Enteroendocrine cells and 5-HT availability are altered in mucosa of guinea pigs with TNBS ileitis

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1Gastrointestinal, Neuroscience, and Mucosal Inflammation Research Groups, Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada T2N 4N1; and 2Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, Vermont 05405

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O’Hara, Jennifer R., Winnie Ho, David R. Linden, Gary M. Mawe, and Keith A. Sharkey. Enteroendocrine cells and 5-HT availability are altered in mucosa of guinea pigs with TNBS ileitis. Am J Physiol Gastrointest Liver Physiol 287: G998–G1007, 2004.—Enteroendocrine cells act as sensory transducers, releasing 5-HT and numerous peptides that are involved in regulating motility, secretion, and gut sensation. The action of mucosal 5-HT is terminated by a 5-HT reuptake transporter (SERT). In this study, we examined the hypothesis that ileitis leads to changes in enteroendocrine cell populations and mucosal 5-HT availability. Ileitis was induced in guinea pigs by intraluminal injection of 2,4,6-trinitrobenzenesulfonic acid and experiments were conducted 3, 7, and 14 days after treatment. The number of somatostatin, neurotensin, and 5-HT-immunoreactive cells increased at 3 and 7 days of ileitis, respectively, whereas no significant changes in the numbers of cholecystokinin, glucagon-like peptide-2, glucose-dependent insulinotropic peptide, and peptide YY-immunoreactive cells were observed. Chemical stimulation of the inflamed mucosa with sodium deoxycholic acid significantly increased 5-HT release compared with basal release. Mechanical stimulation of the mucosa potentiated the effect of the chemical stimuli at day 7. Enteroendocrine cell numbers increased significantly during the time course of inflammation. Thus changes in enteroendocrine cell and 5-HT availability could contribute to the altered motility and secretion associated with intestinal inflammation by disrupting mucosal signaling to enteric nerves involved in peristaltic and secretory reflexes.

inflammatory bowel disease; sensory transduction; motility; secretion; neurotensin; somatostatin

INTESTINAL MOTILITY AND SECRETION are initiated by luminal factors that activate intrinsic and extrinsic primary afferent nerves involved in peristaltic and secretory reflexes (21, 22). Enteroendocrine cells function as mucosal transducers to initiate reflex responses to mechanical and chemical stimulation of the mucosa. These cells transfer information regarding the contents of the lumen to nerve fibers lying in close proximity to the basolateral surface of the epithelium (21).

Bülbring and colleagues (6, 7) first proposed that a subset of enteroendocrine cells, known as enterochromaffin (EC) cells, release 5-HT in response to increases in intraluminal pressure. The 5-HT released from EC cells can stimulate both intrinsic and extrinsic primary afferent (sensory) neurons, via at least three different 5-HT receptors, 5-HT3, 5-HT4 and 5-HT1P (2, 28, 29, 46, 62). Activation of the 5-HT receptors is thought to depolarize afferent nerve terminals, which can lead to a variety of responses including the initiation of peristalsis, secretion, and gut sensation (2, 29, 46, 62). The actions of 5-HT are terminated by reuptake into epithelial cells via the high affinity 5-HT selective reuptake transporter (SERT) (12, 59). The epithelial SERT is identical to that found in the brain and enteric nervous system (32).

In addition to the 5-HT-containing EC cells, there are at least 14 other subpopulations of enteroendocrine cells (55, 56). The secretory products synthesized and released from the different subsets of enteroendocrine cells act as paracrine and/or endocrine mediators that modulate gastrointestinal function. In particular, neurotensin (N cells), somatostatin (Som; D cells), cholecystokinin (CCK; I cells), peptide tyrosine tyrosine (PYY; L cells), glucagon-like peptide-2 (GLP-2; L cells), and glucose-dependent insulinotropic peptide (GIP; K cells) have been reported to be involved in gastrointestinal motility, secretion, and/or cell proliferation (30, 33, 37, 39, 44, 57, 60).

Inflammatory bowel disease (IBD), which includes Crohn’s disease and ulcerative colitis, is associated with altered motility, secretion, and gut sensation (1, 10, 14, 34, 58). Therefore, changes in enteroendocrine cell populations and/or their secretory products could contribute to the symptoms associated with IBD by altering the luminal signaling to extrinsic and intrinsic primary afferent nerves. Previous studies (31, 45) have demonstrated increased numbers of EC cells and 5-HT content in animal models of colitis. An increased number of EC cells has also been demonstrated in human Crohn’s ileitis (3). However, it is unknown whether the change in EC cell numbers during Crohn’s ileitis is associated with an increase in the availability, release, and/or reuptake of 5-HT. Furthermore, changes in other types of enteroendocrine cells have yet to be systematically quantified in animal models of ileitis.

Therefore, the aim of this study was to examine mucosal 5-HT availability in an experimental model of ileitis in the guinea pig. To determine whether mucosal 5-HT availability is altered in inflammation, we used a 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of ileitis and measured the number of 5-HT-immunoreactive EC cells, basal and stimulated release of 5-HT from EC cells, and mucosal 5-HT content. SERT immunoreactivity was examined to determine whether 5-HT reuptake and inactivation was affected by inflammation. We also quantified neurotensin, Som, CCK, PYY, GLP-2, and GIP-immunoreactive enteroendocrine cells in control and inflamed guinea pig ileum to examine the effect of inflammation on these enteroendocrine cell subpopulations. Our results suggest...
that there are significant changes to enteroendocrine cell populations in TNBS ileitis, and this is associated with altered mucosal 5-HT signaling in the inflamed ileum.

**MATERIALS AND METHODS**

*Animal preparations.* Male albino guinea pigs (Charles River, Montreal, Canada) weighing 200–300 g were housed in a temperature-controlled room. The animals were maintained on a normal 12:12-h light-dark cycle and were allowed access to food and water ad libitum. All methods used in this study were approved by the University of Calgary Animal Care Council and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

To induce inflammation in the ileum, fasted guinea pigs were anesthetized with halothane (induced at 4%, maintained on 2.5–3% in oxygen). A midline laparotomy was performed and the distal ileum was identified and exteriorized. TNBS (0.5 ml; Sigma-Aldrich; 30 mg/ml in 30% EtOH) was then injected into the lumen of the ileum ~5 cm from the ileocecal junction using a 30-gauge syringe. The ileum was replaced into the abdominal cavity and the incision was sutured. Three different control groups were also examined: the first group, vehicle controls, were assessed by injecting 0.5 ml of 30% EtOH into the distal ileum; the second group of controls were similarly treated with the exception that 0.5 ml physiological saline (0.9% NaCl) was injected into the distal ileum; and the third group of animals remained naïve.

Animals were maintained in a controlled environment for 3, 7, or 14 days after surgery. At the time of tissue collection, animals were anesthetized with an overdose of pentobarbital sodium and exsanguinated. The distal ileum was then removed and used for experimental studies.

**Assessment of inflammation.** Inflammation induced by administration of TNBS into the lumen of the small intestine has previously been demonstrated as inducing a transmural inflammation analogous to human Crohn’s disease (43). The TNBS acts as a hapten, eliciting a Th1-mediated immune response characterized by the presence of proinflammatory cytokines that are also involved in the development of spontaneous Crohn’s disease (43, 54). The severity of ileitis was assessed by measuring changes in the weight of the animals and examining macroscopic damage to the mucosa. Animals were weighed before administration of TNBS, EtOH, or saline and daily after surgery. After animals were euthanized, the ileum was removed, opened along the mesenteric border, and examined macroscopically. The criteria used for scoring gross morphological damage have been described previously (38, 40, 41). Briefly, the total score of mucosal damage included the presence and severity of adhesions (score 0–2); the maximum thickness of the ileal wall (in mm); and the extent of inflammation, ulceration, and hyperemia (score 0–10).

**Immunohistochemistry.** Ileal segments to be used for immunohistochemistry were opened along the mesenteric border, stapled flat with mucosa side up, and fixed overnight at 4°C in Zamboni’s fixative (2% paraformaldehyde, 15% picric acid, pH 7.4). Samples were then transferred to 20% sucrose in PBS overnight at 4°C. Transverse and circumferential segments from each animal were embedded with the mucosa oriented in the same direction, in OCT compound (Miles, Elkhart, IN). Sections of ileum (12 µm) were cut on a cryostat, thaw-mounted onto poly-d-lysine (PDL)-coated slides, and stored at −20°C until use.

Changes in enteroendocrine cell populations and SERT expression were examined in sections of saline-treated control and inflamed ileum at 3, 7, and 14 days after the administration of saline or TNBS. EtOH-treated controls and naïve animals were examined at 3 and 7 days after injection of EtOH or fasting, respectively. Tissue sections were washed with PBS containing 0.1% Triton X-100 (3 × 10 min), followed by incubation in primary antiserum (Table 1) for 48 h at 4°C. The sections were washed again with PBS containing 0.1% Triton X-100 and incubated with secondary antibodies (Table 1) for 1 h at room temperature. Secondary antibodies were visualized with secondary antibodies (Table 1) for 1 h at room temperature. Secondary antibodies were visualized with fluorescence microscopy. Photographs were taken using a digital imaging system consisting of a digital camera (Sensys; Photometrics, Tucson, Arizona) and image analysis software (V for Windows; Digital Optics, Auckland, New Zealand). Photographs of SERT immunofluorescence were taken at the same exposure time and magnification (×40).

**Measurement of 5-HT content in the ileum.** A segment of fresh ileum (1 × 5 cm) was removed, and the mucosa was gently scraped off, collected, and weighed. The mucosal samples were homogenized in 0.2 M perchloric acid (10 µl/mg mucosa) and centrifuged at 10,000 g for 5 min at 4°C. The supernatant was filtered through a 0.22-µm filter, neutralized with equal volumes of 1 M borate buffer (pH 9.25) and centrifuged at 10,000 g for 1 min. The 5-HT content of an aliquot of each sample was analyzed with an enzyme immunoassay kit used according to the manufacturer’s instructions (Beckman Coulter, Fullerton, CA).

**Measurement of mucosal 5-HT release in the ileum.** The ileum was opened along the mesentery and cut into six segments (1 × 0.5 cm). The segments were pinned flat, mucosal side up, in a Sylgard-coated six-well dish containing 37°C HEPES solution (in mM: 110 NaCl; 5.4 KCl; 1.8 CaCl2; 2 H2O; 1 MgCl2·6 H2O; 60 sucrose; 5 glucose; 20 HEPES). After a 15-min incubation period, the bathing solution was replaced by 3 ml of normal HEPES solution or 3 ml of the bile acid (5 mM sodium deoxycholate) (47). To mechanically stimulate the mucosa, segments of ileum were gently stroked in a circumferential direction with a rounded glass probe (diameter ~2 mm) at a rate of 8 strokes/min for a total of 15 min (31). To represent the contents of the

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Som, somatostatin; GIP, glucose-independent insulinotropic peptide; GLP-2, glucagon-like peptide-2; PYY, peptide tyrosine tyrosine; SERT, serotonin reuptake transporter; CRB, Cambridge Research Biochemicals,Billingham Cleveland, UK; UBC, University of British Columbia, Vancouver, Canada; CURE, Center for Ulcer Research and Education.
small intestine, a combination of mechanical stimulation of the mucosa and bile acid was applied. The mucosal stimulation is analogous to the passage of the fluid contents through the lumen, whereas bile acids are absorbed as the contents move through the small intestine. Basal levels of 5-HT release were determined by leaving preparations undisturbed in the bathing solution for 15 min. The 5-HT released into the bathing solution was measured with an enzyme immunoassay kit used according to the manufacturer’s instructions (Beckman Coulter, Fullerton, CA).

**Data analysis.** The data are presented as means ± SE for n animals. Intensity of SERT immunofluorescence was measured by using the Scion Image program, and statistical comparisons were conducted with GraphPad Prism software (Version 3.03, GraphPad Software, San Diego, CA). Comparisons between three or more groups were done with a one-way ANOVA followed by a Dunnett’s multiple comparison test. Data with a nonparametric distribution were analyzed by a Kruskal-Wallis Test followed by a Dunnett’s multiple comparison test. P < 0.05 was considered statistically significant.

**RESULTS**

**TNBS-induced inflammation.** Administration of TNBS into the lumen of the ileum caused regional inflammation characterized by ulceration, adhesions, hyperemia, and changes to mucosal architecture that were similar to previously described animal models of TNBS-induced ileitis (38, 40, 41). Initially after surgery, TNBS-treated guinea pigs lost weight, but began to regain it after 1–2 days. In contrast, saline-treated control animals remained at a stable weight or gained weight after surgery (Fig. 1A). The macroscopic damage scores from saline-treated controls were not significantly different at 3, 7, and 14 days after injection of saline; therefore, the data at each time point were pooled. Macroscopic damage scores obtained at 3, 7, and 14 days after administration of TNBS demonstrated significant mucosal damage on day 3. At this time, muscle hypertrophy and destruction of the mucosa were evident. By the seventh day of inflammation, the extent of mucosal damage was reduced and villus structure was restored, indicating recovery was in progress; however, muscle thickening and minor adhesions were still evident (Fig. 1B; *P < 0.05 compared with saline-treated controls) (saline-treated controls, n = 12; EtOH-treated controls, n = 6; 3-day TNBS n = 6; 7-day TNBS, n = 6; 14-day TNBS, n = 6). At 14 days after injection of TNBS, macroscopic evaluation of the mucosa revealed no significant difference from control tissue.

Examination of tissue from the EtOH-treated animals demonstrated no significant difference in macroscopic appearance of the mucosa at 3 and 7 days after injection of EtOH and the data from each time point were pooled. Macroscopic damage scores of the EtOH-treated tissue revealed no difference in muscle thickness or mucosal architecture compared with saline-treated controls (Fig. 1B) or naive controls (data not shown).

**EC cell numbers.** 5-HT immunoreactive EC cells were quantified in sections of control and inflamed ileum. The mean number of EC cells distributed throughout 10 villus-encrypt units in each section was calculated (Fig. 2A). The number of EC cells increased over the first 7 days of inflammation. At 7 days of inflammation, the number of EC cells was significantly greater compared with saline-treated controls (Fig. 2B; *P = 0.01) (saline-treated controls, n = 12; 3-day TNBS, n = 6; 7-day TNBS, n = 6; 14-day TNBS, n = 6). To determine whether a general increase in epithelial cell numbers contributed to the increase in EC cell numbers, a hematoxylin and eosin stain was performed. The average number of epithelial cells per villus was not significantly different in the saline-treated control tissue compared with the TNBS-treated tissue (data not shown). Fourteen days after the administration of TNBS, the number of EC cells was not significantly different from the saline-treated controls. In addition, the number of 5-HT-immunoreactive EC cells in the EtOH-treated animals (n = 6) and naive controls (n = 3) was comparable to the number of cells in the saline-treated controls (data not shown).

**Mucosal 5-HT content.** An increase in the number of EC cells, the site of synthesis, and storage of 5-HT, could lead to an increase in the mucosal content of 5-HT. 5-HT content in control and inflamed guinea pig ileum was measured by enzyme immunoassay (Fig. 3A; saline-treated controls, n = 6; 3-day TNBS, n = 6; 7-day TNBS, n = 6). The 5-HT content of the ileum was expressed as a function of wet weight of mucosa, as well as a function of area (cm²) of ileum to account for the infiltration of inflammatory mediators. In both parameters, there was no significant difference in 5-HT content between the TNBS-treated tissues and control preparations.

**Fig. 1.** A: 2,4,6-trinitrobenzenesulfonic acid (TNBS)-treated animals initially lost weight after injection of TNBS but gained weight 1–2 days after surgery. In contrast, saline-treated animals remained at a stable weight after surgery. B: macroscopic damage scores were significantly greater at 3 and 7 days of inflammation compared with saline and EtOH-treated controls. *P < 0.05 compared with controls (means ± SE); Kruskal-Wallis test followed by a Dunnett’s multiple comparison test; saline-treated controls, n = 12; EtOH-treated controls, n = 6; 3-day TNBS n = 6; 7-day TNBS, n = 6; 14-day TNBS, n = 6.
To stimulate the release of 5-HT from EC cells. Under basal segments of ileum under basal and stimulated conditions. 5-HT, we measured 5-HT release from the mucosa in isolated EC cells was associated with an increase in the secretion of 5-HT release. To test whether the increase in the number of enterochromaffin (EC) cells in sections of control vs. 3-, 7-, and 14-day inflamed ileum. Scale bar = 50 μm. B: number of 5-HT-immunoreactive EC cells in saline-treated control tissue did not differ at 3, 7, and 14 days; therefore, the data from each time point are pooled. The number of EC cells per 10 villus-crypt units increased over the first 7 days of ileitis and returned to baseline levels by day 14. *P = 0.01 compared with control (means ± SE); one-way ANOVA followed by Dunnett’s multiple comparison test (saline-treated controls, n = 12; 3-day TNBS, n = 6; 7-day TNBS, n = 6; 14-day TNBS, n = 6).

5-HT release. To test whether the increase in the number of EC cells was associated with an increase in the secretion of 5-HT, we measured 5-HT release from the mucosa in isolated segments of ileum under basal and stimulated conditions. Sodium deoxycholic acid and mechanical stroking were used to stimulate the release of 5-HT from EC cells. Under basal conditions, the amount of 5-HT released did not differ between saline-treated control and inflamed tissue (Fig. 3; basal release: saline-treated control, n = 7; 3-day TNBS; n = 7; 7-day TNBS, n = 8). The release of 5-HT from saline-treated control tissue was significantly increased in response to chemical (n = 6) and mechanical stimuli (n = 7), as well as a combination of the two stimuli (n = 6). In tissues from TNBS-treated animals, 5-HT release at 3 days was significantly increased in response to the bile acid alone (n = 8), and in combination with the mechanical stimulus (n = 9) compared with basal conditions. 5-HT release in response to the combined stimuli was significantly greater in the 3-day ileum vs. the saline-treated control ileum (Fig. 3; P < 0.05). However, mechanical stimulation alone (n = 8) did not significantly alter 5-HT release. At 7 days after administration of TNBS, 5-HT release was significantly increased in response to bile acid (n = 6) compared with basal 5-HT release. Mechanical stimulation (n = 6) failed to elicit a response in the 7-day inflamed tissue compared with basal 5-HT release. However, the mechanical stimulus appeared to potentiate the effect of the bile acid (n = 6). 5-HT release in response to the combined stimuli was significantly greater in the 7-day inflamed tissue compared with the saline-treated controls (Fig. 3; P < 0.05).

5-HT reuptake transporter. The 5-HT reuptake transporter (SERT) removes 5-HT from the extracellular space by transporting 5-HT into epithelial cells; therefore, decreased reuptake of 5-HT may contribute to the increased 5-HT release observed. We examined the possibility that SERT is down-regulated in the inflamed ileum. To determine whether the expression of SERT was altered during inflammation, SERT protein was evaluated by immunohistochemistry. In the saline-treated control ileum, relatively intense SERT immunoreactivity was observed throughout the epithelial cell layer and in the myenteric plexus. Compared with that of control tissue, mucosal SERT immunoreactivity was reduced at 3 days of inflammation and was virtually absent by day 7 (Fig. 4A). At 14 days after injection of TNBS, SERT immunoreactivity in the mucosa had returned to control levels. SERT expression in the myenteric plexus was similar in the ileum of saline and TNBS-treated guinea pigs (Fig. 4B). The intensity of SERT immunoreactivity in the saline-treated control and inflamed ileum were quantitatively compared, and the mean intensity of immunofluorescence in the inflamed mucosa was significantly reduced compared with saline-treated control levels (Fig. 4C; P < 0.05).

Enterodocrine cell numbers. To determine whether the changes in enterodocrine cell numbers were limited to the 5-HT-containing EC cell type, several other subpopulations of enterodocrine cells in saline-treated control vs. inflamed guinea pig ileum were assessed by immunohistochemistry. The enterodocrine cells were quantified as described for the 5-HT-containing EC cells. The numbers of PYY-, GIP-, GLP-2-, and CCK-containing enterodocrine cells were not significantly different in the ileum of the saline-treated control vs. inflamed ileum. However, the numbers of neurotensin and Som-immunoreactive enterodocrine cells were significantly increased at 3 days of inflammation compared with saline-treated guinea pigs (Fig. 5; P < 0.05) (neurotensin: saline-treated controls, n = 11; 3-day TNBS, n = 4; 7-day TNBS, n = 4; 14-day TNBS, n = 4; Som: saline-treated controls, n = 12; 3-day TNBS, n = 6; 7-day TNBS, n = 6; 14-day TNBS, n = 5). On days 7 and 14, the number of neurotensin and Som-containing cells were not significantly different from control values.
DISCUSSION

The aim of this investigation was to examine enteroendocrine cell populations and determine whether mucosal 5-HT availability is altered in the inflamed small intestine. Data from this study demonstrate that TNBS-induced ileitis is associated with an increase in the number of 5-HT-immunoreactive EC cells, a decrease in mucosal SERT immunoreactivity, and an increase in mucosal 5-HT release. We have also shown that the epithelial changes are not limited to the 5-HT-immunoreactive enteric cell type, because changes in the number of neurotensin-immunoreactive N cells and Som-immunoreactive D cells during inflammation were also demonstrated. However, no significant changes to the GIP-, GLP-2-, CCK- and PYY-immunoreactive enteroendocrine cell populations were observed.

The majority of the body’s 5-HT is localized to secretory granules of EC cells (23), and previous studies (20, 31, 45) have demonstrated that the number of 5-HT-immunoreactive EC cells is increased in human and animal models of the inflamed large intestine. Our data from the small intestine are similar to the TNBS-induced colitis model in that we observed an increased number of EC cells in TNBS-induced ileitis. The EC cell hyperplasia observed in previous studies (31, 45) was associated with an increase in mucosal 5-HT content. In the TNBS model of colitis, Linden et al. (31) demonstrated a decrease in 5-HT content per unit wet weight of tissue. However, 5-HT content increased when expressed as a function of unit length of colon. In contrast, Magro et al. (35) demonstrated a decrease in tissue levels of 5-HT in both ulcerative colitis and Crohn’s colitis patients. In the present study, no significant change in 5-HT content was observed in the inflamed small intestine.

Previous studies (5) examining the colonic mucosa of patients with irritable bowel syndrome (IBS) have demonstrated...
an increased number of 5-HT-immunoreactive EC cells vs. controls, which was apparently associated with a lower colonic mucosal 5-HT content in the IBS patients. Thus an increased number of EC cells may not directly correlate with an increase in mucosal 5-HT content. This could be due to a number of factors including decreased synthesis of 5-HT due to alterations in tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT. This is supported in a study by Coates et al. (13), demonstrating reduced 5-HT content that correlated with significantly lower levels of tryptophan hydroxylase in the colonic mucosa of patients with ulcerative colitis and IBS, whereas EC cell numbers were decreased only in patients with severe ulcerative colitis. The incorporation of sites of inflammation and ulceration in the tissue samples assessed could also contribute to the lower 5-HT content observed in the present study. The areas of inflammation often consisted of mucosal damage with destruction of villi, particularly at 3 days of inflammation. Consequently, lower mucosal 5-HT content may be observed.

Regional differences in the intestinal segment being examined could also contribute to any variation observed in the TNBS model of ileitis vs. the TNBS model of colitis. It appears that the time course and severity of the inflammatory response to TNBS in the ileum differs from that in the colon (36, 42, 43). One possible explanation is the variation in the bacterial flora throughout the gastrointestinal tract. The small intestine is a relatively sterile environment compared with the extensive bacterial load in the colon (50) and this may influence the time course and severity of inflammation. Furthermore, a recent study (17) demonstrated the expression of toll-like receptors (TLRs), involved in the recognition of bacterial components on murine and human enteroendocrine cell lines. The same group also reported the expression of TLR1, TLR2, and TLR4 by 5-HT-immunoreactive EC cells in primary human intestinal tissue sections. Further studies examining the effect of bacteria and on the 5-HT signaling system may determine whether the intestinal flora has a role in the inconsistencies between the ileum and colon.

EC cells, acting as mucosal transducers, release 5-HT in response to various luminal stimuli including distortion of the mucosa, bile acids, nutrients, and toxins (24, 46, 47, 51). Therefore, EC cell hyperplasia during inflammation could lead to enhanced release of 5-HT in response to luminal stimuli. This is supported by the observation that the maximum increase in both EC cell numbers and stimulated 5-HT release was at 7 days after injection of TNBS. At the 7-day time point, mechanical stimulation of the mucosa appeared to potentiate the effect of the bile acid. The amount of 5-HT released from the inflamed tissue was significantly greater than the amount released from the saline-treated control tissue in response to the same stimuli. In contrast to the 7-day inflamed tissue, potentiation was not observed at 3 days of inflammation. It is not yet known why this is the case; however, we speculate that the
degree of inflammation at 3 days precludes the maximum stimulatory effect that is observed at 7 days. This may be due to the extensive infiltration of inflammatory mediators at the more acute stage of inflammation.

Interestingly, release of 5-HT in response to mechanical stimulation of the inflamed mucosa was not significantly different from the saline-treated controls, whereas the mechanical stimulation significantly increased 5-HT release compared with basal release in the control ileum. It should be emphasized that the release of 5-HT from EC cells involves a complex mechanism of regulation. EC cells appear to be endowed with a number of different receptors including stimulatory \( \beta \)-adrenoceptors, cholinergic and 5-HT\(_3\) receptors, as well as inhibitory purinoreceptors, \( \alpha_2 \)-adrenoceptors, GABA, histamine H\(_3\), and 5-HT\(_2\) receptors (48, 49). Mechanical stimulation of the mucosa may stimulate extrinsic and intrinsic primary afferent nerves that contain sensory elements activated by stretch or mechanical forces (15, 52). Modulation of neurotransmitter release during inflammation could contribute to the changes in 5-HT release from EC cells in response to mechanical stimulation of the mucosa. Furthermore, mechanical stimulation can release additional mediators from epithelial cells and EC cells that have a paracrine or autocrine regulation of 5-HT release (15). For instance, stroking of the mucosa stimulates the release of ATP from epithelial and EC cells that can subsequently stimulate purinoreceptors located on the EC cells (15). It is possible that intestinal inflammation alters the mechanically stimulated release of other mediators, such as ATP, subsequently leading to aberrant 5-HT release.

An additional factor that could contribute to the observed increase in 5-HT release during inflammation is a decreased reuptake of 5-HT by SERT. Enzymes known to metabolize 5-HT include monoamine oxidase and glucuronyl transferase, both of which are located intracellularly (4). At physiological \( \text{pH} \), 5-HT is highly charged and is unable to readily cross the plasma membrane (12). Therefore, in order for the actions of 5-HT to be terminated, it must be transported into the cells that possess the catabolic enzymes. A decreased ability of cells to take up 5-HT could lead to an increased concentration of 5-HT in the extracellular space and prolonged exposure to the 5-HT receptors located on primary afferent nerves. Our results indicate that reuptake of 5-HT may be decreased during inflammation as SERT immunoreactivity was virtually absent by day 7 of inflammation. The observation that SERT expression is downregulated during inflammation is consistent with previous studies in a guinea pig TNBS model of colitis (31), human ulcerative colitis (13), and IBS (13).

Collectively, these observations suggest that during ileitis there is an increased availability of 5-HT to primary afferent nerves that are located adjacent to the basolateral membrane of the epithelium. The increased availability is most likely due to EC cell hyperplasia and decreased reuptake of 5-HT, leading to increased 5-HT release into the extracellular space. At this time, the physiological role of 5-HT in TNBS-induced ileitis is
unknown. However, 5-HT has been demonstrated to activate intrinsic and extrinsic primary afferent nerves involved in the peristaltic and secretory reflexes (2, 25–27, 29, 46). Therefore, the increased release of 5-HT could enhance activation of these primary afferent nerves, thereby contributing to increased motility and/or secretion during inflammation. Conversely, excessive amounts of 5-HT could lead to receptor desensitization and a reduced activation of the primary afferent terminals leading to decreased motility and/or secretion. This concept is supported in the study by Chen et al. (11) who demonstrated alternating periods of diarrhea and constipation in SERT knockout mice. Therefore, depending on the state of the receptor, the increased availability of 5-HT during inflammation could contribute to the altered motility and secretion associated with IBD. Furthermore, Martinolle et al. (36) examined longitudinal and circular muscle contractile responses to serotonergic receptor stimulation in guinea pigs with TNBS-induced ileitis. In the circular muscle preparations from the inflamed animals, the contractile response to exogenously applied 5-HT was significantly reduced compared with controls. They suggest that the hyporesponsiveness of the circular muscle layer could be due to nerve and/or muscle receptor desensitization that is, in part, caused by the excessive release of inflammatory mediators.

Whereas 5-HT has been emerging as an important mediator in IBD, other enteroendocrine cells and their respective secretory products have also been demonstrated to undergo changes during inflammation. In the colon of patients with IBD, as well as in a Schistosoma mansoni model of inflammation, a reduced number of Som-containing D cells has been demonstrated (18, 61). In addition, there is evidence that neuropeptide content in colonic inflammation is increased and the neuropeptide antagonist, SR-48,692, inhibits toxin A-induced colitis (9). This has led to the hypothesis that neuropeptide is a proinflammatory peptide in colonic inflammation (9). Mice with a nonfunctional T-cell receptor spontaneously develop inflammation of the colon, and these mice demonstrate a decreased expression of neuropeptide, cholecystokinin, and 5-HT-containing cells (53). Furthermore, in a DSS-induced model of mouse colitis, GLP-2 content in the colon was reduced by 50% compared with control tissue (19).

In the present study, we demonstrated an increase in the number of neuropeptide-immunoreactive N cells and Som-immunoreactive D cells at day 3 of inflammation, whereas the maximal increase in 5-HT-immunoreactive EC cells occurred at 7 days of inflammation. There were no significant changes observed in the GIP, GLP-2, CCK, or PYY-immunoreactive enteroendocrine cells at any time point of inflammation. Taken together, these observations indicate that the changes in enteroendocrine cell populations are not due to a general increase or decrease in total epithelial cell number; instead, it suggests that there is a complex regulation of enteroendocrine cell development and differentiation, and this process may be altered during inflammation.

The functional implication of the increased numbers of N and D cells are unknown; however, previous studies (9) have demonstrated elevated levels of neuropeptide during toxin A-induced intestinal inflammation. The increased levels of neuropeptide preceded the toxin A-induced changes in secretion and mucosal permeability (9). Therefore, the increased number of N cells could lead to increased levels of the proinflammatory peptide, thereby contributing to the altered secretion and mucosal permeability associated with inflammation. In contrast, Som is an inhibitory anti-inflammatory peptide that depresses the inflammatory response to various stimuli (8). The altered number of D cells may be a compensatory mechanism that leads to increased mucosal Som levels in an attempt to limit the extent of the inflammatory response elicited by the increased levels of neuropeptide.

In conclusion, data from the present study indicate that the cellular structures responsible for the synthesis, storage, and secretion of 5-HT, neuropeptide, and Som are altered during ileitis. Given the role of enteroendocrine cells in mucosal sensory transduction (6, 7, 22), the inflammation-induced alterations in the number of these cells could lead to aberrant mucosal signaling to extrinsic and intrinsic primary afferent nerves during inflammation, thereby contributing to the altered motility and secretion that is associated with IBD. Therefore, it is possible that 5-HT, Som, and/or neuropeptide signaling pathways could provide useful therapeutic targets for IBD. However, further examination of their role in the inflammatory process is required.

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