Analysis of real-time serotonin (5-HT) availability during experimental colitis in mouse

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Bertrand PP, Barajas-Espinosa A, Neshat S, Bertrand RL, Lomax AE. Analysis of real-time serotonin (5-HT) availability during experimental colitis in mouse. Am J Physiol Gastrointest Liver Physiol 298: G446–G455, 2010. First published December 17, 2009; doi:10.1152/ajpgi.00318.2009.—Serotonin (5-HT)-containing enterochromaffin (EC) cells of the intestine transduce chemical and mechanical stimuli from the intestinal lumen by releasing 5-HT on to afferent nerve terminals. Dysfunctional mucus 5-HT signaling has been implicated in heightened visceral sensitivity and altered motility in patients with inflammatory bowel disease and in animal models. Our aim was to characterize the release and uptake of 5-HT in the mouse dextran sulfate sodium (DSS; 5% wt/vol) model of colitis. We made electrochemical recordings and used an ELISA assay to determine mucus 5-HT release and uptake in untreated mice and mice with DSS-induced colitis. Peak and steady-state 5-HT concentrations were measured before and during blockade of the serotonin reuptake transporter (SERT) with 1 μM fluoxetine. Electrochemical recordings showed that colons from DSS-treated mice had roughly twice the steady-state levels of extracellular 5-HT and compression-evoked 5-HT release compared with untreated mice. Fluoxetine doubled the compression-evoked and steady-state 5-HT levels in control and DSS mice. These data were supported by ELISA assays, which showed enhanced 5-HT release during colitis, by immunohistochemical analyses, which showed increases in EC cell numbers, and by real-time PCR, which identified a decrease in SERT mRNA expression in the mucosa during colitis. These data are the first to demonstrate 5-HT release close to its release site and near its site of action during DSS-colitis. We conclude that DSS-colitis increases 5-HT availability primarily by an increase in the numbers of EC cells and/or of content of 5-HT in these EC cells.

Inflammatory bowel diseases (IBD) such as Crohn’s disease and ulcerative colitis (UC) are characterized by inflammatory damage of gastrointestinal (GI) tissue, leading to symptoms of altered motility, pain, and discomfort (36, 41). Several recent studies have suggested a role for dysfunctional enteric neural reflexes and visceral sensation in symptom generation during IBD (7, 10, 18, 21, 22, 30, 38). Equally important is the role of the serotonin (5-HT)-containing enterochromaffin (EC) cells, which function as sensory transduction elements in the GI mucosa (5, 12), responding to chemical and mechanical stimuli by releasing 5-HT onto afferent nerve terminals to initiate GI reflexes and modulate visceral perception (14). As such, these cells are ideally placed to contribute to neuronal dysfunction and many of the symptoms of IBD. This has led to a great deal of interest in the effects of inflammation on EC cells (16, 22, 25, 35).

Acute GI inflammation can cause profound changes in the content and release of 5-HT from EC cells and the subsequent reuptake of 5-HT into the surrounding epithelial tissues. In early studies of patients with UC, the numbers of EC cells decreased (e.g., 37), whereas, in later studies, the numbers increased (e.g., 11). More recently, Coates et al. (9) have found that the number of EC cells decreased in patients with severe UC but found no change in mild UC. In a mouse model of trinitrobenzene sulfonic acid (TNBS)-induced colitis, Linden et al. (20) showed that 5-HT availability was enhanced because of a decrease in serotonin reuptake transporter (SERT)-dependent 5-HT uptake, with no change in EC cell numbers. SERT expression was also reduced in studies using TNBS models of guinea pig colitis and ileitis, but this was coupled with an increase in EC cell numbers (19, 31). TNBS induces predominantly a T helper-1 (Th1) cytokine-driven colitis thought to resemble Crohn’s disease. No studies have examined the dextran sulfate sodium (DSS) model of mouse colitis, which is considered less severe and is a mixed Th1/Th2 cytokine-mediated colitis (29, 39). Recent electrochemical studies in healthy mucosa have shown that it is possible to determine 5-HT release kinetics (2, 3) and SERT-dependent 5-HT uptake (6). Therefore, the aim of the present study was to determine the real-time availability of 5-HT in a mouse model of DSS-induced colitis.

Materials and Methods

Ethical Approval and Preparation

Experimental protocols were approved by the Queen’s University Animal Care Committee and conformed to the Guidelines of the Canadian Council of Animal Care. Male CD-1 (outbred) mice weighing 25–35 g were obtained from Charles River Laboratories (Montreal, QC, Canada). Colitis was induced by the addition of 5% wt/vol DSS to the drinking water for 5 days (from day zero), followed by normal drinking water for 2–3 days (36, 39); control mice received normal drinking water. During and after DSS treatment, animals were monitored for signs of pain, altered feeding habits, or weight loss, but none were observed. Mice were euthanized by isoflurane overdose followed by cervical transection and exsanguination. The severity of inflammation in the proximal colon was confirmed by myeloperoxidase assay as previously described (26).

Distal colons from control mice or mice with colitis were removed and placed in physiological saline (Krebs: in mM: 126 NaCl, 2.5 NaH2PO4, 1.2 MgCl2, 2.5 CaCl2, 5 KCl, 25 NaHCO3, and 11 glucose) that was bubbled with 95% O2-5% CO2. The colon was opened along 15 mm wide by 25 mm in length) was placed in a small-volume (3 ml) recording chamber lined with a silicone elastomer (Sylgard 184; Dow Corning, Midland, Ontario, Canada) and placed in a small-volume (3 ml) recording chamber lined with a silicone elastomer (Sylgard 184; Dow Corning, Midland, Ontario, Canada).
The preparation used. Preparations of colon were visualized at \( \times 20 \) magnification on a dissecting microscope. Carbon fiber electrodes were placed above or touching the mucosa (Fig. 1A) using a mechanical micromanipulator (MP-1; Narishige Scientific Instruments, Tokyo, Japan).

**Electrochemistry**

Electrochemical recordings were made in amperometry mode as described in detail previously (2, 3, 6). In brief, a 7-\( \mu \)m carbon fiber was insulated with a glass micropipette (Clark Electromedical Instruments, Kent, UK) with \( \sim 200 \) \( \mu \)m left exposed for recordings. This exposed fiber was coated with Nafion (Sigma-Aldrich, MO) to repel the metabolite 5-hydroxyindoleacetic acid. Each electrode was calibrated, and stability over time was checked with 2–10 \( \mu \)M 5-HT (Fig. 2A). 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 (Fig. 1). In this instance, the carbon fiber acts as a very fine von Frey hair and bends with a consistent amount of force. For example, a von Frey hair of 10 mg of breaking force is the minimum used to activate extrinsic colonic afferents (24). 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 concentrations of 5-HT were recorded following decay of the signal to a flat baseline or 15 s had been reached (Fig. 2B). Concentrations of 5-HT were calculated using individual electrode calibrations. The time to 50% (\( t_{50} \)) and 80% (\( t_{80} \)) decay to SS were also calculated. Cyclic voltammetry techniques were used to verify the identity of 5-HT release from the mucosa by the voltage at which it oxidizes (Fig. 2C). Current recordings were made using a VA-10 amplifier (NPI Electronics, Tamm, Germany) with a Ag/AgCl ground, digitized at 20 kHz (Digidata 1440), recorded on a personal computer, and filtered with a 60-Hz notch filter and a five-time substitution average using PClamp 10 (MDS Analytical Technologies, Mississauga, ON, Canada), and then analyzed with Origin 7.5 (MicroCal, Northampton, MA).

**ELISA, RT-PCR, and EC Cell Counts**

**ELISA for 5-HT.** Tissue samples were taken from animals that had also been used for the electrochemical experiments. Distal colon was divided into 4 \( \times 0.5 \) cm\(^2 \) segments and pinned mucosa up in Sylgard-lined wells of a six-well plate. Preparations were incubated at 37°C in oxygenated Krebs for 15 min and the solutions changed to 3 ml of either normal HEPES-buffered saline (HBS; in mM: 110 NaCl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 60 sucrose, 5 glucose, 20 HEPES) or HBS with fluoxetine (1 \( \mu \)M). Tissues were equilibrated for 15 min, and then a 1-ml aliquot was taken and frozen at \(-20^\circ\)C for ELISA. Following three washes in normal HBS or HBS plus fluoxetine, tissues were incubated for a further 15 min while we mechanically stimulated the mucosa with a plastic rod (0.3-cm tip diameter) at a frequency of 8 strokes/min. One-milliliter aliquots of bath solution were again taken and frozen, and the wet weight of the tissue was recorded. ELISAs were performed for detection of 5-HT in accordance with the manufacturer’s instructions (Beckman Coulter, Fullerton, CA), and the concentration of 5-HT was normalized to the tissue wet weight (19).

**Quantitative real-time RT-PCR analysis of SERT expression.** Quantitative real-time RT-PCR was performed on RNA extracted from colonic mucosal scrapings (\( \sim 100 \) mg of starting tissue). RNA was extracted from control mice and mice with DSS-colitis using the TRIzol method (Invitrogen, Carlsbad, CA). RNA was then repurified using LiCl Precipitation Solution (cat. no. 9480; Ambion, Austin, TX). cDNA was reverse transcribed from 5 \( \mu \)g total RNA using Superscript III Reverse Transcriptase (Invitrogen, Burlington, Northampton, MA) and amplified using a Power SYBR Green RNA-to-cDNA master mix (Invitrogen, Carlsbad, CA). Primers for SERT were designed with PrimerBlast (NCBI) and were as follows: sense primer: 5’-ATGCTGGAATGCTGGGATGG-3’; antisense primer: 5’-TACGCTGATTCTACGCTGATT-3’. qRT-PCR was performed using a StepOne Plus Real-Time PCR System (ThermoFisher Scientific). Expression was normalized to 18S expression and the samples were analyzed using the Delta Delta Ct method (20).

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The target (SERT) to reference (villin; an epithelial brush-border protein; Ref. 13) ratio was calculated using Realquant software (Roche, Laval, QC, Canada). The following primers were used: Villin (138 bp amplicon; NM_009509) forward: GCTTCTTCGATGGTGACTGCT, reverse: TGTGTGGTGTAGATGGCAGC; SERT (136 bp amplicon; NM_010484) forward: GGAACATCTGGCGTTTTCC, reverse: ATTTCCGGTGTTACTGGCCCA.

Immunohistochemistry. Immunohistochemical analyses were made as described in detail previously (e.g., 23). Briefly, segments of distal colon were pinned loosely on balsa wood and immersed in fixative (4% paraformaldehyde, 3% sucrose in 0.1 M PBS) for 3.5 h at 4°C. Tissue was rinsed four times at room temperature with PBS and immersed into cold 30% sucrose overnight at 4°C then embedded in optimal cutting temperature (OCT) compound (Miles, Elkhart, IN). Sections (10 μm) were cut on a cryostat, thaw mounted onto glass slides (Fisherbrand Superfrost plus), and stored at −80°C. Before immunolabeling, slides were thawed, and excess OCT compound was washed off. Tissues were incubated at room temperature in a humid chamber for an hour in 4% normal horse serum diluted in 0.1 M PBS with 0.5% Triton X-100 and subsequently incubated in the first primary antiserum diluted in 0.1 M PBS (rabbit anti-5-HT antiserum; 1:5,000; Immunostar, Hudson, WI). The following day, slides were washed and incubated in goat anti-rabbit IgG AlexaFluor 488 (1 h, room temperature, 1:500, Invitrogen) and then washed in PBS and incubated overnight at 4°C in goat anti-c-Kit (1:200; Santa Cruz Biotechnology, Santa Cruz, CA). Finally, tissues were washed and labeled with donkey anti-goat IgG AlexaFluor 555 (1 h, room temperature, 1:200, Invitrogen). This secondary antibody was washed off and tissue covered with buffered glycerol and coverslipped.

Immunoreactivity was analyzed with an epifluorescence microscope (Olympus BX51). Only cells that were within the epithelial border and that were c-Kit negative and 5-HT positive were counted as EC cells. The number of 5-HT immunoreactive EC cells in each of four nonadjacent fields of view were quantified per colon sample, as well as the number of crypts in each, and from that the average number of EC cells per crypt was calculated.

Statistics

Population data are presented as means ± SE. The n value refers to the number of animals studied. A Student’s t-test was used to make single comparisons and was paired or unpaired as noted. Multiple comparisons were done using an ANOVA followed by a post hoc t-test using the Bonferroni-Holm’s step-down test to judge significance of individual comparisons (15). The Holm’s method is stepwise and sequentially rejective, which corrects for errors that occur with repeated t-tests. A Wilcoxon signed-rank test was used for nonparametric data (e.g., percent of control). A value of P ≤ 0.05 was taken as the cut off for statistical significance.

RESULTS

Characterization of 5-HT Release and Uptake from Untreated Mouse Colon

SS and compression-evoked release of 5-HT. EC cells release 5-HT at rest, and this has been used as one measure of 5-HT availability. In untreated mouse colon, we assessed the extracellular 5-HT levels, which represent the ongoing release of 5-HT from the EC cells (termed SS levels) using electrochemical amperometry techniques. The 5-HT oxidation current at SS was measured from multiple sites on the mucosa surface (Fig. 1B) and was found to be consistent within a single preparation and between preparations; thus bias was minimized when choosing recording sites. Using 5-HT calibration data specific to each electrode, we calculated that the mean SS
current detected was equivalent to 1.8 ± 0.5 μM 5-HT (n = 9; Fig. 2B).
EC cells are known to release 5-HT during mechanical stimulation (e.g., 3, 8), and this can also be taken as a measure of 5-HT availability. As established previously in guinea pig ileum (3), the amount of compression-evoked 5-HT released from the untreated mouse colon was determined by using the recording electrode to compress the mucosal epithelium while simultaneously measuring the 5-HT oxidation current. This mucosal compression-evoked release of 5-HT was calculated to be a mean of 4.0 ± 0.7 μM (n = 9; Fig. 2B). The compression-evoked 5-HT oxidation current peaked shortly after the electrode contacted the mucosa (time to peak: 1.5 ± 0.4 s; n = 9) and decayed back to SS levels within 15 s. Overall, the mean peak level of compression-evoked release of 5-HT was higher than the mean SS level (peak: 4.0 ± 0.7 μM; SS: 1.8 ± 0.5 μM; n = 9; P = 0.006; ANOVA with post hoc paired t-test using Holm’s; Fig. 3). These peak and SS measurements were paired, and, when the peak value was taken as a percentage of the SS value from individual experiments, the average peak levels were 267% of SS levels (n = 9; P = 0.005; Wilcoxon signed-rank test used for all percentages).

**SERT-dependent uptake of 5-HT from untreated tissues.** 5-HT availability in the GI tract is determined by release from EC cells and uptake via SERT, which is located on both basal and apical aspects of the epithelial cells (e.g. 13). The difference between 5-HT levels measured before and during SERT blockade with a maximally effective concentration of the selective SERT inhibitor fluoxetine (1 μM) (40) was taken as an estimate of SERT function. We measured the peak and SS 5-HT oxidation current during compression of the mucosa before (Fig. 3A) and during (Fig. 3B) SERT inhibition. During SERT blockade, the peak compression-evoked level of 5-HT was higher than the SS level (peak: 8.0 ± 1.6 μM; SS: 2.9 ± 0.4 μM; n = 9; P = 0.003; ANOVA with post hoc paired t-test using Holm’s), as occurred in control recordings. More importantly, in the presence of fluoxetine, there was a significant increase in the peak amount of 5-HT detected with compression, when measured as both mean concentration (control: 4.0 ± 0.7 μM; fluoxetine: 8.0 ± 1.6 μM; n = 9; P < 0.05; ANOVA with post hoc paired t-test using Holm’s) and when...
individual experiments were expressed as a percent of control (mean: 235%, P < 0.05; Fig. 3C). Fluoxetine also significantly increased the SS levels of 5-HT detected (control: 1.8 ± 0.5 μM; fluoxetine: 2.9 ± 0.4 μM; n = 9; P < 0.05; ANOVA with post hoc paired t-test using Holm’s; Fig. 3C) to 205% of control (n = 9; P = 0.006). Thus the effect of fluoxetine in the uninflamed mouse distal colon was to double the levels of extracellular 5-HT detected at SS and during peak compression-evoked release.

Changes to the Availability of 5-HT in DSS-Colitis

Mice that were exposed to DSS in their drinking water exhibited well-characterized symptoms of colitis, including an increase in proximal colon myeloperoxidase activity (control: 0.97 ± 0.27 U/mg tissue, n = 9; DSS: 5.6 ± 1.7 U/mg tissue, n = 11; P = 0.02, unpaired t-test). In the distal colon, there was a dramatic increase in the levels of extracellular 5-HT detected with carbon fiber electrodes from DSS-treated animals (Fig. 4, A and B). Peak compression-evoked 5-HT release was significantly increased in DSS-treated mice (9.5 ± 1.4 μM; n = 11) compared with untreated mice (4.0 ± 0.7 μM; n = 9; P = 0.001; ANOVA with post hoc paired t-test using Holm’s). SS levels of 5-HT were also significantly increased in DSS-treated mice (control: 1.8 ± 0.5 μM, n = 9; DSS: 3.1 ± 0.5 μM, n = 11; P = 0.037; ANOVA with post hoc t-test using Holm’s; Fig. 4C). As with untreated mice, peak 5-HT levels in DSS-treated mice were always greater than SS levels (peak: 9.5 ± 1.4 μM; SS: 3.1 ± 0.5 μM; n = 11; P = 0.001; ANOVA with post hoc paired t-test using Holm’s). When the variability of the measurements was compared between treatments, it was found that the average SE was not significantly different between any group tested (ANOVA with post hoc paired t-test using Holm’s). Overall, the concentrations of 5-HT detected in DSS-treated mice were twice that detected in untreated mice.

SERT-dependent uptake of 5-HT from inflamed tissues. In DSS-treated animals, fluoxetine significantly increased the peak compression-evoked 5-HT detected from 9.5 ± 1.4 μM to 20.8 ± 2.1 μM (n = 11; P = 0.001; ANOVA with post hoc paired t-test using Holm’s). This was also a significant increase when measurements during fluoxetine were taken as a percent of the paired control levels (mean: 284%; n = 11; P = 0.002; Fig. 4C). Fluoxetine also caused a statistically signifi-
cant increase in SS 5-HT levels in DSS-treated mice when measured as a mean 5-HT concentration (DSS alone: 3.1 ± 0.5 μM; DSS + fluoxetine: 6.6 ± 0.9 μM; n = 11; P = 0.004; ANOVA with post hoc paired t-test using Holm’s) or as percent of control (318%; n = 11; P = 0.002). Peak 5-HT levels (20.8 ± 2.1 μM) were always greater than SS levels (6.6 ± 0.9 μM; n = 11; P = 0.001; ANOVA with post hoc paired t-test using Holm’s). Overall, the proportion of fluoxetine-sensitive uptake of 5-HT was the same in DSS-treated mice compared with untreated mice.

We hypothesized that SERT activity would contribute to the kinetics of the compression-evoked 5-HT response. In untreated mice, compression-evoked 5-HT release decayed to SS levels during the approximate 15-s contact of the electrode with the mucosa. On average, the time to 50% decay (t50) was 2.4 ± 0.3 s, and 80% decay (t80) was 5.1 ± 0.6 s; addition of fluoxetine did not change this (t50: 2.0 ± 0.3 s, P = 0.21; t80: 4.6 ± 0.6 s, P = 0.31; n = 9; ANOVA with post hoc paired t-test using Holm’s). In DSS-treated mice the t50 was 2.3 ± 0.2 s, and t80 was 5.3 ± 0.4 s, which were not significantly different compared with untreated mice (P = 0.27; 0.12). However, in the presence of fluoxetine, the t80 in DSS-treated mice increased to 6.8 ± 0.7 s, which was significantly increased compared with DSS-treated mice without fluoxetine (P = 0.027; ANOVA with post hoc paired t-test using Holm’s), untreated mice without fluoxetine (P = 0.04; ANOVA with post hoc r-test using Holm’s), and untreated mice plus fluoxetine (P = 0.01; ANOVA with post hoc t-test using Holm’s). These data suggest that diffusion of released 5-HT away from the electrode and into the bulk solution, rather than SERT-dependent mucosal uptake, was the primary determinant of 5-HT decay kinetics. However, the contribution of SERT may be increased in the inflamed colon.

**ELISA Measurement of Mucosal 5-HT Availability**

Our electrochemical measurements provide real-time data about levels of extracellular 5-HT from multiple discreet regions of the mucosa. To determine the average release of 5-HT from entire preparations over a period of 15 min, we turned to an ELISA-based assay. On average, the colons from untreated mice released 0.16 ± 0.04 pM of 5-HT per gram of tissue (g.t) into the medium (n = 5) in the absence of mechanical stimulation of the mucosa. These resting levels of 5-HT are qualitatively similar to the SS levels described above for the electrochemistry experiments. The levels of 5-HT released were significantly increased (1.04 ± 0.37 pM/g.t; n = 6; P = 0.023; ANOVA with post hoc paired t-test using Holm’s) by mechanical stimulation of the mucosal surface with a plastic rod (Fig. 5A). Following addition of fluoxetine to the medium, there was no significant increase in the unstimulated levels of 5-HT (0.34 ± 0.11 pM/g.t; n = 6; P = 0.064) or the stimulated levels (1.89 ± 0.55 pM/g.t; n = 6; P = 0.096) compared with control. When comparing unstimulated and stimulated data in the presence of fluoxetine, there was a significant increase in 5-HT released into the medium during stimulation (from 0.34

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**Fig. 5.** ELISA measurements of 5-HT release and SERT function in untreated and DSS-treated mice. Bar graphs showing control (open) and fluoxetine (1 μM; shaded)-treated preparations. A: untreated mice (n = 6) showed significantly higher stimulated 5-HT levels than unstimulated levels with or without fluoxetine present (*P < 0.05; paired t-test with Holm’s). Fluoxetine had no significant effect on 5-HT release in mechanically stimulated or unstimulated preparations. B: in DSS-treated mice (n = 5), mechanical stimulation increased the levels of 5-HT release in the absence of fluoxetine but not in its presence. Fluoxetine significantly increased stimulated 5-HT release but not unstimulated 5-HT release.
to 1.89 pM/g.t; \( P = 0.007 \); ANOVA with post hoc paired \( t \)-test using Holm's).

In DSS-treated mice (Fig. 5B), unstimulated tissues released 1.61 ± 0.47 pM/g.t of 5-HT \((n = 5)\). As in untreated mice, mechanical stimulation caused a significant increase in 5-HT released into the medium \((5.07 \pm 1.58\) pM/g.t; 400% of control; \( n = 5; P = 0.02 \); ANOVA with post hoc paired \( t \)-test using Holm’s). Fluoxetine did not change the amount of 5-HT released from unstimulated preparations \((1.61 \pm 0.32\) pM/g.t; \( n = 5; P = 0.42 \)) or from stimulated preparations \((5.07 \pm 2.09\) pM/g.t; \( n = 5; P = 0.26 \)). In contrast, mechanical stimulation in the presence of fluoxetine caused a significant increase in 5-HT release from in DSS-treated mice \((1.50 \pm 0.32 \) to 6.64 pM/g.t; \( n = 5; P = 0.01; \) ANOVA with post hoc paired \( t \)-test using Holm’s). Compared with untreated mice, all measures of 5-HT release, as measured by ELISA and electrochemistry, were increased in DSS-treated mice.

**EC Cell Numbers**

To examine the cause of the increased levels of 5-HT detected electrochemically and by ELISA in DSS-treated mice, we double labeled untreated and DSS-treated colon against 5-HT and c-Kit using immunohistochemical techniques (Fig. 6A). Counts were made of 5-HT immunoreactive EC cells, which were not c-Kit positive (differentiating them from mast cells) and quantified per mucosal crypt. DSS-induced colitis resulted in a significant increase in the number of EC cells/crypt [untreated: 0.84 ± 0.11 \((n = 5)\); DSS: 1.60 ± 0.35 \((n = 6)\); \( P = 0.013 \); unpaired \( t \)-test].

**SERT mRNA**

Our electrochemical studies of 5-HT release suggested that the fluoxetine-sensitive uptake of 5-HT was unchanged by DSS-treatment. However, previous studies of TNBS-induced...
colitis and ileitis in guinea-pigs reported a decrease in SERT activity and immunoreactivity during inflammation. To further examine this, we used quantitative real-time RT-PCR to determine the levels of SERT mRNA expression in colonic mucosa (Fig. 6B). In contrast to our functional assays of SERT activity, the mucosal levels of SERT mRNA, normalized to villin mRNA, were reduced in DSS-treated mice on day 7 of DSS-colitis compared with untreated mice (P < 0.05; n = 6 each, unpaired t-test). A similar decrease in mucosal SERT mRNA expression was also detected when expression was normalized to GAPDH (data not shown).

**DISCUSSION**

The main finding of this study was that mucosal 5-HT availability was increased in a mouse model of DSS-induced colitis. The levels of 5-HT were determined locally using real-time electrochemical techniques and assessed over whole preparations using an ELISA assay, both of which showed that 5-HT availability was increased by inflammation. These data were supported by increased counts of EC cells, in agreement with previous studies of animal models of IBD (e.g., 19, 32). The function of SERT appeared preserved in DSS-treated colons as measured electrochemically, but was this was associated with a decrease in SERT mRNA. This is the first study to measure 5-HT concentrations close to its release site and near its site of action from inflamed tissue and demonstrates that 5-HT levels are increased during DSS-colitis.

**Inflammation Increases 5-HT Levels**

In the present study, the levels of extracellular 5-HT near the mucosa epithelium were assessed electrochemically. We made two distinct measures of 5-HT availability, active release and SS. Active release was due to compression of the epithelium with the electrode and yielded the peak 5-HT level. Following this, the actions of SERT and diffusion lowered the concentration of 5-HT to a SS level. The same SS level could also be measured without compression when the electrode was brought into gentle contact with the mucosa. We found that the peak levels of 5-HT released from untreated colon were 4 μM in response to compression, whereas SS levels were about 2 μM. Interestingly, these levels are lower than found in the mouse ileum (compression-evoked −10 μM; SS −6 μM) (4) or in the ileum of other species (2, 6) (for review see Ref. 5). Despite relatively low 5-HT levels in untreated tissues, electrochemical measurements revealed a clear increase in the levels of extracellular 5-HT from inflamed colon. The levels of compression-evoked 5-HT release were doubled, as were SS levels found at the mucosal surface. When tissues from the same animals were analyzed using an ELISA assay for 5-HT, a similar story emerged. 5-HT levels were increased in both stimulated (peak) and unstimulated (SS) conditions during colitis.

Changes to the mucosal surface are seen during DSS-colitis (27), and this could cause an accumulation of 5-HT. The increase in 5-HT levels detected in this study represents both a functional increase (compression evoked 5-HT release) and an increase in the levels of 5-HT “trapped” at the mucosal surface. Such an accumulation would be reflected in raised SS concentrations like those we detected with our electrochemical techniques, but it would not affect the peak of compression evoked 5-HT release. One intriguing possibility is that 5-HT at the mucosal surface could reflect raised concentrations of 5-HT in the lamina propria. We speculate that a higher concentration in the lamina propria could cause the concentration at the surface to increase because of increased mucosal permeability seen during DSS-colitis (17).

Changes to the mucosal surface are also suggested by our electrochemical measurements of 5-HT decay kinetics. In the rat ileum, we have previously shown that the decay of the 5-HT signal is a reliable means of inferring SERT activity (6); however, in the present study, we found in the untreated mouse colon that decay was mainly attributable to diffusion of 5-HT away from the electrode. This suggests that the simpler mucosal morphology of the colon may limit the actions of SERT at the mucosa surface. SERT-dependent uptake became more important during inflammation, which supports the idea that the mucosal architecture is changed during inflammation.

A strength of the present study was the use of two independent techniques to measure 5-HT availability. In our hands, both techniques showed a clear increase in 5-HT availability in inflamed tissues. Interestingly, we noted that there was a good correlation between the control and fluoxetine data from the two techniques in paired preparations from untreated animals but not in preparations from DSS-treated animals (data not shown). There were also some qualitative differences in the data gathered. The first and most obvious difference was that the electrochemical recordings (made close to the 5-HT release site) reported higher 5-HT concentrations than did ELISA (made after 5-HT release and dilution in the medium). The electrochemical recordings also had a higher temporal resolution; measurements were made within a few seconds, whereas each ELISA measurement reflected the release and reuptake of 5-HT over several minutes of incubation. As a result, our electrochemical recordings were able to reveal a further effect of SERT on 5-HT release kinetics not seen in the ELISA data. Overall, the two analyses yielded complementary data and showed that 5-HT levels were increased locally and globally across the whole preparation.

**How Does 5-HT Availability Increase in DSS-colitis?**

The extracellular levels of 5-HT are increased by the release of 5-HT from EC cells and reduced by the reuptake of 5-HT by SERT. Changes to either release or uptake could potentially affect the levels of 5-HT measured in the present study. Inflammation has been associated with an increase in the number of EC cells in some animal models (19, 32, 33) and in patients with UC (11), especially when EC cells are expressed vs. the number of crypts. However, some studies have found no changes in the numbers of EC cells in inflamed animal tissue (e.g., 20) or have found a reduction in EC cell numbers in patients with severe UC (e.g., 9). In the present study, we estimated the numbers of EC cells by immunohistochemical localization of 5-HT. We found an increase in the number of EC cells per crypt from inflamed colon compared with untreated tissues. This increase could be due to a greater proliferation of EC cells (32) or, alternatively, a higher content of 5-HT might make the EC cells easier to detect. In either case, this would be a major contributor to the increased 5-HT availability seen during inflammation in the present study.

A reduction in SERT activity could also account for the increased 5-HT levels seen here. Previous studies looking at
animal models of colitis (19, 20) and patients with UC (9) have shown decreases in SERT expression. In agreement with these previous studies, our quantitative RT-PCR data showed a decreased SERT expression. In contrast, our electrochemical data showed very similar effects of fluoxetine in untreated mice and during colitis, suggesting that the functions of SERT are maintained despite a decrease in mRNA expression. In the present study, we estimated SERT function by measuring levels of 5-HT before and during blockade of SERT with fluoxetine. In untreated tissues, blockade of SERT caused the peak and SS 5-HT levels to double, similar to the doubling in levels seen in inflamed tissues. Furthermore, addition of fluoxetine to inflamed tissues revealed an effect on the kinetics of the compression-evoked 5-HT release that was not evident in untreated tissues. We also examined the effects of fluoxetine using the ELISA assay but did not detect a significant difference between the SS levels of 5-HT in untreated and inflamed tissue. For stimulated release, we detected SERT activity, but the levels were the same for both untreated and inflamed tissues, a finding that was mirrored by our electrochemical data. Our ELISA data is similar to what has been seen in other studies such as Linden et al. (19, 20), who found that fluoxetine in the incubation medium did not increase 5-HT levels from untreated or inflamed tissues.

Together, these data suggest that SERT function may not have been impaired at day 7 following DSS-colitis despite a reduction in SERT mRNA at this time. Western blot analysis of SERT protein was attempted but was inconclusive because the three antibodies tested proved not to be reliable in mouse colon. Overall, the simplest explanation is that the increase in the numbers of EC cells and/or of content of 5-HT in these EC cells is the reason for the increase in peak and SS levels of 5-HT seen. We cannot exclude the possibility that SERT function was compromised, and, if so, this would also contribute to the increase in 5-HT availability.

**Potential Physiological Significance**

The present study is the first to accurately measure the absolute concentrations of 5-HT near the inflamed mucosa and, by extension, near the afferent nerve terminals that control GI function and sensation (16). The increased level of 5-HT is important because it may substantially alter the activation or desensitization of 5-HT receptors on afferent nerve terminals or on the EC cells themselves. For example, the peak concentrations of 5-HT measured from untreated tissues were ~4 μM, high enough to activate the low-affinity 5-HT3 receptor (34). These levels are almost doubled in inflamed tissues, suggesting that 5-HT3 receptors might become desensitized or that a larger population of 5-HT3 receptors further from the site of release might become activated. This latter scenario is consistent with data showing hypersensitivity in animal models of inflammation (16) and with clinical data (1, 28).

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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