Abnormalities of 5-Hydroxytryptamine Metabolism in Irritable Bowel Syndrome

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Background & Aims: **5-hydroxytryptamine-3 (5-HT_3) receptor antagonists improve symptoms in patients with diarrhea-predominant irritable bowel syndrome (D-IBS), 5-HT_4 agonists help those with constipation-predominant IBS (C-IBS). These data suggest excess or deficiency in 5-HT in D-IBS or C-IBS, respectively. Mucosal 5-HT–containing enterochromaffin cells (EC) are increased in postinfectious IBS (PI-IBS). Our aim was to define the postprandial release of 5-HT in PI-IBS and C-IBS patients and to relate this to mucosal 5-HT turnover.** ***Methods:*** **Fifteen PI-IBS patients with diarrhea-predominant symptoms, 15 C-IBS patients, and 15 healthy controls underwent serial (platelet-poor) plasma 5-HT measurement for 3 hours after a standard 520-kcal meal. Rectal biopsy specimens were assayed for 5-HT and its metabolite 5-hydroxindoleacetic acid (5-HIAA). Colonic transit was measured using radio-opaque markers.** ***Results:*** **Colonic transit was prolonged in C-IBS patients (mean ± SEM) (49.4 ± 3.8 h) compared with PI-IBS (26.7 ± 4.5) and control patients (34.1 ± 4.5) ($P < .02$). Release of 5-HT assessed by area under the curve (AUC) of platelet-poor plasma 5-HT from 0 to 180 minutes postprandially was significantly lower in C-IBS patients (2593 ± 309 mmol/L · min) compared with P-IBS (5623 ± 721) and control patients (4822 ± 598) ($P < .001$). PI-IBS patients showed significantly higher peak postprandial plasma 5-HT values (median, range) (71.7, 43.4–125.3) ng/L compared with C-IBS patients (31.2, 15.2–40.5) and control patients (43.6, 26.7–50.1) ($P < .01$). Mucosal 5-HT turnover as assessed by mucosal 5-HIAA/5-HT ratio was decreased in both C-IBS and PI-IBS patients, .14 (.01–.6) and .21 (.02–2.5), respectively, compared with control patients 1.12 (.17–3.1) ($P < .002$).** ***Conclusions:*** **C-IBS patients show impaired postprandial 5-HT release whereas PI-IBS patients have higher peak levels, abnormalities that may be related to their different symptoms.**

Irritable bowel syndrome (IBS) is one of the most common causes of gastroenterologic consultations,[1,2] yet its causes remain poorly understood. Recent therapeutic successes with serotonin (5-hydroxytryptamine [5-HT])-modulating agents[3,4] have encouraged a re-examination of the role of 5-HT in IBS. Since the pioneering work of Bulbring and Crema,[5] it has been known that 5-HT plays a key role in gut motility. Their work and that of others also established the importance of 5-HT in mediating intestinal and pancreatic secretions.[6–8] Most of the 5-HT within the gut is stored within the enteroendocrine cells, historically known for their brown staining with dichromate as enterochromaffin (EC) cells. These act as transducers, releasing 5-HT in response to a range of luminal stimuli including nutrients, pressure, or toxins.[6,9,10] The 5-HT released acts on mucosal nerves within the lamina propria, activating secretomotor responses and also signaling gut conditions to the central nervous system via visceral afferents.[11] Although most of these mechanisms have been defined in experimental animals, more recently, treatment trials in both healthy volunteers[12] and IBS patients[13,14] have confirmed its importance in controlling gastrointestinal function. 5-HT_3 antagonists decrease urgency and frequency in diarrhea-predominant IBS (D-IBS) and improve stool consistency,[15] whereas 5-HT_4 agonists stimulate bowel frequency and decrease stool consistency.[14,16]

Interest in this area has been stimulated further by the recognition that a proportion of D-IBS patients develop their symptoms acutely after an infectious illness, the so-called *postinfectious IBS* (PI-IBS). Serial rectal biopsy specimens from a cohort of patients recovering from *Campylobacter jejuni* enterocolitis have shown a significant increase in 5-HT–containing EC cells[17] that appears to persist in those who go on to develop PI-IBS.[18] Further studies have indicated an increase in the messenger RNA for the inflammatory cytokine interleukin-1β.[19] Further-

Abbreviations used in this paper: AUC, area under the curve; C-IBS, constipation-predominant irritable bowel syndrome; D-IBS, diarrhea-predominant irritable bowel syndrome; EC, enterochromaffin cells; 5-HIAA, 5-hydroxindoleacetic acid; 5-HT, 5-hydroxytryptamine; IBS, irritable bowel syndrome; PI-IBS, postinfectious irritable bowel syndrome; SERT, serotonin transporter; SSRI, selective serotonin re-uptake inhibitor.

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more, animal studies have indicated that nonspecific inflammation causes not only EC cell hyperplasia but also decreased expression of the serotonin transporter (SERT). This leads to increased 5-HT availability at the mucosal level in response to mechanical stimuli.[20]

Measuring 5-HT release in humans is difficult because 5-HT released in the mucosa is taken up avidly, first locally by enterocytes and then in the portal blood by hepatocytes. What little remains to enter the systemic circulation then is taken up rapidly and stored in platelets. Although only about .1% of 5-HT released can be measured as free 5-HT within the plasma, early pilot studies have indicated that this measure is increased in D-IBS patients.[21] A more substantial recent study showed that D-IBS patients who experienced symptoms during the test meal showed a significant increase compared with those who did not experience symptoms.[22]

Based on the earlier-described evidence we hypothesized that patients with PI-IBS would have increased postprandial 5-HT release and 5-HT turnover at the mucosal level and that this should relate to accelerated colonic transit. Our aim, therefore, was to assess 5-HT content and turnover within the gut mucosa and to relate this to its release and colonic transit in PI-IBS and to compare this with asymptomatic healthy controls, as well as constipation-predominant IBS (C-IBS) as a disease control.

Materials and Methods

IBS Patients and Healthy Controls

Thirty patients who met the Rome II criteria for IBS[23] and who had completed a full negative evaluation for other diseases in the University Hospital, Nottingham Gastroenterology Outpatient Clinic were included. Over the duration of the study 133 IBS patients were seen. After applying the exclusions listed later there were 51 eligible patients, of whom 30 chose to take part. The most common reason for not participating was inability to take time off from work. The evaluation included a detailed history, examination, sigmoidoscopy and biopsy examination, full blood count, hematinics, electrolytes, anti-endomysial antibody, thyroid function, calcium, liver function tests, and, if relevant, colonoscopy, barium follow-through, ^{75}SeHCAT (selenium homocholic acid taurine) scanning, and duodenal biopsy examination. Patients with a positive lactose tolerance test whose symptoms responded to a lactose-free diet were excluded. Fifteen patients developed symptoms of D-IBS acutely after an episode of gastroenteritis and met our previously published criteria for PI-IBS. We defined PI-IBS as new bowel symptoms developing in a previously asymptomatic individual immediately after an acute illness characterized by 2 or more of the following: diarrhea, vomiting, fever, or positive stool culture.[24] Fifteen patients had C-IBS characterized by hard infrequent stools. The onset of the IBS symptoms in this group was not postinfectious. We also recruited 15 healthy volunteers without gastrointestinal symptoms or disease through advertisements placed around the University Hospital (control group). Volunteers had a general examination and blood was taken for full blood count, hematinics, thyroid function, liver function, calcium, C-reactive protein, and anti-endomysial antibody counts.

Exclusion criteria were as follows: pregnancy; other significant illness other than IBS, bleeding, or clotting disorders; gastrointestinal surgery (other than appendectomy or cholecystectomy); history of alcohol or drug dependence; any medication that may alter gastrointestinal motility (especially prokinetics, serotonin reuptake antagonists, tricyclic antidepressants, and opiates). None of our patients had a body mass index of $\leq$17 or $\geq$30 kg/m^2. Volunteers were excluded if they had taken part in any other clinical study within the previous 3 months or if they met the Rome II criteria for IBS. The study was approved by the University Hospital, Nottingham, and the University of Nottingham Medical School Ethics committees. All subjects gave written informed consent.

Study Protocol

After screening, patients attended on 2 study days. On study day 1, questionnaires were completed and patients underwent a sigmoidoscopy and rectal biopsy examination. A diary was given to ensure that the marker capsules for assessing colonic transit were taken on the 3 days before study day 2. On study day 2, patients attended after an overnight fast, an 18-gauge intravenous cannula was placed in a large antecubital fossa vein, and a plain abdominal radiograph was obtained. At least 2 hours after inserting the intravenous cannula, patients ingested the test meal and serial blood samples were collected.

Rectal biopsy specimens were obtained using a rigid sigmoidoscope without bowel preparation. Biopsy specimens were taken 10 cm from the anal verge by using endoscopic biopsy forceps (FB-13K-1; Olympus, Tokyo, Japan). Two specimens were taken for H&E and immunohistochemical staining and 2 further biopsy specimens were immediately snap-frozen in liquid nitrogen and then stored at -80°C for subsequent mucosal 5-HT quantification. 5-HT–containing cells were counted on coded slides within 4 nonoverlapping high-power fields (final magnification, 200×) on a single microscope by one person (S.D.) who was blind to clinical details. Patients completed questionnaires for habitual postprandial symptoms of pain, loose stools, and urgency, in addition to previous diagnoses or treatment of food intolerance, anxiety, or depression.[25]

Colonic transit was measured using the simplified technique described by Metcalf et al.[26] Two gelatin capsules, each enclosing 10 plastic marker pellets coated with barium, were taken at 8 AM for 3 days before a plain abdominal radiograph was taken at 9 AM on day 4. Colonic transit in hours was estimated by multiplying the number of visible markers on the radiograph × 1.2.