Hormonal Regulation of Human Lower Esophageal Sphincter Competence: Interaction of Gastrin and Secretin

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ABSTRACT The interaction of gastrin and secretin, in the regulation of human lower esophageal sphincter competence, was studied in 54 normal subjects. A dose-response curve, for the lower esophageal sphincter, was constructed from the rapid intravenous injections of synthetic gastrin I (amino acid sequence 2–17). This curve was sigmoid shaped and showed a peak response that was 460.0 ±24.0% (mean ±2 SE) of the initial sphincter pressure, at a dose of 0.7 µg/kg of gastrin I. Secretin, either endogenously released by duodenal acidification, or exogenously administered as a single intravenous injection, markedly reduced the peak response of the sphincter to gastrin I. To ascertain the character of this inhibition, a gastrin I dose-response curve was obtained during a continuous intravenous secretin infusion. This curve showed a parallel shift to the right, with the maximal sphincter response to gastrin I still attainable at higher doses. A sphincter, endogenously stimulated by gastrin, showed a dose-related reduction in pressure with rapid intravenous injections of secretin. At the level of resting sphincter pressure, response to secretin diminished, and larger doses were required for comparable reduction in pressure. These studies indicate: (*a*) Secretin interacts with gastrin in the physiological regulation of human lower esophageal sphincter competence; (*b*) Secretin is a sensitive inhibitor to gastrin stimulation of the lower esophageal sphincter; (*c*) This inhibitory effect of secretin is competitive in character.

INTRODUCTION

Although competence at the human gastroesophageal junction is dependent on the strength of closure of the physiological lower esophageal sphincter (1–4), the factors which regulate this strength are poorly understood. Recently, it has been shown that the gastrointestinal hormone, gastrin, has a physiological role in the control of sphincter strength (5, 6). Since a well-known relationship exists between gastrin and secretin in the hormonal control of gastric acid secretion (7–12) and gastric motor function (13–14), it seemed possible that these hormones also interact to regulate lower esophageal sphincter competence. This study is designed to quantitatively assess the interaction of endogenous and exogenous gastrin and secretin in the regulation of lower esophageal sphincter competence in man.

METHODS

Intraluminal pressure was transmitted by three water-filled polyvinyl tubes, 1.4 mm internal diameter, to external transducers (Statham P23BB, Statham Instruments, Inc., Los Angeles, Calif.). These pressures were graphed on a multichannel Beckman curvilinear ink-writing recorder (Beckman Instruments, Inc., Fullerton, Calif.). Recording tubes were arranged to measure intraluminal pressures at three points 5 cm apart through side orifaces 1.2 mm in diameter. Gastric aspiration and infusion were done through a fourth tube having multiple perforations over an 8 cm distal segment. The four tubes were combined into a single assembly with an outside diam of 5 mm. The midpoint of the multiperforated area was 16 cm distal to the recording oriface at which lower esophageal sphincter pressure was recorded. A separate mercury-weighted, single-lumen, polyvinyl tube, of 1.4 mm internal diameter, was placed in the second portion of duodenum, for those studies requiring duodenal acidification. The position of this tube was verified by fluoroscopy.

Manometric studies were done in 54 normal patients resting quietly in the supine position. Informed consent was obtained in all patients. Belt pneumographs around the chest and over the larynx were used to monitor respirations and swallowing, respectively. The recording assembly was positioned and anchored so that pressures were simultaneously recorded from the esophagus, lower esophageal sphincter, and the stomach. The pressure-recording tubes were infused with distilled water by a syringe pump at a constant rate of 1.2 ml/min.
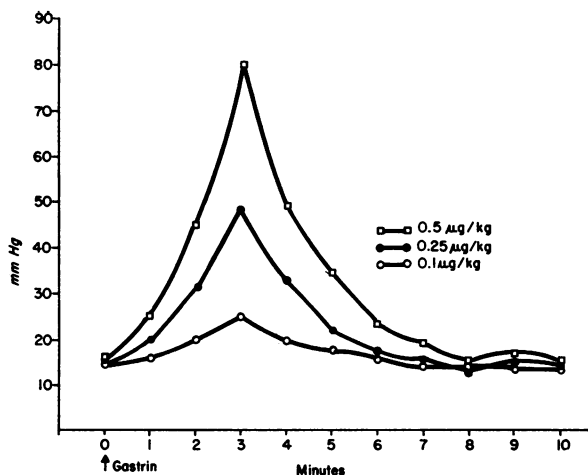
FIGURE 1 Comparison of the lower esophageal sphincter response to three 30-sec intravenous injections of synthetic gastrin I, amino acid sequence 2–17, in a single patient studied on 3 separate days. Each point represents the mean pressure for a 1 min period.

Gastrin I,[1] amino acid sequence 2–17 (Hexadecapeptide Amide),[2] and purified porcine secretin[3] were given intravenously, either as a continuous infusion or as a single 30 sec injection through an indwelling anticubital catheter. Dose-response curves were constructed with single injections during a single study period. Multiple gastrin I injections were occasionally given on a single day, but successive injections were separated by a 60 min interval.

Hydrogen ion activity of the gastric contents was altered by instillation of either acid (0.1 N hydrochloric acid) or alkali (0.1 N sodium hydroxide or 0.1 N sodium bicarbonate), by constant infusion at a rate of 12 mEq per 15 min. This rate was adequate to keep an aspirated sample of gastric contents at pH 1.5 with acid instillations, or greater than pH 7.0 with alkali instillations.

Hydrogen ion activity of the duodenal contents was altered by instillation of acid (0.1 N hydrochloric acid) directly into the duodenum, by constant infusion, at a rate of 18 mEq per 15 min. This rate was adequate to keep a duodenal aspirate below pH 3.0. A Beckman glass electrode was used to measure pH.

Phenol red, at a concentration of 50 mg/liter, was placed in the duodenal infusate to measure reflux of duodenal contents into the stomach. The vol of each 15 min sample of gastric juice was measured, and the phenol red in alkaline solution was determined by spectrophotometer at 560 mu. The phenol red concentration in gastric contents, expressed as a per cent of the concentration of phenol red in the duodenal infusate, was used to quantitate duodenal reflux into the stomach.

Lower esophageal sphincter pressures were recorded as millimeters of mercury, with the mean gastric fundic pressure used as the zero reference. The values of pressure reported, are the mean values recorded over 1 min interval.

[1] FDA approval, IND 6363.

[2] Imperial Chemical Industries Ltd., Alderley Park, Cheshire, England.

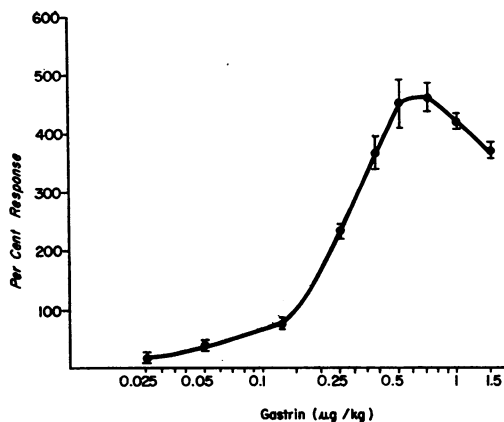[3] GIH Research Unit, Karolinska Institute, Stockholm, Sweden.

FIGURE 2 Dose-response curve of change in lower esophageal sphincter pressure expressed as a per cent of initial resting sphincter pressure against log-dose of gastrin I in μg/kg. At each point is the mean and two standard errors of the mean for 13 normal patients.

RESULTS

In Fig. 1 are shown pressures recorded from a single normal patient studied on 3 separate days during intravenous, 30-sec injections of synthetic gastrin I. Each dose of gastrin I caused sphincter pressure to rise promptly. The response peaked at 3 min and returned to normal within 10 min. The magnitude of the peak response at 3 min is directly proportional to the amount of gastrin I. So that a comparison could be made between this peak response in different patients, it was further expressed as a per cent response of the preinjection sphincter pressure. For example, in this patient, at a preinjection sphincter pressure of 15 mm Hg, 0.5 μg/kg of gastrin I, increased sphincter pressure
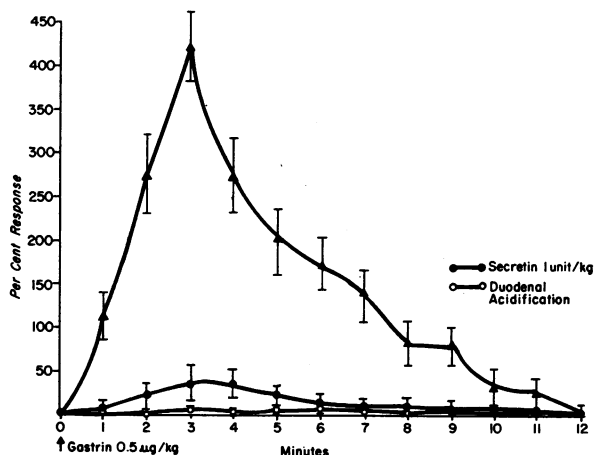

FIGURE 3 Lower esophageal sphincter response in seven patients given 0.5 μg/kg of gastrin I alone as compared to an identical dose of gastrin I given during duodenal acidification at 18 mEq/15 min and directly after intravenous secretin (1 U/kg).
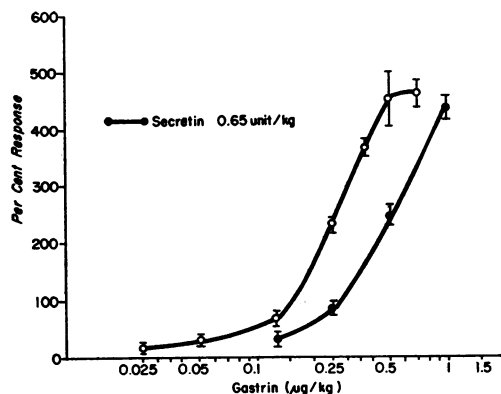
FIGURE 4 Dose-response curves of lower esophageal sphincter pressure expressed as a per cent of initial resting sphincter pressure against log-dose of gastrin I in μg/kg. The curves are those of gastrin I given alone to 13 patients and gastrin I given against a constant intravenous infusion of secretin at 0.65 U/kg per hr to seven patients. Although the dose-response curve performed during secretin infusion is shifted to higher doses of gastrin I, maximal sphincter response is still attained.

FIGURE 6 Comparison of lower esophageal sphincter pressure in a single patient given three different 30 sec intravenous injections of secretin during endogenous gastrin release with gastric deacidification with 0.1 N NaOH at 12 mEq/15 min. Each point represents the mean pressure for a 1 min period.

to a peak value of 80 mm Hg. The change in pressure of 65 mm Hg is 433.3% of the initial pressure of 15 mm Hg. The peak sphincter response at each gastrin I dose was used to calculate a sphincter dose-response curve in 13 patients as shown in Fig. 2. The log dose-response curve is sigmoid shaped with a threshold dose of 0.025
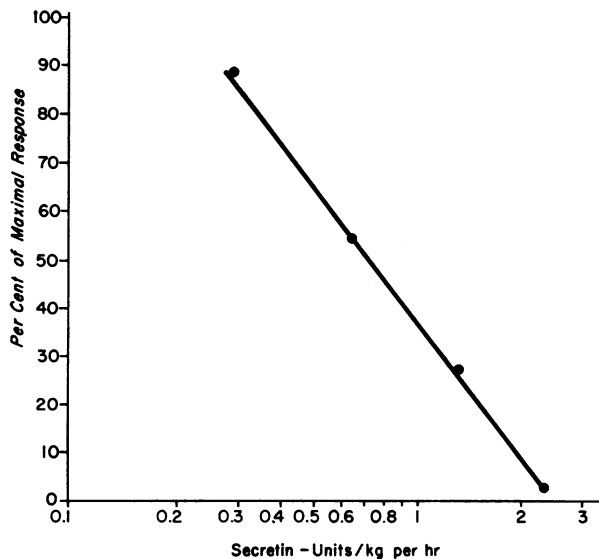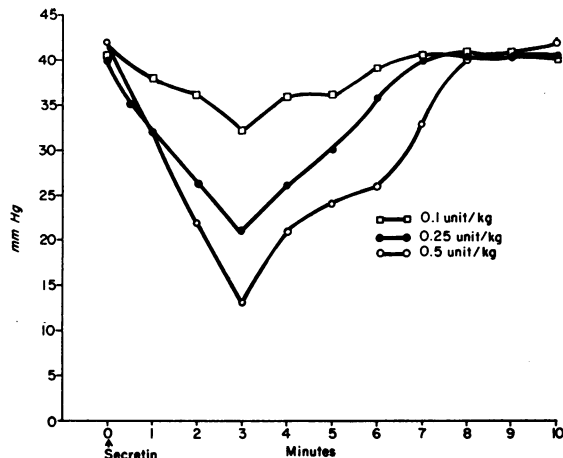

FIGURE 5 Lower esophageal sphincter response to 0.5 μg/kg of gastrin I in a single patient during a continuous infusion of secretin at four different rates on 4 different days. Sphincter response during each rate of secretin infusion is expressed as a per cent of the maximal response to 0.5 μg/kg of gastrin I given alone in this patient. Secretin at 2.3 U/kg per hr almost totally abolished the maximal sphincter response to gastrin I.

μg/kg and a maximum response of 460.0% ±24.0% (mean ±2 SE) of resting sphincter, at a dose of 0.7 μg/kg. At higher doses, the sphincter response declines below the maximum response. These injections of intravenous gastrin I were not accompanied by any systemic side effects.

To see if this increase in pressure, following the intravenous injection of gastrin I, is influenced by endogenous and exogenous secretin, the following studies were performed. Gastrin I, at a dose (0.5 μg/kg) which gave a near-maximal response, was given to the same seven patients (1) alone, (2) during endogenous secretin released by duodenal acidification, and (3) directly following a 30 sec intravenous injection of secretin, at 1 U/kg. As shown in Fig. 3, duodenal acidification and exogenous secretin reduced the sphincter response for 0.5 μg/kg of gastrin I to 8.0% ±4.0% and 37.0% ±22.0%, respectively. Reflux of duodenal contents into the stomach during duodenal acidification was minimal. Phenol red concentration, in the gastric aspirate, was only 1.8% ±0.6% (mean ±2 SE) of the concentration in the duodenal infusate. This indicates that less than 0.5 cc of duodenal infusate refluxed into the stomach per 15 min infusion period.

In Fig. 4, the response of the lower esophageal sphincter to 30 sec injections of gastrin I, in 13 patients, is compared to the response to gastrin I, in 7 patients, during the constant infusion of secretin at 0.65 U/kg per hr. During the infusion of secretin, the gastrin I dose-response curve, although shifted to the right, remained parallel to the original curve. The dose of gastrin I required to obtain a threshold re-
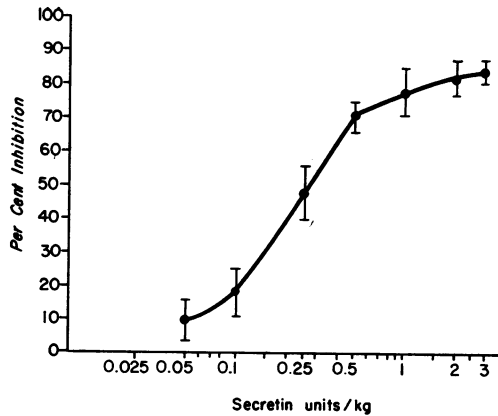
FIGURE 7 Log dose-response curve of the per cent inhibition of lower esophageal sphincter pressure (stimulated by endogenous gastrin release with 0.1 N NaOH at 12 mEq/15 min) against dose of 30 sec intravenous injections of secretin. At each point is the mean and two standard errors of the mean for seven patients.

sponse is greater during the infusion of secretin. The peak response to gastrin I is still attainable, but higher doses of gastrin I are now required.

Fig. 5 illustrates the sphincter response to four 0.5 μg/kg, 30 sec injections of gastrin I. A single patient was studied on separate days during four different rates of constant secretin infusion ranging from 0.30 to 2.3 U/kg per hr. Each increase in the rate of secretin infusion caused a progressively greater inhibition of the gastrin I response. These rates of secretin infusion gave no change in the preinfusion level of sphincter pressure. Duodenal acidification with 0.1 N HCl at a rate of 18 mEq per 15 min, in this patient, caused an inhibition of the sphincter response to 0.5 μg/kg of gastrin I, which resembled the response of the sphincter to an identical gastrin I dose during secretin infusion at 2.3 U/kg per hr.

Fig. 6 shows the effect of three different 30-sec intravenous injections of secretin on lower esophageal sphincter pressure, in one patient, studied on 3 separate days, during the endogenous release of gastrin. An initial sphincter pressure of 17 mm Hg was brought to and maintained at 42 mm Hg by alkalinizing (0.1 N NaOH) a previously acidified stomach (0.1 N HCl). Each injection of secretin caused a prompt drop in pressure which reached its nadir in 3 min and returned to the preinjection level within 10 min. The degree of inhibition was directly proportional to the dose of secretin given and was further expressed as a per cent of the sphincter pressure prior to injection.

In Fig. 7, a dose-response curve was constructed for seven patients, plotting the per cent inhibition of the gastrin-stimulated increased sphincter pressure against the secretin dose, in U/kg. The curve is sigmoid shaped

and demonstrates a threshold and peak-response. At a dose of 0.5 U/kg and a 70.7% ±4.0% reduction in sphincter pressure, the curve breaks sharply. From this point, large doses of secretin are needed to give relatively small changes in sphincter pressure.

In Fig. 8, secretin dose-response curves are recorded in the same seven patients beginning at (a) resting unstimulated sphincter pressure, and (b) sphincter pressure stimulated by endogenous gastrin release. The secretin dose-response curve constructed during gastrin stimulation, shows a precipitous drop in pressure at doses of secretin up to 0.5 U/kg. Thereafter, larger doses of secretin are needed to obtain relatively small changes in pressure. This inflection point in the dose-response curve, occurs at the level of resting unstimulated sphincter pressure and is identical with the point noted in Fig. 7. A secretin dose-response curve, beginning at the level of resting unstimulated sphincter pressure, shows no significant change with doses of secretin up to 1 U/kg. At higher doses of secretin, pressure falls along a curve similar to that obtained against the gastrin stimulated sphincter.

To further study the effect of endogenous secretin release on sphincter pressure, duodenal acidification with 0.1 N HCl at 18 mEq/15 min was performed in seven patients beginning at (a) resting unstimulated sphincter pressure, and (b) sphincter pressure stimu-
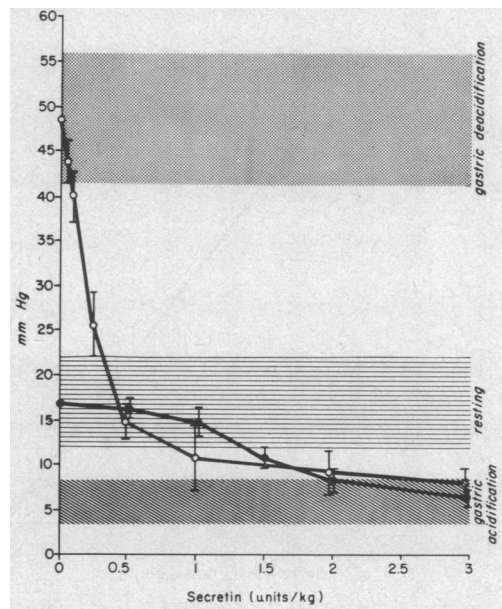

FIGURE 8 Dose-response curves for secretin (30 sec intravenous injections) plotted in seven patients beginning at resting unstimulated sphincter pressure and at the level of pressure attained by endogenous gastrin stimulation of the sphincter. Mean and a two standard error range is indicated for sphincter pressures during gastric deacidification, resting and gastric acidification.

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lated by endogenous gastrin release. Duodenal acidification performed on a gastrin stimulated sphincter reduced pressure by 66% ±6.1% to a level of 16.6 ±2.9 mm Hg. No statistical difference $(P > 0.05)$ was found when comparing this pressure to the unstimulated resting sphincter pressure of 16.9 ±4.8 mm Hg, recorded prior to endogenous gastrin release. Duodenal acidification decreased unstimulated resting sphincter pressure by 8.0% ±2.0% (1.4 ±0.4 mm Hg), to a level of pressure that again shows no statistical difference from the initial pressure $(P > 0.05)$.

DISCUSSION

The purpose of this study was to see if secretin interacts with gastrin in the hormonal regulation of human lower esophageal sphincter competence. The results clearly show that both the endogenous release and the exogenous intravenous administration of secretin markedly inhibit the action of gastrin on the lower esophageal sphincter, but have considerably less effect in lowering the normal level of resting sphincter pressure. Although the action of endogenous and exogenous gastrin on the human lower esophageal sphincter has been qualitatively demonstrated previously (5, 6), the ability to show an interaction of gastrin with secretin required quantitative data for both hormonal effects. Since studies were performed in humans, the difficulty in isolating acid from the duodenum during gastrin administration prompted use of the rapid intravenous injection of gastrin I. The sphincteric action of gastrin I is rapid in onset, easily quantitated, and terminated before acid secretion, reaching the duodenum, could be a significant factor (15). The use of the peak sphincter response to gastrin I is supported by pharmacological studies (16), which show that a single, rapidly administered, intravenous injection of a drug, gives a peak response directly proportional to the dose. Expression of this peak response, as a per cent of the sphincter pressure at which the response is elicited, is based on a similar relation of sphincter response to change in intra-abdominal pressure (17). In both responses, the magnitude change in sphincter pressure is directly related to the initial sphincter pressure. Therefore, comparison of an absolute change in pressure, for multiple patients at different initial pressures, is eliminated. The response is normalized for the group by its expression as a per cent of its initial value. Now, a dose-response curve for gastrin I can be constructed and used to quantitate sphincter response in all patients. The gastrin I log dose-response curve (Fig. 2), is a characteristic sigmoid shaped curve with a threshold dose, a phase of linear response and a maximal response of 460% of resting sphincter pressure at 0.7 µg/kg. Beyond the dose which gave a maximal response,

successively greater doses gave a diminishing response. This phenomenon, known as autoinhibition, has also been found with gastrin acting on other parameters of gastrointestinal function (18, 19). Autoinhibition is thought to be due to saturation of all high affinity excitatory receptors with additional gastrin molecules combining with a low affinity inhibitory receptor (20). Since autoinhibition occurs with high doses of intravenous gastrin I, quantitative interactions of gastrin I with secretin were restricted to the portion of the dose-response curve prior to significant autoinhibition.

The ability of both endogenous and exogenous secretin, to markedly reduce a maximal sphincter response to gastrin I, is shown in Fig. 3. The mechanism and quantitative dynamics of this inhibition, require interpretation of the dose response curves for gastrin I alone, as compared to gastrin I during a constant infusion of secretin. Secretin caused a parallel shift of the gastrin I log dose-response to the right to higher doses. The inhibitory action of secretin was surmountable. Increasing doses of gastrin I ultimately gave a maximal response. These characteristics of antagonism are competitive in nature (12, 20, 21). However, it is fully realized that although a competitive type of antagonism may be dynamically shown, it does not necessarily mean that both hormones are competing for a common receptor site. In fact, it would seem highly unlikely that the dissimilar molecules of gastrin and secretin would act at the same receptor. A chemical interaction, in which secretin inactivated gastrin prior to reaching a hypothetical sphincter receptor, may give identical changes in the log dose-response curve (20). Limited support for this type of interaction is available. Hansky and Cain have shown that secretin lowers the endogenous level of serum gastrin (22). A competitive type of inhibition, with a parallel shift in the log-dose response curve, is also seen in functional antagonism. This is a noncompetitive antagonism in which occupation of one receptor acts to decrease the affinity of the agonist to a second receptor (20). Although studies on acid secretion in dogs show that secretin acts as a noncompetitive inhibitor of gastrin (12), this does not necessarily indicate basic differences in the receptor mechanisms for acid secretion and sphincter function.

The conclusion that this quantitative interaction of secretin and gastrin is physiological is based on two criteria set forth for defining other gastrointestinal hormonal actions as being physiological (12, 23). First, the effect has to be demonstrated for a dose that is submaximal for the primary hormonal response. Second, the hormonal effect, in kind and magnitude, has to be reproduced by endogenous release of hormone. This study satisfies both criteria for the effect of secretin on the human lower esophageal sphincter. The action of

gastrin on the sphincter has previously been shown to satisfy both criteria (5). The demonstration of a physiological action of secretin and gastrin on the sphincter suggests a role for these hormones in the intrinsic regulation of sphincter competence. The increase in sphincter competence with increase in gastrin release, during feeding and increased acid production, seems teleologically correct. The sphincter has been provided with intrinsic adaptive mechanisms for the periods at which an increase in sphincter competence is logically needed. The sphincter not only increases its strength with endogenous gastrin release, but also in response to increase in intra-abdominal pressure. The secretin response, on the other hand, acts to diminish sphincter competence. However, this occurs at a period in the digestive process when secretin is physiologically acting to reduce gastric motility and acid secretion. As its action on the latter two functions is to return them to their basal level (9–14), the action of secretin on sphincter competence seems well adapted to returning this function back to resting competent levels. It is only with pharmacological doses of secretin that sphincter pressure can be brought below normal resting levels.

Since exogenous secretin can produce sphincteric incompetence, the role of secretin in clinical gastroesophageal reflux should be assessed. However, endogenous secretin release, upon both a resting and a gastrin-stimulated sphincter, had a limited ability in lowering pressure to a clinically incompetent level. Although it has been shown, for the first time in man, that an endogenously released gastrointestinal hormone lowers sphincter pressure, it remains for further studies to assign this hormone a role in the pathogenesis of clinical gastroesophageal reflux.

ACKNOWLEDGMENTS

REFERENCES

1. Cohen, S., and L. D. Harris. 1970. Lower esophageal sphincter pressure as an index of lower esophageal sphincter strength. *Gastroenterology.* **58**: 157.
2. Ismail-Beigi, F., P. F. Horton, and C. E. Pope II. 1970. Histological consequences of gastroesophageal reflux in man. *Gastroenterology.* **58**: 163.
3. Pope, C. E., II. 1967. A dynamic test of sphincter strength: its application to the lower esophageal sphincter. *Gastroenterology.* **52**: 779.
4. Winans, C. S., and L. D. Harris. 1967. Quantitation of lower esophageal sphincter competence. *Gastroenterology.* **52**: 773.
5. Castell, D. O., and D. Harris. 1970. Hormonal control of gastroesophageal-sphincter strength. *N. Engl. J. Med.* **282**: 886.
6. Giles, G. R., M. C. Mason, C. Humphries, and C. G. Clark. 1969. Action of gastrin on the lower oesophaceal sphincter in man. *Gut.* **10**: 730.
7. Nakajima, S., and D. F. Magee. 1970. Influences of duodenal acidification on acid and pepsin secretion of the stomach in dogs. *Amer. J. Physiol.* **218**: 545.
8. Tumpson, D. B., and L. R. Johnson. 1969. Effect of secretin and cholecystokinin on the response of the gastric fistula rat to pentagastrin (33835). *Proc. Soc. Exp. Biol. Med.* **131**: 186.
9. Wormsley, K. G., and M. I. Grossman. 1964. Inhibition of gastric acid secretion by secretin and by endogenous acid in the duodenum. *Gastroenterology.* **47**: 72.
10. Johnson, L. R., and M. I. Grossman. 1968. Secretin: The enterogastrone released by acid in the duodenum. *Amer. J. Physiol.* **215**: 885.
11. Nakajima, S., M. Nakamura, and D. F. Magee. 1969. Effect of secretin on gastric acid and pepsin secretin in response to various stimuli. *Amer. J. Physiol.* **216**: 87.
12. Johnson, L. R., and M. I. Grossman. 1969. Characteristics of inhibition of gastric secretion by secretin. *Amer. J. Physiol.* **217**: 1401.
13. Sugawara, K., J. Isaza, J. Curt, and E. R. Woodward. 1969. Effect of secretin and cholecystokinin on gastric motility. *Amer. J. Physiol.* **217**: 1633.
14. Vagne, M., G. F. Stening, F. P. Brooks, and M. I. Grossman. 1968. Synthetic secretin: comparison with natural secretin for potency and spectrum of physiological actions. *Gastroenterology.* **55**: 260.
15. Makhlouf, G. M., J. P. A. McManus, and J. R. Knill. 1968. Quantitative aspects of synergism and inhibition of gastric acid secretion. *Gastroenterology.* **54**: 532.
16. Teorell, T. 1937. Kinetics of distribution of substances administered to the body; extravascular modes of administration. *Arch. Int. Pharmacodynther.* **57**: 205.
17. Cohen, S., and L. D. Harris. 1969. (Abstract) The adaptive response of the lower esophageal sphincter. *Clin. Res.* **17**: 300.
18 Emas, S., and M. I. Grossman. 1967. Differences in responses between dogs and cats to large doses of gastrin on gastric secretion. *Gut.* **8**: 267.
19. Rudick, J., J. L. Werther, M. A. Chapman, D. A. Dreiling, and H. D. Janowitz. 1969. (Abstract). Influence of pentagastrin on mucosal blood flow in canine fundic and antral pouches. *Clin. Res.* **17**: 310.
20. Ariens, E. J. (Editor). Molecular Pharmacology. Academic Press Inc., New York. 1964. Vol. 1.
21. Johnson, L. R., and M. I. Grossman. 1970. Analysis of inhibition of acid secretion by cholecystokinin in dogs. *Amer. J. Physiol.* **218**: 550.
22. Hansky, J., and M. D. Cain. 1969. Radioimmunoassay of gastrin in human serum. *Lancet.* **2**: 1388.
23. Isenberg, J. I., and M. I. Grossman. 1969. Effect of gastrin and sc 15396 on gastric motility in dogs. *Gastroenterology.* **56**: 450.