Cortical processing of human gut sensation: an evoked potential study

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There are important anatomic and physiological differences between the afferent innervation of the proximal and distal organs of the gastrointestinal tract. Proximal gut organs, such as the esophagus and duodenum, develop from the foregut and are innervated jointly by vagal and spinal afferents from the cervical and thoracic spinal cord segments (19). In contrast, distal gut organs such as the rectum develop from the hindgut and are innervated solely by spinal afferents from the sacral spinal cord (19). Despite these differences, electrophysiological studies in animals have shown convergence of afferent pathways from multiple visceral organs onto single cells in both the cortex (4) and thalamus (3). However, differences in the speed, magnitude, or pattern of afferent pathway activation could exist, thereby maintaining the physiological differences in sensation from these organs.

Cortical evoked potentials (CEP) are measurements of the electrical potentials generated by cortical neurons in response to a series of repeated sensory stimuli. These are recorded using scalp surface electrodes. The stimulus-specific CEP occur at a fixed time after each stimulus, whereas other brain activity does not; therefore, by averaging the recorded cortical electrical activity the stimulus-specific CEP can be “extracted” from the brain’s background electrical activity. CEP techniques are now widely used to investigate the integrity of visual, auditory, and somatosensory pathways and have also been used to study both esophageal (2, 5, 8, 12, 14) and rectal afferent pathways (7, 11, 16, 18, 21). There are, however, no published studies that compare CEP from different bowel regions in the same subjects.

We chose to use CEP in this study because its excellent temporal resolution of milliseconds allows characterization of the afferent pathways in addition to providing information about cortical processing. We hypothesized that differences in the afferent innervation of the proximal and distal gut would be reflected by differences in their CEP. To test this hypothesis, we compared the morphologies, latencies, and amplitudes of CEP recorded following electrical stimulation of the esophagus, duodenum, and rectum in healthy volunteers.

**Methods**

**Subjects**

Ten right-handed healthy subjects (1 female, 9 male) with a mean age of 28 yr (range 21–34) were studied after providing written informed consent. None of the subjects had gastrointestinal or neurological symptoms, and none were taking any regular medication. Ethical approval was obtained from the Salford and Trafford research ethics committee.

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Gut Stimulation

Gut stimulation was performed by using a catheter assembly containing platinum bipolar ring electrodes connected to a constant-current, high-voltage stimulator (model DS7; Digitimer, Welwyn Garden City, UK). A square-wave stimulus of 200-ms duration was used in each gut region. This stimulus has previously been demonstrated to be safe, without affecting cardiac rhythm, when bipolar intraoesophageal electrodes are used during esophageal stimulation (6). Previous work has demonstrated that the optimal stimulus intensity for recording esophageal and rectal CEP is 75% of the difference between the sensory and pain thresholds (11, 12). This stimulus intensity was therefore established in each gut region of interest and used for the studies.

Esophageal stimulation. Esophageal stimulation was performed by using a catheter assembly with three pairs of bipolar platinum ring electrodes (2-mm electrodes with an interelectrode distance of 1 cm) sited 5, 12.5, and 20 cm from the tip of the catheter. Solid-state pressure transducers were sited between each electrode pair to enable the catheter to be positioned by using manometric assessment. The catheter was constructed from nylon tubing covered with stainless steel braid insulated with silicone rubber and had an external diameter of 3 mm (Gaeltec, Dunvegan, UK). The catheter was passed either nasally or orally, depending on the subject’s choice. The distal manometric sensor was positioned in the stomach and then slowly withdrawn until the proximal margin of the lower esophageal sphincter was identified. The catheter was then withdrawn so that the distal stimulating electrodes were 5 cm above the lower esophageal sphincter. Esophageal stimulation was then performed by using the distal pair of ring electrodes.

Duodenal stimulation. Duodenal stimulation was performed using the catheter assembly described above. The catheter was passed either nasally or orally, depending on the subject’s choice, until the distal manometric sensor was in the stomach 50–55 cm from the incisors. The subjects were then asked to lie on their right side in a semirecumbent position. The catheter was then slowly advanced while the motility pattern was recorded. When clusters of contraction consistent with phase II of the small bowel migrating motor complex (22) were seen in all three manometric channels, the catheter was secured and duodenal stimulation was performed by using the middle pair of electrodes. Regular contractions at a rate of 3/min were considered to represent antral motor activity.

Rectal stimulation. Rectal stimulation was performed by using a catheter assembly with a single pair of bipolar platinum ring electrodes (2-mm electrodes with an interelectrode distance of 2 cm) sited 2 cm from the tip of the catheter. The catheter was constructed from nylon tubing covered with stainless steel braid insulated with silicone rubber and had an external diameter of 3 mm (Gaeltec). This catheter was attached to a second catheter, with an inflatable latex balloon positioned next to the electrodes. During stimulation, the balloon was inflated by 5–10 ml of air to ensure electrodes–mucosal contact without affecting the CEP characteristics (11, 27).

CEP Recording

CEP were recorded by using silver-silver chloride surface electrodes, with the active electrode positioned at the vertex (Cz) in accordance with the international 10–20 system of electrode placement, the reference electrode positioned on the right earlobe, and the ground electrode positioned on the neck. The electrodes were applied by using electrode paste, and the impedance was kept below 5 kΩ. The CEP data were acquired using a CED 1902 programmable signal conditioner (Cambridge Electronics Design, Cambridge, UK) and an IBM-compatible desktop computer, running SIGAVG software (version 6.04; Cambridge Electronics Design). The data was sampled at a frequency of 2,000 Hz, with an epoch duration of 1,000 ms, of which the first 200 ms was prestimulation time. The amplifier gain was set at 100,000, with online artifact rejection. The band-pass filters were set at 1 and 100 Hz. These parameters are similar to those previously shown to be optimal for recording esophageal and rectal CEP (12, 16). During each stimulus, the impedance between the stimulating bipolar electrodes was monitored and the CEP was rejected if the impedance rose, thereby ensuring adequate mucosa contact.

Protocol

In all subjects, each gut region was studied on a separate day in a randomized order. The catheter was passed, and then the sensory and pain thresholds were determined by increasing the stimulation intensity in steps of 1 mA. All subsequent stimuli were applied at an intensity of 75% of the difference between the sensory and pain thresholds (11, 12). Four runs of 50 stimuli at 0.2 Hz were conducted, with a 10-min rest period between each run. This stimulation has been shown to provide CEP with a reliable and reproducible morphology (12, 16).

Definition of Terms

Sensory threshold. Sensory threshold was defined as the stimulus intensity in milliamperes when the subjects first became aware of any sensation.

Pain threshold. Pain threshold was defined as the stimulus intensity in milliamperes when the subjects first reported pain.

CEP terminology. The peaks of the CEP were designated N for negative deflections and P for positive deflections. The peaks were numbered in order of their appearance after the stimulus. The CEP were displayed according to the standard CEP convention, with positive deflections being displayed downward.

Latency. Latency was defined as the time in milliseconds from the onset of the stimulus to the peak of each CEP component.

Amplitude. Amplitude was defined as the voltage difference in microvolts between consecutive CEP peaks.

Data Analysis

In each subject, the four runs of CEP for each stimulation site were averaged to provide a single trace, which was then assessed by an experienced investigator blinded to the origin of the data. The positive peaks of the CEP were labeled P1 and P2, and the negative peaks were labeled N1 and N2. The latency and amplitude of each CEP component from each subject was used to obtain group mean data for each gut site.

Statistical Comparison

Using Arcus Quickstat software version 1.0 (Addison Wesley Longman), the latencies to each peak and the amplitudes of CEP component evoked from each gut site were compared by using the Wilcoxon’s signed-rank test. A P value of <0.05 was accepted as a statistically significant difference.
RESULTS

Stimulus Intensity

The mean values of the stimulation intensities used for the three gut regions are given in Table 1. The stimulus intensity for the rectum was significantly lower than that for either the esophagus or the duodenum ($P < 0.001$).

Stimulus Perception

At 75% intensity, esophageal sensation was described as a sharp, retrosternal pulse in all subjects. Duodenal sensation was described as a sharp pulse in the periumbilical region, followed by a more diffuse dull sensation that outlasted the sharp pulse, also in the periumbilical region. Rectal sensation was described as a sharp pulse deep within the pelvis.

CEP Morphology

In all subjects, esophageal CEP had similar triphasic morphologies, with three main components: P1, N1, and P2 (Fig. 1). Duodenal CEP were not recorded from two subjects, due to the electrical stimuli not being perceived; in the other eight subjects, the CEP had a triphasic morphology similar to that of esophageal CEP. Rectal CEP in eight subjects were also triphasic and similar to those from the esophagus and duodenum (Fig. 1). However, in two male subjects a distinctly different morphology was seen, consisting of five main components. This pattern was similar for both subjects and reproducible in a repeat study (Fig. 2).

CEP Latency

The mean latencies of the CEP following stimulation of the three regions are given in Table 1. The latency to the first (P1) component of duodenal CEP was significantly longer than the corresponding latency for esophageal CEP ($P = 0.016$). There was no difference in latencies to the later components of esophageal and duodenal CEP ($P = 0.05$). The P1 latency for rectal CEP was shorter than both the esophageal ($P = 0.004$) and duodenal ($P = 0.008$) P1 latency. The N1 and P2 latencies for the rectal CEP were also shorter than the corresponding esophageal and duodenal latencies ($P < 0.05$; see Table 1). However, after a Bonferroni correction for multiple comparisons, only the difference in the P1 latencies between the rectum and esophagus CEP remained significant ($P = 0.03$).

CEP Amplitude

The mean CEP amplitudes following stimulation of the three gut regions are given in Table 1. The CEP amplitude was similar for all three gut regions, with the exception of the P1-N1 amplitude of the duodenal CEP, which was significantly smaller than the corresponding amplitude for the esophagus ($P = 0.04$). However, after a Bonferroni correction, none of the amplitude differences remained significant.

Table 1. Summary of CEP characteristics

<table>
<thead>
<tr>
<th></th>
<th>Esophageal</th>
<th>Duodenal</th>
<th>Rectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulus intensity, mA</td>
<td>71 (62–79)</td>
<td>87 (75–100)</td>
<td>50 (41–59)</td>
</tr>
<tr>
<td>P1 latencies, ms</td>
<td>106 (98–117)</td>
<td>123 (106–150)*</td>
<td>69 (51–92)**</td>
</tr>
<tr>
<td>N1 latencies, ms</td>
<td>157 (149–166)</td>
<td>168 (155–195)</td>
<td>115 (98–134)**</td>
</tr>
<tr>
<td>P2 latencies, ms</td>
<td>228 (219–258)</td>
<td>256 (236–310)</td>
<td>184 (147–236)**</td>
</tr>
<tr>
<td>P1-N1 amplitude, µV</td>
<td>5.7 (4.3–7.7)</td>
<td>5.0 (4.0–7.1)</td>
<td>6.5 (4.0–9.0)</td>
</tr>
<tr>
<td>N1-P2 amplitude, µV</td>
<td>13.4 (9.3–17.9)</td>
<td>12.4 (10.6–14.3)</td>
<td>11.8 (6.4–16.0)</td>
</tr>
</tbody>
</table>

Summary of cortical evoked potential (CEP) characteristics following stimulation of the 3 gut organs are given as median (interquartile range). *$P = 0.004$ vs. esophagus and $P = 0.008$ vs. duodenum. **$P = 0.014$ vs. esophagus and $P = 0.016$ vs. duodenum. ##$P = 0.02$ vs. esophagus and $P = 0.008$ vs. duodenum.
DISCUSSION

The results of our study suggest that anatomic and physiological differences between the different gut organs are reflected by differences in their CEP characteristics. We have also reported for first time CEP evoked by duodenal stimulation.

The esophagus and duodenum have a dual afferent innervation by vagal and spinal afferents. The vagus nerve is predominantly sensory (24) and consists mainly of unmyelinated C fibers and thinly myelinated A-fibers (20, 24, 26). These afferents are sensitive to low-intensity stimuli within the physiological range such as peristalsis and physiological levels of gut distention (20, 26).

Spinal afferents arise from the cervical, thoracic, and lumbar segments of the spinal cord and travel with the sympathetic nerves to the gut (24). Like vagal afferents, spinal afferents are also a mixture of unmyelinated C fibers and thinly myelinated A-fibers and encode a range of sensations, ranging from the innocuous to the noxious (17, 24, 25).

In contrast to the proximal gut, the rectum receives afferent innervation from only the pelvic nerve, which originates from the inferior hypogastric plexus and contains both parasympathetic nerves from the sacral roots and sympathetic nerves from the lumbar roots. As with the proximal gut, rectal afferents are also a mixture of unmyelinated C fibers and thinly myelinated A-fibers (17, 19, 25).

The main difference we have observed between CEP responses evoked from the different gut regions is in the latency of esophageal and rectal CEP. Despite the greater length of afferent pathways from the rectum to the brain, the latency of rectal CEP was shorter than the corresponding latency of the esophageal CEP. One reason for this could be that rectal stimulation activated afferent pathways with faster conduction velocities than esophageal stimulation. Previous investigators have speculated that electrical stimulation in the rectum can activate fast-conducting A-fibers in the pudendal nerve (16), leading to a shorter CEP latency than would occur if CEP were mediated via rectal afferent A-fibers in the pelvic nerve. They reported CEP latencies of 40 ± 2 ms following direct pudendal nerve stimulation, and a similar CEP latency of 34.7 ± 2 ms following rectal stimulation (16). The CEP latency following rectal stimulation in our study was 69 ms, suggesting that activation of pudendal nerve A-fibers is unlikely. This difference in afferent pathways activated in our current study compared with those in previous studies could be due to the use of the rectal balloon in our study. This improves electrode-mucosal contact and allows a smaller stimulating current to be used, which would reduce the risk of stimulating the pudendal nerve (11).

Human studies have demonstrated that both esophageal (8, 13) and rectal CEP (11) are mediated by A-fibers. It could, however, be speculated that rectal sensation is mediated by A-fibers with a faster conduction velocity than those conducting esophageal sensation. Although in humans there are no direct comparisons of the conduction velocities of afferents from different gut organs, the available animal studies suggest that rectal (23) and esophageal (26) A-fibers have a similar conduction velocity. The possibility of a species difference cannot be ruled out; however, this explanation remains speculative and requires further confirmation.

Recording CEP with the active electrode at the vertex and the reference electrode at the ear, as in our study, will not detect subcortical sources. Therefore, an alternative explanation for the differences in CEP latencies could be differences in the representation of these organs at the cortical level. However, this explanation is not supported by animal studies that have demonstrated convergence of esophageal and rectal afferent pathways in the thalamus (3) and cortex (4). Nevertheless, differences in latencies of CEP recorded at the vertex could still result if the electrical activity generated by the early cortical activation following esophageal stimulation did not result in a recordable electrical potential at the vertex. This could occur due to differences in orientation or volume of the cortical neurons representing the esophagus and rectum.

A study of CEP and magnetoencephalography (MEG) responses to electrical esophageal stimulation supports the possibility that differences in cortical neuronal orientation or volume could explain differences in the latencies of CEP recorded from the various gut organs (10). In this study, vertex CEP were recorded following electrical distal esophageal stimulation, with a similar latency to that in our study (98.9 ± 8 ms), whereas MEG responses in the same subjects demonstrated somatosensory cortex activation at 70 ms. It is possible, therefore, to speculate that differences in the volume and/or orientation of the cortex representing the rectum and the esophagus could account for the possibility that the early components of the esophageal CEP were not detected.

The spatial resolution of CEP is limited because the scalp distorts electrical currents, whereas the magnetic component of the electromagnetic field generated by cortical neurons remains unaffected. Recording these magnetic fields by using MEG, therefore, has a greater spatial resolution compared with CEP (9) and allows the volume and orientation of cortex generating the response to a stimulus to be calculated with greater accuracy. Using MEG to study the cortical representation of the esophagus and rectum would help to determine whether the shorter CEP latencies following rectal stimulation are due to differences in the afferent pathways or cortical processing of rectal sensation.

Duodenal CEP had a longer latency than either esophageal or rectal CEP, although this failed to remain significant after the Bonferroni correction. The likely explanation for the latency difference not remaining significant is the smaller number of subjects in whom we could record duodenal CEP, reducing the significance of the difference observed. Despite the considerable overlap in the spinal innervation of the esophagus and duodenum, the peak distribution of

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duodenal afferents is inferior to that of esophageal afferents (19). Therefore, the afferent volley from the duodenum has a longer distance to conduct to the cortex. It would, therefore, be anticipated that the duodenum would have longer CEP latencies than the esophagus.

Our study does not show any differences in the latencies of the later CEP components. These later components probably reflect secondary cortical processing of sensory information and are often more variable than the early components, as in our study.

The CEP morphology from the three gut organs was similar. However, this is not surprising, because the morphology of vertex CEP following stimulation in several sensory modalities is known to be similar (1). Therefore, the similarity in vertex CEP morphology from different gut organs does not imply that the same cortical areas are being activated. We have demonstrated rectal CEP with two different morphologies, as in our previous study (11). The explanation for these two rectal CEP morphologies is unknown, but it has been speculated that these differences may be due to activation of different afferent pathways (15, 16), however, it is equally plausible that these differences could relate to differences in orientation of the cortical neurons activated. Future studies using MEG, which allows assessment of the cortical neuronal volume and orientation, may help to explain the significance of the differing morphologies.

In conclusion, we have shown that it is possible to study afferent pathways from multiple gut organs by using CEP. Our results demonstrate that important differences exist in the latencies of CEP evoked by stimulation of these organs, probably reflecting differences in the characteristics of the afferent pathways and/or cortical representation of the different gut organs. Future studies that combine MEG and CEP will allow a greater understanding of the neurophysiological characteristics of gut afferent pathways in health and in diseases characterized by abnormal perception of gut sensation, such as functional gastrointestinal disorders.

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REFERENCES


