

Inhibition of gastrin release by secretin is mediated by somatostatin in cultured rat antral mucosa.

M M Wolfe, ... , G M Reel, J E McGuigan

J Clin Invest. 1983;72(5):1586-1593. <https://doi.org/10.1172/JCI111117>.

Research Article

Somatostatin-containing cells have been shown to be in close anatomic proximity to gastrin-producing cells in rat antral mucosa. The present studies were directed to examine the effect of secretin on carbachol-stimulated gastrin release and to assess the potential role of somatostatin in mediating this effect. Rat antral mucosa was cultured at 37 degrees C in Krebs-Henseleit buffer, pH 7.4, gassed with 95% O₂-5% CO₂. After 1 h the culture medium was decanted and mucosal gastrin and somatostatin were extracted. Carbachol (2.5 X 10⁻⁶ M) in the culture medium increased gastrin level in the medium from 14.1 +/- 2.5 to 26.9 +/- 3.0 ng/mg tissue protein (P less than 0.02), and decreased somatostatin-like immunoreactivity in the medium from 1.91 +/- 0.28 to 0.62 +/- 0.12 ng/mg (P less than 0.01) and extracted mucosal somatostatin-like immunoreactivity from 2.60 +/- 0.30 to 1.52 +/- 0.16 ng/mg (P less than 0.001). Rat antral mucosa was then cultured in the presence of secretin to determine its effect on carbachol-stimulated gastrin release. Inclusion of secretin (10⁻⁹-10⁻⁷ M) inhibited significantly carbachol-stimulated gastrin release into the medium, decreasing gastrin from 26.9 +/- 3.0 to 13.6 +/- 3.2 ng/mg (10⁻⁹ M secretin) (P less than 0.05), to 11.9 +/- 1.7 ng/mg (10⁻⁸ secretin) (P less than 0.02), and to 10.8 +/- 4.0 ng/mg (10⁻⁷ M secretin) (P less than [...])

Find the latest version:

<https://jci.me/111117/pdf>



Inhibition of Gastrin Release by Secretin Is Mediated by Somatostatin in Cultured Rat Antral Mucosa

M. MICHAEL WOLFE, G. MARSHALL REEL, and JAMES E. MCGUIGAN, *Department of Medicine, University of Florida College of Medicine, Gainesville, Florida 32610*

ABSTRACT Somatostatin-containing cells have been shown to be in close anatomic proximity to gastrin-producing cells in rat antral mucosa. The present studies were directed to examine the effect of secretin on carbachol-stimulated gastrin release and to assess the potential role of somatostatin in mediating this effect. Rat antral mucosa was cultured at 37°C in Krebs-Henseleit buffer, pH 7.4, gassed with 95% O₂-5% CO₂. After 1 h the culture medium was decanted and mucosal gastrin and somatostatin were extracted. Carbachol (2.5 × 10⁻⁶ M) in the culture medium increased gastrin level in the medium from 14.1±2.5 to 26.9±3.0 ng/mg tissue protein (*P* < 0.02), and decreased somatostatin-like immunoreactivity in the medium from 1.91±0.28 to 0.62±0.12 ng/mg (*P* < 0.01) and extracted mucosal somatostatin-like immunoreactivity from 2.60±0.30 to 1.52±0.16 ng/mg (*P* < 0.001). Rat antral mucosa was then cultured in the presence of secretin to determine its effect on carbachol-stimulated gastrin release. Inclusion of secretin (10⁻⁹-10⁻⁷ M) inhibited significantly carbachol-stimulated gastrin release into the medium, decreasing gastrin from 26.9±3.0 to 13.6±3.2 ng/mg (10⁻⁹ M secretin) (*P* < 0.05), to 11.9±1.7 ng/mg (10⁻⁸ secretin) (*P* < 0.02), and to 10.8±4.0 ng/mg (10⁻⁷ M secretin) (*P* < 0.02). Secretin (10⁻⁷ and 10⁻⁸ M) also increased concomitantly culture medium somatostatin concentration. To determine whether secretion inhibition of carbachol-stimulated gastrin release was mediated by somatostatin, antral mucosa was cultured with carbachol, secretin (10⁻⁹-10⁻⁷ M), and antibodies to somatostatin. Inclusion of somatostatin antibodies in the culture medium abolished the capacity of secretin (10⁻⁷ and 10⁻⁸ M) to inhibit carbachol-stimulated gastrin release. Results of these studies indicate (a) that secretin inhibits carbachol-stimulated gastrin release and (b) that under the conditions of these experiments secretin inhibition of gastrin release is mediated, at

least in part, locally through release of antral somatostatin.

INTRODUCTION

Somatostatin, a polypeptide originally isolated from ovine hypothalamus, is a potent inhibitor of gastrin release (1-3). Somatostatin-containing cells have been demonstrated in close proximity to gastrin-producing cells in rat antral mucosa, suggesting a potential local regulatory role for somatostatin (4). More recently, after demonstrating significant increases in basal gastrin release with addition of somatostatin antibodies to incubated rat antral mucosa, Chiba et al. (5) concluded that antral somatostatin exerted its inhibitory effect on gastrin through paracrine pathways. In studies using the isolated perfused rat stomach, stimulation of somatostatin release, accompanied by inhibition of gastrin release, was shown to follow intraarterial infusion of glucagon, vasoactive intestinal peptide (VIP),¹ secretin, and gastric inhibitory peptide (GIP) (6, 7). Recently, secretin and GIP have been shown to be physiologic inhibitors of meal-stimulated gastrin release and gastric acid secretion in dogs (8, 9). Results of these investigations suggest a possible role for gastric somatostatin in mediating the inhibitory effects of certain of these regulatory peptides on gastrin release and gastric acid secretion.

This study was designed to examine the effects of secretin on carbachol-stimulated gastrin release in antral tissue culture and to determine the potential role of somatostatin in mediating these effects.

¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; CCK, cholecystokinin; GIP, gastric inhibitory peptide; GRP, gastrin-releasing peptide; KHB buffer, Krebs-Henseleit bicarbonate buffer; NRS, normal rabbit serum; SHG, synthetic human gastrin I; SLI, somatostatin-like immunoreactivity; VIP, vasoactive intestinal peptide.

Address reprint requests to Dr. M. Michael Wolfe.

Received for publication 21 April 1983 and in revised form 1 July 1983.

METHODS

Preparation of antibodies to somatostatin. Cyclic somatostatin (15-28) (Beckman Instruments, Inc., Palo Alto, CA) was conjugated to bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO), and rabbits were immunized with the somatostatin-BSA conjugate, as previously described (10). Antibodies to somatostatin were identified in serum of each of the immunized rabbits. Antibodies to somatostatin in one antiserum preparation (117-3) after three immunizations were used in the studies described in this report.

The antibody activity of antiserum 117-3 was characterized using ^{125}I -somatostatin, prepared by a modification of the chloramine-T method of Hunter and Greenwood (11). The assay system contained 100 μl of somatostatin antiserum at a final dilution of 1:600,000, 100 μl of ^{125}I -somatostatin (1,500-2,000 cpm), 10 μg rabbit gamma globulin, and varying amounts of somatostatin. All reactants were prepared in 0.05 M potassium phosphate buffer (pH 7.45), which contained 6.7 mM EDTA, aprotinin (Trasyol, Bayer, Leverkusen, FRG) 100 KIU/ml, BSA 1 mg/ml, and sodium azide 200 $\mu\text{g}/\text{ml}$ in a total incubation volume of 1.0 ml. After incubation at 4°C for 48 h, 100 μl of goat anti-rabbit gamma globulin (1:400 final dilution) was added, and tubes were vortexed and incubated at 4°C for 24 h. After centrifugation at 2,000 g at 4°C for 35 min, precipitates (containing antibody-bound ^{125}I -somatostatin) and supernates were separated and counted with an automatic gamma spectrometer (model 1185, Tracor Analytic, Inc., Elk Grove Village, IL).

A Scatchard plot of binding of somatostatin by antibodies to somatostatin was constructed by plotting the ratio of antibody-bound to free immunoreactive ^{125}I -somatostatin against the concentration of antibody-bound somatostatin (Fig. 1). The maximum binding capacity of this preparation of antibodies to somatostatin was obtained by extrapolating the binding curve to a bound/free ratio for ^{125}I -somatostatin to zero (12). The affinity (average intrinsic association constant, K_a) for the antibody preparation for somatostatin was calculated as the reciprocal of the unbound somatostatin concentration in the incubation medium with one-half occupancy of antibody-binding sites by somatostatin at equilibrium (13). Analysis of logit plots for standard calibration curves using unlabeled somatostatin and using ^{125}I -somatostatin indicated that the preparation of antibodies to somatostatin exhibited comparable affinity for radiolabeled and unlabeled somatostatin.

Somatostatin antiserum 117-3 was also examined for potential immunological cross-reactivity with other gastrointestinal peptides and somatostatin fragments. These included purified porcine VIP and 99% pure porcine cholecystokinin-pancreozymin (CCK), obtained from Dr. Viktor Mutt, the Gastrointestinal Hormone Research Laboratory, Karolinska Institute, Stockholm, Sweden; crystalline porcine glucagon, purchased from Eli Lilly & Co., Indianapolis, IN; synthetic human gastrin I (SHG) (G17), obtained from Imperial Chemical Industries, Ltd., Cheshire, England; human big gastrin (G34), a gift from Professor R. A. Gregory, The Physiological Laboratory, University of Liverpool, England; purified porcine gastric inhibitory peptide (GIP), purchased from Professor John C. Brown, University of British Columbia, Vancouver, Canada; and pentagastrin (Peptavlon), obtained from Ayerst Laboratories, New York. CCK octapeptide and synthetic porcine secretin were obtained from E. R. Squibb & Sons, Inc., Princeton, NJ. Somatostatin 28 (1-14) and somatostatin 28 were purchased from Bachem Fine Chemicals, Torrance, CA.

Control rabbit serum used in this study was obtained from

two healthy fasting New Zealand White rabbits. Control serum and somatostatin antiserum were filtered through 0.20- μm plain membrane Nalgene filter units (Nalge Co., Nalgene Labware Div., Rochester, NY) and were stored in sterile containers at -40°C until used in these studies described below.

Tissue culture technique. Antral tissue was obtained from fasting, male Sprague-Dawley rats (200-250 g) and was washed with modified Krebs-Henseleit bicarbonate (KHB) buffer, pH 7.4 (110 mM NaCl, 4.7 mM KCl, 1 mM CaCl_2 , 1.13 mM MgCl_2 , 1.15 mM NaH_2PO_4 , 25 mM NaHCO_3 , 10 mM Hepes, 5.6 mM glucose, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin; all reagents purchased from Fisher Scientific Co., Pittsburgh, PA), containing 2 g/liter BSA. Antral mucosa stripped from the muscularis was sectioned into 225- μm fragments with a Sorvall TC-2 sectioner (DuPont Co., Sorvall Biomedical Div., Newton, CT). Antral mucosal tissue fragments were stabilized during an initial 30-min period in KHB buffer at 37°C, gassed with 95% O_2 -5% CO_2 ; stabilization and test cultures were performed in a Dubnoff metabolic shaking incubator (GCA Precision Scientific Group, Chicago, IL) at 100 oscillations/min. Mucosal fragments were separated from stabilization medium by centrifugation at 400 g for 5 min and were resuspended in either control or test medium. Replicate cultures consisted of 1-ml aliquots of antral tissue fragments and medium, which were cultured for 60 min at 37°C (pH maintained at 7.4) in 12 \times 75-mm polypropylene culture tubes. Media were sampled for radioimmunoassay determination of gastrin and somatostatin concentrations, and antral mucosal tissue was harvested at the end of the incubation period. Antral gastrin and somatostatin were extracted by boiling antral mucosal fragments in 2 ml distilled water, and residual tissue was preserved for subsequent protein determination. Media and antral extracts were stored at -20°C until assayed.

Experimental design. Antral mucosa was incubated under control conditions in KHB buffer containing normal rabbit serum (NRS) at a final dilution of 1:500. The dose-response effects of the cholinergic agent carbamylcholine chloride (carbachol; Sigma Chemical Co.) on antral gastrin release, which were previously examined, indicated that maximal gastrin release was achieved with 2.5×10^{-6} M carbachol. In these studies carbachol was included in the culture medium at a concentration of 2.5×10^{-6} M to determine its effect on gastrin and somatostatin release. The effect of somatostatin on release of antral mucosal gastrin into the culture medium and on carbachol-stimulated gastrin release was examined by inclusion of rabbit somatostatin antiserum (final dilution of 1:500). Effects of secretin on carbachol-stimulated gastrin release were examined in separate experiments in which secretin (10^{-9} to 10^{-7} M) was added to the culture media in the presence of either somatostatin antiserum or NRS (as control).

After a 1-h incubation, antral mucosal fragments were boiled in 2 ml distilled water, and the protein contents of residual tissue sediments were determined by the method of Lowry et al. (14).

Radioimmunoassay determination of gastrin and somatostatin concentrations. Samples of culture medium obtained at 60 min were assayed in triplicate with antibodies to gastrin (56-02), which measure all known bioactive gastrin species, using a previously described method (10). Dilutions of assay reagents were prepared in 0.02 M sodium barbital buffer, pH 8.4, containing 2% normal human serum. All standards and plasma samples were included as 100- μl aliquots in a total incubation volume of 2 ml in 10 \times 75-mm glass tubes. To each standard and sample were added 100 μl gas-

trin antiserum at a final dilution of 1:2,000,000 and 100 μ l of synthetic ^{125}I -SHG (G17) ($\sim 3,500$ – $4,000$ cpm) prepared by a modification of the chloramine-T method of Hunter and Greenwood (11, 15). Serum gastrin concentrations were calculated from calibration curves using SHG (G17) standard concentrations of 1,000 to 8 pg/ml. Sensitivity of the assay was 8 pg/ml (0.8 pg gastrin/incubation tube). Cross-reactivity of the gastrin antiserum (56-02) with porcine CCK was <1% (16).

Somatostatin-like immunoreactivity (SLI) was measured in culture media and antral extracts by radioimmunoassay. The double-antibody method was used for separation of antibody-bound from free ^{125}I -somatostatin. All standards and samples were assayed as 100- μ l aliquots in a total incubation volume of 1 ml. Assays were performed in 10 \times 75-mm glass incubation tubes in triplicate, and SLI was calculated from somatostatin calibration curves using standard concentrations of 1,000 to 8 pg/ml. Sensitivity of the assay was 16 pg/ml (1.6 pg somatostatin/incubation tube).

Statistical analysis. The Student's *t* test for unpaired samples was used to compare data obtained under the various conditions. Statistical significance was assigned if $P < 0.05$.

RESULTS

Characterization of antiserum to somatostatin.

The K_a of the preparation of antibodies to somatostatin (117-3) was $5.2 \times 10^{10} \text{ M}^{-1}$. The total binding capacity of the undiluted antiserum to somatostatin was found to be 2.86 μg somatostatin/ml antiserum (Fig. 1).

Studies examining potential immunological cross-reactivity of other gastrointestinal peptides with somatostatin antibody binding of ^{125}I -somatostatin are indicated in Fig. 2. Studies were performed, as de-

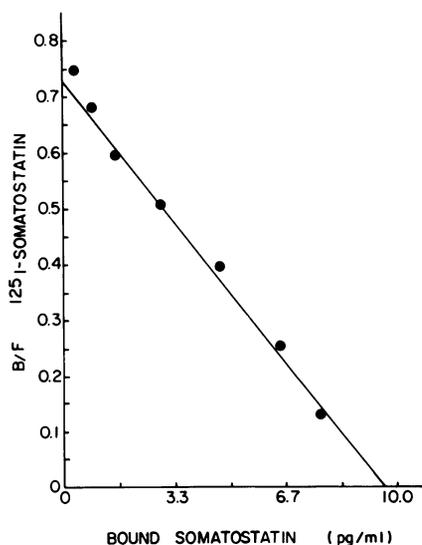


FIGURE 1 Scatchard plot with the ratio of bound/free (B/F) ^{125}I -somatostatin as a function of antibody-bound somatostatin. The somatostatin antiserum was used at a final dilution of 1:300,000. The binding capacity of somatostatin antiserum was 2.86 μg somatostatin/ml antiserum. The K_a was $5.2 \times 10^{10} \text{ M}^{-1}$.

scribed, with each peptide in concentrations from 100 pg/ml to 1.0 μg /ml. Radioimmunoassay of somatostatin demonstrated an intraassay coefficient of variation of 6% and interassay coefficient of variation of 12%. Equivalent equimolar immunoreactivity was exhibited with dihydro-somatostatin 14. There was no detectable immunoreactivity with the aminoterminal tetradecapeptide of somatostatin 28 (somatostatin 28:1-14). There was minimal immunological cross-reactivity with D-tryptophanyl⁸ cyclic somatostatin 14 (index of inhibition with 50% reduction in antibody-bound/antibody-free ^{125}I -somatostatin = 0.0024), indicating antibody specificity for the midportion of the somatostatin (15-28) molecule. There was nearly equivalent immunoreactivity with somatostatin 28 (index of inhibition = 0.75) (Fig. 2). There was no detectable immunological cross-reactivity with any of the other gastrointestinal regulatory peptides examined (Fig. 2).

Effects of carbachol and antibodies to somatostatin on antral gastrin and somatostatin release. In the control cultures, which contained NRS only, media gastrin and SLI were 14 ± 2.5 (SEM) and 1.91 ± 0.28 ng/mg protein, respectively. Inclusion of carbachol $2.5 \times 10^{-6} \text{ M}$ in the culture medium increased media gastrin levels by 93% to 26.9 ± 3.0 ng/mg ($P < 0.02$) (Fig. 3) and decreased media SLI by $\sim 70\%$ to 0.62 ± 0.12 ng/mg ($P < 0.01$) (Table I). Carbachol, which had no significant effect on extracted mucosal gastrin, decreased extracted mucosal SLI from 2.60 ± 0.30 to 1.52 ± 0.16 ng/mg ($P < 0.001$) (Table I).

Incubation of antral mucosa with antibodies to somatostatin significantly increased media gastrin levels, both in the presence and in the absence of carbachol. Media gastrin concentrations increased by $69.3 \pm 17.9\%$ ($P < 0.01$) over control during incubation with antibodies to somatostatin in the absence of carbachol (Fig. 4). Inclusion of antibodies to somatostatin in culture media that contained carbachol increased media gastrin levels by $132 \pm 43.0\%$ ($P < 0.05$) (Fig. 3). This increment was not significantly greater than the increase observed when only somatostatin antiserum was included in the culture media (without carbachol). During incubation with antibodies to somatostatin, both in the presence and absence of carbachol, no unbound immunoreactive somatostatin could be detected in the culture media.

Effects of secretin on carbachol-stimulated gastrin release. Rat antral mucosa was cultured in the presence of secretin (10^{-9} – 10^{-7} M) to determine its effect on carbachol-stimulated gastrin release. At all doses examined secretin inhibited significantly carbachol-stimulated gastrin release into the media, decreasing media gastrin concentrations from 26.9 ± 3.0 to 13.6 ± 3.2 ng/mg protein with 10^{-9} M secretin ($P < 0.05$), to 11.9 ± 1.7 ng/mg with 10^{-8} M secretin ($P < 0.02$), and

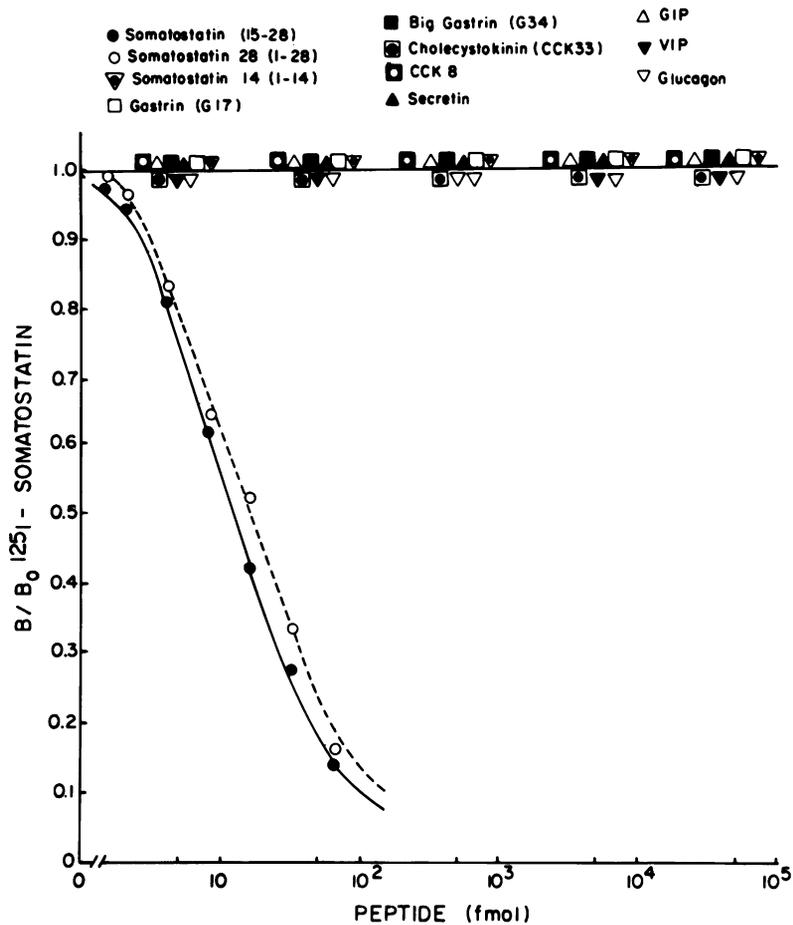


FIGURE 2 Calibration curve examining potential cross-reactivity of gastrointestinal peptides and glucagon with antibodies to somatostatin. With the exception of somatostatin 28 there was no detectable immunological cross-reactivity with any of the peptides studied. Relative to the potency of cyclic somatostatin (15-28), somatostatin inhibited antibody binding of ^{125}I -somatostatin by $\sim 75\%$.

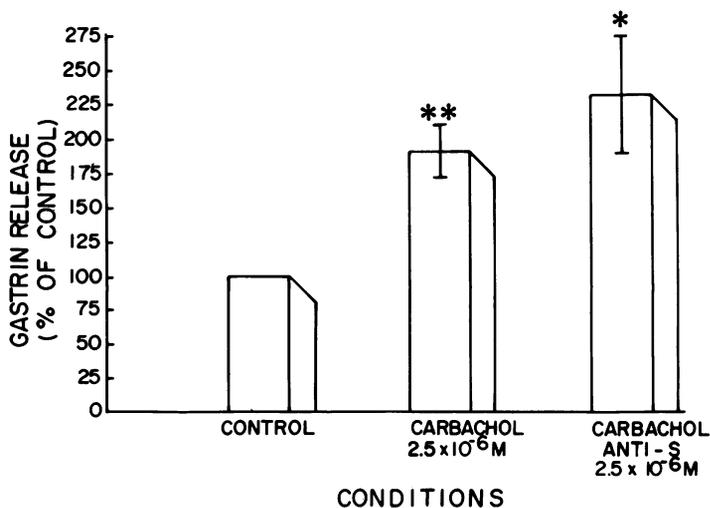


FIGURE 3 Media gastrin levels under control conditions and with culture media containing carbachol $2.5 \times 10^{-6} \text{ M}$. Results are expressed as percent change over control \pm SEM. * $P < 0.05$; ** $P < 0.01$.

TABLE I
Culture Medium and Antral Tissue Somatostatin

Condition	Culture medium	Antral tissue
	ng/mg protein	
Control	1.91±0.28	2.60±0.30
Carbachol 2.5×10^{-6}	0.62±0.12*	1.52±0.16†
Anti-S	ND	5.00±0.82‡
Carbachol plus anti-S	ND	2.94±0.30

Results are expressed as the mean±SEM of 12 replicates. ND, none detected; anti-S, antibodies to somatostatin.

* $P < 0.01$.

† $P < 0.001$.

‡ $P < 0.02$ compared with control conditions.

to 10.8 ± 4.0 ng/mg with 10^{-7} M secretin ($P < 0.02$) (Fig. 5). In addition, inclusion of secretin (10^{-8} and 10^{-7} M) in culture medium containing carbachol resulted in concomitant increases in media SLI.

To determine whether inhibition of carbachol-stimulated gastrin release was mediated by somatostatin, antral mucosa was cultured in the presence of carbachol, secretin (10^{-9} – 10^{-7} M), and antibodies to somatostatin. Antibodies to somatostatin abolished the capacity of secretin (10^{-8} and 10^{-7} M) to inhibit carbachol-stimulated gastrin release: in the presence of 10^{-8} M secretin, gastrin in the culture media increased from 11.9 ± 1.7 (in the absence of somatostatin antiserum) to 22.7 ± 3.8 ng/mg ($P < 0.05$) in the presence of antibodies to somatostatin and, at 10^{-7} M, secretin culture media gastrin increased from 10.8 ± 4.0 to 28.1 ± 4.4 ng/mg ($P < 0.02$) (Fig. 6). During incubation with antibodies to somatostatin in the presence of secretin, no somatostatin could be detected in the culture media.

DISCUSSION

Due to its widespread location and its multiple and diverse inhibitory activities, somatostatin has been proposed as a likely potential candidate for local paracrine regulation of various endocrine cells. Somatostatin has been shown to inhibit hypothalamic growth hormone release (17) and thyrotropin-releasing hormone-induced secretion of thyroid-stimulating hormone (18). In pancreatic islets somatostatin cells occur in close proximity to both α - and β -cells, and somatostatin inhibits directly both basal and stimulated insulin and glucagon release (19). Recently, somatostatin was identified in cholinergic postganglionic neurons of the cardiac vagus, where it inhibits the cardiac pacemaker and atrial muscle (20). Somatostatin, a potent inhibitor of gastric acid secretion, is found in abundant quantities in the mucosa of the body and antrum of the

stomach (5). Somatostatin inhibits acid secretion both by direct inhibition of parietal cells (21) and indirectly through inhibition of gastrin release (1–3, 21).

The diversity of actions of somatostatin and the vast array of chemical regulators that influence gastrin release in intact animals make it advantageous to investigate the effects of somatostatin on gastrin-secreting cells by means of in vitro methods. Antral mucosal tissue culture, which permits direct assessment of gastrin-producing cell function, has been used to demonstrate the effects of the cholinergic agent carbachol on gastrin synthesis and secretion (22, 23). Recently, Chiba et al. (5) examined the effects of somatostatin antibodies on basal gastrin release using anesthetized rats, the isolated perfused rat stomach, and incubated rat antral mucosal culture (5). Although basal gastrin concentrations were not affected in intact animals or in the isolated perfused rat stomach, they found that addition of antisomatostatin γ -globulin to incubated rat antral mucosa increased gastrin release (5).

In this study, addition of somatostatin antiserum to rat antral mucosal culture enhanced basal gastrin release into the culture medium (Fig. 4). These findings are consistent with the aforementioned studies of Chiba et al. (5) and provide further support for a local regulatory role for somatostatin in regulation of gastrin release. The results are also consistent with an intraluminal mode of transmission and action for somatostatin. Inclusion of carbachol 2.5×10^{-6} M in the culture medium significantly increased release of gastrin into the culture medium (Fig. 3) and concomitantly decreased both culture media and tissue SLI (Table I). These observations are in accord with and extend the findings of Harty and McGuigan (23), who reported carbachol-stimulated gastrin synthesis and release in

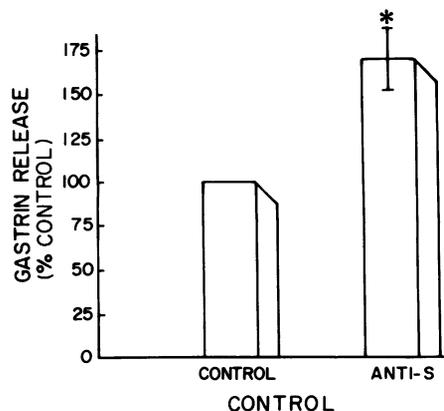


FIGURE 4 Media gastrin levels under control conditions and with culture media containing somatostatin antiserum (anti-S). Results are expressed as percent change over control±SEM. * $P < 0.01$.

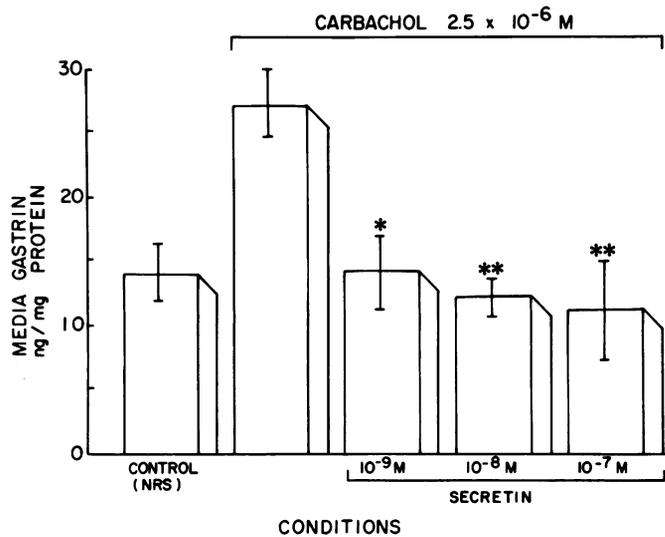


FIGURE 5 Media gastrin concentrations under control conditions and with culture media containing carbachol 2.5×10^{-6} M alone or both carbachol and secretin 10^{-9} – 10^{-7} M. Results are expressed as nanograms gastrin per milligram protein \pm SEM. * $P < 0.05$; ** $P < 0.02$.

rat antral organ culture, and its progressive inhibition by increasing concentrations of atropine in the culture media. They also corroborate previous studies performed using the isolated perfused rat stomach, reporting carbachol-stimulated gastrin release associated with inhibition of somatostatin release (24–26).

Previous studies have shown that members of the secretin family of gastrointestinal regulatory pep-

tides—secretin, glucagon, VIP, and GIP—possess the capacity to inhibit gastrin release and gastric acid secretion (6, 8, 9). In addition, stimulation of somatostatin release, associated with simultaneous inhibition of gastrin release, has been demonstrated in the isolated perfused rat stomach after intraarterial infusion of secretin, glucagon, VIP, and GIP (6, 7). These studies suggest a potential important role for gastric somato-

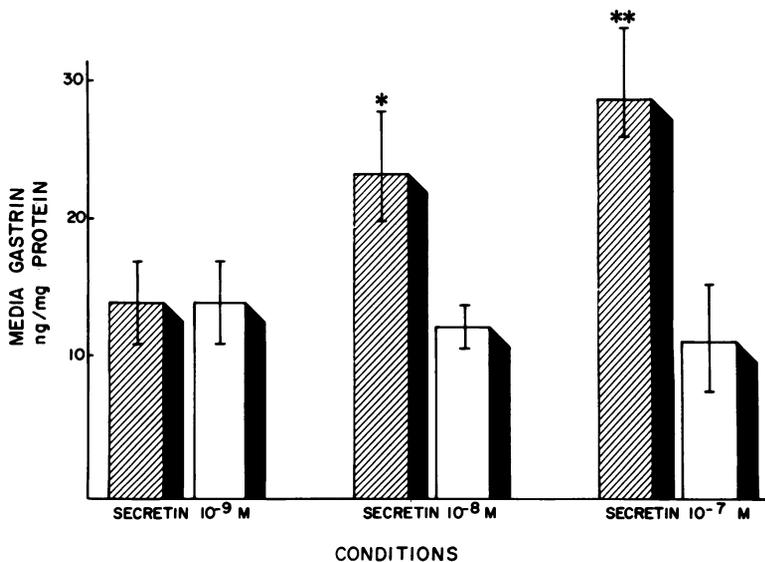


FIGURE 6 Media gastrin concentrations obtained during antral tissue culture with carbachol 2.5×10^{-6} M and secretin 10^{-9} – 10^{-7} M, containing either somatostatin antiserum (anti-S, ▨) or NRS (□). Results are expressed as nanograms gastrin per milligram protein \pm SEM. * $P < 0.05$; ** $P < 0.02$.

statin in mediating the inhibitory effects of members of the secretin family of gastrointestinal peptide hormones on gastrin release and gastric acid secretion. In the present study, when secretin was included in culture media, carbachol-stimulated gastrin release into the media was reduced significantly, whereas SLI in the culture media was increased (Fig. 5). To determine whether inhibition of carbachol-stimulated gastrin release was mediated by somatostatin, antral mucosa was cultured in the presence of carbachol, secretin, and specific antibodies to somatostatin. Inclusion of antibodies to somatostatin in excess in the culture medium abolished the capacity of secretin (10^{-7} and 10^{-8}) to inhibit carbachol-stimulated gastrin release (Fig. 6). These results differ from studies by Saffouri et al. (27) using the isolated perfused rat stomach, who found that although intraarterial infusion of secretin increased somatostatin secretion and decreased gastrin secretion, addition of somatostatin antiserum did not prevent inhibition of gastrin secretion. They concluded that secretin inhibited gastrin release directly, by a mechanism independent of secretion of somatostatin (28).

Our study supports the conclusion that inhibition of gastrin release by secretin is indeed mediated, at least in part, by somatostatin. Differences in results obtained in this study from those reported by Saffouri et al. (27) may be explained by differences in methods used. Chiba et al. (5), whose studies indicated that somatostatin did appear to function as a paracrine regulator

of gastrin release, concluded that, when investigating peptide interactions involving somatostatin, antral culture techniques may be preferable to the isolated perfused rat stomach. A direct effect of secretin as an inhibitor of gastrin release cannot be excluded by the present experiment, since inhibition of carbachol-stimulated gastrin release by 10^{-9} M secretin (the lowest concentration used) was not abolished by inclusion of somatostatin antiserum in the culture medium (Fig. 6).

DuVal et al. (28) recently proposed a model by which gastrin-releasing peptide (GRP), the mammalian equivalent of bombesin, and somatostatin act in concert to regulate antral gastrin secretion. They postulated that cholinergic stimulation of gastrin release was exerted through release of GRP (by a neural mechanism) and that inhibition of gastrin release was effected by antral somatostatin (28). The present studies provide support for this hypothesis, in that carbachol-stimulated gastrin release was accompanied by simultaneous decreases in culture media and tissue somatostatin (Fig. 3 and Table I). Fig. 7 depicts an extension of the proposed model of DuVal et al. (28), into which results of this experiment have been incorporated, and in which we suggest that physiologic inhibition of gastrin release by regulatory peptides, such as secretin and GIP, may be mediated by somatostatin, as well as by their direct effect on the gastrin-producing cell.

In conclusion, the results of these studies indicate that secretin inhibits carbachol-stimulated gastrin release and that this inhibition is mediated, at least in

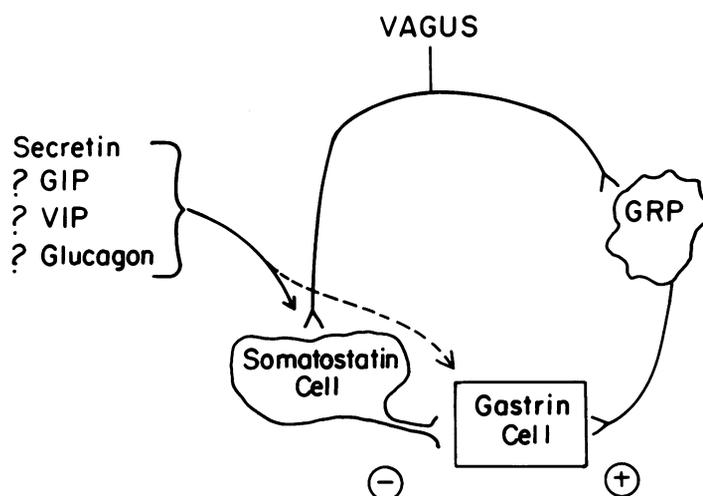


FIGURE 7 Proposed model describing factors involved in the control of gastrin from the gastrin-producing cell. The model represents an expansion of that proposed by DuVal et al. (28), and shows that cholinergic stimulation, under vagal control, of gastrin release is exerted via release of GRP (neural mechanism) and concomitant inhibition of antral somatostatin (local mechanism). In the present model the effects of secretin, a physiologic inhibitor of gastrin release, and possibly other gastrin inhibitors, are mediated through local release of somatostatin, as well as through direct effects on the gastrin-producing cell.

part, locally through release of antral somatostatin. Further investigation is required to define the complex neural and hormonal interrelationships involved in the control of gastrin release and in the physiologic regulation of gastric acid secretion.

ACKNOWLEDGMENTS

The authors wish to thank Dr. R. F. Harty for his expert technical advice and assistance and J. Wilhite for assistance in the preparation of this manuscript.

This study was supported, in part, by Biomedical Research support grant S07 RR05362-21 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health. Dr. Wolfe is the recipient of Clinical Investigator Award AM 00147 from the National Institutes of Health.

REFERENCES

1. Bloom, S. R., C. H. Mortimer, and M. O. Thorner. 1974. Inhibition of gastrin and gastric acid secretion by growth hormone release-inhibiting hormone. *Lancet*. II:1106-1109.
2. Hayes, J. R., D. G. Johnson, D. Koerker, and R. H. Williams. 1975. Inhibition of gastrin release by somatostatin in vitro. *Endocrinology*. 96:1374-1376.
3. Harty, R. F., D. G. Maico, and J. E. McGuigan. 1981. Somatostatin inhibition of basal and carbachol-stimulated gastrin release in rat antral organ culture. *Gastroenterology*. 81:707-712.
4. Larsson, L.-I., N. Goltermann, L. DeMagistris, J. F. Rehfeld, and T. W. Schwartz. 1979. Somatostatin cell processes as pathways for paracrine secretion. *Science (Wash. DC)*. 205:1393-1395.
5. Chiba, T., S. Kadowaki, T. Taminato, K. Chihara, Y. Seino, S. Matsukura, and T. Fujita. 1981. Effect of antisomatostatin γ -globulin on gastrin release in rats. *Gastroenterology*. 81:321-326.
6. 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.
7. McIntosh, C. H. S., R. A. Pederson, H. Koop, and J. C. Brown. 1981. Gastric inhibitory polypeptide-stimulated secretion of somatostatin-like immunoreactivity from the stomach: inhibition by acetylcholine or vagal stimulation. *Can. J. Physiol. Pharmacol.* 59:468-472.
8. Chey, W. Y., M. S. Kim, K. Y. Lee, and T. M. Chang. 1981. Secretin is an enterogastrone in the dog. *Am. J. Physiol.* 240:G239-244.
9. Wolfe, M. M., M. P. Hocking, D. G. Maico, and J. E. McGuigan. 1983. Effects of antibodies to gastric inhibitory peptide on gastric acid secretion and gastrin release in the dog. *Gastroenterology*. 84:941-948.
10. McGuigan, J. E., and M. M. Wolfe. 1982. Gastrin radioimmunoassay. *Clin. Chem.* 28:368-373.
11. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)*. 194:495-496.
12. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci.* 51:660-672.
13. Klotz, I. M. 1946. The application of the law of mass action to binding by proteins. Interactions with calcium. *Arch. Biochem.* 9:109-117.
14. Lowry, O. H., H. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
15. Rehfeld, J. F., F. Stadil, and B. Rubin. 1972. Production and evaluation of antibodies for the radioimmunoassay of gastrin. *Scand. J. Clin. Lab. Invest.* 30:221-232.
16. McGuigan, J. E. 1970. Production and characterization of specific antibodies to gastrin with no cross-reactivity with cholecystokinin-pancreozymin. *Am. J. Med. Sci.* 260:139-149.
17. Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin. 1973. Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science (Wash. DC)*. 179:77-79.
18. Carr, D., A. Gomez-Pan, D. R. Weightman, V. C. M. Roy, R. Hall, G. M. Besser, M. O. Thorner, A. S. McNeilly, A. V. Schally, A. J. Kastin, and D. H. Coy. 1975. Growth hormone release inhibitory hormone: actions on thyrotrophin and prolactin secretion after thyrotrophin-releasing hormone. *Br. Med. J.* 3:67-69.
19. Gerich, J. E., M. A. Charles, and G. M. Grodsky. 1976. Regulation of pancreatic insulin and glucagon secretion. *Annu. Rev. Physiol.* 38:353-388.
20. Campbell, G., I. L. Gibbins, J. L. Morris, J. B. Furness, M. Costa, J. R. Oliver, A. M. Beardsley, and R. Murphy. 1982. Somatostatin is contained in and released from cholinergic nerves in the heart of the toad *Bufo marinus*. *Neuroscience*. 7:2013-2023.
21. Konturek, S. J., J. Tasler, M. Cieszkowski, D. H. Coy, and A. V. Schally. 1976. Effect of growth hormone release-inhibiting hormone on gastric secretion, mucosal blood flow, and serum gastrin. *Gastroenterology*. 70:737-741.
22. Harty, R. F., J. C. van der Vijver, and J. E. McGuigan. 1977. Stimulation of gastrin secretion and synthesis in antral organ culture. *J. Clin. Invest.* 60:51-60.
23. Harty, R. F., and J. E. McGuigan. 1980. Effects of carbachol and atropine on gastrin secretion and synthesis in rat antral organ culture. *Gastroenterology*. 78:925-930.
24. Saffouri, B., G. C. Weir, K. N. Bitar, and G. M. Makhlof. 1980. Gastrin and somatostatin secretion by perfused rat stomach: functional linkage of antral peptides. *Am. J. Physiol.* 238:G495-501.
25. Lefebvre, P. J., A. S. Luyckx, and A. H. Brassinne. 1981. Inhibition by somatostatin of carbamylcholine-induced gastrin and glucagon release from the isolated perfused canine stomach. *Gut*. 22:793-797.
26. Martindale, R., G. L. Kauffman, S. Levin, J. H. Walsh, and T. Yamada. 1982. Differential regulation of gastrin and somatostatin secretion from isolated perfused rat stomachs. *Gastroenterology*. 83:240-244.
27. Saffouri, B., J. W. DuVal, A. Arimura, and G. M. Makhlof. 1981. Effects of VIP and secretin on gastrin and somatostatin secretion by the isolated rat stomach. *Gastroenterology*. 80:1267a. (Abstr.)
28. DuVal, J. W., B. Saffouri, G. C. Weir, J. H. Walsh, A. Arimura, and G. M. Makhlof. 1981. Stimulation of gastrin and somatostatin secretion from isolated rat stomach by bombesin. *Am. J. Physiol.* 241:G242-247.