A cortical evoked potential study of afferents mediating human esophageal sensation

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FUNCTIONAL GASTROINTESTINAL disorders such as irritable bowel syndrome and noncardiac chest pain affect between 10 and 20% of the general population (37). Despite their high prevalence, the pathophysiology of these conditions is unknown. One of the most common clinical findings in these patients is hypersensitivity to visceral stimulation (30). The mechanisms responsible for this visceral hypersensitivity remain unclear; however, it has been postulated that symptoms are due to either hypersensitive gut afferent nerves or abnormal cortical processing of visceral sensation (30). Until recently, the lack of suitable noninvasive neurophysiological techniques to assess brain-gut interactions has prevented investigation of either possibility.

Cortical evoked potentials (CEP) are the electrical manifestation of the brain’s response to an external stimulus. They are recorded via electrodes placed on the scalp and represent the sequence of negative and positive voltage changes generated in the brain following the arrival of a sensory stimulus (29). Initial CEP components reflect the characteristics ofafferent pathways, whereas subsequent components relate to specific steps in cortical sensory processing. The technique is used routinely in clinical practice to assess the integrity of auditory, visual, and somatosensory systems (10, 12, 18). CEP may also be recorded following stimulation of the gastrointestinal tract (3, 4, 8, 15), which allows objective assessment of the mechanisms of human visceral sensation in health and disease.

CEP have been successfully recorded in response to both electrical and mechanical stimulation of the esophagus (3, 8, 15, 16, 21, 23). However, exactly by what route the afferents that mediate esophageal CEP pass to the brain remains controversial. This is because the esophagus receives dual sensory innervation from both vagal and spinal afferents (34, 35). Some investigators have speculated that the vagus is the sole mediator of esophageal CEP (24, 25) because CEP acquired in response to direct vagal stimulation have a similar morphology to esophageal CEP (38). Furthermore, CEP acquired in patients with a spinal cord injury are similar to those acquired in normal subjects, suggesting that intact spinal pathways are not necessary to elicit esophageal CEP (13).

We have provided evidence for the involvement of spinal afferents in the mediation of esophageal CEP because topographic mapping studies indicated that the cortical source of the early CEP component was in the primary somatosensory cortex (3), which only receives spinal and not vagal afferents. These findings have now been supported further by both magnetoen-
cerephalography (17) and positron emission tomography studies (2).

Further debate surrounds the fiber types that mediate esophageal CEP to different stimulation modalities. Several authors have suggested that CEP to electrical stimulation are mediated by thinly myelinated Aδ-fibers (15, 26), whereas CEP to balloon distension are mediated via slower conducting, unmyelinated C-fibers (23, 36). This has been based on estimations of the conduction velocity of the response and by the fact that the latency of CEP components following electrical stimulation is significantly shorter than following mechanical stimulation. However, the methodologies used by different groups to acquire esophageal CEP differ considerably, and this may very well have contributed to the variability in the reported CEP data between different groups.

We have recently established the optimal stimulation and recording parameters for recording esophageal CEP to both electrical and mechanical stimulation and have shown that it is possible to record highly reproducible CEP to both stimulation modalities (21, 22). To date, however, a direct comparison of CEP using these optimal parameters with both techniques in the same subjects has not been performed. It was therefore the aim of this study to compare the characteristics of the cortical responses elicited by electrical and mechanical stimulation of the healthy human esophagus in the same subjects and to use this information to evaluate the afferents that mediate esophageal sensation.

METHODS

Subjects

We recruited healthy volunteers free of any gastrointestinal, cardiac, or neurological disorders, none of whom was taking any medication at the time of the study. Informed written consent was obtained from all volunteers, and The Salford Area Health Authority Ethics Committee approved the experimental protocols. Standard esophageal manometry was performed on each volunteer to exclude any esophageal motor disorder and to identify the distance between the lower esophageal sphincter (LES) and the incisors.

Esophageal Mechanical Stimulation

Mechanical stimulation of the esophagus was performed using a 2-cm-long silicone balloon (Medasil Surgical, Leeds, UK) sited 10 cm from the tip of a polyvinyl catheter (4-mm diameter; Cook UK, Letchworth, UK). The balloon was connected to a specially constructed pump (Medical Physics Department, Hope Hospital, Salford, UK) sited 10 cm from the tip of an intraluminal catheter (external diameter = 3 mm). The catheter was constructed from nylon tubing covered with stainless steel braid and sheathed in silicone rubber (Gaeltex, Dunvegan, UK). The electrodes were connected to a constant-current, high-voltage stimulator (Model DST7; Digitimer, Welwyn Garden City, UK). The stimulator was triggered using a laboratory interface (CED 1401plus). The interelectrode impedance was monitored throughout, and a value of <10 kΩ was taken as evidence of good mucosal contact. Electrical stimuli were applied using a 200-μs-duration square-wave pulse.

CEP Recording

CEP were recorded using two Ag-AgCl surface electrodes, which were applied to the scalp with electrode paste (Elefix, Nihon Kohden, Japan). With the use of the international 10–20 system of electroencephalograph electrode placement (27), the active electrode was positioned at Cz (vertex) and the reference electrode was positioned on the left ear lobe. An additional ground electrode was positioned on the neck. Recordings were performed in a quiet room with the subject semirecumbent, awake with eyes open, and asked to minimize eye movements and swallowing.

The data were acquired using a CED 1902 programmable signal condition (Cambridge Electronics Design). Display and analysis utilized the SIGAVG program v. 6.04 and Signal for Windows v. 1.72 (Cambridge Electronic Design). The amplifier gain was set at 100,000, and the recording sensitivity was 25 μV. The bandpass filter settings were 1–100 Hz, and a 50-Hz notch filter was utilized, if needed, to reduce interference from the main electrical supply. The sampling rate was 2,000 Hz, and the recording epoch was 1 s in duration. The first 200 ms of the epoch was prestimulation time. Each individual epoch was saved, and the average of the run could be viewed during acquisition. An automatic artifact rejection facility was employed to prevent contamination from eye blinks and swallows. Before each recording, scalp electrode impedance was reduced to <3 kΩ by applying a preparation paste (Omniprep, Weaver & Aurora).

Experimental Protocols

Experiment 1: CEP characteristics. To compare the characteristics of CEP obtained to mechanical and electrical stimulation, we studied six healthy volunteers (5 male, 1 female; mean age = 31.6 yr, age range = 21–47 yr). At the beginning of the study, the catheter was passed perorally into the esophagus and positioned so that the electrodes or the mid-balloon level was 5 cm above the LES.

CEP morphology. CEP to electrical and mechanical stimulation were recorded from each of the six subjects on separate days, within the same week, using the average of 200 stimuli acquired in 4 runs of 50 stimuli. A 10-min rest period was left between each run. Stimulation was performed at a frequency of 0.2 Hz and at an intensity that was 75% of the subjects’ maximum tolerated value. The parameters used were se-
lected on the basis of our previous work to provide optimal CEP responses (21, 22).

Effect of Stimulation Intensity. To demonstrate the effect of stimulation intensity on the characteristics of the CEP response to electrical and mechanical stimulation, the average of 200 stimuli were acquired at 5 stimulation intensities ranging from sensory threshold to pain threshold in 3 subjects (2 male, 1 female; mean age = 37 yr, age range = 39–47 yr) and the data were compared. Sensory threshold was labeled as 0% and maximum tolerated intensity as 100%. Stimulation intensities that were 25, 50, and 75% of the difference between sensory threshold and maximum tolerated intensity were then identified. For example, if sensory threshold was reported at 30 mA and maximum tolerated intensity at 70 mA, then 25, 50, and 75% were calculated as 15, 35, and 52.5 mA, respectively. The CEP was acquired using the optimal stimulation parameters described above on three separate occasions, at least two days apart, at the same time of the day, for each modality.

Experiment 2: CEP conduction velocity. To estimate the conduction velocity of the pathways involved in the mediation of electrical and mechanical CEP, we studied 6 healthy volunteers (5 male, 1 female; mean age = 31.6 yr, age range = 21–47 yr). CEP to electrical and mechanical stimulation were recorded on separate days, within the same week, at 5 cm and 15 cm above the LES. The same recording and stimulation parameters were used as described in experiment 1A. Stimulation intensity was first determined at the distal site, and the same intensity was then used for the proximal site.

Definition of Terms

Latency is the interval between the onset of the stimulus and the peak of each potential. Values are expressed in milliseconds.

Amplitude is the potential difference between the maximal positive and the maximal negative deflection. Values are expressed in microvolts.

Interpeak latency is the interval between consecutive peaks. Values are expressed in milliseconds.

Sensory threshold is the intensity at which a stimulus was first perceived. Maximum tolerated intensity is the lowest stimulation intensity at which the subject described pain.

Data Analysis

All recordings are displayed using common neurophysiological convention, i.e., a negative potential is displayed as an upward deflection. For each experimental protocol, the average CEP to 200 stimuli were analyzed.

Experiment 1A. Group mean values for amplitude and latency were calculated for both modalities and compared. The difference between the latency of each CEP component for each stimulation modality was calculated by subtracting the latency of the mechanical component from that of the corresponding electrical component. Electrical and mechanical CEP acquired in the same subject were superimposed to compare morphology.

Experiment 1B. For each stimulation modality, group mean values were calculated for amplitude and latency of each component at each of the five intensities. Data are displayed as group means ± SD.

Experiment 1C. Intrasubject variability was assessed by comparing values for amplitude and latency in each individual on three separate occasions for each modality. Intersubject variability was assessed by comparing values for amplitude and latency across all three individuals for each occasion for both modalities.

Experiment 2. The latency of the first positive (P1) component was compared for CEP elicited via both modalities at 5 cm and 15 cm above the LES. The latency difference between the two sites allowed the conduction velocity of the response to be calculated. Values are displayed as group means ± SD and expressed in meters per second.

Statistical Analysis

Descriptive statistical analysis was performed using Arcus Quickstat (Biomedical version 1.0; Addison Wesley Longman, Research Solutions). In experiment 1 we used a Shapiro-Wilk test for normality and a paired two-tailed Student’s t-test. We calculated the coefficient of variance of the values for amplitude and latency in experiment 1C to demonstrate intra- and intersubject variability of CEP components.

RESULTS

Perception of Stimuli by Subjects

Electrical stimulation was described as a sharp, nonpainful pulse felt retrosternally. Mechanical stimulation was felt as a strong, nonpainful pulse retrosternally, duller in nature and more long lasting. Triphasic CEP were recorded in response to both stimulation modalities in all subjects. An illustrative example of these responses in one subject is shown in Fig. 1. The three components seen consistently were labeled P1 (first positive), N1 (first negative), and P2 (second negative).

Experiment 1

Experiment 1A: CEP characteristics. All values for peak latency, amplitude, and interpeak latency are...
shown in Table 1. The morphology of the CEP response was similar in all subjects, with the P1-N1-P2 complex being present in each subject for both modalities (Fig. 2).

LATENCY. The peak latencies for P1, N1, and P2 components were significantly longer for mechanical stimulation compared with electrical stimulation ($P < 0.005$).

AMPLITUDE. The amplitudes of the P1-N1 and N1-P2 components were significantly larger for electrical stimulation compared with mechanical stimulation ($P < 0.05$) for similar stimulation intensities.

INTERPEAK LATENCIES. There was no significant difference between interpeak latencies for either the P1-N1 or N1-P2 components ($P > 0.3$).

The latency difference for the corresponding mechanical and electrical CEP components were similar ($P > 0.2$). The values were $P1 = 48 \pm 6.7$ ms, $N1 = 53.3 \pm 6.8$ ms, and $P2 = 42.9 \pm 9.2$ ms, respectively.

**Experiment 1B: stimulation intensity.** The latency of CEP components decreased and their amplitude increased as stimulation intensity increased both for electrical and mechanical stimulation (Figs. 3, 4, and 5).

**Experiment 1C: reproducibility.** The intra- and intersubject variability of the latency of CEP components was similar for both modalities (Table 2). For electrical stimulation, the intersubject coefficient of variance was <0.1 for all three components. For mechanical stimulation, the intersubject coefficient of variance was <0.1 for the P1 and N1 components, but the P2 component showed a variability of 0.2. Intra- and intersubject amplitude variability was more pronounced (40–60%) but similar for each modality.

**Experiment 2: CEP Conduction Velocity**

The conduction velocity of the CEP response was $7.9 \pm 1.9$ m/s for mechanical stimulation and $8.6 \pm 2.3$ m/s for electrical stimulation.

**DISCUSSION**

The results of our study show that the morphology, interpeak latencies, and conduction velocity of CEP responses to both electrical and mechanical stimulation are similar. This implies that similar neural pathways and cortical processing are likely to be involved in...
the mediation of both mechanical and electrical CEP. There were, however, significant differences in the peak latencies and amplitudes of CEP components elicited by the two modalities.

There is currently a debate in the literature about the nature of the afferents that mediate esophageal CEP. Some authors (23, 38) have suggested that CEP to both electrical and mechanical stimulation are mainly mediated via vagal afferents, because CEP elicited by electrical stimulation of the esophagus and direct vagal stimulation in humans have a similar morphology. However, there are several reasons to question this assertion. First, it has been shown that, due to the close proximity of the vagal electrodes to the surrounding somatic tissue, stimulation of somatic afferents also occurs because the desired signal can often be contaminated by myogenic potentials (20). It is therefore likely that somatic spinal afferents also contribute to the CEP responses described. Second, CEP with similar morphology can be obtained following electrical stimulation of the esophagus and the skin over the anterior chest wall (16). Because stimulation of the skin will activate spinal afferents, it could therefore be similarly argued that spinal afferents are involved in the mediation of esophageal CEP.

Further evidence used to support the vagal afferent route is the finding that esophageal CEP to mechanical stimulation in patients with a C6/C7 spinal cord injury were similar to those seen in normal subjects (13). However, as the animal data suggest (11, 19) and the authors themselves concluded, this may only mean that a C6/C7 spinal cord lesion does not interrupt all the spinal afferents that mediate esophageal sensation (13).

The arguments in favor of a spinal afferent route for esophageal perception and pain are based mainly on animal data that show that vagal afferents saturate at low intensities, whereas spinal afferents respond to intensities ranging from subthreshold to nociception (32, 34, 35). Furthermore, our previous work has shown that stimulus perception and CEP thresholds are identical to both mechanical and electrical stimulation (21, 22) and that CEP amplitudes increase and latencies decrease with increasing stimulus intensity (21, 22, 24, 36). In this study we have demonstrated that this relationship between stimulation intensity and the amplitude and latency of CEP is consistent for both modalities. Since thresholds for activation of spinal and vagal afferents appear to be similar (32), it seems implausible that the strongly perceived stimulation intensities needed to elicit reproducible CEP would preferentially activate only vagal or spinal pathways.

Studies of the central processing of human esophageal sensation using functional brain imaging techniques such as positron emission tomography (2) and magnetoencephalography (17) have demonstrated that both nonnociceptive and nociceptive esophageal stimulation activate the primary somatosensory cortex, an area that receives projections from spinal but not vagal afferents (9). In addition, however, the studies also show activation of the insula and the limbic cortices, brain areas that receive both spinal and vagal projections. Therefore, it is likely that both vagal and spinal pathways contribute to the esophageal CEP responses to electrical and mechanical stimulation. The fact that the morphology and the interpeak latencies of CEP to both modalities were similar strongly supports the argument that they are mediated by similar pathways.

The longer latencies of CEP components to mechanical compared with electrical stimulation has led some authors to suggest that the two responses are mediated via different afferent fiber types (23, 36). In addition, several groups have looked at the conduction velocity of CEP by stimulating two esophageal sites (14, 15, 23). All studies using electrical stimulation have shown that esophageal CEP are mediated by afferents with conduction velocities between 7 and 11 m/s, indicating mediation by thinly myelinated Aδ-fibers (15, 23). However, studies using mechanical stimulation have shown that afferents with conduction velocities that
range from 1.7 to 8 m/s mediate esophageal CEP, implicating either unmyelinated C- or Aδ-fibers (14, 23).

There are, however, several other explanations for the apparent differences between conduction velocities for mechanical and electrical CEP. First, the inflation pump used in the studies by Hollerbach et al. (23) and DeVault et al. (14) had a flow rate of 170 ml/s. Because the flow rate was constant, the rise time to maximum inflation for different balloon volumes was variable; a 1-ml difference in the eventual balloon volume would therefore produce a 6-ms difference in rise time. Because balloon volumes required to induce similar sensory endpoints at the two esophageal stimulation sites differed, and because the rise time of balloon inflation was variable, using the peak latencies to estimate conduction velocity will give an inaccurate estimation of the true conduction velocity. Second, the proximal esophageal stimulation site used in these two studies was almost certainly within the striated muscle portion of the esophagus (28). Because there are differences in the innervation and cortical projections of striated and smooth muscle esophagus (3, 17, 28), comparing data from the proximal and distal sites to calculate conduction velocities may not be valid.

We attempted to control for these variables by keeping the rise time to maximum balloon inflation constant for any given volume and using the same stimulation intensity at both esophageal sites. In addition, the two sites we stimulated within the esophagus are both within the smooth muscle portion of the esophageal body (28). The conduction velocities obtained us-
ing these parameters were 7.9 ± 1.9 m/s for mechanical stimulation and 8.6 ± 2.3 m/s for electrical stimulation, indicating that the first CEP component (P1) for both stimulation modalities is probably mediated via myelinated Aδ-fibers.

This is not to say that unmyelinated C-fibers are not also activated by mechanical and electrical stimulation. Indeed, animal studies show that both Aδ- and C-fibers are present in spinal and vagal afferents, and both have similar thresholds of activation to esophageal distension (32). However, because Aδ-fibers have faster conduction velocities, it would be expected that they would mediate the first CEP potential.

Studies of somatosensory CEP elicited by laser stimulation have shown that it is extremely difficult to record reliable cortical responses to pure C-fiber stimulation due to the enormous latency jitter of C-fiber-evoked cortical responses (1, 5–7, 39). The first laser-evoked cortical potential components are mediated by Aδ-fibers occurring at a latency of 240–370 ms (6). The C-fiber-mediated components, known as ultra-late components, that occur between 1,050 and 1,250 ms are not apparent without blockade of Aδ-fiber transmission (6). The C-fiber-mediated CEP components have a standard deviation of 150 ms, which makes conventional signal-averaging techniques ineffective and requires the use of an iterative latency correction filter (40). This makes it unlikely that esophageal CEP we recorded without blockade of Aδ afferents and with conventional signal averaging are primarily mediated by C-fibers in humans.

A further reason why C-fiber transmission of the CEP responses is unlikely is because the latency difference of the P1 components to electrical and mechanical stimulation, which in our study was consistently 50 ms, is much less than would be expected if mechanical stimulation was mediated only via unmyelinated C-fibers. Our data lead us to suggest that the differences in the P1 latencies can be explained simply and adequately by differences in time between triggering of stimulation onset and the development of an adequate esophageal stimulus. In the case of electrical stimulation, this delay will be very short; for mechanical stimulation the delay will be much longer because the

Table 2. Intra- and intersubject coefficient of variance for CEP component latencies and amplitudes to both mechanical and electrical stimulation

<table>
<thead>
<tr>
<th>Component</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mechanical</td>
<td>Electrical</td>
<td>Mechanical</td>
<td>Electrical</td>
</tr>
<tr>
<td>P1</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>N1</td>
<td>0.025</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>P2</td>
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<td>0.02</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>P1-N1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.37</td>
</tr>
<tr>
<td>N1-P2</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Fig. 5. Group mean data (+ SD) from 3 subjects for amplitude of CEP components acquired with electrical (A) and mechanical (B) stimulation at intensities ranging from sensory threshold (0%) to maximum tolerated intensity (100%). An increase in amplitude occurs with increasing stimulation intensity.
mechanical pump has to deliver sufficient air into the intraluminal balloon for esophageal distension to occur and for the esophageal distension to then induce afferent neural activation. The fact that the latency differences between the two modalities were similar for all three CEP components indicates that all of the components are probably responsive to the initial afferent volley arriving at the cortex and that the later components probably represent secondary cortical processing of the initial afferent volley and not activation of different fiber types.

The differences in amplitude of CEP components to electrical and mechanical stimulation in our study are again consistent with the animal data. Although esophageal distension will activate only those afferents that are mechanosensitive, electrical stimulation will activate all afferents regardless of modality (32). Because the amplitude of the CEP components is directly related to the number of afferents contributing to the signal, then it would be expected that CEP to electrical stimulation would be larger than CEP to mechanical stimulation, as was found in our study.

These amplitude differences raise an intriguing potential for the application of CEP in the assessment of clinical disorders. It is known that a large proportion of afferent fibers [both myelinated and unmyelinated fibers (31–33)] are normally mechanosensitive in healthy subjects (silent nociceptors) and that some of these fibers become mechanically sensitive in conditions such as inflammation (32). Although electrical stimulation would be expected to activate all of these fibers regardless of modality and pathological condition, CEP components to mechanical stimulation may become enhanced only in injury, due to the recruitment of the additional, previously insensitive, afferent fibers. This may mean that changes in mechanical CEP could be used to monitor abnormalities in inflammatory conditions. Electrical CEP, on the other hand, might be better suited to detecting abnormalities in conditions in which visceral hypersensitivity is thought to occur as a result of central rather than peripheral sensitization.

In conclusion, we have shown evidence to indicate that CEP to mechanical and electrical stimulation of the esophagus are mediated by similar afferent pathways, most likely a combination of both vagal and spinal afferents, and that the early components of the CEP to both stimulation modalities are mediated via Aδ-fibers. The combined use of both may have an important role in the assessment of esophageal sensory processing in disease states.

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Q. Aziz is a Medical Research Council Clinician Scientist.

REFERENCES


