Altered 5-Hydroxytryptamine Signaling in Patients With Constipation- and Diarrhea-Predominant Irritable Bowel Syndrome

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Background & Aims: **Evidence suggests that postprandial platelet-depleted plasma 5-hydroxytryptamine (5-HT) concentrations may be abnormal in irritable bowel syndrome (IBS). However, interpretation of the data has been hampered by the variable methodology and rather small numbers used in previous studies. Therefore, the aim of this study was to measure concentrations of platelet-depleted plasma 5-HT and its metabolite 5-HIAA under fasting and fed conditions in a large group of patients with diarrhea-predominant (d-) and constipation-predominant (c-) IBS, compared with controls. The ratio of plasma 5-HIAA:5-HT and platelet stores was also assessed.** ***Methods:*** **Twenty-nine c-IBS patients (aged, 19–53 years), 55 d-IBS patients (aged, 19–52 years), and 35 healthy volunteers (aged, 18–46 years) had platelet-depleted plasma 5-HT/5-HIAA concentrations measured using reverse-phase, high-performance liquid chromatography with fluorimetric detection before and after a standard meal.** ***Results:*** **d-IBS patients had raised platelet-depleted plasma 5-HT concentrations under fasting and fed conditions ($P < .05$). However, the postprandial relative to fasting concentration was similar to controls. In contrast, c-IBS patients failed to show an increase in platelet-depleted plasma 5-HT concentration with meal ingestion compared with controls ($P < .01$). c-IBS was associated with decreased 5-HIAA ($P < .01$) but normal 5-HIAA:5-HT ratio and d-IBS with normal 5-HIAA concentrations but reduced 5-HIAA:5-HT ratio ($P < .005$). C-IBS but not d-IBS patients had increased platelet 5-HT.** ***Conclusions:*** **These results support the concept that d-IBS is characterized by reduced 5-HT reuptake, whereas impaired release may be a feature of c-IBS. These results also provide a rational basis for current pharmacologic approaches involving modulation of different 5-HT receptors in c- and d-IBS.**

Irritable bowel syndrome (IBS) is associated with both motor and sensory abnormalities of the gastrointestinal tract. These can vary depending on the bowel habit subtype of the patient, with those exhibiting diarrhea having increased colonic motility, particularly the number of high-amplitude propagating contractions (HAPCs) and transit,[1–3] and those with constipation having reduced colonic motility, HAPCs, and transit[2–6] compared with healthy controls. Both patient subgroups, however, can demonstrate visceral hypersensitivity,[7] although only those with constipation appear to sometimes exhibit hyposensitivity to balloon distension.[7–9]

5-Hydroxytryptamine (5-HT) released from the enterochromaffin (EC) cells of the gastrointestinal (GI) tract and acting through various receptor subtypes located on submucosal and myenteric neurons of the enteric nervous system is known to play a key role in the normal functioning of the gastrointestinal tract.[10–13] Depending on the type of receptor subtype and its location, stimulation can result in, for example, smooth muscle relaxation through its interaction with 5-HT_4 or 5-HT_{1D} receptors on inhibitory nitrergic neurons or contraction via interaction with 5-HT_4 or 5-HT_3 receptors on cholinergic neurons.[10–13] 5-HT may also modulate visceral sensation either via a direct effect on perception or through modulation of gastrointestinal tone and phasic activity. Activation of 5-HT_3 receptors on vagal afferents and 5-HT_3 and possibly 5-HT_4 receptors on either spinal afferents or nerves closely coupled with these nerves are thought to mediate the possible direct effects of 5-HT on visceral perception.[10–13]

5-HT found in the blood is almost entirely derived from the EC cells of the GI tract,[14] and we and others have shown that the concentration of this amine,[15,16] along with its metabolite 5-hydroxyindoleacetic acid

Abbreviations used in this paper: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; EC, enterochromaffin; c-IBS, constipation-predominant IBS; d-IBS, diarrhea-predominant; HV, healthy volunteers; PI-IBS, postinfectious IBS.

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(5-HIAA),[16] are elevated in platelet-depleted plasma following meal ingestion in patients with diarrhea-predominant IBS (d-IBS) compared with healthy controls. Under fasting conditions, 5-HT was either undetectable[15] or of a concentration that was not significantly different from healthy controls.[16] However, using a linear modeling approach to take into account various factors, such as fasting concentrations, the latter study showed that fasting 5-HT concentration significantly affected concentrations recorded under fed conditions, such that the postprandial relative to fasting concentration of 5-HT appeared to be no different between d-IBS patients and healthy controls.[16] These observations suggest that, despite this being a relatively large study (39 d-IBS patients and 20 healthy volunteers), it may not have been sufficiently powered to detect differences under fasting conditions and that d-IBS patients may generally have high concentrations of platelet-depleted plasma 5-HT rather than an exaggerated 5-HT response to meal ingestion. The additional observation that platelet concentration of 5-HT was higher in d-IBS patients compared with healthy controls[16] appeared to support this view because platelets are unable to synthesize 5-HT and therefore acquire it directly by active transport from the circulation.[13,17]

In contrast, a recent study has suggested that patients with constipation-predominant IBS (c-IBS) may have lower concentrations of plasma 5-HT following meal ingestion compared with healthy controls and patients with postinfectious IBS (PI-IBS).[18] Interestingly, platelet 5-HT concentration was also shown to be higher in c-IBS patients compared with PI-IBS patients but not statistically different from healthy controls.[18] However, this study was relatively small (15 subjects per group) and did not report fasting 5-HT concentration, postprandial relative to fasting concentration of 5-HT (ie, the 5-HT response to meal ingestion), plasma 5-HIAA concentration, or the ratio of plasma 5-HIAA:5-HT or make comparisons with d-IBS patients.

Therefore, the aim of this study was to assess both fasting and fed concentrations of platelet-depleted plasma 5-HT to determine the 5-HT response to meal ingestion in a large number of patients with c- and d-IBS compared with healthy controls. To explore possible mechanisms of altered 5-HT signaling, platelet-depleted plasma 5-HIAA concentrations along with the ratio of 5-HIAA:5-HT and platelet 5-HT stores were also assessed.

Materials and Methods

Subjects

The study was carried out on 29 patients with c-IBS (aged, 19–53 years; 3 male) and 55 patients with d-IBS (aged, 19–52 years; 5 male), diagnosed according to the Rome II criteria[19] together with 35 healthy volunteers (HV) (aged, 18–46 years; 6 male). Patients were recruited from the outpatient departments of the University Hospitals of South Manchester (tertiary patients excluded), Manchester, United Kingdom; local general practices; advertisement in regional newspapers; and an existing departmental volunteer pool of patients. No subject had coexistent disease, and all had normal hematology, biochemistry, urinalysis, and sigmoidoscopy, together with a normal colonoscopy or barium enema if aged over 40 years. All healthy subjects had normal laboratory investigations (as above) and negative toxicology for substances of abuse. Subjects were excluded for the following: had a history of gastrointestinal surgery (other than appendectomy and hiatus hernia repair); gastrointestinal symptoms related to or exacerbated by consumption of milk or milk products; or were taking drugs that might modify either gastrointestinal function or the 5-HT system, such as analgesic medication, tranquillizers, or antidepressants. Female subjects were excluded if they were pregnant, breast-feeding, or hysterectomized, and all were postpubertal and premenopausal. Because there is evidence to suggest that steroid ovarian hormones might affect the 5-HT system,[20,21] all female subjects were studied during the luteal phase of the menstrual cycle (high progesterone and estrogen) or while taking combined (nonphased) estrogen/progesterone contraceptive medication. All medications and cigarette smoking were prohibited for 48 hours prior to the study, and alcohol and caffeine-containing drinks were stopped 24 hours before the study. All subjects drank below the recommended safe alcohol limit (<21 units/week), smoked <5 cigarettes per day, and had not participated in a clinical trial of any drug within the previous 30 days. The study was approved by South Manchester Medical Research Ethics Committee, and all subjects gave written informed consent.

Study Design

After an overnight fast, subjects attended the Neurogastroenterology Unit, and an arm vein was cannulated. Nine milliliters of blood was taken via EDTA vacutainer at 0 hours for platelet count and 5-HT/5-HIAA analysis. Additional 5-mL blood samples for 5-HT and 5-HIAA analysis were taken at hourly intervals for 2 hours under fasting conditions and at half-hour intervals for a further 4 hours following ingestion of a standard carbohydrate-rich meal, consisting of 200 g spaghetti (Heinz; Stockley Park, Uxbridge, United Kingdom), 2 medium slices of toast, a jam and fresh cream scone (Marks and Spencer, London, United Kingdom), and 200 mL water (totaling 65.5 g carbohydrate, 12 g protein, 16 g fat, calorie content of 457 kcal), which was consumed within 10 minutes.[16]

Symptomatology was assessed on attendance at the laboratory with the question "Is your IBS active at the moment?" In addition, at hourly intervals (0, 1, 2, 3 hours . . .) throughout the study, questions targeting the presence and severity of abdominal pain/discomfort, bloating, and bowel urgency were asked, eg, "In the past hour, have you experienced abdominal pain/discomfort?" If the subjects reported "yes," they were then asked to grade the severity of that symptom using the scale 1 = mild, 2 = moderate, 3 = intense, and 4 = severe. A worsening of symptoms with meal ingestion was defined as an increase in symptom score of at least 1.[16]

Analysis of Platelet-Depleted Plasma Concentrations of 5-HT and 5-HIAA

The collected blood (t = −2 to 4 hours postmeal) was transferred to tubes containing 0.5 mL 3.12% trisodium citrate and centrifuged (room temperature) twice to ensure no platelet contamination of supernatant; initially at 2500 rpm for 10 minutes and then at 4000 rpm for a further 10 minutes. Platelet-depleted plasma was aspirated, and duplicate samples were stored at −70°C for later batch analysis. 5-HT and 5-HIAA concentrations were measured in duplicate using reverse-phase, high-performance liquid chromatography (HPLC) with fluorimetric detection.[16,22,23] All samples were analyzed blind to subject status. The sensitivity of the HPLC system for the detection of 5-HT and 5-HIAA was as previously described.[16]

Analysis of Platelet 5-HT Concentrations

The blood collected at t = −2 hours was transferred to a tube containing 0.9 mL 3.12% trisodium citrate and initially spun only once at 900 rpm for 5 minutes. One aliquot of aspirated, platelet-rich plasma was then used to assess platelet count (Advia Centaur Analyser; Bayer Ltd, Berkshire, United Kingdom), whereas additional duplicate samples were used for 5-HT analysis of platelet-rich plasma, and following further centrifugation (as above) for analysis of platelet-depleted plasma 5-HT/5-HIAA concentrations.[16]

Data and Statistical Analysis

The following end points were analyzed for 5-HT and 5-HIAA platelet-depleted plasma concentrations: (1) fasting concentration, calculated as the average of the preprandial measurements; and (2) ratio to fasting baseline area under the postprandial concentration/time curve. This was calculated as the area under the postprandial concentration curve vs time divided by fasting concentration multiplied by 4. (3) The area under the postprandial concentration/time curve (not referenced to fasting baseline) and (4) peak postprandial concentration. Other end points included platelet 5-HT concentration and fasting and fed ratio of 5-HIAA:5-HT.

Linear models were fitted to the log transformation of the above variables.[16] All models included terms for subject group and age, and the analysis of ratio to fasting baseline area under the curve and peak postprandial concentration also included a term for fasting concentration. All models were checked for the validity of model assumptions. In addition, the time to peak postprandial concentration was analyzed for both platelet-depleted plasma 5-HT and 5-HIAA using the Cox proportional hazards regression analysis, which adjusts for censored observations (ie, peaks that did not occur within the time frame of the study).

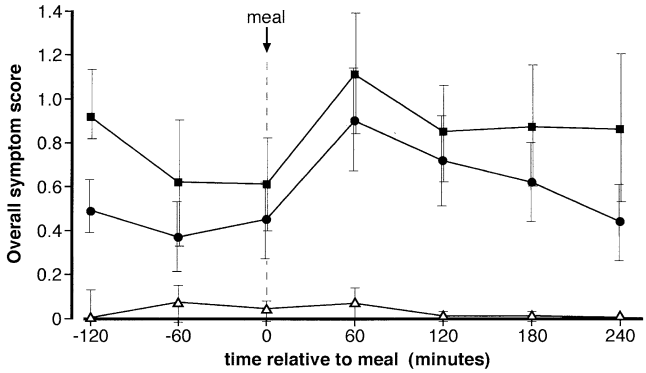

Figure 1. Profile of overall symptom score with respect to meal ingestion (t = 0) in patients with constipation (■)- and diarrhea (●)-predominant IBS and in healthy controls (△). Data are mean and 95% confidence interval.

The sum of the individual symptom scores for pain, urgency, and bloating at each time point were used to calculate an overall hourly symptom score, from which the mean premeal (2 hours) and postmeal (4 hours) symptom scores were then calculated for each subject. Differences in fasting and fed symptom scores among the 3 groups were assessed using the analysis of variance (ANOVA) test followed by Student 2-sample *t* test. In addition, the change in mean symptom score pre- to postmeal (ie, symptom worsening) was calculated, and statistical differences among groups were evaluated using analysis of variance (ANOVA) and Student 2-sample *t* test.

Results

Symptomatology

Ninety-six percent (53 of 55) of d-IBS and 90% (26 of 29) of c-IBS patients reported that their IBS was active at the time of the investigation. Of the c-IBS patients, 92% reported a bowel frequency of 1–3 per week and 8% a frequency of <1 per week, with most describing their stool consistency as varying from normal to hard. Of the d-IBS patients, 81% reported a bowel frequency of 3–5 per day and 19% a bowel frequency of >5 per day, and all reported their stool consistency as generally loose to watery.

Assessment of symptoms on the study day showed that, as expected, both c- and d-IBS patients had significantly worse symptomatology under both fasting and fed conditions compared with HV ($P < .05$) (Figure 1). In addition, symptoms worsened with meal ingestion in d- (+ 0.26) and c- (+ 0.31) IBS patients compared with

Table 1. Comparison of Platelet-Depleted Plasma 5-hydroxytryptamine Concentrations Among Patients With Constipation-Predominant Irritable Bowel Syndrome, Diarrhea-Predominant Irritable Bowel Syndrome, and Healthy Volunteers

5-HT concentration	HV (n = 35)	d-IBS (n = 55)	c-IBS (n = 29)	*P* value (all)
Fasting (*nmol/L*)	21.31	27.44	22.42	.040
Ratio to HV		1.27 (1.02–1.58)[a]	1.06 (0.80–1.40)	
Ratio to d-IBS			0.80 (0.64–1.00)[a]	
Fed (*nmol/L*)	28.53	41.55	18.74	<.001
Ratio to HV		1.46 (1.07–1.98)[a]	0.66 (0.48–0.91)[b]	
Ratio to d-IBS			0.45 (0.33–0.61)[c]	
Fed AUC	1.52	1.48	1.00	.003
Ratio to HV		0.99 (0.76–1.29)	0.65 (0.50–0.86)[b]	
Ratio to d-IBS			0.68 (0.52–0.88)[b]	
Fed peak (*nmol/L*)	64.59	57.80	25.92	<.001
Ratio to HV		0.91 (0.65–1.28)	0.40 (0.27–0.59)[c]	
Ratio to d-IBS			0.44 (0.31–0.63)[c]	

NOTE. Results expressed as adjusted geometric mean (95% confidence interval).
AUC, area under the postprandial concentration/time curve referenced to fasting baseline levels.
[a]$P \leq .05$ for the difference between the groups.
[b]$P \leq .01$ for the difference between the groups.
[c]$P \leq .001$ for the difference between the groups.

HV (−0.03; mean difference d-IBS from HV, 0.29; 95% CI difference: 0.07–0.51, $P = .009$; mean difference c-IBS from HV, 0.34; 95% CI difference: 0.13–0.56, $P = .002$). There was no difference in symptom worsening with meal ingestion between patient subgroups (mean difference d-IBS from c-IBS, 0.05; 95% CI difference: −0.23–0.33, $P = .718$) (Figure 1). Postprandial exacerbation of symptomatology was reported by 80% (44 of 55) of d-IBS and 97% (28 of 29) c-IBS patients.

Platelet-Depleted Plasma 5-HT Concentrations

Under fasting conditions, d-IBS patients had significantly higher platelet-depleted plasma 5-HT concentrations than both c-IBS patients ($P < .05$) and HV ($P < .05$). There was no difference in fasting platelet-depleted plasma 5-HT concentrations between c-IBS patients and HV (Table 1, Figure 2*A*).

Ingestion of the meal significantly increased platelet-depleted plasma 5-HT concentrations in both d-IBS and HV ($P < .001$) (Figures 2*A* and 3*A*), although the magnitude of the increase (ie, ratio to fasting baseline area under the postprandial concentration/time curve) and peak 5-HT concentration was no different between the 2 groups (Table 1). However, the area under the postprandial 5-HT concentration/time curve only (not referenced to fasting) was significantly higher in d-IBS patients compared with HV ($P = .018$) (Table 1, Figure 2*A*). Conversely, c-IBS patients showed no increase in platelet-depleted plasma 5-HT concentration with meal ingestion ($P = .831$), with concentrations remaining similar to those seen under fasting conditions (Figures 2*A* and 3*A*), such that the area under the postprandial 5-HT concentration/time curve (referenced or not to fasting concentrations), together with peak 5-HT concentrations, were significantly lower than those seen in both HV and d-IBS patients ($P < .01$) (Table 1, Figure 2*A*).

Interestingly, platelet-depleted plasma 5-HT concentration postmeal did not always show a gradual increase followed by decrease with time because 11 (38%) c-IBS, 6 (11%) d-IBS, and 5 (14%) healthy subjects exhibited repeated sharp rises and falls in 5-HT concentration throughout the postmeal period (overall difference, $P = .008$; c-IBS > d-IBS and HV, $P < .05$) (Figure 3*A*).

There was no statistically significant difference in the time taken to reach peak platelet-depleted plasma 5-HT concentration following meal ingestion among d-IBS (median, 180; range, 30–240 minutes), c-IBS (median, 210; range, 30–240 minutes), and healthy subjects (median, 210; range, 30–240 minutes) (Figure 4).

Platelet-Depleted Plasma 5-HIAA Concentrations

Under fasting conditions, platelet-depleted plasma 5-HIAA concentration was significantly lower in c-IBS patients compared with both HV ($P < .001$) and d-IBS patients ($P < .001$) (Table 2, Figure 2*B*). There were no differences in fasting 5-HIAA concentrations between d-IBS patients and HV (Table 2, Figure 2*B*).

Meal ingestion was associated with a significant increase in platelet-depleted plasma 5-HIAA concentration in both d-IBS patients and HV ($P < .001$) but not in c-IBS patients (Figures 2*B* and 3*B*). Thus, the area under the postprandial 5-HIAA concentration/time curve (referenced or not to fasting) and peak 5-HIAA concentra-

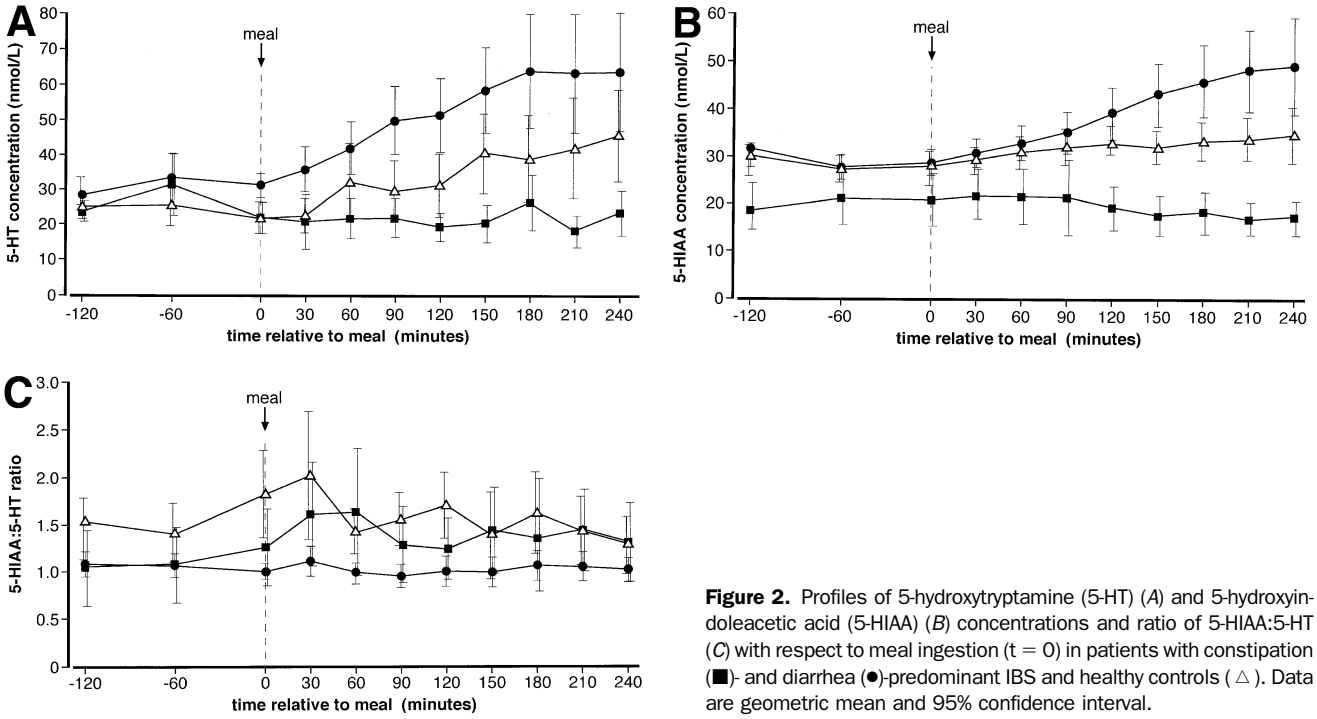

Figure 2. Profiles of 5-hydroxytryptamine (5-HT) (*A*) and 5-hydroxyindoleacetic acid (5-HIAA) (*B*) concentrations and ratio of 5-HIAA:5-HT (*C*) with respect to meal ingestion (t = 0) in patients with constipation (■)- and diarrhea (●)-predominant IBS and healthy controls (△). Data are geometric mean and 95% confidence interval.

tion were significantly lower in c-IBS patients compared with both d-IBS patients ($P < .05$) and HV ($P < .05$) (Table 2, Figures 2*B* and 3*B*). There was no difference in any of the parameters between d-IBS patients and HV (Table 2).

The time taken to reach peak platelet-depleted plasma 5-HIAA concentration following meal ingestion was no different among groups (d-IBS: median, 180; range, 30–240 minutes; c-IBS: median, 120; 30–240 minutes; controls: median, 150; 30–240 minutes).

5-HIAA:5-HT Ratio

Under fasting conditions, the ratio of 5-HIAA:5-HT was significantly lower in both d- ($P < .001$) and c-IBS ($P < .01$) patients compared with HV. There was no difference in the 5-HIAA:5-HT ratio between d- and c-IBS patients (Table 3, Figure 2*C*).

Under fed conditions, the ratio of 5-HIAA:5-HT (area under the postprandial 5-HIAA:5-HT concentration/time curve not referenced to fasting) was significantly lower in d-IBS patients compared with HV ($P < .001$) (Table 3). There was no difference between c-IBS patients and HV or between c- and d-IBS patients (Table 3).

Platelet 5-HT Concentration

Platelet stores of 5-HT were significantly larger in c-IBS patients compared with both d-IBS patients and HV (ratio c-IBS:d-IBS: 1.93; 95% CI: 1.78–4.0, $P < .001$; c-IBS:HV: 2.0; 95% CI: 1.67–4.36, $P < .001$). However, no difference in platelet 5-HT concentration was apparent between d-IBS patients and HV in this study (d-IBS:HV: 1.15; 95% CI: −0.44 to 1.30, $P = .329$) (Figure 5).

Discussion

These results extend those of our previous study by showing that patients with d-IBS have raised concentrations of plasma 5-HT under fasting as well as fed conditions, such that the postprandial relative to fasting concentration is no different from controls. In contrast, c-IBS patients appeared to exhibit no or limited 5-HT response to meal ingestion with plasma concentrations remaining similar to those seen under fasting conditions. The ratio of postprandial plasma 5-HIAA:5-HT concentration appeared to be normal in c-IBS but reduced in d-IBS.

Our observations that fasting and fed 5-HT concentrations were elevated, but that the postprandial relative to fasting 5-HT and 5-HIAA concentrations in d-IBS patients were no different from HV, suggests that these patients might have a disorder primarily of metabolism and/or reuptake rather than synthesis and/or release of 5-HT. This is supported by recent studies investigating genetic polymorphisms of the serotonin reuptake transporter (SERT), which have shown that d-IBS patients exhibit an increased frequency of the short/short (s/s)

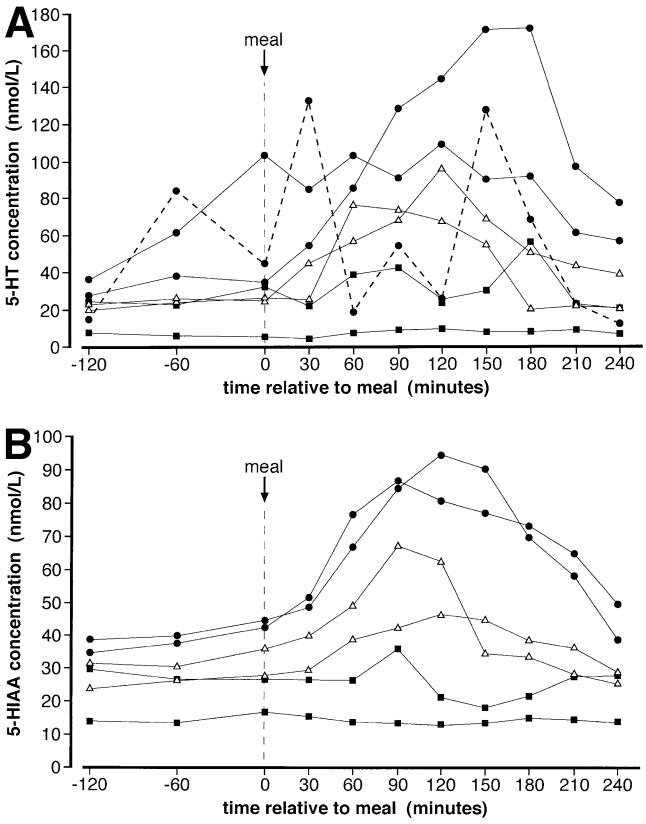

Figure 3. Typical profiles of 5-hydroxytryptamine (5-HT) (*A*) and 5-hydroxyindoleacetic acid (5-HIAA) (*B*) concentrations in individual patients with constipation (■)- and diarrhea (●)-predominant IBS and healthy controls (△). The *dashed line* represents a profile seen in an individual who exhibited repeated sharp rises and falls in plasma 5-HT concentration with time. The latter pattern of 5-HT profile was seen in 38% of c-IBS, 11% of d-IBS, and 14% of HV (overall difference, $P = .008$).

homozygous[24] or short/long (s/l) heterozygous[25] type allele of the SERT promoter gene, both of which are associated with less transporter protein expression and serotonin reuptake.[26,27] Indeed, other studies have shown reduced expression of the transporter protein, which increases the availability of 5-HT in these patients.[28,29] Furthermore, this is the first study to show convincingly that ingestion of a carbohydrate-rich meal is associated with an increase in plasma 5-HT concentration in d-IBS patients that is of a similar magnitude to that seen in healthy controls and supports Coates et al's[29] recent observation that EC cell 5-HT release from mechanically stimulated mucosal biopsy specimens is normal in these patients. This appears to be the case, despite some reports of reduced synthesis and EC cell content of 5-HT,[29,30] which might explain the lower mucosal 5-HT concentration previously recorded in this subgroup.[29–31] Coates et al[29] concluded that the normal release, despite reduced EC cell content of 5-HT in d-IBS patients, must suggest that there is normally a surplus of 5-HT in the system that is available for release. Maybe the general increased availability of 5-HT in d-IBS leads to a compensatory reduction in 5-HT synthesis in these patients. One further possible explanation for the elevated concentrations of plasma 5-HT seen in d-IBS, especially under fasting conditions, is platelet activation. However, the methodology used in the present study was no different from that of our previous study,[16] in which β-thromboglobulin, a marker of platelet activation, was found to be undetectable in the plasma samples. Furthermore, because recruitment and assessment of the subjects was random, and blood collection and HPLC analysis were blind to subject status and similar for all 3 groups, it seems unlikely that technical difficulties might have led to greater platelet activation in the d-IBS subgroup alone. In addition, we are not aware of any evidence to suggest that platelet activation is more likely to occur in d-IBS patients compared with the other 2 groups, and this is supported by the observation that platelet 5-HT concentration was similar in d-IBS patients and healthy controls.

In contrast, we have shown that patients with c-IBS have no or only a limited 5-HT response to meal ingestion, with plasma concentrations remaining very similar to those recorded under fasting conditions. These findings are consistent with Dunlop et al's[18] preliminary observations that postprandial plasma 5-HT concentrations are significantly lower in c-IBS patients compared with healthy controls and PI-IBS patients. However, lack of measurement of fasting 5-HT concentration in their study[18] prevented assessment of the 5-HT response to meal ingestion in c-IBS. Together with the fact that postprandial 5-HIAA concentration is also significantly lower, but the ratio of 5-HIAA:5-HT no different than HV, our data suggest that c-IBS may be a disorder that primarily arises from reduced EC cell release of 5-HT. In addition, the observation that platelet-depleted plasma 5-HT concentration was more likely to show repeated sharp rises and falls throughout the course of the post-

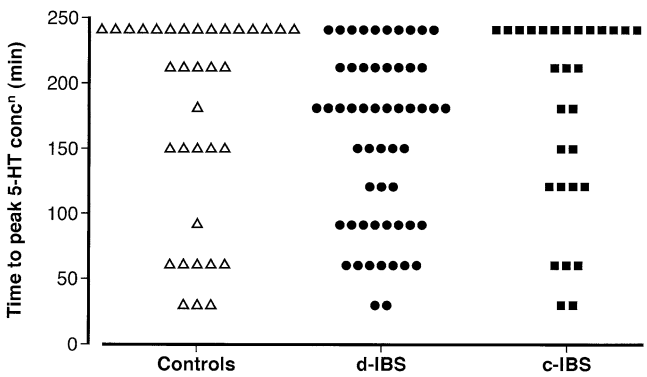

Figure 4. Individual plots of values for the time to peak 5-hydroxytryptamine (5-HT) concentration in patients with constipation (■)- and diarrhea (●)-predominant IBS and healthy controls (△).

Table 2. Comparison of Platelet-Depleted Plasma 5-Hydroxyindoleacetic Acid Concentrations Among Patients With Constipation-Predominant Irritable Bowel Syndrome, Diarrhea-Predominant Irritable Bowel Syndrome, and Healthy Volunteers

5-HIAA concentration	HV (n = 35)	d-IBS (n = 55)	c-IBS (n = 29)	*P* value (all)
Fasting (*nmol/L*)	27.00	25.71	17.51	<.001
Ratio to HV		0.96 (0.81–1.14)	0.64 (0.50–0.83)[a]	
Ratio to d-IBS			0.68 (0.54–0.85)[a]	
Fed (*nmol/L*)	31.13	35.06	16.15	<.001
Ratio to HV		1.13 (0.92–1.38)	0.52 (0.41–0.66)[a]	
Ratio to d-IBS			0.46 (0.36–0.59)[a]	
Fed AUC	1.19	1.31	0.90	<.001
Ratio to HV		1.09 (0.94–1.26)	0.74 (0.61–0.90)[b]	
Ratio to d-IBS			0.70 (0.59–0.83)[a]	
Fed peak (*nmol/L*)	37.37	40.77	31.37	.035
Ratio to HV		1.10 (0.91–1.32)	0.81 (0.65–1.00)[c]	
Ratio to d-IBS			0.77 (0.62–0.95)[c]	

NOTE. Results expressed as adjusted geometric mean (95% confidence interval).
AUC, area under the postprandial concentration/time curve referenced to fasting baseline levels.
[a]$P \leq .001$ for the difference between the groups.
[b]$P \leq .01$ for the difference between the groups.
[c]$P \leq .05$ for the difference between the groups.

prandial period in c-IBS patients compared with both d-IBS patients and healthy controls may also be suggestive of a problem with EC cell 5-HT release in this group. However, it is also possible, although unlikely (see previous discussion on β-thromboglobulin), that the latter profile just reflects an increased likelihood of detecting platelet activation because of the limited EC cell 5-HT release and the increased frequency of 5-HT measurement postmeal compared with fasting conditions. Furthermore, our conclusion that EC cell 5-HT release may be abnormally reduced in c-IBS is consistent with previous observations that mucosal concentrations of 5-HT are elevated in these patients[31–33] but does not preclude other findings that there may also be an increased frequency of the s/s homozygous type allele of the SERT promoter gene[25] along with reduced transporter protein expression,[29] 5-HT synthesis,[29] and EC cell 5-HT content[29,34] in c-IBS. A reduction in 5-HT reuptake would not be expected significantly to affect plasma 5-HT concentration if there were initially no or limited release of 5-HT in response to a stimulus. One study has reported that EC cell 5-HT release is normal in c-IBS,[29] but the study technique involved mechanically agitating mucosal biopsy specimens in a vortex machine, which may have been a more potent stimulus than the physiologic stimulus of meal ingestion used in this study.

One limitation of this study is the interpretation of the meaning of the ratio of the concentration of platelet-depleted plasma 5-HIAA to 5-HT. Plasma 5-HT originates from the mucosal EC cells, whereas the concentration of plasma 5-HIAA reflects the relative activity of the enzyme monoamine oxidase, not only in the platelets but also in other tissues such as the liver and lungs. Thus, the ratio of the concentrations of platelet-depleted plasma 5-HIAA to 5-HT should only be viewed as a surrogate measure of 5-HT turnover. A more appropriate measure of 5-HT turnover might have been the ratio of the concentrations of mucosal 5-HIAA to 5-HT.

The different levels of availability of 5-HT found in d- and c-IBS patients could be considered to be consistent

Table 3. Comparison of Ratio of Plasma 5-HIAA:5-HT Among Patients With Constipation-Predominant Irritable Bowel Syndrome, Diarrhea-Predominant Irritable Bowel Syndrome, and Healthy Volunteers

Ratio 5-HIAA:5-HT	HV (n = 35)	d-IBS (n = 55)	c-IBS (n = 29)	*P* value (all)
Fasting	1.44	0.98	0.90	.001
Ratio to HV		0.71 (0.58–0.87)[a]	0.62 (0.46–0.85)[b]	
Ratio to d-IBS			0.92 (0.72–1.19)	
Fed	1.38	0.95	1.14	.003
Ratio to HV		0.69 (0.57–0.82)[a]	0.83 (0.62–1.10)	
Ratio to d-IBS			1.20 (0.95–1.52)	

NOTE. Results expressed as adjusted geometric mean (95% confidence interval).
AUC, area under the postprandial concentration/time curve referenced to fasting baseline levels
[a]$P \leq .001$ for the difference between the groups.
[b]$P \leq .01$ for the difference between the groups.

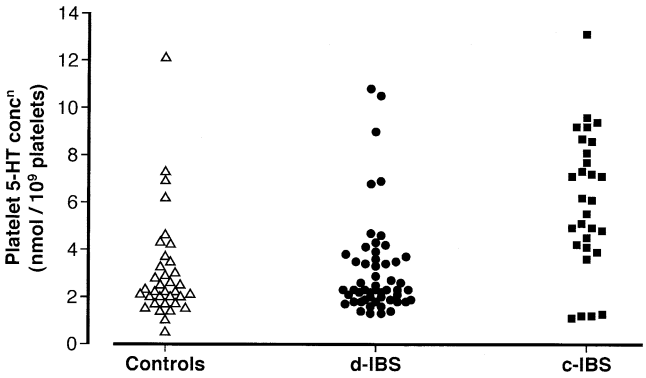

Figure 5. Individual plots of values for platelet 5-hydroxytryptamine (5-HT) concentration in patients with constipation (■)- and diarrhea (●)-predominant IBS and healthy controls (△).

with the various motor abnormalities sometimes seen in these 2 IBS subgroups,[1–6] but whether they represent the cause or are the consequence of these motility patterns cannot be determined from this study. The time to peak 5-HT and 5-HIAA concentration was similar in both d- and c-IBS patients compared with healthy controls, which implies that the difference in postprandial 5-HT concentration is not solely because of variations in transit, but that 5-HT is causing, at least in part, the motility patterns associated with these IBS subgroups. Abnormalities in gastrointestinal transit tend to be modest in patients with IBS,[35,36] and, therefore, it seems unlikely that differences in transit could have been responsible for the general, elevated concentrations of plasma 5-HT seen in d-IBS patients and, especially, the complete lack of increase in plasma 5-HT seen with meal ingestion in some c-IBS patients. Furthermore, the considerable intersubject variation in platelet-depleted plasma 5-HT concentration and resulting large number of IBS patients needed to be studied to show significant differences from healthy controls may explain why many manometry studies have been unable to identify differences in GI motility between IBS patients and healthy controls[35–38] because they have either been underpowered and/or not differentiated between c- and d-IBS patients.

The similar overall symptom profile (average of pain, bloating, and urgency scores) reported by both d- and c-IBS patients despite disparate plasma 5-HT concentrations might suggest that 5-HT is predominantly driving bowel habit rather than visceral symptoms such as pain and bloating. This is supported by the observation that the use of selective serotonin reuptake inhibitors is frequently associated with an increased incidence of diarrhea[39,40] but not always an improvement in symptoms.[41] However, it must also be remembered that the gut has only a limited repertoire of sensations that can be induced by varying pathophysiologies, and, thus, excessive availability of 5-HT through its associated effects on physiology might still increase symptomatology, as suggested in our previous study.[16] In the present study, the number of patients in each bowel habit subgroup who did not experience symptoms was too small to make sound comparisons with those who did report symptoms.

In contrast to our previous study, platelet 5-HT concentration in d-IBS patients was no different from healthy controls. This might again be explained by the power of our previous study[16] being too small to detect accurately the differences in platelet 5-HT stores. Closer examination of the data[16] suggests that the increased stores seen in d-IBS may have resulted from a few outlying data points. Furthermore, our present observations are not necessarily inconsistent with the growing evidence of reduced 5-HT reuptake in these patients[24,25,28,29] because they might indicate that the increased plasma 5-HT concentrations seen in d-IBS is sufficient to maintain normal platelet concentration. Interestingly, despite there being a significant reduction in exposure of the platelets to 5-HT because of decreased postprandial platelet-depleted plasma 5-HT concentration in c-IBS patients, platelet 5-HT concentration appeared to be higher in these patients than in both d-IBS and healthy subjects. This finding is in accord with Dunlop et al,[18] although the reason for these high platelet 5-HT levels is unclear.

In conclusion, this is the largest study to date to compare postprandial platelet-depleted plasma 5-HT concentrations in patients with d- and c-IBS with healthy controls. The substantial number of subjects studied has also allowed more accurate assessment of fasting 5-HT concentrations and the 5-HT response to meal ingestion, whereas the concomitant measurement of platelet-depleted plasma 5-HIAA concentration aids understanding of possible mechanisms involved in altered 5-HT signaling. Our data suggest, for the first time, that d-IBS patients probably have generally raised concentrations of 5-HT in their plasma as a consequence of reduced metabolism and uptake but a 5-HT response to meal ingestion that is similar to healthy controls. Conversely, c-IBS patients appear to have a very limited 5-HT response to meal ingestion as demonstrated by the low concentrations of both 5-HT and 5-HIAA. These observations, along with those of previous studies, suggests that d- and c-IBS may be primarily disorders of reduced 5-HT reuptake and release, respectively.

References

1. Chey WY, Jin HO, Lee MH, Sun SW, Lee KY. Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. Am J Gastroenterol 2001;96:1499–1506.

2. Connell AM. The motility of the pelvic colon. II. Paradoxical motility in diarrhea and constipation. Gut 1962;3:342–348.
3. Cann PA, Read NW, Brown C, Hobson N, Holdsworth CD. Irritable bowel syndrome: relationship of disorders in the transit of a single solid meal to symptom patterns. Gut 1983;24: 405–411.
4. Bazzocchi G, Ellis J, Villanueva-Meyer J, Jing J, Reddy SN, Mena I, Snape WJ Jr. Postprandial colonic transit and motor activity in chronic constipation. Gastroenterology 1990;98: 686–693.
5. Bassotti G, Chistolini F, Marinozzi G, Morelli A. Abnormal colonic propagated activity in patients with slow transit constipation and constipation-predominant irritable bowel syndrome. Digestion 2003;68:178–183.
6. Bassotti G, Chiarioni G, Vantini I, Betti C, Fusaro C, Pelli MA, Morelli A. Anorectal manometric abnormalities and colonic propulsive impairment in patients with severe chronic idiopathic constipation. Dig Dis Sci 1994;39:1558–1564.
7. Houghton LA. Evidence of abnormal rectal sensitivity in IBS. In: Camilleri M, Spiller R, eds. Irritable bowel syndrome—diagnosis and treatment. Edinburgh: W. B. Saunders, 2002:69–76.
8. Harraf F, Schmulson M, Saba L, Niazi N, Fass R, Munakata J, Diehl D, Mertz H, Naliboff B, Mayer EA. Subtypes of constipation predominant irritable bowel syndrome based on rectal perception. Gut 1998;43:388–394.
9. Hammonds R, Houghton LA, Whorwell PJ. Urge and no-urge constipation predominant irritable bowel syndrome (IBS): sensory dysfunction of the whole gut (abstr). Gastroenterology 2000; 118(Suppl 2):830.
10. Kim D-Y, Camilleri M. Serotonin: a mediator of the brain-gut connection. Am J Gastroenterol 2000;95:2698–2709.
11. Spiller RC. Effects of serotonin on intestinal secretion and motility. Curr Opin Gastroenterol 2001;17:99–103.
12. Tack J, Sarnelli G. Serotonergic modulation of visceral sensation: upper gastrointestinal tract. Gut 2002;51(Suppl 1):i77–i80.
13. Gershon MD. Importance of serotonergic mechanisms in gastrointestinal motility and sensation. In: Camilleri M, Spiller RC, eds. Irritable bowel syndrome—diagnosis and treatment. Edinburgh: W. B. Saunders, 2002:95–115.
14. Erspamer V, Testini A. Observations on the release and turnover rate of 5-hydroxytryptamine in the gastrointestinal tract. J Pharm Pharmacol 1959;11:618–623.
15. Bearcroft CP, Perrett D, Farthing MJG. Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. Gut 1998;42:42–46.
16. Houghton LA, Atkinson W, Whitaker RP, Whorwell PJ, Rimmer MJ. Increased platelet depleted plasma 5-hydroxytryptamine concentration following meal ingestion in symptomatic female subjects with diarrhoea predominant irritable bowel syndrome. Gut 2003; 52:663–670.
17. Da Prada M, Tranzer JP, Pletscher A. Storage of 5-hydroxytryptamine in human blood platelets. Experimentia 1972;28:1328–1329.
18. Dunlop SP, Coleman NS, Blackshaw PE, Perkins AC, Singh G, Marsden CA, Spiller RC. Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. Clin Gastroenterol Hepatol 2005;3:349–357.
19. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. Gut 1999;45(Suppl 11):42–47.
20. Bethea CL, Pecins-Thompson M, Schutzer WE. Ovarian steroids and serotonin neural function. Mol Neurobiol 1998;18: 87–123.
21. Atkinson W, Houghton LA, Whorwell PJ, Whitaker P. Gender differences in plasma 5-hydroxytryptamine concentration in diarrhoea predominant irritable bowel syndrome: influence of the menstrual cycle (abstr). Gastroenterology 2003;124(Suppl 1):A388, M1639.
22. Whitaker RP, Dursun SM, Davies T. Measurement of platelet rich plasma 5-HT and platelet poor plasma 5-HIAA and 5-HT in normal adults (abstr). Ann Clin Biochem 1996;537:341.
23. Dursun SM, Szemis A, Andrews H, Whitaker P, Reveley MA. Effects of clozapine and typical antipsychotic drugs on plasma 5-HT turnover and impulsivity in patients with schizophrenia: a cross-sectional study. J Psychiatr Neurosci 2000;25:347–352.
24. Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M, Knaggs A, Asquith S, Taylor I, Bahari B, Crocker N, Rallan R, Varsani S, Montgomery D, Alpers DH, Dukes GE, Purvis I, Hicks GA. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. Gut 2004;53:1452–1458.
25. Pata C, Erdal ME, Derici E, Yazar A, Kanik A, Ulu O. Serotonin transporter gene polymorphism in irritable bowel syndrome. Am J Gastroenterol 2002;97:1780–1784.
26. Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, Lesch KP. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621–2624.
27. Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR, Del Proposto ZS, Hill E, Cassin BJ, Watson SJ, Cook EH. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am J Psychiatry 1998;155:207–213.
28. Bellini M, Rappelli L, Blandizzi C, Costa F, Stasi C, Colucci R, Giannaccini G, Marazziti D, Betti L, Baroni S, Mumolo MG, Marchi S, Del Tacca M. Platelet serotonin transporter in patients with diarrhea-predominant irritable bowel syndrome both before and after treatment with alosetron. Am J Gastroenterol 2003;98: 2705–2711.
29. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin re-uptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004;126:1657–1664.
30. Bose M, Nickols C, Greenwald S, Feakins R, Perrett D, Farthing M. 5-hydroxytryptamine (5-HT) levels in colonic mucosa in the irritable bowel syndrome (IBS): assessment by high performance liquid chromatography (HPLC) (abstr). Gut 2001;48:A57.
31. Miwa J, Echizen H, Matsueda K, Umeda N. Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhoea-predominant patients and subjects with normal bowel habit. Digestion 2001;63:188–194.
32. Zhao RH, Baig MK, Mack J, Abramson S, Woodhouse S, Wexner SD. Altered serotonin immunoreactivities in the left colon of patients with colonic inertia. Colorectal Dis 2002;4:56–60.
33. Lincoln J, Crowe R, Kamm MA, Burnstock G, Lennard-Jones JE. Serotonin and 5-hydroxyindoleacetic acid are increased in the sigmoid colon in severe idiopathic constipation. Gastroenterology 1990;98:1219–1225.
34. Baig MK, Zhao RH, Woodhouse SL, Abramson S, Weiss JJ, Nogueras JJ, Wexner SD. Variability in serotonin and enterochromaffin cells in patients with colonic inertia and idiopathic diarrhoea as compared to normal controls. Colorectal Dis 2002;4: 348–354.
35. Quigley EMM. Disturbances in small bowel motility. In: Houghton LA, Whorwell PJ, eds. Irritable bowel syndrome. Baillieres clinical gastroenterology. Vol. 13. No. 3. London: Baillieres Tindall, 1999:385–395.
36. Spiller RC. Disturbances in large bowel motility. In: Houghton LA, Whorwell PJ, eds. Irritable bowel syndrome. Baillieres clinical gastroenterology. Vol. 13. No. 3. London: Baillieres Tindall, 1999:397–413.

37. McKee DP, Quigley EMM. Intestinal motility in irritable bowel syndrome: is IBS a motility disorder? Part 1. Definition of IBS and colonic motility. Dig Dis Sci 1993;38:1761–1772.
38. Houghton LA. Sensory dysfunction and the irritable bowel syndrome. In: Houghton LA, Whorwell PJ, eds. Irritable bowel syndrome. Baillieres clinical gastroenterology. Vol. 13. No 3. London: Baillieres Tindall, 1999:415–427.
39. Gorard DA, Libby GW, Farthing MJG. Influence of antidepressants on whole gut and orocaecal transit times in health and irritable bowel syndrome. Aliment Pharmacol Ther 1994;8: 159–166.
40. Spigset O. Adverse reactions of selective serotonin re-uptake inhibitors: reports from a spontaneous reporting system. Drug Saf 1999;20:277–287.
41. Kuiken SD, Tytgat GN, Boeckxstaens GE. The selective serotonin re-uptake inhibitor fluoxetine does not change rectal sensitivity and symptoms in patients with irritable bowel syndrome: a double-blind, randomised, placebo-controlled study. Clin Gastroenterol Hepatol 2003;1:219–228.

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Ehlers and Danlos of the Ehlers–Danlos Syndrome

Edvard Lauritz Ehlers

Edvard Lauritz Ehlers (1863–1937) was born in Copenhagen where his father was mayor. He qualified in medicine in 1891, then pursued further studies in Berlin, Breslau, Vienna, and Paris. In 1906, he was appointed chief of the dermatology clinic at the Fredericks Hospital in his native city. From 1911 until his retirement in 1932, he directed the dermatology service at the Kummunehospitalet (Municipal Hospital) in Copenhagen. Much of his research dealt with leprosy and syphilis. Henri-Alexandre Danlos (1844–1912) was born in Paris where he was awarded his doctoral degree in 1874. In 1890, he suffered a prolonged, painful illness and became withdrawn and depressed. Once recovered, he was appointed to the staff of the Saint Louis Hospital where he conducted research in the treatment of syphilis and various diseases of the skin. Ehlers maintained a close association with his colleagues in Paris. In 1899, at a meeting of the Paris Society of Syphilogy and Dermatology, he described the case of a 21-year-old law student with lifelong laxity of joints, hemarthrosis, and hyperextensible skin. At a 1908 meeting of the same society, Danlos commented on a similar case. Subsequent reports remarked the frequency of gastrointestinal bleeding due to congenital connective tissue disruption. It has been suggested that the virtuosity of the Italian violinist Nicolo Paganini might be attributed to a forme-fruste expression of what came to be called the Ehlers–Danlos syndrome.

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