Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis

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Am J Physiol Gastrointest Liver Physiol 285: G207–G216, 2003. First published March 19, 2003; 10.1152/ajpgi.00488.2002.—5-HT released from enterochromaffin cells acts on enteric nerves to initiate motor reflexes. 5-HT’s actions are terminated by a serotonin reuptake transporter (SERT). In this study, we tested the hypothesis that inflammation leads to altered mucosal 5-HT signaling. Colitis was induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS), and experiments were conducted on day 6. 5-HT content, number of 5-HT-immunoreactive cells, and the proportion of epithelial cells that were 5-HT-immunoreactive increased twofold in colitis. The amount of 5-HT released under basal and stimulated conditions was significantly increased in colitis. SERT inhibition increased the 5-HT concentration in media bathing-stimulated control tissue to a level comparable to that of the stimulated colitis tissue. mRNA encoding SERT and SERT immunoreactivity were reduced during inflammation. Slower propulsion and reduced sensitivity to 5-HT receptor antagonism were observed in colitis. These data suggest that colitis alters 5-HT signaling by increasing 5-HT availability while decreasing 5-HT reuptake. Altered 5-HT availability may contribute to the dysmotility of inflammatory bowel disease, possibly due to desensitization of 5-HT receptors.

inflammatory bowel disease; serotonin transporter; enterochromaffin; enteroendocrine; motility

ENTEROCHROMAFFIN (EC) cells function as sensory transducers that initiate motor and secretory reflexes in response to chemical or mechanical stimulation of the gastrointestinal mucosa (4, 5, 9, 12, 14, 27). Bülbbring and colleagues (4, 5) first proposed that EC cells might be mucosal transducers, in part because they found that an intact mucosa is required to initiate a peristaltic reflex. This concept has been supported by the demonstration that mucosal stimulation releases serotonin (5-HT) from EC cells, which in turn directly activates processes of submucosal and myenteric afferent neurons to initiate peristalsis and mucosal secretion (2, 9, 13, 14, 19, 27, 29).

Linden, David R., Jing-Xian Chen, Michael D. Gershon, Keith A. Sharkey, and Gary M. Mawe. Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 285: G207–G216, 2003. First published March 19, 2003; 10.1152/ajpgi.00488.2002.—5-HT released from enterochromaffin cells acts on enteric nerves to initiate motor reflexes. 5-HT’s actions are terminated by a serotonin reuptake transporter (SERT). In this study, we tested the hypothesis that inflammation leads to altered mucosal 5-HT signaling. Colitis was induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS), and experiments were conducted on day 6. 5-HT content, number of 5-HT-immunoreactive cells, and the proportion of epithelial cells that were 5-HT-immunoreactive increased twofold in colitis. The amount of 5-HT released under basal and stimulated conditions was significantly increased in colitis. SERT inhibition increased the 5-HT concentration in media bathing-stimulated control tissue to a level comparable to that of the stimulated colitis tissue. mRNA encoding SERT and SERT immunoreactivity were reduced during inflammation. Slower propulsion and reduced sensitivity to 5-HT receptor antagonism were observed in colitis. These data suggest that colitis alters 5-HT signaling by increasing 5-HT availability while decreasing 5-HT reuptake. Altered 5-HT availability may contribute to the dysmotility of inflammatory bowel disease, possibly due to desensitization of 5-HT receptors.

Whereas enteroendocrine cells are known to contain numerous neuroactive molecules, including cholecystokinin and neurotensin (6, 10, 32), it is the 5-HT that is released from EC cells that has been identified as a critical mediator of the peristaltic reflex. 5-HT activates both intrinsic (via 5-HT1P, 5-HT3, and 5-HT4 receptors) and extrinsic (via 5-HT3 receptors) primary afferent neurons. Pharmacological blockade of any one of these three 5-HT receptors diminishes propulsive motor activity, and simultaneous antagonism of all of these receptors eliminates peristaltic reflex activity (14, 18, 33, 35). After it is released from EC cells, mucosal 5-HT is inactivated by uptake into epithelial cells (8, 35). This uptake is mediated by a plasma-membranous 5-HT transporter identical to the 5-HT transporter expressed by neurons (15).

The present study was designed to determine whether inflammation leads to changes in the availability of 5-HT from EC cells. Because 5-HT is important in initiating motor activity in the bowel, and inflammation of the bowel is associated with decreased motility (20, 25, 30, 31), a change in the bioavailability of mucosal 5-HT could contribute to the dysmotility during inflammation. We used 2,4,6-trinitrobenzene sulfonic acid (TNBS) to induce colitis in the guinea pig, and we evaluated 5-HT signaling in the inflamed and control colon. The 5-HT-mediated initiation of peristalsis was assessed with a dual 5-HT3/5-HT4 receptor antagonist. We also measured the mucosal content of 5-HT, the number of EC cells, the basal and stimulation-induced release of 5-HT, and the amount of mRNA encoding serotonin reuptake transporter (SERT). Our observations suggest that there is both a substantial increase in the availability of 5-HT and a decrease in the inactivation of released 5-HT in the inflamed colon.

MATERIALS AND METHODS

Animal preparations. All methods used in this study were approved by the Animal Care and Use Committee of the University of Vermont. Adult guinea pigs (Charles River, Montreal, Canada) of either sex, weighing 250–350 g, were housed in metal cages with soft bedding. Animals had access...
to food and water ad libitum and were maintained at 23–24°C on a 12:12-h light-dark cycle.

To induce inflammation in the distal colon, guinea pigs were anesthetized with isoflurane (induced at 4%, maintained on 2–2.5% in oxygen). TNBS (0.5 ml; Fluka, Milwaukee, WI; 30 mg/ml) in 30% (vol/vol) ethanol was then administered into the lumen of the colon through a polyethylene catheter inserted via the rectum 7 cm proximal to the anus. Control animals were either similarly treated with 0.5 ml of physiological saline (0.9% NaCl) instead of TNBS, or they remained naïve to treatment. Animals were maintained in a controlled environment for 6 days after intracolonic injection. This period was chosen in preliminary experiments because guinea pigs were able to gain weight after treatment (an outward sign that the animals were in the chronic phase of inflammation), and at this time point, the epithelial barrier was intact. This period of time is consistent with the models of TNBS-induced chronic inflammation in the rat (23) and mouse (24).

At the time of tissue collection, animals were deeply anesthetized with isoflurane and perfused transcardially with ice-cold PBS (0.1 M pH 7.4). The distal colon, identified as the part of the colon between the hypogastric flexure and the pelvic brim, was removed and used for experimental studies.

Assessment of colonic inflammation. The severity of colitis was assessed in three ways: 1) changes in the weight of the animals, 2) scoring of macroscopic damage, and 3) histological evaluation. Animals were weighed before the administration of TNBS or saline and daily after the procedure. After animals were euthanized, the colon was removed and examined macroscopically. Criteria used for scoring gross morphological damage have been described previously (22). Briefly, the scoring of macroscopic damage takes into account the presence and severity of adhesions (score 0–2), the maximum thickness of the bowel wall (in mm), and the absence or presence of diarrhea (0–1). In addition, a score was also assigned on the basis of the extent of mucosal damage. These scores ranged from normal (score of 0) to a score of 10 depending on the presence and extent of hyperemia and ulceration.

Colonic samples for histology were fixed overnight at 4°C in 0.1 M PBS containing 4% paraformaldehyde, and 0.2% picric acid. Samples were then transferred to 30% sucrose in PBS overnight at 4°C. Colonic segments from different groups of animals were embedded in OCT compound (Miles, Elkhardt, IN) in the same blocks, so that further processing occurred under identical conditions. Transverse sections of the colon (10 µm) were cut on a cryostat-microtome, thaw-mounted onto gelatin-coated slides, and stored at −20°C until used. Tissue sections were dehydrated and stained with hematoxylin and eosin. Histological scoring was on the basis of a semiquantitative scoring system in which the following features were considered and scored as follows: extent of destruction of normal mucosal architecture (0 = normal; 3 = maximal damage), presence and degree of cellular infiltration (0 = normal; 3 = maximal infiltration), extent of muscle thickening (0 = normal; 3 = maximal thickness), presence or absence of crypt abscesses (0 = absent; 1 = present) and the presence or absence of goblet cell mucus (0 = absent; 1 = present). In each case, a numerical score was assigned with a maximum score of 11. Three tissue sections from each animal were assessed (each separated by at least 500 µm) and averaged to obtain a mean histopathological score.

Mucosal epithelial architecture and 5-HT immunoreactivity. Changes in the EC cell population of the colonic epithelium were quantified by immunocytochemically demonstrating 5-HT in transverse sections of the colon. Tissue sections were prepared as described above. Endogenous peroxidase activity in the tissue sections was blocked by incubation for 30 min in a solution of hydrogen peroxide (0.3%) in methanol followed by three 15-min washes with PBS. The sections were subsequently incubated for 2 h at room temperature with PBS containing 0.5% Triton X-100 and 4% normal goat serum. This solution was removed, and the sections were incubated overnight at room temperature with a 1:80 dilution of rabbit anti-5-HT antiserum (Immunotech, Marseille, France) in PBS containing 4% normal goat serum and 0.5% Triton X-100. The sections were then washed with PBS (3 × 15 min), incubated with a 1:400 dilution of biotin-conjugated goat anti-rabbit serum (Jackson ImmunoResearch, West Grove, PA) in PBS containing 0.5% Triton X-100 for 2 h. The sections were washed again (3 × 15 min) with PBS and incubated with a 1:400 dilution of strepavidin-conjugated peroxidase (Kirkegaard and Perry, Gaithersburg, MD) in PBS containing 0.5% Triton X-100 for 2 h. The sections were finally rinsed thoroughly and processed for 2 min with a diaminobenzidine reagent kit according to the manufacturer’s instructions (Kirkegaard and Perry) and washed (3 × 15 min) with PBS. Finally, the sections were counterstained with hematoxylin and dehydrated so that the total number of epithelial cells in a given region could be determined.

The total circumferential length of the muscularis mucosa in each transverse section was measured at a magnification of ×40. Epithelial cell hyper- or hypoplasia was determined by counting the number of stained nuclei of epithelial cells for a given circumferential length of colon, usually 4–7 glands, and quantified as the number of epithelial cells per gland. 5-HT-containing epithelial cells were counted and normalized as functions of the circumferential length of colon and the proportion of epithelial cells. Goblet cells were identified morphologically and quantified in a similar manner. These determinations were made at a magnification of ×400 from three random locations in each transverse section, taking care to ensure that the area contained intact glands. Three tissue sections, at least 500 µm apart, were assessed from each animal.

Measurement of the 5-HT content of the colon. A segment of colon was removed from the animal and homogenized in 0.5 ml of iced 0.2 M perchloric acid, and centrifuged at 10,000 g for 5 min. The supernatant was neutralized with 0.5 ml of 1.0 M borax buffer (pH 9.25), and centrifuged at 10,000 g for 1 min. The 5-HT content of an aliquot of the sample was analyzed by enzyme immunoassay with a kit used according to the manufacturer’s instructions (Beckman Coulter, Fullerton, CA). The 5-HT content of the tissue was expressed as a function of wet weight (in mg), and also as a function of the length (in cm) of the colon to compensate for the increased protein and mass due to edema and infiltration of inflammatory cells that occurs as a result of inflammation.

Measurement of the release of 5-HT from the mucosa of the colon. Segments of colon (0.5 cm) were opened along the mesenteric border and pinned flat, mucosal side up, in a Sylgard-coated six-well dish containing aerated 37°C Krebs solution. The bathing solution was replaced by 3 ml of normal Krebs solution or Krebs solution that contained fluoxetine (10 µM). To stimulate the mucosa, preparations were gently rubbed in a circumferential direction with a 3-mm glass probe at a rate of ~1 Hz for a duration of 4.5 min at intervals of 5 min for a total of 15 min. Basal release of 5-HT was measured by leaving preparations undisturbed in the bathing solution for 15 min. The 5-HT released into the bathing solution was measured by enzyme immunoassay as described above.
Measurement of mRNA encoding SERT. The amount of mRNA encoding SERT was quantified by using real-time RT-PCR. Segments of distal colon were removed from the animal and rapidly homogenized in Tris-buffered saline (Sigma, St. Louis, MO) for the extraction of total RNA. Samples of cDNA, in a volume of 20 μl, were generated by RT with 3 μg total RNA, 6 μg random primers, 1 mM deoxynucleotides, 40 units of RNase OUT, and 200 units of Superscript II Plus RNase H−RT, according to the manufacturer’s instructions (Invitrogen). cDNA (1 μl) was employed to quantify mRNA encoding β-actin, which was used as a nonregulated reference gene to which the expression of SERT could be normalized. cDNA (3 μl) was employed to quantify cDNA encoding SERT. A Light-Cycler instrument (Roche) was used for real-time PCR. The appearance of the double-stranded DNA product during amplification was detected in real time by measuring the result of amplification with a melting curve analysis. Cloned cDNAs encoding β-actin or SERT were amplified with the same sense and antisense target cDNAs and were used as the reference materials for quantifying cDNA. Primers used for amplification were based on the GenBank sequence, X63253 and were as follows: sense, 5'-TACATGGCGGAGATGA-3'; antisense, 5'-CCATAGAACCAGACA-3' (nucleotides 1268–1283); antisense, 5'-CCATAGAACCAGACA-3' (nucleotides 1650–1665)(8, 35). The relative expression of SERT in each sample was normalized to that of β-actin.

5-HT transporter immunoreactivity. Tissue sections were prepared and immunostained as described above with the exception that the primary antiserum was rabbit anti-SERT (1:5,000), a generous gift from Dr. Robert Blakely (Vanderbilt University), and the secondary antiserum was Cy3-conjugated goat anti-rabbit (1:400; Molecular Probes, Eugene, Oregon). Sections were counterstained with Citifluor and examined with an Olympus AX70 fluorescence microphotometer.

In vitro pellet propulsion. Methods used to measure the rate of propulsion of fecal pellets in the isolated distal colon of the guinea pig have been described previously (18). Briefly, a segment of distal colon with attached mesentry was removed from the animal, placed in a Sylgard-coated chamber, and straightened by placing pins every 2 cm along the mesentery. The isolated colon was maintained in aerated, circulating (10 ml/min) 37°C Krebs solution (in mM: 121 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 1.2 NaH2PO4, and 8 glucose, aerated with 95% O2-5% CO2). After an equilibration period of 15 min, during which natural pellets were expelled from the colon, silicon-coated fecal pellets were placed into the lumen at the oral end of the colonic segment and moved ~1 cm along the segment with a glass rod. The rate of motility for each trial was calculated by recording the time taken by the isolated colon to propel the pellet a distance of 3 cm in the center of each segment. The mean rate of propulsion was calculated from at least five trials for each condition, conducted with 5-min rest periods between trials. The dual 5-HT2/5-HT4 antagonist SDZ-205–557 was added to the circulating solution in increasing concentrations. After the cumulative concentration-response relationship was ascertained, the preparation was washed with normal Krebs solution, and the experiment was repeated. In each case, the rate of pellet propulsion returned essentially to that seen at the end of the experiments.

Data analyses. All experiments were replicated with tissue from the same animal and mean values were recorded. The data presented are means ± SE for n animals. Statistical analyses were performed by using Prism software (version 3.0a; GraphPad Software, San Diego, CA) or StatView (version 5.0.1; SAS Software, Cary, NC). Comparisons between two groups were made parametrically (t-test) or nonparametrically (Mann-Whitney U-test) as appropriate. Comparisons among three or more groups were done with one-, two-, or three-way ANOVA followed by the Newman-Keuls, Bonferroni’s, or Student-Newman-Keuls multiple comparisons tests, respectively. Differences between means at a level of P < 0.05 were considered to be significant. Nonlinear sigmoidal concentration-response regression curves were plotted and IC50 values were determined by using Prism software.

RESULTS

TNBS-induced inflammation. A single administration of TNBS in ethanol into the lumen of the guinea pig distal colon consistently caused regional inflammation characterized by ulceration, hyperemia, adhesions, edema, and changes to mucosal architecture similar to previous descriptions of TNBS-induced intestinal inflammation in the rat (23) and mouse (24). All TNBS-treated animals initially lost weight after the administration of TNBS but began to gain weight again after 2–3 days (Fig. 1A). In contrast, control animals, which received intracolonic saline, did not lose weight but rather remained at the preprocedure weight for 1 day and gained weight normally thereafter (Fig. 1A). Macroscopic and histological damage scores, obtained 6 days after application of TNBS, revealed that the colon of TNBS-treated animals was significantly damaged at this time point although the animals had recovered the ability to gain weight and had an intact epithelial barrier (Fig. 1, B and C). The combination of weight gain and intact epithelial barrier in the face of severe damage to the colon 6 days after the instillation of TNBS is consistent with the induction of a chronic inflammatory state in that organ. Tissue was therefore collected 6 days after the administration of TNBS in all subsequent experiments.

Colonic serotonin content is increased in colitis. The 5-HT content of the colon was measured to test the hypothesis that TNBS colitis is associated with changes in 5-HT availability. The colons were homogenized, and 5-HT was measured by enzyme immun assay (Fig. 2). When examined as a function of tissue weight of tissue sample, there was a significant reduction in the 5-HT content of the inflamed colon compared with control (P < 0.05, t-test). Because inflammation is associated with an increase in protein and mass due to edema and infiltration of inflammatory cells, 5-HT content was also measured as a function of length (in cm). This analysis revealed that the amount of 5-HT per unit length of distal colon was significantly increased in TNBS colitis compared with control (P < 0.05, t-test).

EC cells are more numerous in the inflamed colon. Most of the 5-HT in the colon is synthesized, stored, and secreted by EC cells (34). To determine whether the inflammation-induced increase in the 5-HT content of the colon was associated with an increase in the number of EC cells, 5-HT-immunoreactive mucosal epithelial cells were quantified in transverse sections of...
control and inflamed distal colon (Fig. 3A). The number of 5-HT-immunoreactive EC cells was evaluated per millimeter length of muscularis mucosa. Although inflammation was associated with an increase in the volume of the mucosal layer, this normalization was still possible, because the total circumference of the muscularis mucosa per section in the inflamed colon was not different from that of saline-treated controls (control, 8.8 ± 0.8 mm; TNBS, 10.2 ± 0.6 mm; n = 6 and 4, respectively; P > 0.05, t-test). The number of 5-HT-immunoreactive EC cells was increased twofold in sections of colon from TNBS-treated animals compared with saline-treated controls (Fig. 3B; P < 0.05, t-test).

Concurrent with an increase in the number of 5-HT-immunoreactive EC cells, dramatic changes occurred in the mucosal architecture of the colon. The glands in inflamed colon were significantly longer than saline-treated controls (Fig. 4A; P < 0.05, t-test), which is consistent with an increase in the total number of epithelial cells per gland (Fig. 4B; P < 0.05, t-test). It is possible that an increase in the number of 5-HT-containing EC cells was simply due to an increased production, or decreased apoptosis, of all epithelial cells. Although there was an increase in the total number of epithelial cells, the proportion of goblet cells was unchanged in colon samples from TNBS animals compared with control sections (Fig. 4C; P > 0.05, t-test). In contrast, the proportion of total epithelial cells that contain 5-HT was significantly increased in the inflamed colon (Fig. 4D; P < 0.05, t-test).

**Release of 5-HT is increased in colitis.** To determine whether the inflammation-induced increases observed in the content of 5-HT and the number of EC cells were associated with similar changes in the actual release of 5-HT after stimulation, we measured the amount of 5-HT released from the isolated colon into the ambient solution. The secretion of 5-HT was measured under basal conditions or in response to mechanical stimulation of the mucosa of normal and inflamed preparations. Effects of the inhibition of SERT on the 5-HT content of the ambient medium were also ascertained by studying the release of 5-HT in the presence or absence of fluoxetine. Results of these experiments are

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**Fig. 1.** Inflammation is induced by a single intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS). A: TNBS animals lost weight initially but began gaining weight at a normal rate 2 days after TNBS, whereas control animals did not lose weight. B: gross damage score (system explained in MATERIALS AND METHODS) was significantly increased in TNBS compared with controls. C: histopathology score (system explained in MATERIALS AND METHODS) was also significantly elevated in TNBS animals compared with control animals. *P < 0.001 compared with controls; t-test.

**Fig. 2.** A: when examined as a function of wet weight of tissue sample, there was a significant reduction in the 5-HT content of the inflamed colon compared with control. B: when examined as a function of length, the amount of 5-HT was significantly increased in TNBS colitis compared with control. *P < 0.05 compared with saline controls; t-test.
shown in Fig. 5. In the absence of fluoxetine, the 5-HT concentration in the ambient medium was significantly greater for TNBS-treated tissues than for control tissues; this was true, both for the basal release of 5-HT and for that detected after mucosal stimulation ($P < 0.05$, three-way ANOVA, Student-Newman-Keuls multiple comparisons test).

In the presence of 1 µM fluoxetine, the basal secretion of 5-HT was comparable to that seen in the absence of fluoxetine both for colons of TNBS-treated and control guinea pigs. After mucosal stimulation, however, the presence or absence of fluoxetine made a difference. More 5-HT was detected in the ambient medium after stimulation of the mucosa of the colons of

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**Fig. 3.** Enterochromaffin (EC) cells are more numerous in the inflamed colon. *A:* representative micrographs illustrating 5-HT immunoreactive EC cells (arrows) in sections of control and TNBS colons. Scale bar = 50 µm. *B:* number of 5-HT-immunoreactive EC cells per length of muscularis mucosa was increased over twofold in sections of colon from TNBS animals compared with controls. Normalization per length of muscularis mucosa was possible because the total circumferential length of the muscularis mucosa per section in the inflamed colon was not different than saline-treated controls (see EC cells are more numerous in the inflamed colon). *$P < 0.05$* compared with controls; *t*-test.

**Fig. 4.** The glands in the TNBS colon were significantly longer than control (*A*), and contained significantly more epithelial cells (*B*). Although there was an increase in the total number of epithelial cells, the proportion of goblet cells was unchanged in colon samples from inflamed animals compared with control (*C*). In contrast, the proportion of total epithelial cells that contained 5-HT was significantly increased in the inflamed colon (*D*). *$P < 0.05$* compared with controls; *t*-test.
and D fluoxetine did not significantly affect the amounts of 5-HT detected after mucosal stimulation of the control colon. When the activity of SERT was inhibited pharmacologically, therefore, the release of 5-HT from stimulated control tissue was comparable to that released from stimulated inflamed tissue. This is consistent with the possibility that SERT may not be as effective in the inflamed as in the control colon.

**Decreased expression of the 5-HT transporter in colitis.** Because the findings of the 5-HT release experiments were consistent with the possibility of a SERT defect in inflamed tissue, we tested the hypothesis that SERT is downregulated in colitis. To determine whether the expression of SERT was altered in the inflamed colon, real-time RT-PCR was used to quantify mRNA encoding SERT in colons from guinea pigs treated with TNBS and in those of control animals. Transcripts encoding SERT were normalized to those encoding β-actin, a nonregulated protein. The ratio of mRNA encoding SERT to that of β-actin was significantly lower in the inflamed than in the control colon. TNBS-induced colitis (0.11 ± 0.04 fg SERT/ pg β-actin, n = 4) was associated with a 10-fold decrease relative to control (1.1 ± 0.7 fg SERT/ pg β-actin, n = 5; P < 0.05, Mann-Whitney U-test) in the SERT/ β-actin ratio of mRNA.

SERT protein was evaluated immunocytochemically to verify that changes in transcription were also reflected in expression of SERT protein. Compared with that of control tissue (n = 6), SERT immunoreactivity was decreased in the mucosa of the colons of guinea pigs treated with TNBS (n = 5); however, SERT immunoreactivity in the myenteric plexus was comparable in the colons of TNBS-treated and control guinea pigs (Fig. 6). The immunocytochemical data are consistent with the data obtained by quantitative RT-PCR and indicate that the mucosa of the inflamed colon expresses less SERT than does that of the control colon.

**TNBS colitis slows colonic propulsion.** Collectively, the data described above suggest that colitis is associated with increased 5-HT levels and 5-HT release, and diminished 5-HT reuptake. The effect of colitis on fecal pellet propulsion was tested with the idea that elevated 5-HT levels in the lamina propria should lead to
altered motility because fecal pellet propulsion is activated by the release of 5-HT from EC cells (13).

The rate of propulsion of artificial fecal pellets in isolated segments of the distal colon was used as a measure of colonic motility. The colons of TNBS-treated animals propelled pellets at significantly slower rates than did the isolated colons of naïve animals (Fig. 7A; \( P < 0.05 \), two-way ANOVA for repeated measures, Bonferroni’s multiple comparisons test). Previous studies (14, 18) have demonstrated that the rate of propulsion of pellets in the isolated guinea pig distal colon is dependent on the activation of multiple 5-HT receptors. Kadokawa et al. (18) used SDZ-205–557 at a concentration of 10 \( \mu \)M, which antagonizes both 5-HT\(_3\) and 5-HT\(_4\) receptors. In the present study, a variety of concentrations of SDZ-205–557 were used to determine whether serotonergic signaling via 5-HT\(_3\) and/or 5-HT\(_4\) receptors is altered in the inflamed colon. In colons from control animals, SDZ-205–557 was found to reduce the rate at which pellets were propelled in a concentration-dependent manner (Fig. 7A).

The concentration of SDZ-205–557 needed to half-maximally inhibit propulsion (IC\(_{50}\)) was 4 ± 1 \( \mu \)M (n = 5). SDZ-205–557 was significantly less potent in inhibiting propulsion of pellets in colonic segments from TNBS-treated animals than it was in inhibiting propulsion in control animals (IC\(_{50}\): 16 ± 5 \( \mu \)M; n = 5; \( P < 0.05 \), t-test).

Statistical analysis of the SDZ-205–557 inhibition curves revealed a significant interaction between the effect of animal treatment and the effect of different SDZ-205–557 concentrations (\( P < 0.05 \), two-way ANOVA for repeated measures); therefore, the raw data were normalized to percentage of basal propulsion rate (Fig. 7B). This analysis revealed that, in addition to a lower potency of SDZ-205–557 in TNBS animals, lower concentrations of SDZ-205–557 (0.1 and 0.3 \( \mu \)M) actually enhanced the basal rate of pellet propulsion in the isolated colons (\( P < 0.05 \), two-way ANOVA for repeated measures, Newman-Keuls multiple comparisons test).

Decreased potency of the 5-HT\(_{3/4}\) receptor antagonist is consistent with the data described above indicating that more 5-HT is available to receptors in the inflamed colon. It seemed counterintuitive that an increased availability of 5-HT, the transmitter involved in initiating peristalsis, would be the cause of the decreased rate of colonic motility detected during chronic inflammation. We thus tested the hypothesis that increasing the amount of free 5-HT available to receptors can lead directly to a decrease in the rate of propulsion of fecal pellets in the isolated colon. Exogenously applied 5-HT caused the propulsion rate to decrease significantly (1.0 \( \mu \)M: 0.9 ± 0.1 mm/s, n = 3; 10 \( \mu \)M: 0.9 ± 0.2 mm/s, n = 4) compared with basal controls (2.0 ± 0.2 mm/s, n = 4; \( P < 0.05 \), t-test). Similarly, inhibition of SERT by fluoxetine (1 \( \mu \)M) (8), which effectively increases the availability to receptors of free endogenous 5-HT, significantly reduced the colonic propulsion rate (1.1 ± 0.2 mm/s, n = 4) compared with basal controls (2.3 ± 0.2 mm/s, n = 4; \( P < 0.05 \), t-test). These findings of the effects of fluoxetine and exogenous 5-HT on the propulsion of artificial pellets in the isolated guinea pig colon are consistent with prior observations (35).

**DISCUSSION**

The present investigation was conducted to test the hypothesis that the availability of 5-HT to its receptors is altered in the mucosa of the inflamed colon. In these studies, TNBS was used to induce colitis in the guinea pig distal colon. Although the concentration of TNBS and ethanol was less than those used to induce inflammation in the rat (23) or mouse (24), the response of the guinea pig colon to TNBS (in extent and duration of inflammation) was comparable to those of the other rodents. TNBS-induced colonic inflammation in the guinea pig is thus as viable and reproducible a model of inflammatory bowel disease (IBD) as it is in other species.

Using the guinea pig TNBS model of colitis, we made the novel observation that TNBS-induced colitis is associated with an increase in the bioavailability of 5-HT from mucosal epithelial cells. EC cell hyperplasia occurs during inflammation and is likely to be the major contributing factor to the increase in the content of 5-HT in the inflamed colon, which was also docu-

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**Fig. 7.** Colonic propulsion and 5-HT receptor antagonism were significantly reduced in the inflamed colon. A: colons of TNBS animals had significantly slower pellet propulsion rates (\( P < 0.05 \), two-way ANOVA for repeated measures, Bonferroni’s multiple comparisons test). The dual 5-HT\(_{3/4}\) receptor antagonist, SDZ-205–557, reduced pellet propulsion rates in colons from both groups in a concentration-dependent manner (IC\(_{50}\) values: control, 4 ± 1 \( \mu \)M, n = 5; TNBS, 16 ± 5 \( \mu \)M, n = 5; \( P < 0.05 \), t-test). B: normalization of data to percentage of basal propulsion rate revealed an increase in the propulsion rates of colons from TNBS animals at low concentrations of SDZ-205–557 (at 0.1 and 0.3 \( \mu \)M SDZ-205–557, \( P < 0.05 \), two-way ANOVA for repeated measures, Bonferroni’s multiple comparisons test).
mented. The supposition that the inflammation-induced increase in the number of EC cells is responsible for the increase in the content of 5-HT was supported by the observation that a parallel twofold increase was found in each. These data are consistent with findings from a rat model of colitis indicating increased 5-HT content and EC cell numbers (26). In the present study, the rise in the store of EC cell 5-HT was complemented by an increase in 5-HT release from the inflamed colon and by an evident decrease in the amount of SERT in the mucosa of the inflamed colon. The amount of mRNA encoding SERT and SERT-immunoreactive protein was found to be reduced in the inflamed colon. Collectively, these data suggest that more 5-HT is available to be released in the inflamed colon and that which is released should remain in the extracellular space longer, because the uptake mechanism responsible for the inactivation of 5-HT is compromised. Responses in the mucosa of the inflamed colon that are 5-HT mediated would thus be expected to be potentiated relative to similar responses in the normal colonic mucosa. There is a limit, however, as to how much potentiation is possible in terms of physiological responses. Receptors desensitize when they are stimulated with concentrations of agonists that are excessive in amount and/or duration of exposure. Receptor desensitization, therefore, is also to be anticipated in the inflamed colon in which 5-HT receptors are likely to be exposed to higher-than-normal concentrations of 5-HT for greater-than-normal periods of time.

A decreased capacity of the mucosal epithelium to take up released 5-HT due to the inflammation-associated reduction in SERT expression, as well as the hyperplasia of EC cells, may contribute to the evident increase in the release of 5-HT from the inflamed colon. Fluoxetine, which is a selective SERT inhibitor, increased the recoverable amount of 5-HT in the media bathing stimulated segments of control colon to levels comparable to those found in media bathing stimulated segments of inflamed colon. The release of 5-HT from the inflamed colon, unlike that of the control, was unaffected by the presence or absence of SERT. The inability of fluoxetine to affect the measured release of 5-HT from either resting or stimulated segments of inflamed colon could be explained by the reduced expression of SERT in the inflamed colon. Fluoxetine can only demonstrate an effect of SERT inhibition on tissue that contains enough SERT to make that inhibition manifest.

Previous studies (25, 30) have demonstrated diminished contractile activity in IBD. In the present study, the rate of propulsion of pellets in the colon was significantly reduced by the presence of inflammation. This result was at first surprising, because the combination of an increased store and release of 5-HT with a reduced ability of the inflamed colon to inactivate it by uptake was expected to potentiate the action of 5-HT. Because 5-HT initiates the peristaltic reflex, potentiation of its action was expected to speed propulsion. That logic, however, assumes that receptors do not become desensitized by their excessive exposure to 5-HT in the inflamed colon. Nevertheless, the 5-HT receptors involved in the initiation of the peristaltic reflex appear to desensitize readily and do so in vitro when SERT is inhibited and in vivo when SERT is lacking. In vitro, fluoxetine at low concentrations has previously been found to enhance the propulsion of pellets by the isolated distal colon but to decrease propulsion at higher concentrations when SERT inhibition becomes complete (35, present study). When 5-HT receptors are desensitized and the peristaltic reflex is impaired, the gut still responds to electrical stimulation, acetylcholine, and other agonists, illustrating the specificity of desensitization induced by SERT inhibition for 5-HT. In vivo, transgenic mice that lack SERT display an alternating pattern of diarrhea and constipation (7). The diarrhea is evidently due to the potentiation of 5-HT associated with the lack of SERT, whereas the constipation occurs because of transient episodes of receptor desensitization. The inflammation-associated slowing of colonic motility is thus likely to be caused by the overloading of 5-HT receptors with consequent desensitization due to the decreased expression of SERT in the 5-HT-enriched inflamed bowel. This hypothesis is supported by the observation that the rate of propulsion of pellets by the isolated colon is decreased by exogenous 5-HT and by inhibiting 5-HT reuptake (35, present study).

Increased availability of 5-HT to its receptors can also explain the decreased ability of SDZ-205–557 to inhibit propulsion in the inflamed colon. The decrease in potency is to be expected for a competitive antagonist facing increased competition from an endogenous agonist. The inflammation-induced increase in 5-HT and simultaneous decrease in SERT expression would both tend to increase the amount of endogenous 5-HT available to compete with and decrease the efficacy of SDZ-205–557. The observed increase in content, basal release, and stimulated release of 5-HT supports the idea that it is an increase in competition from endogenous 5-HT that accounts for the insensitivity of propulsion in the inflamed colon to inhibition by SDZ-205–557. Whether or not inflammation causes additional changes to occur in the affinity and/or expression of 5-HT receptors has not been examined in the present study and thus requires subsequent examination.

The enhanced propulsion caused by exposing the isolated colon to relatively low concentrations of SDZ-205–557 is an interesting observation that deserves further examination. It is likely that increased basal release of 5-HT in the inflamed bowel would desensitize 5-HT receptors on enteric neurons and contribute to stasis by decreasing the efficacy of receptors necessary for normal transmission. If so, SDZ-205–557 could release the receptors from the desensitized state, rendering them available to be activated when exposed to 5-HT.

Changes in the EC cell population and in 5-HT content have been reported in association with IBD, and there has been some inconsistency in the findings of these studies. Some reports indicate that 5-HT is increased in IBD, because EC cells are increased in
IBD when measured as cells per colonic gland (3) or by measuring the area of 5-HT immunoreactivity in the mucosal layer (11). Other studies have reported a decrease in 5-HT in IBD when measured as cells per colonic gland (1) or measured as 5-HT content per wet weight of tissue (21). Whereas, in the present study, a decrease in 5-HT content per wet weight of tissue was detected in the inflamed colon, the 5-HT content was significantly increased as a function of length. The decrease in 5-HT per wet weight of tissue in colitis detected in this study and in human IBD (21) may be a result of an increased mass of the inflamed tissue due to edema and lymphocytic infiltration. Measuring 5-HT content as a function of length may be more indicative of the changes that occur in colitis, because there is an increase in the EC cell population in the inflamed colon and the magnitude of this change was comparable to the increase in 5-HT per length of colon.

Taken together, the available data suggest that the increase in 5-HT that is detected in colitis may involve EC cell hyperplasia. Normal turnover of mucosal epithelium in the alimentary canal occurs rapidly via the coordination of proliferation, differentiation, migration, and apoptosis of epithelial cells (for review see Ref. 17). Enteroctyes, goblet cells, and enteroendocrine cells of the colon, all derive from a common stem cell (15). Differentiation as enteroctyes appears to be the default cell fate, because secretory cells (i.e., goblet cells and enteroendocrine cells) are positively selected by a mechanism dependent on the Math1 transcription factor (36). Several types of enteroendocrine cells are ultimately formed, including the 5-HT-containing EC cells. The determination of enteroendocrine cell fate involves a complex cascade of differentiation, where final fate determinations can occur from several distinct precursor lines (28). In the present study, inflammation was associated with an increase in the proportion of EC cells, whereas the proportion of goblet cells was unchanged; therefore, it is possible that the expression of the EC cell phenotype is selectively promoted during the restoration of the epithelial lining, either by the natural process of repair or by the presence of inflammatory mediators during inflammation. Many factors outside the scope of the present study, including growth factors and neuropeptides, may contribute to this process (for review see Ref. 16).

In conclusion, our findings support the idea that altered 5-HT signaling may contribute to the dysmotility associated with IBD. If the TNBS model mimics IBD, then the inflammation-induced changes observed in the guinea pig bowel may also be anticipated to occur in the inflamed human bowel. These changes, including an increase in the number of EC cells, a corresponding increase in the availability and release of 5-HT, and an accompanying decrease in the ability to terminate the action of 5-HT due to reduced SERT expression, all would tend to potentiate the effects of 5-HT on its receptors. The result of this potentiation could be to enhance or inhibit motility, depending on whether or not the degree of 5-HT potentiation is sufficient to desensitize receptors. The state of receptors can change, allowing abnormal motility patterns to alternate as they do in mice that lack SERT (7). 5-HT transmission, therefore, may represent a useful target for pharmacological manipulation to restore normal motor patterns in IBD.

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