

Reciprocal Regulation of Antral Gastrin and Somatostatin Gene Expression by Omeprazole-induced Achlorhydria

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Abstract

Gastric acid exerts a feedback inhibition on the secretion of gastrin from antral G cells. This study examines whether gastrin gene expression is also regulated by changes in gastric pH. Achlorhydria was induced in rats by the gastric H⁺/K⁺ ATPase inhibitor, omeprazole (100 μmol/kg). This resulted in fourfold increases in both serum gastrin (within 2 h) and gastrin mRNA levels (after 24 h).

Antral somatostatin D cells probably act as chemoreceptors for gastric acid to mediate a paracrine inhibition on gastrin secretion from adjacent G cells. Omeprazole-induced achlorhydria reduced D-cell activity as shown by a threefold decrease in antral somatostatin mRNA levels that began after 24 h. Exogenous administration of the somatostatin analogue SMS 201-995 (10 μg/kg) prevented both the hypergastrinemia and the increase in gastrin mRNA levels caused by omeprazole-induced achlorhydria. Exogenous somatostatin, however, did not influence the decrease in antral somatostatin mRNA levels seen with achlorhydria.

These data, therefore, support the hypothesis that antral D cells act as chemoreceptors for changes in gastric pH, and modulates somatostatin secretion and synthesis to mediate a paracrine inhibition on gastrin gene expression in adjacent G cells.

Introduction

Secretion of gastrin is inhibited by gastric acid, and this serves as a negative feedback control that prevents excess acid secretion (1). Achlorhydria, as is seen in atrophic gastritis, releases antral gastrin cells (G cells) from this inhibition and consequently causes marked hypergastrinemia and G-cell hyperplasia (1–3). Surgical ablation of gastric acid secretion in rats by fundectomy produces similar changes (4) and has also been shown to stimulate gastrin synthesis (5). Chronic blockade of acid secretion with the H⁺/K⁺ ATPase antagonist omeprazole results in hypergastrinemia and, with prolonged treatment, in G-cell hyperplasia (6–8). These results suggest that achlorhydria may also stimulate gastrin gene expression in G cells. Rapidly inducing achlorhydria with omeprazole allows this question to be addressed without the complication of G cell hyperplasia and should, therefore, illuminate the physiological mechanisms that regulate gastrin gene expression.

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1. Abbreviations used in this paper: POMC, proopiomelanocortin.

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Substantial evidence supports the hypothesis that gastric acid inhibits gastrin secretion through somatostatin released from antral D cells (9, 10). Somatostatin is a potent inhibitor of gastrin release (11). Antral D cells have characteristic cytoplasmic projections onto G cells (12, 13) that may act as a pathway for paracrine inhibition by somatostatin. Since gastric acid stimulates somatostatin release (14–16), gastrin secretion is thought to be inhibited by somatostatin released locally from these cytoplasmic processes. The inhibition of gastrin secretion by antral somatostatin thus represents a probable example of paracrine regulation. Measurement of changes in somatostatin mRNA levels with changes in gastric pH may be of value in evaluating the D cell's role as a paracrine regulator of antral G cells, since these mRNA levels directly reflect D cell activity.

Although somatostatin's inhibitory action on gastrin secretion is well established, it is not known whether somatostatin also inhibits gastrin gene expression. In the pituitary, where somatostatin also mediates a similar feedback cycle involving the inhibition of hormone secretion (17), short term incubation with somatostatin does not inhibit growth hormone gene transcription (18). This suggests a dissociation between somatostatin's ability to inhibit pituitary hormone secretion and its effects on hormone synthesis. Consequently, somatostatin may not necessarily inhibit gastrin gene expression despite its well described effects on gastrin secretion.

Methods

Omeprazole treatment. Male Sprague-Dawley rats weighing 180–220 g were fed regular laboratory chow ad lib. Omeprazole dissolved in 100 μmol/kg PEG 2000 (concentration 20 mg/ml) was injected intraperitoneally twice daily at 12-h intervals (0.2 ml PEG in 1 ml 0.15% NaHCO₃). Control rats received intraperitoneal injections of 0.2 mls PEG alone in 1 ml NaHCO₃. After an overnight fast, rats were anesthetized with ether, 1.5 ml of their blood was drawn by cardiac puncture, and then they were killed. The antrum was excised, and a similar weight of corpus from the greater curvature adjacent to the sacculus margin was excised. The duodenum, excluding a 2-mm rim adjacent to the pylorus, was also removed. Tissues were immediately placed in guanidium thiocyanate lysis buffer (18) and homogenized using a polytron (Brinkmann Instruments Co., Westbury, NY).

RNA preparation. RNA was prepared by the lithium chloride precipitation method of Cathala et al. (19) and the concentration of RNA was measured spectrophotometrically by the absorbance at 260 Å. The efficiency of RNA recovery, determined by adding ³H-labeled SP6 sense gastrin cRNA, was between 80–85%.

RNA probe synthesis. ³²P-labeled RNA probes were synthesized using SP6 RNA polymerase (20) transcribed from rat gastrin (21) and somatostatin (22) cDNA templates. Synthetic oligonucleotides complementary to rat actin mRNA (TGCTGCAGTCTAGAGGATCCGAATTCG) and 18S subunit ribosomal RNA were end labeled with ³²P γ ATP using polynucleotide kinase (10⁸ cpm/μg sp act).

Quantitation of gastrin and somatostatin mRNA by dot-blot analysis. Total RNA was immobilized on to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) using a dot-blot procedure (23). As an

internal correction for RNA loading variations, dot-blot filters were hybridized with a ^{32}P -labeled synthetic oligonucleotide that is complementary to 18S ribosomal RNA. The baked filters were prehybridized at 43°C for several hours in sealed plastic bags containing 5 ml of 5× standard saline citrate (SSC), 0.2% Ficoll, 0.2% polyvinyl pyrrolidone, 0.2% BSA, 50 mM sodium phosphate buffer, pH 7.0, 1% SDS, and 0.1% sodium pyrophosphate-sonicated salmon sperm DNA (100 µg/ml). The ^{32}P -labeled probe was added (10⁶ cpm/ml) and the mixture was hybridized at 55°C for 15 h. Filters were then washed in 0.2× SSC, 1% SDS at 55°C three times for 20 min each time. Filters were exposed to x-ray film (Kodak AR-10) for 6 and 18 h. Exposed film was developed and fluorograms were quantitated by scanning with a laser densitometer (LKB Instruments, Inc., Gaithersburg, MD) using an area integrator. The ribosomal RNA probe was then melted off by heating blots at 95°C in 0.1× SSC for 15 min. The blots were then exposed overnight to confirm the melting of the probe.

Dot blots were then rehybridized with ^{32}P -labeled RNA SP6 probes that were complementary to rat gastrin or somatostatin mRNA. Prehybridization and hybridization were performed as above with the following exceptions: hybridization solution contained 50% formamide, and 0.1% SDS, prehybridization temperature was 45°C, and hybridization temperature was 65°C. After hybridizing for 15 h, filters were washed with 0.1× SSC and 0.1% SDS three times for 20 min each time. Filters were then exposed for 6 and 18 h at room temperature. Individual dots were then cut out and the radioactivity was determined by scintillation spectrometry. The autoradiograms were then quantitated by densitometric scanning.

Northern blot analysis. RNA samples were denatured, (24) then electrophoresed on 1.4% agarose formaldehyde gels (24, 25) and were then transferred to nitrocellulose filters (26). After Northern transfer, the nitrocellulose filters were baked in a vacuum oven at 80°C for 2 h. Blots were hybridized as above with ^{32}P -labeled gastrin and somatostatin probes at 65°C for 16 h. Blots were washed in 0.2× SSC, 0.1% SDS at 65°C three times for 20 min each time.

Gastrin RIA. Gastrin was measured using rabbit antisera 2604 (1/100,000 dilution) raised against synthetic human heptadecapeptide gastrin (gastrin 17) (27). Tyrosine monoiodinated human gastrin 17 tracer was used in all assays (28) and synthetic human gastrin 17 was used as a standard. RIAs using 2604 react with component I, gastrin 34, and gastrin 17, sulfated and nonsulfated, with equal potency.

Somatostatin RIA. Somatostatin was extracted from tissues as described by Patel and Reichlin (29). Diluted extracts were incubated with somatostatin antiserum and 3,000 cpm of ^{125}I -Tyr¹¹ somatostatin (1M161; Amersham Corp., Arlington Heights, IL) in 50 mM sodium phosphate buffer, pH 7.2, containing 0.3% BSA and 10 mM EDTA at 4°C overnight. The somatostatin antiserum was purchased from Amersham Corp. (cat no N 1611) and diluted following the supplier's instructions. Damage was < 7% and binding in the absence of competing peptide was 60%. Somatostatin concentration was normalized to the protein concentration that was measured by the method of Bradford (1976) (30).

Immunohistochemistry. Antral tissue was excised from omeprazole and control-treated rats, immersed in OCT 4583 (Miles Scientific Div., Naperville, IL), and frozen at -70°C. Frozen tissue sections were cut, fixed, and then incubated with a gastrin-specific antisera (2604) at a dilution of 1/100 after which they were incubated with FITC-labeled protein A (KPL 125000, Cat F51-5). At least 15 adjacent fields were counted on serial sections stained with gastrin antisera. The area density is expressed as the number of cells per centimeter of mucosa.

Statistical analysis. Data were analyzed for significant differences using unpaired Student's *t* test. Confidence levels were of $P < 0.01$.

Results

Male Sprague-Dawley rats were treated with omeprazole (100 µmol/kg) twice daily for 5 d, in parallel with matched controls that were injected with the PEG carrier alone. In both groups blood was drawn for gastrin RIA and RNA was isolated for

mRNA determination. Treatment with omeprazole resulted in a marked hypergastrinemia (Fig. 1 A), serum gastrin being fivefold higher in omeprazole-treated rats. Omeprazole treatment also increased gastrin mRNA levels as shown by Northern blot analysis (Fig. 1 C) and dot-blot quantitation (Fig. 2 B). Northern blot analysis of antral RNA (Fig. 1 C) shows a single gastrin mRNA species (~ 570 nucleotides). The intensity of gastrin mRNA hybridization was increased in RNA from omeprazole-treated rats compared with control. Densitometric quantitation showed that omeprazole induced an increase in gastrin mRNA levels. Gastrin mRNA levels were 0.11±0.05 O.D. units for control RNA and 0.66±0.07 O.D. units for omeprazole-treated RNA, mean±SE, $n = 10$, $P < 0.01$. To determine whether the increase in gastrin mRNA induced by omeprazole was specific, actin mRNA levels were measured on the same blotted RNA samples (Fig. 1 B). In contrast to gastrin mRNA, actin mRNA levels were not significantly increased in RNA from omeprazole-treated rats. Actin mRNA levels were 0.29±0.03 O.D. units for control RNA vs. 0.33±0.08 O.D. units for omeprazole-treated RNA (mean±SE, $n = 10$). The calculated ratio of the gastrin to actin mRNA levels in RNA from the omeprazole-treated group was 2.39±0.56 O.D. units, which was significantly greater than that of the control group, 0.35±0.08 O.D. units (mean±SE, $n = 10$, $P < 0.01$).

To measure more accurately the changes in gastrin mRNA levels with omeprazole treatment, antral RNA from 14 omeprazole-treated rats was quantitated by dot-blot analysis and compared with controls (Fig. 2). 10, 5, and 2.5 µg of total antral RNA was immobilized on nitrocellulose and hybridized to a gastrin cRNA probe. A representative autoradiogram is shown in Fig. 2 A. As a control for nonspecific hybridization of the RNA probe to ribosomal RNA, corpus RNA was also blotted and hybridized in the same filter. There was no significant binding of the gastrin probe to corpus RNA under the hybridization and washing conditions used (Fig. 2 A). Hybridization of the gastrin probe to antral RNA samples was quantitated both by scanning autoradiograms with a soft laser densitometer (LKB Instruments) and by directly measuring the ^{32}P radioactivity bound to the filters by Cherenkov counting in a liquid scintillation counter. Both methods gave nearly identical results for the increase in gastrin mRNA with omeprazole treatment. The amount of gastrin mRNA hybridized was directly proportional to the quantity of total RNA bound to the filter. At all quantities of bound RNA, gastrin mRNA levels were significantly greater in omeprazole-treated rats (~ 3.5-fold higher). To exclude a systematic difference in the binding of omeprazole and control RNA samples to the filters, a ^{32}P -labeled synthetic oligonucleotide that was complementary to the 18S subunit of ribosomal RNA was hybridized to the filters before hybridization with the gastrin probe. Fig. 2 C illustrates the autoradiogram of the 18S ribosomal probe hybridized to filters shown in Fig. 2 A. Hybridization was quantitated by scanning with a densitometer and plotted against the amount of RNA that was loaded. Ribosomal RNA bound quantitatively to the nitrocellulose and the levels of ribosomal RNA bound in omeprazole-treated samples was not greater than in control RNA samples. This implies that the concentration of gastrin mRNA transcripts is greater in omeprazole-treated rats.

This observed increase in gastrin mRNA could result from increases in the number of gastrin cells (G-cell hyperplasia) or

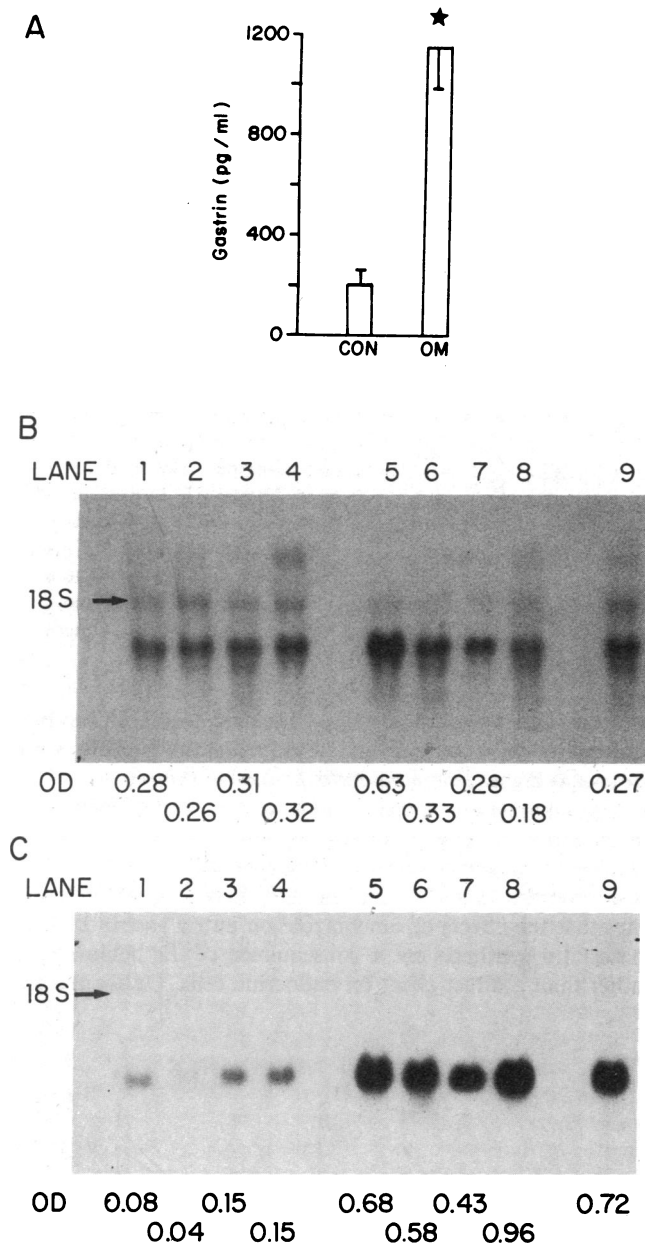


Figure 1. (A) Serum gastrin concentrations in rats treated with a 5-d course of omeprazole injections, 100 $\mu\text{mol/kg}$ *bis die*, *i.p.* (OM). Control rats (CON) were injected with the PEG carrier alone (0.2 ml/1 ml). Mean \pm SE, $n = 14$. * $P < 0.01$. (B) Northern blot analysis of actin mRNA levels in total antral RNA 20 μg isolated from omeprazole-treated rats (lanes 5–9) and control rats (lanes 1–4). Blot was hybridized with ^{32}P -labeled oligonucleotides that were complementary to rat actin cDNA in 5 \times SSC, 10% SDS for 16 h at 55 $^{\circ}\text{C}$. The blot was washed in 0.2 SSC, 1% SDS at 55 $^{\circ}\text{C}$ three times for 20 min each time. Exposure was for 8 h. Actin mRNA was quantitated by scanning densitometry of the autoradiogram and is shown below each lane in arbitrary absorbance units. (C) Northern blot analysis of gastrin mRNA levels. The filter shown in B was boiled in water to melt off the actin probe. The filter was hybridized with a rat gastrin cRNA probe in 50% formamide, 5 \times SSC, 0.1% SDS at 65 $^{\circ}\text{C}$ for 16 h. Blot was washed at 65 $^{\circ}\text{C}$ in 0.2 SSC, 0.1% SDS three times for 20 min each time. Exposure was for 2 h. Gastrin mRNA levels were determined by densitometric scanning of the autoradiogram and are shown below each lane in arbitrary absorbance units.

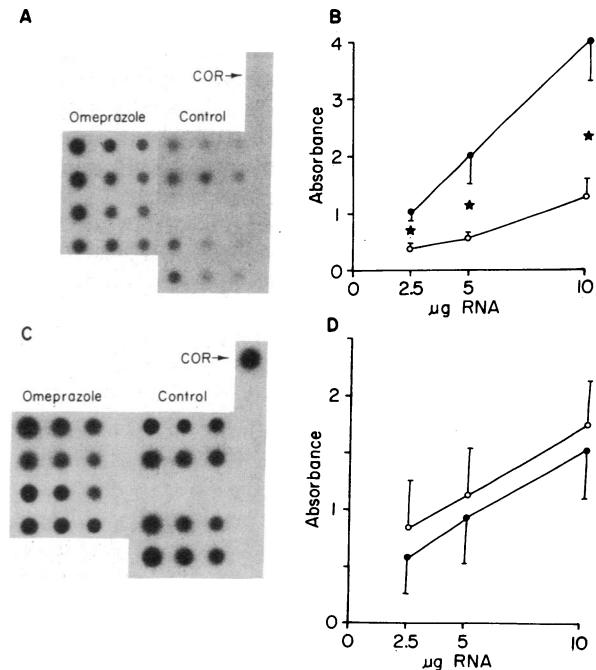


Figure 2. (A) Autoradiogram of a dot blot quantitating gastrin mRNA levels in antral RNA isolated from omeprazole- and control-treated rats. 10, 5, and 2.5 μg of total antral RNA was immobilized onto nitrocellulose filters, hybridized with a ^{32}P -rat gastrin cRNA probe in 50% formamide 5 \times SSC, 0.1% SDS at 65 $^{\circ}\text{C}$ for 16 h, and washed at 65 $^{\circ}\text{C}$ in 0.2 \times SSC, 0.1% SDS three times for 20 min each time. Filters were exposed for 2 h. Shown in the inset (COR) is 10 μg of rat corpus RNA immobilized on the filter as indicated by the arrow. (B) Densitometric quantitation of autoradiograms shown in A together with three other additional RNA samples in each group. Autoradiograms were scanned with an LKB soft laser densitometer. Results are expressed as arbitrary absorbance units, mean \pm SE, $n = 14$. \bullet , omeprazole; \circ , control; *, $P < 0.01$. (C) Autoradiogram of the antral RNA dot blot showed in Fig. 2A after having been hybridized to a ^{32}P -labeled synthetic oligonucleotide probe complementary to 18S ribosomal RNA at 55 $^{\circ}\text{C}$ in 5 \times SSC for 16 h and washed at 55 $^{\circ}\text{C}$ in 0.2 \times SSC. Exposure was 12 h at -70°C . Shown in the inset (COR) is 10 μg of rat corpus RNA immobilized onto the filter as indicated by the arrow. (D) Densitometric quantitation of 18S ribosomal RNA levels in antral RNA of the autoradiogram shown in C. Mean \pm SE, $n = 14$. \bullet , omeprazole; \circ , control.

from an increased number of gastrin mRNA transcripts per G cell (increased gene expression). Previous studies have shown that prolonged omeprazole treatment (10 wk) results in G cell hyperplasia (6). To determine whether omeprazole treatment for < 1 wk resulted in G-cell hyperplasia, G-cell numbers were determined by quantitative immunohistochemistry. Antral G-cell number was not significantly increased after 5 d of treatment with omeprazole (445 ± 83 cells/cm mucosal length vs. controls (415 ± 60 cells/cm mucosal length, mean \pm SE, $n = 5$ rats, 15 fields per animal). This implies that omeprazole treatment for 5 d increases gastrin mRNA by stimulating gastrin gene expression rather than by stimulating G-cell hyperplasia.

In contrast to the increase in gastrin secretion and synthesis, omeprazole treatment for 5 d resulted in a decrease in antral somatostatin gene expression. RNA from control and

omeprazole-treated rats was subjected to Northern blot analysis by hybridization with a rat somatostatin cRNA probe (Fig. 3 A). A single mRNA species of 670 nucleotides was detected, which is consistent with previous observations (31). The intensity of somatostatin hybridization to RNA from omeprazole-treated rats was lower than that of control RNA samples (Fig. 3 B) as quantitated by densitometric scanning. To determine whether the decrease in somatostatin mRNA was specific, actin mRNA levels on the same filters were also determined. The somatostatin mRNA levels for each sample were expressed as a ratio of the corresponding actin mRNA levels for each sample (Fig. 3 B). The somatostatin mRNA to actin mRNA ratio was nearly threefold lower in RNA from omeprazole-treated rats compared with RNA from control-treated rats. The decreased somatostatin synthesis was also demonstrated by decreased somatostatin peptide concentration in antral extracts. (Fig. 3 C). Dot-blot analysis was used to quantitate somatostatin mRNA levels more precisely (Fig. 4). There was a linear relationship between the amount of RNA that was loaded and the somatostatin mRNA levels. At all quantities of RNA loaded, somatostatin mRNA levels were significantly lower in RNA from omeprazole-treated rats compared with

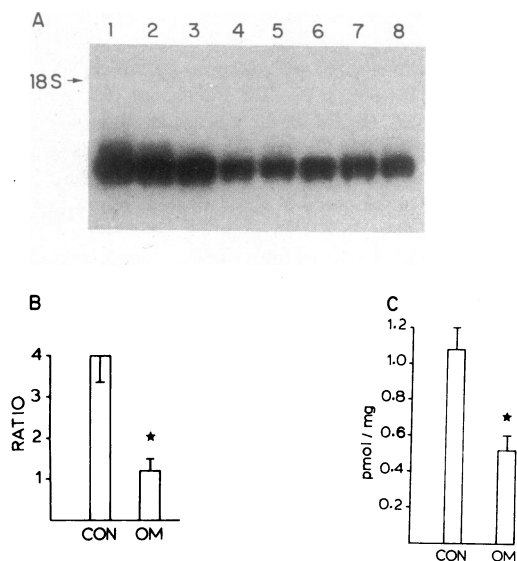


Figure 3. (A) Autoradiogram of a Northern blot measuring somatostatin mRNA in 20 µg total antral RNA isolated from omeprazole-treated (lanes 4–6), and control-treated rats (lanes 1–3). Lanes 7 and 8 show antral RNA from rats treated with both omeprazole and somatostatin analogue SMS 201-995, 10 µg/kg *bis die* (OM and SMS). Blot was hybridized for 16 h with rat somatostatin cRNA probe at 65°C in 50% formamide, 5× SSC and washed 0.2× SSC 0.1% SDS at 65°C. Blots were exposed for 12 h at room temperature. (B) The ratio of somatostatin to actin mRNA levels in antral RNA isolated from omeprazole-treated rats (OM) and control rats (CON). Northern blots were hybridized as in Fig. 1 to a rat actin probe and then autoradiographed. After melting off the actin probe, the filter was then hybridized with a rat somatostatin cRNA probe and then reautoradiographed. Somatostatin and actin mRNA levels were quantitated by scanning densitometry. The ratio of somatostatin to actin levels in each RNA sample was calculated. Results are expressed as the mean±SE, *n* = 10. **P* < 0.01. (C) Somatostatin peptide concentrations in antral extracts from omeprazole- (OM) and control- (CON) treated rats (mean±SE, *n* = 14) **P* < 0.01.

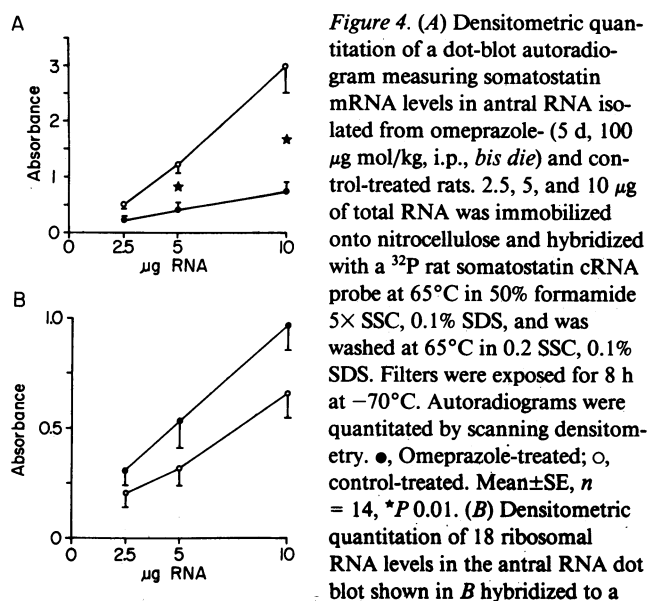


Figure 4. (A) Densitometric quantitation of a dot-blot autoradiogram measuring somatostatin mRNA levels in antral RNA isolated from omeprazole- (5 d, 100 µg mol/kg, *i.p.*, *bis die*) and control-treated rats. 2.5, 5, and 10 µg of total RNA was immobilized onto nitrocellulose and hybridized with a ³²P rat somatostatin cRNA probe at 65°C in 50% formamide 5× SSC, 0.1% SDS, and was washed at 65°C in 0.2 SSC, 0.1% SDS. Filters were exposed for 8 h at -70°C. Autoradiograms were quantitated by scanning densitometry. ●, Omeprazole-treated; ○, control-treated. Mean±SE, *n* = 14, **P* 0.01. (B) Densitometric quantitation of 18 ribosomal RNA levels in the antral RNA dot blot shown in B hybridized to a

³²P-labeled synthetic oligonucleotide complementary to 18S ribosomal RNA as in Fig. 2. Exposure was for 12 h at 70°C. ●, Omeprazole treated; ○, control treated. Mean±SE, *n* = 14. **P* < 0.01.

controls. This was not due to systematically less RNA binding to the filter since the levels of ribosomal RNA bindings were somewhat higher in the omeprazole-treated samples. The changes in gastrin and somatostatin mRNA with omeprazole treatment are restricted to the antrum, as no changes in duodenal gastrin and somatostatin mRNA levels were observed (Fig. 5) with omeprazole treatment. This implies that the effects of omeprazole on antral gastrin and somatostatin synthesis are a consequence of the achlorhydria rather than a direct effect on endocrine cells. Unlike the an-

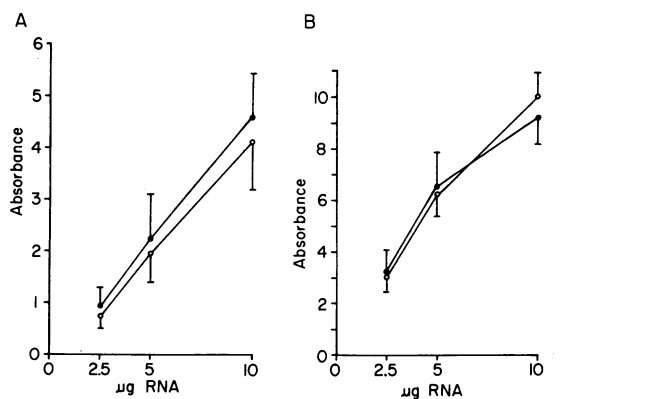


Figure 5. (A) Duodenal gastrin mRNA levels in total RNA isolated from omeprazole (●) and control (○) rats quantitated by dot-blot analysis; 2.5, 5, 10 µg total RNA immobilized on nitrocellulose was hybridized with a rat gastrin cRNA probe and was washed as in Fig. 2 A. Autoradiogram was exposed for 3 d at -70°C. Autoradiograms were quantitated by densitometric scanning. Results are expressed in arbitrary absorbance units, mean±SE. (B) Duodenal somatostatin mRNA levels in RNA isolated from omeprazole- (●) and control- (○) treated rats quantitated by dot-blot analysis. A duplicate filter of Fig. 3 A was hybridized to a ³²P rat somatostatin cRNA probe and was washed under the conditions discussed in Fig. 2 A. Autoradiograms were exposed for 12 h and quantitated by densitometric scanning. Results are expressed in arbitrary absorbance units; mean±SE.

trum, duodenal endocrine cells are not exposed to an acid environment, the pH of duodenal contents being maintained near neutrality by pancreatic bicarbonate secretion. Omeprazole-induced achlorhydria is therefore unlikely to have any effect on duodenal pH, since the reduced acidity of the gastric contents that empty into the duodenum are balanced by reduced secretin-mediated stimulation of pancreatic bicarbonate secretion.

To determine the time course of the stimulation of gastrin secretion and synthesis induced by omeprazole, serum gastrin and gastrin mRNA levels were determined in rats treated with omeprazole for 1, 2, 4, and 12 h after a single intravenous injection or 12 h after 1-, 2-, or 5-d course of intraperitoneal injections given twice daily (8 a.m. and 5 p.m.). The levels of gastrin and somatostatin mRNA in antral RNA were quantitated by both Northern blot (Fig. 6) and dot-blot analyses (Fig. 7). Although serum gastrin was elevated 2 h after a single injection of omeprazole, more than 24 h were required for a significant increase in gastrin mRNA levels (Figs. 6 and 7 B). The decrease in somatostatin mRNA levels in the antrum was also delayed, occurring 24 h after omeprazole-induced achlorhydria (Figs. 6 B and 7 A).

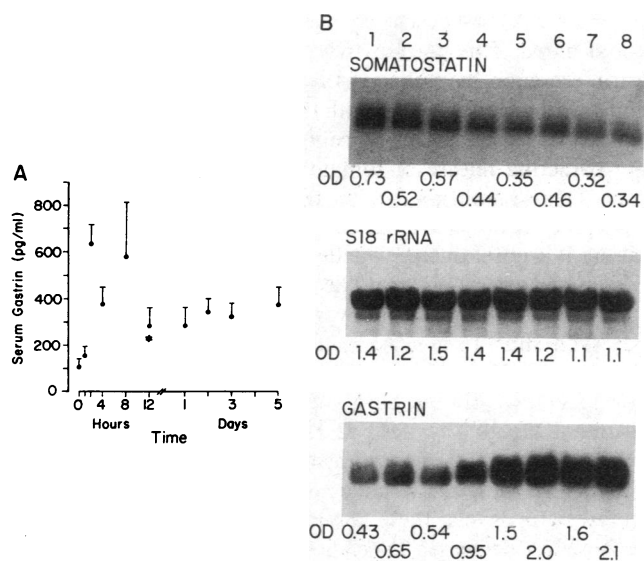


Figure 6. (A) Serum gastrin concentrations at different durations of omeprazole treatment. Serum gastrins were determined 1, 2, 4, and 12 h after a single intravenous injection of 100 $\mu\text{mol/kg}$ omeprazole or 12 h after 1-, 2-, or 5-d course of twice daily intraperitoneal injections (8 a.m. and 6 p.m.). Results are expressed as mean \pm SE, * P < 0.01. $n = 4$ for 1, 2, and 4 h. $n = 8$ for day 1, 2, and 5. (B) Autoradiogram of a Northern blot of 20 μg total antral RNA from rats treated with omeprazole (100 $\mu\text{g/kg}$ *bis die*) for 1 d (lanes 3 and 4), 2 d (lanes 5 and 6), and 5 d (lanes 7 and 8) compared with untreated controls (lanes 1 and 2). The blot was hybridized first with a somatostatin cRNA probe (conditions as discussed in Fig. 4 A) with an exposure of 12 h at -70°C , then reprobated with the 18S ribosomal RNA probe (conditions as discussed in Fig. 2 C), with an exposure of 16 h at -70° , then it was probed with a rat gastrin cRNA under conditions as discussed in Fig. 2 A. Exposure went on for 5 h at room temperature. Densitometric quantitation of somatostatin, ribosomal, and gastrin RNA levels are shown below each autoradiogram and are expressed in arbitrary absorbance units.

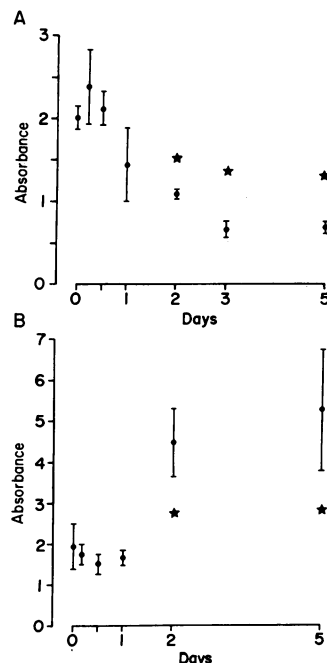


Figure 7. Time course of changes in somatostatin (A) and gastrin (B) mRNA levels in antral mRNA with omeprazole-induced achlorhydria. Antral RNA was isolated from rats 4 and 12 h after an omeprazole injection (100 $\mu\text{mol/kg}$, i.v., *bis die*) and 1, 2, 3, and 5 d after omeprazole 100- μg , *bis die* intraperitoneal injection, and compared with mRNA levels in untreated controls. mRNA levels were quantitated by dot-blot hybridization as described in Fig. 2 A and Fig. 4 B, respectively. Results are expressed as mean \pm SE, $n = 4$. * P < 0.01.

If decreased somatostatin release causes the increased levels of gastrin mRNA seen with omeprazole-induced achlorhydria, then administration of an amount of exogenous somatostatin sufficient to increase local somatostatin levels should prevent the increase in gastrin mRNA. To test this hypothesis, omeprazole-treated and control rats were injected with the long-acting somatostatin analogue SMS 201-995 (10 $\mu\text{g/kg}$ twice daily for 5 d) (32). Antral RNA was isolated and gastrin mRNA levels were determined by Northern and quantitative dot-blot analysis. Exogenous somatostatin prevented both the increase in serum gastrin and gastrin mRNA that resulted from omeprazole treatment (Fig. 8). Somatostatin injections, however, did not change serum-gastrin concentrations or gastrin mRNA levels in control rats. Furthermore, exogenous somatostatin did not prevent the decrease in antral somatostatin mRNA (Fig. 3 A, lanes 7 and 8) or peptide levels (Fig. 8 C) seen after omeprazole treatment. This excludes the trivial explanation for the inhibition of gastrin gene expression by exogenous somatostatin after omeprazole treatment, namely that somatostatin prevents omeprazole-induced achlorhydria, an effect reported by de Graef et al. (33).

Discussion

Although the regulation of gastrin secretion is well understood (1), few studies have investigated the physiological factors that control gastrin gene expression. A previous study suggested that surgically induced achlorhydria stimulated gastrin biosynthesis and increased gastrin secretion as well (5). The rapid and complete blockade of gastric acid secretion with omeprazole offers a convenient approach with which the effects of short term achlorhydria on gastrin gene expression can be demonstrated. Omeprazole-induced achlorhydria stimulates a specific increase in antral gastrin gene expression without any effect on duodenal gastrin mRNA levels. Although gastrin secretion is stimulated within 2 h of an omeprazole injection, the

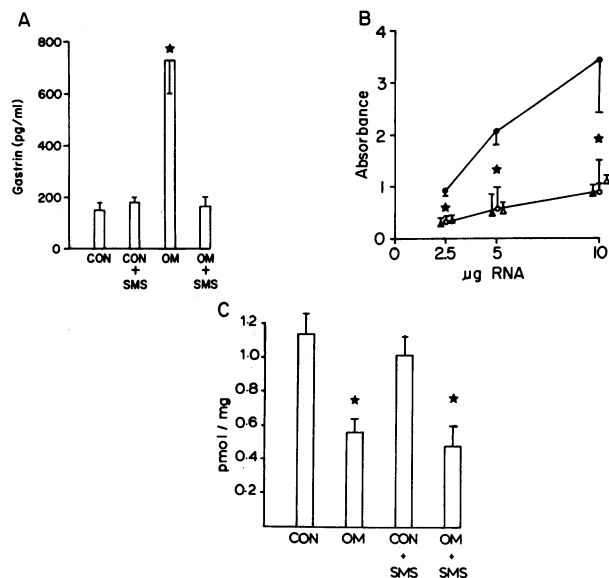


Figure 8. (A) Serum gastrin concentrations after 5-d treatment with omeprazole alone (100 $\mu\text{mol/kg}$ *bis die*, i.p.) alone (OM), PEG 4000 carrier alone (CON), or, combined with the somatostatin analogue SMS 201-998 (10 $\mu\text{g/kg}$ *bis die* i.p.), (CON + SMS and OM + SMS). Mean \pm SE, $n = 6$. * $P < 0.01$. (B) Gastrin mRNA levels in antral RNA after treatment with omeprazole alone (●) or omeprazole plus somatostatin (▲) compared with control-treated PEG carrier alone (○) or combined with somatostatin (△). 2.5, 5, and 10 μg total RNA was dot blotted onto nitrocellulose, hybridized to a rat gastrin cRNA probe as in Fig. 2, and quantitated by densitometric scanning of autoradiograms. Results are expressed in arbitrary absorbance units. Mean \pm SE, $n = 6$. * $P < 0.01$. (C) Antral somatostatin peptide concentrations after a 5-d treatment with omeprazole alone (100 $\mu\text{mol/kg}$ *bis die*, i.p.) (OM), PEG 4000 carrier alone (CON), or combined with the somatostatin analogue SMS 201-998 (10 $\mu\text{g/kg}$ *bis die*) (CON + SMS and OM + SMS). Mean \pm SE. * $P < 0.01$.

increase in gastrin mRNA is only seen after 24 h of achlorhydria. A similar delay in the rise in proopiomelanocortin (POMC)¹ mRNA levels when compared with the stimulation of adrenocorticotrophic hormone secretion is also seen in pituitary corticotrophs when they are released from glucocorticoid inhibition by adrenalectomy (34). Nevertheless, POMC gene transcription was maximally elevated within 1 h of adrenalectomy (34), which implies that stimulation of gene activity occurs concurrently with the increased secretion. The slow accumulation of mRNA results from the fact that the gene transcription rate is small compared with the stability of the cytoplasmic pool of POMC mRNA. Although further studies are necessary to demonstrate that achlorhydria stimulates gastrin gene transcription and that this is concomitant with the stimulated secretion, it is likely that the delayed rise in gastrin mRNA levels also reflects the stability and size of the cellular pool of gastrin mRNA. It is also possible that the increase in gastrin mRNA with achlorhydria results from a selective increase in gastrin mRNA stability.

Reduced inhibition by somatostatin may mediate the increase in gastrin mRNA levels that result from omeprazole-induced achlorhydria. As reviewed above, substantial evidence suggests that gastric acid inhibits gastrin secretion through stimulating the local release of somatostatin. Chronic achlorhydria induced by omeprazole treatment for 4 wk decreases

antral D-cell density and tissue content of somatostatin possibly through decreased stimulation by gastric acid (6, 7). There is also decreased somatostatin release from the isolated perfused stomach of rats treated with omeprazole for 10 wk (35). This reciprocal relationship between somatostatin and gastrin with chronic omeprazole treatment suggests that decreased somatostatin inhibition causes the hypergastrinemia.

Other studies, however, show that blocking gastric acid secretion may cause hypergastrinemia without demonstrable changes in antral somatostatin. Less complete suppression of gastric acid secretion by ranitidine results in chronic hypergastrinemia without reducing mucosal D-cell density or tissue content of somatostatin (6). Reduced D-cell activity was only seen with the higher gastric pH caused by the complete achlorhydria that was induced by omeprazole (6). Furthermore, studies using shorter term omeprazole treatment have shown that changes in G-cell activity precede effects on D-cell activity. Koop et al. (35) reported that omeprazole treatment for 7 d results in increased gastrin release without a decrease in stimulated somatostatin release. Jensen et al. (1987) have also examined the effect of gastric pH changes on gastrin and somatostatin concentrations in venous blood by draining the antrum in anesthetized, atropinized human subjects during surgery (36). Although reduced acidity increased venous gastrin, and increased acidity reduced gastrin levels, these were not coupled to reciprocal changes in somatostatin levels in venous blood. This suggests that gastric pH changes can alter gastrin secretion by pathways that do not necessarily involve somatostatin. The difficulty with this type of study, however, is that venous somatostatin concentrations do not directly measure paracrine release of somatostatin into the extracellular space. This is emphasized by the failure in this study to observe a difference between arterial and venous somatostatin concentration. It is questionable whether changes in paracrine release with altered gastric pH could be measured in these patients.

In contrast to the above work, the present study suggests that even short-term achlorhydria induced by omeprazole stimulates gastrin secretion and gene expression through reduced somatostatin inhibition of G-cell activity. Omeprazole treatment causes a twofold decrease in somatostatin mRNA levels in the antral mucosa associated with the increase in gastrin mRNA levels. Unlike measuring changes in venous somatostatin levels, these somatostatin mRNA changes directly reflect decreased D-cell activity and imply that there is decreased synthesis and release of somatostatin with achlorhydria. Furthermore, in contrast to Koop et al. (35), the omeprazole-induced decrease in somatostatin mRNA levels occurred at the same time as the increase in gastrin mRNA levels. More direct evidence that somatostatin regulates antral gastrin gene expression is provided by the observation in this study that exogenous somatostatin prevents the stimulation of gastrin mRNA levels by omeprazole.

Although this observed reduced somatostatin synthesis is consistent with reduced G-cell inhibition, the decreased somatostatin mRNA levels are only seen after the onset of the hypergastrinemia. However, the relationship between the onset of increased G-cell secretion and the fall in somatostatin mRNA levels is indirect. The proposed paracrine model postulates that achlorhydria results in both decreased somatostatin secretion and decreased somatostatin gene transcription. It is the kinetics of the decrease in somatostatin secretion that

determines the time course of G-cell effects. The time course of somatostatin mRNA changes, on the other hand, is determined by both the decrease in transcription and the somatostatin mRNA half life. Like the mRNA that encodes other regulatory peptides, the half lives of gastrin and somatostatin mRNA are long. In gastrin- and somatostatin-expressing islet cell lines, actinomycin chase studies have shown that mRNA half lives are > 12 h (unpublished observations). Consequently, the decrease in somatostatin mRNA levels with omeprazole is not seen until after 24 h. Furthermore, the half lives of both somatostatin and gastrin mRNAs are independent variables; the time courses of the two mRNAs are only indirectly related to each other, even though the changes in gene transcription may occur at the same time.

This study, therefore, suggests that somatostatin specifically inhibits gastrin mRNA accumulation as well as gastrin secretion in antral G cells, by regulating either gastrin gene transcription or mRNA stability. In contrast to the inhibition of hormone secretion, effects of somatostatin on gene expression have not been well described. Baringa et al. (18), however, reported that somatostatin does not inhibit growth hormone gene transcription stimulated by growth hormone-releasing factor even though somatostatin inhibited both growth hormone secretion and the rise in cAMP which was shown to stimulate growth hormone gene transcription. This suggests that the inhibitory effects of somatostatin on secretion may not necessarily be linked to its effects on gene transcription. There are many differences between the present study and Baringa et al. in respect to our explanations of the different effects of somatostatin on gastrin and growth hormone gene expression. Baringa et al. studied the changes in growth hormone gene transcription only after brief exposure to somatostatin. By contrast, the present study examined somatostatin's effects on gastrin gene expression after treatment for longer periods. Furthermore, the present in vivo study cannot determine whether the change in gastrin mRNA levels results from a direct effect of somatostatin on G cells or if it is an indirect consequence of somatostatin inhibiting the release of G-cell stimuli (such as gastrin-releasing peptide) from mucosal nerve terminals. Further studies are therefore required to show whether somatostatin inhibits gastrin gene expression by a direct cellular action and to define the intracellular events that mediate somatostatin effects on gastrin gene expression.

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