Serotonin Signaling Is Altered in Irritable Bowel Syndrome With Diarrhea but Not in Functional Dyspepsia in Pediatric Age Patients

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BACKGROUND & AIMS: In adults, irritable bowel syndrome (IBS) and functional dyspepsia (FD) are chronic conditions that often start during childhood. We investigated mucosal serotonin (5-HT) signaling in children with the idea that data from subjects with a shorter history may improve our understanding of underlying pathophysiological mechanisms. **METHODS:** Ninety-eight children undergoing gastroscopy or colonoscopy were studied prospectively. Biopsy specimens were evaluated for inflammation, enterochromaffin cell numbers, 5-HT content, and messenger RNA (mRNA) levels for the synthetic enzyme, tryptophan hydroxylase 1, and the serotonin transporter (SERT) were assessed by quantitative real-time reverse-transcription polymerase chain reaction. **RESULTS:** Data from 12 children with IBS and 17 with FD were compared with age-matched controls (12 with rectal biopsies and 12 with gastric biopsies) and with subjects with organic disorders. In patients with FD, a small number of immune cells were observed in the gastric mucosa in half of the patients, but no abnormalities with respect to the 5-HT pathway were identified. In patients with IBS, no differences were detected between patients and controls regarding intraepithelial lymphocytes and CD3+ cells in the lamina propria although all patients showed at least a slight inflammatory infiltrate. In the IBS samples, higher 5-HT content ($P < .01$) and lower SERT mRNA ($P < .05$) were detected as compared with controls. Severe inflammation in the colonic mucosa had a high impact on 5-HT signaling with a significant decrease in enterochromaffin cells ($P < .01$) and 5-HT content ($P < .01$) and a high SERT mRNA expression ($P < .01$). **CONCLUSIONS:** These results confirm the role of 5-HT signaling in IBS in children and argue against such a role in FD.

Keywords: Children; Abdominal Pain; Irritable Bowel Syndrome; Functional Dyspepsia; Serotonin; Enterochromaffin Cells; Serotonin Reuptake Transporter; Visceral Sensitivity.

Functional gastrointestinal disorders (FGID) are defined as recurrent symptoms unexplained by structural or biochemical anomalies. Fifteen percent of school-age children are affected by abdominal pain secondary to FGID.[1]

Irritable bowel syndrome (IBS) and functional dyspepsia (FD) as defined by the Rome III criteria are the most frequent painful FGID in children.[2] The pathophysiology of FGID is not clearly understood; however, since 2001, several different groups[3–7] have reported that rectal hypersensitivity is found in 75%–100% of children with IBS, although in adults its prevalence varies from 20%[8] to 94%[9] across studies. This suggests that visceral hypersensitivity is a more reliable marker in children than in adults, and that in adults the chronic conditions with symptoms persisting for years could affect and alter the initial pathophysiological mechanisms. In that context, studying young patients, with a shorter history than adults, may increase the possibility of showing the role of underlying pathophysiologic mechanisms.

Altered mucosal serotonin (5-HT) signaling is one of several hypothetical mechanisms underlying FGIDs. 5-HT is synthesized by enterochromaffin cells (ECs), which use tryptophan hydroxylase-1 (TpH-1) as their rate-limiting enzyme in the biosynthesis of 5-HT.[10] 5-HT is released locally and acts on specific receptors located on nearby nerve fibers and cells in the lamina propria. The 5-HT selective reuptake transporter (SERT) terminates the actions of 5-HT by removing it from the interstitial space.[11] Organic cation transporter-1 (OCT-1) is a low-affinity 5-HT transporter and is expressed in the human GI tract.[12] OCT-1 is thought to contribute to the inactivation of 5-HT when SERT is absent or deficient.[13,14] Studying 5-HT signaling in the intestinal mucosa in children with FGID is of importance for several reasons: (1) 5-HT plays a critical role in the regulation of gastrointestinal motility, secretion, and sensation through specific receptors that are widely expressed within the intrinsic primary afferent neurons, on smooth muscle cells, enterocytes, and extrinsic afferent nerve fibers[10]; (2) changes in 5-HT

Abbreviations used in this paper: EC, enterochromaffin cells; FD, functional dyspepsia; FGID, functional gastrointestinal disorders; 5-HT, serotonin; IBS, irritable bowel syndrome; OCT-1, organic cation transporter-1; QPGS, Questionnaire on Pediatric Gastrointestinal Symptoms in Children; SERT, serotonin transporter; TpH-1, tryptophan hydroxylase-1.

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signaling have been reported in blood[15,16] and colonic mucosa[17,18] of adults with IBS with conflicting results for the latter; and (3) despite the withdrawal of tegaserod and alosetron, both acting on 5-HT_4 and 5-HT_3 receptors that were shown beneficial in IBS in adults, several drugs with an action on the 5-HT signaling, such as 5-HT_{2b}-receptor antagonist or TpH-1 inhibitor, are potentially useful in the treatment of IBS and are under development.[19,20]

By studying young patients with a short duration of symptoms, this study was designed to test the hypothesis that, in children with IBS or FD, 5-HT signaling is altered in the digestive mucosa.

Materials and Methods

Subjects

Gastric or colonic tissues samples were obtained prospectively from children aged 8 to 18 years for whom a colonoscopy or gastroscopy was required in their evaluation. Potential subjects were excluded from the study if they had an acute intestinal infection (acute gastroenteritis) during the 4 weeks preceding the examination. The day of the procedure, all children completed a validated questionnaire, the Questionnaire on Pediatric Gastrointestinal Symptoms in Children (QPGS), which evaluates gastrointestinal symptoms and was adapted to the Rome III diagnostic criteria of FGID in children.[21,22]

Six gastric (corpus) and/or colonic (rectal) biopsy specimens were obtained in mucosa free of ulcerative or aphthous lesions, and each sample was placed immediately into a prepared Eppendorf tube, weighed, and processed for histology, immunohistology, 5-HT content dosage, and RNA extraction.

All patients were reassessed 3 months after the procedures to determine their final diagnosis. The final diagnosis of each participant was established by studying the patient's file, and if necessary, in collaboration with the patient's physician after examination of the QPGS. Participants were classified into 1 of 2 groups: (1) patients with IBS or FD according to Rome III criteria, and (2) subjects with nonfunctional (organic) GI disorder.

According to the responses regarding the symptoms provided in the QPGS (in which abdominal pain was reported or not) the subjects with nonfunctional GI disorders were divided further into the following groups: (1) subjects with painful nonfunctional GI disorder, and (2) subjects with nonpainful nonfunctional GI disorder.

The protocol was approved by the institutional ethics committee and appropriate consent was obtained from all participants; consent was signed by the parents or legal guardians and by the child himself/herself if 14 years or older before the procedure.

Measure of 5-HT Content in Gastric or Rectal Mucosa

The 5-HT content in the homogenized biopsy specimen from each individual was analyzed using an enzyme immunoassay kit according to the manufacturer's instructions (Beckman Coulter; Mississauga, Ontario, Canada).

Inflammation Assessment

Inflammation is known to influence 5-HT signaling[23]; therefore, the mucosal inflammatory condition was determined for each individual included in the study. H&E-stained tissue, 4-μm–thick paraffin sections, from each individual in the study was blindly assigned an inflammatory activity score on a scale of 0–3 by an experienced pathologist (N.P.). The scoring system was as follows: 0, normal number of inflammatory cells in the lamina propria with no active inflammation; 1, minimally increased number of inflammatory cells in the lamina propria and/or a rare neutrophil/eosinophil with the crypt epithelium; 2, mild increased number of inflammatory cells in the lamina propria and/or mild active inflammation; 3, significantly/severely increased number of inflammatory cells in the lamina propria and/or moderate/severe active inflammation. Scores of 0 and 1 were considered to be within the range of normal. Scores of 2 and 3 were considered to be histopathologically inflamed. To further characterize the inflammatory infiltrate in the digestive mucosa of children with FGID, we quantified lymphocytes, neutrophils, and eosinophils in the colonic and gastric mucosa of patients with FD and IBS and compared them with controls. Cells in the lamina propria were assessed as the total number of inflammatory cells within 3–5 nonoverlapping high-power fields at 40× magnification, with H&E staining. Cells were classified as lymphocytes, neutrophils, or eosinophils according to their typical morphology and staining properties. Fields containing lymphoid aggregates were excluded. Lymphocytes and intraepithelial lymphocytes were counted as CD3-positive cells.

Immunohistochemistry

One biopsy from each subject was fixed for 2–3 hours in 2% paraformaldehyde/0.2% picric acid and transferred in 70% ethanol at 4°C. Immunohistochemistry techniques were performed on an automate (NextES IHC, Ventana), and indirect immunoperoxidase staining was performed (see the Supplementary Materials and Methods section).

Lymphocytes were counted in the lamina propria as CD3-positive cells within 3–5 nonoverlapping high-power fields at 40× magnification. Intraepithelial lymphocytes were enumerated as CD3-positive cells per 100 epithelial cells. Numbers of enteroendocrine cells (chromogranin A

Table 1. Demographics and Diagnoses of the 57 Subjects Who Underwent a Gastroscopy

Diagnosis	Number	Sex, M/F	Median age, *y* (range)	Inflammation status (grade of inflammation:number of patients)
FD	17	7/10	14 (10–17)	(0):7 (1):9 (2):1
Controls	40	18/22		
Nonpainful GI disorder	19	9/10	14 (8–18)	(0):7
Eosinophilic esophagitis/GER	7			(1):9
Polyps screening/normal gastroscopy	5			(2):2
Crohn's disease	4			(3):1
Celiac disease	3			
Painful GI disorder	21	9/12	14 (8–18)	(0):6
Eosinophilic esophagitis/GER	8			(1):10
Crohn's disease	9			(2):5
Gallbladder lithiasis	1			
Celiac disease	1			
Helicobacter pylori gastritis	2			

GER, gastroesophageal reflux.

immunoreactive) and EC cells (serotonin immunoreactive) were evaluated at the 40× magnification on the entire section using a quantitative score (number of positive cells divided by number of glandular epithelial cells). All counts were performed blindly by an experienced pathologist (N.P.).

Measurement of RNA

One specimen from each individual was placed in a tube containing RNAlater (Qiagen, Mississauga, Ontario). Reverse-transcription and quantitative real-time polymerase chain reaction were performed for SERT, OCT-1, and TpH-1 in each sample and were normalized by the geometric mean of 3 reference genes (18S, phosphomannomutase 1, and hypoxanthine phosphoribosyltransferase 1)[24] (see the Supplementary Materials and Methods section).

Statistical Analyses

Summary data are expressed as means (± standard deviation) of normally distributed data and medians (25th–75th percentile) for nonnormally distributed data. N values represent the number of subjects included in the data set. Comparisons between the patients (FD or IBS) and the controls used the Student *t* test or the Mann–Whitney *U* test to compare means or medians, respectively, and the chi-square test for count data. A one-way analysis of variance with a Tukey post hoc test was used to compare values from inflamed biopsies obtained from subjects with organic disorders and values from noninflamed biopsies obtained from individuals with organic disorders or controls. The Spearman test was used to correlate the different variables. Significance was expressed at a P of less than .05.

Results

Patients

A total of 118 subjects were recruited for the study (Supplementary Figure 1). Among them, specimens were not collected in 6 for technical reasons (ulcerations or aphthous lesions in the mucosa). In 14 participants who underwent endoscopy, the diagnosis of FGID was performed but neither IBS nor FD was confirmed by the Rome III criteria; these subjects therefore were excluded from the analysis. Ninety-eight children (42 boys; mean age, 13.9 y; range, 8–18 y) finally were included for the study. Fifty-one colonic and 57 gastric specimens were collected. Subject demographics and diagnoses are summarized in Tables 1 and 2. Although female patients were more numerous in the FGID group, there was no difference regarding the sex ratio between patients and control subjects (χ^2 test, $P > .05$). All patients with IBS and FD fulfilled the Rome III criteria. Although the pediatric Rome III criteria do not separate the different subtypes of IBS, we classified the patients according to their predominant alteration of transit as reported in the QPGS: 10 patients reported diarrhea-predominant IBS and 2 reported alternating IBS. Supplementary Table 1 provides symptom severity assessed by QPGS at the time of investigation. All patients with IBS underwent colonic biopsies and all patients with FD underwent gastric biopsies.

Controls

Patients were compared with age-matched subjects with nonfunctional GI disorder in whom colonic or gastric biopsies were obtained. These subjects were divided into 2 groups: those reporting no painful symptoms and those with painful complaints as reported in the questionnaires. Specimens (gastric biopsies, n = 12 [5

Table 2. Demographics and Diagnoses of the 51 Subjects Who Underwent a Colonoscopy

Diagnosis	Number	Sex, M/F	Median age, *y* (range)	Inflammation Status (grade of inflammation:number of patients)
IBS	12	3/9	14 (11–16)	(0):1
				(1):11
Controls	39	19/20		(0):4
Nonpainful GI disorder	24	12/12	14 (8–18)	(1):13
Polyps screening/normal colonoscopy	14			(2):5
Crohn's disease	10			(3):2
				(0):3
Painful GI disorder	15	7/8	14 (8–18)	(1):6
Crohn's disease	15			(2):1
				(3):5

males and 7 females]; colonic biopsies, n=12 [7 males and 5 females]) from subjects whose diagnosis was not inflammatory bowel disease or celiac disease, who reported no painful complaints, and for whom the analysis of the specimens revealed an inflammation grade of 0 or 1 were included in the control group. The others were considered as subjects with organic diseases. Because gastric specimens from subjects with nonfunctional GI disease predominantly were noninflamed (Table 1), the number of gastric biopsies with inflammation grading of 2 or greater was too low to perform statistical analysis. The results obtained with these 8 biopsies were not included in the analysis.

Inflammation in IBS and FD

Analysis of the inflammatory condition of biopsy specimens collected in children with IBS showed that 11 of 12 patients had a minimal inflammation (grade 1), and the remaining specimen had a complete absence of inflammation (grade 0) (Table 2). In the stomach, the proportion was slightly different with biopsies from 7 of 17 FD patients having no inflammation (grade 0), 9 of 17 showing minimal inflammation (grade 1), and 1 of 17 showing moderate inflammation (grade 2) (Table 1). No differences were detected between patients and controls regarding intraepithelial lymphocytes and CD3+ cells in the lamina propria (Table 3), although all patients showed at least a slight inflammatory infiltrate in the lamina propria. Neutrophils were not detected in sections from any of the subjects in any of the groups.

ECs and 5-HT Immunoreactive Cells

In patients with FD, enteroendocrine (chromogranin-positive) cells and EC (5-HT–positive) counts in the gastric mucosa were similar to those of controls and subjects with organic disease (Figure 1*A* and *B*).

In patients with IBS, enteroendocrine cell counts in the colonic mucosa were comparable with those of controls. However, the presence of severe inflammation (grade, ≥2) in specimens of subjects with organic disease led to a significant decrease of chromogranin immunoreactive cells as compared with controls and with subjects with noninflamed organic diseases (Figure 1*C*). A similar pattern was detected for EC counts (Figure 1*D*).

Serotonin Content

In patients with FD, the 5-HT content of the gastric mucosa was similar to controls and to subjects with organic disease (Figure 2*A*).

In patients with IBS, the 5-HT content of the rectal mucosa was higher than controls (Figure 2*B*). Inflammation appeared to influence the 5-HT content in the rectal mucosa because significantly lower 5-HT concentrations were detected in the inflamed specimens from subjects with organic disease than controls and noninflamed organic diseases (Figure 2*B*).

TpH-1 Messenger RNA

TpH-1 is the rate-limiting enzyme of 5-HT synthesis in EC cells.[10] In the patients with FD, transcript levels for TpH-1 in gastric biopsies were similar to those

Table 3. Characterization of Mucosal Inflammation in Patients With IBS or FD

	IBS	Controls	IBS vs controls	FD	Controls	FD vs controls
IEL	6.0 (4.1–14.6)	6.1 (1.0–10.2)	NS	8.5 (4.2–14.3)	6.8 (3.2–10.3)	NS
Lymphocytes	48.5 (18–126)	44.2 (29–104)	NS	43.7 (20–60)	49 (17–87)	NS
Neutrophils	0.0 (0–2)	0.0 (0–1)	NS	0.0 (0–1)	0.0 (0–1)	NS
Eosinophils	0.0 (0–0)	0.0 (0–1)	NS	0.0 (0–0)	0.0 (0–0)	NS

NOTE. Data are expressed as median and range. Intraepithelial lymphocytes are expressed per 100 epithelial cells and lymphocytes, neutrophils, and eosinophils are expressed as the number of cells per high-power field.
IEL, intraepithelial lymphocytes.

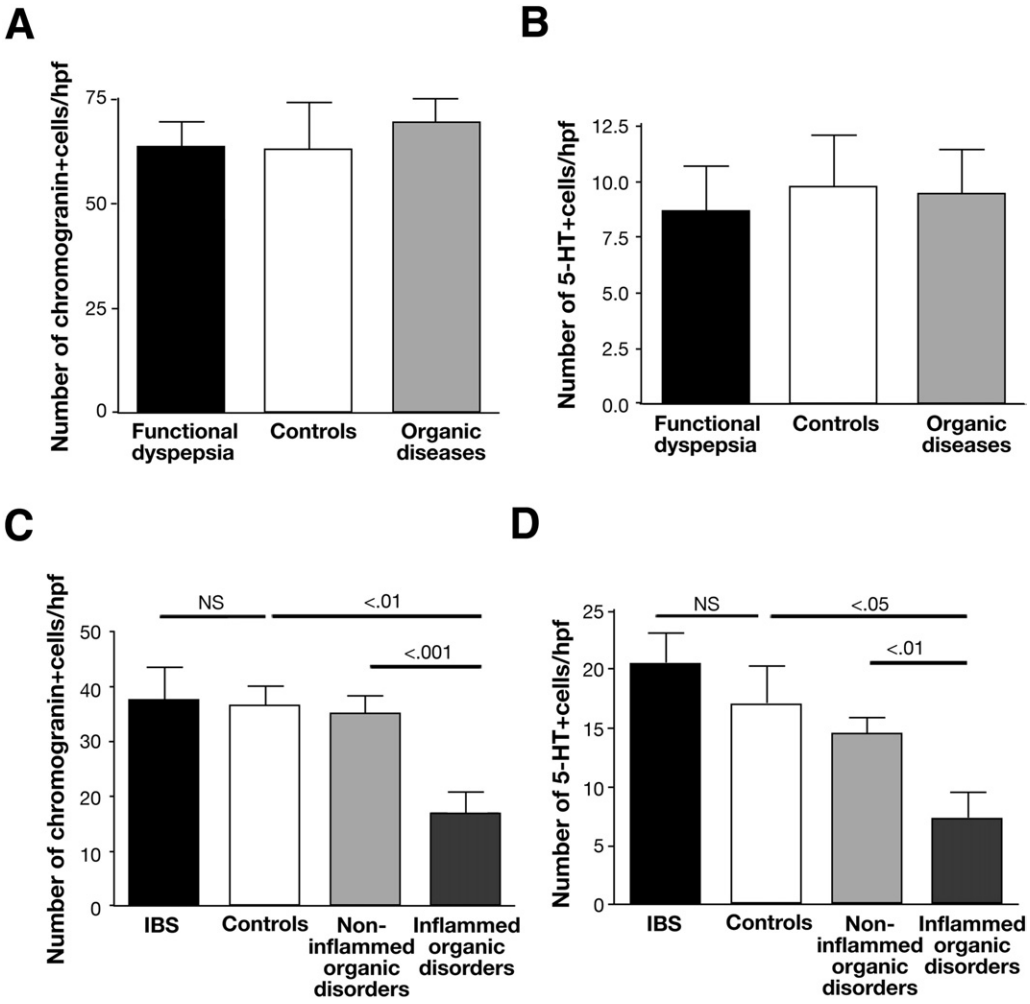

Figure 1. (*A*) Enteroendocrine (chromogranin-positive) cell and (*B*) enterochromaffin (5-HT–positive) cell counts in the gastric mucosa of patients with FD are similar as compared with control subjects and subjects with organic diseases. (*C*) Enteroendocrine (chromogranin-positive) cell and (*D*) enterochromaffin (5-HT–positive) cell counts in the rectal mucosa of patients with IBS, control subjects, and subjects with organic diseases with inflammation grading greater than 2 (inflamed organic disorders) and without inflammation (noninflamed organic disorders). No significant differences were detected in enteroendocrine and enterochromaffin cell counts of samples from patients with IBS as compared with controls. Biopsies with inflammation grading greater than 2 obtained from subjects with organic disease showed significant lower enteroendocrine and enterochromaffin cell counts as compared with controls and biopsies without inflammation (grade, <1).

measured in controls (median [interquartile range] of relative expression of TpH-1 for FD, 5.65 [2.5–7.1] vs 2.67 [1.62–3.7] for controls; $P > .05$).

In patients with IBS there was no difference between patients and controls and with noninflamed controls (Figure 3). However, the presence of severe inflammation (grade, ≥2) in specimens of subjects with organic disease led to a significant overexpression of TpH-1 messenger RNA (mRNA) as compared with subjects with noninflamed organic diseases (Figure 3), suggesting that inflammation itself increases TpH-1 mRNA expression.

SERT mRNA

In patients with FD, SERT mRNA expression in the gastric specimens was comparable with controls and subjects with organic diseases (median [interquartile range] of relative expression of SERT for FD, 2.49 [1.93–3.7] vs 2.67 [1.97–3.1] for controls; $P > .05$).

In rectal mucosa of the patients with IBS, the SERT mRNA level was significantly lower than in controls (Figure 4). The presence of severe inflammation (grade, ≥2) in specimens of subjects with organic disease led to a significant overexpression of SERT mRNA as compared with subjects with noninflamed organic diseases (Figure 4).

OCT-1 mRNA

Because OCT-1 contributes to the inactivation of 5-HT in the absence of SERT, we examined OCT-1 mRNA expression to further find a mechanism to explain the higher content in 5-HT in IBS and to explore a possible involvement of 5-HT signaling in FD. No difference was seen in the gastric mucosa of patients with FD as compared

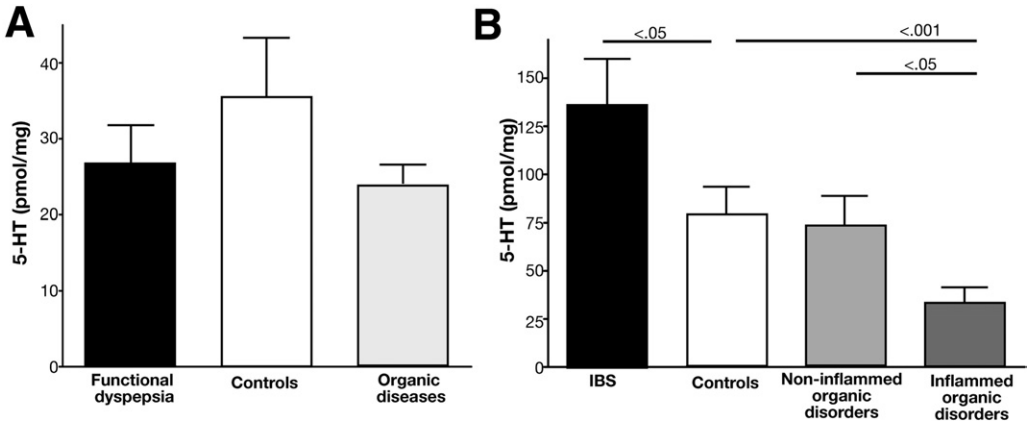

Figure 2. (*A*) 5-HT content in the gastric mucosa of patients with FD is similar as compared with control subjects and subjects with organic diseases. (*B*) 5-HT content in the rectal mucosa of patients with IBS, control subjects, and subjects with organic diseases with inflammation grading greater than 2 (inflamed organic disorders) and without inflammation (noninflamed organic disorders). 5-HT content is significantly higher in the rectal mucosa of patients with IBS as compared with control subjects. 5-HT levels are significantly lower in biopsies obtained from subjects with inflamed organic disorders as compared with controls and biopsies from noninflamed organic disorders.

with controls either inflamed or not (median [interquartile range] of relative expression of OCT-1 for FD, 1.23 [0.95–1.51] vs 1.41 [1.13–1.64] for controls; $P > .05$).

Similarly, OCT-1 mRNA expression was identical in the rectal mucosa of patients with IBS and controls and subjects with organic disease with or without inflammation (Supplementary Figure 2).

Correlation Between SERT mRNA and 5-HT Content

A significant inverse correlation also was found between SERT mRNA and 5-HT content in the colonic specimens ($r = -0.47$; $P = .0003$) (Supplementary Figure 3). No such correlation was found in the gastric specimens.

Discussion

IBS and FD are highly prevalent in children, but, as in adults, the pathophysiology is not yet fully under-

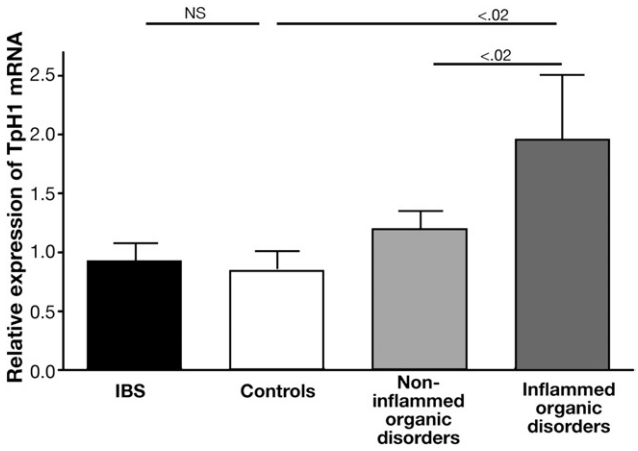

Figure 3. Relative expression of TpH-1 mRNA in the rectal mucosa of patients with IBS, control subjects, and subjects with organic diseases with inflammation grading greater than 2 (inflamed organic disorders) and without inflammation (noninflamed organic disorders). No significant differences were detected in TpH-1 transcript levels of samples from patients with IBS as compared with controls. TpH-1 transcript levels are significantly higher in biopsies obtained from subjects with inflamed organic disorders as compared with controls and biopsies from noninflamed organic disorders.

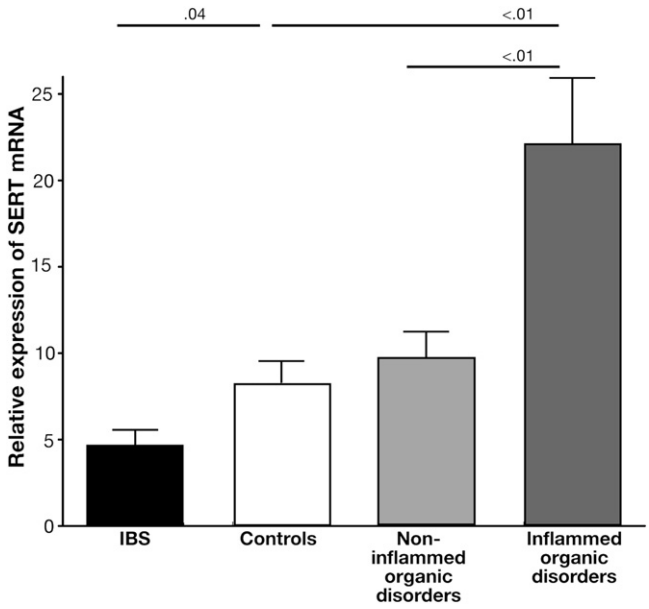

Figure 4. Relative expression of SERT mRNA in the rectal mucosa of patients with IBS, control subjects, and subjects with organic diseases with inflammation grading greater than 2 (inflamed organic disorders) and without inflammation (noninflamed organic disorders). A significantly lower level of SERT transcript was detected in samples from patients with IBS as compared with controls. SERT transcript levels are significantly increased in biopsies obtained from subjects with inflamed organic disorders as compared with controls and biopsies from noninflamed organic disorders.

stood. We conducted a prospective study to test the hypothesis that mucosal abnormalities are present in children with FD and IBS. We designed our study protocol in such a way that we were able to analyze not only controls with nonfunctional GI (organic) disorders, but also ideal controls for which endoscopic procedures were performed for conditions not related to inflammatory and/or painful GI disorders such as colonic polyps screening.

FGID in adults is a chronic condition with symptoms that, in a majority of patients, began during childhood and persisted for years.[25–27] They therefore can be considered as a continuum from childhood to adulthood. One can suggest that the protracted evolution could affect and alter the initial pathophysiologic mechanisms in such a way that a clear demonstration of involvement of 5-HT signaling is not easy and may explain the discrepancies in the literature.[17,18] In that context, studying young patients, with a shorter history of symptoms than adults, increases the possibility of showing the pathophysiological mechanisms as illustrated by the higher prevalence of rectal hypersensitivity in children with IBS across studies from several independent groups[3–7] than in adults.[8,9,28]

In children with FD, a low inflammation grade in the gastric mucosa was detected in half of the patients, but no abnormalities with respect to the 5-HT pathway were identified as compared with controls. Our data argue against a role of mucosal 5-HT in the symptoms of FD and are consistent with the lack of efficacy of pharmacologic 5-HT signaling modulation in such patients.[10] Other investigators similarly failed to show any role of altered mucosal 5-HT biosynthetic and uptake capacity in upper abdominal symptoms in idiopathic gastroparesis.[29] However, all gastric specimens evaluated in the present study were obtained from the corpus, a region known to have a low number of EC cells. The paucity of these cells may hamper the detection of any potential change occurring in FD or organic disorders relative to controls. Whether similar results may be obtained in other regions of the upper GI tract is debatable because, for example, SERT, TpH-1, and EC cells vary significantly in the different regions of the stomach and in the duodenum.[30]

In children with IBS, we detected immune cells in the rectal mucosa in the majority of cases and a higher availability of 5-HT with higher 5-HT content and lower SERT mRNA than in control subjects. Low-grade inflammation has been reported in the enteric ganglia[31] and in the mucosa[31,32] of adult patients with IBS, and recent studies outline the key role of the mucosal inflammation in driving possibly local modifications promoting peripheral sensitization in adults.[33–35] The role of a GI infectious episode before the IBS symptoms was not specifically assessed in the patients included in the present study because of the major recall bias of such a retrospective question.[36,37]

We did not find a significant difference in enteroendocrine cells and ECs between IBS and controls similar to previous adult studies.[17,38] However, we found in the IBS patients a higher mucosal 5-HT availability than in controls. This higher availability was not owing to a higher synthesis rate as shown by the normal mRNA expression of TpH-1, the rate-limiting enzyme of 5-HT synthesis in ECs. However, recent data show that TpH-2, the "brain" isoform of TpH, also is present in enteric neurons and may participate in 5-HT synthesis in the GI tract.[39] Because we used mucosal biopsies that are superficial and do not contain submucosal or myenteric neuronal bodies, TpH-2 mRNA was not quantifiable and we cannot exclude the possibility that the higher 5-HT mucosal content in IBS patients may not be related to higher TpH-2 expression. One possible mechanism to explain the higher 5-HT availability is the lower SERT mRNA expression in IBS rectal mucosa found in our population. The significant correlation found between 5-HT content and SERT mRNA in the colonic specimens highly reinforces this possible explanation. The low expression of SERT mRNA in IBS patients reported here was not caused by a differential expression of the reporter genes as compared with the controls. Moreover, because of discrepancies in the literature, we paid particular attention to the quantification of the mRNA and used, as suggested in the most recent recommendations, 3 reporter genes and their geometric mean. SERT mRNA expression in IBS has been reported previously in the adult literature with conflicting results. Coates et al[17] reported a significant reduction of SERT mRNA expression in colonic mucosa of adult patients with IBS. The results were not replicated by Camilleri et al,[18] and Kerckhoffs et al[40] found conversely an increased expression of SERT mRNA in the duodenal mucosa of IBS patients. On the other hand, reports of increased postprandial plasma 5-HT concentrations in diarrhea-predominant IBS[16,41] are consistent with a decreased capacity of the intestinal epithelial cells to take up 5-HT. To further examine the 5-HT uptake capacity in these samples, we studied the expression of OCT-1 mRNA, which transports 5-HT when SERT is absent. We did not find any difference between the patients and the controls, suggesting that OCT-1 is neither defective nor overexpressed in the conditions examined here. SERT gene polymorphisms, which may be associated with diarrhea-predominant IBS in adult females,[42] also could be involved in the modulation of 5-HT signaling in children with IBS.

In the present study, we report that colonic mucosal inflammation has a high impact on 5-HT signaling in children. Despite a high TpH-1 mRNA expression, we report low 5-HT availability in the subjects with high-

grade inflammation in the rectal mucosa with a significant decrease in EC cells and 5-HT content and a high SERT mRNA expression. The low 5-HT content is not owing to a loss of tissue because the biopsies were not performed into ulcerative zones; the high SERT mRNA expression is not related to low expression of reporter genes as previously mentioned.

All patients with a high inflammatory grade were afflicted by Crohn's disease. Recent animal studies have shown that 5-HT plays a key role in inflammation[43,44] but conflicting results have been reported in inflammatory colitis with some investigators reporting increased ECs and 5-HT content[45,46] whereas others reported a decrease in ECs and 5-HT.[17,47–49] Our present data are in keeping with the work of Motomura et al[50] showing that T helper-1 response, which characterizes Crohn's disease, has been shown to provoke a decrease of ECs and 5-HT availability. Increased SERT transcripts in inflammation was an unexpected finding because decreased SERT mRNA levels have been reported in ulcerative colitis[17] and in animal models of inflammatory colitis.[51] On the other hand, Minderhoud et al[52] did not find any difference in SERT mRNA in either the ileum or in the colon in patients with Crohn's disease as compared with controls, and a recent large study reported that SERT mRNA was significantly higher in the ileal mucosa of patients with Crohn's disease.[53] Moreover, our data on SERT mRNA are consistent with the lower 5-HT content measured in the inflamed colonic biopsies. The exact role played by 5-HT and the regulation of its synthesis and uptake during the course of inflammation and the influence of anti-inflammatory drugs are unknown. The apparent discrepancy between the high level of TpH-1 mRNA and the low 5-HT content reported here requires further studies.

Potential limitations of this study are related to the relatively low number of patients included. However, despite the difficulties in obtaining tissue specimens in children, we were able to recruit children who can be considered appropriate controls because they were asymptomatic regarding painful symptoms and were investigated for nonpainful and noninflammatory GI disorders. Their specimens were controlled carefully for inflammatory status. We also are aware that the participants were recruited from a tertiary pediatric center and therefore those with IBS and FD may be at the more severe end of the spectrum of the functional disorders. If this is related to increased involvement of 5-HT signaling, it may result in an overestimation of the difference in IBS patients and controls but argues definitely against the potential role of 5-HT in FD. However, we cannot conclude that the present findings are valid for all pediatric patients with FGID in the community. Finally it should be noted that these results do not necessarily apply to constipation-predominant IBS patients because the present study was not designed to specifically include constipated patients.

Because this study was conducted in children and adolescents for whom the functional symptoms are of shorter duration than adults, we believe that the present results are of great value and definitely confirm the role of 5-HT in IBS and argue against such a role in FD. However, we are aware that FGID in childhood could not be explained exclusively by a biological model but rather is the result, as in adults, of complex interactions between biological (5-HT signaling) and social, familial, and psychological traits.

These results provide important data that should further encourage the development of new treatments oriented toward a lower 5-HT availability in patients with IBS such as TpH-1 inhibitors[54] and 5-HT uptake enhancer.[20]

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2010.03.032.

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Conflicts of interest

The authors disclose no conflicts.

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