The Genesis of Lower Esophageal Sphincter Pressure: Its Identification through the Use of Gastrin Antiserum

William Lipshutz, … , William Hughes, Sidney Cohen

Research Article

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The Genesis of Lower Esophageal

Sphincter Pressure: Its Identification through

the Use of Gastrin Antiserum

WILLIAM LIPSHUTZ, WILLIAM HUGHES, and SIDNEY COHEN

From the Gastrointestinal Section, Department of Medicine, Hospital of the University of Pennsylvania, and Veterans Administration Hospital, Philadelphia, Pennsylvania 19104

A B S T R A C T The purpose of this study was to evaluate the role of gastrin in the genesis of lower esophageal sphincter (LES) pressure by the use of a high titer gastrin antiserum. Intravenous infusions of increasing amounts of rabbit gastrin antiserum, but not control antiserum, produced graded reductions in the resting LES pressure in anesthetized opossums. A maximal inhibition in LES pressure of 80.0 ±3.1% (mean ±SE) was achieved when gastrin antiserum was administered in an amount estimated to bind almost all endogenous circulating gastrin in the opossum. Gastrin antiserum also inhibited the LES response to endogenous gastrin release (gastric deacidification) and to exogenous intravenous administration of gastrin I. The inhibition of the LES response to exogenous gastrin I by gastrin antiserum could be eliminated by giving excess gastrin I. Studies performed in vitro showed that gastrin antiserum inhibited the contractile response of LES circular muscle to gastrin I, but not to acetylcholine. These studies indicate that gastrin antiserum: (*a*) specifically antagonized the response of LES circular muscle to gastrin, in vitro; (*b*) diminished the LES response to the endogenous release and to the exogenous administration of gastrin; and (*c*) markedly reduced the resting level of LES pressure. We conclude that endogenous gastrin is the major determinant of resting LES pressure.

INTRODUCTION

The intrinsic strength of the physiological lower esophageal sphincter (LES)[1] is the major determinant of

competence at the gastroesophageal junction in man (1–5). Recent studies have focused on the neural (6, 7) and humoral (8–11) factors that augment LES pressure above its resting level. However, the genesis of the resting LES strength itself is not known. Differences in the resting LES strength distinguish normals from patients with gastroesophageal reflux (2–5). In addition, the absolute magnitude of the LES response to neural and humoral stimuli is dependent upon the preexisting LES pressure (6–9).

Recent investigations have implicated gastrin in the origin of resting LES strength. It has been shown that, in man, a major portion of the resting LES pressure can be suppressed by the reduction in endogenous gastrin release by gastric acidification (8–10). Also the marked in vitro sensitivity of LES circular muscle to gastrin compared to adjacent esophageal and gastric muscle further suggested an important role for this hormone as a determinant of sphincter function (12). It was the purpose of this study to evaluate the role of endogenous gastrin in the determination of LES strength in a suitable animal model, the opossum (13), by the use of a high titer gastrin antiserum.

METHODS

Antibodies to human synthetic gastrin I (HSG 1) (Imperial Chemical Industries Ltd., Alderley Park, Cheshire, England) were obtained following the technique of McGuigan and Trudeau (14, 15). HSG 1, amino acid sequence 2–17, conjugated to bovine serum albumin, was emulsified in Freund's adjuvant and injected into the foot pads of rabbits. Immunizations were carried out at 1, 5, and 11 months after the initial immunization. A high titer gastrin antiserum was obtained 10 days after the fourth injection in one rabbit, and was used for all studies. The globulin

[1] *Abbreviations used in this paper:* CCK-PZ, cholecysto-kinin-pancreozymin; HSG 1, human synthetic gastrin I; LES, lower esophageal sphincter.

fraction of the antiserum was precipitated by adding an equal volume of saturated aqueous ammonium sulfate. The precipitate containing the gastrin antibodies was solubilized in phosphate-buffered saline and dialyzed against this same solution. The globulin preparation was brought to a volume equal to the original volume of serum, and its antigastrin activity was characterized in a double antibody radioimmunoassay system.

The assay system contained varying known amounts of HSG 1 (1–17), a constant trace amount of HSG 1 (2–17) labeled with ^{125}I, 10.0 μg of rabbit gamma globulin, and rabbit gastrin antiserum (as prepared above) in a dilution of 1:100,000. The reactants were in a solution of 1.0% ovalbumin, 0.01 M Na EDTA, 0.15 M NaCl, and 0.01 M KHPO$_4$ at pH 7.4, in a total volume of 1.0 ml. After incubation at 4°C for 3 days, excess goat antibody to rabbit gammaglobulin was added to precipitate the soluble gastrin antigen antibody complexes. The radioimmunoassay calibration diagram of HSG 1 is shown in Fig. 1.

Analysis of a logit plot of the standard curve showed that the gastrin antibody had similar affinity for labeled HSG 1 (2–17) and unlabeled HSG 1 (1–17) (16). Therefore, the amount of gastrin bound by the antibody could be estimated by multiplying the total amount of unlabeled gastrin in the assay system by the per cent of immunoreactive labeled HSG 1 that was bound. A Scatchard plot (17) of gastrin binding constructed by plotting the bound/free ratio of immunoreactive HSG 1-^{125}I against the concentration of bound unlabeled gastrin is shown in Fig. 2. The maximum binding capacity of the antibody was obtained by extrapolating the binding curve to a HSG 1-^{125}I bound/free ratio of zero. The average affinity of the antibody population for gastrin was calculated as the reciprocal of the free gastrin concentration in the system when one-half of the antibody binding sites were occupied by gastrin (18).

Control antiserum was obtained from a rabbit immunized serially with an unrelated antigen, thyroid-stimulating hormone, emulsified in Freund's adjuvant. This serum was processed in a manner similar to that of the gastrin antiserum.

Studies in vivo. All studies were performed on the opossum, *Didelphis virginiana,* an animal with an esophagus and LES similar to those in man (13). Studies were done on adult opossums of both sexes, selected by weight (2.3–2.7 kg). Anesthesia with intraperitoneal pentobarbital, 40 mg/kg, was administered, and the animals were strapped supine to an animal board for studies in vivo. Esophageal manometric studies were performed with water-filled polyvinyl tubes, 1.4 mm internal diameter, connected to external transducers (Statham P23BB; Statham Instruments, Inc., Los Angeles, Calif.). Intraluminal pressures were graphed on a Beckman multichannel, curvilinear, ink-writing recorder (Beckman Instruments, Inc., Fullerton, Calif.). Recording tubes were arranged as a fixed unit to record intraluminal pressures at three points, 5 cm apart, through side orifices, 1.2 mm in diameter. After the recording assembly was passed by mouth, all orifices were positioned in the stomach. The recording assembly was withdrawn at 0.5-cm intervals, with measurements being obtained at each level for a 1 min period. After this manometric evaluation, the recording assembly was positioned and anchored at the lower jaw, so that pressures were recorded simultaneously from esophagus, LES, and stomach. Each recording tube was continuously perfused with distilled water by an infusion pump at a rate of 1.2 ml/min. The stomach was
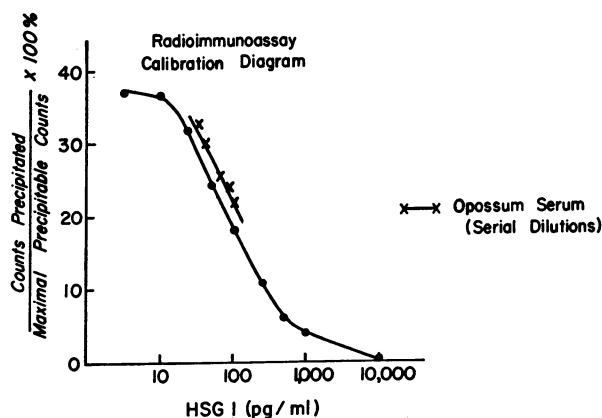

FIGURE 1 Radioimmunoassay calibration diagrams using HSG 1 (●) and opossum serum (X). Per cent of immunoreactive gastrin-^{125}I bound was plotted as a function of increasing amounts of unlabeled HSG 1. Maximum precipitability of labeled gastrin with excess gastrin antiserum was 44%. The precipitation curves of diluted opossum serum and HSG 1 were parallel, indicating similar immunoreactivity.

intermittently aspirated through the distal orifice during the course of each study period.

The hydrogen ion activity of the gastric contents was increased by the instillation of 5 mEq of acid (0.1 N HCl) and decreased by the instillation of 5 mEq of alkali (0.1 N NaOH) as a single, 10 min infusion. This infusion was adequate to keep an aspirated sample of gastric contents at pH 1.5 with acid instillation, or greater than pH 7.0 with alkali instillation. A Beckman glass electrode (Beckman Instruments, Inc.) was used to measure pH.

Lower esophageal sphincter pressures were recorded in millimeters Hg with the gastric fundal pressure used as a zero reference. The mid-respiratory pressure, as recorded from the zone of maximal pressure, was reported as the
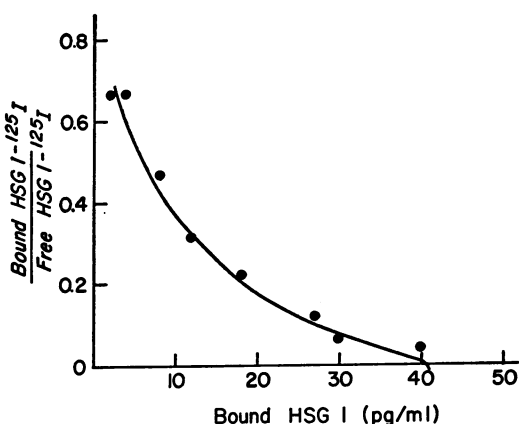

FIGURE 2 Gastrin binding curve. The ratio of bound/free HSG 1-^{125}I was expressed as a function of bound unlabeled HSG 1. The gastrin antiserum was used at a dilution of 1:100,000. Binding capacity of the original gastrin antiserum was 4.2 μg HSG 1/ml. The average antibody affinity constant, K$_A$, was 1.7 × 10^{-10} M^{-1}.
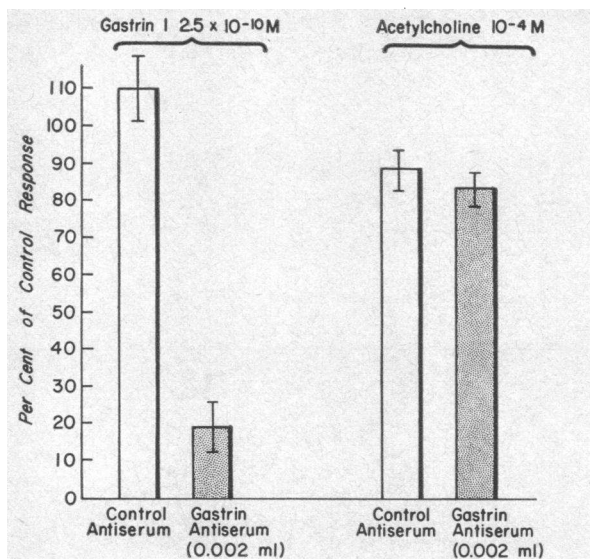
FIGURE 3 Per cent of the maximum gastrin I and acetyl-choline response in the presence of control serum and 0.002 ml of gastrin antiserum on the LES circular muscle in vitro. Gastrin antiserum reduced the peak gastrin I response to 19.0 ±7.3% of its initial value but did not significantly affect the response to acetylcholine. Control serum did not significantly affect the response to either gastrin or acetyl-choline. Each bar represents the mean ±SEM of 10 observations carried out on LES muscle from four animals.

average value obtained during a 1 min interval. LES pressure was continuously recorded and a complete pull-through of the middle recording orifice was obtained at each 5 min interval. During prolonged study periods, the esophageal and gastric catheters were not perfused.

All intravenous injections were given through an indwelling femoral venous cannula, and all blood samples were obtained from a femoral arterial cannula in the opposite extremity.

Gastrin antisera in varying dilutions were administered in a 1.0 ml volume through the venous cannula. Control serum was administered in the same volume to all animals that received injections of gastrin antiserum. Single injections of 1.0 ml and 0.1 ml of the antiserum were administered in a single study period. Multiple injections of the antiserum were given with quantities less than 0.1 ml during a single study period.

Gastrin I, amino acid sequence 2–17, was administered as a rapid intravenous injection, over a 30 sec period, in a dose of 1.0 μg/kg. This dose of gastrin I was previously shown to produce a maximal response on the LES, when full dose-response curves were constructed (12). In the experiments where gastrin I and gastrin antiserum were given in combination, equal volumes of gastrin I and antiserum were mixed for 15 min, at room temperature, before intravenous injection. The response to gastrin I, in the presence of either the control or the gastrin antiserum, was expressed as a per cent of the response to gastrin I alone, in the same animal.

Studies in vitro. After the location of the LES by manometry, four animals were killed by intravenous nembutal. Circular muscle strips from the manometrically defined LES were studied in vitro utilizing methods previously described in detail (12). Two to three strips of LES circular muscle were studied simultaneously in individual muscle chambers containing 20 ml of Krebs-Ringer solution, maintained at 37°–38°C and bubbled with 95% O_2 and 5% CO_2. The isometric tension developed by the circular muscle layer was determined using force transducers (Grass Ft .03C; Grass Instrument Co., Quincy, Mass.) whose outputs were graphed on a Beckman recorder (Beckman Instruments, Inc.). Each muscle was studied at its length of optimal tension development, L_o, as determined by length-tension diagrams.

Gastrin I was used at a molar concentration (2.5 × 10⁻¹⁰ M) previously shown to produce the peak response on LES circular muscle (12). 0.002 ml of antiserum was estimated to bind approximately 8.0 ng of the 10.0 ng of gastrin (2.5 × 10⁻¹⁰ M) in the 20 ml bath and was used in these studies. The gastrin and gastrin antiserum were incubated in a 1 ml volume for 15 min before their addition to the bath. Control antiserum and gastrin I were given in a similar manner. The response to gastrin I, alone or in combination with antisera, was recorded over a 15 min period. The peak tension obtained during this period was tabulated. The response to 10⁻⁴ M acetylcholine was similarly tested. The response to gastrin I and acetylcholine in the presence of control and gastrin antisera was expressed as a per cent of the response to either gastrin I or acetylcholine, alone, on the same muscle strip.

Statistical significance was determined using Student's *t* test.

RESULTS

Serial dilutions of opossum serum were tested in an assay using HSG 1 for reference standards. The radio-calibration diagram of opossum serum was parallel to the calibration diagram of HSG 1 (Fig. 1) and indicated that gastrin was present in the blood of the fasting opossum and was similar to HSG 1 in its immuno-reactivity with the antibody. A mean serum level of 174.0 ±14.5 pg/ml (mean ±SEM) was measured in the arterial blood of 10 fasting, anesthetized, and intubated animals. There was no significant difference in gastrin levels in peripheral arterial and venous blood. The pH of the gastric contents in these animals ranged from 2.5 to 4.6.

Studies were performed to estimate the binding capacity of the gastrin antiserum and to evaluate its cross-reactivity. As shown in Fig. 2, the rabbit antiserum to gastrin I had a maximum binding capacity of 4.2 μg/ml of antiserum. The gastrin antiserum was tested for cross-reactivity with pentagastrin, cholecysto-kinin-pancreozymin (CCK-PZ), and secretin in binding inhibition studies. The concentration of these compounds required to inhibit 70% of binding of HSG-¹²⁵I was divided into the concentration of HSG 1 that produced the same degree of inhibition. This gave the molar inhibitory potency of the compounds. The molar inhibitory potency of HSG 1 was 1.0; for pentagastrin it was 0.082; and for CCK-PZ it was 0.063. Secretin did not inhibit the binding of labeled gastrin. The cross-reactivity with pentagastrin and CCK-PZ suggested that the
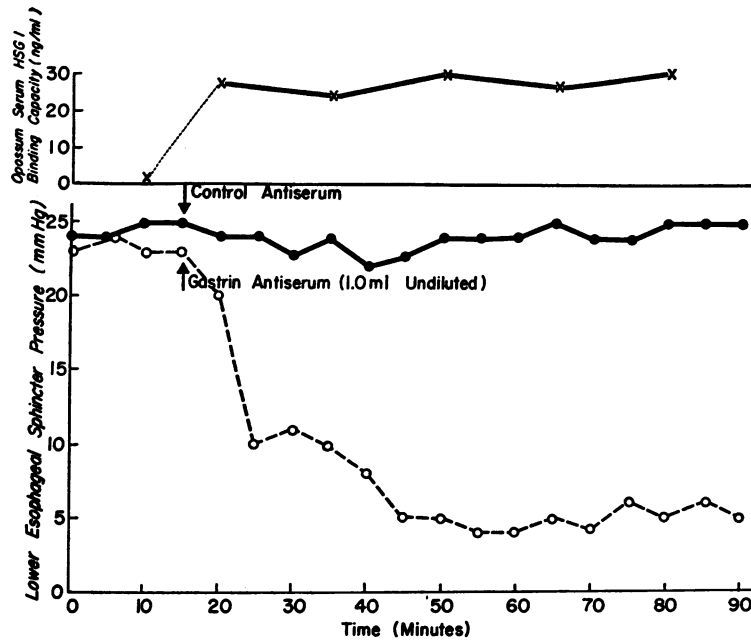
FIGURE 4 The effect of 1.0 ml of control serum and 1.0 ml of gastrin antiserum on the resting lower esophageal sphincter pressure of a single animal. Binding capacity of opossum serum for HSG 1 after the intravenous injection of gastrin antiserum, shown at the top, remained unchanged while LES pressure was reduced.

antibody principally bound the carboxy terminal end of the gastrin molecule which shared its terminal amino acid sequence with pentagastrin and CCK-PZ.

Initial physiological studies were performed to determine the specificity of the effect of gastrin antiserum on the LES circular muscle, in vitro. In Fig. 3 is shown the isometric tension developed by the circular muscle of the LES at its length of optimal tension development, Lo, to gastrin I and to acetylcholine. The peak response of the LES circular muscle to gastrin I occurred at a dose of 2.5×10^{-10} M, and represented 10.0 ± 0.8 g of active tension. This response was unaltered by control serum $(P > 0.05)$, but was markedly reduced by 0.002 ml of gastrin antiserum $(P < 0.001)$. The peak response to acetylcholine occurred at a dose of 10^{-4} moles/liter and represented 23.0 ± 1.2 g of active tension. A slight reduction that was not statistically significant occurred in the response to acetylcholine in the presence of both control and gastrin antisera $(P > 0.05)$. After the demonstration of a specific inactivation of gastrin by the gastrin antiserum, studies were performed in vivo.

In all animals, intraluminal manometry demonstrated the presence of a 1.5 ± 0.2 cm zone of elevated pressure at the junction of esophagus and stomach. The mean mid-respiratory pressure within this zone was 35.0 ± 4.2

mm Hg. A consistent decrease in pressure was recorded from this high pressure zone when deglutition was initiated. This zone of elevated pressure represented the lower esophageal sphincter.

The effect of 1.0 ml of gastrin antiserum and control antiserum on the resting LES pressure of a single animal is shown in Fig. 4. After the intravenous injection of 1.0 ml of gastrin antiserum, LES pressure dropped precipitously within 10 min, reached a nadir of pressure at 30 min, and remained at this nadir for the full 75 min of recording. The initial pressure of 23.0 mm Hg was reduced to a nadir of pressure of 4.0 mm Hg and represented an 83.0% reduction. Control antiserum had no effect upon resting LES pressure. The persistence of antibody activity was determined in serial samples of arterial blood obtained at periodic intervals after the intravenous injection of 1.0 ml of gastrin antiserum. The capacity of these serial blood samples to bind gastrin was measured and is shown at the top of Fig. 4. The gastrin-binding capacity of the opossum blood was unchanged while LES pressure remained at its nadir.

In Fig. 5 are shown the results of increasing amounts of gastrin antiserum upon resting LES pressure. Each point represents the mean ±SEM for five separate determinations. The control antiserum and 0.0001 ml of the

gastrin antiserum had no significant effect upon LES pressure ($P > 0.05$). Increasing amounts of antisera, 0.001, 0.01, 0.1, and 1.0 ml produced a graded inhibition in resting LES pressure. The maximum inhibition was obtained with 2.0 ml of undiluted antiserum and represented a 80.0 ±3.1% reduction in resting LES pressure. This inhibition in resting LES pressure was similar to a 74.6 ±4.1% reduction obtained in eight animals upon suppression of endogenous gastrin release by exogenous gastric acidification with 0.1 N HCl.

The amount of opossum blood gastrin bound by gastrin antibody in vivo could not be assayed directly. In lieu of a method for direct measurement an estimation of the amount of gastrin bound by antibody was made using the immunoassay-determined values for antibody affinity and capacity. To determine the concentration of the administered antibody in the intravascular space of the opossum, we estimated the blood volume to be 5% of total body weight in kilograms. The mean fasting serum gastrin level before antisera injection was determined by radioimmunoassay to be 174.0 ±14.5 pg/ml. The concentration of endogenous gastrin bound by the gastrin antisera was obtained from the following equation: $K_A = [x]/((2 [Ab] - [x]) ([gastrin] - [x]))$. K_A was the average affinity of antibody, 1.7×10^{10} M^{-1}. [Ab] was the estimated concentration of the antibody in the blood volume of the opossum. [Gastrin] was the mean fasting serum gastrin level measured in the animals used in these studies, and [x] was the calculated amount of circulating gastrin bound by the antibody. By this calculation approximately 10% of blood gastrin was bound by 0.001 ml of antiserum. The estimated percentage of gastrin bound by graded quantities of antisera (Fig. 6) appeared to be related to the per cent inhibition of resting LES pressure (Fig. 5).

The effect of control and gastrin antiserum on the LES response to endogenous gastrin release is shown
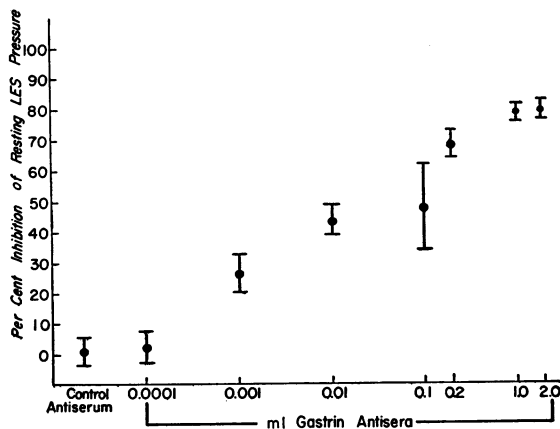
FIGURE 5 Per cent inhibition of resting LES pressure as a function of increasing amounts of gastrin antisera.
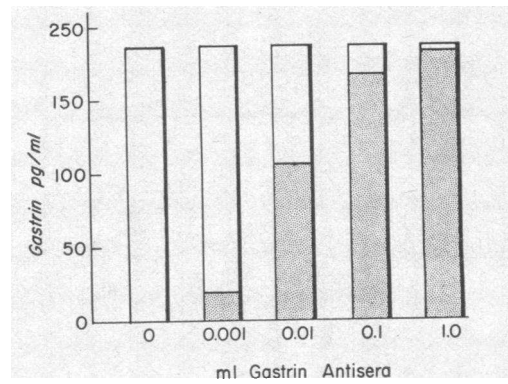
FIGURE 6 Estimation of the amount of circulating opossum gastrin, bound (stippled bars) and free (open bars), after the intravenous administration of increasing amounts of gastrin antisera. All calculations were based on a mean fasting gastrin level of 174 pg/ml and a mean blood volume of 125 ml in 10 animals. The lowest amount of gastrin antiserum 0.001 ml, that decreased resting LES pressure, was estimated to bind 18 pg/ml of circulating opossum gastrin. 1 ml of antiserum was estimated to bind almost all circulating gastrin.

in Fig. 7. Acidification of the stomach with 0.1 N HCl resulted in a prompt decrease in LES pressure. The decrease in pressure was obtained within the 5 min after acid instillation. The pressure in this animal decreased from a resting level of 29.0 mm Hg to 8.0 mm Hg and represented a 73.0% reduction in resting LES pressure. When the acid was removed and 0.1 N NaOH was instilled into the stomach, a prompt elevation in LES pressure was evident within 5 min and reached a peak pressure of 45.0 mm Hg at 15 min. The administration of 1.0 ml of control antiserum did not effect this rise in LES pressure. However, the intravenous administration of 1.0 ml of gastrin antiserum, 10 min before gastric deacidification, prevented the normal rise in LES pressure. The increase in pressure after gastric deacidification in five animals was 38.7 ±2.5 mm Hg. Control serum did not affect this increase in LES pressure, 37.5 ±2.0 mm Hg. The administration of 1.0 ml of gastrin antiserum reduced the increase in LES pressure to 3.0 ±0.4 mm Hg. This represented a significant inhibition ($P < 0.001$) in the response of the LES to endogenous release of gastrin produced by deacidification.

The effect of gastrin antiserum on the LES response to exogenous gastrin I is shown in Fig. 8. The peak response of the LES to exogenous gastrin I occurred at a dose of 1.0 µg/kg and represented a 83.2 ±10.1 mm Hg increase in LES pressure above the preinjection level. Control serum and gastrin antiserum less than 0.01 ml had no effect upon the LES response to this dose of gastrin I. 0.2 ml of the gastrin antiserum re-

duced the peak response to gastrin I to 34.1 ±6.2% of its control value ($P < 0.001$). 1 ml of gastrin antiserum reduced the peak response to gastrin I to 9.3 ±8.0% of its control value ($P < 0.001$). The gastrin I response in the presence of 0.2 ml of gastrin antiserum was not inhibited when an additional 1.0 μg of gastrin was also given.

DISCUSSION

These studies indicated that gastrin antiserum: (a) specifically antagonized the response of LES circular muscle to gastrin, in vitro; (b) diminished the LES response to the endogenous release and to the exogenous administration of gastrin; and (c) markedly reduced the resting level of LES pressure. We propose that endogenous gastrin is the major determinant of resting LES competence.

The validity of these studies in determining the genesis of resting LES strength is based on the ability of the gastrin antiserum to bind endogenous opossum gastrin and, in so doing, render it biologically inactive. We initially determined that opossum gastrin was immunoreactively similar to human gastrin I, as shown by the parallelism of their precipitation curves in the radioimmunoassay.

The initial physiological studies were carried out in vitro to demonstrate the effect of gastrin antiserum on the LES circular muscle response to gastrin I and acetylcholine. Gastrin antiserum, after its short incubation with gastrin I, diminished the contractile response of the circular muscle to this hormone. The gastrin antiserum did not alter the muscle response to another agonist, acetylcholine. These in vitro studies indicated that gastrin antiserum could render gastrin biologically inactive without altering the capacity of the circular muscle to contract to other stimuli.

After the characterization of the immunoreactivity of opossum gastrin and the demonstration that gastrin antiserum could specifically inactivate gastrin, studies were carried out in vivo. Graded amounts of gastrin antiserum administered to animals of similar weight showed a reduction in resting LES pressure with as little as 0.001 ml of gastrin antiserum. This amount of gastrin antiserum was estimated to bind a significant portion of circulating opossum gastrin as shown in Fig. 6. With administration of increasing quantities of the gastrin antiserum, greater decreases in LES pressure were obtained, until a maximal inhibition of 80.0 ±3.1% was noted with 2 ml of gastrin antiserum. Therefore, a graded reduction in resting LES pressure
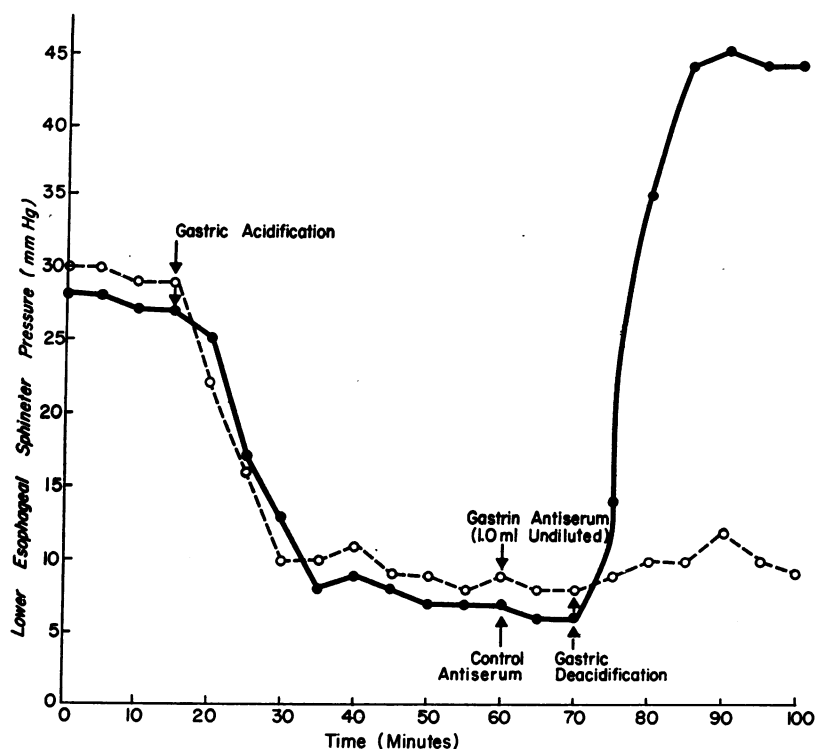

Figure 7 Response of the LES to gastric acidification and to gastric deacidification in a single animal. The response to deacidification was tested after the administration of control antiserum and gastrin antiserum.
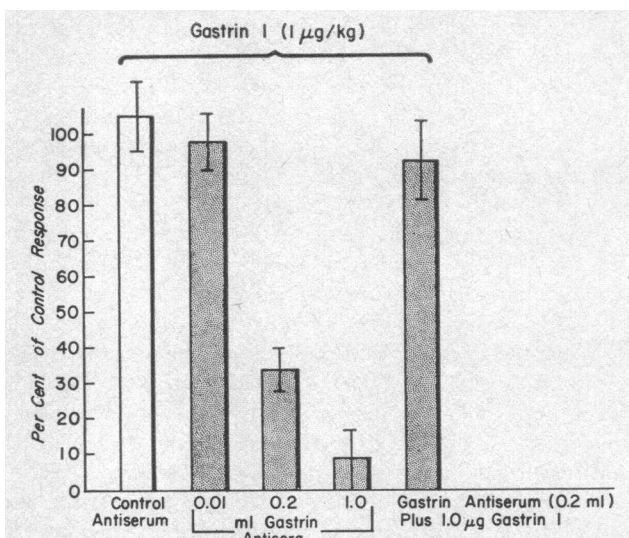
Gastrin I (1 μg/kg)

Per Cent of Control Response

100
90
80
70
60
50
40
30
20
10
0

Control Antiserum | 0.01 | 0.2 | 1.0 | Gastrin Antiserum (0.2 ml) Plus 1.0 μg Gastrin I
ml Gastrin Antisera

FIGURE 8 Effect of gastrin antisera on the peak LES response to exogenous gastrin I (1.0 μg/kg). Per cent of the control response to gastrin I was plotted as a function of increasing amounts of gastrin antisera. Gastrin antisera (0.2 ml and 1.0 ml) produced a graded decrease in the LES response to gastrin I. An additional 1.0 μg of gastrin I maintained the peak response at its control level in the presence of 0.2 ml of gastrin antiserum. Each bar represents the mean ±SEM of five observations made in separate animals.

was produced by binding increasing amounts of circulating opossum gastrin. The decrease in LES pressure (Fig. 5) appeared to be related to the estimated bound gastrin (Fig. 6). However, a precise correlation of this relationship was not possible as the estimation of the amount of gastrin bound by the antibody was based on calculations of antibody affinity and capacity measured in the assay system which was incubated at 4°C for 4 days. The higher temperatures and shorter periods of incubation in the in vitro and in vivo studies most likely resulted in less gastrin being bound than we estimated. The cross-reactivity of the antiserum with CCK-PZ raised the possibility that binding of CCK-PZ could be significant in the presence of the larger amounts of antibody and might affect LES strength. However, as exogenous CCK-PZ appeared to have an inhibitory effect on sphincter pressure,[2] this possibility could not account creasing amounts of antibody for the progressive fall in sphincter pressure with in-

To confirm the observed effect of gastrin antiserum upon resting LES pressure, studies were performed during periods of known endogenous and exogenous gastrin stimulation of the sphincter. The response of the LES to the endogenous release of gastrin, by gastric deacidification, was almost completely abolished by the

gastrin antiserum and unaffected by control antiserum. We concluded that the failure of the LES to respond to the endogenous release of gastrin was due to the gastrin antiserum binding endogenously released gastrin and preventing its physiological action on the LES. The possibility that gastrin antiserum inhibited release of gastrin seemed unlikely but could not be ruled out.

The effect of gastrin antiserum upon the response of the LES to exogenous gastrin I allowed quantification of the antiserum inhibition of a known magnitude of gastrin stimulation. Gastrin antiserum, but not control antiserum, produced a decrease in the LES response to the peak dose of gastrin I (1 μg/kg). The decrease in LES response to gastrin I was related to the quantity of gastrin antiserum administered. When an additional 1.0 μg of gastrin was given to compensate for the gastrin bound by the 0.2 ml of gastrin antiserum, the response to gastrin I was restored to its control value.

Gastrin antiserum had been previously used to inhibit the acid secretory response to both endogenously released (19) and exogenously administered gastrin (20). Likewise, active immunization of rats to gastrin tetrapeptide produced almost total resistance of the acid secretory response to exogenous gastrin (21). The antibodies used in these studies, as in the present study, reacted with the carboxy terminal end of the gastrin molecule, the physiologically active site of the hormone. In all studies utilizing both passive and active immunization, the basal gastric acid secretion was unaltered in the rat (19–21). By contrast, gastrin antiserum markedly inhibited resting LES pressure in the fasting opossum. These data, although obtained in different species, suggested that gastrin played a major role in the genesis of resting LES pressure, and only a minor role in basal gastric acid secretion.

Competence at the gastroesophageal junction in man is dependent upon the intrinsic strength of the physiological lower esophageal sphincter (1–5). Previous studies have suggested the importance of gastrin in determining resting sphincter competence. Acidification of the gastric antrum consistently decreased resting LES pressure by 68% of its initial level in normal subjects (8, 9). In patients with achalasia, it has been shown that the elevated resting LES pressure was dependent upon the increased sensitivity of the LES to gastrin (9). Likewise, it has been suggested that the diminished LES pressure seen in patients with gastroesophageal reflux was due to decreased gastrin effect (22). In vitro observations have demonstrated that LES circular muscle has a greater sensitivity to gastrin than the adjacent circular muscles of the esophagus and antrum (12). Although each of these findings supported the importance of gastrin in the maintenance of resting LES strength, they did not provide conclusive

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[2] Cohen, S., and W. Lipshutz. Unpublished observation.

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evidence. The data in this study provided this proof. By utilizing a highly specific technique of gastrin inactivation in the anesthetized opossum, we have shown that gastrin was the major determinant of resting LES pressure.

ACKNOWLEDGMENTS

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