capsaicin treated animals (n = 5). Lower left hand panel, somatostatin mRNA from antrum of control (lanes *1–4*) or capsaicin-treated (lanes *5–8*) rats. Lower right hand panel, corresponding bar graph of somatostatin mRNA abundance in antrum of control (n = 5) or capsaicin-treated (n = 5) rats. □, Control; ▨, capsaicin.

spinal afferents, while intrinsic neurons of the stomach are unaffected. The decrease in corpus somatostatin mRNA that was found in capsaicin-treated rats suggests that gastric afferents play a role in controlling mucosal endocrine cell function. The observation that antibodies to CGRP are also associated with reduced corpus somatostatin mRNA abundance indicates that this peptide is very likely one of the mediators of gastric afferent neurons. In principle CGRP could act as a transmitter at either central or peripheral terminals. However, it seems unlikely that antibody would penetrate to central terminals in sufficient quantities to exert neutralizing actions. In contrast there is abundant evidence for the release of peptides from the peripheral terminals of primary afferents and the peptides so released would be considerably more accessible to neutralizing antibody.

Many CGRP-containing primary afferent neurons also contain substance P, and the two peptides are presumably co-released on stimulation of these neurons (30–34). It may well be that there are cooperative actions between CGRP and other afferent factors. Direct evidence for this comes from the observation that infusion of CGRP increases corpus somatostatin mRNA abundance in control but not in capsaicin-treated rats. It is plausible to suppose that in control rats CGRP increases somatostatin mRNA by interacting with some other factor, perhaps a tachykinin, produced by small diameter afferent neurons; whereas in capsaicin-treated rats, in which these factors are absent, the activity of CGRP is reduced correspondingly.

The changes in corpus somatostatin mRNA abundance with capsaicin pretreatment, and in the presence of CGRP antibodies, can be considered specific since in the same circumstances there were no changes in gastrin and somatostatin mRNA in antrum, or for that matter in HDC mRNA which occurs in a different endocrine cell population (ECL cell) of the corpus mucosa. When the present observations are taken together with reports (*a*) that CGRP is a potent releaser of gastric somatostatin (11–13), and (*b*) that there are marked ultrastructural changes in a population of gastric endocrine cells in capsaicin-treated rats (34), it is clear that CGRP released from primary afferent terminals in the stomach is potentially a major mediator of corpus somatostatin cell function. Moreover, Inui et al. (35) have shown that capsaicin-induced changes in gastric somatostatin secretion are mediated by CGRP.

The spinal afferent innervation of the gut is involved in the transmission of pain sensation to the central nervous system. But in recent years it has become clear that the same afferents may also play a part in maintaining gastric mucosal defense mechanisms, and in regulating upper gastrointestinal tract motility. In both the latter instances, there is now evidence to suggest that CGRP is a peripheral transmitter of gastric afferent neurons: thus CGRP is released in the stomach after afferent stimulation (36), and neutralizing antibodies to CGRP both enhance the damaging effects of intragastric ethanol, and reverse the inhibition of gastric emptying by acid and hyperos-

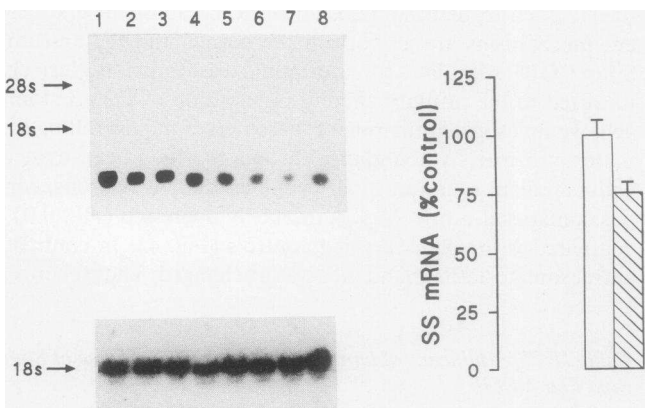

Figure 5. Northern blot analysis of somatostatin mRNA in corpus of control and CGRP immunized rats. Upper left hand panel, somatostatin mRNA in corpus of control (lanes *1–4*) or CGRP-immunized (lanes *5–8*) animals. Lower left hand panel, the same membrane stripped and rehybridized with an end-labeled oligonucleotide probe to the 18s ribosomal subunit. Right hand panel, somatostatin mRNA abundance in control (n = 6) and CGRP-immunized (n = 4) rats. □, Control; ▨, CGRP.

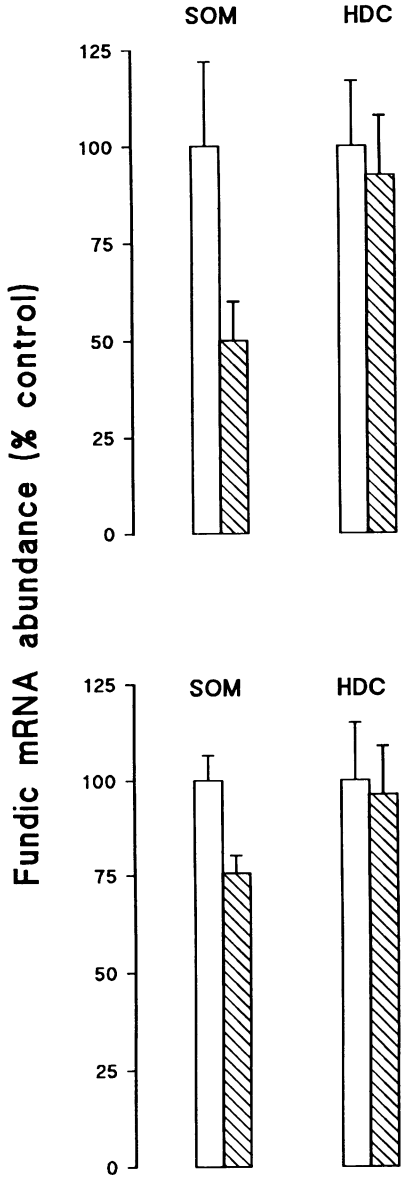

Figure 6. Somatostatin and HDC mRNA abundance in acid-secreting stomach of capsaicin-treated and CGRP-immunized rats. Upper panel, somatostatin and HDC mRNA abundance in control (expressed as 100%, □; $n = 5$) and neonatally capsaicin-treated animals (▨; $n = 5$). Lower panel, somatostatin and HDC mRNA abundance in control (□; $n = 5$) and CGRP-immunized rats (▨; $n = 5$).

molal solutions (21, 22). Moreover, CGRP is a potent inhibitor of gastric acid secretion (which at least in part, is likely to be due to release of somatostatin) (37–39). The data therefore support the notion that gastric CGRP acts at several different levels to protect (*a*) the gastric mucosa from noxious stimuli including acid, and (*b*) the small intestine from potentially damaging substances in the gastric lumen, by delaying their emptying.

Previous studies have established that reduction of gastric acid leads to an increase in gastrin mRNA, and a corresponding decrease in antral somatostatin mRNA (19, 20, 29). Withdrawal of food from rats for 24 h or longer decreases antral gastrin stores, gastrin mRNA, and plasma gastrin, but does not reduce basal acid secretion, and these effects are reversed by inhibition of acid secretion by omeprazole (23). It appears that in the absence of food in the stomach there is unrestrained stimulation of antral D cell function by luminal acid which in turn leads to depression of G cell function. The failure of the corpus and antral somatostatin mRNA pools to change in parallel during fasting and omeprazole-treatment was striking. Indeed there was a decrease in corpus somatostatin mRNA with fasting which was not reversed by omeprazole-treatment. It should be noted that the mRNA for β actin in both antrum and corpus also decreased to a similar extent after food withdrawal and was not reversed by omeprazole. Since β actin is a widely distributed cytoskeletal protein it seems plausible to suppose that with prolonged food withdrawal there is depression of protein synthesis (reflected in decreased mRNA) in many systems, implying that changes in gastric corpus somatostatin mRNA are not specifically related to the state of the luminal contents.

It is generally thought that the primary function of antral D cells is control of nearby G cells, while corpus D cells regulate directly or indirectly parietal cell function (1, 4). Inhibition of acid secretion is a common endpoint in both circumstances, and perhaps for this reason previous studies have not for the most part attempted to make detailed comparison of the factors controlling antral and corpus D cells. The present findings suggest this is an important distinction. It remains to be shown how the relevant factors act at a cellular level to control somatostatin mRNA abundance; these factors could include regulation of gene expression, or by analogy with recent studies on gastrin by regulating mRNA stability (20, 40). Changes in somatostatin mRNA abundance may not be directly reflected in changed tissue stores (20); it is however, clear that factors which influence gastric somatostatin mRNA are able to act over relatively short periods (29), and that the same factors also rapidly influence somatostatin secretion (3, 12, 13, 35) supporting the idea that the two are functionally linked.

References

1. Walsh, J. H. 1988. Peptides as regulators of gastric acid secretion. *Annu. Rev. Physiol.* 50:41–63.

2. Walsh, J. H., C. T. Richardson, and J. S. Fordtran. 1975. pH dependence of acid secretion and gastrin release in normal and ulcer subjects. *J. Clin. Invest.* 55:462–468.

3. Saffouri, B., G. C. Weir, K. N. Bitar, and G. M. Makhlouf. 1980. Gastrin and somatostatin secretion by perfused rat stomach: functional linkage of antral peptides. *Am. J. Physiol.* 238:G495–G501.

4. Yamada, T., and T. Chiba. 1989. Somatostatin. *In* Handbook of Physiology. The Gastrointestinal System. G. M. Makhlouf, editor. American Physiological Society, Bethesda, MD. 431–453.

5. Short, G. M., J. W. Doyle, and M. M. Wolfe. 1985. Effect of antibodies to somatostatin on acid secretion and gastrin release by the isolated perfused rat stomach. *Gastroenterology.* 88:984–988.

6. Schubert, M. L., N. F. Edwards, A. Arimura, and G. M. Makhlouf. 1987. Paracrine regulation of gastric acid secretion by fundic somatostatin. *Am. J. Physiol.* 252:G485–G490.

7. Soll, A. H., T. Yamada, J. Park, and L. P. Thomas. 1984. Release of somatostatinlike immunoreactivity from canine fundic mucosal cells in primary culture. *Am. J. Physiol.* 247:G558–G566.

8. Yamada, T., A. H. Soll, J. Park, and J. Elashoff. 1984. Autonomic regulation of somatostatin release: studies with primary cultures of canine fundic mucosal cells. *Am. J. Physiol.* 247:G567–G573.

9. Chiba, T., T. Taminato, S. Kadowaki, H. Abe, K. Chihara, Y. Seino, S. Matsukura, and T. Fujita. 1980. Effects of glucagon, secretin and vasoactive intestinal polypeptide on gastric somatostatin and gastrin release from isolated perfused rat stomach. *Gastroenterology.* 79:67–71.

10. Holst, J. J., S. L. Jensen, S. Knuhtsen, O. V. Nielsen, and J. F. Rehfeld. 1983. Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. *Am. J. Physiol.* 244:G515–G522.

11. Bunnett, N. W., W. S. Helton, H. T. Debas, and J. W. Ensinck. 1990. CGRP stimulates the release of pro-somatostatin-derived peptides from the gastric fundus. *Am. J. Physiol.* 258:G316–G319.

12. Helton, W. S., M. M. Mulholland, N. W. Bunnett, and H. T. Debas. 1989. Inhibition of gastric and pancreatic secretion in dogs by CGRP: role of somatostatin. *Am. J. Physiol.* 256:G715–G720.

13. Dunning, B. E., and G. J. Taborsky, Jr. 1987. Calcitonin gene-related

peptide: a potent and selective stimulator of gastrointestinal somatostatin secretion. *Endocrinology.* 120:1774–1781.

14. Green, T., and G. J. Dockray. 1988. Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. *Neuroscience.* 25:181–193.

15. Varro, A., T. Green, S. Holmes, and G. J. Dockray. 1988. Calcitonin gene-related peptide in visceral afferent nerve fibres: quantification by radioimmunoassay and determination of axonal transport rates. *Neuroscience.* 26:927–932.

16. Mulderry, P. K., M. A. Ghatei, R. A. Spokes, P. M. Jones, A. M. Pierson, Q. A. Hamid, S. Kanse, S. G. Amara, J. M. Burrin, S. Legon, et al. 1988. Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience.* 25:195–205.

17. Su, H. C., A. E. Bishop, R. F. Power, Y. Hamada, and J. M. Polak. 1987. Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. *J. Neurosci.* 7:2674–2687.

18. Sternini, C., J. R. Reeve, Jr., and N. Brecha. 1987. Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. *Gastroenterology.* 93:852–862.

19. Brand, S. J., and D. Stone. 1988. Reciprocal regulation of antral gastrin and somatostatin gene expression in omeprazole-induced achlorhydria. *J. Clin. Invest.* 82:1059–1066.

20. Wu, S. V., A. Giraud, M. Mogard, K. Sumii, and J. H. Walsh. 1990. Effects of inhibition of gastric secretion on antral gastrin and somatostatin gene expression in rats. *Am. J. Physiol.* 258:G788–G793.

21. Forster, E. R., and G. J. Dockray. 1991. The role of calcitonin gene-related peptide in gastric mucosal protection in the rat. *Exp. Physiol.* 76:623–626.

22. Forster, E. R., and G. J. Dockray. 1991. Immunoneutralization suggests that calcitonin gene related peptide regulates gastric emptying in the rat. *Neurosci. Lett.* 131:5–7.

23. Dimaline, R., D. Evans, A. Varro, and G. J. Dockray. 1991. Reversal by omeprazole of the depression of gastrin cell function by fasting in the rat. *J. Physiol.* 433:483–493.

24. Dockray, G. J., L. Best, and I. L. Taylor. 1977. Immunochemical characterization of gastrin in pancreatic islets of normal and genetically obese mice. *J. Endocrinol.* 72:143–151.

25. Chirgwin, J. J., A. E. Przbyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

26. Goodman, R. H., D. C. Aron, and B. A. Roos. 1983. Rat pre-pro-somatostatin. Structure and processing by microsomal membranes. *J. Biol. Chem.* 258:5570–5573.

27. Dimaline, R., and A. K. Sandvik. 1991. Histidine decarboxylase gene expression in rat fundus is regulated by gastrin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 281:20–22.

28. Montminy, M. R., R. H. Goodman, S. J. Horovitch, and J. F. Habener. 1984. Primary structure of the gene encoding rat preprosomatostatin. *Proc. Natl. Acad. Sci. USA.* 81:3337–3340.

29. Wu, S. V., K. Sumii, A. Tari, M. Mogard, and J. H. Walsh. 1990. Regulation of gastric somatostatin gene expression. *Metab. Clin. Exp.* 9:125–130.

30. Gibbins, I. L., J. B. Furness, M. Costa, I. MacIntyre, C. J. Hillyard, and S. Girgis. 1985. Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. *Neurosci. Lett.* 57:125–130.

31. Lee, Y., Y. Kawai, S. Shiosaka, K. Takami, H. Kiyama, C. J. Hillyard, S. Girgis, I. MacIntyre, P. C. Emson, and M. Tohyama. 1985. Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: Immunohistochemical analysis. *Brain Res.* 330:194–196.

32. Gulbenkian, S., A. Merighi, J. Wharton, I. M. Varndell, and J. M. Polak. 1986. Ultrastructural evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig. *J. Neurocytol.* 15:535–542.

33. Wiesenfeld-Hallin, Z., T. Hokfelt, J. M. Lundberg, W. G. Forssman, M. Reinecke, F. A. Tschopp, and J. A. Fischer, J. A. 1984. Immunoreactive calcitonin gene-related peptide and substance P coexist in sensory neurons in spinal cord and interact in spinal behavioural responses of the rat. *Neurosci. Lett.* 52:199–204.

34. Pfeiffer, C. J., and S. Evangelista. 1991. Gastric and jejunal ultrastructure in capsaicin-treated rats with and without experimental ulcer. *In* Sensory Nerves and Neuropeptides in Gastroenterology. M. Costa, G. Surrenti, S. Gorini, C. A. Maggi, and A. Meli, editors. Plenum Publishing Corp., New York. 85–96.

35. Inui, T., Y. Kinoshita, A. Yamaguchi, T. Yamatani, and T. Chiba. 1991. Linkage between capsaicin-stimulated calcitonin gene-related peptide and somatostatin release in rat stomach. *Am. J. Physiol.* 261:G770–G774.

36. Holzer, P., B. M. Peskar, B. A. Peskar, and R. Amann. 1990. Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach. *Neurosci. Lett.* 108:195–200.

37. Pappas, T., H. T. Debas, J. H. Walsh, J. Rivier, and Y. Tache. 1986. Calcitonin gene related peptide-induced selective inhibition of gastric acid secretion in dogs. *Am. J. Physiol.* 250:G127–G133.

38. Beglinger, C., W. Born, P. Hildebrand, J. W. Ensinck, F. Burkhardt, J. A. Fischer, and K. Gyr. 1988. Calcitonin gene-related peptides I and II and calcitonin: distinct effects on gastric acid secretion in humans. *Gastroenterology.* 95:958–965.

39. Tache, Y., T. Pappas, M. Lauffenburger, Y. Goto, J. H. Walsh, and H. Debas. 1984. Calcitonin gene-related peptide: potent peripheral inhibitor of gastric acid secretion in rats and dogs. *Gastroenterology.* 87:344–349.

40. Karnik, P. S., and M. M. Wolfe. 1990. Somatostatin stimulates gastrin mRNA turnover in dog antral mucosa. *J. Biol. Chem.* 265:2550–2555.