Central neural mechanisms mediating human visceral hypersensitivity

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1Section of Gastrointestinal Sciences, University of Manchester, Hope Hospital, Salford M6 8HD; 2Neurosciences Research Institute, Aston University, Birmingham B4 7ET, United Kingdom; 3Department of Anesthesia and Critical Care, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

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Sarkar, Sanchoy, Anthony R. Hobson, Paul L. Furlong, Clifford J. Woolf, David G. Thompson, and Qasim Aziz. Central neural mechanisms mediating human visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 281: G1196–G1202, 2001.—Although visceral hypersensitivity is thought to be important in generating symptoms in functional gastrointestinal disorders, the neural mechanisms involved are poorly understood. We recently showed that central sensitization (hyperrexcitability of spinal cord sensory neurones) may play an important role. In this study, we demonstrate that after a 30-min infusion of 0.15 M HCl acid into the healthy human distal esophagus, we see a reduction in the pain threshold to electrical stimulation of the non-acid-exposed proximal esophagus (9.6 ± 2.4 mA) and a concurrent reduction in the latency of the N1 and P2 components of the esophageal evoked potentials (EEP) from this region (10.4 ± 2.3 and 15.8 ± 5.3 ms, respectively). This reduced EEP latency indicates a central increase in afferent pathway velocity and therefore suggests that hyperrexcitability within the central visceral pain pathway contributes to the hypersensitivity within the proximal, non-acid-exposed esophagus (secondary hyperalgesia/allodynia). These findings provide the first electrophysiological evidence that central sensitization contributes to human visceral hypersensitivity.

The etiology of functional gastrointestinal disorders (FGD) such as noncardiac chest pain (NCCP) remains unclear. The most consistent and often only finding is visceral hypersensitivity, which is characterized by a reduced pain threshold to experimental stimulation of the organ believed to be the source of the symptoms (15, 32). The neurophysiological basis for this visceral hypersensitivity remains unclear in contrast to the basis for somatic hypersensitivity, which has been well described (39, 41, 42, 45).

After somatic injury, hypersensitivity occurs with two important properties. First, pain is induced by innocuous stimuli (allodynia) and exaggerated in severity to noxious stimuli (hyperalgesia). Second, the hypersensitivity is diffuse, being present not only at the site of injury (primary allodynia/hyperalgesia) but also in the surrounding healthy tissue (secondary alldynia/hyperalgesia). The mechanisms responsible for the generation of primary and secondary allodynia/hyperalgesia are quite different. Primary alldynia/hyperalgesia results from an increase in the transduction sensitivity of high threshold C and Aδ nociceptive fibers innervating the site of injury (peripheral sensitization) (25, 30, 41, 42). In contrast, secondary alldynia/hyperalgesia results from an increase in excitability of dorsal horn neurones of the spinal cord (central sensitization), which is induced by nociceptive C fiber activation at the site of injury.

Central sensitization is sustained by phosphorylation of N-methyl-D-aspartate (NMDA) receptors expressed in dorsal horn neurones (42, 44, 45, 48, 49), which induces changes in the receptor kinetic properties and increases its sensitivity to synthetically released glutamate (7, 50). This leads to an increase in the excitability and the receptive fields of the spinal neurones and results in recruitment and amplification of both nonnociceptive and nociceptive inputs from the adjacent healthy tissue (31, 46, 47). Evidence that central sensitization is a component of somatic hyperexcitability has been provided by well-established models of human somatic pain. Cutaneous nociceptor activation with capsaicin or mustard oil induces secondary alldynia/hyperalgesia with demonstrable increases in afferent pathway sensitivity (23, 24), which are prevented or reduced by prior administration of NMDA receptor antagonists (20, 21).

Animal studies involving direct electrophysiological recordings from spinal neurones suggest that central sensitization may also be an important mechanism in visceral hypersensitivity (13, 14, 28). Similar studies in humans have not so far been possible because of a lack of suitable noninvasive neurophysiological techniques to assess visceral afferent pathways.

We have now developed a human model of esophageal hypersensitivity, in which acid exposure of the
distal human esophagus leads to the development of hypersensitivity in the proximal non-acid-exposed esophagus (secondary allodynia) (33). Although the mechanism responsible for this is likely to be central sensitization, there is no experimental evidence to show a change in the responsiveness of central neurons.

It is now possible to study the central processing of esophageal afferents by recording cortical evoked potentials (EP) in response to esophageal stimulation (10, 11, 16, 19, 37, 40). Cortical EP recording is a noninvasive neurophysiological technique, commonly used to investigate central nervous system (CNS) processing after activation of peripheral nerves (8). Our studies have shown that esophageal-evoked potentials (EEP) can be used to assess the integrity and characteristics of central pathways activated by esophageal afferents and that these responses directly reflect the known properties of esophageal afferents reported in previous animal studies (18).

The aim of our study was therefore to investigate the mechanisms underlying acid induced hypersensitivity of the human esophagus. We tested the hypothesis that if the secondary allodynia after acid exposure of the human esophagus results from central sensitization, then the increase in excitability within the central afferent pathway would be manifest as an increase in the conduction velocity of the EEP elicited from the non-acid-exposed esophagus.

METHODS

Subjects

Ten healthy volunteers (9 male), mean age 30 yr (range 21–48 yr), were studied on two different occasions at least 1 wk apart. All had normal esophageal motor function as shown by esophageal manometry, had no previous or current upper gastrointestinal (GI) symptoms, and were not taking any medication. The study protocol was presented to, and approved by, the Salford and Trafford Health Authority Ethics Committee, and informed written consent was obtained from each subject.

Esophageal Stimulation

Electrical stimulation of the esophagus was performed using a pair of platinum, bipolar ring electrodes (2-mm electrodes with an interelectrode distance of 1 cm) sited 20 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 (Gaeltec, Dunvegan, UK). The electrodes were connected to a constant current stimulator (model DS7, Digitimer, Welwyn Garden City, UK). The interelectrode impedance was adequately recorded (2). A reference electrode was positioned on the left ear lobe, and a ground electrode was placed on the neck. Scalp electrode impedance was <5 kΩ throughout the study, and recordings were performed in a quiet room with the subject semirecumbent, awake with eyes open, and asked to minimize eye movements.

For data acquisition, a CED 1902 programmable signal conditioner (Cambridge Electronics Design) was used. Display and analysis used the SIGAVG program, version 6.04 (Cambridge Electronics 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 used to reduce interference from the mains 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 the prestimulation time. An automatic artifact reject was used to prevent contamination from eye blinks and swallows. After each stimulation, individual epochs of data were saved, and the average of the run could be viewed during acquisition.

Acid/Saline Infusion

HCl or NaCl (0.15 M) was infused into the lower esophagus, 3 cm above the lower esophageal sphincter (LES), through a 1-mm-diameter catheter at a rate of 8 ml/min via an infusion pump (IMED 960 Volumetric, Milton Trading Estate, Abingdon, UK). This concentration of HCl used is greater than that used in the Bernstein test (0.1 M) (4) because somatic studies indicate that nociceptive fiber activation is essential for the induction of hypersensitivity (36) and our previous studies have shown that this concentration is sufficient to induce pain in healthy subjects (33), thus reflecting esophageal nociceptive fiber activation.

pH Monitoring

Esophageal intraluminal pH was monitored with a two-channel monocrystal antimony pH probe (991–9011, Synectics Medical, Enfield, UK). The pH electrodes were 15 cm apart and connected to an ambulatory pH recorder (Synectics Mark III, Digitrapper, Synectics Medical). A reference electrode was attached to the subject’s neck. At the beginning and end of each study, the pH electrodes were calibrated by using standard solutions of pH 1 and 7. Analysis of the recorded data was performed with commercially available computer software (Oesophagogastrosoft 1987, Synectics Medical).

Assessment of Esophageal Sensation to Electrical Stimulation

Sensation intensity. Sensation was scored using a six-point categorical rating scale (1, unaware; 2, slight sensation; 3, definite sensation; 4, slight discomfort; 5, uncomfortable; and 6, painful). There is a direct relationship between the score and the values for amplitude and latency of EEP components, with the amplitude increasing and latency decreasing with increasing sensation (16, 17). Subjects were also asked to describe sensory and pain thresholds using a list of words from the McGill Questionnaire (29).

Threshold determination. The sensory (score 2) and pain thresholds (score 6) were determined three times, increasing...
the intensity in increments of 2 mA. The mean of these three readings was calculated, then the 75% value, in mA, of the difference between sensory threshold (0%) and pain threshold (100%) was elucidated. This method has previously been shown to provide adequate stimulation of esophageal afferents, as values for latency do not decrease significantly with greater stimulation intensities (16, 17).

**Definition of Terms**

**EEP latency.** The interval, in milliseconds, between the onset of the stimulus and the peak of each potential.

**EEP amplitude.** The potential difference, in microvolts, between the maximal positive and maximal negative deflection.

**Sensory threshold.** The minimum value, in milliamps, at which the subject first perceived the stimulus (sensation score 2).

**Pain threshold.** The minimum value, in milliamps, at which the subject first described the stimulus as painful (sensation score 6).

**Data Analysis**

**Effect of acid and saline on sensation score.** For each subject, the mean sensation score for the four EEP runs before and after the infusion of acid and saline was determined. Comparisons of the sensation scores before and after the infusion were made using Wilcoxon paired sample test.

**Effect of acid and saline on pain threshold.** The Wilcoxon paired sample test was used to make comparisons of pain threshold pre- and postinfusion for the acid and saline studies.

**Effect of acid and saline on EEP.** The EEP data acquired both before and after the infusion were analyzed by an independent operator experienced in neurophysiological analysis, who remained unaware of the origin of the data. For each data set, the latencies and amplitudes were measured. Comparisons of latencies and amplitudes before and after each infusion were made using the Wilcoxon paired sample test. Values in the text are expressed as means ± SE.

**RESULTS**

**Sensation Evoked by Electrical Stimulation**

Upper esophageal sensation was located between the suprasternal notch and the sternal angle (dermatome T1 and T2). The most commonly used words to describe sensory threshold were “pulsing” and “tingling,” whereas for pain threshold they were “sharp,” “stabbing,” and “throbbing.”

**Sensations Evoked by Acid Infusion**

In all subjects, a burning pain was induced in the lower chest within 15 min of the commencement of acid infusion, which resolved within 30 min of stopping the infusion. No symptoms were induced by the saline infusion.

**Esophageal pH**

Acid infusion always reduced pH to <2 in the lower esophagus, whereas the pH in the upper esophagus always remained >5, indicating that no acid exposure occurred at the site of stimulation. Saline infusion induced no reduction in intraluminal pH in either the lower or the upper esophagus.

**Pain Threshold**

The effects of acid and saline infusion on esophageal pain threshold in all ten subjects are demonstrated in Fig. 1. After acid infusion there was a reduction in mean pain threshold from 78.3 ± 8.4 to 68.7 ± 8.7 mA (reduction of 9.6 ± 2.4 mA, P = 0.01), whereas after saline infusion there was no change in threshold (pre-infusion, 70.5 ± 7.9 mA; postinfusion, 74.4 ± 7.7 mA; P = 0.2).

**Sensation Intensity**

There was an increase in the sensation score of the preestablished 75% stimulus intensity after acid infusion from 4 ± 0.1 to 4.7 ± 0.2 (P = 0.01), indicating that sensation within the proximal esophagus had intensified after acidification of the distal esophagus. After saline infusion there was no change in the sensation score (P = 0.2).

**EEP**

**Morphology.** Figure 2 shows the morphology of the potentials obtained from each subject. EEP consisted of a triphasic morphology with two positive (P1, P2) and one negative (N1) components. This morphology was consistent before and after both acid and saline infusions.

**Latency.** The results demonstrating the effect of acid and saline on EEP latency are shown in Fig. 3. Acid. There was a reduction in latency of the N1 component of 10.4 ± 2.3 ms (P < 0.01) and of the P2 component of 15.8 ± 5.3 ms (P < 0.01) with no change in the P1 component (P = 0.5). However, comparison with the P1 component after saline infusion revealed a relative reduction in latency of 9.6 ± 3.0 ms (P = 0.03) after acid infusion.
SALINE. In contrast, after saline infusion there was an increase in latency of the P1 component of $7.3 \pm 2.7$ ms ($P = 0.01$) and of the N1 component of $3.9 \pm 1.3$ ms ($P = 0.03$), with no change in the P2 component ($P = 0.2$) (Fig. 3B).

Amplitude. ACID. After acid infusion there was no change in the EEP amplitude ($P = 0.1$), with P1-N1 and N1-P2 components being $6.9 \pm 1.6$ and $12.7 \pm 2 \mu V$, respectively.

SALINE. After saline infusion, there was a reduction in the EEP amplitude of the N1-P2 component from $7.3 \pm 2.1$ to $5.5 \pm 1.7 \mu V$ and the P1-N1 component from $12.3 \pm 3.3$ to $8.1 \pm 1.7 \mu V$, but this was not statistically significant.

DISCUSSION

Stimulation of the GI tract at intensities innocuous to healthy controls can often reproduce symptoms in patients with FGD (26, 27, 32). The presence of a heightened responsiveness to GI stimulation (visceral hypersensitivity) in FGD is, therefore, treated as an important biological marker of the condition; however, its mechanisms remain uncertain. In this study, we have shown that experimental acidification of the healthy human distal esophagus induces hypersensitivity in the proximal, non-acid-exposed esophagus (secondary allodynia) that is associated with a concurrent increase in the EEP conduction velocity. These findings provide the first electrophysiological evidence that an increase in central visceral afferent pathway sensitivity is important mechanistically in generating visceral hypersensitivity in humans.

Using the model of esophageal hypersensitivity outlined in this study, we previously showed that the mechanism of this increased central afferent pathway sensitivity is likely to be central sensitization of spinal dorsal horn neurones. This is because, despite confinement of acid to the distal esophagus, hyperalgesia and allodynia are induced not only in the adjacent non-acid-exposed proximal esophagus, but also in the area

Fig. 1. Shows the individual data in all 10 subjects for effect of distal esophageal acid (A) or saline (B) infusion on proximal esophageal pain threshold. After acid infusion, there is a reduction in pain threshold in the non-acid-exposed esophagus, suggesting secondary allodynia/hyperalgesia is induced. After the infusion of the control (saline), no reduction in pain threshold occurs.

Fig. 2. A representative esophageal-evoked potential (EEP) response before and after an infusion of either acid (A) or saline (B) in 1 individual. It is evident that, although there are no significant differences after saline infusion (Fig. 3B), after acid infusion there are reductions in EEP latencies, suggesting an increase in sensitivity within the afferent pathway (Fig. 3A). No such changes are seen after saline infusion.
Fig. 3. The effect of lower esophageal acid or saline infusion on latency of EEP acquired from the upper esophagus. A: a reduction in latency of negative (N1) and positive (P2) components evoked by stimulation of the upper esophagus after acid infusion in the lower esophagus. B: an increase in the P1 and N1 latencies acquired from the same region after saline infusion.

of somatic referral on the anterior chest wall (33). This concurrent visceral and somatic pain hypersensitivity, which is well outside the area of acid exposure, must be due to altered sensory processing within the CNS, with central sensitization being the most probable explanation (45). In addition, we have shown that central sensitization may be important in mediating visceral hypersensitivity in functional gut disorders, because patients with NCCP have more pronounced secondary esophageal allodynia after esophageal acidification compared with healthy subjects (33).

Previous studies of visceral hypersensitivity have relied on descriptive methods of reporting visceral sensation (43). Although great care is taken to prevent subjective factors from introducing response bias, no truly objective measures of sensation have so far been reported. We recently showed that EEP are a robust and reproducible method of studying esophageal sensory pathways. Our studies have shown that, as stimulation intensity and sensory perception increases, there is an associated reduction in the latency and increase in amplitude of the EEP components (16–18). This phenomenon is common across all evoked potential modalities and reflects the recruitment of an increasing number of afferents with faster conduction velocities (1). It also allows a correlation of an increase in the reported sensation with an objective, neurophysiological measure, thus reducing the inherent response bias commonly encountered in clinical evaluation of sensation. The fact that after acid and not after saline infusion an increase in sensory perception of esophageal stimulation occurred together with a reduction in the latency of the EEP provides objective evidence that subject reporting bias was not a contributory factor in our study.

Although the observed reduction in evoked potential latency confirms that painful esophageal acidification induces measurable changes in the central visceral afferent pain pathway, further interpretation of the results may help us to understand the mechanisms and cortical regions that contribute to the development of visceral hypersensitivity. A reduction in the latency of an evoked potential component can occur as a consequence of changes occurring at several levels of the central pain pathway. This has been elegantly demonstrated in studies of somatic pain using laser stimulation of the skin (5). Like esophageal stimulation, laser stimulation selectively activates nociceptive afferents, namely thinly myelinated Aδ- and unmyelinated C fibers. The resultant laser evoked potential response shares several other characteristics with EEP in that it habituates over time and is maximally recorded at the vertex and that changes in the latency of components can occur with alteration of the level of vigilance/attention afforded to the stimulus (1, 5).

Because subjects described our sensitizing stimulus as painful, it might be suggested that the change in latency seen after esophageal acidification was due to a shift in the subject’s vigilance toward the esophageal stimulus. However, somatic evoked potential studies have shown that shifts in vigilance/attention can affect the latency of both early and late evoked potential components, probably due to the priming of modality-specific neurones, like those found in the primary sensory cortex (S1), to enhance their functional efficiency (9). Because we only observed changes in the later N1 and P2 components, whereas the early P1 latency remained relatively stable, increased vigilance/attention toward the postacid esophageal stimulus is unlikely to explain the changes seen in our study. However, this mechanism may help to explain the habituation of the EEP after saline infusion during which an increase in the latency of all EEP components occurred, most likely due to a decrease in subject’s attention toward a repetitive stimulus over a long study period.

Previous studies of evoked potentials in other sensory systems have shown that a reduction in evoked potential latency can also occur either due to enhanced recruitment of primary afferents or via an increase in sensitivity of the central afferent pathway (6, 38). The effect seen in our study must be within the central afferent pathway, because there was no acid exposure in the region from which the reduction in EEP latency was demonstrated, so direct changes in the transduction properties of primary afferent neurones rendering them hypersensitive (41) are unlikely to have occurred. An increase in central afferent pathway sensitivity may occur either as a result of an increase in its excitability or via the reduction of inhibitory influences. The reduction in EEP latency in our study is
consistent with central sensitization, as by definition this is an increase in spinal cord excitability induced by C fiber sensory inflow that is manifested as either hyperresponsiveness and/or increase in receptive fields of spinal neurones (46, 47). In our study, acid in the distal esophagus may have triggered C fibers within this region either directly, by activating acid-sensitive ion channels on esophageal afferents, or indirectly, via C fiber activation secondary to an inflammatory reaction in response to esophageal injury. Such changes would result in activity-dependent neuronal plasticity of spinal neurones innervating the distal esophagus (central sensitization) and would allow both previously subthreshold (novel afferent recruitment) and threshold inputs from the proximal non-acid-exposed esophagus to be amplified and induce secondary allodynia and hyperalgesia, respectively.

Functional brain imaging techniques such as functional magnetic resonance imaging and magnetoencephalography have identified a complex network of cortical regions that process gastrointestinal sensation (3, 12, 35). After the induction of central sensitization in our study, the resultant increase in the afferent signal arriving at the cortex would have differential effects, depending on the function of each particular cortical region. For instance, the primary somatosensory cortex (S1) is thought to process the sensory discriminatory aspects of pain and therefore does not play an important role in encoding the intensity of a stimulus (34). However, lesion studies have shown that damage to the secondary somatosensory cortex (S2) and insula can impair a subject’s ability to perceive a painful stimulus and that these regions would play a far more important role in the encoding of pain intensity (34). Although the exact cortical correlates of the EEP response have yet to be satisfactorily delineated, S1, S2, and insula are all activated after esophageal stimulation and are likely to contribute to the EEP response (3, 12, 35). A differential response to increasing stimulation intensities in these regions may help to explain why latency reduction was observed in some of the EEP components (N1/P2) but not others (P1) after acid infusion.

Although we have clearly shown reduction in proximal esophageal EEP latency after distal esophageal acidification, secondary allodynia was not accompanied by an increase in EEP amplitude. This is similar to findings from other evoked potential studies that have shown that reduction in latency is far more sensitive than increased amplitude for demonstrating afferent recruitment (1). This is probably because amplitude is affected by more confounding variables, such as habituation and signal-to-noise quality of evoked potential recording, than is latency (1). Furthermore, it is also known that once the maximum receptor threshold is reached, further increases in stimulation intensity do not increase evoked potential amplitude, despite an increase in perception of the stimulus. This is because the increased perception of the stimulus results from an increase in the frequency of receptor firing rather than an increase in magnitude in receptor response. This is reflected by the reduction in evoked potential latency without a concomitant increase in amplitude, as demonstrated in our study.

In conclusion, the reduction in pain threshold and EEP latency from stimulation of the non-acid-exposed esophagus indicates that secondary allodynia results from central mechanisms involving the recruitment of novel sensory afferent fibers and/or from the increase in excitability within the sensory afferent pathway. Both explanations are consistent with our assertion that central sensitization contributes to visceral hypersensitivity in humans. Our ability to model visceral hypersensitivity in humans and detect changes in the sensitivity of central afferent pathways provides us with a unique opportunity to explore the pathophysiology of FGD. This approach will also allow us to objectively examine the effects of future therapies, such as pharmacological intervention with drugs that specifically block central sensitization (i.e., NMDA receptor antagonists) in patients with central sensitization-mediated visceral hypersensitivity.

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