Interaction of gastrin I and secretin on gastrointestinal circular muscle

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LIPSHUTZ, WILLIAM, AND SIDNEY COHEN. Interaction of gastrin I and secretin on gastrointestinal circular muscle. Am. J. Physiol. 222(3) : 775-781. 1972.—The effect of gastrin I and secretin was studied on the in vitro circular smooth muscle of the lower esophageal sphincter (LES), antrum, and pylorus of the opossum. Each muscle was studied at its length of optimal tension development, L₀, as determined by length-tension diagrams. At L₀, full-dose response curves were constructed for gastrin I and secretin, alone and in combination. Gastrin I stimulated LES and antral muscle, and secretin produced a selective competitive type of antagonism to this gastrin I response. Pyloric circular muscle did not respond to gastrin I. Secretin, over a wide dose range, had little effect on antral and LES muscle. Pyloric circular muscle was stimulated by secretin, and gastrin I produced a selective, noncompetitive type of antagonism to this secretin response. We conclude that the action and interaction of gastrin and secretin on upper gastrointestinal circular smooth muscle differ at each region. It is suggested that these hormonal effects that have been demonstrated in vitro determine, in part, the motor function of these separate anatomic regions in vivo.

METHODS

Studies were performed on 30 adult opossums of both sexes, weighing 2.5-5.0 kg. The methods outlined here have previously been described in detail (14). The animals were anesthetized with 40 mg/kg of intraperitoneal Nembutal and strapped supine to an animal board for studies in vivo. Esophageal manometric studies utilizing an infused, open-tipped catheter system were performed in all animals. A triple-lumen polyvinyl tube (1.4 mm id with three side orifices 1.2 mm in diam) was passed through the mouth into the stomach. Each catheter was continuously infused with distilled water by an infusion pump at the rate of 1.2 ml/min. Intraluminal pressure was transmitted to external transducers (Statham P23BB), whose output was graphed on a Beckman curvilinear, ink-writing recorder. After all orifices were in the stomach, the tube assembly was withdrawn at 0.5-cm intervals, and pressures were recorded at each level for 1 min. Deglutition was initiated by stroking the posterior oropharynx. After completion of the manometric study, the recording tube was anchored at the lower jaw and positioned with the middle recording orifice in the LES. All animals were then killed by intravenous Nembutal. The LES, as determined by manometry, was identified and marked. The LES was within 2 cm of the esophageal junction, the entire stomach, and duodenum were mobilized and freed from the surrounding tissues. The midesophagus and distal duodenum were ligated, and the upper gastrointestinal tract from midesophagus to distal duodenum was excised, washed in Krebs-Ringer solution with the composition in millimoles per liter: Na⁺, 138.6; K⁺ 4.6; Ca²⁺, 2.5; Mg²⁺, 1.2; Cl⁻, 126.2; HCO₃⁻, 21.9; PO₄³⁻ 1.2; glucose, 49.6, at 37-38 C, and transferred to an
organ bath of Krebs-Ringer solution, bubbled with 95% O\textsubscript{2} and 5% CO\textsubscript{2}, and maintained at 37–38 C. The esophagus was separated from the stomach at the anatomic gastroesophageal junction where the narrow esophagus flares into the stomach. The pylorus was identified as a distinct prominent anatomic ring at the junction of duodenum and antrum. The pyloric ring was separated from the antrum proximally and from the duodenum distally. The mucosa from each region was removed to the level of the submucosa. The antral circular muscle strips were taken 2 cm proximal to the pylorus.

Each anatomic region was subsequently identified histologically by lining epithelium. The LES was lined by squamous epithelium with the junction of columnar epithelium with the junction of columnar epithelium within 1 cm distal to it. The antrum and pylorus were lined by columnar epithelium with gastric glands. No Brunner’s glands or intestinal villi were present in the pyloric sections. All anatomic regions contained a well-defined inner circular and outer longitudinal muscle layer. The circular layer in the pyloric strips was increased as compared to the other areas. The muscle layers were distinct with no intermixing of fibers. Each area was studied so that the force generated would be that of the circular layer only.

Circular smooth muscle strips, 0.5 cm wide and 1.0 cm long, were cut from each anatomic region. Each muscle was mounted in a 20-ml bath of Krebs-Ringer solution, bubbled with 95% O\textsubscript{2} and 5% CO\textsubscript{2}, and kept at 37–38 C. All strips were arranged to record the isometric tension of the circular smooth muscle of each region. One end of the muscle was attached to an inflexible wire which was hooked to an external force transducer (Grass FT-05C). The other end was attached to a metal rod which could be raised and lowered by adjustment of a screw micrometer. The outputs of the transducers were graphed on a Beckman curvilinear ink-writing recorder. Four to six strips were studied simultaneously.

Following a 30-min equilibration period, length tension curves were constructed on muscles from each anatomic region. The length of the muscle was progressively increased by adjusting the screw micrometer until the first increase in passive tension was noted. The length just prior to the development of a passive tension was designated the initial length at zero passive tension. From this point, the length was increased by the adjustment of the screw micrometer. The passive tension and the peak active tension produced by chemical stimulation with 10\textsuperscript{-4} M acetylcholine is shown in upper set of curves. Each point represents mean ± S.E for 20 separate experiments. SEM values for passive tension measurements are ±0.1 to ±0.5 g. Length of maximum active tension development, L\textsubscript{o}, is different for muscles from different anatomic regions.

gastrin I (amino acid sequence 2-17) (Imperial Chemical Industries, Ltd.; Alderley Park, Cheshire, England) mol wt 2,089, and secretin (GIF Research Unit, Karolinska Institute, Stockholm, Sweden) mol wt 3,055 (16), were solubilized with Krebs-Ringer solution, and 1-ml volumes were added to a 20-ml bath to obtain the final molar concentration as noted. The dose-response curves were constructed using individual doses. No cumulative doses were given. The response to each drug was recorded over a 10-min period. The peak tension recorded at each dose within this period was used to construct the dose-response curves. LES muscle showed no spontaneous activity. The peak spontaneous contractions of the gastric and pyloric muscles during control periods were quantified and subtracted from the peak tension recorded at each dose. Stimulation of antral and pyloric muscle consisted of an increase in the amplitude of the spontaneous contractions with low concentrations of the hormone. At higher concentrations, the entire base-line pressure became elevated. LES muscle showed a single phasic contraction with hormonal stimulation. In the experiments where gastrin and secretin were used in combination, the antagonist was added to the bath 5 min prior to the addition of the agonist. At least 15 min were allowed between experiments. The molar concentration of secretin was calculated using 3.4 units of secretin as being equal to 1 μg (21).

Direct muscle stimulation with 143.6 mM KCl was obtained at the L\textsubscript{o} for each muscle. The response to other

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**Fig. 1.** Length-tension curves for lower esophageal sphincter, stomach, and pylorus. Tension in grams corrected for muscle weight is plotted as a function of percent increase in muscle length. Passive tension is shown on lower set of curves, and active tension produced by chemical stimulation with 10\textsuperscript{-4} M acetylcholine is shown in upper set of curves. Each point represents mean ± S.E for 20 separate experiments. SEM values for passive tension measurements are ±0.1 to ±0.5 g. Length of maximum active tension development, L\textsubscript{o}, is different for muscles from different anatomic regions.
agonists, acetylcholine, histamine, norepinephrine (NE), and dimethylpiperazinium (DMPP), was determined using each drug at a concentration that produced a barely maximal response. All muscles were lightly blotted and weighed at the termination of the experiment. All active tensions have been corrected for muscle weight.

RESULTS

The data reported in this study are computed on the basis of peak responses to each agonist or to each paired agonist and antagonist combination. The comparison of the peak responses of each muscle at the length of optimal tension development, Lₒₒ, allows comparison of muscles with dissimilar mechanical characteristics at an identical point on their respective length-tension diagrams. By studying the muscle at Lₒₒ, a dose-related response was obtained for each agonist. Since the response is dose related and reproducible, the effect of an antagonist is readily quantified. The construction of length-tension diagrams with 10⁻⁴ M acetylcholine chloride did not alter the subsequent response of the muscle to other agonists. Muscle set at Lₒₒ, as previously determined from length-tension diagrams, responded in an identical manner to the hormones, as when 10⁻⁴ M acetylcholine was given initially.

In Table I, the maximum absolute responses of muscle from each area are compared for gastrin I, secretin, and KCl (143.6 mM) depolarization. The mean weights of the muscles are also shown. The responses to both gastrin I and secretin are less than those to KCl depolarization at each anatomic region. The muscle response to KCl depolarization, corrected for weight, is not statistically different for the three anatomic regions (P > .05). The maximum response of the muscles from the three areas to gastrin I differed significantly (P < .001). The maximum response to secretin showed a significant difference between the pylorus and the two other areas (P < .001). The LES and antral muscles did not differ in their response to secretin (P > .05).

The full log dose-response curves for circular muscle from each anatomic region to synthetic gastrin I are shown in Fig. 2. The response of the muscle from each region is expressed as a percent of the response of the circular muscle of the LES, the anatomic area that gave the maximal active tension. Gastrin I produced contraction of the LES and antral muscles. The circular muscle of the pylorus showed a slight decrease in the amplitude of its spontaneous contractions. The threshold dose for gastrin I on the LES muscle was less than that for the other regions. The maximum active tension for LES muscle to gastrin I was greater than the maximum active tension for the other muscles, and it occurred at a lower concentration of gastrin I.

The log dose-response curves for secretin are shown in Fig. 3. The response of the circular muscle from each anatomic region is expressed as a percent of the peak response for the circular muscle of the pylorus. Secretin gave an increase in the amplitude of the spontaneous contractions of the circular muscle of the pylorus. Muscles from the other anatomic areas showed a minimal response at higher concentrations of secretin.

The dose-response curves of the circular muscle of the LES for gastrin I alone and in the presence of secretin (3.0 X 10⁻¹⁹ M) are shown in Fig. 4. Secretin is used at a molar concentration that does not alter the tension of LES muscle. In the presence of secretin, the concentration of gastrin I required for a threshold response is increased. The linear phases of the dose-response curves are parallel, and the same maximum response to gastrin I is attained at a higher concentration of gastrin I. No significant difference was found between the slopes of the steep linear phase of each dose-response curve (P > .05).

In Fig. 5 are shown the dose-response curves for antral muscle to gastrin I, alone, and in the presence of 1.6 X 10⁻¹⁸ M secretin. This dose of secretin does not alter the amplitude of spontaneous contractions. In the presence of

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TABLE 1. Comparison of maximum absolute responses of muscle from each area

<table>
<thead>
<tr>
<th></th>
<th>Lower Esophageal Sphincter</th>
<th>Antrum</th>
<th>Pylorus</th>
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<tbody>
<tr>
<td>Peak active tension to gastrin I, g</td>
<td>10.0 ± 0.8</td>
<td>4.61 ± 0.98</td>
<td>-0.7 ± 0.2</td>
</tr>
<tr>
<td>Peak active tension to secretin, g</td>
<td>0.23 ± 0.06</td>
<td>0.32 ± 0.11</td>
<td>4.55 ± 0.95</td>
</tr>
<tr>
<td>Peak active tension (g) to KCl depolarization (143.6 mM)</td>
<td>22.5 ± 2.5</td>
<td>20.8 ± 1.7</td>
<td>17.6 ± 2.5</td>
</tr>
<tr>
<td>Muscle wt, mg</td>
<td>38.6 ± 4.8</td>
<td>42.2 ± 4.6</td>
<td>88.4 ± 3.8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of 20 separate determinations. Active tensions are corrected for muscle weight.
secretin, the dose of gastrin I required for a threshold response is greater. The steeply sloped linear portions of the dose-response curves are parallel, and the same maximum response to gastrin I is still attained in the presence of secretin. No significant difference in the slopes of the linear phase of the curves was noted (P > .05).

In Fig. 6 are shown the dose-response curves of the pyloric circular muscle for secretin alone, and in the presence of $10^{-16}$ M gastrin I. This dose of gastrin I does not change the amplitude of spontaneous contractions of the circular muscle of the pylorus. The threshold response to secretin is shifted to the right, but the linear phases of the dose-response curves are not parallel. The same peak response to secretin could not be reached in the presence of gastrin I. The peak response of the pyloric muscle to secretin ($3 \times 10^{-8}$ M) in the presence of gastrin I ($10^{-16}$ M) is 67% of its control response. The slopes of the linear portion of each dose-response curve showed a statistically significant difference (P < .01).

In Fig. 7 are shown the effects of secretin on other agonists that act on LES circular muscle. Secretin, at a concentration that reduces the maximum gastrin I response to 6.9 ± 3.0% (mean ± SE) of its control response, has no effect on the maximal stimulatory muscle response to dimethylphenylpiperazinium $10^{-4}$ M, acetylcholine $10^{-4}$ M, histamine $3 \times 10^{-7}$ M, and norepinephrine $10^{-4}$ M. Similar selectivity of
hormonal inhibition on the antrum and pylorus was obtained and is illustrated in Fig. 8.

The gastrointestinal hormone that gave almost complete inhibition of either gastrin or secretin was further tested in a 10^4-fold greater concentration against other agonists in five separate experiments. No significant diminution in the response to other agonists was noted. Secretin, 3 × 10^-14 M, did not significantly alter the LES muscle response to ACh, histamine, DMPP, and NE (P > .05). Secretin, 10^-7 M, did not alter the antral muscle response, and gastrin, 5 × 10^-11 M, did not alter the pyloric muscle response to other agonists (P > .05).

DISCUSSION

The purpose of this study is to investigate the response, in vitro, of the circular smooth muscle of the LES, antrum, and pylorus to gastrin I and secretin, when administered both individually and in combination. The opossum was selected as the experimental animal because of the anatomic and physiologic similarity of its lower esophageal sphincter to that in man (4). The data in this study illustrate several points. First, circular muscle of each anatomic area shows specific dose-response characteristics to each hormone. Second, stimulation of muscle from each region by either gastrin I or secretin is antagonized by the other hormone. Third, antagonism of one hormone for the other is selective and does not alter the response of the muscle to other agonists. Each finding is discussed in detail.

To quantify differences in response to gastrin I and secretin, full dose-response curves were constructed for the circular muscle of the LES, antrum, and pylorus. The response of each muscle was determined at its respective length of optimal tension development. This standardization allows for differences in length-tension characteristics of the muscles from each anatomic region. An arbitrary selection of a passive tension or change in length for all muscles would place each at a different point on its respective length-tension curve. Comparison of dose-response curves at an arbitrary length may therefore give incorrect results. If a muscle is not studied at its L0, its response to an agonist will be diminished. A comparison of the responses of different muscles at dissimilar points on their length-tension curves might indicate contractile differences that would be falsely attributed to the response of the agonist. The determination of L0 standardizes the responses of each muscle and corrects for intrinsic differences in the mechanical properties of different muscles. KCl depolarization at L0 indicates that the maximum tension developed by each muscle, when corrected for weight, is similar.

Having normalized each muscle for intrinsic differences in its length-tension properties and having shown equal maximum contractile ability to KCl depolarization, the response of the muscles to the different hormones was then studied. The circular muscle of the three anatomic areas differed in its responses to both gastrin I and secretin. Gastrin I stimulated the circular muscle of the lower esophageal sphincter and antrum, but did not affect the pyloric muscle. Secretin alone had little effect upon antral and LES muscle, but did stimulate pyloric sphincter muscle. These differences in response to gastrin I and secretin represent intrinsic differences in the behavior of muscle from each region.

The hormone that did not elicit an independent effect at a given anatomic area was able to antagonize the contraction produced by the other hormone. The characteristics of the
antagonism differed at each region. Secretin antagonism to the action of gastrin I on the circular muscle of the LES and gastric antrum was characterized by: 1) a shift to a higher threshold dose; 2) a parallel shift to the right of the linear portion of the dose-response curve; and 3) the ability to attain the same maximum response in the presence of the antagonist. These characteristics define a competitive type of antagonism, but do not necessarily indicate hormonal interaction at a single receptor (1). A single receptor for both hormones would be unlikely because of dissimilar hormonal structures. There are at least two alternative explanations for the observed interaction of these hormones on muscle contraction. First, secretin could either chemically alter or reduce the maximal response to the other hormone, do not decrease the affinity of the agonist for a second receptor (1).

It has been reported. Secretin does not inhibit histamine-stimulated acid secretion (12). The antagonism of one hormone for the other is selective. Secretin and gastrin I, at concentrations which markedly reduce the maximal response to the other hormone, do not affect the response to other agonists. Similar selectivity for the secretin antagonism of gastrin-stimulated acid secretion has been reported. Secretin does not inhibit histamine-stimulated acid secretion (12).

These observations can be compared with studies performed in vivo. In man and in the opossum, gastrin contracts the LES (3, 6, 14) and the antrum (8, 10, 15, 18). Secretin antagonizes the effect of gastrin at both areas (6, 20, 21). In dogs, endogenous secretin released by duodenal acidification contracts the pylorus (2). Although the study in dogs suggested physiological sphincteric properties for the pylorus, this finding is not agreed upon. It is generally accepted that the pylorus functions only as part of an antural peristaltic pump mechanism without separate sphincteric properties (5). The observed differences in response between the gastric antrum and pylorus in vitro to gastrin and secretin suggest that these areas may actually have independent functions in vivo, contrary to the present description of the motor behavior of the stomach.

The data in this study support the gastrointestinal hormone-receptor hypothesis proposed by Grossman (9). This hypothesis suggests a single-hormone receptor with two sites for the two hormonal structural prototypes, gastrin and secretin. The data in this study indicate that both gastrin and secretin act at each anatomic region. The selectivity of the interaction of these hormones suggests that the receptor sites for gastrin and secretin are related. The differences in response of the muscles from each anatomic region are determined by the interaction of the hormone with its receptor. The factors which determine the response elicited from each receptor are unknown.

In conclusion, we have shown that circular muscle of different anatomic regions has a specific response to both gastrin and secretin. The response to each hormone and to each hormone combination may, in part, determine the physiologic motor function of these regions in vivo.

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