Gut perception in humans is modulated by interacting gut stimuli

ANNA M. ACCARINO, FERNANDO AZPIROZ, AND JUAN-R MALAGELADA
Digestive System Research Unit, Hospital General Vall d’Hebron,
Autonomous University of Barcelona, 08035-Barcelona, Spain

Received 19 April 2001; accepted in final form 15 October 2001

Gust perception in humans is modulated by interacting gut stimuli. Am J Physiol Gastrointest Liver Physiol 282: G220–G225, 2002. First published October 10, 2001; 10.1152/ajpgi.00161.2001—Digestive symptoms depend on multiple interacting gut stimuli, but integration of visceral afferent traffic is poorly understood. Our aim was to elucidate the contribution of simultaneous intestinal stimuli to conscious perception. In 17 healthy subjects, we performed stimulus-response trials of jejunal distensions (1-min duration at 5-min intervals in 8-ml increments) either alone or with a background electrical stimulus, and stimulus-response trials of electrical stimuli (1-min duration at 5-min intervals in 6-mA steps) either alone or with a background intestinal distension. The four stimulus-response trials were performed concomitantly applying the different types of stimuli in random order. Perception was measured on a scale of 0 to 6. Background stimulation markedly increased perception of test stimuli, reducing tolerance from 44 ± 3 to 32 ± 3 ml and from 67 ± 6 to 33 ± 4 mA (P < 0.05 for both). However, whereas jejunal distensions below the perception threshold did not modify perception of the background stimulus (4 ± 1% change; not significant), unperceived electrical stimuli exerted a sensitizing effect and increased perception of the background distension up to uncomfortable levels (111 ± 40% increment; P < 0.05). In conclusion, activation of different pools of jejunal afferents produces summative effects on perception, and this sensitizing effect can be exerted by unperceived stimulation of mechanosensitive jejunal afferents.

MATERIAL AND METHODS

Participants. Seventeen healthy individuals without gastrointestinal symptoms (11 men, 6 women; age range: 20–31 yr) participated in the study after giving their written informed consent. Participants were not experienced at this kind of the study and were not on an athletic training program. The protocol for the study had been previously approved by the Institutional Review Board of the Hospital General Vall d’Hebron.

Tube assembly. An intraluminal probe was used to deliver intestinal stimuli (intestinal distension and electrical nerve stimulation) and also to record local responses (myoelectric and phasic pressure activity). The probe consisted of a polyvinyl tube (5 mm OD, 3 mm ID) with two bipolar electrodes, because it may occur both under normal and pathological conditions.

Address for reprint requests and other correspondence: F. Azpiroz,
Digestive System Research Unit, Hospital General Vall d’Hebron,
08035 Barcelona, Spain (E-mail: fernando.azpiroz@wol.es).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
two manometric ports, and an inflatable balloon at its distal tip. The two bipolar electrodes were built into the rim of suction holes located 5 and 10 cm from the tip balloon; the two manometric ports opened opposite the electrodes. The electromanometric probe has been previously described in detail (2, 5).

Intestinal stimulii. Intestinal distension was produced by inflation with a predetermined volume of air of the distal balloon, located 15 cm distal to the angle of Treitz. The balloon was inflated with a syringe by the investigator at a median rate of 10 ml/s (7- to 13-ml/s range). At the end of distension, the balloon was completely deflated.

The intraluminal pressure at each distending volume tested was determined to calculate the compliance of the jejunal wall. Before each study, the intraballoon pressure with inflation volumes tested in 8-ml increments up to 56 ml was measured in vitro. We used high-compliance latex balloons made with condoms, and intrinsic intraballoon pressure did not exceed 25 mmHg at any volume tested. To calculate the actual pressure acting on the jejunal wall, the intrinsic pressure was determined in vitro for the same distending volume. The intraballoon pressure recorded during jejunal distension in vivo. Intestinal compliance was expressed as the volume/pressure ratio.

Transmucosal electrical nerve stimulation in the intestine was produced by a constant-current stimulator (model 15EO1 Digitism, Dantec, Skovlunde, Denmark) connected to the distal electrode (10 cm caudal to the angle of Treitz), with 100-μs rectangular pulses at a frequency of 15 Hz with a predetermined intensity. The central lumen of the tube was connected to a suction pump to ensure firm contact of the tube to the gut wall.

Intestinal recording. Intestinal myoelectrical activity was recorded via the intraluminal electrodes. Spike activity was recorded with a time constant of 0.03 s and an upper frequency cutoff of 30 Hz. To record intestinal phasic pressure activity, the manometric catheters were perfused with distilled water via a low-compliance pneumohydraulic pump (0.1 ml/min perfusion rate) and were connected to pressure transducers (model DTX, Gould, Oxnard, CA). The tip balloon (deflated) was also connected to a pressure transducer. Intra-balloon pressure during balloon inflation as well as spike activity and phasic pressure activity at the two jejunal sites were recorded on a paper polygraph (Dynograph Recorder R611, SensorMedics, Anaheim, CA).

Perception questionnaire. A graded questionnaire was used to measure the intensity and type of sensations perceived, and an anatomical questionnaire was used to measure the location and extension of the perceived sensations. The graded questionnaire included seven graphic rating scales (5, 9) graded from 0 (no perception) to 6 (pain) specifically for scoring 1) abdominal pressure/bloating, 2) cramp/colic sensation, 3) stinging sensation, 4) paresthesia/flutterlike sensation, 5) warmth sensation, 6) feeling of emptiness, and 7) other types of sensation (to be specified), respectively. All participants received standard instructions specifying that a score of 0 represented no perception at all, a score of 5 represented discomfort, and score of 6 represented a painful sensation, which was not intended and was to be instantaneously reported for immediate discontinuation of the stimulus. Any sensation (1 or more simultaneously) had to be scored on the respective scale based on its intensity of perception, and orientative descriptors were provided indicating that a score of 1 represented vague perception of mild sensation, a score of 2 represented definite perception of mild sensation, and scores of 3 and 4 represented vague and definite sensation perception of moderate sensation, respectively. Participants were also told that, if appropriate, they could mark half-unit scores on the scale. Therefore, 12 intensity grades were actually provided. This type of questionnaire has been extensively used and has been validated in detail by showing 1) the reproducibility of repeat stimuli, 2) the stimulus-related perception using graded stimulation, 3) significant and reproducible changes in perception under different experimental conditions, and 4) differential responses to various stimuli even though the sensations elicited were undistinguishable (1–6, 8, 9, 17, 18).

The anatomical questionnaire showed the abdomen divided into nine areas corresponding to epigastrium, periumbilical area, hypogastrium, both hypochondria, flanks, and ileal fossae, and participants were instructed to mark the location where the sensations were perceived, i.e., abdominal or extraabdominal area(s).

Both questionnaires were fully explained to the participants before the study. Participants were told that intestinal stimuli would be tested without their knowledge of the type or the moment when the stimuli were applied and that after each blind stimulus (including sham stimuli), the investigator would ask them to describe in the questionnaires any sensation perceived during the preceding minute.

Procedure and experimental design. Participants were orally intubated at 8:00 AM after an overnight fast. The intestinal tube assembly was positioned under fluoroscopic control with the orad electrode and the tip balloon located 5 and 15 cm distal to the angle of Treitz, respectively. The studies were conducted in a quiet, isolated room. The subjects were placed supine in bed at an angle of 30° to the horizontal and were asked to relax comfortably. The tube was then connected to the stimulation and recording systems.

After a 20-min stabilization period, we first determined in each individual the distending volume and the intensity of electrical stimuli in the intestine that induced perception. Phasic stimuli of 10-s duration were tested in either 8-ml or 6-ml increments at 20-s intervals up to the respective level of perception. After a 5-min interval, the same stimuli were applied for 1 min at 5-min intervals. If, in one individual, perception exceeded score of 4 at retesting, the magnitude of the stimulus was readjusted to induce clear perception without discomfort (scores 2–4). Mean values (±SE) of individual perception levels were 28 ± 1 ml for distension and 49 ± 4 mA for electrical stimulation.

In the main studies, four stimulus-response trials were performed up to the level that first induced a perception score of 5 or more; that is, the upper limit of each trial was established at the discomfort threshold. In each stimulus-response trial, a different combination of electrical and mechanical stimuli was tested: we tested intestinal distensions in 8-ml increments both 1) alone (with sham electrical stimuli) and 2) with background electrical stimuli applied simultaneously at the perception level previously determined (49 ± 4 mA); we also tested electrical stimuli in 6-mA increments both 3) alone (with sham distensions) and 4) with background distensions applied simultaneously at the perception level previously determined (28 ± 1 ml). Each type of stimulus was applied in ascending order, but the four trials were performed concomitantly, applying the different types of stimuli in random order. Individual stimuli were applied for 1 min at 5-min intervals without participants knowing either the moment of application or the type of stimulus applied. After each stimulus, participants were asked to fill out the perception questionnaires. No stimuli were applied during or within 10 min after the end of a phase III of the interdigestive motor complex recorded in the intestine, and
any stimulus performed within the 10-min period before the onset of a phase III was repeated afterwards.

Data analysis. Perception of each stimulus was measured by the score in the graded questionnaire; when more than one sensation was scored, only the highest score was computed for comparisons. For each type of stimulus, the threshold for discomfort was defined as the lowest stimulus that produced discomfort (perception score of 5 or more); for both perception and electrical stimulation applied alone, the threshold for perception was defined as the lowest stimulus (distending volume or electrical intensity) that produced any kind of perception (perception above score of 0). Because the highest stimulus tested in each trial (upper limit) differed among individuals, the stimulus-response curves were normalized based on the discomfort and perception thresholds as follows. When either distensions or electrical stimuli were applied alone, dose-response curves were calculated by plotting the perception scores at four magnitudes of stimulation: 1) at the threshold for discomfort, 2) at the threshold for perception, and 3) one step below, i.e., unperceived stimuli at the subthreshold for perception. For mechanical stimuli with a background electrical stimulus, the dose-response curve was constructed by plotting the perception scores 1) at the discomfort threshold, 2) at the volume corresponding to the perception threshold when distensions were applied alone, 3) one step below, i.e., volume corresponding to subthreshold for perception of distensions alone, and 4) at 0 ml distension, i.e., background electrical stimulus alone. For electrical stimuli with background distensions, the dose-response curve was constructed by plotting the perception scores 1) at the discomfort threshold, 2) at an intensity level one-half the discomfort threshold in each subject, and 3) at 0 mA stimulation, i.e., background distension alone; in the absence of other significant reference points, the midpoint of the stimulus-response curves (intensity one-half the discomfort threshold) was arbitrarily chosen to represent the configuration of the curve.

The relative frequency of the different sensations elicited by each type of stimulus in each subject was calculated as percent distribution. In each subject, we also counted the number of different sensations elicited by each stimulus, and the average number of sensations elicited by each type of stimulation was then calculated. In the anatomical questionnaire, we measured the percentage of stimuli referred over more than one abdominal region.

To calculate the effect of the stimuli on phasic electromechanical activity in the jejunum, we measured the number of phasic pressure waves >20 mmHg/min recorded by manometry and the number of spikes per minute recorded by electromyography during the 2-min period preceding the stimulus (prestimulation period), during the stimulus (stimulation period), and during the 2-min period after the stimulus (poststimulation period).

Statistical analysis. We compared in each subject the intensity-related responses: low stimuli were unperceived, and perception increased from the threshold for perception up to the threshold for discomfort (Fig. 1). When the same distensions were applied with a concomitant background electrical stimulus, perception was significantly higher (Fig. 1). Background electrical stimulation applied alone (with 0 ml distending volume) induced mild perception, and concomitant application of small distending volumes (up to a level corresponding to the distension subthreshold for perception) did not modify perception (not significant vs. 0 ml distension). However, with distending volumes corresponding to the perception threshold and above, perception appeared summative: the same distending volumes that were tested in each subject both with and without background electrical stimuli induced 78 ± 29% higher perception scores in the former case (P < 0.05). Background electrical stimulation reduced the tolerance of distensions, and the threshold for discomfort was reached with significantly smaller volumes (P < 0.05).

Perception of transmucosal electrical nerve stimulation. Electrical stimulation of the jejunum applied without background distension also induced intensity-related responses: low stimuli were unperceived, and perception increased from the threshold for perception up to the threshold for discomfort (Fig. 2). When elec-

RESULTS

Perception of jejunal distension. Jejunal distension applied without background electrical stimulus induced volume-related perception: small volumes were unperceived, and perception increased from the threshold for perception up to the threshold for discomfort (Fig. 1). When the same distensions were applied with a concomitant background electrical stimulus, perception was significantly higher (Fig. 1). Background electrical stimulation applied alone (with 0 ml distending volume) induced mild perception, and concomitant application of small distending volumes (up to a level corresponding to the distension subthreshold for perception) did not modify perception (not significant vs. 0 ml distension). However, with distending volumes corresponding to the perception threshold and above, perception appeared summative: the same distending volumes that were tested in each subject both with and without background electrical stimuli induced 78 ± 29% higher perception scores in the former case (P < 0.05). Background electrical stimulation reduced the tolerance of distensions, and the threshold for discomfort was reached with significantly smaller volumes (P < 0.05).

Perception of transmucosal electrical nerve stimulation. Electrical stimulation of the jejunum applied without background distension also induced intensity-related responses: low stimuli were unperceived, and perception increased from the threshold for perception up to the threshold for discomfort (Fig. 2). When elec-

Fig. 1. Perception of jejunal distension. Stimulus-related perception of jejunal distension tested without and with concomitant background electrical stimuli at fixed intensity. Values are means ± SE. The distending volume at the discomfort threshold was significantly smaller with than without background electrical stimulation, and perception of distending volumes above the perception threshold was significantly higher when a background electrical stimulus was simultaneously applied (*P < 0.05 for both).
Jejunal stimuli were applied with a concomitant background distension, perception was significantly higher (Fig. 2). Background distension applied alone (without 0 mA electrical stimulation) induced mild perception. Concomitant application of very low, unperceived electrical stimuli (corresponding to intensities below the perception threshold) significantly increased perception up to levels of discomfort ($P < 0.05$ vs. 0 mA). The intensity of electrical stimulation at the discomfort threshold was significantly smaller with than without background distension ($P < 0.05$), and, conversely, electrical stimuli below the perception threshold significantly increased perception of the background distension up to the level of discomfort ($P < 0.05$ vs. 0 mA).

**Symptoms induced by jejunal stimulation.** Jejunal distension induced perception of clinical-type abdominal sensations (Fig. 3). Interestingly, transmucosal electrical nerve stimulation induced, by and large, similar sensations; only a small proportion of the stimuli induced paresthesia or flutterlike sensation. A similar symptom pattern was also induced by distensions and electrical stimuli with background distension (Fig. 3). Most stimuli (97%) induced only one sensation, without differences for the different types of stimulation.

Abdominal symptoms induced by jejunal stimuli, regardless of the type of stimulus, were most frequently perceived in the epigastrium, perumbilical area, and left flank ($27 \pm 5\%$, $37 \pm 7\%$, and $21 \pm 4\%$, respectively; pooled data for electrical stimuli and distensions both without and with background stimulation). The extension of the referral area was also similar for all kinds of stimuli: the percentage of the stimuli perceived over more than one area was $20 \pm 9\%$ and $17 \pm 7\%$ for distensions without and with background electrical stimulation, respectively, and $19 \pm 7\%$ and $19 \pm 9\%$ for electrical stimuli without and with background distension, respectively (not significant).

**Jejunal compliance.** Jejunal compliance, assessed during balloon distension, was not modified by simultaneous electrical stimulation. Intraballoon pressures were found virtually unchanged ($2 \pm 1\%$ change; not significant) when comparing the distending volumes tested both alone and with background electrical stimuli in each subject. Likewise, when distensions were applied as background stimuli, intraballoon pressure was not modified by the increasing intensity of electrical stimuli: at 6 mA, intraballoon pressure was $34 \pm 2$ mmHg, and at the threshold for discomfort ($35 \pm 5$ mA), the pressure was $36 \pm 2$ mmHg (not significant vs. 6 mA).

**Effect of jejunal stimuli on phasic motility and electromyographic activity.** Jejunal distension with high volumes produced inhibition of phasic pressure activity that was only apparent adjacent to the balloon, i.e., at the recording site located 5 cm proximal to the balloon, but not at a distance of 10 cm (Table 1). When distensions were applied with background electrical stimuli, motor inhibition was lost (Table 1), probably because the threshold for discomfort was reduced and hence the highest volumes could not be further tested. Transmucosal electrical nerve stimulation at any level tested,
with a wide range between the distensions elicit dose-related perception effect and increases perception of well-tolerated by low, unperceived electrical stimuli exerts a sensitizing role. Interestingly, nonspecific stimulation of intestinal afferents by low, unperceived electrical stimuli exerts a sensitizing role. As previously shown (1, 2), both electrical stimuli and balloon distensions elicit dose-related perception with a wide range between the “threshold for perception” and the “threshold for discomfort.” By combining electrical and mechanical stimuli, a synergistic effect between simultaneous, yet different, stimuli became apparent. Indeed, background electrical stimuli reduced the tolerance of distension, and, likewise, background distension reduced the tolerance of electrical stimulation. Conversely, because a whole range of stimuli up to the level of discomfort were tested, we could also analyze the effect of stimuli both below and above the perception threshold on perception of the background stimulus. This analysis further demonstrated specific interactions depending on the mechanism of stimulation. Mechanical stimuli, only when applied at perceivable levels, increased perception of the background electrical stimulation and thus manifested additive effects, whereas unperceived distensions had no effect. By contrast, electrical stimuli below the perception threshold exerted a sensitizing role and potentiated perception of the background mechanical stimulation even to the level of discomfort. These results are important because they show that unperceived stimuli can potentiate perception of a different concurrent stimulus.

It is not clear by which mechanism these phenomena of sensitization occur. However, we can confidently rule out that local motor effects were involved, because electrical stimuli had no effect on intestinal compliance: at the same distending volumes, intraballoon pressures were similar with and without background electrical stimuli; likewise, during background distension, intraballoon pressures were unchanged at any intensity level of the electrical stimuli tested. Furthermore, electrical stimuli, either with or without background distension, did not modify intestinal phasic motor activity measured by manometry. Indeed, only the distensions at the highest level tested, i.e., at the threshold for discomfort, induced an inhibitory effect on phasic motility, and with combined electrical stimulation, this effect was not achieved because the highest volumes were not further tolerated.

Different types of stimuli in the somatic territory activate specific subsets of afferents (9, 12). However, extrapolations from somatic to visceral sensitivity are uncertain, because the types of afferents innervating the gut have not yet been completely characterized (16). Nevertheless, electrical and mechanical stimulation of the gut induces, to some extent, different responses, suggesting that different pathways are activated. We have consistently shown, in the present as well as in previous studies (2), that mechanical stimulation activates intestinointestinal inhibitory reflexes that inhibit phasic motility, whereas electrical stimulation does not. Furthermore, electrical and mechanical stimuli have differential effects on perception in patients with the irritable bowel syndrome who are hypersensitive to mechanical and normosensitive to electrical stimulation (2). Intestinal fat infusion also has distinct effects: fat increases perception of intestinal distension but not of transmucosal electrical nerve stimulation (3). True, both stimuli induce a similar sensory experience, but this could be related to the limited sensory expression of the intestine, with simi-

### Table 1. Effect of jejunal stimuli on manometrically recorded phasic pressure activity

<table>
<thead>
<tr>
<th></th>
<th>5 cm orad, wave/min</th>
<th>10 cm orad, wave/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Stimulus</td>
</tr>
<tr>
<td>Distension alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perception threshold</td>
<td>1.4±0.3</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td>Discomfort threshold</td>
<td>1.3±0.5</td>
<td>0.1±0.1*</td>
</tr>
<tr>
<td>Distension plus background TENS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perception threshold</td>
<td>1.7±0.5</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Discomfort threshold</td>
<td>2.0±0.5</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>TENS plus background distension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perception threshold</td>
<td>1.8±0.5</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td>Discomfort threshold</td>
<td>1.8±0.5</td>
<td>1.4±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Manometry recorded 5 and 10 cm orad to the tip of the tube during 2-min periods before (pre), during (stimulus), and after (post) intestinal distensions and transmucosal electrical nerve stimulation (TENS); distending balloon located at the tip of the tube and stimulating electrode 5 cm orad. *P < 0.05 vs. prestimulation period.
lar sensations being induced independently of the type of stimulus and the region stimulated (5).

The increase in perception induced by combined electrical and mechanical stimuli is similar to the spatial summation phenomena produced by simultaneous intestinal distensions (17, 18). Summation phenomena occur independently of whether stimuli are applied to afferents innervating close or distant segments of the gut. Indeed, the same effects are observed with distensions applied at different distances along the small bowel (18). However, we previously showed that transcutaneous electrical nerve stimulation applied on the hand reduces perception of gut distension (7). This phenomenon is conceivably related to the counterirritation analgesia induced by somatic stimuli (10, 19) that has been shown to reduce somatic nociception via a supraspinal circuitry with inhibitory control on spinal transmission and potentially also at higher levels of the sensory pathways (7, 11).

Particularly intriguing is the mechanism of intestinal hypersensitivity induced by unperceived electrical gut stimulation. Visceral perception is mediated by splanchnic spinal fibers (15, 1), but the type of pathways activated by unperceived electrical stimuli is unknown, and activation of vagal afferents cannot be ruled out. In this regard, electrical stimulation of vagal afferents has been shown to modulate sensory pathways, and, specifically, low-intensity stimuli produce a facilitation, concordant with the increase in perception observed in our experiments (15, 14, 13).

Our results may bear pathophysiological significance, because they provide experimental support for the concept that unperceived intestinal stimuli may exert sensitizing effects. Sensitizing stimuli may be associated with relatively common phenomena such as low-level luminal irritation, gas, or spastic events. Upregulation of such sensitizing mechanisms could be a putative cause of the visceral hypersensitivity possibly involved in the origin of unexplained abdominal symptoms in patients with functional gastrointestinal disorders.

We thank A. Aparici and M. Casaus for technical support, G. Santaliestra for secretarial assistance, and C. O’Hara for English language editing of the manuscript.

This work was supported in part by the Spanish Ministry of Education (Dirección General de Enseñanza Superior del Ministerio de Educación y Cultura, PM 97–0096) and the National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-57064.

REFERENCES


