Neurophysiological evaluation of healthy human anorectal sensation

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THE PATHOPHYSIOLOGICAL MECHANISMS responsible for disordered lower gastrointestinal (GI) function remain unclear. It is now well established that the regulatory control of GI function occurs both locally (intrinsic nervous system) and centrally (extrinsic nervous system) at the spinal cord, brain stem, and cerebral cortex. Alterations to any aspect of this brain-gut axis, whether motor, sensory, or psychosocial, can lead to significant GI dysfunction. In the anorectum the majority of tests available to assess function are limited to measurements of anorectal sensory function but provide virtually no detailed information about the neurophysiological properties of the extrinsic afferent pathways. Detailed assessment of these pathways isincreasingly being recognized as important especially in patients with functional GI disorders (FGDs) such as irritable bowel syndrome, who are suspected to have a primary sensory deficit that causes them to perceive normal innocuous physiological gut stimuli as being noxious often in conjunction with lower abdominal pain and intestinal dysmotility (24). The causes of these symptoms remain elusive, but current speculations include visceral hypersensitivity, caused either by a barrage of noxious stimuli increasing the input to neurons in the dorsal horn resulting in central sensitization or by psychological factors causing hypervigilance to bodily sensation. As a result, previously innocuous stimuli become capable of evoking pain (allodynia) and noxious stimuli produce increased pain (hyperalgesia).

This hypersensitivity has been described in patients with irritable bowel syndrome (5, 19, 20) in response to balloon distension. However, the exact trigger for its development is unknown. Thus a quantitative assessment of sensory perception with reproducible methods would be valuable to assess abnormal pain perception and help understand the mechanisms responsible for symptom generation.

Detailed assessment of sensory function in the anorectum is complicated by the convergence of two different types of innervation: visceral afferents in the rectum and the somatosensory pudendal nerve in the anal canal. Accurate evaluation is reliant on an artificial stimulus that attempts to mimic normal physiological processes enabling activation of specific afferent pathways with a strong enough intensity to induce conscious sensation. Various methods have been used to induce visceral sensation; these include barostat (9), rapid balloon distension (7), and electrical (10) and thermal stimulation (6). After delivery of the stimulus there is then a reliance on the subjects’ ability to accurately and reproducibly describe the sensation experienced. Although great care is taken to eliminate subjective factors from introducing response bias it remains difficult to objectively measure sensation in patients using descriptive methods alone. In an attempt to reduce this subjectivity the recording of cortical evoked potentials has been developed as a method of examining the anorectal afferent pathway in conjunction with descriptive measures of reporting pain. This technique employs a brief sensory stimulus in the organ of interest, in this case the anorectum, whereas cortical responses are measured by recording the EEG via surface electrodes placed on the scalp. However, gut stimulation with barostat, manual balloon inflation, or thermal probe cannot be easily utilized for this technique because the onset time of the induced stimulus is too slow. The remaining methods that are available for inducing visceral sensation to measure evoked potentials are therefore electrical stimulation (ES) and rapid balloon distension (RBD) (10), but the reproducibility of these potentials are therefore electrical stimulation (ES) and rapid balloon distension (RBD) (10), but the reproducibility of these...
tests and details of their stimulus characteristics remain to be established. The aim of our study was therefore to perform a comprehensive evaluation of anorectal sensation in healthy subjects by assessing anorectal sensory and motor function and in the same group of subjects assess the visceral afferent pathway by means of rectal evoked potentials (REP). We specifically aimed 1) to assess tolerance and type of sensation evoked by ES and RBD and to establish within- and between-occasion (separate days) reproducibility and 2) to measure REPs using the most reproducible test for inducing painful sensations (as determined by aim 1).

**MATERIALS AND METHODS**

**Subjects**

Eight individuals were recruited [4 women, 4 men; median (range) age 35 yr (18–48 yr)]. All were healthy adults with normal anorectal manometry (as determined by this study), had no current or previous GI symptoms, and were not on any medication. Female subjects were studied during the follicular stage of the menstrual cycle to reduce the potential gonadal hormonal influence on nociceptive processing (8). The study protocol was approved by the Salford and Trafford Health Authority Research Ethics Committee, and informed, written consent was obtained from all subjects.

**Physiological Techniques**

**Anorectal manometry.** This was performed using two separate solid-state custom-designed 3-mm diameter catheters (Standard Instruments, Karlsruhe, Germany). The rectal catheter had four sensors positioned longitudinally along the catheter spaced 1, 3, 5, and 15 cm from the 0 reference point (Fig. 1A). A 6-cm section of silicone tubing was tied onto the catheter with catgut so that it was positioned over the fourth sensor and could be used as a rectal inflation device. The anal catheter (Fig. 1B) had four sensors positioned in a circumferential array at 90° to each other 1 cm from the 0 reference point. Signals were amplified (Standard Instruments) passed via a PowerLab system 16SP (AD Instruments) to a computer running PowerLab software sampling at 10 kHz.

**Electrical stimulation.** This was delivered to the rectum and anal canal via a catheter constructed from polyurethane and nonmagnetic metal materials that are galvanically plated with pure gold at the stimulation zone (Standard Instruments). The average diameter of the tubing is 4.6 mm (14 French) increasing to 4.9 mm where the catheter houses two pairs of ring electrodes (inter-electrode distance 2 cm). The catheter was connected to a constant-current stimulator (model DS7A, Digitimer) where the available current was limited by a preset (safety) value of 200 V. The stimulator was attached to a trigger generator (model DG2, Digitimer) that enabled a preset stimulation frequency to be delivered.

**Rapid balloon distension.** A 33-cm Ryles tube CH/FG16 (5.3 mm diameter) (Pennine Healthcare, Derby, UK) with a 3-cm latex balloon secured with Marsilk 2/0 to one end was used. The catheter was attached via a three-way tap to a specially designed inflator device (Medical Physics Department, Hope Hospital, Manchester, UK) that was capable of rapidly distending the balloon. Anal or rectal stimuli were delivered to the subjects via the catheter at a frequency of 0.5 Hz, with an inflation time of 250-ms duration, at intensities between 0 and 30 pounds per square inch (psi) (0–206 kPa), which gave the equivalent balloon volume of 0–140 ml.

**Volume of Air Within the Balloon**

The volume of air within the balloon was derived from a laboratory-based measurement from the driving pressure of the RBD pump and the volume measured within the balloon catheter calculated by polynomial second order nonlinear regression and yielded a curve with excellent fit ($r^2 = 0.99$).

**Manual balloon distension.** A preassembled catheter consisting of a 2-mm-diameter polyethylene tube (Lectro-cath, Vygon), with an attached 5-cm-long latex balloon was used for rectal balloon inflation manually. The catheter was attached to a three-way tap and 100-ml syringe.

**Rectal evoked potentials.** These were recorded using two silver-silver chloride surface electrodes, which were applied to the scalp with electrode paste (Elefix, Nihon Kohden). These data were acquired using a CED 1902 programmable signal conditioner (Cambridge Electronic Design).
bridge 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 mV. The band-pass 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 4 KHz, and the recording epoch was 1 s in duration. The first 200 ms of the epoch constituted 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 as well as visual inspection of each sweep.

**Stimulation Perception and Intensity Rating**

The stimulation delivered was assessed for urge intensity, pain intensity, and unpleasantness using a visual analog score (VAS). Word anchors for pain and urge were described as weak, mild, moderate, strong, and intense. The word anchors for the unpleasantness VAS were mild, discomforting, distressing, horrible, and excruciating.

To assess the multidimensional qualities of anorectal pain subjects were asked to describe the pain or maximum sensation experienced using a list of words from the shortened form of the McGill Questionnaire (18) and draw on the location on a body map.

**Individual Anxiety Assessment**

The Spielberger State-Trait Anxiety Inventory (STAI) (12) was used to assess subjects’ anxiety levels. This is considered the definitive instrument for measuring anxiety in adults and clearly differentiates between the temporary condition of “state anxiety” (STAI-S) and the more general and longstanding quality of “trait anxiety” (STAI-T). The STAI-S evaluates how respondents feel at a particular time and is a sensitive indicator of changes in transitory anxiety experienced by the subject, such as during one particular type of stimulation. Both STAIT and TRAIT assessment consists of completing a 20-point weighted questionnaire.

**Experimental Protocols**

Subject preparation. To avoid any irritant-induced anorectal sensitization, no bowel preparation was used; however, subjects were asked to empty their rectum before starting the study. Subjects were positioned in the left lateral position with knees and hips flexed to 90° for the manometry and sensory assessments. During measurement of REP subjects sat semirecumbent in a reclining chair. On each testing occasion (3 in total) all procedures were performed in the same order: manometry, manual balloon distension, ES, then RBD.

Preliminary studies. CATHETER POSITION. An initial study was performed to determine whether ES mucosal contact was affected by the presence of a closely aligned inflated balloon. Stimulations were performed to determine the electrical sensory threshold and pain threshold with six conditions: 1) no balloon present alongside catheter, 2) empty balloon, and balloon inflated with 3) 5 ml, 4) 10 ml, 5) 15 ml, and 6) 20 ml of air inside. There was no significant difference in rectal sensation during ES with the stimulating catheter on its own or with the stimulating catheter with a balloon present, either empty or containing air. For the rest of the study no balloon was used.

STIMULATION FREQUENCY. A further preliminary study was performed using ES and RBD to determine the rate of stimulation to be used for the sensory assessments. Stimulation rates of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1, 2, 3, and 4 Hz were tested for determining sensory threshold and pain maximum tolerated sensation. Stimulation frequencies above 0.5 Hz reduced the level at which pain maximum tolerated sensation was reported, increased the urge with RBD, induced urge with ES, and added more variability to the results. As a result, stimulation intensities above 0.5 Hz were not used in this study.

Anorectal manometry. CATHETER PLACEMENT. The rectal catheter was lubricated, inserted, and orientated such that reference point 0 was at the anal verge, allowing the sensors to be positioned with a posterior orientation. This then enabled one sensor to record rectal pressure and three sensors to record anal canal pressure. The anal catheter was positioned in the high-pressure zone of the anal canal. Once each probe was in the correct position, surgical tape was applied to secure it firmly in place. Subjects were left to rest for 10 min to allow the traces to stabilize, and the catheter was not touched again until the pressure measurements had been acquired. All measurements were repeated five times. Initially, resting anorectal pressures were recorded for a 20-s period. Subjects were then asked to perform a cough maneuver in which they produced a short sharp cough as hard as possible. Pressures were recorded in the rectum and anal canal. Subjects were then asked to bear down, as if to simulate defecation. After this the rectal inhibitory reflex was then evaluated by rapidly inflating the rectal balloon to examine the integrity of the myenteric plexus between the rectum and anal canal. This catheter was then removed and the anal catheter was positioned within the high-pressure zone in the anal canal. Subjects were again rested, and after the pressures had stabilized they were initially instructed to squeeze the anal sphincter as tightly as possible and to hold it until asked to relax. Strong verbal encouragement was given and a rest period of 30 s was allowed between each contraction. After this the subjects were asked to squeeze as tightly as possible and to maintain the squeeze for as long as possible. This duration and anorectal pressure profile was recorded. This catheter was then removed.

**Measurement of Sensory Perception**

**Electrical stimulation. RECTAL SENSORY THRESHOLD.** The electrical stimulating catheter was taped in position so that one pair of electrodes was positioned in the anal canal and one in the rectum (2 and 10 cm from anal verge). The catheter was then attached to the stimulator. For both anal and rectal ES stimuli were delivered to the subjects at a frequency of 0.5 Hz, with square wave pulses of 500-μs duration, at intensities between 0 and 100 mA. Rectal sensory threshold (RST) was defined as the stimulus intensity when the subjects first became aware of a definite sensation within the rectum using an ascending method (2-mA increments). This was repeated five times, and a mean RST was calculated for each subject.

RECTAL PAIN THRESHOLD. The stimulus intensity was then increased from RST in 2-mA increments until rectal pain threshold (RPT) was reached; this was defined as the stimulus intensity at which the subjects first reported pain. This was repeated five times, and a mean RPT was calculated for each subject.

After RST and RPT were determined, stimulation intensities that were 25, 50, and 75% of the difference between sensory threshold (ST) and maximum tolerated intensity were then identified. For example, if ST was reported at 20 mA and maximum tolerated intensity at 80 mA, then 25, 50, and 75% were calculated as 35, 50, and 65 mA, respectively. This methodology has previously been validated (11) so that despite variability in intersubject sensory and maximum tolerated thresholds the stimulation intensity increased by the same factor and thus allows for comparison across subjects.

Stimulations were then delivered to the subjects in both an ascending and randomized order, five stimulations at each of the five levels (ST, 25%, 50%, 75%, and pain). For all modalities in the rectum and anal canal stimulations were delivered by the same protocol.

ANAL SPHINCTER SENSORY THRESHOLD. Sphincter sensory threshold (SST) was defined as the stimulus intensity when the subjects first became aware of a definite sensation when using an ascending electrical stimulation method (0.2-mA increments). This was repeated five times, and a mean SST was calculated for each subject.

ANAL SPHINCTER PAIN THRESHOLD. The stimulus intensity was increased from SST in 0.2-mA increments until sphincter pain threshold (SPT) was reached; this was defined as the stimulus intensity...
when the subjects first reported pain. This was repeated five times, and a mean SPT was calculated for each subject.

Rapid balloon distension. RECTAL SENSORY THRESHOLD. The catheter was positioned in the rectum 10 cm proximal to the anal verge and taped in position. This was then attached via a three-way tap to the inflator device. RST was determined by ascending balloon distensions of 1-psi increments to when the subjects first became aware of a definite sensation. This was repeated five times, and a mean RST was calculated for each subject.

RECTAL PAIN THRESHOLD. The stimulus intensity was increased from ST in 1-psi increments until RPT was reached; this was defined as the stimulus intensity when the subjects first reported pain or maximum tolerated stimulation. This was repeated five times, and a mean SPT was calculated for each subject.

ANAL SPHINCTER SENSORY THRESHOLD. The catheter was withdrawn into the anal canal and positioned 1 cm proximal to the anal verge. SST was determined similar to the rectum, with ascending balloon distensions of 1-psi increments to when the subjects first became aware of a definite sensation. This was repeated five times, and a mean SST was calculated for each subject.

ANAL SPHINCTER PAIN. The stimulus intensity was increased from SST in 1-psi increments until SPT was reached; this was defined as the stimulus intensity when the subjects first reported pain or maximum tolerated stimulation. This was repeated five times, and a mean SPT was calculated for each subject.

Manual balloon distension. Because manual balloon distension is the standard method of determining rectal sensation, this methodology was also examined. The preprepared catheter was lubricated, gently inserted into the rectum, and taped in position so that the balloon lay 10 cm from the anal verge. The catheter was attached to a syringe via a three-way tap and inflated at a rate of ~10 ml/s up to a maximum volume of 300 ml. During this, subjects were provided with a chart and were asked to rate the sensations at three levels: first sensation, desire to defecate, and maximum tolerated volume. The volumes of air in the balloon catheter in the rectum were recorded at each stage, and subjects were asked to describe the sensation at maximum tolerated volume. This was repeated five times.

Rectal evoked potentials. After the anorectal physiology studies, all subjects went on to a rectal evoked potential study using ES, which appeared to the most robust stimulus. All recordings were performed with the subject semirecumbent and awake with eyes open and fixed onto a stationary object. Subjects were requested to minimize eye movements and swallowing. Before attachment of the electrodes, the scalp was cleaned with alcohol wipes and scalp impedance was maintained at <5 kΩ by applying a preparation paste (Omniprep, Weaver & Aurora). With the use of the international 10–20 system of electroencephalograph electrode placement (14), the active electrode was positioned at the vertex and the reference electrode was positioned on the ear lobe. An additional ground electrode was positioned on the neck. The rectal stimulating catheter was then repositioned 10 cm proximal to the anal verge and attached to the electrical stimulating device described earlier. RST and RPT were determined, and RST was labeled as 0% and RPT as 100%. Stimulus intensities that were 25, 50, and 75% of the difference between RST and RPT were then identified. In total, the subjects received 200 stimulations at each of the five levels, which were then averaged to enhance signal clarity. This was split into four runs, two on each testing occasion (1 wk apart). This was done because four runs in one testing session (1,000 stimuli) were too long for the subject to maintain a comfortable posture, minimize eye movements, and attend to the stimulus. Amplitudes and latencies were examined by averaging the 200 stimuli acquired at the five stimulation intensities. Previous work has shown this to be the optimal number of stimuli needed to achieve the best signal-to-noise ratio and good clarity of all the cortical evoked potentials components (11).

Stimulation perception and intensity rating. During the ES and RBD studies, subjects were asked to rate the stimulation delivered by using a VAS and were assessed for their ability to grade the stimulations by scoring of the sensations at the time every stimulation occurred. To minimize the effects of anticipation, subjects received stimulations both in an ascending stepwise fashion and in a randomized order.

Individual anxiety assessment. All subjects completed a STAI questionnaire at the beginning of all of the studies and a STAI-S during each different mode of stimulation at the time of pain/maximum stimulation.

Data Analysis

Recorded data were transferred to Graph Pad Prism (San Diego, CA) statistical program for nonparametric analysis. Repeatability of tests was assessed by the standard deviation of repeat measurements and the intraclass correlation coefficient (ICC) (3, 4). ICC was used for the assessment of sensation because the two techniques (ES and RBD) have different measurement scales. Polynomial second order nonlinear regression was used to determine the volume within the balloon at a known pump driving pressure. All data are expressed as median (range). All REP recordings are displayed by using common neurophysiological convention, i.e., a negative potential is displayed as an upward deflection (13). For each protocol the average REP for 200 stimulations were analyzed. Synchronized real-time data (i.e., volume, mA, and perception ratings) from the anorectal stimulations were analyzed to assess the relationship between the size of the stimulus and the magnitude of the perception rating. The level of significance for all calculations was set at the 95% confidence level ($P < 0.05$).

RESULTS

All subjects completed all the protocol without adverse events.

Manometry

All subjects demonstrated normal anorectal manometry according to previously published data (2, 21). Anal sphincter pressures [maximum anal resting pressure; median (range)] was 62 mmHg (56–78 mmHg). Maximum squeeze pressure was 189 mmHg (96–352), and sustained squeeze pressure was 86 mmHg (26–145 mmHg). The median (range) duration of the sustained squeeze was 39 s (19–118 s). Pressures recorded were lower in women but not significantly different from that in men. Rectoanal inhibitory response was present in all subjects. The median (range) volume of rectal air required to elicit the anal relaxation was 30 ml (20–40 ml). The median (range) reduction in pressure that was measured in the anal canal was 22 mmHg (11–50 mmHg).

Sensory Perception

Reproducibility with ES and RBD. BETWEEN OCCASION. Both ES and RBD of the rectum demonstrated acceptable levels of reproducibility (ICC > 0.6) over three separate occasions (Fig. 2) at pain and maximum tolerated volume but was highly variable at sensory threshold (Table 1). ES of the sphincter was reproducible at pain (Fig. 3) but was highly variable at sensory threshold (ICC 0.12). RBD of the sphincter was also highly variable at both pain (ICC 0.09) and sensory threshold (ICC 0.1) (Table 1).

WITHIN OCCASION. For both RBD and ES within-occasion reproducibility was good for both sphincter and rectum (Table 1).
Reproducibility with manual balloon distension. Both reports of maximum tolerated volume and the sensation of the desire to defecate demonstrated similar and clinically acceptable levels of reproducibility over three separate occasions (Table 2). By contrast, the volume to first sensation had low ICC, indicating poor levels of reproducibility. Within-occasion reproducibility was similarly poor (Table 2).

Stimulus characteristics. ES and RBD provoked different sensations both within the rectum and sphincter. By use of the McGill Pain Questionnaire, ES in the rectum was described as a sharp burning sensation located posteriorly at the upper edge of the natal cleft whereas ES in the sphincter was described as a sharp pricking local sensation. In contrast, RBD was described as a duller pulsing sensation in both rectum and sphincter, more longer lasting than ES but not as clearly localized. ES invoked pain in all subjects; by contrast, RBD rarely caused pain and more commonly produced an overwhelming urge to defecate (Fig. 4). In the rectum all subjects reported significantly higher scores for unpleasantness during RBD than ES ($P < 0.01$).

Volume of air within the balloon. The volume of air median (range) within the balloon to produce the maximum tolerated sensation for RBD rectum was 82 ml (63–120 ml), for RBD sphincter it was 77 ml (24–87 ml), and with manual balloon inflation of the rectum it was 168 ml (106–304 ml).

<table>
<thead>
<tr>
<th>Stimulation Mode</th>
<th>Stimulation Site</th>
<th>ST</th>
<th>SDRM</th>
<th>Pain/MTV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ICC</td>
<td>SDRM</td>
<td>ICC</td>
</tr>
<tr>
<td><strong>Between occasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>Rectum</td>
<td>0.25</td>
<td>5.9 mA</td>
<td>0.82</td>
</tr>
<tr>
<td>ES</td>
<td>Sphincter</td>
<td>0.12</td>
<td>2.3 mA</td>
<td>0.79</td>
</tr>
<tr>
<td>RBD</td>
<td>Rectum</td>
<td>0.37</td>
<td>0.5 psi</td>
<td>0.73</td>
</tr>
<tr>
<td>RBD</td>
<td>Sphincter</td>
<td>0.10</td>
<td>2 psi</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Within occasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>Rectum</td>
<td>0.9</td>
<td>1.7 mA</td>
<td>0.95</td>
</tr>
<tr>
<td>ES</td>
<td>Sphincter</td>
<td>0.95</td>
<td>0.49 mA</td>
<td>0.97</td>
</tr>
<tr>
<td>RBD</td>
<td>Rectum</td>
<td>0.6</td>
<td>0.4 psi</td>
<td>0.88</td>
</tr>
<tr>
<td>RBD</td>
<td>Sphincter</td>
<td>0.96</td>
<td>0.39 psi</td>
<td>0.9</td>
</tr>
</tbody>
</table>

ES, electrical stimulation; RBD, rapid balloon distention. ICC, intraclass correlation coefficient; SDRM, standard deviation repeat measurements; ST, sensory threshold; MTV, maximum tolerated volume. ICC > 0.6 indicates good reproducibility.
Manual balloon inflation and RBD produced similar sensations; however, the duration of the sensation lasted longer with manual balloon inflation than with the RBD technique.

**Individual Anxiety Assessment**

All subjects had a baseline STAIT that was within the normal limits for the adult working population with a median (range) STAIT score 32 (24–38). There was no difference between the prestudy baseline STAIS score on each visit. The STAIS score was significantly higher ($P < 0.05$) during rapid balloon distension of the rectum [median (range) 36 (25–57)] than at prestudy baseline [median (range) 32 (22–39)] (Fig. 5). There was no significant change in STAIS score during RBD of the sphincter, ES of the sphincter, and ES of the rectum compared with prestudy baseline STAIS score.

**Rectal Evoked Potentials**

All REPs reported below refer to ES, because this represented the most robust visceral stimulus for producing pain without the additional influence of increased anxiety.

**REP waveform.** The majority of subjects had similar waveform morphology, apart from one subject who demonstrated an inverted pattern. This pattern was not the early-onset response (<40 ms) as described previously by Loening-Baucke et al. (17) but the late-onset response. Interestingly, this particular subject showed a similar peculiarity when esophageal cortical evoked potential was measured in a previous study, perhaps demonstrating a degree of asymmetrical cortical representation for visceral sensation highlighted with measurements taken from a midline electrode. Without further detailed topographical studies it would be impossible to comment further on the origin of this individual response. There were some individual differences with regard to the early-onset, multiphasic waveforms (<50 ms). Because the primary aim of the study was to assess the REP with regard to stimulus intensity in response to rectal stimulation, the first peak reported in this study was taken as the first clearly detectable positive peak uncontaminated by stimulation artifacts (40 ms poststimulus onset). An illustrative example of the waveform from one subject at each of the five stimulation intensities can be seen in Fig. 6. The

Table 2. Between- and within-occasion reproducibility for manual balloon distension of the rectum at sensory threshold, desire to defecate, and maximum tolerated volume

<table>
<thead>
<tr>
<th>Stimulation Mode</th>
<th>Stimulation Site: Rectum</th>
<th>Sensory Threshold</th>
<th>Desire to Defecate</th>
<th>Maximum Tolerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Balloon</td>
<td>Between occasion</td>
<td>0.08</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Within occasion</td>
<td>0.5</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Fig. 3. Values for sphincter sensory threshold for ES (A) and RBD (C) and pain/maximum tolerated sensation with ES (B) and RBD (D) are reported for the 8 individual subjects on 3 separate occasions. Values reported are the electrical stimulator output (mA) and balloon inflation volume (ml) at the time the subject reported the sensation.
median (range) latency of P1 was 79 ms (47–132 ms) with painful rectal stimulation. The first peak was followed by a broader negative component (N1) with a median (range) latency of 123 ms (96–159 ms) followed by a second positive peak (P2) with a median (range) latency of 189 ms (99–250 ms). Median (range) amplitude of P1–N1, N1–P2 was 7.9 μV (4–17 μV) and 19 μV (14–62 μV).

Stimulation intensity. There was a clear dose-response relationship between intensity of the rectal stimulus and the magnitude of the REP response (Fig. 6). Spearman’s correlation demonstrated a negative linear relationship between latency of the REP and stimulation intensity and a positive linear relationship between the amplitude of P1–N1 and N1–P2 and stimulation intensity (Table 3).

Perception and reporting of stimuli by subjects. As the stimulation intensity increased from sensory threshold to pain the level of reported pain increased in a linear fashion (Fig. 4; Spearman’s correlation r = 1; P < 0.0001).

DISCUSSION

This study provides the most definitive quantitative data to date concerning the perception of anorectal sensation in healthy subjects using two stimulation techniques, ES and RBD. Our results demonstrate that ES provides a robust method of inducing visceral pain whereas RBD (which generally did not produce pain) consistently produced an overwhelming urge to defecate. Although the variability of sensation assessed by both techniques between occasions was similar, overall ES had better reproducibility and was able to reliably produce pain within both the rectum and anal canal. Thus ES was selected as the stimulus of choice for measuring evoked potentials, a minimally invasive method that enabled quantitative characterization of the neurophysiological properties of the rectal afferent pathway.

Reproducibility

Any useful test of function must first be shown to be reproducible. Thus consideration of reproducibility is important, particularly if one is trying to obtain a longitudinal assessment of anorectal function. Previous work looking at repeatability of sensation in the lower GI tract has mainly focused on the rectum by using the barostat (2, 9). Chan et al. (6) reported good repeatability using manual balloon distension in healthy volunteers but only reported on maximum tolerated volume. By comparison, in patients at least electrical stimulation of the anal canal has previously been reported as a reproducible technique (22). From our present study we found that assessments of sensations using manual balloon distension, RBD, and ES are reproducible to varying degrees, depending on the intensity of the stimulation. For example, ES provides the most reproducible technique for assessing pain in the rectum and anal canal. By contrast, RBD can be used specifically for measuring sensation in the rectum but is not satisfactorily reproducible within the anal canal. Manual bal-
loon distension in the rectum is reproducible at both urge to defecate and maximum tolerated volume but not at first sensation. For all techniques, maximum sensation (pain) levels gave the most robust results.

Comparison of the Two Different Stimulation Modalities

Most observers believe that distending stimuli activate sensory pathways and induce perception by specific activation of mechanoreceptors in the gut wall whereas ES induces similar perception by nonspecific activation of all afferent pathways including afferent nociceptive pathways. Thus the fact that we induced pain with ES (at high stimulation intensities) in both the rectum and anal canal supports this idea. Furthermore, whereas perception scores were very similar during ES in the anal canal and rectum the intensity of the stimulus delivered to induce the same perception score was much lower in the anal canal. This may be accounted for by the fact that the anal canal is believed to have a greater variety of afferent nerve endings than the rectum. In contrast, RBD even at the highest level of urgency and unpleasantness rarely produced the perception of pain. It maybe that rapid rectal distension is not able to reach painful levels as the perception of extreme urgency overrides the progression of sensation, with a need to defecate, so preventing further assessment. Interestingly, RBD in the anal canal produced a similar response to the rectum with comparable levels of unpleasantness and urge. However, although both approaches, ES and RBD, provide complementary information about two independent sensations, direct comparisons between these different stimulation modalities are difficult because the duration of the sensation is very different. It is likely that RBD and ES provide unique evaluation properties for visceral sensation. ES provides a definite painful stimulation and so may be more applicable in pain studies. Moreover, the ease with which sensation can be quantified and the fact that there are fewer technical pitfalls (size of the balloon, speed of inflation, compliance of the balloon material) makes it a more preferable test to balloon distension. By contrast, RBD appears to be more of an urge sensation-inducing stimulus similar to a volume of feces passing through the rectum. As a result, we would recommend that in future studies both techniques should be used for assessment of anorectal function.

Table 3. Group data for amplitude and latency correlations with stimulation intensity

<table>
<thead>
<tr>
<th>Correlation</th>
<th>R Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation intensity and P1 latency</td>
<td>-0.89</td>
<td>0.03</td>
</tr>
<tr>
<td>Stimulation intensity and N1 latency</td>
<td>-0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Stimulation intensity and P2 latency</td>
<td>-1</td>
<td>0.0083</td>
</tr>
<tr>
<td>Stimulation intensity and N3 latency</td>
<td>-1</td>
<td>0.003</td>
</tr>
<tr>
<td>Stimulation intensity and P1–N1 amplitude</td>
<td>0.9</td>
<td>0.0083</td>
</tr>
<tr>
<td>Stimulation intensity and N1–P2 amplitude</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P1 and P2, first and second positive peaks, respectively; N1 and N3, first and third negative components, respectively.

Stimulation Perception Rating

Similar to a previous study (15), we have reported a close relationship between the perception of unpleasantness and pain and unpleasantness and urge. Interestingly, the unpleasantness associated with the RBD and urge was higher than with ES and pain, and the STAIS score was significantly higher than baseline. Although it is easy to imagine that artificially induced pain and urge sensations are...
not pleasant experiences, one may expect direct pain to cause greater unpleasantness than straightforward physiological sensation such as the urge to defecate. Perhaps in the research environment two factors need to be considered. First, pain induced experimentally may not have the same associations of fear as that experienced with true illness. Second, the unpleasantness associated with the sensation of urge to defecate may be disproportionately higher in the laboratory environment where the potential for involuntary (witnessed) defecation causes higher levels of anxiety in the subject.

Measurement of REP

The rectum has a complex innervation with visceral afferent fibers traveling within both sympathetic and parasympathetic (spinal afferent) nerves (1). Specific stimulation of these via the rectum is complicated by the close proximity of somatic nerves that may well be activated by high-intensity rectal stimulation such as ES. Thus when measuring cortical activity in response to visceral stimulation it is important to consider how any cross stimulation might contaminate early REP morphology (23). Because visceral afferents have been shown to consist of mainly small myelinated Aδ (conduction velocity 5–25 m/s) and unmyelinated C (conduction velocity <2.5 m/s) (13), it is reasonable to conclude that cerebral activation as a result of solely visceral stimulation would not occur before 40–50 ms. REP responses that occur before this are likely to be mediated by aβ-fiber somatic afferents. These early REP responses have stimulation characteristics that encode nonpainful stimuli but saturate at stimulation levels well below pain threshold. Thus the REP amplitude and latency measurements in this study were analyzed from 40 ms onward, and these components correlated well with increases in subjective intensity reports, encoding both painful and nonpainful levels of stimulation. Subtle changes in the stimulus characteristics might prove useful to assess aβ-fiber afferents in isolation, which may be important in sensitization states because this population of afferents can undergo phenotypic changes after the development of central sensitization (25). These factors are important to consider when interpreting the REP response.

In conclusion, this study shows that both ES and RBD are reproducible as tests of rectal sensory function. However, the sensations experienced with each technique vary markedly, probably reflecting differences in peripheral receptors stimulated and/or the central processing of the sensory input (16, 17). Combined with assessment of anorectal physiology, two different ratings of sensation may be valuable in conjunction with novel neuroimaging techniques to provide insight into the neurophysiological characteristics of visceral afferent pathways in both health and disease. These observations have important implications particularly for longitudinal studies that involve detailed anorectal sensory assessment in both research studies and clinical practice.

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GRANTS

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REFERENCES