Serotonin availability in rat colon is reduced during a Western diet model of obesity

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Serotonin availability in rat colon is reduced during a Western diet model of obesity. Am J Physiol Gastrointest Liver Physiol 303: G424–G434, 2012. First published May 17, 2012; doi:10.1152/ajpgi.00048.2012.—Constipation and slowed transit are associated with diet-induced obesity, although the mechanisms by which this occurs are unclear. Enterochromaffin (EC) cells within the intestinal epithelium respond to mechanical stimulation with the release of serotonin [5-hydroxytryptamine (5-HT)], which promotes transit. Thus our aim was to characterize 5-HT availability in the rat colon of a physiologically relevant model of diet-induced obesity. EC cell numbers were determined immunohistochemically in chow-fed (CF) and Western diet-fed (WD) rats, while electrochemical methods were used to measure mechanically evoked (peak) and steady-state (SS) 5-HT levels. Fluoxetine was used to block the 5-HT reuptake transporter (SERT), and the levels of mRNA for tryptophan hydroxylase 1 and SERT were determined by quantitative PCR, and SERT protein was determined by Western blot. In WD rats, there was a significant decrease in the total number of EC cells per crypt (0.86 ± 0.06 and 0.71 ± 0.05 in CF and WD, respectively), which was supported by a reduction in the levels of 5-HT in WD rats (2.9 ± 1.0 and 2.5 ± 0.2 μM at SS and peak, respectively) compared with CF rats (7.3 ± 0.4 and 18.4 ± 3.4 μM at SS and peak, respectively). SERT-dependent uptake of 5-HT was unchanged, which was supported by a lack of change in SERT protein levels. In WD rats, there was no change in tryptophan hydroxylase 1 mRNA but an increase in SERT mRNA. In conclusion, our data show that foods typical of a WD are associated with decreased 5-HT availability in rat colon. Decreased 5-HT availability is driven primarily by a reduction in the numbers and/or 5-HT content of EC cells, which are likely to be associated with decreased intestinal motility in vivo.

5-hydroxytryptamine; constipation; electrochemistry; diet-induced obesity; serotonin reuptake transporter

MATERIALS AND METHODS

Diet and sample collection. Male Sprague-Dawley rats (~200 g body wt at 6–7 wk of age) were obtained from Animal Resources Centre (Perth, WA, Australia). Experimental protocols were approved by the Animal Experimentation Ethics Committee of the University of New South Wales. Animals were randomly divided into two groups. For 16–20 wk, the first group was fed a standard rat chow [11 kJ/g, 14% fat; Gordon’s Specialty Stockfeeds, Yanderra, NSW, Australia; chow-fed (CF)] and the second group was fed a highly palatable cafeteria-style diet, which consisted of high-fat foods of a known caloric content [average 15.33 kJ/g, Western diet (WD)] (14, 19, 36). The WD consisted of foods such as meat pies, cakes, and deep-fried...
potatoes, in addition to standard chow. The WD, which was ~60% carbohydrate and 32% fat, can be considered a “medium”-fat diet (64); the CF diet was 65% carbohydrate and 14% fat. The veterinary staff monitored animals for signs of disease. Body weight was measured weekly, and 24-h food intake (kJ) was measured every 2–3 wk. At the end of the diet period, rats were anesthetized with thiopentone sodium (100 mg/kg ip; Abbott, Kurnell, NSW, Australia), and blood samples were taken by cardiac puncture, with blood glucose measured immediately (Accu-Chek Advantage, Roche Diagnostics Australia, Castle Hill, NSW, Australia). Rats were then euthanized by guillotine, and left retroperitoneal and testicular fat were removed and weighed, and segments of colon were removed for assay. The distal colon was chosen for this study, as this region is primarily responsible for the transit of fecal pellets and corresponds to the area of greatest constipation in humans with slowed transit (47).

Immunohistochemistry. Immunohistochemical analyses were carried out as described previously (9, 14). Briefly, 1-cm segments of colon were removed from rats and immersed in fixative (4% paraformaldehyde in PBS, pH 7.4) for 4 h at 4°C. Tissues were subjected to three 5-min rinse cycles at room temperature with PBS, immersed in cold 20% sucrose overnight at 4°C, and embedded in Tissue-Tek OCT compound (Sakura Finetek, ProSciTek, Thuringowa, QLD, Australia). Sections (sections: 10–14 mm) were cut on a cryostat, thaw-mounted onto 0.3% gelatin-coated glass slides (Fisherbrand Superfrost Plus), and stored at −80°C if required. Before they were immunolabeled, slides were thawed and subjected to three 10-min wash cycles in 0.1 M PBS with 0.5% Triton X-100 (Sigma-Aldrich). Tissue sections were blocked with 1% normal donkey serum (NDS) in 0.1 M PBS with 0.1% Triton X-100 for 1 h at room temperature in a humid chamber, briefly washed, and subsequently incubated with rabbit anti-5-HT primary antibody (1:5,000 dilution; Immunostar, Hudson, WI) in 1% NDS in 0.1 M PBS with 0.1% Triton X-100 overnight at 4°C. On the following day, slides were washed and incubated with donkey anti-rabbit IgG Cy3 secondary antibody (1:200 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h at room temperature in a humid chamber and then washed. Tissue sections were incubated with goat anti-c-Kit primary antibody (1:400 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) in 1% NDS in 0.1 M PBS with 0.1% Triton X-100 overnight at 4°C to detect mast cells, washed, and incubated with donkey anti-goat fluoroscein isothiocyanate secondary antibody (1:200 dilution; Jackson ImmunoResearch Laboratories) for 2 h at room temperature. They were then washed and covered with buffered glycerol, and coverslips were applied.

Immunoreactivity was analyzed with an epifluorescence microscope (model BH-2, Olympus, Tokyo, Japan). c-Kit is a cell surface cytokine receptor for stem cell factor, which, in the gut, can be used as a marker for mast cells and most interstitial cells of Cajal. In rodents, mast cells and EC cells contain 5-HT, but the absence of c-Kit staining in a 5-HT-positive cell suggests that it is an EC cell. Thus, only cells that were within the epithelial border and were c-Kit-negative and 5-HT-positive were counted as EC cells.

The number of 5-HT-immunoreactive EC cells in each of two nonadjacent fields of view at ×40 magnification was quantified per sample, as well as the number of crypts in each, and the average number of EC cells per crypt was calculated. EC cells can be classified as open or closed to the lumen and with one or more processes (35, 45, 61). In the present study, EC cells were categorized as follows: those with typical wineglass shape [I process that opens to the lumen and is the most abundant type in the ileum (14)], those with a single process that does not contact the lumen (closed), and those with multiple processes. The multipolar cells were grouped together, as they could be difficult to distinguish between open and closed types; in general, there appeared to be about half of each type (P. P. Bertrand, personal observation).

Electrochemistry. Electrochemical recordings were made in amperometry mode, as described previously (7, 8, 12). Briefly, a 7-μm carbon fiber was insulated with a glass micropipette (Clark Electromedical Instruments, Kent, UK), with ~200 μm left exposed for recordings. This exposed fiber was coated with Nafion (Sigma-Aldrich, St. Louis, MO) to repel the metabolite 5-hydroxyindoleacetic acid, and the electrode was calibrated and stability over time was checked with 2–10 μM 5-HT. In addition to recording 5-HT oxidation current at rest [steady state (SS)], the carbon fiber electrode was also used to compress the mucosal epithelium with 1–2 mg of force, yielding a peak level of 5-HT release. In this instance, the carbon fiber acts as a very fine von Frey hair and bends with a consistent amount of force. Contact between the carbon fiber electrode and the mucosa ensured that we maintained the same distance from mucosa to electrode, and recordings were made at three to six locations per preparation to reduce the variability in recordings.

SS levels of 5-HT were recorded following decay of the signal to a flat baseline or when 15 s had been reached. Concentrations of 5-HT were calculated using individual electrode calibrations. Cyclic voltammetry techniques were used to verify the identity of 5-HT release from the mucosa by the voltage at which it oxidizes. Current recordings were made using a VA-10 amplifier (NPI Electronics, Tamm, Germany) with a Ag-AgCl ground, digitized at 20 kHz (Digidata 1200), recorded on a personal computer, and filtered with a 50-Hz notch filter and a 5 × substitution average using PClamp 9 (MDS Analytical Technologies, Sunnyvale, CA) and then analyzed with PClamp 9 and graphed with Origin 7.5 (MicroCal, Northampton, MA).

Real-time quantitative PCR analysis of mRNA expression. Extraction of total RNA from samples of whole colon from CF or WD rats was accomplished using the TRIzol method (Invitrogen, Mulgrave, VIC, Australia) followed by a DNase treatment (3 U at 37°C for 20 min) to remove residual DNA. Single-strand cDNA was reverse-transcribed from 2 μg of total RNA using a SuperScript III first-strand synthesis system (Invitrogen) and random hexamers (50 ng/μl) at 1 cycle of 25°C for 10 min, 50°C for 50 min, and 85°C for 5 min.

Real-time quantitative PCR was carried out to determine mRNA expression of SERT, tryptophan hydroxylase 1 (TPH1) enzyme, villin (intestinal epithelium brush border protein), and GAPDH using Real MasterMix SYBR ROX (5 PRIME, Quantum-Scientific, Murarrie, QLD, Australia). Each PCR was performed in a volume of 25 μl containing 500 ng of single-strand cDNA, 0.05 U of HotMaster Taq polymerase, 4 mM magnesium acetate, 0.4 mM dNTP with dUTP, SYBR Green 1 dye, and a pair of gene-specific primers for which the optimal concentrations were predetermined. GAPDH and villin were used as housekeeping genes (HKGs), and in each real-time PCR assay, a designated calibration RNA (from 1 control rat colon) was used to allow inter-run comparisons. The PCR amplification conditions were cycle were carried out during gradual temperature elevation from 60°C to 95°C. Oligonucleotide primers used for real-time PCR were as follows: SERT 5′-attgagacacaccgtctga-3′ (forward) and 5'-gtggggacaccttgta-3′ (reverse)), TPH1 5′-caagagaaacagcct-c3′ (forward) and 5′-cagctccacaaactca-3′ (reverse)), villin 5′-caagctctcatacacc-3′ (forward) and 5′-gcaaggtaacactctag-3′ (reverse)), GAPDH 5′-gtggggacaccttgta-3′ (forward) and 5′-ggtaagatggtgagttt-3′ (reverse)).

The mRNA level for each gene was expressed as fold change, in which each target gene was normalized to GAPDH or villin and expressed relative to the calibrator using the following formula: fold change = 2−ΔΔCt, where ΔΔCt = [Ct(target) − Ct(HKG)]sample − [Ct(target) − Ct(HKG)]calibrator (59).

Western blotting for SERT. Approximately 50 mg of whole colon (n = 4 from each group) were frozen in liquid nitrogen and, if needed, stored at −80°C. Tissues were ground in liquid nitrogen using a pestle and mortar, resuspended in PBS, pH 7.4, containing complete protease inhibitor cocktail (Roche, Castle Hill, NSW, Australia) and 0.1% Triton X-100, and centrifuged (3,000 g, 4°C, 5 min). The supernatant was removed and placed on ice, divided into aliquots, snap-frozen in liquid nitrogen, and stored at −80°C. Protein concen-
tation of the samples was determined using the Bradford protein assay (Bio-Rad, Gladesville, NSW, Australia).

Aliquots of protein extracts (20 μg of protein) were dissolved in lithium dodecyl sulfate sample buffer (0.5% lithium dodecyl sulfate, 62.5 mM Tris-HCl, 2.5% glycerol, and 0.125 mM EDTA, pH 8.5) for 10 min at 70°C. The samples were separated by electrophoresis in bis-Tris polyacrylamide gels using MOPS-SDS running buffer and electroblotted onto polyvinylidene difluoride membranes overnight at 4°C according to the recommendations of the manufacturer (Invitrogen). After transfer, membranes were thoroughly washed, blocked, and probed with anti-SERT primary antibody (ImmunoStar) overnight at 4°C. Specific binding was visualized using alkaline phosphatase-conjugated secondary antibody and chemiluminescence according to the instructions of the manufacturer (Invitrogen). Membranes were then stripped and reprobed with anti-actin antibody (Sigma-Aldrich) for 2 h at room temperature, and specific binding was visualized as described above. The intensity of the band corresponding to SERT or actin protein expression was determined using Photoshop CS3 Extended softward (Adobe Systems, San Jose, CA), normalized against sample-specific Coomassie-stained protein bands, and statistically analyzed using Prism 5 software (GraphPad, La Jolla, CA).

Solutions. All reagents were purchased from Sigma-Aldrich unless otherwise noted. Fluoxetine was made up as a 10 mM stock solution in ethanol, stored at room temperature, and diluted into physiological saline on the day of the experiment. 5-HT was made up fresh from powder on the day of the experiment or frozen as a 10 mM solution in distilled H₂O.

Statistics. Population data are presented as means ± SE, with the range and/or median given where appropriate. In all cases, n refers to the number of animals used, while the number of repetitions is given when more than one response from a single animal was studied. Student’s t-test was used for single comparisons of parametric data, and Wilcoxon’s signed-rank test was used for nonparametric data (such as percentages); these tests were paired or unpaired as noted. Tests were one-tailed where a strong prediction existed. For multiple comparisons, a one-way ANOVA with Tukey-Kramer post hoc test was used for parametric data and Kruskal-Wallis test with Dunn’s post hoc test was used for nonparametric data. P < 0.05 was taken as the cutoff for statistical significance.

RESULTS

Effect of WD on weight. As we previously reported (14), during the diet period, the average daily energy intake was significantly increased in animals maintained on the WD compared with CF [675 ± 23 (n = 16) vs. 308 ± 12 kJ·rat⁻¹·day⁻¹ (n = 14), P < 0.001]. For the present study, at the end of the diet period, body weight (563 ± 13 and 747 ± 23 g in CF and WD, respectively, P < 0.001, unpaired t-test; Table 1) and fat mass (kidney, liver, and retroperitoneal and testicular fat; Table 1) were increased in WD animals. Blood glucose was also significantly increased from 8.9 ± 0.5 mM in CF rats to 10.2 ± 0.5 mM in WD rats (P = 0.036, unpaired t-test; Table 1).

Effect of WD on colonic EC cell numbers. Sections of full-thickness colon from CF and WD rats were double-labeled against 5-HT and c-Kit using immunohistochemical techniques (Fig. 1). 5-HT-immunoreactive EC cells that were not c-Kit-positive (differentiating them from mast cells) were counted and quantified per mucosal crypt. Colonic EC cells had a variety of morphologies, as previously reported (35, 45, 61). For counting purposes, EC cells were classified as those with a typical wineglass shape (1 process open to the lumen; Fig. 1A), those with a single process (closed; Fig. 1C), or those with multiple (open and closed) processes (Fig. 1, B and D); the total number of all EC cells and crypts was then calculated. The number of total EC cells per crypt decreased significantly from 0.86 ± 0.06 in CF to 0.71 ± 0.04 in WD colon (n = 6 and 5, respectively, P = 0.028, unpaired t-test; Fig. 2, A and B). When EC cells were analyzed on the basis of shape, there was a significant decrease in cells with multiple processes (0.42 ± 0.04 and 0.27 ± 0.04 EC cells/crypt in CF and WD, respectively, P < 0.05) but no change in the numbers of wineglass or single-process EC cells (P > 0.05, ANOVA with Tukey’s post hoc test).

A change in diet has been shown to change mucosal architecture (1, 65), including signs of inflammation (25). Thus, in the present study, we analyzed sections from the colon of CF and WD rats and looked at overall mucosal and muscle thickness. We found no significant difference in mucosal thickness (291 ± 21 and 291 ± 32 μm in CF and WD, respectively, n = 5 each, P = 0.988, unpaired t-test) or a combination of longitudinal and circular muscle thickness (288 ± 51 and 275 ± 26 μm in CF and WD, respectively, n = 5 each, P = 0.847, unpaired t-test). We also restained some sections with hematoxylin-eosin and counted the number of polymorphonuclear leukocytes (including neutrophils, eosinophils, and basophils) in the lamina propria. In CF colon, there were 6.3 ± 0.6 polymorphonuclear leukocytes per field (5 fields/section, 1 section/animal, n = 3), which was not significantly different from WD colon, which had 6.1 ± 0.9 polymorphonuclear leukocytes per field (5 fields/section, 1 section/animal, n = 3). In WD colon, there were 6.3 ± 0.6 polymorphonuclear leukocytes per field (5 fields/section, 1 section/animal, n = 3), which was not significantly different from 78 ± 6 goblet cells/mm of crypt length in CF colon (7–10 crypts per section, 1 section/animal, n = 3), which was not significantly different from 78 ± 6 goblet cells/mm of crypt length in WD colon (n = 3, P = 0.791, unpaired t-test).

Characterization of 5-HT release from rat distal colon. To determine if there were consequences of the decreased numbers of EC cells detected immunohistochemically in WD rats, we examined 5-HT availability using real-time electrochemical methods. EC cells release 5-HT at rest, and this has become an important measure of 5-HT availability (10). The SS levels of 5-HT were measured from three to six sites on the mucosal surface using electrochemical amperometry techniques. Calibration data specific to each electrode allowed us to calculate that the oxidation current generated was, on average, equivalent to 7.3 ± 0.4 μM 5-HT in CF rats (n = 5; Fig. 3), while in WD rats, levels were significantly reduced to 2.9 ± 1.0 μM 5-HT (36% of CF rats, n = 5, P = 0.001, unpaired t-test; Fig. 3B).

Table 1. Diet, weight, adiposity, and metabolic parameters of rats fed chow or maintained on a Western diet for 16-20 wk

<table>
<thead>
<tr>
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<th>CF</th>
<th>WD</th>
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<tr>
<td><strong>Body weight, g</strong></td>
<td>563 ± 13</td>
<td>747 ± 23*</td>
</tr>
<tr>
<td><strong>Kidney fat, g</strong></td>
<td>1.6 ± 0.1</td>
<td>1.9 ± 0.1*</td>
</tr>
<tr>
<td><strong>Liver fat, g</strong></td>
<td>19.7 ± 0.7</td>
<td>24.0 ± 1.1*</td>
</tr>
<tr>
<td><strong>Retroperitoneal (left) fat, g</strong></td>
<td>7.1 ± 0.6</td>
<td>20.9 ± 1.7*</td>
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<tr>
<td><strong>Testicular fat, g</strong></td>
<td>6.2 ± 0.7</td>
<td>19.5 ± 2.4*</td>
</tr>
<tr>
<td><strong>Blood glucose, mM</strong></td>
<td>8.9 ± 0.5</td>
<td>10.2 ± 0.5*</td>
</tr>
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Values are means ± SE; n = 13 chow-fed (CF) and 14 Western diet-fed (WD) mice. *Significantly different from CF (P < 0.05, unpaired t-test).
EC cells are known to release 5-HT during mechanical stimulation (13); therefore, using the recording electrode to compress the mucosal epithelium, we determined the peak compression-evoked 5-HT release. Compression-evoked 5-HT release was also significantly decreased from 18.4 ± 3.4 μM in CF rats (n = 5) to 10.5 ± 2.6 μM in WD rats (57% of CF rats, n = 5, P = 0.036, unpaired t-test; Fig. 3B). To rule out any contribution of movement artifacts to the oxidation current, we confirmed that when the electrode potential was held at 0 mV, no oxidation current was detected during compression of the mucosa (data not shown).

**SERT-dependent uptake of 5-HT during obesity.** The uptake of 5-HT by SERT-dependent mechanisms is a key factor in controlling 5-HT availability in the GI tract, and uptake can be inferred using electrochemical techniques (12). The difference between 5-HT levels before and during SERT blockade with 1 μM fluoxetine was taken as an estimate of SERT function. At rest, the average SS 5-HT levels were significantly increased from 3.7 ± 0.5 μM in control to 15.7 ± 5.5 μM with fluoxetine (n = 5, P = 0.047, paired t-test). The average compression-evoked peak levels of 5-HT were also significantly increased from 11.5 ± 2.7 μM in control to 30.9 ± 8.2 μM with fluoxetine (n = 5, P = 0.018, paired t-test). The capacity for SERT-dependent uptake of 5-HT was similar in WD rats. Blockade of SERT significantly increased the SS 5-HT levels from 2.7 ± 0.4 μM in control to 5.8 ± 1.6 μM with fluoxetine (n = 5, P = 0.035, paired t-test). The average peak levels of 5-HT were also significantly increased from 8.5 ± 1.3 μM in control to 14.7 ± 2.1 μM with fluoxetine (n = 5, P = 0.007, paired t-test; data not shown).

To determine whether the WD affected the magnitude of SERT-dependent uptake of 5-HT, the effect of fluoxetine was compared between WD and CF rats. To reduce interexperiment variability, fluoxetine data were expressed as a percentage of control for individual experiments, and these percentages were averaged. In CF rats, fluoxetine caused a robust increase of SS (362 ± 83%, P = 0.034) and peak (267 ± 25% of control, P = 0.004, 1-sample t-test for each) 5-HT levels. In WD rats, fluoxetine increased SS (221 ± 36%, P = 0.029) and peak (176 ± 21% of control, P = 0.024, 1-sample t-test for each) 5-HT levels, which were not significantly different from CF rats (P > 0.05, Kruskal-Wallis with Dunn’s post hoc test; data not shown).

**Effect of diet composition.** We were interested in whether the fat content of the WD was the most important contributor to the increase in 5-HT availability. To test this, we examined rats fed a medium-fat pellet diet (22% fat chow; Specialty Feeds; compared with 32% fat in WD) for 16–20 wk and compared these animals with age-matched control rats fed a standard chow diet. Body weights of rats fed the control and medium-fat diets were 642 ± 16 g (n = 4) and 723 ± 30 g (n = 3), respectively, which represents a significant increase (P = 0.360). In contrast to WD rats, medium-fat pellet-fed rats did not show a change in 5-HT availability. SS levels were not changed with 2.0 ± 0.3 μM 5-HT in CF rats and 1.6 ± 0.7 μM 5-HT in medium-fat pellet-fed rats (n = 4 and 3, respectively, P = 0.247; data not shown). Peak compression-evoked 5-HT release was also not changed with 6.2 ± 1.3 μM 5-HT in CF rats and 4.9 ± 0.4 μM 5-HT in medium-fat pellet-fed rats (n = 4 and 3, respectively, P = 0.218, unpaired t-test; data not shown).
shown). Together, these data suggest that the fat content of the WD, although higher than the CF, may not be the most important component driving changes in 5-HT.

Levels of TPH1 and SERT mRNA. Our immunohistochemical studies show a decrease in the number of EC cells, while our electrochemical studies suggest a decrease in 5-HT availability. We used quantitative real-time RT-PCR to determine the levels of TPH1 mRNA expression (Fig. 4A). TPH1 is the rate-limiting enzyme in the 5-HT synthesis pathway, and we predicted that fewer EC cells and decreased levels of 5-HT would be reflected by less TPH1. However, no change was detected in the levels of TPH1 mRNA in WD rat colon when normalized to GAPDH mRNA \( [1.16 \pm 0.39 \, (n = 8) \text{ and } 2.22 \pm 0.86 \, (n = 6)] \) in CF and WD, \( P = 0.245 \), unpaired \( t \)-test] or to villin mRNA \( [2.73 \pm 0.73 \, (n = 7) \text{ and } 4.39 \pm 1.09 \, (n = 5)] \) in CF and WD, respectively, \( P = 0.109 \); Fig. 4A].

We predicted that an increase in SERT might contribute to the decreased in 5-HT availability. The mucosal levels of SERT mRNA in rat colon, normalized to GAPDH mRNA, were significantly increased in WD rat colon \( [0.11 \pm 0.02 \, (n = 9) \text{ and } 0.17 \pm 0.04 \, (n = 6)] \) in CF and WD, respectively, \( P = 0.044 \), unpaired \( t \)-test; Fig. 4B] and significantly increased compared with villin \( [0.32 \pm 0.04 \, (n = 10) \text{ and } 0.53 \pm 0.07 \, (n = 7)] \) in CF and WD, respectively, \( P = 0.008 \), unpaired \( t \)-test; Fig. 4B]. There was no change in villin mRNA compared with GAPDH \( [3.02 \pm 0.47 \, (n = 10) \text{ and } 3.52 \pm 1.01 \, (n = 7)] \) for villin and GAPDH, respectively, \( P = 0.313 \), unpaired \( t \)-test; Fig. 4C].

SERT protein. To further examine the role of SERT in determining 5-HT availability, Western blots were used to quantify the amount of SERT protein in relation to actin and Coomassie staining, which were used as loading controls; the loading controls were also compared with each other. In WD rat colon, the ratio of SERT to actin was \( 0.84 \pm 0.08 \), which was unchanged compared with \( 0.79 \pm 0.07 \) in CF rats \( (n = 4 \text{ and } 3, \text{ respectively}, \, P > 0.05 \); Fig. 5A). Similarly, when the ratio of
SERT to Coomassie protein was examined, there was no change $[8.96 \pm 0.72 \,(n = 4) \text{ and } 8.91 \pm 1.44 \,(n = 3)]$ in WD and CF, respectively, $P > 0.05$; Fig. 5B]. For the same samples, actin was compared with Coomassie protein and was found to be unchanged $[10.7 \pm 0.52 \,(n = 4) \text{ and } 11.09 \pm 0.82 \,(n = 3)]$ in WD and CF, respectively, $P > 0.05$, ANOVA with Tukey’s post hoc test; Fig. 5C].

**DISCUSSION**

The main finding of this study was that the availability of 5-HT at the mucosal surface was decreased in the colon of a rat model of diet-induced obesity. The numbers of EC cells were decreased during obesity as assessed immunohistochemically and were accompanied by a decrease in 5-HT availability recorded by real-time electrochemical measurements. In contrast, quantitative RT-PCR showed no changes in the levels of mRNA for TPH1. Our electrochemical data also showed no change in SERT-dependent uptake of 5-HT, which was supported by a lack of change in SERT protein levels in Western blots, although quantitative RT-PCR showed an increase in SERT mRNA. This is the first study to demonstrate a decrease in 5-HT availability in the colon during diet-induced obesity and to characterize the molecular changes in 5-HT signaling associated with a detrimental Western-style diet.

**Diet-induced obesity decreases 5-HT levels.** In the present study, measurements from the colon of WD-fed rats revealed a clear decrease in 5-HT availability. Our electrochemical data showed that SS 5-HT levels and peak compression-evoked 5-HT release were lower at the mucosal surface of WD than CF rats (36% vs. 57%). The levels of 5-HT at the mucosal surface are controlled by several factors, including the active release of 5-HT from EC cells, which is controlled by the numbers of EC cells and the amount of 5-HT contained within them. After 5-HT is released, its diffusion into the surrounding solution and reuptake by SERT contribute to overall 5-HT availability (12).

The numbers of EC cells in the colon have been assessed in several genetic models of obesity and diabetes. The numbers of colonic EC cells have been found to be decreased in the leptin-deficient ob/ob rat (66), the leptin receptor-deficient db/db mouse (60), and an acute hyperglycemia model (dexta-
methasone-treated rats) (34). Similarly, in the present study, we show a reduction in EC cell numbers in the colon of WD rats, while the numbers of mature goblet cells were unchanged. We would predict that a reduction in EC cells would be associated with a decrease in TPH1, the rate-limiting enzyme in the 5-HT synthesis pathway. In contrast, we saw no change in the expression of TPH1. However, because TPH1 is expressed predominantly by the EC cells, we can account for their reduced number by applying a small correction factor. This correction acts to increase the apparent levels of TPH1 and caused our TPH1 vs. villin comparison to approach statistical significance. Although speculative, this could suggest that the amount of TPH1 per EC cell increased, perhaps in compensation for the overall reduction in EC cell numbers. Interestingly, the morphological characteristics of the EC cells shifted from cells with more processes in CF rats to those with fewer processes in WD rats, but no change between open and closed types was apparent. Because the role of these processes in colonic EC cells is not clear, it is not possible to speculate about the significance of this change. Taken together, the reduction in EC cell numbers provides one explanation for the decrease in 5-HT availability, which we measured electrochemically.

SERT is localized to the plasmalemma of all GI epithelial cells (33, 52) and is under the control of a novel promoter that is specific for the intestinal epithelium (49). An increase in SERT function could account for some of the decrease in 5-HT availability. Indeed, quantitative RT-PCR showed an increase
in SERT mRNA in the colon of the obese rat compared with the housekeeping gene GAPDH and the brush border protein villin. Villin was not significantly changed in WD colon, and we have evidence that calponin and smoothelin are unchanged in WD rat colon (11), which suggests that any obesity-related remodeling is minor. This is supported by our anatomic observations showing that the thicknesses of the mucosal and muscle layers were not changed in WD rat colon. In contrast, in our electrochemical experiments, the function of SERT was unchanged in WD rats, and quantitative Western blot data showed no change in SERT protein compared with actin or Coomassie protein levels. Taken together, these data suggest that increased SERT function does not account for the reduction in 5-HT availability at 16–20 wk of WD but may be important in the longer term if increases in SERT mRNA translate into increased function.

How does diet-induced obesity decrease 5-HT availability? There is evidence that obesity is a mild inflammatory disease (3, 20, 30, 62), and a high-fat diet has been associated with changes in the intestinal microflora (25, 38), which in turn have been linked to inflammation (25, 51). Thus it is reasonable to consider the present findings in light of the effects of inflammation on the decrease in 5-HT availability in the present study. In the ileum, 2,4,6-trinitrobenzene sulfonic acid treatment has been associated with EC cell hyperplasia (55) and increased 5-HT availability (54). Similarly, in the colon, 5-HT availability has been shown to be increased in a guinea pig model of 2,4,6-trinitrobenzene sulfonic acid-induced colitis (48), a rat model of dextran sodium sulfate-induced colitis (56), and a mouse model of dextran sodium sulfate-induced colitis (9). Increased 5-HT is not a passive consequence of the inflammatory process but can act to worsen colitis, as has been shown following the knockout of SERT (which increases 5-HT), which increases damage (16), while the knockout of TPH1 (which lowers 5-HT) results in reduced damage (32). In contrast, the present study has shown a reduction in EC cell numbers and a reduction in 5-HT availability in the colon of obese rats. In a recent study, De La Serre et al. (25) showed that a high-fat diet is associated with inflammation in the rat ileum, and we showed that this is associated with an increase in 5-HT availability (14), but Hyland et al. (41) did not report increases in inflammatory markers in rat colon fed a medium-fat diet. Indeed, our data show that one of the hallmarks of inflammation, a thickening of the colonic wall, was not present in WD rat colon, and the combined numbers of neutrophils, eosinophils, and basophils were not increased. Taken together, this suggests that inflammatory changes, similar to those seen in colitis, do not account for the reduction in 5-HT availability in WD rat colon.

Another hypothesis is that the WD has a direct effect on the proliferation of the EC cells (65). The fat content of the diet used in the present study was increased (32%); thus, to determine if this alone was responsible for the decreased 5-HT availability, we examined rats fed a medium-fat (22%) pellet diet (which had near-normal levels of protein and sugar) for 16 wk. Despite their significant weight gain over the CF controls, in WD rats, availability of 5-HT in the colon did not change, although these data are from a small sample size. While we could speculate that the higher fat content of the WD reached a threshold that the pellet diet failed to reach, our previous data show that the fat content of the medium-fat pellet diet is sufficient to act on the ileal EC cells (14). Thus the simplest interpretation is that the fat content of the WD was not the most important component driving the decrease in 5-HT availability.

In the WD used in the present study, sugar content was also increased and, correspondingly, fewer calories were derived from protein. It has been recently shown that a high-protein diet causes altered colonocyte metabolism and morphology (1); thus we could speculate that a reduced-protein diet may also alter colonocytes (and, presumably, colonic EC cells). There is also evidence that EC cells have sensory transduction machinery for direct response to sugars and amino acids through taste receptors such as T1Rs (28, 72, 73). However, the only nutrients in the colon are likely to be those generated by bacteria (e.g., short-chain fatty acids) or those that are indigestible in the small intestine, such as oligosaccharides (39). Interestingly, the trisaccharide raffinose tastes about one-quarter as sweet as sucrose (15) and, thus, may interact with taste receptors in the intestine, as do other sugars (53). Although this mechanism could theoretically provide a direct effect of dietary sugar on colon EC cells, it seems unlikely to play a major role in the present study. The WD contained few foods with a high oligosaccharide content (e.g., beans and cabbage); rather, it was high in simple sugars such as fructose. This, coupled with the relatively small number of EC cells in the colon that are open to the lumen and, thus, might contact luminal sugars, suggests that another mechanism is needed to explain the reduction in 5-HT availability seen here. Taken together, it seems that no one idea satisfactorily explains the reduction in 5-HT availability in the WD rats, although the fact that genetic models of obesity also show a reduction in EC cells (34, 60, 66) suggests that this a robust finding across different models of obesity.
Physiological relevance of decreased 5-HT. Obesity and a high-fat diet have been associated with changes in GI function, such as constipation, and in the levels of intestinal hormones secreted (23, 31, 71). Animal models of obesity have also shown changes in intestinal secretion (42), gastric emptying (2), and motility (18, 44), although there are conflicting reports as to whether there is an increase or a decrease in transit and/or mixing (reviewed in Ref. 31). A low 5-HT availability, as observed in the present study, could reduce colonic motility during obesity and cause constipation. Indeed, we observed that in vitro colonic transit of natural pellets appeared slowed, but we were unable to quantify this because of technical difficulties (personal observations). Our findings in the colon are in stark contrast to our recent study in the ileum, where a WD was associated with an increase in 5-HT availability and a greater number of EC cells (14). It seems noteworthy that although the same components of the system were affected, the ileum and colon have shown opposite responses to the same diet. This suggests that one simple mechanism, such as inflammation or a specific component of the diet, is unlikely to account for all the changes.

It is known that 5-HT stimulates colonic motility (68), with evidence for the involvement of 5-HT3 receptors (69) and 5-HT4 receptors (40). In addition, current treatments with prokinetics target 5-HT receptors, and new serotonergic treatments are in development (67). Our findings suggest that treatment with 5-HT4 receptor agonists might be more effective in the colon of an obese individual, where endogenous 5-HT is low. Conversely, an agonist might be less effective in the ileum, where levels of 5-HT are already high. Future studies that focus on alterations to colonic transit under different dietary conditions and on the effects of serotonergic agonists such as renzapride or prucalopride are needed (67). Furthermore, we can speculate that reduced transit during a high intake of food may be beneficial in the short term. An important function of the transverse and descending colon in humans is storage of fecal matter, allowing the choice of time and place for elimination. However, during long-term overeating, it may be that this increased storage turns into functional constipation. Thus, although a low fiber content of the WD has generally been blamed for constipation in obese individuals (24), we provide evidence that changes to the endocrine control of the colon, especially a decreased availability of 5-HT, may also contribute. Conclusions. The findings of this study demonstrate that 5-HT availability is decreased in the colon of WD-fed rats. The number of EC cells was reduced during obesity, and this was associated with a reduction in the levels of 5-HT, but not TPH1, mRNA. SERT-dependent uptake of 5-HT and SERT protein levels were unchanged, while SERT mRNA was increased. Taken together, these data suggest that the WD reduced 5-HT availability in the colon by a reduction in EC cell numbers, which is likely to be associated with decreased motility or sensation in vivo.

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AUTHOR CONTRIBUTIONS


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