Influence of acute tryptophan depletion on gastric sensorimotor function in humans

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Geeraerts B, Van Oudenhove L, Boesmans W, Vos R, Vanden Berghe P, Tack J. Influence of acute tryptophan depletion on gastric sensorimotor function in humans. Am J Physiol Gastrointest Liver Physiol 300: G228–G235, 2011. First published September 30, 2010; doi:10.1152/ajpgi.00020.2010.—Peripheral serotonin (5-hydroxytryptamine; 5-HT) is involved in the regulation of gastrointestinal motility and sensation, whereas centrally it plays a role in mood regulation. A dysfunctional serotonergic system might provide a plausible link between functional dyspepsia symptoms and its high psychosocial comorbidity such as anxiety and depression. The aim of this study was to evaluate the effect of decreased 5-HT synthesis by acute tryptophan depletion (ATD) on gastric sensorimotor function and nutrient tolerance, anxiety scores, and gastrointestinal mucosal 5-HT concentrations in healthy volunteers. All subjects were studied under a control condition and during ATD. Gastric sensorimotor function and nutrient tolerance were assessed using a barostat (n = 16, mean age 28.8 ± 1.4 yr) and a satiety drinking test (n = 13, mean age 27.3 ± 1.4 yr). Anxiety during the barostat was evaluated using State-Trait Anxiety Inventory (STAI) questionnaire. 5-HT concentrations were measured in fundic and duodenal mucosal biopsies by means of ELISA and immunohistochemistry. ATD significantly decreased plasma tryptophan levels compared with control in every experiment. ATD did not affect gastric sensitivity and compliance but decreased the sensation of nausea during balloon distension (AUC: 17.4 ± 4.3 vs. 11.4 ± 3.4 mm·mmHg, P = 0.030). ATD enhanced the postprandial volume increase (ANOVA, P < 0.05), but this was not accompanied by augmented nutrient tolerance (848 ± 110 vs. 837 ± 99 ml, nonsignificant). ATD had no effect on STAI state anxiety scores. No evidence was found for an effect on the number of enterochromaffin cells, but ATD reduced 5-HT levels in the duodenal mucosa. ATD alters gastric postprandial motor function and distension-induced nausea. These findings confirm involvement of 5-HT in the control of gastric accommodation and sensitivity.

THE PATHOPHYSIOLOGY OF FUNCTIONAL DYSPESIA (FD) is incompletely understood. A multifactorial conceptual disease model has been postulated providing the influence of biological, psychological, and social factors (9). The brain-gut axis allows bidirectional input and links emotional and cognitive centers of the brain with peripheral functioning of the gastrointestinal (GI) tract and vice versa. This biopsychosocial conceptualization is believed to play a major role in the pathogenesis of functional GI disorders, such as FD. FD is associated with visceral hypersensitivity and with a high comorbidity of psychiatric disorders, in particular depression and anxiety disorders (51).

Serotonin (5-hydroxytryptamine; 5-HT) is a biogenic amine that functions as a neurotransmitter and is located predominantly in the enterochromaffin cells (EC) scattered in the mucosa of the GI tract (95% of body 5-HT). Besides this large mucosal source, 5-HT is also released by a subpopulation of enteric neurons. Although the existence of these serotonergic neurons was subject to debate for a long time, recent evidence (12, 27) confirmed, at least in rodents, the early reports on the role of 5-HT as a neurotransmitter in the enteric nervous system (ENS) (11). In the periphery, 5-HT is involved in the regulation of GI secretion, motility, and perception (15, 24), whereas in the central nervous system, where about 5% of body 5-HT is located, it plays a pivotal role in the regulation of mood and cognition (3). 5-HT can be considered a key component of the brain-gut axis model.

The exact role of serotonin in this model, however, is poorly understood. This is mainly due to the presence of many serotonin receptors in the GI tract and the lack of suitable and selective antagonists for use in humans. Information on the physiological role of serotonin in humans has mainly been obtained from the use of selective serotonin reuptake inhibitors (SSRIs). Increased serotonin availability following SSRI administration has no clear-cut effects on sensation throughout the entire GI tract, but effects on GI motility seem to be more consistent (15).

Acute tryptophan depletion (ATD) is a well-established method to temporarily reduce 5-HT synthesis by decreasing the availability of its precursor, tryptophan. This can be achieved by administration of an amino acid mixture devoid of tryptophan (10, 31). ATD has primarily been used in psychiatric research to investigate the role of the central 5-HT system in patients with affective disorders (depression and anxiety) (1). Recently, ATD was also shown to affect GI physiology by delaying gastric emptying (48) and enhancing visceral pain perception during rectal balloon distension (20).

The objectives of this study were 1) to evaluate the effect of ATD on gastric sensorimotor function and nutrient tolerance in healthy volunteers, 2) to investigate whether ATD affects anxiety scores during gastric sensitivity testing, and 3) to investigate whether ATD is associated with altered 5-HT levels in the GI tract.

MATERIALS AND METHODS

Study Subjects

Healthy volunteers without symptoms or a history of GI disease, and who were not taking any medication, were recruited for this study. In addition, participants reporting any history of psychiatric disease or a positive first-degree psychiatric family history were excluded (38). Written informed consent was obtained from each participant. The

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protocol was approved by the Ethics Committee of the University Hospital.

Study populations differed between the different experiments. All subjects participated in the barostat studies. Subsets of these subjects also participated in the satiety drinking test and endoscopy with biopsies protocols. Within each experiment, all subjects were studied on two occasions with at least a 7-day interval. All measurements started 5 h after ingestion of the amino acid mixture (1).

**Acute Tryptophan Depletion**

The amino acid mixtures were prepared and coded for a double-blind controlled study by the pharmacy of the University Hospital. The tryptophan (TRP)-deficient TRP-deficient amino acid mixture consisted of 15 amino acids, including 5 large neutral amino acids (LNAs) (38). The control mixture contained the same amino acids enriched with TRP to prevent a decrease in TRP levels. After an overnight fast, the subjects consumed the amino acid mixtures dissolved in 200 ml of water as quickly as possible. Plasma TRP levels, the plasma ratio TRP/ΣLNAA (sum of tyrosine, leucine, phenylalanine, isoleucine, and valine) and urinary 5-hydroxyindoleacetic acid (5-HIAA) levels (which is the major 5-HT metabolite) were measured at baseline, time (t) = 5 h, and t = 7 h. These biochemical analyses were performed by the Laboratory Medicine department of the University Hospital Gasthuisberg (Leuven, Belgium).

**Gastric Barostat**

Sixteen healthy volunteers [age 28.8 ± 1.4 yr; 11 females; body mass index (BMI) 22.8 ± 0.7 kg/m²] were enrolled in this part of the investigation. All underwent the procedure twice, at least 1 wk apart, after ATD or control pretreatment in a double-blind crossover mode. After an overnight fast of at least 12 h, and 5 h after ingestion of the amino acid mixture, a double-lumen polyvinyl tube (Salem sump tube 14 Ch; Sherwood Medical, Petit Rechaim, Belgium) with an adherent polyethylene bag (maximal volume 1,200 ml; maximum diameter 17 cm) was introduced through the mouth and secured to the subject’s chin with adhesive tape. The correct position of the bag in the gastric fundus was checked fluoroscopically.

The polyvinyl tube was then connected to a programmable barostat device (Synectics Visceral Stimulator, Stockholm, Sweden). To unfold the bag, it was inflated with a fixed volume of 300 ml for 2 min with the subject in a recumbent position and again deflated completely. The subjects were then positioned in a comfortable sitting position with the knees bent (80°) and the trunk upright in a specifically designed bed. After a 30-min adaptation period, the minimal distending pressure (MDP) was determined by increasing the intrabag pressure with 1 mmHg every 3 min, until a volume of 30 ml or more was reached (42).

Subsequently, isobaric distensions were performed in stepwise increments of 2 mmHg starting from MDP, each lasting for 2 min, while the corresponding intragastric volume was recorded. Subjects were instructed to score their perception of upper abdominal sensations at the end of every distending step. They used both a global graphic rating scale that combined verbal descriptors on a scale graded 0 to 6 (42) and a 10-cm visual analog scale (VAS) to indicate the intensity of nine epigastric symptoms (discomfort, pain, fullness, bloating, satiety, nausea, epigastric burning, belching, and heartburn). The end point of this distension sequence was established at an intrabag volume of 1,500 ml or when the subjects reported discomfort or pain (score of 5 or 6). Pressure-volume curves were obtained from this stepwise distension sequence. The slope of this curve (obtained by linear regression) was used to quantify gastric compliance, since this was previously shown to provide the best fit (33). The study protocol is summarized in Fig. 1.

After the distension sequence, pressure level was set at MDP + 2 mmHg for 90 min and gastric tone was continuously monitored. After the first 30 min, a standardized liquid meal (200 ml, 300 kcal, 13% proteins, 48% carbohydrates, 39% lipids, Nutridrink; Nutricia, Bornem, Belgium) was administered. Barostat measurements continued for 60 min after the start of meal ingestion.

In addition, on each study day, levels of momentary anxiety were assessed using the state version of the State-Trait Anxiety Inventory (STAI) questionnaire (39). These were administered at t = 0 h (before ingestion of the amino acid mixture), t = 2.5 h (intermediate time point), t = 5 h (right before the barostat measurement), and t = 7 h (immediately after cessation of the barostat measurement).

**Satiety Drinking Test**

After an overnight fast, 13 subjects (age 27.3 ± 1.4 yr; 8 females; BMI 23.2 ± 0.7 kg/m²) underwent a drinking test to quantify nutrient tolerance and the occurrence of meal-induced satiety. All subjects underwent the procedure twice, at least 1 wk apart, after ATD or control pretreatment in a double-blind crossover mode. A peristaltic pump (Minipuls 2; Gilson, Villiers-Le-Bel, France) filled one of two beakers at a rate of 15 ml/min with a liquid meal (Nutridrink; Nutricia). Subjects were requested to start drinking 5 h after drinking the amino acid mixture and to maintain intake at the filling rate, thereby alternating the beakers as they were filled and emptied. At 5-min intervals, they were asked to fill out VAS scores for eight epigastric symptoms and to score their satiety using a separate graphic rating scale that combined verbal descriptors on a scale graded 0 to 5 (1, threshold; 5, maximum satiety). Meal intake was stopped when a score of 5 was reached. Participants were monitored during a 3-h follow-up period after reaching maximal satiety. The postprandial evolution of satiety and other meal-related symptoms were evaluated every 15 min using VAS scores. We have previously shown that this test of meal-induced satiation is sensitive to pharmacological intervention (7, 43, 46).

![Fig. 1. Schematic outline of the barostat protocol.](http://ajpgi.physiology.org/10.1152/ajpgi.00490.2010)
In the barostat study, for each 2-min isobaric distending period, the intragastric volume was calculated by averaging the recording. The thresholds for perception and discomfort were computed after the experiments by analyzing the perception score corresponding to each distension step. Perception threshold was defined as the lowest pressure relative to MDP that evoked a perception score of 1 or more and the corresponding volume. Discomfort threshold was defined as the lowest pressure relative to MDP and the corresponding volume that provoked a score of 5 or more. Pressure-volume curves were obtained from the stepwise distensions. Gastric compliance was calculated as the slope and the intercept of the pressure-volume curve obtained during the isobaric distensions. To evaluate gastric tone before and after administration of the meal, mean intraballoonal volume was calculated over consecutive 5-min intervals of the measurement. Gastric accommodation was quantified as the volume increase over the mean preprandial volume during 30 and 60 min postprandially. For the satiety drinking test, the amounts of kilocalories ingested until the occurrence of maximum satiety (score of 5) were calculated as previously reported (45).

Chromogranin A (CgA)- (i.e., enteroendocrine cells; EEC) and 5-HT-positive cells were counted within a field of view (magnification ×40) at random locations from 15 sections of each mucosal biopsy. The results were calculated as cells per 0.1 mm². Counts and analyses were performed by one person (B. Geeraerts) who was blind to treatment details. EC were identified as those positive for both CgA and 5-HT. The codes were matched to the subjects only after all processing was finalized.

### Statistical Analysis

Paired Student’s t-tests were used to assess whether the means of two parameters differed between both groups. Nonparametric tests were applied when necessary. Gastric accommodation data were compared using ANOVA for repeated measures. All data are means ± SE. A *P* value <0.05 was considered to be statistically significant. Statistical evaluations were performed using SAS 9.1 (with E-guide 4.0; SAS Institute, Cary, NC).

### RESULTS

#### Biochemical Parameters

The plasma TRP levels, TRP/LNAA, and urinary 5-HIAA concentrations are displayed in Table 1. Baseline values are comparable under both conditions. ATD was associated with significantly decreased compared with the control condition (all *P* < 0.001). Sex did not affect the influence of ATD on these biochemical parameters.

#### Influence of ATD on Gastric Sensitivity to Distension and Compliance

During the gastric barostat studies, oral intubation was well tolerated by all subjects. The mean MDP was 7.6 ± 0.5 mmHg on the day the control mixture was administered and 8.3 ± 0.3 mmHg on the day of ATD (*P* = 0.11).

During isobaric distensions, ATD had no significant influence on gastric sensitivity parameters. The pressures needed to induce first perception or discomfort and the corresponding intraballoonal volumes remained unaltered. Comparison of VAS scores between ATD and the control condition also did not reveal any altered intensities of pain or discomfort. During stepwise gastric distension, ATD was associated with significantly lower scores for nausea (see Table 2 for details). Gastric compliance, obtained from the pressure-volume curves, re-

### Table 1. Biochemical parameters at baseline and at 5 and 7 h during ATD and control conditions

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 h</th>
<th>7 h</th>
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<tbody>
<tr>
<td>TRP, µM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ATD</td>
<td>52.0 ± 1.8</td>
<td>11.9 ± 1.6*</td>
<td>32.6 ± 3.3*</td>
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<tr>
<td>Control</td>
<td>52.5 ± 2.1</td>
<td>136.2 ± 9.7</td>
<td>75.9 ± 3.3</td>
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<tr>
<td>TRP/LNAA (×100)</td>
<td></td>
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<tr>
<td>ATD</td>
<td>10.2 ± 0.6</td>
<td>1.1 ± 0.2*</td>
<td>3.3 ± 0.4*</td>
</tr>
<tr>
<td>Control</td>
<td>10.2 ± 0.4</td>
<td>13.1 ± 0.9</td>
<td>7.9 ± 0.5</td>
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<tr>
<td>5-HIAA, mg/l</td>
<td></td>
<td></td>
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<tr>
<td>ATD</td>
<td>5.6 ± 0.5</td>
<td>2.2 ± 0.3*</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>3.3 ± 0.3</td>
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Values are means ± SE at baseline (*t* = 0 h), at *t* = 5 h, and at *t* = 7 h during acute tryptophan depletion (ATD) and control conditions. TRP, tryptophan; 5-HIAA, 5-hydroxyindoleacetic acid. *P* < 0.001, between-group differences.
mained unaltered after ATD (52.7 ± 5.6 vs. 51.7 ± 5.8 ml/mmHg, P = 0.86).

Influence of ATD on Gastric Accommodation to a Meal

Preprandial intragastric volumes were similar under both conditions (220 ± 26 vs. 259 ± 29 ml, P = 0.25). Ingestion of the meal caused an immediate relaxation of the proximal stomach in all subjects, reflected by an increase in the balloon volume. During ATD, gastric accommodation was significantly larger (repeated-measures ANOVA, P < 0.05) (Fig. 2), with no influence of sex. In addition, the maximal intraballoon volume increase in the first 30 min postprandially was also significantly higher under ATD (216 ± 42 vs. 321 ± 38 ml, P < 0.05), confirming that the largest difference between both conditions can be observed during the first 30 min, as is also apparent from Fig. 2.

Influence of ATD on Nutrient Tolerance

During the control condition, the amount of liquid nutrient meal ingested until the occurrence of maximum satiety was 848 ± 110 ml (corresponding duration 57 ± 7 min). During ATD, similar volumes were consumed (837 ± 99 ml, P = 0.88). The average duration was 56 ± 7 min. VAS scores for eight meal-induced epigastric symptoms during the drinking test or in the 3-h postprandial follow-up period were not altered (details not shown).

Influence of ATD on GI Mucosal Serotonin Content

To determine whether ATD is associated with an alteration in local 5-HT content, we counted the number of EC in gastric and duodenal mucosal biopsy specimens. Mucosal 5-HT concentration was examined with ELISA. No significant differences in EC numbers in fundic (2.6 ± 0.4 vs. 2.2 ± 0.3, P = 0.25) and duodenal mucosa samples (14.9 ± 3.4 vs. 11.6 ± 1.0, P = 0.11) were found between both conditions (Fig. 3, A and B).

ELISA results revealed that ATD resulted in a significant reduction in the duodenal mucosa 5-HT content (3.3 ± 0.7 vs. 2.2 ± 0.8 μg/g wet tissue, P = 0.02), whereas 5-HT concentrations in the fundus mucosa remained unaltered (1.2 ± 0.6 vs. 1.1 ± 0.3 μg/g wet tissue, P = 0.86) (Fig. 3C). There was no evidence of an influence of sex on the effect of ATD on mucosal 5-HT content.

Influence of ATD on Anxiety Scores

Between both conditions, STAI anxiety scores did not differ at baseline (29.7 ± 1.6 vs. 29.6 ± 1.8, P = 0.96) or at t = 2.5 h (27.7 ± 1.4 vs. 28.9 ± 1.6, P = 0.29), t = 5 h (29.0 ± 1.8 vs. 29.2 ± 1.7, P = 0.87), or t = 7 h (28.3 ± 1.8 vs. 28.2 ± 1.6, P = 0.90).

DISCUSSION

TRP is an essential amino acid that needs to be acquired from dietary resources in humans. Plasma TRP levels are determined by the balance between dietary intake and removal from the plasma by several processes. Apart from its conversion to 5-HT, TRP is a rate-limiting amino acid in protein synthesis; it contributes to the formation of substrates of glycolysis, and about 95% of ingested TRP enters the kynurenine pathway (29). The latter is of increasing interest since metabolites of this pathway are believed to be involved in the pathogenesis of various GI disorders (19). ATD is brought about by the ingestion of an amino acid load devoid of TRP to deplete the body of the amino acid precursor of serotonin (10, 31). In the control condition, TRP is added to the solution. Ingestion of the TRP-depleting mixture stimulates protein synthesis in the liver with incorporation of available plasma TRP. In addition, the LNAAs compete with TRP for transport across the blood-brain barrier and reduce TRP entry into the brain, thereby decreasing TRP content in the central nervous system. The ATD paradigm is based on the hypothesis that reducing plasma TRP produces a consequent reduction in 5-HT synthesis, which has been confirmed by a large body of preclinical and clinical evidence (for review see Ref. 17). The plasma ratio TRP/LNAA has been reported to be an accurate predictor of brain TRP levels.

In the present study, we confirmed that plasma TRP values decreased in all subjects as a result of ATD, whereas the plasma TRP values in the control group were increased, due to the composition of the control mixture, which is not perfectly balanced for TRP. For the present study, this may be beneficial since we studied the effect of alteration in TRP levels. We also observed an expected massive decrease in TRP/LNAA as a result of ATD. We observed that ATD enhanced gastric accommodation and decreased induction of nausea during gastric distention.

Our findings regarding the role of 5-HT in the regulation of the accommodation reflex may seem surprising, since most 5-HT receptor agonists that have been studied in humans (such as sumatriptan, MKC-733, cisapride, and tegaserod) have been shown to enhance gastric accommodation to a meal in healthy volunteers (6, 41, 43, 44, 46, 48). Studies in the guinea pig proximal stomach, on the other hand, show involvement of 5-HT receptors in both inhibitory and relaxatory circuits, with the nitrergic nerves expressing 5-HT1P receptors and cholinergic nerves expressing 5-HT3 receptors (23). However, because ATD decreases 5-HT availability without specificity for any receptor, its effects would be expected to be the opposite to that of acute SSRI administration (which increases 5-HT availability in a non-receptor-specific way) rather than specific 5-HT agonists. In a recent study, we demonstrated that acute intravenous administration of the SSRI citalopram reduces...
gastric accommodation, which is consistent with the enhancement of accommodation induced by ATD in the present study (18a). However, we also previously showed that a 5-day pretreatment with the SSRI paroxetine enhances gastric accommodation in healthy volunteers (40). This may be due to receptor desensitization after 5 days of SSRI pretreatment as opposed to a mere increase in synaptic 5-HT availability after acute interventions including ATD and intravenous citalopram.

Gastric accommodation has been shown to correlate well with the maximum tolerated volume during a slow caloric drinking test (43). However, in the present study, enhanced gastric accommodation was not reflected by increased nutrient availability.

Fig. 3. Influence of ATD on mucosal serotonin (5-hydroxytryptamine; 5-HT) content. A: the presence of enterochromaffin cells (EC) in mucosal specimens was revealed by immunohistochemistry. EC were counted when immunoreactive for both chromogranin A (CgA; green) and 5-HT (red). Nuclei were stained using 4,6-diamino-2-phenylindole (blue). Bar, 25 μm. B: the number of EC in fundic (left) and duodenal mucosal specimens (right) was not significantly altered after ATD. C: whereas ATD had no effect on the mucosal 5-HT concentration in specimens taken from the fundus, ATD decreased the 5-HT concentration in the duodenal mucosal specimens. *P < 0.05 vs. control. (Student’s t-test).
Tryptophan depletion and gastric function

Our observation regarding the role of 5-HT in the generation of nausea is less surprising. We observed that ATD reduced the sensation of nausea during gastric balloon distension. In clinical practice, 5-HT₃ antagonists such as ondansetron and granisetron are used on a daily basis to reduce chemotherapy-induced nausea in patients receiving cancer treatment (34). Taking into account the lack of effect of ATD on mechanosensitivity and compliance of the stomach, it seems that 5-HT is mainly involved in mediating chemosensitivity and not mechanosensitivity in the upper GI tract.

Our data show no effect of ATD on anxiety scores during gastric barostat measurements. Although a gastric barostat study is considered an invasive and stressful investigation, this did not increase anxiety levels in combination with ATD. This observation is in line with several studies assessing anxiety ratings following ATD, with most of these studies finding very little (if any) effect of ATD on anxiety ratings (4, 10, 28, 36, 37, 53). Other studies have applied an anxiety-provoking challenge given at the time of maximal TRP depletion. These include not only pharmacological panicogenic agents, such as CO₂ inhalation (5 and 35%) and yohimbine administration, but also behavioral challenges, such as a simulated public speaking paradigm (16, 21, 25, 26, 35). In healthy volunteers, ATD in combination with specifically designed anxiety challenges only produced small increases in anxiety or nervousness. The mood effects of ATD depend on the characteristics of the individual tested: women, subjects with baseline depression scores at the upper end of the normal range, and subjects who have a family history of affective illness are reported to have an increased vulnerability to ATD-induced mood alterations (for review see Ref. 1).

The mechanism through which ATD influences gastric motor function is not known. Because 5-HT has been shown to be involved in the reflex control of gastric motility, it is conceivable that lower 5-HT availability in the GI tract, induced by ATD, influences vagally mediated gastric accommodation and motility (18, 30, 47). ATD-induced changes in 5-HT content in EC or in the ENS could in theory underlie the observed changes. We have measured fundic and duodenal 5-HT concentrations and found that the duodenal mucosa 5-HT content was significantly decreased, although not completely depleted, after standard ATD induction. Although the number of EC tends to be lower in the duodenum, a significant effect could not be found. To fully unravel the effects of ATD on GI function, future studies are needed to better understand the physiology of EC cells. The production of 5-HT in these cells is catalyzed, in a rate-limited fashion, by TRP hydroxylase isoform 1 (TPH1) (15, 56). 5-HT is stored in large, dense core vesicles and small synaptic-like vesicles and released upon mechanical or chemical stimulation (32). The mechanism and/or pathway by which uptake of TRP occurs is, however, not known. The epithelial amino acid transporter system (B₀AT₁, solute carrier 6A19, SLC6A19) that mediates the absorption of orally ingested TRP has been shown to be present on the apical membrane of enterocytes, but it is not known whether EC also express this transporter. As a consequence, it is unclear whether EC absorb TRP themselves directly from the lumen or whether they collect it in a paracrine fashion from neighboring enterocytes. The lack of effect on the number of EC indicates that ATD is, as expected, insufficient to induce a complete depletion of 5-HT in these cells. Probably, more specific interventions are needed to deplete 5-HT from EC, such as knockout or selective inhibition of TPH-1 (22, 52, 55). Taken together, these results indicate that the effect of ATD on gastric accommodation that we observed is most likely attributable to interference with other than mucosal sources of 5-HT.

Apart from EC, a population of neurons also residing in the ENS are known to contain serotonin (12, 13). These serotonergic neurons use TRP hydroxylase isoform 2 (TPH2; sometimes referred to as the “brain” form of the enzyme) to synthesize 5-HT but also are proposed to transport 5-HT from the extracellular milieu (11). Intrinsic serotonergic neurons tend to be highly conserved among species, which makes it likely that, as in other species, they also function as interneurons and participate in mediating propagating contractile complexes in the human ENS (11, 12, 14, 27). Importantly, because TRP has been shown to be transported into enteric neurons, 5-HT turnover in serotonergic neurons is likely to be faster than its mucosal counterpart (2, 14). It is therefore conceivable that a reduction in the rather low 5-HT content in enteric neurons has a more dramatic consequence and is at the origin of the effects we detected. Given the marginal changes in mucosal 5-HT content, the effects on gastric motility observed in the current study could well be considered as important evidence that serotonergic neurons play a crucial role in human GI physiology. Unfortunately, mucosal biopsies fail to provide information on 5-HT content in the human ENS, strongly limiting our ability to determine the relative contribution of 5-HT from mucosal or neuronal sources to the regulation of GI motility and the brain-gut axis in humans.

In addition, it is also conceivable that post-ATD GI motor changes are of central origin. Selective inhibitors of TPH-1 may allow us to better differentiate the role of peripheral and central 5-HT receptors in gastric motor control in humans (55).

A possible limitation in this study is the regeneration of TRP levels under ATD due to massive nutrient intake during the drinking test, which may provide an explanation for the absence of an expected difference in nutrient tolerance. To test the rate of replenishment, we took additional blood samples from the first five participating volunteers. During the drinking test under ATD, TRP levels amounted to 24.3, 36.6, and 52.6% of the baseline value at 15, 30, and 60 min after initiation of the drinking, respectively. The average duration of drinking test was ~1 h. Furthermore, the ATD approach is by its very nature limited to acute modulation of 5-HT levels; therefore, this study does not permit conclusions on the influence of more chronic changes in 5-HT levels, as may be the case in various disease states, on gastric sensorimotor function, nutrient tolerance, and/or symptom levels.

In conclusion, ATD does not affect gastric sensitivity during balloon distension, but it enhances the postprandial accommodation reflex, although this is not reflected by increased nutrient tolerance during a standardized drinking test. Although the influence of central mechanisms cannot be excluded, it is likely that the lowered peripheral 5-HT content, possibly most dramatic in enteric serotonergic neurons, has a role in the gastrointestinal motor changes during and/or after ATD. Further studies in health and functional dyspepsia are needed to elucidate the exact role of serotonin...
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at various levels of the brain-gut axis, from ENS neurons to the brain.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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