

Gastric atrial natriuretic peptide regulates endocrine secretion in antrum and fundus of human and rat stomach

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Gower, W. R. Jr., S. Premaratne, R. W. McCuen, A. Arimura, Q. McAfee, and M. L. Schubert. Gastric atrial natriuretic peptide regulates endocrine secretion in antrum and fundus of human and rat stomach. *Am J Physiol Gastrointest Liver Physiol* 284: G638–G645, 2003; 10.1152/ajpgi.00427.2002.—Atrial natriuretic peptide (ANP) is present in gastric mucosa and preferentially binds to two subtypes of natriuretic peptide receptors (NPR), NPR-A and NPR-C. The present study examines the role of endogenous ANP in regulating endocrine secretion in rat and human stomachs. NPR-A protein expression and transcripts were identified in rat antral and fundic mucosa by Western blot and RT-PCR. In superfused rat and human antral and fundic segments, ANP (0.1 pM to 0.1 μ M) caused a concentration-dependent increase in somatostatin secretion. In antrum, this was accompanied by a decrease in gastrin, and in fundus, this was accompanied by a decrease in histamine secretion. Changes in gastrin and histamine secretion reflected changes in somatostatin secretion and were abolished by somatostatin antibody. The NPR-A receptor antagonist anantin 1) inhibited basal somatostatin secretion and 2) abolished the somatostatin, gastrin, and histamine responses to ANP. We conclude that endogenous ANP, acting via the NPR-A receptor, stimulates somatostatin secretion from both antrum and fundus of rat and human stomach. Stimulation of somatostatin secretion is coupled to inhibition of gastrin secretion in the antrum and inhibition of histamine secretion in the fundus.

somatostatin; gastrin; histamine; atrial natriuretic factor; natriuretic peptide receptor; hormone; peptide; guanylyl cyclase-A

ATRIAL NATRIURETIC PEPTIDE (ANP), also known as atrial natriuretic factor, a 28-amino acid polypeptide first identified in cardiac atrial myocytes, is also present in a variety of extracardiac tissues including the stomach (9, 10, 14, 29). ANP preferentially binds to two subtypes of natriuretic peptide receptors (NPR), NPR-A [also known as guanylyl cyclase-A (GC-A) or NPR1] and NPR-C (also known as NPR3) (14). NPR-A, a transmembrane cell surface receptor with ligand-dependent guanylyl cyclase activity, mediates the biolog-

ical effects of ANP in kidney, adrenal, and vascular tissues (13, 14, 27). NPR-C, a transmembrane cell surface receptor lacking guanylyl cyclase activity, originally thought to act primarily as a natriuretic peptide clearance receptor, has been reported in some tissues to inhibit adenylate cyclase activity (11) and stimulate phosphoinositide hydrolysis (3).

Although ANP secreted from atrial myocytes into the circulation is thought to act systemically to elicit natriuresis and diuresis in the kidney, the fact that ANP and functional ANP receptors are frequently coexpressed in the same tissues suggests that ANP may have local physiological paracrine roles related to the specific organ system within which it is produced (12, 18, 28).

In the stomach, ANP has been reported to relax smooth muscle cells (6) and either inhibit or stimulate acid secretion (26). In anesthetized rats, low doses of ANP augmented vagally induced acid secretion, whereas higher doses reduced vagally induced acid secretion as well as that induced by carbachol (26). With the use of ribonuclease protection analysis and RT-PCR, we have detected the expression of ANP mRNA in antral and fundic mucosa of rat stomach, with the antrum having threefold higher levels of ANP transcripts than the fundus (10). In the antrum, the ANP-expressing cells were identified as enterochromaffin cells by dual immunohistochemistry and colorimetric in situ hybridization (10). Localization of ANP to antral and fundic mucosa has led us to postulate that ANP may physiologically regulate gastric endocrine secretion in a paracrine fashion. It should be noted that studies in conscious animals, in which agonists are administered systemically, cannot distinguish between central and peripheral actions of ANP and do not necessarily reflect the action of ANP released in the stomach.

In the present study, we have used antral and fundic segments obtained from human and rat stomach, preparations that retain intact neural and paracrine pathways, to examine the role of ANP on somatostatin,

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gastrin, and histamine secretion. The results indicate that endogenous ANP, acting via the NPR-A receptor, stimulates somatostatin secretion from both antrum and fundus of human and rat stomach. In the antrum, stimulation of somatostatin secretion is coupled to inhibition of gastrin secretion, and in the fundus, it is coupled to inhibition of histamine secretion.

MATERIALS AND METHODS

Western blotting. Forty micrograms of tissue protein extract from full thickness and the mucosal layer of antrum and fundus from Sprague-Dawley rats, measured by using the bicinchonic acid (BCA) protein assay kit (Pierce; Rockford, IL), was loaded onto each lane of a discontinuous 3.5/7.5% SDS-PAGE gel (Bio-Rad; Hercules, CA), separated by electrophoresis, and then transblotted onto a nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ) for 85 min at 0.5 A in Towbin buffer. Blots were blocked for 1 h at room temperature in a 5% solution of dry milk and then incubated for 1 h in a 5% solution of bovine serum albumin (Fraction V; Fischer Scientific, Fair Lawn, NJ) in Tris-buffered saline that contained a 1:2,500 dilution of A035 polyclonal antibody directed against the COOH terminus of the NPR-A receptor protein (generously provided by Dr. David L. Garbers, University of Texas Southwestern, Dallas, TX). After being washed with Tris-buffered saline, the membrane was incubated for 1 h at room temperature in a solution of dry milk with a 1:15,000 dilution of horseradish peroxidase conjugated goat anti-rabbit IgG antibody (Amersham Life Sciences, Arlington Heights, IL). After a second washing with Tris-buffered saline, the bands were identified by enhanced chemiluminescence reagents (ECL Plus Kit; Amersham Pharmacia Biotech) and visualized in a luminescent image analyzer (model LAS-1000; Fujifilm, Tokyo, Japan). Specificity was revealed by the presence of a signal in rat lung (positive control) and absence of a signal with normal rabbit serum, rabbit IgG, and after preabsorption of the NPR-A antibody with NPR-A protein (27).

RT-PCR. Total cellular RNA was extracted from antral and fundic mucosa of Sprague-Dawley rats with the use of oligo(dT)-linked paramagnetic beads according to the protocol provided in the Dynabeads mRNA Direct Kit (Dyna, Lake Success, NY). Single-strand cDNA was synthesized with the use of a RT reaction solution containing (in mM) 50 Tris·HCl, 75 KCl, 3 MgCl₂, 10 dithiothreitol, and 0.5 dNTP, with 0.5 µg oligo(dT) primers (Boehringer-Mannheim, Indianapolis, IN) and 200 units Superscript II RT (GIBCO-BRL, Gaithersburg, MD). The mixture was incubated at 42°C for 60 min followed by 15 min at 70°C to inactivate the enzyme. The cDNA product was amplified by PCR for 30 cycles (94°C for 1 min, 55°C for 30 s, and 72°C for 30 s) followed by a final extension cycle (72°C at 10 min). Optimal primers for NPR-A were chosen with the use of Vector NIT (Informax, Bethesda, MD). The upstream primer was 5'-GAGAACAGCAGCAACATCCT-3' and the downstream primer was 5'-AGCGCAGCATCCAGTAGT-3' (IDT Technologies, Coralville, IA). The expected length of the PCR product was 454 bp. The amplified products were analyzed by ethidium bromide-stained agarose gel electrophoresis and the DNA extracted with the use of the Gene Clean Spin Kit (Qbiogene, Carlsbad, CA). The PCR product was cloned into topoisomerase (TOPO) vector (Invitrogen, Carlsbad, CA) and subjected to blue/white colony selection. The extracted DNA was sequenced by Commonwealth Biotechnologies (Richmond, VA) and identified by using the Gene Blast program. In negative

control studies, cDNA was omitted to control for amplification of contaminating RNA or DNA.

Superfusion of antral and fundic segments. The studies were performed on superfused segments of rat and human antrum and fundus as previously described (25, 31). Sprague-Dawley rats, weighing 250–400 g, were deprived of food overnight but allowed free access to water containing 10% glucose. The animals were anesthetized with 20% urethane (0.25 ml/50 g body wt) injected intraperitoneally. The serosal and muscle layers were partly removed to improve drug diffusion, and a segment, ~1 cm², was obtained from each region. Each piece of tissue (average weight: antrum, 178 ± 8 mg; fundus, 254 ± 10 mg) was cut into 6–8 segments, washed with saline, and placed on a porous grid separating the two halves of a minichamber (Swinnex 25, 1.4-ml volume; Millipore, Bedford, MA).

A similar procedure was used for human segments. Briefly, human stomachs, obtained after total gastrectomy for adenocarcinoma of the cardia or body, were immediately placed in ice-cold Krebs bicarbonate superfusion solution, gassed with 95% O₂-5% CO₂, and transported to pathology. Segments, ~6 cm², of visually normal fundus and antrum were removed by scalpel and cut into small pieces (average weight: antrum, 225 ± 8 mg; fundus, 342 ± 14 mg), washed with saline, and placed in the superfusion minichamber. After the experiment, both the unused and used tissues were fixed, embedded in paraffin, and double stained with eosin in 95% ethanol and hematoxylin in water; all segments were confirmed to be histologically normal.

For both rat and human tissues, Krebs bicarbonate solution containing 0.2% bovine serum albumin, 4% dextran, and 4.5 mM glucose was perfused into the bottom of the chamber at the rate of 1 ml/min, and the effluent was collected via a catheter leading from a small aperture at the top of the chamber. The perfusate was gassed with 95% O₂-5% CO₂. Drugs were delivered at the rate of 0.1 ml/min via a side arm close to the inlet. The entire preparation was contained within a chamber maintained at 37°C.

Protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee, McGuire Research and Development Committee, and McGuire Research Human Studies Subcommittee.

Experimental design for superfused segments. A 30-min equilibration period was followed by an 80-min sampling period. The sampling period consisted of a 30-min control basal period, a 20-min period, during which ANP (Bachem; King of Prussia, PA) was superfused at various concentrations (0.1 pM to 0.1 µM) either alone or in combination with the competitive NPR-A receptor antagonist anantoin (Bachem) (5, 17, 32) or the axonal blocker tetrodotoxin, and a final 30-min control period. In some experiments, somatostatin antibody (S775, raised by A. Arimura; final dilution 1:300) or normal rabbit serum (final dilution 1:300) was superfused for 30 min before superfusion with ANP (10 nM). In other experiments, anantoin (0.1 µM) or the specific NPR-C agonist, cANP4–23 (0.1 pM to 1 µM) (Bachem) (1, 5, 15) was perfused for 20 min.

A single cluster of segments was separately obtained from the antrum and fundus of each rat. Several clusters of segments were obtained from human mucosa; each cluster was tested with different agents. One-milliliter samples of the superfusate were obtained at 5-min intervals and stored in 0.5-ml aliquots at –20°C for subsequent measurement of somatostatin, gastrin, and histamine concentrations by radioimmunoassay.

Radioimmunoassay. Somatostatin concentration was measured in duplicate by radioimmunoassay as described in

detail previously (20). Somatostatin antibody 1001 (final dilution 1:66,000) was a gift from Dr. T. Yamada and Dr. J. DelValle (University of Michigan). ^{125}I -labeled somatostatin was purchased from New England Nuclear (Boston, MA). The limit of detection was 5 pg/ml of sample, and the IC_{50} was 47 ± 9 pg/ml of sample (mean \pm SD; $n = 7$ assays). Interassay and intraassay coefficients of variability were 12 and 8%, respectively.

Gastrin concentration was measured in duplicate with the use of radioimmunoassay as described previously (20). Gastrin antibody 1611 (final dilution, 1:100,000) was a gift from Dr. J. Walsh and provided by CURE/UCLA/DDC Antibody/RIA Core. ^{125}I -labeled gastrin was purchased from New England Nuclear. The limit of detection was 1 pg/ml of sample, and the IC_{50} was 53 ± 7 pg/ml of sample (mean \pm SD; $n = 5$ assays). Interassay and intraassay coefficients of variation were 6 and 8%, respectively.

Histamine concentration was measured in duplicate with the use of a commercial radioimmunoassay kit (Amac, Westbrook, ME) as previously described (31). The kit includes tubes coated with monoclonal antibody against acylated histamine, acylating agent, and ^{125}I -labeled histamine as tracer. The limit of detection was 0.1 nM histamine, and the IC_{50} was 7 ± 1 nM of sample (mean \pm SD; $n = 6$ assays). Interassay and intraassay coefficients of variability were 10 and 7%, respectively.

Data analysis. Somatostatin, gastrin, and histamine secretion were expressed as the mean increase or decrease in picograms or nanomoles per minute or as percent change from the preceding basal level during the 5 min immediately preceding the experimental period. Changes in secretion were tested for significance by using Student's *t*-test for unpaired values. All values are given as means \pm SE of *n* experiments on different animals. Concentrations eliciting EC_{50} were calculated by using sigmoidal plot logistics.

RESULTS

Western blot analysis of NPR-A in gastric antrum and fundus. Western blotting was performed to identify the presence of specific polypeptides corresponding to NPR-A in rat antral and fundic mucosa. The NPR-A antibody detected a distinct band of ~ 120 kDa in protein lysates prepared from rat lung (positive control), full thickness antrum and fundus, and mucosa of antrum and fundus (Fig. 1). Rabbit serum, rabbit IgG, and preabsorption of the NPR-A antibody with NPR-A protein yielded no band (negative control).

Identification of NPR-A expression in gastric antral and fundic mucosa by RT-PCR, cloning, and sequence analysis. With the use of NPR-A-specific primers, a distinct RT-PCR product of the predicted size (454 bp) was obtained from rat antral and fundic mucosa (Fig. 2). The NPR-A-specific products were cloned into TOPO vector and sequenced in both directions, each yielding a 454-bp sequence 100% identical with rat NPR-A. Control experiments without cDNA did not yield PCR products.

Basal somatostatin, gastrin, and histamine secretion from rat and human segments. In rat and human antral mucosal segments, mean basal somatostatin and gastrin secretion were reproducible between experiments and reverted to initial control levels at the end of the experimental period (rat: somatostatin secretion, 31 ± 4 and 33 ± 6 pg/min; gastrin secretion,

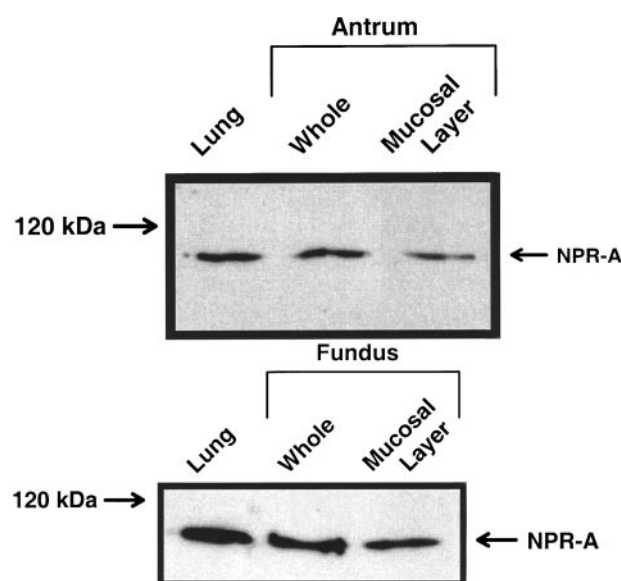


Fig. 1. Western blot analysis of natriuretic peptide receptor (NPR)-A in rat tissue. The NPR-A antibody recognized a single band of molecular weight ~ 120 kDa on the immunoblots of lung (positive control), whole antrum and fundus, and mucosa of antrum and fundus.

241 ± 50 and 243 ± 52 pg/min; human: somatostatin secretion, 12 ± 4 and 13 ± 3 pg/min; gastrin secretion, 68 ± 17 and 67 ± 16 pg/min).

In rat and human fundic mucosal segments, mean basal somatostatin and histamine secretion were also reproducible between experiments and reverted to initial control levels at the end of the experimental period (rat: somatostatin secretion, 34 ± 3 and 35 ± 5 pg/min; histamine secretion, 356 ± 24 and 322 ± 34 nmol/min; human: somatostatin secretion, 35 ± 4 and 31 ± 6 pg/min; histamine secretion, 52 ± 8 and 52 ± 9 nmol/min).

Effect of ANP on somatostatin and gastrin secretion from rat and human antral segments. Superfusion of rat antral segments for 20 min with ANP, in the range of 0.1 pM to 0.1 μM , caused a prompt, reversible, and concentration-dependent increase in somatostatin secretion accompanied by a reciprocal decrease in gastrin secretion (Figs. 3 and 8). The EC_{50} value for stimulation of somatostatin secretion was 3.2×10^{-10} and for inhibition of gastrin secretion was 2.2×10^{-11} . Maximal stimulation of somatostatin secretion ($60 \pm 10\%$ above basal level, $P < 0.005$, $n = 6$) and inhibition of gastrin secretion ($28 \pm 4\%$ below basal level, $P < 0.001$, $n = 6$), expressed as the integrated 20-min response, were obtained at a concentration of 10 nM. Responses were not significantly affected by the axonal blocker tetrodotoxin (5 μM ; $n = 5$).

Superfusion of human antral segments for 20 min with ANP, in the range of 0.1 pM to 0.1 μM , also caused a prompt, reversible, and concentration-dependent increase in somatostatin secretion that was accompanied by a reciprocal decrease in gastrin secretion (Figs. 3 and 4). The EC_{50} value for stimulation of somatostatin secretion was 1.4×10^{-10} and for inhibition of gastrin

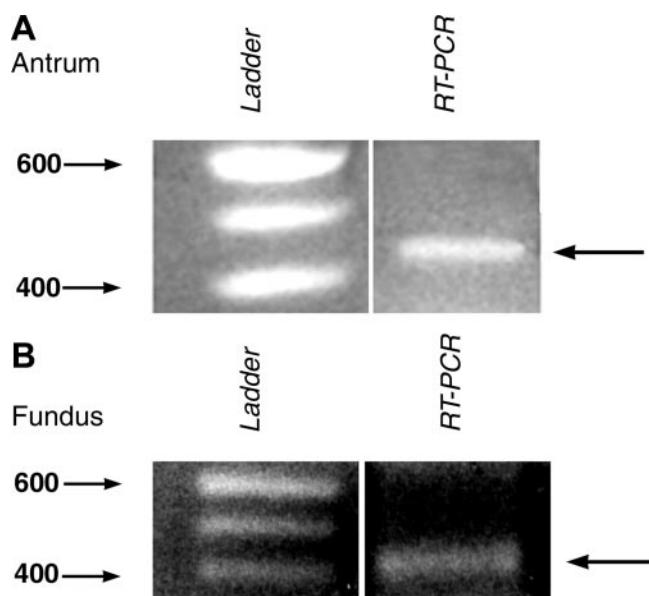


Fig. 2. RT-PCR analysis of NPR-A expression in rat gastric antrum (A) and fundus (B). RT-PCR using rat NPR-A-specific primers yielded a band (arrows) at the expected size for rat NPR-A (454 bp) in rat antral and fundic mucosa.

secretion was 1.9×10^{-11} . Maximal stimulation of somatostatin secretion ($100 \pm 17\%$ above basal level, $P < 0.005$, $n = 6$) and inhibition of gastrin secretion ($54 \pm 1\%$ below basal level, $P < 0.001$, $n = 6$), expressed as the integrated 20-min response, was obtained at a concentration of $0.1 \mu\text{M}$. The responses were not significantly affected by tetrodotoxin ($5 \mu\text{M}$; $n = 5$) (Fig. 4).

In rat and human antral segments, the specific NPR-C receptor agonist cANP4–23 superfused in the range of 0.1 pM to $1.0 \mu\text{M}$ had no significant effect on either somatostatin or gastrin secretion ($n = 4$ – 6 each).

Effect of ANP on somatostatin and histamine secretion from rat and human fundic segments. Superfusion of rat fundic segments for 20 min with ANP, in the range of 0.1 pM to $0.1 \mu\text{M}$, caused a prompt, reversible, and concentration-dependent increase in somatostatin secretion that was accompanied by a reciprocal decrease in histamine secretion (Figs. 5 and 8). The EC_{50} value for stimulation of somatostatin secretion was 1.5×10^{-10} and for inhibition of histamine secretion was 2.6×10^{-11} . Maximal stimulation of somatostatin secretion ($72 \pm 14\%$ above basal level, $P < 0.005$, $n = 6$) and inhibition of histamine secretion ($60 \pm 3\%$ below basal level, $P < 0.001$, $n = 6$), expressed as the integrated 20-min response, were obtained at a concentration of $0.1 \mu\text{M}$. The responses were not significantly affected by the axonal blocker tetrodotoxin ($5 \mu\text{M}$; $n = 5$).

Superfusion of human fundic segments for 20 min with ANP, in the range of 0.1 pM to $0.1 \mu\text{M}$, also caused a prompt, reversible, and concentration-dependent increase in somatostatin secretion accompanied by a reciprocal decrease in histamine secretion (Figs. 4 and 5). The EC_{50} value for stimulation of somatostatin secretion was 1.9×10^{-10} and for inhibition of histamine secretion was 1.7×10^{-10} . Maximal stimulation of somatostatin secretion ($65 \pm 1\%$ above basal level, $P < 0.001$, $n = 6$) and inhibition of histamine secretion ($40 \pm 2\%$ below basal level, $P < 0.001$, $n = 6$) expressed as the integrated 20-min response was obtained at a concentration of $0.1 \mu\text{M}$. The responses were not significantly affected by tetrodotoxin ($5 \mu\text{M}$; $n = 5$) (Fig. 4).

In rat and human fundic segments, the NPR-C receptor agonist, cANP4–23, superfused in the range of 0.1 pM to $1.0 \mu\text{M}$, had no significant effect on either somatostatin or histamine secretion ($n = 4$ – 6 each).

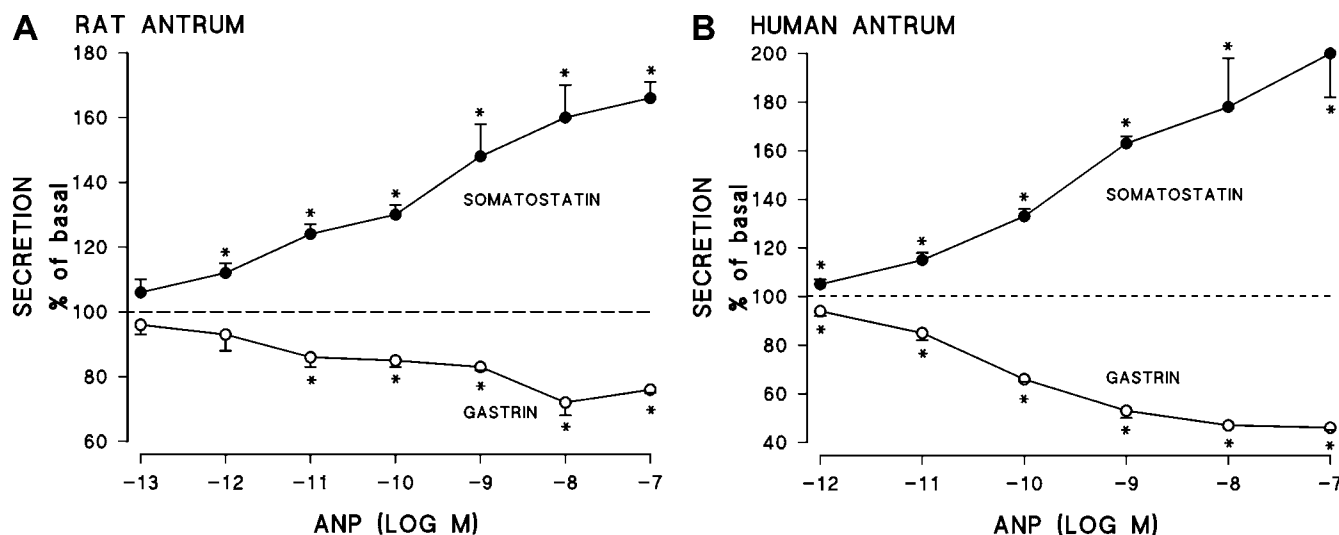


Fig. 3. Effect of atrial natriuretic peptide (ANP) (0.1 pM – $0.1 \mu\text{M}$) on basal somatostatin (●) and gastrin (○) secretion in superperfused rat (A) and human (B) antral segments. Data are means \pm SE of 6 experiments each.

*Significant difference from basal levels at $P < 0.01$.

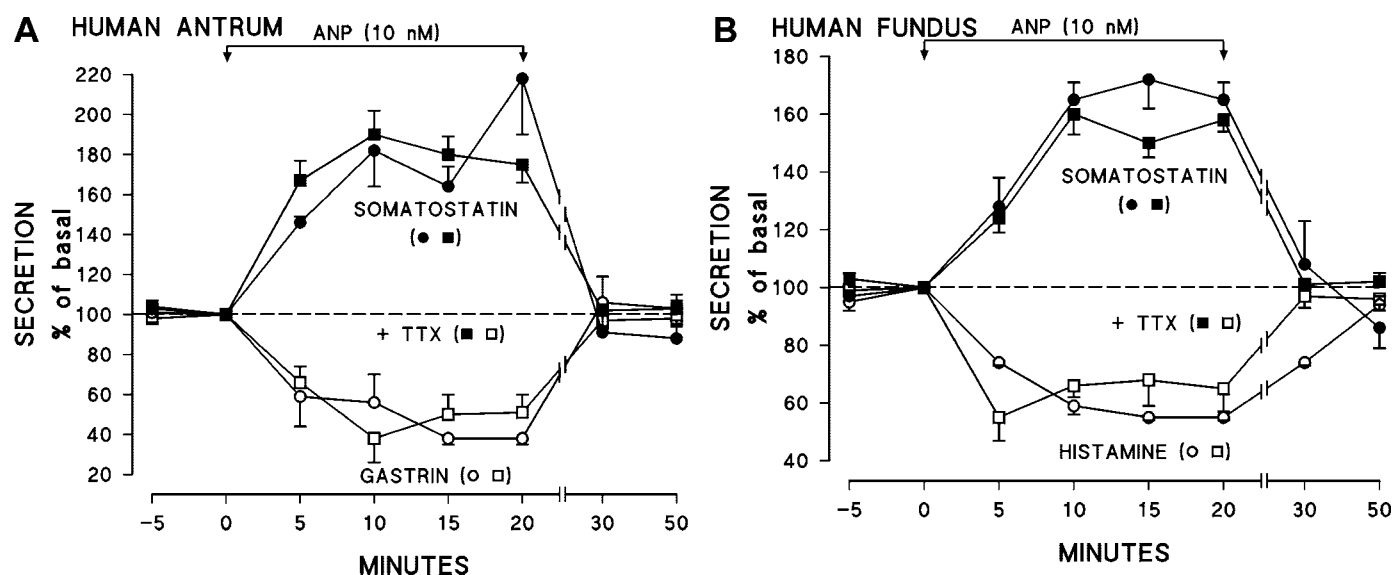


Fig. 4. Time course for the effect of ANP (10 nM) alone and in combination with the axonal blocker tetrodotoxin (TTX; 5 μ M) on basal somatostatin and gastrin secretion in human antral segments (A) and on basal somatostatin and histamine secretion in human fundic segments (B). Dotted line indicates level of basal secretion. Data are means \pm SE of 5–6 experiments each.

Effect of somatostatin antibody on the responses in rat antral and fundic segments. To determine whether the effect of ANP on gastrin secretion in the antrum and histamine secretion in the fundus was mediated by changes in somatostatin secretion, experiments were performed under conditions in which the effect of somatostatin was precluded. Superfusion of rat antral mucosal segments with somatostatin antibody alone (final dilution 1:300) for 30 min, but not normal rabbit serum, caused an increase in gastrin secretion (integrated response: $13 \pm 4\%$ above basal level, $P < 0.02$, $n = 6$) (Fig. 6), confirming previous studies showing that endogenous somatostatin exerts an inhibitory

paracrine influence on gastrin secretion (31). Likewise, superfusion of rat fundic mucosal segments with somatostatin antibody alone, but not normal rabbit serum, caused an increase in histamine secretion (integrated response: $14 \pm 1\%$ above basal level, $P < 0.001$, $n = 6$) (Fig. 6) confirming previous studies showing that endogenous somatostatin exerts an inhibitory paracrine influence on histamine secretion (30).

In the presence of somatostatin antibody, the decrease in gastrin secretion induced by ANP (10 nM) in antral mucosal segments was blocked by 79% ($33 \pm 6\%$ below basal level with ANP plus normal rabbit serum vs. $7 \pm 3\%$ below basal level with ANP plus somatosta-

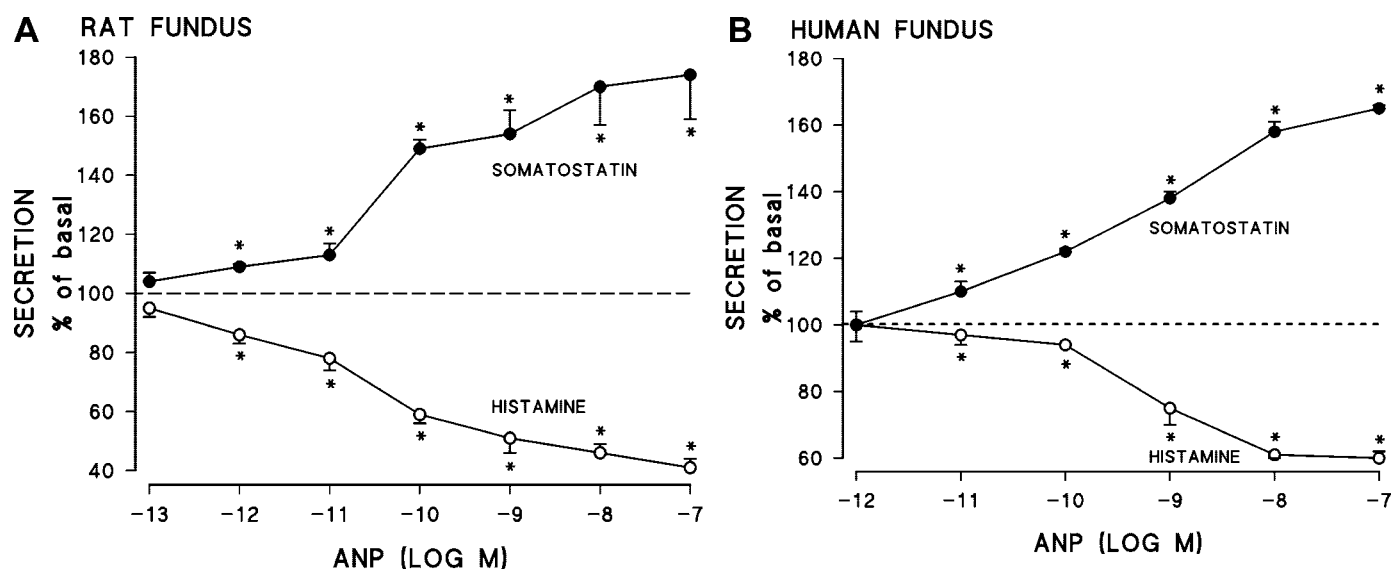


Fig. 5. Effect of ANP (0.1 pM–0.1 μ M) on basal somatostatin (●) and histamine (○) secretion in superfused rat (A) and human (B) fundic segments. Data are means \pm SE of 6 experiments each. *Significant difference from basal levels at $P < 0.01$.

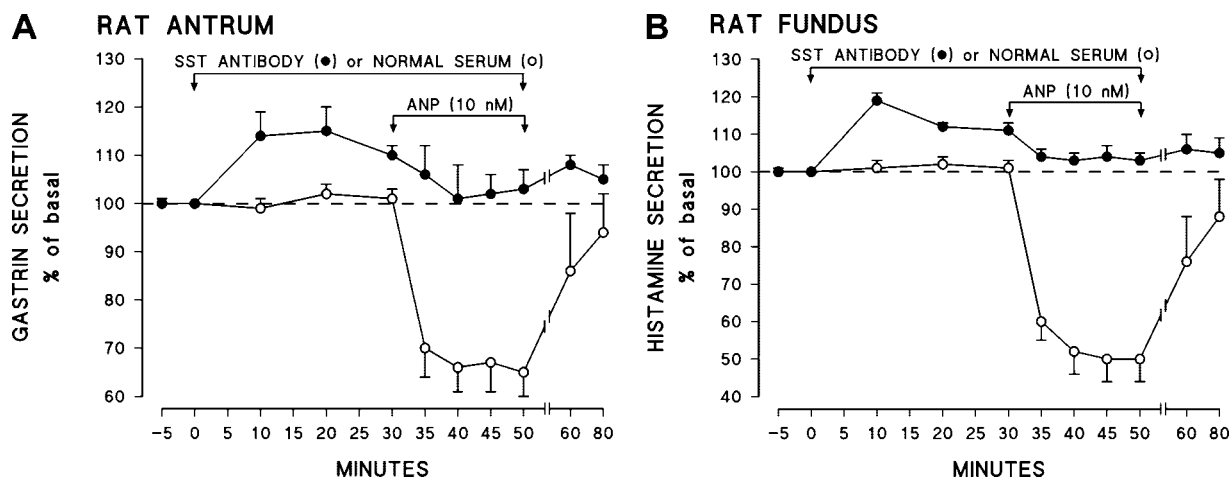


Fig. 6. Time course for the effect of somatostatin antibody (final dilution 1:300; ●) or normal rabbit serum (final dilution 1:300; ○) alone and in combination with ANP (10 nM) on gastrin secretion from rat antral segments (A) and histamine secretion from rat fundic segments (B). Dotted line indicates level of basal secretion. Data are means \pm SE of 6 experiments each.

tin antibody; $P < 0.01$ for the difference) and the decrease in histamine secretion induced by ANP (10 nM) in fundic mucosal segments was blocked by 85% ($47 \pm 6\%$ below basal level with ANP plus normal rabbit serum vs. $7 \pm 1\%$ below basal level with ANP plus somatostatin antibody; $P < 0.001$ for the difference) (Fig. 6), implying that the effects of ANP on gastrin secretion in the antrum and histamine secretion in the fundus were mediated by changes in somatostatin secretion.

Effect of NPR-A receptor antagonist on the responses from rat and human antral and fundic segments. The involvement of NPR-A in ANP-stimulated somatostatin secretion in the antrum and fundus was tested by using the selective NPR-A receptor antagonist anantin (5, 17, 32). Superfusion of rat antral or fundic mucosal segments with anantin (0.1 μ M) for 20 min caused a decrease in somatostatin secretion (antrum integrated response: $12 \pm 3\%$ below basal level; fundus integrated response: $18 \pm 5\%$ below basal level; $P < 0.01$, $n = 6$ each) (Fig. 7). Likewise, superfusion of human antral or fundic mucosal segments with anantin (0.1 μ M) for 20 min caused a decrease in somatostatin secretion (antrum integrated response: $15 \pm 4\%$ below basal level; fundus integrated response: $19 \pm 5\%$ below basal level; $P < 0.02$, $n = 5$ each) (Fig. 7). The results imply that endogenous ANP, acting via the NPR-A receptor, exerts a tonic stimulatory influence on antral and fundic somatostatin secretion in rat and human stomach.

In the presence of the NPR-A receptor antagonist, both the somatostatin and gastrin responses to ANP in rat antrum and the somatostatin and histamine responses to ANP in rat fundus were abolished (Fig. 8), implying that the ANP effects in both regions were mediated via the NPR-A receptor.

DISCUSSION

The presence of ANP in gastric antral and fundic mucosa suggests that ANP may function in a paracrine

manner to regulate gastric endocrine secretion (9, 10, 29). In the antrum, ANP has been localized to enterochromaffin cells, whereas the precise cellular source of ANP in the fundus has not been identified (10). ANP exerts many of its actions through interaction with the NPR-A subtype receptor, a particulate guanylyl cyclase-linked receptor (14). In some tissues, the effects of ANP may be mediated via NPR-C, a transmembrane receptor lacking guanylyl cyclase activity (3, 11).

In the present study, we have identified, by immunoblot and RT-PCR, the presence of NPR-A protein and mRNA in rat gastric antral and fundic mucosa. The results confirm and extend previous observations demonstrating the presence of NPR-A transcripts in antral mucosa and whole fundus of rat stomach (10, 18). The presence of both ANP and its receptor in antral and

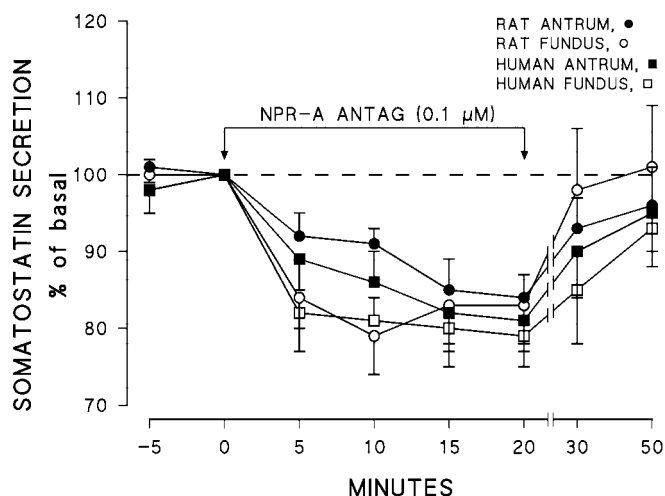


Fig. 7. Time course for the effect of superfusion with the NPR-A antagonist anantin (0.1 μ M) on basal somatostatin secretion from 1) rat antral and fundic segments and 2) human antral and fundic segments. Dotted line indicates level of basal secretion. Data are means \pm SE of 5–6 experiments each.

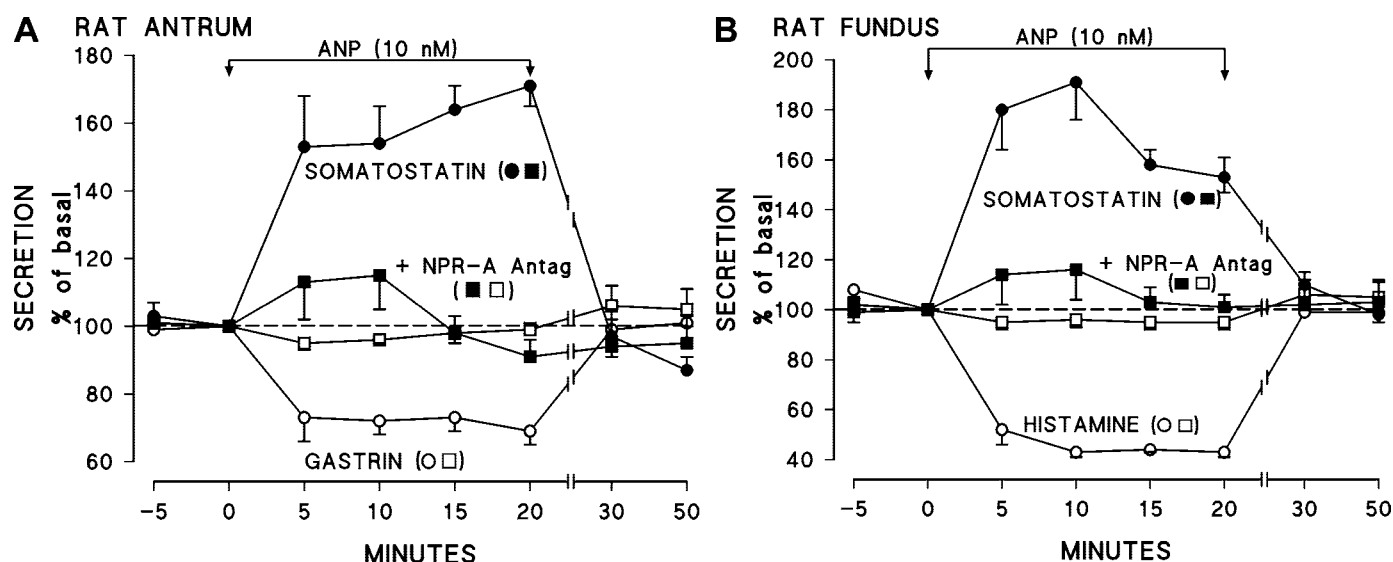


Fig. 8. Time course for the effect of ANP (10 nM) alone and in combination with the NPR-A antagonist anantin (100 nM), on basal somatostatin and gastrin secretion in rat antral segments (A) and on basal somatostatin and histamine secretion in rat fundic segments (B). Dotted line indicates level of basal secretion. Data are means \pm SE of 6 experiments each.

fundic mucosa suggests that ANP, acting via paracrine pathways, may participate in the regulation of gastric endocrine secretion. Results of the present study indicate that endogenous ANP, acting via the NPR-A receptor, exerts a stimulatory paracrine influence on somatostatin secretion from both regions of the stomach. In the antrum, stimulation of somatostatin secretion is coupled to inhibition of gastrin secretion, and, in the fundus, it is coupled to inhibition of histamine secretion. The evidence on which this conclusion is based can be summarized as follows.

First, the NPR-A receptor antagonist anantin, by itself, significantly decreased somatostatin secretion in rat and human antral and fundic segments, implying that endogenous ANP, acting via the NPR-A receptor, exerts a tonic stimulatory influence on somatostatin secretion in both regions. Consistent with this notion, exogenous ANP caused a concentration-dependent stimulation of somatostatin secretion in rat and human antral and fundic segments, whereas the NPR-C agonist cANP4–23, administered at 10-fold higher concentrations than ANP, was without significant effect.

Second, the increase in somatostatin secretion elicited by ANP was accompanied by a reciprocal decrease in gastrin secretion in the antrum and histamine secretion in the fundus. Changes in gastrin and histamine secretion elicited by ANP reflected changes in somatostatin secretion rather than any direct effect of ANP on gastrin or histamine-containing enterochromaffin-like cells. This was evident in experiments in which the influence of somatostatin was eliminated by the addition of somatostatin antibody. The addition of somatostatin antibody, but not normal serum, blocked the gastrin and histamine responses elicited by ANP by 79–85%. It should be noted that somatostatin antibody, by itself, increased gastrin secretion in the antrum and histamine secretion in the fundus, consistent with previous studies demonstrating

that endogenous somatostatin exerts a tonic paracrine inhibitory influence on the secretion of gastrin and histamine (7, 19, 31).

Third, the somatostatin and gastrin responses to ANP in the antrum as well as the somatostatin and histamine responses to ANP in the fundus of rat stomach were blocked by the addition of the selective NPR-A receptor antagonist anantin. Although it has been suggested that ANP can have biological actions mediated also by signaling activity via NPR-C (2, 3, 11, 16), this was not evident in the present study. As noted above, the specific NPR-C receptor agonist cANP4–23 had no significant effect on somatostatin, gastrin, or histamine secretion in rat and human antral and fundic segments.

Finally, the responses to ANP in the antrum and fundus of both rat and human stomach were unaffected by the addition of the axonal blocker tetrodotoxin, suggesting a direct paracrine effect of ANP on somatostatin secretion. This concentration of tetrodotoxin (5 μ M) was previously shown to block neurally mediated peptide secretion in this preparation (21, 23).

The findings that 1) ANP and its receptor NPR-A are present in antral and fundic mucosa and 2) endogenous ANP, acting via the NPR-A receptor, stimulates somatostatin secretion from both regions of the stomach implies that ANP physiologically regulates gastric somatostatin secretion. The precise factors and mechanisms regulating ANP secretion from the stomach, however, are not known. It has been reported that prolonged fasting decreases steady-state ANP mRNA levels in the antrum of adult rats (10). We have recently shown that release of ANP from rat antrum can be modulated by activation of intramural cholinergic and pituitary adenylate cyclase activating peptide (PACAP) neurons (8, 22); cholinergic neurons inhibit, whereas PACAP neurons stimulate, ANP secretion.

Accordingly, it is possible that physiological stimuli, such as luminal protein or mechanical distension, both of which are known to activate intramural neurons (21, 24), may regulate ANP secretion.

In conclusion, the present study demonstrates, for the first time, a physiological role for locally synthesized ANP in the regulation of somatostatin secretion in the antrum and fundus of human and rat stomach. Endogenous ANP, acting via the NPR-A receptor, stimulates somatostatin secretion from both regions of the stomach. Stimulation of antral somatostatin secretion is coupled to inhibition of gastrin secretion, and stimulation of fundic somatostatin secretion is coupled to inhibition of histamine secretion. These paracrine pathways represent one mechanism by which ANP may inhibit acid secretion.

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