Cerebral topography of rectal stimulation using single photon emission computed tomography

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Bouras, Ernest P., Terence J. O’Brien, Michael Camilleri, Michael K. O’Connor, and Brian P. Mullan. Cerebral topography of rectal stimulation using single photon emission computed tomography. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G687–G694, 1999.—Central processing of visceral information in humans is incompletely understood. We aimed to demonstrate the feasibility of single photon emission computed tomography (SPECT) and to quantitate the changes in regional cerebral blood flow during rectal distension. Ten healthy volunteers underwent randomized sham and active rectal distensions on separate days, during which cerebral blood flow was assessed by intravenous technetium-99m ethyl cysteinate dimer (99mTc-ECD) SPECT. Three-dimensional coregistration of brain images was used to quantitate activation in four preselected cerebral foci and two control regions. Paired analysis compared blood flow during sham and active distensions. There was increased right anterior cingulate gyrus activity (6.5 ± 2.9%, P = 0.03) with active rectal distension. A 5.4 ± 2.4% reduction in blood flow in the superior parieto-occipital control region (P = 0.04) suggested blood “redistribution” during stimulation. Marked variability in activation of the frontal cortex, thalamus/basal ganglia complex, and mesiotemporal lobe was noted. Thus rectal distention increases activity in the right anterior cingulate gyrus on average; other foci of cerebral activation are quite variable, suggesting a lack of specific cerebral projections during rectal stimulation.

Methods

Human Subjects

Ten healthy volunteers between the ages of 18 and 65 yr old were recruited by public advertisement. All completed the Bowel Disease Questionnaire (BDQ; Ref. 40) and Symptoms Checklist-90-Revised (SCL-90-R; Ref. 14) to exclude volunteers with significant gastrointestinal symptoms (BDQ) or underlying psychological disorders (SCL-90-R). Individuals with previous abdominal or gastrointestinal surgeries except appendectomy, hernia repair, or cholecystectomy were excluded from the study. All participants were off over-the-counter or prescribed analgesics during the 2 mo before the study, except for occasional use (up to 2 tablets per week of nonopiate analgesics). No analgesics or anti-inflammatory medications were allowed during the 2 mo before the study.

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agents were allowed within 48 h of the studies. The protocol was approved by Mayo’s Institutional Review Board, and written informed consent was obtained from each volunteer.

Perception During Rectal Distension

Rectal distensions (see Experimental Protocol) were performed with the use of an infinitely compliant polyethylene balloon attached to a rigid piston barostat apparatus with an inflation rate of 30 ml/s. Perception scores for the sensations of gas, urgency, and pain were recorded by participants with separate 10-cm visual analog scales after sham and active distension procedures.

SPECT Imaging of Cerebral Blood Flow

Technetium-99m ethyl cysteinate dimer (99mTc-ECD) is a radiotracer that is rapidly taken up by the brain. On crossing the blood-brain barrier, 99mTc-ECD loses its lipophilic characteristics and becomes trapped in a manner that reflects relative blood flow in the different regions of the brain. Thus the resulting distribution of 99mTc-ECD yields information on the status of cerebral blood flow at the time of injection. Because rates of elimination of the radiotracer may vary between regions, the technique requires comparison of sham and active conditions, as well as standardizing times of imaging after injection of tracer.

99mTc-ECD (20 mCi) was administered intravenously after the pressure in the barostat system had reached a distension pressure of 52 mmHg. The half-life of the isotope is such that SPECT images can be acquired up to several hours later. During imaging, each volunteer’s head was securely restrained in a head holder. A dual-headed gamma camera system equipped with ultra-high-resolution fan beam collimators (Helix system, Elscint, Haifa, Israel) acquired images from a 20% energy window centered around the 140 keV photopeak of 99mTc-ECD. From each detector head, 120 images were obtained over 360° in a circular orbit every 3° at 15 s per view and were stored in a 128 × 128 matrix.

The planar images were corrected for uniformity and center of rotation variations and were reconstructed by filtered backprojection, with the use of a Metz filter, with power = 3 and cutoff at 0.4 Nyquist. The reconstructed slices were placed into a 64 × 64 matrix with a voxel size of 3.6 mm. Attenuated correction was applied (Chang method, zero order) with an attenuation coefficient (µ) of 0.10 cm⁻¹. The system resolution was ~7- to 9-mm full-width half-maximum (FWHM). Figure 1 is a representative transaxial brain image taken from one SPECT study. Different amounts of cerebral blood flow are represented by different intensities of color. As the intensity scale indicates, brighter (whiter) colors reflect greater blood flow.

Experimental Protocol (Fig. 2)

After an overnight fast, participants emptied their bowels and remained in a darkened, quiet room for 30 min, after which time a polyethylene balloon was placed in the rectum. Eyes were blindfolded and ear plugs were inserted. After a

Fig. 1. Representative transaxial single photon emission computed tomography (SPECT) brain image. Different amounts of cerebral blood flow are represented by different intensities of color. Scale on left (arbitrary units) illustrates that brighter (whiter) colors reflect greater cerebral blood flow, which is marker for cerebral activity.
30-min equilibration period, the participants were randomized to receive either sham or active balloon distension with the use of the barostat apparatus. $^{99m}$Tc-ECD was injected intravenously once the balloon distension pressure reached 52 mmHg, $-40$ mmHg above minimum distending pressure (selected to be in the range that induced moderate pain in a previous study in which the same method was used; Ref. 17). This pressure was then maintained for 3 min. Participants remained isolated with eyes and ears covered (to limit external stimulation) for a total of 10 min from the onset of balloon distension. After this 10-min study period, the balloon was withdrawn and perception scores were obtained as previously described. All SPECT brain images were acquired within 1 h of radiotracr washout, during rectal distension for sham and active studies. All studies were performed by the same investigator (E. P. Bouras) at the same time of day to avoid diurnal variations in pain perception (7).

Analysis of SPECT Images

The analysis of the SPECT images was performed on an off-line Unix-based workstation with the aid of a commercially available image analysis software package (ANALYZE 7.5, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN).

SPECT to SPECT coregistration. With the use of a Chamber distance-based surface matching technique, 1,000 surface points on a binary image that represented the cerebral area of the SPECT images during sham distension were matched to the corresponding points on a binary image that represented the cerebral area of the SPECT images during active distension. A $4 \times 4$ matrix was calculated that best described the three-dimensional (3-D) transformation of the binary images during sham distension to those during active distension (20). This transformation matrix was then applied to the original SPECT images during sham distension to transform them into the same 3-D space of the images during active distension.

SPECT normalization. All data sets were multiplied by a binary image representing their common cerebral area to remove the extracerebral regions of activity. The mean cerebral pixel intensity of the modified SPECT data sets was then measured and normalized to a mean cerebral intensity of 100 with the following equation.

\[ I_n = I_o \times 100 / \text{mean cerebral pixel intensity} \]

in which $I_n$ is normalized pixel intensity, and $I_o$ is original pixel intensity.

Geometric regions of interest. As shown in Fig. 3, standard regions of interest (ROI) were placed over the mesiotemporal lobe, the thalamus/basal ganglia complex, the ACG, and the frontal cortex. In addition, two control ROI, considered on the basis of current knowledge to be uninvolved in the central processing of afferent visceral information, were assessed. They were located in the inferior and superior region of the parieto-occipital cortex.

With the use of the transaxial images, all ROI were placed bilaterally in the two central horizontal cuts of each structure of interest (identified with neuroanatomic and SPECT atlases; Ref. 31) with the use of standardized, predefined templates. The mean pixel count within each of the ROI was measured for both sham and active images and the percent change calculated as follows.

\[ \% \text{change} = \left( \frac{\text{mean count}_{\text{active scan}} - \text{mean count}_{\text{sham scan}}}{\text{mean count}_{\text{sham scan}}} \right) \times 100 \]

Statistical Analysis

The percent change in $^{99m}$Tc-ECD uptake in each cerebral ROI between the sham and active studies was compared for each volunteer using a two-sided paired t-test. Mean values for the relative change (i.e., increase or decrease) recorded in each ROI during rectal distension were then calculated for the entire group. Unless stated otherwise, data in this paper are reported as means ± SE. A P value of <0.05 was considered significant.

RESULTS

Participants

Ten healthy right-handed volunteers were studied (7 females and 3 males). Mean age was 33.5 ± 3.5 yr old (range 20–46 yr). In all participants, responses on the BDQ were within normal limits. Mean SCL-90-R T scores for all subjects were normal (anxiety 44, depression 46, and somatization 42) with a mean general severity index T score of 42 ± 2.4 (normal <63). No complications were encountered during the studies, and all volunteers successfully completed the protocol.

Perception Scores

Figure 4 shows the median (and interquartile) perception scores for the sensations of gas, urgency, and pain during active rectal distension. There was marked interindividual variability in the subjective perception of the standardized rectal stimulus.

Cerebral Activation

Figure 5 pictorially demonstrates cerebral activation during rectal distension for sham and active studies. The bottom left shows a subtraction assessment of the sham from the active distension study. Seen in bright yellow, activated areas (demonstrating uptake of $^{99m}$Tc-ECD greater than 2 standard deviations above the mean value for uptake in the entire brain) can be superimposed onto the SPECT images, yielding an
Figure 3. Quantitation of radioactivity (blood flow) in brain regions involved in visceral sensation. Geometric regions of interest were placed bilaterally in the two central horizontal cuts of each structure with use of standardized, predefined templates. We assessed mesiotemporal lobe, thalamus/basal ganglia complex, anterior cingulate gyrus, and frontal cortex, as well as 2 control areas: one located in inferior region of parieto-occipital cortex (control1) and other in superior region (control2).

Figure 4. Perception scores during rectal distension. Data represent median values with 25th to 75th percentile ranges shown.

**DISCUSSION**

SPECT imaging demonstrates focal cerebral activation in the right ACG during rectal stimulation in healthy humans. We believe this novel technique also illustrates several important points to be considered in the interpretation of past studies and the development of future studies in the emerging field of neuroenteric sensory research.

The cerebral foci evaluated in this study were chosen on the basis of previous investigations and a review of the literature. The ACG, with its extensive connections to various regions of the brain, impacts several aspects of human behavior, including nociception, autonomic function, and emotional responses (as an integral part of the limbic system). It is also involved in other
affective and cognitive functions, such as conditioned emotional learning, motivational assessments, assigning emotional vigor to internal and external stimuli, information processing, and response selection (15, 42). Recent data further suggest that the perceived unpleasantness of noxious stimuli closely parallels changes in ACG activity (33). Specifically, increased unpleasantness corresponds to increased ACG activity.

Together with the ACG, the frontal cortex has been implicated as an active cerebral site during visceral pain (36, 37), with the frontal cortex regulating emotional responses in light of previous experiences. The frontal cortex is activated during visceral and somatic pain in both healthy individuals and patients (6, 9, 16, 36, 37). Linked to other brain regions thought to play a part in sensation, the frontal cortex appears to be associated with the cognitive evaluation of painful sensations (12), verbalization and emotional vocalization (15), recall of negatively charged affective memories (32), and behavioral responses to painful stimuli (29). Silverman et al. (39) further suggested a hypervigilance network involving the right frontal cortex in patients with IBS that was not found in healthy subjects.

As the thalamus receives projections from the ascending spinothalamic and spinoreticular neural pathways that carry afferent visceral information, it likely plays a role in the processing of visceral sensation. As with somatic pain, PET imaging studies of regional brain activation during visceral pain have documented thalamic activation (36, 37). Neuroanatomic experiments indicate that the basal ganglia receive nociceptive information via several different pathways. Nociceptive information may be modulated through a variety of neurotransmitters (10), such as opiates, dopamine,
In our study were chosen on the basis of their apparent lack of involvement in visceral sensation. Despite these findings, we are unaware of definite evidence documenting simple physiological shunting of flow.

There was marked interindividual variability in response to a standardized rectal distension stimulus, both in sensation perception and cerebral activation. An important difference between this study and others investigating rectal distension (6, 39) is that the stimulus, rather than the quality or perception of the sensation, was standardized in our study. By standardizing the nature of the stimulus, it was possible to demonstrate that the central processing (cerebral activation) and conscious awareness (perception) of identical visceral stimuli can be different between healthy individuals.

Our results show that the frontal cortex was activated in several healthy individuals, consistent with findings of other studies investigating both visceral and somatic stimulation (5, 6, 9, 16). Differences in frontal cortical activation between healthy subjects and IBS patients suggested a mechanism for heightened perception in IBS patients, with the frontal cortex serving as part of a hypervigilance network (39). However, a difference in frontal activation between health and irritable bowel syndrome was observed in response to an anticipated rather than an actual painful rectal distension. This emphasizes the importance of avoiding order effect and distension protocols that may introduce a bias, as recommended by Naliboff and Mayer (30). No order effect was noted in our study.

Avoidance of hypervigilance is a particularly difficult problem in this form of research. Our approach to provide a standardized stimulus that was not noxious or alerting to the participants may have contributed to the variation in the brain activation observed in our study. In fact, a recent study on somatic pain, in which stimuli of variable intensity were used, also showed differential patterns of brain activation (13). It is also difficult to standardize the intensity of perceived sensations without bias introduced by the participant’s expectations or hypervigilance (44).

Our experience with SPECT suggests it has several attractive features that are similar to PET imaging. First, the resolution in this system approaches 7 mm, which is similar to that obtained with conventional PET imaging. Second, the strategy described in this paper, including 3-D coregistration, improves the accuracy of the SPECT analysis because the images during sham and active interventions can be more accurately aligned. Third, an emerging field of research demonstrates the utility of binding radioligands to neurotransmitters (4, 18, 21, 22, 26). Combining this technology with SPECT (or PET) provides a unique opportunity to investigate the complex interactions among neurotransmitters and their receptors, both in health and disease states. Such studies may lead to a better understanding of the potential role of various neurotransmitters in visceral sensation or the central effects of several pharmacological agents on visceral sensation.

The major limitation of this current study is the ability to perform only one study per day (given the
required time for radioligand washout). Functional magnetic resonance imaging (fMRI) has emerged as a promising, developing technology within the field of functional neuroimaging. The main advantage is one of improved temporal resolution compared with SPECT or PET, though there are some practical limitations with fMRI. SPECT is more widely available than fMRI or PET, and it is a relatively inexpensive functional neuroimaging technique, rendering it suitable for physiological studies or pharmodynamic experiments.

Refinements of the technique and analysis paradigms similar to those used in this study may also enhance the potential of SPECT imaging in the study of visceral sensation. For example, MRI coregistration could precisely identify subareas of activation, and further studies will help identify which transaxial slices provide the most representative changes in regional brain activation. To avoid bias in the present study, we arbitrarily predetermined the slices to be used in the analysis as the two central cuts through each anatomic area of interest. Nevertheless, this technique shares the limitation of PET in providing only static images during the time of greatest radiotracer delivery to the brain; static images are unlike the dynamic process of brain activity. It is, therefore, possible to underestimate smaller foci of activation with PET or SPECT, and it is also possible that limitations of current methodology for quantitatively specific brain regions with a more accurate region of interest program may account for some of the negative results in these imaging studies. For example, it is surprising that studies using PET (39) or SPECT failed to demonstrate thalamic activation despite the report of painful sensation.

In summary, our data suggest that SPECT, with novel methods to align coregistered images and quantify differences after normalization of isotope load, identifies regions of cerebral activation during rectal distension in humans. Although significant right ACG activation was found in the entire group, the foci of cerebral activation were quite variable among individuals exposed to a standard distension stimulus used in an attempt to avoid hypervigilance or perceptual bias; in our study, 2 of 10 healthy subjects demonstrated activation in the right frontal cortex, and 5 of 10 demonstrated activation in the left frontal cortex. These data, however, contrast the previous report that frontal activation is restricted to patients with IBS (39). Preliminary data using fMRI confirm that several other cortical regions (e.g., right and left dorsolateral prefrontal, orbitofrontal, and insular) and thalamus are activated during rectal distension in healthy subjects (5, 11). Further neuroenteric sensory research is needed to more precisely characterize cerebral function during visceral sensation. We may then gain a better understanding of the potential differences between health and disease states. However, this field of research remains challenging in view of the methodological difficulties, the potential ease with which the data can be biased by the nature of the stimulus, and the relatively simplistic assumption that blood flow mark-

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REFERENCES


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