Is Gastrin a Major Determinant of Basal Lower Esophageal Sphincter Pressure?

A DOUBLE-BLIND CONTROLLED STUDY USING HIGH TITER GASTRIN ANTISERUM

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ABSTRACT Studies were performed in anesthetized opossums to determine the influence of binding of circulating gastrin with a high titer gastrin antiserum on lower esophageal sphincter pressure. Gastrin antiserum or control antiserum was administered intravenously in successive doses of 0.02, 0.1, and 0.5 ml/kg on separate days. The lower esophageal sphincter pressures were measured for 1 h before and for 1 h after antiserum administration. The control serum caused no binding of opossum circulating gastrin, nor did it modify lower esophageal sphincter pressure. On the other hand, the administration of gastrin antiserum resulted in the binding of 85-90% of circulating gastrin, but it did not reduce sphincter pressure. A continuous infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of synthetic human gastrin I caused a significant ($P < 0.05$) increase in the sphincter pressure, a 30-fold increase in gastric acid output, and a fourfold increase in immunoreactive gastrin in the opossum blood. Prior treatment with 0.1 ml/kg of gastrin antiserum antagonized and 0.2 ml/kg of the antiserum abolished the gastrin-stimulated gastric acid secretion and the stimulating effect of gastrin on lower esophageal sphincter pressure. However, neither dose of antiserum modified basal lower esophageal sphincter pressure. It is concluded that circulating gastrin may not be an important determinant of basal sphincter pressure.

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INTRODUCTION

The role of gastrin in the physiologic regulation of lower esophageal sphincter pressure (LESP)[1] is controversial (1-8). Some observers have proposed that circulating gastrin (3, 4) is the major determinant of LESP. This hypothesis of gastrin closure of lower esophageal sphincter (LES) has been used as a model: (*a*) to explain the pathogenesis of a variety of disorders of the LES such as sphincter incompetence in reflux esophagitis (9-11) and sphincter hypertension in achalasia (11, 12); (*b*) to explain the mechanism of inhibitory action of secretin (3) and cholecystokinin (13) on LESP; and (*c*) to estimate endogenous gastrin activity in vivo (14). On the other hand, others (5-7) have recently questioned some of the evidence which formed the basis of the hypothesis that gastrin has a major role in the physiological regulation of LESP.

To gain additional insight into the regulation of the LESP, we invesigated the influence of antibody binding of circulating gastrin on LESP by studying: (*a*) the effect of administration of high titer antiserum against gastrin on the LESP in the opossum in a double-blind study; and (*b*) the influence of the antiserum on the effects of gastrin infusion on LESP and gastric acid output.

[1] *Abbreviations used in this paper:* HGI, human gastrin I; K_a, average intrinsic association constant; LES, lower esophageal sphincter; LESP, lower esophageal sphincter pressure.

METHODS

Preparation of antiserum. Antibodies to human gastrin I (HGI) were produced in randomly bred New Zealand white rabbits which were repeatedly immunized with HGI (residues 2–17) (Imperial Chemical Industries, Ltd., Alderley Park, Cheshire, England) conjugated to the protein Limulus hemocyanin utilizing 1-ethyldimethylaminopropyl carbodiimide as previously described (15). The HGI-protein conjugate was emulsified in complete Freund's adjuvant for injection into the footpads of the rabbits. Immunizations were performed using 2 mg of the HGI-protein conjugate, and the immunizations were repeated 2, 6, and 9 mo after initial immunization. One high titer antiserum to gastrin (59-04) which was obtained from one rabbit 12 days after the fourth immunization was used for all studies. The globulin fraction of the antiserum was prepared by precipitation with an equal volume of saturated aqueous ammonium sulfate at 4°C. The antibody-containing globulin precipitate was solubilized in phosphate-buffered saline (0.15 M NaCl-0.01 M potassium phosphate, pH 7.4) and dialyzed against 2 liters of phosphate-buffered saline (with two buffer changes) for 48 h at 4°C. The antibody-containing globulin fraction was adjusted to a volume equal to the original serum volume by addition of phosphate-buffered saline, and the antibody activity was then characterized utilizing ^{125}I-HGI residues 1–17 (^{125}I-HGI). The assay system contained varying amounts of synthetic HGI residues 1–17, a constant trace amount of ^{125}I-HGI and the antibody-containing globulin fraction (as described above) in a final dilution of 1:100,000. All reactants were prepared in a solution containing 1:50 normal human serum in 0.02 M barbital buffer, pH 8.4 in a total volume of 1.0 ml. After incubation at 4°C for 3 days, at which time equilibrium was achieved, antibody-bound ^{125}I-HGI was separated from antibody-free ^{125}I-HGI by addition of 40 mg of the anion-binding resin IRP 58 M (Rohm and Haas Co., Philadelphia, Pa.) 100–400 mesh in 0.2 ml of 0.02 M barbital buffer, pH 8.4. The tubes were then mixed in a rapid vortex mixer (Scientific Industries, Inc., Queens Village, N. Y.) for 3–4 s and centrifuged at 600 *g* for 10 min at 4°C. Simultaneous radioimmunoassay measurements of serially diluted opossum serum were made to compare relative immunoreactivity of antibodies with synthetic HGI and with immunoreactive gastrin in the serum of the opossum (Fig. 1).

Analysis of logit plots for standard calibration curves indicated that the preparation of antibodies to gastrin exhibited comparable affinities for radiolabeled and nonradiolabeled HGI. A Scatchard plot of binding of gastrin by antibodies to gastrin was constructed by plotting the ratio of antibody-bound-to-free immunoreactive ^{125}I-HGI against the concentration of antibody-bound unlabeled gastrin, as shown in Fig. 2. The maximum binding capacity of this preparation of antibodies to gastrin was obtained by extrapolating the binding curve to a bound-free ratio for ^{125}I-HGI to zero. The affinity (average intrinsic association constant) (K_a) for the antibody preparation for gastrin was calculated as the reciprocal of the unbound gastrin concentration in the incubation medium with one-half occupancy of antibody-binding sites by gastrin at equilibrium. This globulin fraction that contains antibodies to gastrin will be referred to subsequently as gastrin antiserum.

Control antiserum utilized in this study was obtained from a rabbit immunized in the same manner with 2 mg of Limulus hemocyanin that had not been conjugated to gastrin. Ammonium sulfate fractionation of this serum sample was performed identically with that for the antiserum obtained from the rabbit immunized with gastrin-protein conjugate.

Experimental design and techniques. All studies were performed on adult opossums (*Didelphis virginiana*) of both sexes, weighing 2.6–3.2 kg. These animals were

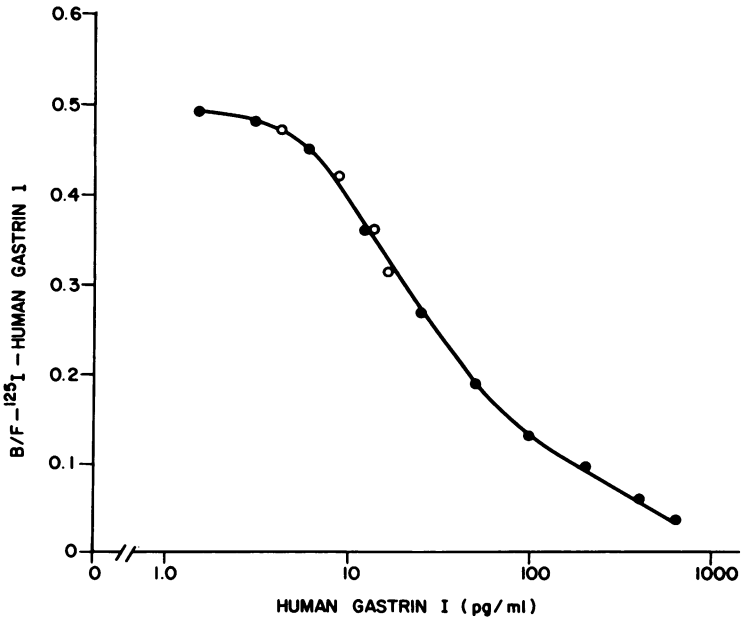

FIGURE 1 Radioimmunoassay calibration diagrams using HGI (●) and opossum serum (○). B/F ratios of immunoreactive ^{125}I-HGI were plotted against increasing amounts of unlabeled HGI. The calibration curves for diluted opossum serum and HGI were parallel.

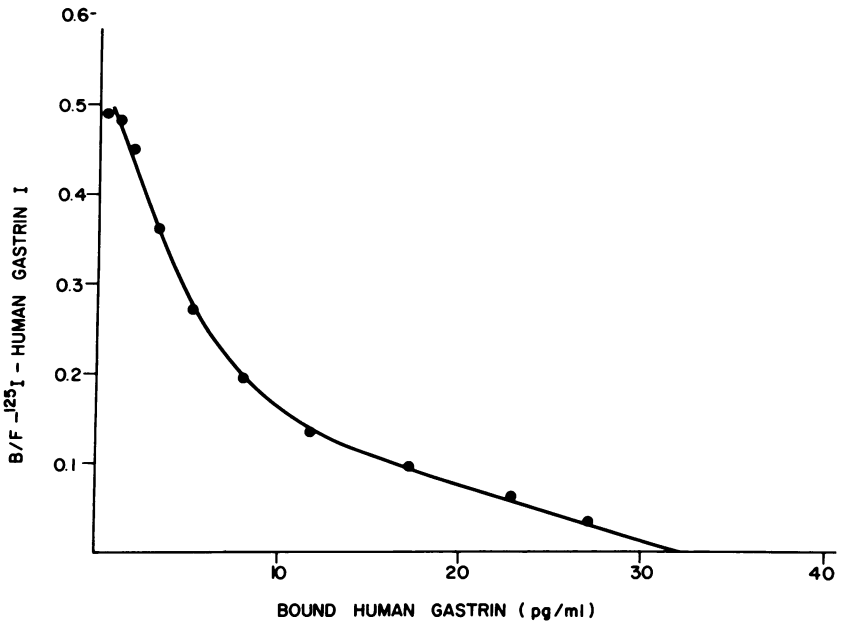

FIGURE 2 Scatchard plot with the ratio of B/F ^{125}I-HGI expressed as a function of bound unlabeled HGI. The gastrin antiserum was used at a final dilution of 1 : 100,000. The binding capacity of the original gastrin antiserum was 3.2 μg HGI/ml antiserum. The K_a was 5.85 $\times 10^{10}$ M^{-1}.

bred and raised at the Animal Science and Technology Branch, National Institute of Environmental Health Sciences, Triangle Park, N. C. and were free of any gastrointestinal parasites. The animals were anesthetized with intramuscular ketamine (5–15 mg/kg) and were strapped supine on an animal board. The anesthesia was maintained with small intravenous supplements of phentobarbital (5–10 mg) as required. Because these animals were used repeatedly on separate days, care was taken not to produce deep anesthesia. In these animals respiratory depression did not occur, and respiratory assistance was not required. Superficial reflexes and leg withdrawal with pinching were not abolished.

LESP was measured with water-filled polyvinyl catheters (Becton-Dickinson and Co., East Rutherford, N. J., model 6179; ID = 0.86 mm, OD = 1.12 mm) connected to pressure transducers (Statham model P23Db; Statham Instruments, Inc., Oxnard, Calif.). The catheters were continuously perfused with boiled water through a side opening at a rate of 9 ml/h, using polyethylene tubing (Clay Adams model PE10; ID = 0.28 mm and OD = 0.61 mm) and a continuous infusion pump (B. Braun Apparatgebau, Melsungen, West Germany). Each catheter was 50 cm long and had a 1.0-mm side opening and closed tip. This manometric system had low compliance; the rate of pressure increase with sudden occlusion of the catheter was over 300 mm Hg.[2] The catheter assembly consisting of three recording units with openings 1 cm apart was introduced into the animal's stomach through the mouth. Intraluminal pressures were recorded in a Beckman recorder (Dynagraph 411, Beckman Instruments, Inc., Fullerton, Calif.). The catheter assembly was gradually withdrawn at 2-mm intervals from the stomach to define the profile of the high pressure zone of the LES. The catheter was then positioned and fixed so that the middle lead recorded from the highest point of the high pressure zone. The sphincter pressures were measured in millimeters Hg with reference to atmospheric pressure. The pressure at the height of respiratory excursion was reported as the sphincter pressure. The values of sphincter pressure were obtained from the pressure records without any knowledge of the type of treatment received by the animals. The pressure was read at every 5 min interval. If the animal happened to swallow at that time, however, the pressure recorded was that measured just before swallowing. Gastric acid secretions were collected continuously with a soft polyvinyl tubing (ID = 2 mm; OD = 2.5 mm; Pharmaseal Laboratories, Glendale, Calif.) permanently glued to 4 polyvinyl catheters (ID = 0.86 mm; OD = 1.12 mm; Becton Dickinson, model 6179). The polyvinyl tube for gastric aspiration extended 10 cm beyond the most proximal catheter opening. The other catheter openings were 1, 2, and 5.5 cm distal to the proximal catheter opening. The widest outside diameter of the assembly was 4 mm. The tube for gastric aspiration had 10 holes at 0.5-cm intervals to facilitate collection of gastric contents.

The gastric juice was collected continuously in 15-min portions with constant aspiration at a pressure of 5 mm Hg using a Stedman pump (American Gastroscope Makers, Inc., New York, model 2590). The volume of gastric contents was recorded, and acid output was estimated by the method of Moore and Scarlata (16) after determination of the pH of the samples to an accuracy of 0.001 pH unit, using Sargent-Welch model NX pH meter (Sargent-Welch Scientific Company, Skokie, Ill.). All intravenous injections were administered through an indwelling femoral or brachial cannula, and all blood samples were obtained from a venous or arterial cannula on the opposite side.

[2] Actual $\Delta P/\Delta T$ was 100 mm Hg in 0.1 s and 200 mm Hg in 0.35 s.

The gastrin antiserum and control serum were supplied as unknowns A and B. The opossums were paired on the basis of identical sex and comparable weights. One animal in each pair was treated with A, and the other was treated with B. The animals were fasted overnight (10–12 h) on the study days. Each animal received three successive doses of 0.02, 0.1, and 0.5 ml/kg of the antiserum treatments on different days. The sphincter pressures were recorded for 1 h before and 1 h after the administration of each dose of antiserum. The treatments were decoded after the results of the sphincter pressures had been analyzed.

HGI heptadecapeptide was administered as a slow continuous infusion in the dose of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ in 30 ml of saline using a Holter pump (model 911, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). Control infusion of 30 ml of saline for 1 h was given before gastrin infusion. Gastric secretions were collected in 15-min portions, and LESP's were measured during this period with a complete pull-through technique. These studies were repeated twice in the same animals on 2 separate days after treatment with 0.1 and 0.2 ml/kg of gastrin antiserum administered 15 min before the studies.

Measurement of antibody binding of gastrin in opossum serum. The proportion of gastrin in the opossum serum bound by antibodies to gastrin was determined by two methods. The first method, which was identical with that used by Lipshutz and associates (4), utilized an estimation of the amount of gastrin bound to antibody by applying empirically determined values for affinity of antibody to gastrin and the capacity of antibody to bind gastrin as measured by in vitro immunoassay binding determinations. For these calculations the concentrations of administered antibody in the intravascular space of the opossum were determined assuming dilution of intravenously injected gastrin antibodies in an estimated blood volume of 5% of body weight. The mean fasting serum gastrin concentration for the opossums before antiserum injection was determined by radioimmunoassay to be 77±16 (SEM) pg/ml. The concentration of endogenous gastrin bound by gastrin antibodies was obtained by the following equation: $K_a = [x]/\{(2\ [Ab] - [x])\ ([\text{gastrin}] - [x])\}$. K_a was the average intrinsic association constant (affinity) of the antibody preparation for gastrin. $[Ab]$ was the estimated molar concentration of the antibody in the circulation of the opossum. [Gastrin] was the mean molar-fasting serum gastrin concentration measured in the animals used in these studies, and $[x]$ was the calculated molar concentration of circulating gastrin bound by antibodies to gastrin. The second method was a direct in vitro measurement of antibody-bound gastrin. In this method sera obtained 1 h after administration of each antiserum preparation were serially diluted (1:4, 1:8, 1:16, 1:32) in the buffer-protein system for radioimmunoassay. 0.2 ml of each dilution of each sample was then incubated in the presence of trace amounts of ^{125}I-HCl (3 pg), and the proportions of antibody-bound and antibody-free ^{125}I-HGI were identified as described for the radioimmunoassay calibration system. Determination of antibody binding of gastrin by undiluted opossum serum was made by extrapolating the percent antibody binding to zero dilution.

The globulin fraction that contained antibodies to gastrin was also examined for its immunological cross-reactivity with the carboxyl-terminal tetrapeptide amide of gastrin, pentagastrin, pure porcine, cholecystokinin-pancreozymin, and secretin. The molar concentrations of these peptides required to inhibit 75% of binding of ^{125}I-HGI was divided by the concentration of HGI that produced the same degree of inhibition of antibody binding; this calculation yielded the molar inhibitory potency of these peptides in respect to gastrin.

Statistical analysis. Statistical analysis was performed by calculating Student's t test for unpaired and paired observations.

RESULTS

Characterization of antiserum to gastrin. The K_a of the preparation of antibodies for gastrin, as determined by a Scatchard analysis (17) of gastrin binding (Fig. 2), was 5.85×10^{10} M^{-1}. Using this same plot, the total binding capacity of the undiluted antiserum to gastrin was 3.2 μg gastrin/ml gastrin antiserum. Serial dilutions of fasting (basal) opossum serum were examined in the gastrin radioimmunoassay using serial dilutions of HGI as reference standards for construction of this calibration curve, and comparisons were made with those of serially diluted opossum serum. The radioimmunoassay calibration diagram for diluted opossum serum was parallel to that for HGI (Fig. 1), a finding that is consistent with the similar immunoreactivity of fasting serum gastrin in the opossum to that of synthetic HGI. The molar inhibitory potency with this antibody preparation for HGI was designated as 1.0. The inhibitory potency was 0.05 for the carboxyl-terminal tetrapeptide amide of gastrin, 0.075 for pentagastrin, and 0.07 for cholecystokinin-pancreozymin. There was no evidence of immunological cross-reactivity of this antibody preparation with the structurally unrelated gastrointestinal peptide hormone secretin.

Effects of gastrin antiserum and control serum on LESP and gastrin binding

Effect on basal LESP. Fig. 3 shows effects of gastrin antiserum and control serum in the dose of 0.02 ml/kg on LESP in the first pair of animals. Spontaneous fluctuations in sphincter pressure were observed, but there was no obvious reduction in the LESP with antigastrin serum administration. The mean±SE sphincter pressure was 35.2±1.7 mm Hg before and 46.7±1.1 mm Hg after the antiserum administration. The LESP was 22.5±2.6 mm Hg before and 22.8±1.4 mm Hg after administration of control serum.

These experiments were repeated on separate days using successive doses of 0.1 and 0.5 ml/kg of the sera. The mean±SE values of LESP, before and after serum administration in different animals, have been summarized in Table I. There were considerable variations in the LESP that were statistically significant in different study periods in most of the animals. However, there was no difference between animals treated with the antigastrin serum and those treated with control serum.

The cumulative mean values of LESP in the three animals treated with gastrin antiserum and the other

TABLE I

Effect of Antigastrin and Control Serum on LESP and Serum Gastrin Binding in the Opossum Blood in Different Animals and after Different Doses of Serum

Pair no.	Dose of serum*	Antigastrin serum				Control serum			
		LESP‡		Unbound gastrin		LESP‡		Unbound gastrin	
		Before	After	Before	After	Before	After	Before	After
				%				%	
I:	0.02 ml/kg	35.2 ±1.7	46.7§ ±1.13	100	14	22.5 ±2.5	22.8 ±1.4	100	100
	Plus 0.1 ml/kg	33.3 ±1.4	31.4 ±1.6	—	—	21.1 ±1.9	24.3 ±1.2	—	—
	Plus 0.5 ml/kg	50.7§ ±2.1	45.1§ ±2.2	0	5	31.5§ ±1.5	39.9§ ±2.1	100	100
II:	0.02 ml/kg	30.6 ±2.8	38.8 ±6.7	100	10	23.5 ±1.2	13.7§ ±0.9	100	100
	Plus 0.1 ml/kg	30.7 ±1.8	35.9§ ±2.6	—	—	32.5§ ±3.2	28.8§ ±2.2	—	—
	Plus 0.5 ml/kg	29.2 ±1.4	23.2§ ±0.9	14	4	46.4§ ±6.1	57.8§ ±4.2	100	100
III:	0.02 ml/kg	45.6 ±2.1	39.7 ±5.8	100	16	50.6 ±5.5	75.3§ ±6.9	100	100
	Plus 0.1 ml/kg	45.6 ±2.0	54.4§ ±3.0	—	—	70.2 ±6.1	51.3 ±5.9	—	—
	Plus 0.5 ml/kg	52.3§ ±1.5	45.2 ±2.1	13	11	20.2§ ±3.9	26.2§ ±2.8	100	100

* Studies with different doses were performed on different days.

‡ Each value represents mean±SE of 13 observations obtained every 5 min for a 1-h period.

§ Indicates that the value was statistically different from LESP before any serum administration.

three animals treated with control serum in different doses have been shown in Fig. 4. There was no significant difference in the basal LESP with any serum treatment.

Antibody binding of gastrin in serum by administered antibodies was 82% after the first injection of gastrin antiserum (0.02 ml/kg), 95% after the second injection (0.10 ml/kg), and 99% after the third injection (0.50 ml/kg).

Direct determination of the binding of circulating gastrin by antiserum administered to the opossum revealed no detectable serum binding of gastrin in the opossums before treatment and in those animals receiving the control antiserum (antiserum preparation B). In contrast, substantial antibody binding of gastrin was demonstrated consistently after each injection of the gastrin antiserum preparation. After the initial injection of gastrin antiserum (0.02 ml/kg) antibody binding of gastrin averaged 87% (range 84-90%). After the second injection of antibodies to gastrin (0.10 ml/kg), antibody binding of gastrin averaged 92% (range 86-103%). After the final injection of antibodies to gastrin (0.50 ml/kg), antibody binding averaged 95.5% (range 95-96%). Table I summarizes the percent of unbound serum gastrin in different animals at different study periods. The basal gastrin level before any treatment was 84 pg/ml (49-170) and 67.3 pg/ml (56-77) in animals subsequently treated with antigastrin and control serum, respectively.

Effects of gastrin infusion on LESP, acid secretion, and serum gastrin levels

Effects on LESP. Table II summarizes the mean values of LESP in different animals obtained with four pull-throughs (one during each 15 min period) during the saline infusion and four pull-throughs during the infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ gastrin I. Gastrin infusion caused a significant increase ($P < 0.02$) in the basal sphincter pressure.

Effect on gastric acid secretion. Basal- and gastrin-stimulated acid secretion in the four animals are also

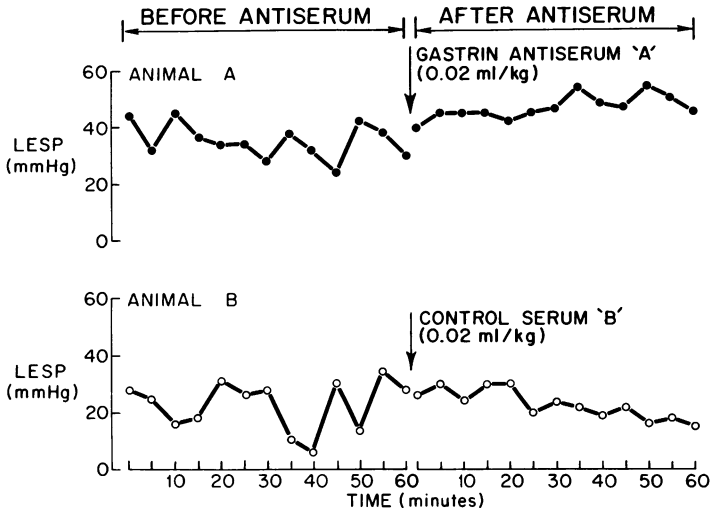

FIGURE 3 The response of LESP to gastrin antiserum (A) and control serum administration (B). Each dot represents the sphincter pressure at every 5 min interval for 1 h before and for 1 h after the serum treatment. Values in this experiment were obtained in the first pair of animals.

summarized in Table II. The mean basal acid output was 3.1±1.9 μeq/h and increased to 97.6±38.6 μeq/h during the infusion of gastrin ($P < 0.02$).

Effects of gastrin infusion on serum gastrin levels in opossums. The serum gastrin concentrations in the four opossums before intravenous infusion of gastrin was 67.2 ±5.7 pg/ml. Serum gastrin concentrations were measured at 15-min intervals during the 60-min infusion of HGI (0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$). The mean serum gastrin concentration during intravenous infusion with gastrin was 262.0±49.6 pg/ml; 30 min after stopping the infusion of gastrin, serum gastrin concentrations were 108.2±9.3 pg/ml.

Influence of gastrin antiserum on basal- and gastrin-stimulated LESP and acid secretion

Influence on LES. The mean±SE basal LESP (during saline infusion) in the four animals after 0.1 ml/kg antiserum was 28.2±3.2 mm Hg, and after 0.2 ml/kg antiserum the sphincter pressure was 28.4±3.4 mm Hg. Neither of these values was significantly different from control values ($P > 0.05$; Fig. 4).

The mean sphincter pressure during the infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ gastrin I after pretreatment with 0.1 ml/kg gastrin antiserum was 30.4±7.2 mm Hg, and after pretreatment with 0.2 ml/kg antiserum the mean sphincter pressure was 32.1±1.1 mm Hg (Fig. 5).

TABLE II

Effect of Continuous Intravenous Infusion of Gastrin I (0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$) on LESP and Gastric Acid Output in Different Animals

Animal identification no.	LESP		Gastric acid output	
	Saline infusion	Gastrin I (0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$)	Saline infusion	Gastrin I (0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$)
	*mm Hg**		*μeq/h*	
91	28	42	4.6	198
93	24	46	0	118
94	25	30	7.8	29.9
95	27	37	0	44.7
Mean±SE	26±0.9	38.7±3.4	3.1±1.9	97.6±38.6
P value		<0.02		<0.02

* Each value is a mean of four observations at 15-min intervals.

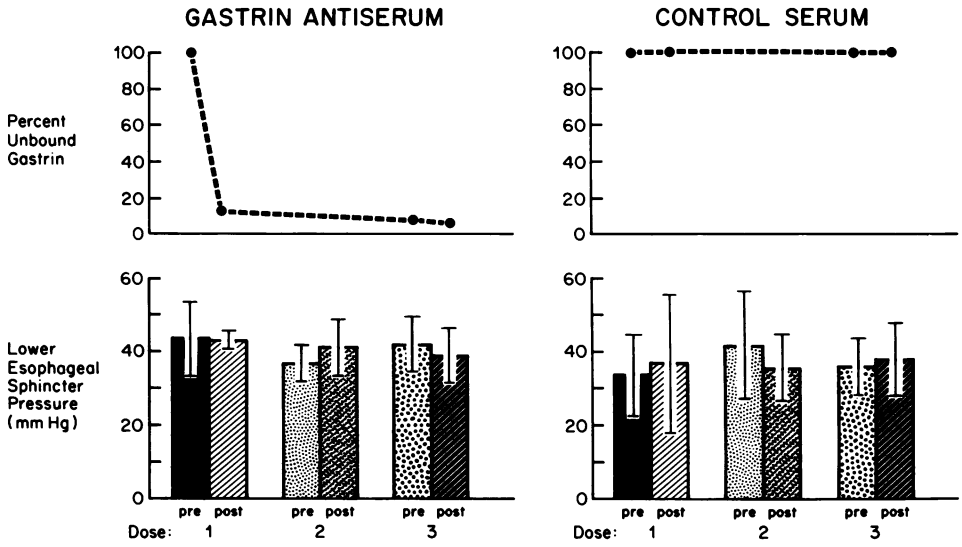

FIGURE 4 LESP and percent unbound gastrin in the opossum serum after treatment with gastrin antiserum and control serum. The animals received three successive doses of 0.02, 0.1, and 0.5 ml serum/kg (designated dose 1, 2, and 3, respectively) on separate days. Lower panel: the bar heights indicate mean±SE of the sphincter pressures before (pre) and after (post) the administration of the sera. The sphincter pressure for each animal was obtained by averaging pressures at 5-min intervals. Note that none of the doses of gastrin antiserum or control serum significantly altered the LESP ($P > 0.05$ for all values). Upper panel: each point shows the mean value of percent unbound gastrin in the opossum serum as determined in vitro (see text for details). Note that 0.02 ml/kg gastrin antiserum caused over 85% binding, and the third dose caused 95.5% binding of the circulating opossum gastrin. On the other hand, control serum did not cause any binding.

Neither of these values was significantly different from the control values during the saline infusion ($P > 0.05$).

Influence on gastric acid secretion. Basal gastric acid outputs were 7.0±6.6 (mean±SE) and 8.2±8 μeq/h after administration of 0.1 and 0.2 ml/kg antiserum, respectively. The gastrin-stimulated acid secretion was 42.7±22.8 μeq/h after the administration of 0.1 ml/kg and 4.0±2.0 μeq/h after 0.2 ml/kg gastrin antiserum. Thus, 0.1 ml/kg antiserum reduced and 0.2 ml/kg antiserum treatment abolished the gastric acid secretory response to administration of gastrin I (Fig. 6).

Influence of intravenous injection of gastrin antiserum on gastrin binding in opossum serum. The percent binding of circulating opossum gastrin was determined in

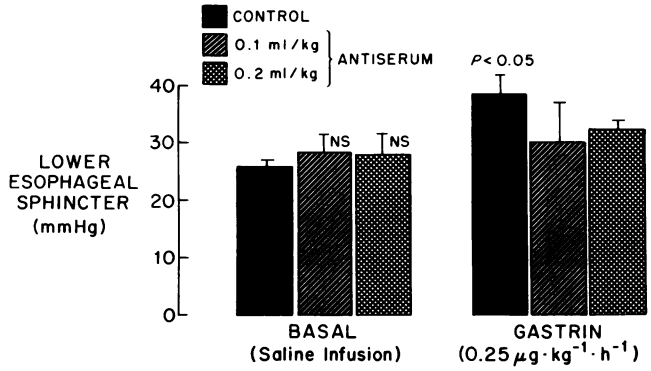

FIGURE 5 Influence of gastrin antiserum on basal- and gastrin-stimulated sphincter pressure. Note that basal sphincter pressure (during saline infusion) was not modified by 0.1 or 0.2 ml/kg of gastrin antiserum. Sphincter pressure during the infusion of gastrin was significantly higher than that during the control period ($P < 0.05$). Increase in LESP with gastrin I was abolished by antiserum treatment.

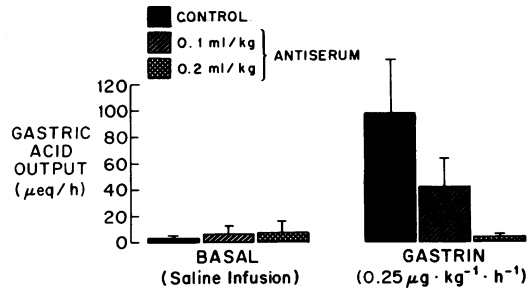

FIGURE 6 Influence of gastrin antiserum on basal- and gastrin-stimulated gastric acid output. Note that 0.1 ml/kg of gastrin antiserum inhibited and 0.2 ml/kg of the antiserum abolished the gastrin-stimulated gastric acid output. Basal acid output was not abolished by the antiserum.

vitro after antiserum treatment and after the animals had also received an infusion of HGI (0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$). The percent binding of circulating gastrin was 88.4± 3.6% after the administration of 0.1 ml/kg and 99.7± 1.7% after the administration of 0.2 ml/kg (Fig. 7).

DISCUSSION

These studies demonstrate that intravenous administration of a high titer antiserum against HGI in the opossum: (*a*) caused binding of a large proportion of circulating opossum gastrin; (*b*) antagonized the increase in LESP and stimulation of gastric acid secretion in response to exogenous HGI; but (*c*) did not alter the resting LESP.

Our observation that gastrin antiserum administration did not reduce resting LESP is different from that of Lipshutz et al. (4) who reported a dose-related reduction in LESP with graded dose of high titer gastrin antiserum. The reason for differences in results in the two studies is not clear. In both studies, (*a*) high titer antiserum was raised in the rabbits against HGI; and (*b*) opossums were used as experimental animals. However, there were some differences in the design of the experiments. (*a*) The present study was performed in a double-blind fashion; the nature of the serum (gastrin antiserum or control serum) was not known at the time of the study, and sphincter pressures and binding of the circulating gastrin in the opossum blood were measured and interpreted without knowledge of the type of treatment. (*b*) We studied repeatedly the same animals after treatment with successively large doses of antiserum, whereas Lipshutz et al. used different sets of animals for different doses of antiserum. (*c*) For recording sphincter pressures, we used infusions at a rate which was one-eighth the rate used previously (4). This difference in the infusion rate could not explain the observed differences in the two studies. Although the measured amplitude of the contractions in the body of the esophagus (because of their transient nature) is markedly influenced by the infusion rate (18, 19), the sphincter pressure measurements can be recorded accurately over a wide range of infusion rates (20, 21). Moreover, because of the low compliance of our system, even at the infusion rate of 9 ml/h, the rate of pressure increase with sudden occlusion of catheter was over 300 mm Hg/s, a rate that is more than adequate to measure sphincter pressures accurately (19). (*d*) We used ketamine and phenobarbital as the anesthetic agents, whereas phenobarbital anesthesia was used before (4), and the animals in the present study were anesthetized lightly. We have previously shown that the depth of anesthesia does not appear to exert a significant effect on the basal sphincter pressure (22). Thus, although there were minor differences in the two studies, they do not appear to explain the differences in the results obtained.

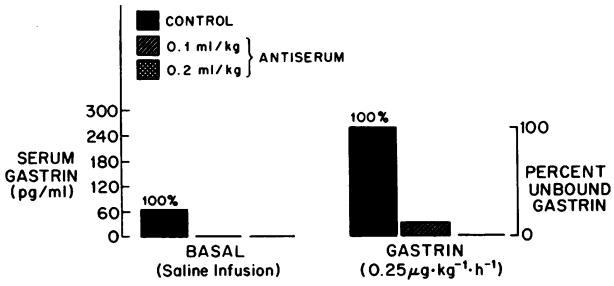

FIGURE 7 Influence of gastrin antiserum on gastrin binding after gastrin infusion. Note that infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ HGI caused a fourfold increase in serum gastrin. Immunoreactive gastrin could not be determined after antiserum treatment. However, the percent unbound gastrin after 0.1 ml/kg of antiserum was 11.6%; after 0.2 ml/kg of antiserum, it was 0.3%.

The validity of conclusions from these studies rests on the assumption that intravenously administered gastrin antiserum effectively bound the circulating opossum gastrin and, moreover, rendered it biologically inactive. We found, as was found previously (4), that the binding of the opossum gastrin by HGI antiserum was similar to binding of HGI. Antiserum in the dose of 0.02 ml/kg caused binding of 84-90% of circulating gastrin in different animals. Additional and cumulatively larger doses of antiserum resulted in greater gastrin binding. Lipshutz et al. (4) did not measure gastrin binding directly in the blood of opossums treated with antiserum. However, they estimated that an intravenous dose of 0.01 ml given to each opossum would result in approximately 60% binding of circulating gastrin and that 0.1 ml antiserum would cause 90% binding of circulating opossum gastrin.

Bioinactivity of gastrin bound by gastrin antiserum was shown by Lipshutz et al. (4) as follows: incubation of HGI with antiserum in vitro for 15 min antagonized

the effect of HGI on opossum LES strips in vitro and on the LESP in vivo. We have shown that the pretreatment of animals with the antiserum antagonized the effects of continuous infusion of HGI on the LES and gastric acid secretion. The blocking effects of gastrin antiserum treatment on LESP responses to gastrin infusion has not been reported before, but such blocking effects of antiserum to the active carboxyl-terminal gastrin tetrapeptide on gastric acid stimulation with gastrin in the rate have been reported previously (23, 24).

If the assumption concerning antibody binding of circulating opossum gastrin is correct, we can conclude that circulating gastrin may not be an important determinant of basal LESP. This conclusion receives further support from the following observations: (*a*) gastrin exerts its effect on the LES by action on cholinergic neurons (9, 25) and small doses of atropine, which antagonize the effect of a dose of gastrin that produced maximal contraction of LES, did not reduce basal LESP (9). Such a decrease in pressure would be expected if gastrin were responsible for basal LESP (26). (*b*) Dodds et al. found no correlation between basal gastrin levels and LESP in different subjects nor in the same subjects on different days (27). (*c*) A twofold increase in immunoreactive gastrin in human blood produced by continuous administration of HGI caused only a 3–4-mm Hg increase in LESP (28). (*d*) Circulating gastrin in the basal state in many species is big gastrin or big big gastrin (29, 30). The biological significance or potency of big big gastrin is not known at present; however, the endogenous potency of big gastrin in respect to acid secretion is approximately one-sixth that of heptadecapeptide gastrin I (31).

Even though the resting basal LES pressure may not be dependent upon the gastrin circulating in the basal state, changes in serum gastrin levels with physiological stimulation may modulate sphincter pressure. Our studies have shown that continuous infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ caused a 12-mm Hg increase in basal LESP and a 25-fold increase in gastric acid secretion. Walker et al. (7) have shown that continuous intravenous infusion of pentagastrin in man in doses that caused more than half-maximal stimulation of gastric acid secretion produced an 8-10 mm Hg increase of (about 30%) in LESP. A protein meal causes maximal stimulation of gastric acid secretion (32) and about a 10-12 mm Hg increase in LESP (33). However, the increase in LESP in response to a protein meal cannot be explained exclusively on the basis of gastrin release because of the following considerations. The LES response to feeding is disproportionately large when compared with gastrin release: a protein meal in man usually increases the circulating gastrin level by no more than twofold (33, 34), whereas infusion of 0.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$ HGI in the opossum, which induced a comparable increase in LESP, caused a fourfold increase in basal gastrin level. Studies in man have shown that a twofold increase in circulating gastrin level occurred with an intravenous infusion of 0.12 $\mu g \cdot kg^{-1} \cdot h^{-1}$ caused only a 4-mm Hg increase in resting LESP (28). Furthermore, the predominant form of serum gastrin released in response to a protein meal is big gastrin which is biologically less active than HGI (31). Increases in LESP with gastric alkalinization have been assumed in the past to be due to increases in serum gastrin concentrations (1, 33), and this assumption has been used to provide evidence for the potential role of gastrin in the control of LESP (1, 4, 8). Direct measurements of serum gastrin levels, however, have failed to demonstrate increases in serum gastrin with antral alkalinization (34). These observations suggest that variations in circulating gastrin levels in physiologic states may not exert a major regulatory role in the control of LESP. Whether gastrin plays a more subtle or supportive role in the control of LESP remains to be elucidated.

The physiological and clinical importance of the observations reported in this study regarding the role of gastrin in the regulation of LESP are obvious: (*a*) it has been suggested that secretin (3) and cholecystokinin (13) may inhibit LESP by competing for excitatory receptor sites with circulating gastrin. If circulating gastrin is not responsible for the genesis of LESP, then an alternative mechanism of inhibitory effect of such hormone must be considered. (*b*) Defects in gastrin release and gastrin sensitivity have been proposed as the basis of LES incompetence (10, 11) and LES hypertension in achalasia (11–13), respectively. If gastrin is not a major determinant of LESP, then concepts about the pathogenesis of LES disorders need to be revised, and alternative pathogenetic mechanisms must be investigated to provide a basis for rational therapy.

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