Regional differences of the effects of acetylcholine in the human gastric circular muscle

Dong Hyun Sinn,1,* Byung-Hoon Min,1,* Eun-ju Ko,2 Ji Yeon Lee,2 Jae J. Kim,1 Jong Chul Rhee,1 Sung Kim,1 Sean M. Ward,4 and Poong-Lyul Rhee1

1Department of Medicine, 2Biomedical Research Institute, and 3Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; and 4Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada

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Sinn DH, Min BH, Ko E, Lee JY, Kim JJ, Rhee JC, Kim S, Ward SM, Rhee PL. Regional differences of the effects of acetylcholine in the human gastric circular muscle. Am J Physiol Gastrointest Liver Physiol 299: G1198–G1203, 2010. First published August 26, 2010; doi:10.1152/ajpgi.00523.2009.—The motor functions of the stomach have traditionally been regarded to have regional differences. However, to date there have been only a few data investigating whether such regional differences in motor function exist in the human stomach. The aims of the present study were to examine the spontaneous activity and responses to acetylcholine in the anatomically defined regions of human stomach. Human gastric circular muscle tissues from fundus, corpus, and antrum were obtained from 25 patients (14 men, 11 women with a mean age of 55.2 yr; 36–74 yr) undergoing gastrectomy for gastric cancers. Isometric force measurements were performed by using muscle strips from the different regions of the human stomach under basal conditions and in response to the exogenous application of acetylcholine. Spontaneous phasic contractions were observed in all human gastric smooth muscles. However, the responses to acetylcholine displayed regional differences. In the gastric antrum, there was a dose-dependent increase in the peak contraction, contractile frequency, and amplitude of contraction after acetylcholine exposure (up to 1 μM). However, there was no significant change in the basal tone. In the corpus and fundus, acetylcholine induced a dose-dependent increase in the peak contraction and basal tone. However, there was no significant change in the contractile frequency or amplitude of contraction. In conclusion, the response of human gastric circular muscle to acetylcholine displayed regional differences between the antrum and the corpus and fundus. This finding suggested the presence of distinct functional regions in the human stomach.

stomach; acetylcholine; regional differences

THE MAIN FUNCTION OF GASTRIC motility is to accommodate and store an ingested meal, grind down or triturate solid particles, and then empty the contents of the meal into the duodenum in a carefully controlled and regulated fashion. To accomplish these discrete motor functions, the stomach needs to demonstrate a degree of regional specialization.

Anatomically, the stomach can be divided into regions that can be defined by anatomic landmarks. The cardia is a small area of the stomach immediately adjacent to its junction with the esophagus. The fundus projects upward, above the cardia and gastroesophageal junction. The corpus is located immediately below and continuous with the fundus. The incisura angularis, a fixed, sharp indentation two-thirds of the distance along the stomach at the lesser curvature, marks the caudal aspect of the gastric corpus. The gastric antrum ranges from incisura angularis to the pyloric sphincter.

In functional terms, regional specializations of the stomach have been observed in animal models. Using the dog model, Morgan et al. (9) demonstrated differences in the contractile behavior of various parts of the stomach. These regional variations in contractile behavior may be attributed to the intrinsic properties of gastric smooth muscle cells and differences in the distribution of interstitial cells of Cajal (ICC) that provide pacemaker activity and function as critical mediators in enteric motor activity (4, 12). However, to date there have been only a few studies supporting regional specialization in the contractile behavior of the human stomach (8).

Acetylcholine is a neurotransmitter that is synthesized in cholinergic neurons and is the principal excitatory neurotransmitter in the gastrointestinal tract (3). In the present study we sought to determine whether there were regional differences in basal spontaneous activity in anatomically defined regions of the human stomach. We also sought to determine whether there were regional differences in response to acetylcholine along the stomach, which might explain the different functional roles of the gastric fundus, corpus, and antrum.

MATERIALS AND METHODS

Patients. Human gastric circular muscles were obtained from 25 patients (14 men, 11 women with a mean age of 55.2 yr; 36–74 yr) undergoing gastrectomy for gastric cancers. Thirteen patients underwent subtotal gastrectomy and 12 patients underwent total gastrectomy. The mean body mass index (kg/m2) was 23.8 ± 4.0. Three patients were taking antihypertensive medication to control blood pressure. None of the patients had fasting serum glucose levels exceeding 126 mg/dl, had abnormal results after a 75-g oral glucose tolerance test, or were using insulin or oral antihyperglycemic drug.

The stomach was divided by an incision across the region of the incisura angularis. After gastrectomy, circular muscle strips were obtained from the regions macroscopically not invaded by cancer. The sites where the gastric tissues were obtained were carefully mapped by the surgeon into the gastric fundus, corpus, or antrum. The surgeon was instructed on mapping of the exact gastric sites prior to the procedure. Written, informed consent was obtained from all participating subjects. Experiments were performed in accordance with the Declaration of Helsinki. The institutional review board of Samsung Medical Center approved the study protocol.

Isometric force measurements. Mechanical experiments were performed by using standard organ baths techniques within 18 h after resection. Gastric muscle strips (10 from fundus, 8 from corpus, and
result of acetylcholine (Sigma Chemical, St. Louis, MO). The dose-dependent effects of acetylcholine were evaluated using Clampfit software (Molecular Devices, pClamp version 10, Sunnyvale, CA).

Responses to acetylcholine. Circular muscle strips were examined for their contractile responses to acetylcholine (Sigma Chemical, St. Louis, MO). The dose-dependent effects of acetylcholine were evaluated 20 min after exposure to acetylcholine with progressive increases of acetylcholine dose (1 nM–1.0 μM). After the strips were exposed to acetylcholine, there was a 20-min period during which the strips were washed with KRB before exposure to next drug concentration. In the analysis of dose-dependent effects of acetylcholine, the half-maximally effective concentration (ED$_{50}$) was determined for fundus, corpus, and antrum. To calculate ED$_{50}$, the data were fit with Hill’s equation.

Data analysis. Data are expressed as means ± SE. The $n$ values reported in the text refer to the number of muscle strips used for each experiment. Each muscle strip used for $n$ values was taken from a different patient. We analyzed 1) peak contraction, 2) basal tone, 3) frequency of phasic contraction, and 4) amplitude of phasic contractions. The contractions were expressed in grams and/or percent change of the contractions from baseline. The Kruskal-Wallis test or Mann-Whitney rank sum test was used to evaluate differences in the data. The Wilcoxon signed rank-sum test was used to evaluate changes in parameters after exposure to acetylcholine. A $P$ value less than 0.05 was considered significant.

RESULTS

Regional differences in the spontaneous contractile activity of the human stomach. All gastric muscle strips showed spontaneous phasic contractions. Examples of these activities from fundus, corpus, and antrum are illustrated in Fig. 1. There were significant differences in the peak contraction and basal tone in the three regions examined. However, no significant difference was observed in the frequency or the amplitude of phasic contractions in each of the three regions (Table 1).

Spontaneous activity and effects of acetylcholine on gastric fundus. Figure 2A shows a typical isometric force measurement from the gastric fundus showing spontaneous activity and the concentration-dependent effects of acetylcholine. The gastric fundus developed a basal tone averaging 0.85 ± 0.09 g. Superimposed on this tone, spontaneous activity consisting of regular phasic contractions averaging 0.15 ± 0.03 g in amplitude occurred at a frequency of 4.23 ± 0.32 cycles/min. Application of acetylcholine (1 nM–1.0 μM) induced a dose-dependent significant increase in the peak contractions. The mean peak contractions were 1.05 ± 0.12, 1.09 ± 0.11, 1.13 ± 0.11, 1.35 ± 0.14, 1.45 ± 0.13, 1.81 ± 0.15, and 1.88 ± 0.14 g with 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3A). The ED$_{50}$ value for acetylcholine-induced contraction in fundus was 357.3. There was also a significant increase in the basal tone at concentrations starting at 1 nM of acetylcholine. The mean basal tones were 0.88 ± 0.09, 0.92 ± 0.10, 0.95 ± 0.10, 1.16 ± 0.13, 1.25 ± 0.12, 1.55 ± 0.17, and 1.62 ± 0.16 g at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3B). However, acetylcholine did not cause a dose-dependent increase in the frequency or the amplitude of contractions in the fundus (Fig. 3, C and D).

Spontaneous activity and effects of acetylcholine on gastric corpus. Figure 2B shows a typical tracing from the corpus revealing spontaneous activity and the dose-dependent effects of acetylcholine. In the corpus, basal spontaneous activity consisted of basal tone averaging 0.59 ± 0.09 g. Superimposed

Table 1. Characteristics of spontaneous contractions of human gastric circular muscles according to stomach regions

<table>
<thead>
<tr>
<th>Value</th>
<th>Fundus ($n = 10$)</th>
<th>Corpus ($n = 8$)</th>
<th>Antrum ($n = 7$)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak contraction, g</td>
<td>1.01 ± 0.12</td>
<td>0.82 ± 0.11</td>
<td>0.50 ± 0.19</td>
<td>0.018</td>
</tr>
<tr>
<td>Basal tone, g</td>
<td>0.85 ± 0.09</td>
<td>0.59 ± 0.09</td>
<td>0.36 ± 0.08</td>
<td>0.008</td>
</tr>
<tr>
<td>Frequency, min$^{-1}$</td>
<td>4.23 ± 0.32</td>
<td>3.93 ± 0.26</td>
<td>4.87 ± 0.28</td>
<td>0.085</td>
</tr>
<tr>
<td>Amplitude, g</td>
<td>0.15 ± 0.03</td>
<td>0.22 ± 0.06</td>
<td>0.13 ± 0.05</td>
<td>0.507</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Fig. 2. Typical tracing of acetylcholine response according to stomach region. The response pattern was different according to stomach region. An example of this activity from the fundus (A), corpus (B), and antrum (C).
on this basal tone were spontaneous phasic contractions averaging 0.22 ± 0.06 g in amplitude occurring at a frequency of 3.93 ± 0.26 cycles/min. Application of acetylcholine (1 nM–1 μM) caused a dose-dependent significant increase in the peak contractions that were evident at concentrations of 5 nM or greater. The mean peak contractions were 0.84 ± 0.11, 0.92 ± 0.12, 0.96 ± 0.12, 1.22 ± 0.16, 1.32 ± 0.19, 1.62 ± 0.25, and 1.72 ± 0.26 g at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3A). The ED50 value for acetylcholine-induced contraction in corpus was 358.3. acetylcholine also caused a significant increase in the basal tone at concentrations of 10 nM or greater. The mean basal tone were 0.60 ± 0.09, 0.66 ± 0.10, 0.68 ± 0.09, 0.90 ± 0.11, 0.88 ± 0.11, 1.15 ± 0.17, and 1.21 ± 0.17 g at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3B). However, acetylcholine did not cause a dose-dependent increase in the frequency or the amplitude of contractions in the gastric corpus (Fig. 3, C and D).

**Spontaneous activity and effects of acetylcholine on gastric antrum.** Basal spontaneous activity of the gastric antrum is shown in Fig. 2C. In the gastric antrum basal spontaneous activity consisted of a nominal tone of 0.36 ± 0.08 g. Superimposed on this were spontaneous phasic contractions that averaged 0.13 ± 0.05 g in amplitude and occurred at a frequency of 4.87 ± 0.28 cycles/min. Application of acetylcholine (1 nM–1 μM) caused a dose-dependent increase in the peak contractions that was evident at concentrations of 50 nM or greater. The mean peak contractions were 0.54 ± 0.10, 0.73 ± 0.25, 0.72 ± 0.25, 0.90 ± 0.28, 1.00 ± 0.27, 1.37 ± 0.41, and 1.48 ± 0.47 g at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3A). The ED50 value for acetylcholine-induced contraction in antrum was 139.4. Interestingly, acetylcholine did not produce a significant change in the basal tone (Fig. 3B) but caused a concentration-dependent significant increase in the frequency of contractions at concentrations of 5 nM or greater (Fig. 3C). The contraction frequency was 4.83 ± 0.28, 5.55 ± 0.76, 5.85 ± 0.66, 6.48 ± 0.90, 6.37 ± 0.85, 6.62 ± 0.99, and 6.77 ± 0.98 min⁻¹ at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3C). acetylcholine also caused a significant increase in the amplitude of contractions at concentrations of 50 nM or greater. The mean amplitude of contraction were 0.10 ± 0.08, 0.39 ± 0.26, 0.39 ± 0.25, 0.53 ± 0.28, 0.60 ± 0.27, 0.90 ± 0.43, and 0.98 ± 0.51 g at 1 nM, 5 nM, 10 nM, 50 nM, 0.1 μM, 0.5 μM, and 1 μM, respectively (Fig. 3D).

Fig. 3. Change in peak contraction (A), basal tone (B), frequency (C), and amplitude (D) according to stomach region. The response to acetylcholine of the human gastric fundus and corpus was different from that of the human gastric antrum. *Statistically significant changes compared with baseline value, P < 0.05.

**Figures:**
- A: Peak contraction (g) vs. Acetylcholine concentration.
- B: Basal tone (g) vs. Acetylcholine concentration.
- C: Frequency (min⁻¹) vs. Acetylcholine concentration.
- D: Amplitude (g) vs. Acetylcholine concentration.
Effects of age or sex on basal activity and responses to acetylcholine in different regions of the stomach. Peak contraction, basal tone, frequency, or amplitude of contraction did not differ by sex (male vs. female, \( P > 0.05 \) for all comparisons) or age (age <60 vs. age \( \geq 60 \), \( P > 0.05 \) for all comparisons). Although sample size was relatively small, the responses to acetylcholine that were observed in the different stomach regions were also similar by sex (male vs. female) and age (age <60 vs. age \( \geq 60 \)).

DISCUSSION

In the present study we examined the regional differences in basal activity and responses to acetylcholine in human stomach. The results of this study showed that basal characteristics of spontaneous contraction and the response pattern to exogenous acetylcholine are different according to the stomach region. In the present study, the response patterns of the gastric fundus and corpus to exogenous acetylcholine were relatively similar each other. However, gastric antrum showed different response patterns from those of the gastric fundus and corpus. In the gastric fundus and corpus, significant dose-dependent increase was found in both peak contraction and basal tone. However, no significant alteration was observed in the frequency or amplitude of contraction. In the antrum, acetylcholine induced a significant increase in the peak contraction, contractile frequency, and amplitude of contractions, but no significant change was found in the basal tone. These results provide the direct and robust evidence supporting different motor activities in different regions of the human stomach.

To date, there have been only a few studies reporting the regional differences of the effects of acetylcholine in the human stomach. One study showed that the fundus exhibited purely tonic spontaneous activity and a tonic contraction pattern after application of acetylcholine whereas the activity in the circular antrum was purely phasic. In the same study, the corpus and longitudinal antrum demonstrated a combination of tonic and phasic contractions (8). Currently, the exact mechanism of different effect of acetylcholine in fundus, corpus, and antrum is unclear. Li et al. (7) recently provided the plausible explanation for different effects of acetylcholine. In their study, regional difference was observed in the distribution of high- and low-affinity binding sites to muscarinic receptors in the smooth muscles of human stomach.

Gastric emptying is a one of main functions of gastric motility. Gastric emptying is known to be dependent on the interplay between the propulsive forces generated by the tonic contractions of the proximal stomach and the resistance caused by the antrum and pylorus. For low-calorie liquids, fluid emptying occurs by a tonic pressure pump mediated by fluctuations in fundic tone (5, 11). For solid meals or high-calorie mixed meals, emptying requires redistribution and trituration, which requires antral contractions (10). These activities require regional specializations along the stomach. The findings of our study provide an explanation for the differences in contractile behavior between the proximal and distal parts of the stomach during gastric emptying. The gastric fundus and corpus responded to acetylcholine by changes in muscle tone, whereas the gastric antrum responded by increases in the amplitude of phasic contractions. The gastric antrum also showed chronotropic effects, which might explain replacement of random contractions, the fed pattern, with cyclical patterns of migrating motor complexes during food ingestion (6). In a murine model, activation of muscarinic receptors in the gastric antrum was associated with an increased frequency of antral slow waves (2).

The present study had several limitations. First, this study analyzed the mechanical characteristics of gastric smooth muscle only with circular muscle strip. In the preliminary experiment, however, we did not see significant difference in mechanical characteristics between circular and longitudinal muscle strips. Therefore, we focused on circular contraction in this study. Second, morphological and physiological studies in animals have shown that intramuscular interstitial cells of Cajal (ICC-IM) are mediators of excitatory and inhibitory enteric motor neurotransmission in the stomach (1, 2, 13). Song et al. (12), using samples taken from different regions of the murine stomach, demonstrated that the distribution of ICC-IM differed by stomach region. They also found that when ICCs were present, robust cholinergic and nitrergic neural responses were observed, and when ICCs were absent, these responses were greatly attenuated or not observed. Since our study did not include immunohistochemical evaluation of the ICCs, the question remains whether the observed differences in our study were related to differences in distribution of the ICCs in the human stomach. This needs further clarification.

In conclusion, the response of human gastric circular muscle to acetylcholine displayed regional differences between the antrum and the corpus and fundus. These findings provided the direct evidence supporting regional specializations in motor activities and suggested the presence of distinct functional regions in human stomach.

GRANTS

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DISCLOSURES

The authors have no conflict of interest to disclose in relation to this article.

REFERENCES


