Effect of Atropine on Vagal Release of Gastrin and Pancreatic Polypeptide

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A B S T R A C T We studied the effect of several doses of atropine on the serum gastrin and pancreatic polypeptide responses to vagal stimulation in healthy human subjects. Vagal stimulation was induced by sham feeding. To eliminate the effect of gastric acidity on gastrin release, gastric pH was held constant (pH 5) and acid secretion was measured by intragastric titration. Although a small dose of atropine (2.3 μg/kg) significantly inhibited the acid secretory response and completely abolished the pancreatic polypeptide response to sham feeding, this dose of atropine significantly enhanced the gastrin response. Higher atropine doses (7.0 and 21.0 μg/kg) had effects on gastrin and pancreatic polypeptide release which were similar to the 2.3-μg/kg dose. Atropine (0.78 and 2.3 μg/kg) without sham feeding significantly inhibited basal acid secretion and also led to significant increases in serum gastrin above basal levels. The gastrin response to sham feeding with 2.3 μg/kg atropine was significantly greater than the sum of the gastrin responses to sham feeding alone and to 2.3 μg/kg atropine alone, indicating potentiation of vagal gastrin release by atropine. We conclude: (a) Unlike vagally mediated gastric acid secretion and pancreatic polypeptide release which can be blocked by atropine, vagal gastrin release is potentiated by atropine. This observation suggests the existence of a vagal-cholinergic pathway which normally (i.e., in the absence of atropine) inhibits gastrin release. (b) Because atropine (without sham feeding) increased basal gastrin levels, it is likely that the cholinergic pathway which inhibits gastrin release is active even when the vagus nerve is not stimulated by sham feeding.

INTRODUCTION

The effect of atropine on vagally mediated gastrin release in man is poorly understood. In a recent study,

the rise in serum gastrin concentration induced by insulin hypoglycemia was further increased by atropine premedication (1). In addition, several workers have shown that atropine increases the serum gastrin concentration after an eaten meal (2-4). These observations, together with the fact that basal and postprandial gastrin levels rise after vagotomy (5-7), have led to speculation that the vagus nerve can inhibit, as well as stimulate, gastrin release.

For several reasons, however, none of these observations proves that the vagus nerve actually inhibits gastrin release under normal circumstances. First, studies using insulin hypoglycemia as a vagal stimulant must be interpreted with caution because there is evidence that hypoglycemic gastrin release is nonvagal (8-10). For example, the gastrin response to hypoglycemia persists after a complete vagotomy (8, 9). Second, meal studies that have been done with atropine (2-4) are not definitive because atropine reduces acid secretion, and the resulting higher antral pH would facilitate gastrin release in response to any stimulant. This criticism is also pertinent to vagotomy studies in which gastric pH was not controlled. Third, many studies that have been done with atropine are inconclusive because the effect of atropine alone was not studied. If atropine alone increased serum gastrin concentrations, higher meal- or insulin-stimulated gastrin levels would be expected after atropine simply on an additive basis.

The purpose of our studies was to evaluate more definitively the effect of atropine on vagal gastrin release in man. To do this, we employed a method of vagal stimulation that acts only through the vagus nerve: sham feeding (11, 12). Antral pH was held constant by in vivo intragastric titration during the course of the experiments so that changes in acid secretion could not affect gastrin release. We evaluated the effect of several doses of atropine in combination with sham feeding as well as the effect of atropine alone on serum gastrin concentration. For comparison, the

effect of atropine on vagal release of another hormone, human pancreatic polypeptide, was also studied.

METHODS

Subjects. 10 normal human subjects participated in these studies. Six were men and four were women. Their mean age was 29 yr (range, 20–46 yr). Subjects fasted for at least 10 h before each study. Their mean (±SEM) peak acid output to 6.0 μg/kg pentagastrin subcutaneously (Peptavlon, Ayerst Laboratories, New York) was 37.5±5.4 meq/h. Studies were approved by a Human Research Review Committee and informed consent was obtained from each subject.

Intubation and intragastric titration technique. In all experiments a radiopaque triple lumen nasogastric tube was used. Tubes 1 and 2 terminated 14 cm proximal to the tip of tube 3. Under fluoroscopic guidance the triple lumen tube was positioned so that the tip of tube 3 was in the gastric antrum. To initiate each experiment 50 ml saline (300 mosm/kg), adjusted to pH 5.0, was infused into the stomach through tube 3. This was followed by a continuous intragastric saline infusion (pH 5.0) through tube 1 at a rate of 4.2 ml/min (Brinkman Peristalic Pump, Desaga Inc., Heidelberg, Germany). Gastric pH was held constant and acid secretion measured by in vivo intragastric titration. Every 2 min, 0.5 ml gastric fluid was removed through tube 3 and the pH was determined. Sodium bicarbonate (0.3 N) was infused into the stomach through tube 2 at a rate sufficient to maintain gastric pH at 5.0. The number of milliequivalents of bicarbonate added is equal to the number of milliequivalents of acid secreted (13). Previous studies have shown (14), and it was confirmed again in this study, that this continuous intragastric saline infusion has no effect on basal serum gastrin concentrations.

Atropine. After a 60-min control period, atropine sulfate (Wyeth Laboratories, Philadelphia, Pa.) was injected intramuscularly in doses of 0 (saline control), 0.78, 2.3, 7.0, and 21.0 μg/kg total body weight. One atropine dose was given per study day, and the order in which the atropine doses were given was randomized.

Sham feeding (SF).[1] 15 min after atropine (or saline) was injected, subjects chewed for 30 min, but did not swallow, an appetizing meal consisting of sirloin steak, french-fried potatoes, and water (15). Meals were cooked in a separate building so that subjects could not see or smell food until time for SF. Subjects were trained in preliminary experiments not to swallow food. During all experiments, gastric aspirates were examined for swallowed food and none was found.

Gastrin and human pancreatic polypeptide (HPP) measurement. Venous blood was collected through an indwelling catheter kept open by a slow intravenous 0.15 M NaCl infusion. Blood was allowed to clot and serum was stored at −20°C until assayed.

Serum gastrin concentrations were measured by radioimmunoassay. All samples were tested in duplicate in the same assay. Antibody 1296, rabbit antigastrin prepared by immunization with gastrin conjugated to bovine serum albumin, was used at a final dilution of 1:300,000. Human heptadecapeptide gastrins (HG-17-I and HG-17-II) and human big gastrins (HG-34-I and HG-34-II) are measured with this antiserum, big gastrins being approximately two-thirds as potent as heptadecapeptides (16). Cross reactivity with porcine cholecystokinin was <5% (17). With natural G-17-I as a standard, results are expressed as the rise (in picograms per milliliter) above average basal levels. Basal serum gastrin was

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[1] *Abbreviations used in this paper*: HPP, human pancreatic polypeptide; IGR, integrated gastrin response(s); IPPR, integrated HPP response(s); SF, sham feeding.

measured at 15 min and immediately before atropine injection. Mean basal gastrin levels varied 5 pg/ml or less at these time periods and were therefore averaged. Serum gastrin was also measured at 15, 22.5, 30, 37.5, 45, 60, and 75 min after atropine.

Serum HPP concentration was also measured by radioimmunoassay. Antiserum against HPP (a generous gift of Dr. R. A. Chance, Eli Lilly & Co., Indianapolis, Ind.) was used at a final dilution of 1:1,250,000. Bovine pancreatic polypeptide was labeled by modification of the chloramine T method (18), and the tracer was pruified on a 1 × 100-cm Sephadex G-50 superfine column (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). Highly purified HPP was used as a standard for all the assays. Serum was assayed at a final dilution of 1:10. Separation was performed with charcoal, and a control tube without antibody was used to correct for nonspecific binding. No displacement of tracer from antiserum was found with concentrations of 100 nmol/liter of little gastrin, big gastrin, secretin or vasoactive intestinal peptide, or with 10 nmol/liter of monocomponent insulin, pancreatic glucagon, or cholecystokinin. The experimental detection limit was 34 pg/ml of serum. The ratio of bound:free labeled bovine pancreatic polypeptide was inhibited by 50% at a concentration of 17 pg/ml HPP in the incubation tube. The intra-assay precision was 4% and the interassay precision 8%. Recovery of HPP added to serum deviated no more than 17% from the expected values over the range of 40–340 pg/ml serum. Mean (±SE) basal HPP levels with this assay were 90±8 pg/ml.

Statistical analysis. All results are expressed as mean±1 SE. Differences were determined by paired t test, and values <0.05 were considered significant (19). Integrated gastrin and integrated pancreatic polypeptide responses were calculated as described (20).

RESULTS

Gastrin

SF without atropine. As shown in Fig. 1 (left panel), SF without atropine led to significant rises in serum gastrin concentrations. By the end of SF (i.e., at 45 min), serum gastrin had risen 15±4 pg/ml ($P < 0.005$).

Atropine without SF. Four subjects were given 0.78 or 2.3 μg/kg atropine, without SF, on two separate test days in random order. As shown in Table I, gastrin levels increased in all four subjects in response to the 0.78-μg/kg dose. The peak gastrin rise averaged 10±2.6 pg/ml and was significant ($P < 0.02$). The 2.3-μg/kg dose led to even larger gastrin rises in the four subjects, averaging 16±4.1 pg/ml ($P < 0.025$). This peak rise in response to 2.3 μg/kg was significantly greater than the response to 0.78 μg/kg ($P < 0.05$).

The center panel of Fig. 1 shows the gastrin responses to 2.3 μg/kg for the nine subjects. The peak gastrin increment was reached 45 min after atropine was injected and averaged 18±8 pg/ml above basal levels ($P < 0.05$).

SF with atropine. The solid line in the right panel of Fig. 1 shows the rises in serum gastrin levels that occurred when 2.3 μg/kg atropine was injected 15 min before SF. The peak gastrin increment, reached at 30 min, averaged 42±13 pg/ml ($P < 0.01$). The gastrin
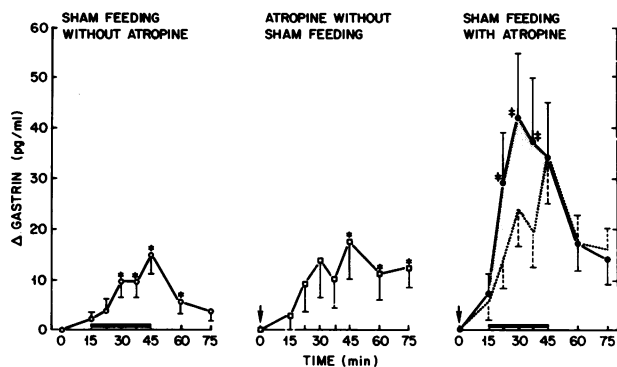
FIGURE 1 Mean (±1 SE) gastrin rises above basal levels (Δ gastrin) after SF without atropine (left panel), 2.3 μg/kg atropine without SF (center panel), or SF with 2.3 μg/kg atropine (solid line in right panel) in nine subjects. Atropine was injected at 0 min (see arrows). SF was started at 15 min and ended at 45 min (see bars). Both SF without atropine and atropine without SF led to significant gastrin rises (shown as asterisks). Gastrin rises after SF with atropine (right panel) were significantly greater (shown as double daggers) than the sum of the rises after SF alone plus atropine alone (dashed line, right panel) at several sampling intervals. Shaded area represents the extent to which atropine potentiated the gastrin response to SF (see text).
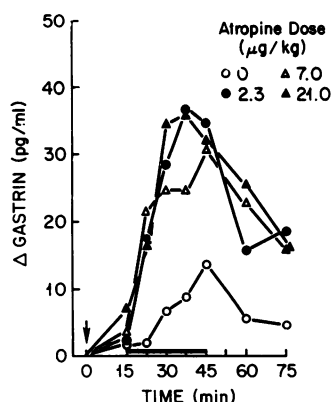

FIGURE 2 Mean gastrin rise above basal levels (Δ gastrin) in nine subjects. Eight of these nine subjects also had all three studies shown in Fig. 1. Atropine (or saline control) was injected at 0 min (see arrow). SF was started at 15 min and ended at 45 min (see bar). Gastrin rises after SF with 2.3, 7.0, or 21 μg/kg atropine were significantly greater than after SF without atropine (open circles) at most sampling intervals after 15 min.

rises after SF with atropine were significantly greater (at 22.5, 30, and 37.5 min) than the sum of the rises after SF alone plus atropine alone (shown as a dashed line in Fig. 1, right panel). Therefore, atropine not only increased basal serum gastrin levels (center panel) but also potentiated the gastrin response to SF (right panel). In this context, potentiation means that the response to a combined stimulus is significantly greater than the sum of the responses to the stimuli given separately. The extent to which atropine potentiated the gastrin response to SF is shown as a shaded area in Fig. 1.

As shown in Fig. 2, 7 and 21 μg/kg atropine increased serum gastrin concentrations during SF to approximately the same degree as did 2.3 μg/kg. Peak gastrin increments averaged 37, 31, and 36 pg/ml for the 2.3-, 7-, and 21-μg/kg atropine doses, respectively. The integrated gastrin response(s) (IGR) to SF with atropine

were more than threefold greater than the IGR to SF without atropine ($P < 0.05$ for each dose) (Table II). IGR after SF with 2.3, 7, or 21 μg/kg atropine were not significantly different from each other.

HPP

SF led to significant rises in serum HPP concentrations from basal levels of 89±10 pg/ml to a peak level of 136±20 pg/ml at 37.5 min ($P < 0.01$). The integrated HPP response(s) (IPPR) to SF averaged 1.40±0.38 ng·min/ml (Table II). Atropine (2.3 μg/kg) completely abolished the IPPR to SF. In fact, the negative IPPR in Table II indicates that this dose of atropine reduced HPP below basal levels. Higher atropine doses had similar effects on HPP release during SF. IPPR after SF with 2.3, 7, or 21 μg/kg atropine were not significantly different from each other.

Gastric acid secretion and gastric volume

As shown in Table II, the rate of gastric acid secretion in response to SF was progressively suppressed by increasing doses of atropine. In addition (although not shown in Table II), 2.3 μg/kg atropine without SF significantly reduced basal acid secretion in all nine subjects ($P < 0.005$). In the four subjects studied with 0.78 μg/kg, atropine significantly reduced basal acid secretion ($P < 0.01$). It should be recalled that gastric pH was kept constant, in spite of different rates of acid secretion, by intragastric titration with sodium bicarbonate.

The volume of fluid aspirated from the stomach at the end of the experiment (final gastric volume) increased in a dose-related fashion (Table II).

TABLE I
Peak Rises in Serum Gastrin Concentrations above Basal Levels in Response to 0.78 or 2.3 μg/kg Atropine in Four Subjects

Patients	0.78 μg/kg	2.3 μg/kg
	pg/ml	
J.H.	16	28
N.H.	4	12
H.D.	12	14
R.S.	8	10
Mean±SE	10±2.6	16±4.1

TABLE II
Effect of Atropine on Responses to SF in Nine Normal Subjects

Atropine dose	IGR*	IPPR*	Acid‡	Final gastric volume	Pulse
µg/kg	ng·min/ml	ng·min/ml	meq/h	ml	per min
0	0.42±0.15	1.40±0.38	18.1±3.3	39±5	67±2
2.3	1.50±0.55§	−0.60±0.30	10.8±1.5§	60±9§	62±3§
7	1.37±0.54§	−1.06±0.30	7.7±1.7§	79±15§	72±4§
21	1.66±0.48§	−1.36±0.58	4.7±1.4§	175±24§	97±3§

* IGR and IPPR from 0 to 75 min.
‡ Acid secretion during and for 30 min after SF.
§ $P < 0.05$ vs. 0 atropine dose (saline control).

Symptoms and pulse rate. No subject reported symptoms after 0 (control), 0.78, 2.3, or 7.0 µg/kg atropine. All subjects experienced dryness of the mouth and a few noted mild lethargy after the 21-µg/kg atropine dose. The 2.3-µg/kg atropine dose slightly slowed, and the higher doses accelerated the pulse rate (Table II). The 0.78-µg/kg atropine dose had no effect on pulse rate.

DISCUSSION

A major finding in this study was that small doses of atropine (0.78 and 2.3 µg/kg) led to graded and significant rises in basal serum gastrin levels. Although these doses of atropine significantly inhibited gastric acid secretion, the effect of atropine on gastrin release was not mediated by a decrease in gastric acidity, because gastric pH was controlled by intragastric titration. Assuming that atropine suppressed gastric secretion by blocking cholinergic-muscarinic receptors in the stomach (21), it is most likely that atropine increased gastrin levels by blocking a cholinergic pathway which normally (i.e., in the absence of atropine) inhibits gastrin release in the basal state.

Previous workers have not found an increase in basal serum gastrin concentrations after atropine (2, 3, 22), and some actually found a decrease (4, 23). However, our experiments cannot be compared directly with previous studies because we used smaller doses of atropine and because antral pH was not held constant in these other studies. In addition, it is possible that the slight amount of gastric distention in our studies (≅50 ml) at pH 5.0 initiated local or vagovagal reflexes, which in the presence of atropine, resulted in antral gastrin release.

Atropine did more than raise basal gastrin concentrations. The shaded area in Fig. 1 represents the extent to which 2.3 µg/kg atropine potentiated the gastrin response to SF and therefore the extent to which the vagus had inhibited gastrin release during SF without atropine. Thus, when activated by SF, the vagus nerve appears to suppress gastrin release by a

cholinergic, atropine-sensitive pathway. The exact location of the putative vagal-cholinergic pathway that inhibits gastrin release is not clear from these studies, although it is convenient to envision an inhibitory neuron in the vicinity of antral gastrin cells.

Because 2.3 µg/kg atropine slowed the pulse rate slightly, the possibility that this dose of atropine was acting as a parasympathomimetic drug (24), rather than as a muscarinic antagonist, needs to be considered. However, we feel that this dose of atropine was not acting as a cholinergic agonist drug for several reasons. First, administration of cholinergic agonist drugs does not release gastrin (25–28). Second, 2.3 µg/kg atropine abolished vagal release of pancreatic polypeptide, a hormone known to be released by vagal-cholinergic stimulation (29). Third, atropine doses >2.3 µg/kg, which led to clear-cut antimuscarinic effects (tachycardia, dry mouth), had similar effects on gastrin and HPP release as the 2.3-µg/kg dose, which favors a similar pharmacologic mode of action. And fourth, a dose of atropine <2.3 µg/kg released gastrin and inhibited acid secretion without changing the pulse rate. This latter observation also suggests that the inhibitory pathway for gastrin release and the gastric parietal cells are more sensitive to atropine than the heart.

Because SF without atropine led to significant rises in serum gastrin concentrations, it is apparent that the vagus nerve stimulates, as well as inhibits, gastrin release. We found that atropine doses as high as 21 µg/kg led to an increase in the amount of gastrin released during SF. This suggests that the vagus releases gastrin by a pathway that is atropine-resistant and that may therefore be noncholinergic.

To summarize, vagal activation by SF stimulates the release of HPP and gastrin. In contrast to vagally mediated HPP release and gastric acid secretion, which are inhibited by atropine, vagal gastrin release is potentiated by atropine, supporting the existence of a vagal-cholinergic inhibitory pathway for gastrin release. In addition, because atropine alone significantly increased serum gastrin above basal

levels, this putative inhibitory cholinergic pathway may be active even when the vagus nerve is not stimulated by SF.

REFERENCES

1. Farooq, O., and J. H. Walsh. 1975. Atropine enhances serum gastrin response to insulin in man. *Gastroenterology.* **68:** 662–666.
2. Korman, M. G., C. Soveny, and J. Hansky. 1971. Serum gastrin in duodenal ulcer. Basal levels and effect of food and atropine. *Gut.* **12:** 899–902.
3. Walsh, J. H., R. S. Yalow, and S. A. Berson. 1971. The effect of atropine on plasma gastrin response to feeding. *Gastroenterology.* **60:** 16–21.
4. Becker, H. D., D. D. Reeder, and J. C. Thompson. 1974. Effect of atropine on basal and food-stimulated serum gastrin levels in man. *Surgery (St. Louis).* **75:** 701–704.
5. McGuigan, J. E., and W. L. Trudeau. 1972. Serum gastrin levels before and after vagotomy and pyloroplasty or vagotomy and antrectomy. *N. Engl. J. Med.* **296:** 184–188.
6. Kronborg, O., F. Stadil, J. Rehfeld, and P. M. Christiansen. 1973. Relationship between serum gastrin concentrations and gastric acid secretion in duodenal ulcer patients before and after selective and highly selective vagotomy. *Scand. J. Gastroenterol.* **8:** 491–496.
7. Jaffe, B. M., B. G. Clendinneu, R. J. Clark, and J. A. Williams. 1974. The effect of selective and proximal gastric vagotomy on serum gastrin. *Gastroenterology.* **66:** 944–953.
8. Stadil, F. 1972. Effect of vagotomy on gastrin release during insulin hypoglycemia in ulcer patients. *Scand. J. Gastroenterol.* **7:** 225–231.
9. Stadil, F., and J. F. Rehfeld. 1974. Gastrin response to insulin after selective, highly selective, and truncal vagotomy. *Scand. J. Gastroenterol.* **66:** 7–15.
10. Christensen, K. D., and F. Stadil. 1976. On the beta-adrenergic contribution to the gastric acid and gastrin responses to hypoglycemia in man. *Scand. J. Gastroenterol. Suppl.* **37:** 81–86.
11. Pavlov, I. P. 1910. The centrifugal (efferent) nerves to the gastric glands and of the pancreas. *In* The Work of the Digestive Glands. Translated by W. H. Thompson. Charles Griffin and Company, Ltd., Philadelphia. 2nd Edition. Lecture III. 48–59.
12. Farrell, J. I. 1928. Contributions to the physiology of gastric secretion. The vagi as the sole efferent pathway for the cephalic phase of gastric secretion. *Am. J. Physiol.* **85:** 685–687.
13. Fordtran, J. S., and J. H. Walsh. 1973. Gastric acid secretion rate and buffer content of the stomach after eating.

Results in normal subjects and in patients with duodenal ulcer. *J. Clin. Invest.* **52:** 645–657.
14. Feldman, M., J. H. Walsh, H. C. Wong, and C. T. Richardson. 1978. Role of gastrin heptadecapeptide in the acid secretory response to amino acids in man. *J. Clin. Invest.* **61:** 308–313.
15. Richardson, C. T., J. H. Walsh, K. A. Cooper, M. Feldman, and J. S. Fordtran. 1977. Studies on the role of cephalic-vagal stimulation in the acid secretory response to eating in normal human subjects. *J. Clin. Invest.* **60:** 435–441.
16. Walsh, J. H., H. H. Trout, III, and H. T. Debas. 1974. Immunochemical and biological properties of gastrins obtained from different species and of different molecular species of gastrins. *In* Endocrinology of the Gut. W. Y. Chey and F. Brooks, editors. Charles B. Slack, Inc., Thorofare, N. J. 277–289.
17. Dockray, G. J., and J. H. Walsh. 1975. Amino terminal gastrin fragment in serum of Zollinger-Ellison Syndrome patients. *Gastroenterology.* **68:** 222–230.
18. 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.
19. Hoel, P. G. 1962. Introduction to mathematical statistics. John Wiley and Sons, Inc., New York. **11:** 276–279.
20. Richardson, C. T., J. H. Walsh, M. I. Hicks, and J. S. Fordtran. 1976. Studies on the mechanisms of food-stimulated gastric acid secretion in normal human subjects. *J. Clin. Invest.* **58:** 623–631.
21. Innes, I. R., and M. Nickerson. 1975. Atropine, scopolamine, and related antimuscarinic drugs. *In* The Pharmacological Basis of Therapeutics. L. S. Goodman and A. Gilman, editors. Macmillan, Inc., New York. 5th edition. **25:** 516.
22. Schrumpf, E., and J. Myren. 1974. Effect of atropine on plasma concentration of gastrin in fasting subjects. *Scand. J. Gastroenterol.* **9:** 123–125.
23. Ganguli, P. C., and W. M. Hunter. 1972. Radioimmunoassay of gastrin in human plasma. *J. Physiol. (Lond.).* **220:** 499–510.
24. Kottmeier, C. A., and J. S. Gravenstein. 1968. The parasympathomimetic activity of atropine and atropine methylbromide. *Anesthesiology.* **29:** 1125–1133.
25. Vatn, M. H., E. Schrumpf, and J. Myren. 1975. The effect of carbachol and pentagastrin on the gastric secretion of acid, pepsin, and intrinsic factor (IF) in man. *Scand. J. Gastroenterol.* **10:** 55–58.
26. Higgs, R. H., T. J. Humphries, D. O. Castell, and J. E. McGuigan. 1976. Lower esophageal sphincter pressures and serum gastrin levels after cholinergic stimulation. *Am. J. Physiol.* **231:** 1250–1253.
27. McCallum, R. W., M. M. Kline, N. Curry, and R. A. L. Sturdevant. 1975. Comparative effects of metoclopramide and bethanechol on lower esophageal sphincter pressure in reflux patients. *Gastroenterology.* **68:** 1114–1118.
28. Humphries, T. J., R. L. Farrell, D. O. Castell, and J. E. McGuigan. 1974. Serum gastrin response to oral bethanechol. *Gastroenterology.* **66:** 714. (Abstr.)
29. Schwartz, T. W., J. J. Holst, J. Fahrenkrug, S. Lindkaer Jensen, O. V. Nielson, J. F. Rehfeld, and O. B. Schafalitzky de Muckadell. 1978. Vagal, cholinergic regulation of pancreatic polypeptide secretion. *J. Clin. Invest.* **61:** 781–789.