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Activation of Colonic Mucosal 5-HT$_4$ Receptors Accelerates Propulsive Motility and Inhibits Visceral Hypersensitivity

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Abstract

BACKGROUND & AIMS—5-hydroxytryptamine receptor (5-HT$_4$R) agonists promote gastrointestinal motility and attenuate visceral pain, but concerns about adverse reactions have restricted their availability. We tested the hypotheses that 5-HT$_4$ receptors are expressed in the colonic epithelium and that 5-HT$_4$R agonists can act intraluminally to increase motility and reduce visceral hypersensitivity.

METHODS—Mucosal expression of the 5-HT$_4$R was evaluated by reverse-transcriptase polymerase chain reaction and immunohistochemical analysis of tissues from 5-HT$_4$R(BAC)-enhanced green fluorescent protein mice. Amperometry, histology, and short-circuit current measurements were used to study 5-HT, mucus, and Cl^- secretion, respectively. Propulsive motility was measured in guinea pig distal colon, and visceromotor responses were recorded in a rat model of colonic hypersensitivity. 5-HT$_4$R compounds included cisapride, tegaserod, naronapride, SB204070, and GR113808.

RESULTS—Mucosal 5-HT$_4$ receptors were present in the small and large intestines. In the distal colon, 5-HT$_4$ receptors were expressed by most epithelial cells, including enterochromaffin and goblet cells. Stimulation of 5-HT$_4$Rs evoked mucosal 5-HT release, goblet cell degranulation, and Cl^- secretion. Luminal administration of 5-HT$_4$R agonists accelerated propulsive motility; a 5-HT$_4$R antagonist blocked this effect. Bath application of 5-HT$_4$R agonists did not affect motility. Oral or intracolonic administration of 5-HT$_4$R agonists attenuated visceral hypersensitivity.

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Conflicts of interest The authors disclose no conflicts.

Intracolonic administration was more potent than oral administration, and was inhibited by a 5-HT_4R antagonist.

CONCLUSIONS—Mucosal 5-HT_4 receptor activation can mediate the prokinetic and antinociceptive actions of 5-HT_4R agonists. Colon-targeted, intraluminal delivery of 5-HT_4R agonists might be used to promote motility and alleviate visceral pain, while restricting systemic bioavailability and resulting adverse side effects.

Keywords

Constipation; ATI-7505; Peristaltic Reflex; Cavitation

5-hydroxytryptamine (5-HT, serotonin) is an important gastrointestinal (GI) signaling molecule involved in motor, secretory, and sensory functions.[1,2] These actions are mediated by a large family of serotonin receptors located within the neural circuitry and on a variety of other cell types in the gut.[3] Of the 5-HT receptors expressed in the intestines, the 5-HT_4 receptor (5-HT_4R) has been one of the most widely studied in regards to GI function, and 5-HT_4R agonists have been developed for the treatment of constipation and visceral pain.[4] Despite clinical effectiveness, the 5-HT_4R agonists tegaserod and cisapride were removed from the market because of concerns related to the possibility of adverse cardiovascular effects.[4]

The 5-HT_4R is a G-protein–coupled receptor that promotes activation of the adenylate cyclase/cyclic adenosine monophosphate (cAMP)/protein kinase A pathway, and can affect various cellular functions including facilitation of neurotransmitter release.[3] Stimulation of presynaptic 5-HT_4Rs on myenteric cholinergic nerve terminals enhances fast excitatory synaptic inputs to neurons and increases neurogenic muscle contractions in the intestines.[5,6] As a result, presynaptic facilitation within the peristaltic reflex circuitry is thought to be responsible for the prokinetic actions of 5-HT_4R agonists. It also is possible that 5-HT_4R agonists act via a mucosal site of action. Luminal application of 5-HT_4R agonists promotes propulsive motility and enhances the ascending contractile and descending relaxatory limbs of the peristaltic reflex.[7,8] However, the existence and distribution of 5-HT_4Rs in the mucosal layer of the intestines have not been investigated directly.

The aim of this investigation was to explore the hypothesis that 5-HT_4Rs are expressed in the colonic mucosa and their activation promotes motility and/or alleviates visceral pain. 5-HT_4Rs have been identified in the GI tracts of a number of species, including human beings. In these studies, we used assays that have been validated previously in mouse, rat, and guinea pig to evaluate functional responses to 5-HT_4R activation. The expression pattern of 5-HT_4Rs in the GI epithelium was determined by quantitative reverse-transcription polymerase chain reaction (RT-PCR) and by evaluation of green fluorescent protein (GFP) immunoreactivity in 5-HT_4R(BAC)-enhanced GFP (eGFP) transgenic mice. Epithelial responses were evaluated by measuring 5-HT_4R agonist–induced 5-HT release, mucus secretion, and ion transport. Colonic propulsive motility was measured in response to luminal vs serosal administration of 5-HT_4R agonists. Finally, we evaluated the effects of luminal administration of 5-HT_4R agonists on the visceromotor response (VMR) to colorectal distension (CRD) in a colonic hypersensitivity model. Our findings indicate that 5-HT_4Rs are expressed in the epithelial layer of the colon, and suggest that targeted activation of these receptors has prokinetic and antinociceptive effects.

Materials and Methods

See Supplementary Materials and Methods section for details of immunohistochemistry protocols, strains and sources of animals used, and physiological solution recipes and reagents.

Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committees of the University of Vermont, Oklahoma City VA Medical Center and University of Oklahoma Health Sciences Center, and the University of Calgary Animal Care Committee. In all cases, animals were euthanized by isoflurane and exsanguination or cervical dislocation.

Human Biopsies

Human tissue biopsy specimens were obtained from patients of the Division of Gastroenterology and Hepatology using protocols approved by the University of Vermont Institutional Review Board. Individuals provided informed consent before their scheduled screening procedures. Mucosal samples were obtained using standard biopsy forceps. Samples were immediately placed in RNA stabilization solution (RNA*later*; Ambion, Austin, TX).

RT-PCR

RNA was extracted from human biopsies and murine full-thickness preparations or mucosal scrapings using the RNeasy Mini Kit (Qiagen, Valencia, CA) and complementary DNA (cDNA) was generated by reverse-transcriptase reaction (Promega, Madison, WI). An Applied Biosystems 7500 Fast Realtime PCR System was used with Fast Universal PCR Master Mix and validated TaqMan Gene Expression Assays for human 5-HT_4R (Hs00410577_m1), mouse 5-HT_4R (Mm00434129_m1), human HPRT1 (Hs99999909_m1), and mouse HPRT1 (Mm00446968_m1) (Applied Biosystems, Foster City, CA). Resulting data were calculated using the standard curve method and the level of 5-HT_4R expression was normalized to HPRT1. HPRT1 expression was consistent across the regions studied. To ensure that mucosal samples did not contain neuronal cell bodies, a subset of human samples were immunostained for anti-human neuronal protein HuC/D and neuron-specific enolase, and no neurons were observed. Furthermore, HuC/D transcript was not detected in cDNA from mouse mucosal samples.

Immunohistochemistry/BAC Transgenic Mice

Tissue samples from 5-HT_4(BAC)-eGFP mice with a Swiss Webster (SW) strain genetic background (kindly provided by Eric Schmidt and Nathaniel Heintz, Rockefeller University) were fixed with 4% paraformaldehyde, paraffin-embedded, and sectioned at 10 μm. The eGFP signal was amplified by GFP immunostaining, which yielded similar, but more intense, fluorescence than emitted by eGFP alone. Sections were examined on an Olympus AX70 fluorescence microscope (Olympus America, Inc, Melville, NY), and some sections were double-stained for 5-HT or mucin 2. Images of microscopic fields were captured with an Optronics MagnaFire digital camera (Optronics, Goleta, CA) using identical exposure settings.

Amperometry

Boron-doped diamond microelectrodes were used for continuous amperometric recordings.[9,10] The holding potential for the electrode was set at 700–750 mV with an Axoclamp-2B amplifier (Axon Instruments, Union City, CA). This potential was determined

previously to oxidize 5-HT at a mass transfer limited rate. Electrical signals were acquired using a MiniDigi 1A (Axon Instruments) interfaced with pClamp software (Molecular Devices, Sunnyvale, CA) on an iMac computer (Apple, Cupertino, CA). Experiments on guinea pigs were performed with the colon bathed in oxygenated (95% O_2, 5% CO_2) Krebs solution at room temperature to minimize muscle contractions (flow rate 2 mL min-1). Mouse experiments were conducted at 37°C. After a 30-minute or longer equilibration period and confirmation of basal 5-HT release, recordings were obtained with the electrode placed 50 μm above the mucosal surface.

Mucus Release

Full-thickness colonic segments were equilibrated for 30 minutes in oxygenated, 37°C Krebs solution, followed by 30 minutes of an experimental condition. Tissues were rinsed and fixed in 10% formalin overnight at 4°C. Preparations were paraffin-embedded and sectioned, and stained with periodic acid– Schiff and Alcian Blue. The percentage of goblet cells that were cavitated was evaluated blindly by counting cavitated and non-cavitated goblet cells at 400× magnification.

Measurement of Ion Transport

Full-thickness preparations were mounted in Ussing chambers bathed in oxygenated Krebs solution warmed to 37°C.[11,12] The tissue was voltage-clamped at 0 mV. Short circuit current (I_{SC}, $\mu A/cm^2$) responses were measured as the maximum increase occurring within 10 minutes of drug application. At the end of each experiment, forskolin (10 μmol/L) was added to confirm tissue viability.

Motility Analysis

A Gastrointestinal Motility Monitor (Catamount Research and Development, Inc, St. Albans, VT) was used to record and analyze the rate of propulsive motility in guinea pig colonic segments.[13] A segment of distal colon was pinned onto a Sylgard-lined (Dow Corning Co, Midland MI) organ bath perfused with recirculating warmed (37°C) and oxygenated Krebs solution at a flow rate of 10 mL min^{-1}. Intraluminally delivered compounds were delivered at a flow rate of 0.25 mL min^{-1}. An epoxy-coated pellet was inserted into the oral end of an isolated segment, and the motility pattern of the pellet was tracked with a digital camera coupled to the Gastrointestinal Motility Monitor computer analysis software. After a 30-minute equilibration period, at least 3 trials were recorded, with a 5-minute recovery between each trial.

Assessment of Colonic Sensitivity

Acclimatized rats were anesthetized with isoflurane (5% induction; 1%–2% maintenance in O_2) and a strain gauge force transducer was sutured to the right external oblique muscle. Twenty minutes after administration of compounds via oral gavage or an intracolonic catheter, rats were infused intracolonically (i.c.) with 1.5 mL 0.6% acetic acid, and a 5-cm balloon catheter was inserted 8 cm into the distal colon. Sixty minutes later, the colorectal balloon was attached to a barostat (Distender Series IIR; G & J Electronics Inc, Toronto, Ontario, Canada) and chart recorder, and VMR was measured as the number of abdominal contractions 10 min^{-1} to randomized isobaric distension pressures (0, 15, 30, 45, and 60 mm Hg). Contractions were scored by a deflection in the chart recording accompanied by observation of abdominal flexion.

Data Analysis

The data presented are means ± standard error of the mean for n animals or subjects. Statistical analyses were performed using GraphPad Prism software (v. 5.0a; GraphPad

Software, San Diego, CA). Differences were determined by unpaired Student *t* test, 1-way analysis of variance, or a 2-way analysis of variance with Bonferroni post-test. A *P* value less than .05 was considered statistically significant.

Results

5-HT$_4$Rs Are Expressed in the Intestinal Epithelium

Murine mucosal 5-HT$_4$R expression—To assess the presence and relative levels of 5-HT$_4$R transcript throughout the GI tract, real-time quantitative RT-PCR was performed in samples from the gastric corpus, duodenum, jejunum, ileum, and proximal and distal colons of SW and BALB/cJ mice. In full-thickness preparations, 5-HT$_4$R transcript was detected from all regions, and when normalized to the endogenous control *Hprt1*, significantly higher levels were found in the distal and proximal colon as compared with more proximal regions of the gut (data not shown). 5-HT$_4$R transcript was not detected in gastric mucosal samples. In intestinal mucosa samples, a clear gradient was observed, with low levels of expression in the small intestine, and highest in the mucosa of the distal colon (SW, Figure 1*A*; Balb/cJ, Supplementary Figure 1). Mucosal 5-HT$_4$ transcript expression also was confirmed in mucosal scrapings from rat and CD1 mouse colon (data not shown). The normalized expression level in the distal colon was higher in mucosal samples than full-thickness samples ($P < .05$), suggesting that the density of expression is highest in the mucosa.

As an additional approach to evaluate the distribution of 5-HT$_4$Rs and to identify the cell types expressing the receptor, we examined tissue from mice expressing eGFP under the regulatory elements for the 5-HT$_4$ promoter (5-HT$_4$[BAC]-eGFP; available: www.gensat.org) and observed a pattern similar to that detected with PCR. In the duodenum and jejunum, GFP-immunoreactive neurons were observed in submucosal and myenteric ganglia, and a small number of cells that appeared to be enteroendocrine cells were detected in the epithelium (Figure 2*A* and *B*). In the ileum, intense GFP immunofluorescence was observed in cells at the base of the crypts, enteroendocrine-like cells, and neurons (Figure 2*C*). In the distal colon, essentially all cells in the epithelium were GFP-immunoreactive (Figure 2*D*). GFP-immunoreactive cells also were observed in the muscularis mucosa, and in enteric ganglia (Figure 2*D*).

To identify the subtypes of epithelial cells that express 5-HT$_4$R, double-labeling was performed in sections from 5-HT$_4$(BAC)-eGFP mouse intestines. Immunostaining antibodies directed against 5-HT and mucin 2 were used to identify 5-HT–containing enterochromaffin (EC) cells (Figure 3*A*), and goblet cells (Figure 4*A*), respectively. In the colon, 5-HT– and mucin 2–immunoreactive cells were also GFP-immunoreactive, indicating that EC cells and goblet cells express the 5-HT$_4$R.

Human mucosal 5-HT$_4$R expression—To assess 5-HT$_4$R transcript in the human GI tract, real-time quantitative RT-PCR was performed in mucosal biopsy specimens from the gastric corpus, duodenum, terminal ileum, proximal colon, and distal colon. Very low levels of 5-HT$_4$R transcript were detected in gastric biopsies (Figure 1*B*). In intestinal samples, 5-HT$_4$R transcript was present in all regions tested, with the highest transcript level in the terminal ileum (Figure 1*B*). In separate studies, 5-HT$_4$R transcript was detected in rectal biopsies (data not shown).

Effects of 5-HT$_4$R Activation on Colonic Epithelial Cells

Mucosal 5-HT$_4$R activation elicits 5-HT release—To test whether activation of 5-HT$_4$Rs on EC cells affects 5-HT release, we used *in vitro* amperometry with diamond-coated microelectrodes calibrated to detect 5-HT as an oxidation current. Cisapride was used

for these studies because tegaserod oxidizes at the same voltage as 5-HT; therefore, tegaserod-mediated oxidation currents cannot be distinguished from serotonergic currents. Upon application of cisapride (1.0 μmol/L), transient increases in the oxidation current were detected in the distal colon of guinea pigs (Figure 3*B–D*) and SW mice (Figure 3*E*). The cisapride-induced response was inhibited by the 5-HT_4R antagonist administration (Figure 3*C–E*), but persisted in the presence of tetrodotoxin (TTX) (0.3 μmol/L). These findings indicate that cisapride elicits 5-HT release from the mucosa by directly activating 5-HT_4Rs on EC cells, rather than via a neural mechanism.

Mucosal 5-HT_4R Activation Elicits Mucus Secretion

Data from 5-HT_4(BAC)-eGFP mouse sections immunostained for mucin 2 suggest that goblet cells express 5-HT_4Rs. Previous work has shown that 5-HT causes mucus secretion by activation of goblet cells, which can be visualized as large vacuoles in the epithelial layer, referred to as *cavitations*, in periodic acid–Schiff and Alcian Blue– stained sections.[14] To determine whether 5-HT_4R activation leads to mucus secretion, preparations from SW mice (Figure 4*B* and *C*) and guinea pig (Supplementary Figure 2) distal colon were exposed to vehicle, 1 μmol/L tegaserod, tegaserod plus TTX (0.3 μmol/L), or tegaserod plus the 5-HT_4R antagonist, GR113808 (1 μmol/L). The proportion of goblet cells that were cavitated was increased in preparations treated with tegaserod or tegaserod and TTX ($P < .001$), and this effect was inhibited by GR113808.

Mucosal 5-HT_4R Activation Elicits Cl^- Secretion

The effects of mucosal vs serosal administration of a 5-HT_4R agonist on I_{SC} were evaluated in full-thickness segments of CD1 mouse duodenum and distal colon mounted in Ussing chambers. In the colon, 1 μmol/L tegaserod increased I_{SC} when applied to the mucosal side of the chamber (Figure 5*A*; $P < .001$), but had no effect when applied serosally ($P = .19$; Figure 5*B*). The peak response to mucosally applied tegaserod was 25 ± 8 $\mu A/cm^2$ (n = 6). This effect was reduced by GR113808 (1 μmol/L; $P < .001$, n = 6; Figure 5*A*). Similar results were obtained in the distal colons of SW mice and guinea pigs (Supplementary Figure 3). The tegaserod-mediated increase in I involves Cl^- SC secretion because tegaserod did not alter the I when Cl^- SC was excluded from the Krebs solution. Furthermore, the response to mucosal application of tegaserod was blocked by TTX, indicating that this response was mediated via a neural mechanism. Mucosal application of tegaserod in the murine duodenum did not alter I_{SC} ($P = .54$; Figure 5*C*).

Effects of 5-HT_4R Agonists on Propulsive Motility and Visceral Hypersensitivity in the Colon

The findings described earlier show that 5-HT_4Rs are widely expressed in the colonic epithelium and that stimulation of these receptors elicits fluid, mucus, and 5-HT secretion. 5-HT_4R agonists promote colonic motility and attenuate visceral hypersensitivity in animal models and human beings.[15,16] Therefore, we investigated whether luminal application of 5-HT_4R agonists could elicit these effects. For these studies, we had access to naronapride (ATI-7505), which is a more selective 5-HT_4R agonist than tegaserod or cisapride.[4,17] Unlike cisapride and tegaserod, naronapride does not interact with other 5-HT_4Rs.[4,17]

Mucosal Administration of a 5-HT_4R Agonist Promotes Colonic Propulsive Motility

Previous studies have shown that luminal 5-HT_4R agonist application accelerates propulsive motility in isolated segments of guinea pig distal colon.[18] To evaluate the effects of mucosal 5-HT_4R activation, as compared with stimulation of myenteric receptors, we tested the effects of luminal vs bath application of naronapride using this model. Luminally applied naronapride (0.1 $\mu mol\ L^{-1}$) increased the rate of propulsive motility (Figure 6*A*), and this

effect was blocked by the 5-HT_4R antagonist, SB204070 (10 nmol L^{-1}). The addition of naronapride to the bathing solution did not alter the rate of propulsive motility (Figure 6*B*). Comparable data were obtained with luminal vs bath application of tegaserod (data not shown).

Visceral Hypersensitivity is Attenuated by Intraluminal Administration of a 5-HT_4R Agonist

Previous studies have shown that colonic infusion of acetic acid enhances VMRs to CRD in awake, freely moving rats,[19] and that intraperitoneal administration of tegaserod suppresses this colonic hypersensitivity in rats.[16] In the current study, we compared the effects of oral vs intracolonic administration of 5-HT_4R agonists. Oral administration of tegaserod attenuated the VMR at both 1- and 10-mg kg^{-1} doses (Figure 7*A*). Intraluminal administration of tegaserod also decreased the VMR at doses of 1 and 0.1 mg kg^{-1}, and this response was blocked by luminal pretreatment with GR113808 (1 mg kg^{-1}) (Figure 7*B*).

To further establish that the antinociceptive effects of tegaserod on colonic hypersensitivity involves mucosal 5-HT_4Rs, the actions of naronapride were tested. Oral administration of naronapride attenuated visceral hypersensitivity at doses of 0.1–30 mg kg^{-1}, with the maximal decrease in VMR observed at 1.0 mg kg^{-1} (Figure 7*C*). Similar to the effects observed with tegaserod, intracolonic administration of naronapride (0.1 and 1 mg kg^{-1}) decreased VMR to CRD at pressures of 15 mmHg and greater (Figure 7*D*). As observed with tegaserod, GR113808 (1 mg kg^{-1} i.c.) blocked the antinociceptive action of naronapride (1 mg kg^{-1} i.c.) (Figure 7*D*).

Discussion

This study was performed to test the hypotheses that 5-HT_4Rs are expressed in the colonic mucosa, and, when activated, promote propulsive motility and attenuate visceral hypersensitivity. Our findings provide novel molecular, morphologic, and physiological evidence for 5-HT_4R expression in the colonic epithelium of mouse, rat, guinea pig, and human beings. Expression of this receptor was found on serotonin-containing EC cells and mucin 2–immunoreactive goblet cells, and activation of mucosal 5-HT_4Rs elicited 5-HT release, mucus release, and increased short-circuit current. Furthermore, luminal administration of 5-HT_4R agonists increased the velocity of propulsive motility and decreased colonic hypersensitivity. Collectively, these studies contribute new knowledge regarding the expression and function of the 5-HT_4R in the colonic mucosa, and support the concept that 5-HT_4R agonists formulated to target the colonic mucosa could provide an effective and safer method of delivery.

It generally is accepted that 5-HT_4R agonists have prokinetic actions and can be used to improve symptoms related to constipation. Emerging data involving more selective 5-HT_4R agonists, including naronapride (ATI-7505), prucalopride, and velusetrag (TD-5108) support this receptor as an effective therapeutic target for promoting gut motility. However, the mechanism of action of these compounds has not been clearly resolved. One possibility is that 5-HT_4R agonists promote motility by stimulating receptors on enteric nerve terminals and increasing neurotransmitter release. It is clear from a number of investigations that 5-HT_4Rs are located on enteric nerve terminals, and 5-HT_4R agonists facilitate synaptic transmission through a presynaptic mechanism.[5,6,20–22] Furthermore, morphologic and molecular studies have shown that 5-HT_4Rs are expressed by enteric neurons.[22–24] Consistent with this model, bath application of cisapride increases peristalsis in the ileum.[6] However, in the current investigation, when 5-HT_4R agonists were applied to the bathing solution, where they would have access to the myenteric plexus, propulsive motility in the distal colon was not detectably altered.

Another possible mechanism for the prokinetic action of 5-HT_4R agonists involves a mucosal site of action. In the current and previous[8,18] studies of 5-HT_4R responses in the colon, agonists increased the rate of propulsive motility when administered intraluminally, and these responses were inhibited by 5-HT_4R antagonists. Furthermore, Grider et al[7] reported that application of tegaserod to the colonic mucosa activates ascending excitatory and descending relaxant peristaltic reflex responses. Yet, until now, a lack of direct evidence for epithelial 5-HT_4Rs has limited acceptance of a mucosal site of action for 5-HT_4R agonists.

The findings reported here indicate that 5-HT_4Rs are expressed in the intestinal mucosa, where they are distributed differentially and expressed by a number of epithelial cell types. These data show that there is a gradient of mucosal 5-HT_4R expression in the murine GI tract, with highest expression in the distal colon where all or most cells appear to express this receptor. 5-HT_4R messenger RNA also was present in human and rat colonic mucosal samples. It is possible that activation of 5-HT_4Rs on colonic epithelial cells could mediate the prokinetic actions of 5-HT_4R agonists. In the current investigation, we show 5-HT_4R expression by EC cells, goblet cells, and enterocytes, and it is conceivable that stimulation of secretion by any or all of these cell types could promote colonic transit. For example, 5-HT release could activate peristaltic reflex activity, mucus release could decrease friction along the epithelial lining, and fluid secretion could soften the stool and facilitate propulsion.

In the current study, 5-HT–immunoreactive EC cells also expressed GFP immunoreactivity in sections from 5-HT_4(BAC)-eGFP transgenic mice, indicating that EC cells in the distal colon express 5-HT_4Rs. The effects of 5-HT_4R activation on 5-HT release have been conducted in the small intestine, where 5-HT_4R agonists are reported to decrease basal 5-HT release.[25,26] However, in this study, using continuous electrochemical recordings with electrodes calibrated for measurement of 5-HT, application of a 5-HT_4R agonist to the colonic mucosa evoked an increase in oxidation current that was TTX-insensitive, and was blocked by a 5-HT_4R antagonist. The concept that 5-HT_4R activation enhances 5-HT release is consistent with the fact that this receptor signals through the cAMP pathway. Furthermore, freshly isolated mammalian ECcells,[27] as well as the EC cell models, BON cells[28] and KRJ-1 cells,[29] promote 5-HT release via cAMP signaling.

Previous studies have shown that 5-HT causes TTX-insensitive mucus secretion and goblet cell cavitation in the rat colon,[14] but the receptor(s) responsible for these actions were not identified. Evidence for mucosal 5-HT_4Rs mediating mucus secretion includes the findings that goblet cells are GFP-immunoreactive in sections from 5-HT_4(BAC)-eGFP transgenic mice, and 5-HT_4R activation increases cavitation in goblet cells. The concept that 5-HT_4R stimulation elicits mucus secretion is supported by previous findings that stimulation of the cAMP/PKA pathway causes mucus secretion in the T84 human colonic adenocarcinoma cell line,[30] which includes goblet-like cells with mucin-containing secretory granules.

Serotonin stimulates chloride secretion in the intestine, and 5-HT_4Rs may contribute to this response.[31,32] In the current study, luminal administration of a 5-HT_4R agonist to the distal colon elicited an increase in Cl^- secretion, whereas serosal application had no effect. Furthermore, in the mouse duodenum, where 5-HT_4R expression was barely detectable, administration of a 5-HT_4R agonist to the mucosa had no effect on I_{SC}. The I_{SC} response in the colon was eliminated in the presence of TTX, indicating that this response was mediated neurally. This was some-what surprising because data from the 5-HT_4(BAC)-eGFP transgenic mice suggest that enterocytes express the 5-HT_4R, and as indicated earlier, this receptor is linked to the cAMP pathway. It is possible that tegaserod elicits Cl^- secretion by activating 5-HT release because EC cell activation leads to a neurally mediated secretory

response.[33] Regardless of the mechanism, these findings are consistent with a prokinetic effect of 5-HT_4R activation, and may contribute to the relief of constipation.

Previous human[15] and animal[16] studies have shown that tegaserod alleviates abdominal pain and discomfort, with the compound administered orally in human beings and intraperitoneally in rats. We report here that intracolonic infusion of tegaserod or naronapride reduced the VMR in a dose-dependent manner when infused into the colon, and the agonists were more potent when administered intracolonically.

There has been considerable debate as to whether the antinociceptive actions of tegaserod are mediated via activation of 5-HT_4Rs and/or antagonism of 5-HT_{2B}Rs.[34,35] 5-HT_{2B}R antagonists suppress VMR responses in Wistar Kyoto rats[35] and in a model of trinitrobenzene sulfonic acid (TNBS)–induced colonic hypersensitivity.[36] Also, tegaserod is an antagonist at the 5-HT_{2B}R, in addition to its more potent action as a 5-HT_4R agonist.[37] However, previous studies of rats with acetic acid–induced colonic hypersensitivity showed that the tegaserod-induced attenuation of VMR was partially inhibited by 5-HT_4R antagonism, but no additional inhibition of the antinociceptive response was observed after co-administration of a 5-HT_{2B}R antagonist.[34] Here, we report that intracolonically administered naronapride, a compound with 1000-fold greater affinity for the 5-HT_4R than other 5-HTRs,[17] caused a decrease in the VMR to CRD in the sensitized colon. Furthermore, the antinociceptive responses to tegaserod and naronapride were blocked by a 5-HT_4R antagonist. Collectively, these findings support the concept that exposure of the colonic mucosa to 5-HT_4R agonists alleviates visceral hypersensitivity, but the precise mechanisms of action are unknown.

Interestingly, although various classes of 5-HT_4R agonists are useful for the treatment of functional gastrointestinal disorders, their efficacies for alleviating upper vs lower GI symptoms appear to vary. For example, cisapride is well known for its effects on gastric emptying and gastroparesis, whereas tegaserod and prucalopride are more recognized for improving colonic transit. Tegaserod is poorly absorbed,[38] and its selectivity for the colon may involve a direct action on mucosal 5-HT_4Rs.[39] Formulation of 5-HT_4R agonists to prevent systemic absorption and deliver the drug effectively to the colonic mucosa may enhance their clinical effects while avoiding systemic bioavailability and potential side effects.

The findings presented here provide evidence for mucosal expression of 5-HT_4Rs in the guinea pig, rat, and human colons, and 3 strains of mice (Supplementary Table 1). Support for 5-HT_4R–mediated 5-HT release and goblet cell degranulation are provided from both SW mice and guinea pigs. Furthermore, mucosal application of a 5-HT_4R agonist elicited an increase in I_{SC} in CD1 and SW mice, and guinea pigs. Collectively, these findings indicate that these epithelial responses are not species-specific effects. By using assays previously used to study 5-HT_4R functions in the gut, we present data showing that luminal administration of 5-HT_4R agonists promotes propulsive motility and suppresses visceral hypersensitivity in guinea pigs and rats, respectively. A limitation of the current study was that the 5-HT_4R was localized in mouse colonic mucosa, but the motility and visceral sensitivity assays were conducted in guinea pigs and rats, respectively. This was performed to maintain consistency with previous studies of 5-HT_4R agonists on gut function and sensation. Additional studies will be required to confirm the mucosal distributions of 5-HT_4Rs and determine whether the mucosal actions of 5-HT_4R agonists are comparable across species, including human beings.

In conclusion, these findings show that 5-HT_4Rs are expressed on a variety of epithelial cells of the colon, and activation of these mucosal 5-HT_4Rs leads to mucus, serotonin, and

fluid secretion. Furthermore, activation of mucosal 5-HT_4Rs promotes propulsive motility and attenuates visceral hypersensitivity, but the precise mechanisms remain to be resolved. Moreover, these data support the novel concept that the colonic mucosa should be explored as an effective target for 5-HT_4R agonists in the treatment of constipation and abdominal pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

BAC	bacterial artificial chromosome
CRD	colorectal distension
EC	enterochromaffin
eGFP	enhanced green fluorescent protein
GFP	green fluorescent protein
GI	gastrointestinal
5-HT	5-hydroxytryptamine or serotonin
5-HT_4R	5-hydroxytryptamine receptor
I_{SC}	short-circuit current
RT-PCR	reverse-transcription polymerase chain reaction
SW	Swiss Webster
TTX	tetrodotoxin
VMR	visceromotor response

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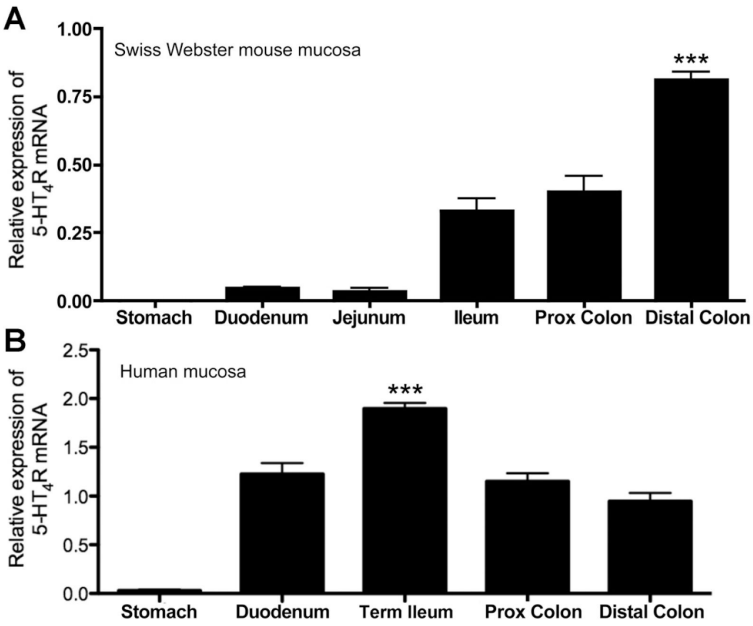

Figure 1.

(*A*) SW mouse mucosal 5-HT_4R transcript levels detected in the distal colon were significantly greater than levels detected in all other regions. The transcript levels in the proximal colon were significantly greater than levels in the duodenum or jejunum ($P < .001$). Data were normalized to HPRT1. (*B*) 5-HT_4R transcript was detected in all regions of the human intestinal mucosa, with the highest level in the terminal ileum. 5-HT_4R transcript was undetectable in murine, and detected at low levels in human gastric mucosal samples. ***$P < .001$ as compared with other regions (n = 3–5 for mouse samples, and 7–12 for human samples).

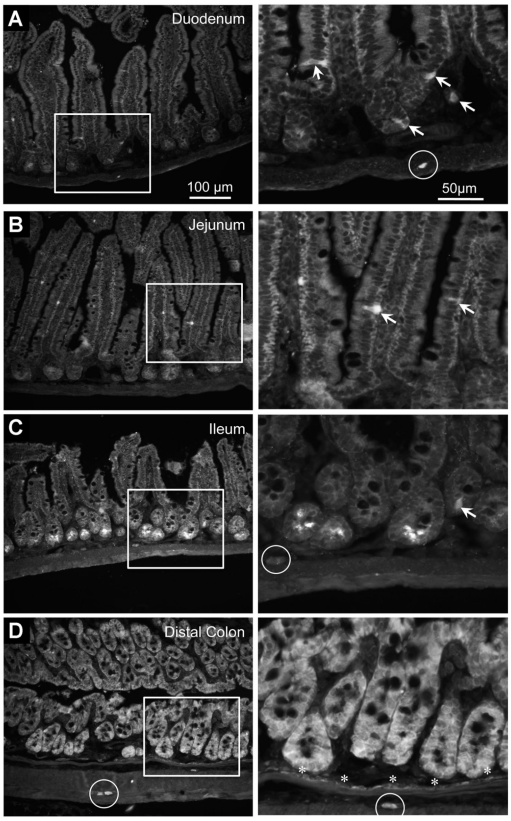

Figure 2.

Photomicrographs of sections from a 5-HT_4R(BAC)-eGFP mouse showing GFP immunoreactivity in the (*A*) duodenum, (*B*) jejunum, (*C*) ileum, and (*D*) distal colon. White boxes indicate regions shown at higher magnification. In the duodenum and jejunum, GFP immunoreactivity was detected in enteric neurons (*circle*) and enteroendocrine cells (*arrows*). In the ileum, GFP immunoreactivity was detected in epithelial cells at the base of crypt glands, in enteroendocrine cells, and in neurons. In the distal colon, GFP immunoreactivity was observed throughout the epithelial layer, in a monolayer of cells along the muscularis mucosa (*asterisks*), and in neurons (*circles*).

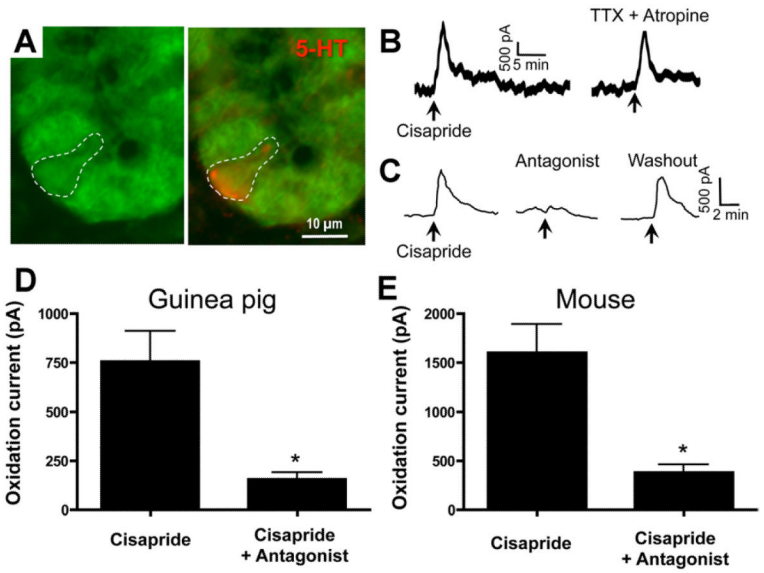

Figure 3.

(*A*) Photomicrographs from a 5-HT_4R(BAC)-eGFP mouse section showing that 5-HT–immunoreactive EC cells are GFP-immunoreactive. (*B*) In guinea pig distal colon, the 5-HT_4R agonist, cisapride (1 μmol/L), increased the oxidation current for 5-HT in a TTX-(0.3 μmol/L) and atropine-(10 μmol/L) insensitive manner. (*C* and *D*) In guinea pig, the cisapride response was blocked by the 5-HT_4R antagonist, SB204070 (0.1 μmol/L) (n = 5–8). (*E*) In SW mice, the cisapride response was blocked by the 5-HT_4R antagonist, GR113808 (1 μmol/L) (n = 5). *$P < .02$ vs cisapride alone.

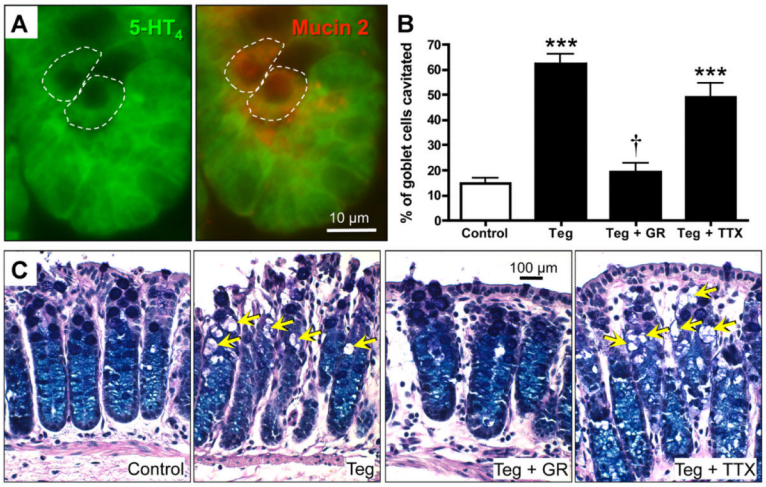

Figure 4.

(*A*) Photomicrographs from a 5-HT_4R(BAC)-eGFP mouse section showing that mucin 2–immunoreactive goblet cells are GFP-immunoreactive. (*B*) Percentage of goblet cells that were cavitated under conditions tested. (*C*) Application of the 5-HT_4R agonist, tegaserod (Teg, 1 μmol/L), to the mucosal surface of the SW mouse distal colon elicited an increase in goblet cell cavitation (*yellow arrows*) in periodic acid–Schiff and Alcian Blue (PAS/AB)–stained sections. The tegaserod response was blocked by the 5-HT_4R antagonist, GR113808 (1 μmol/L), but not by TTX (0.3 μmol/L). ***$P < .001$ vs vehicle; $^{\dagger}P < .001$ vs tegaserod, and >0.05 vs vehicle (n = 5 per group).

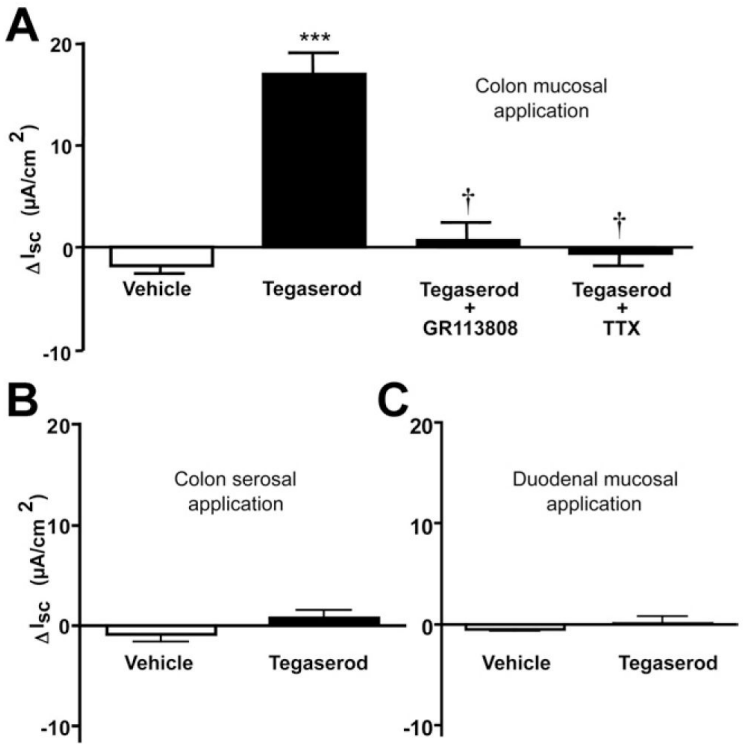

Figure 5.

(*A*) Tegaserod (1 μmol/L) caused a significant increase in I_{SC} that was blocked by the 5-HT_4R antagonist, GR113808 (1 μmol/L), and by neural blockade with TTX (0.3 μmol/L). (*B*) Serosal application of tegaserod did not alter the I_{SC}. (*C*) No response was detected by mucosal application of tegaserod in the duodenum. ***$P < .001$ vs vehicle; †$P < .001$ vs tegaserod (n = 6).

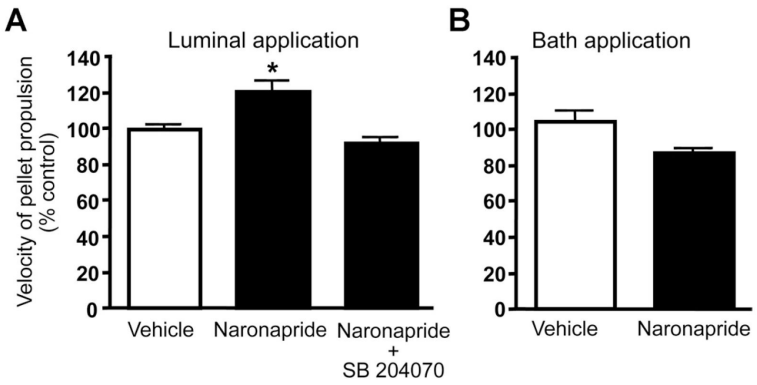

Figure 6.

(*A*) Intraluminal administration of naronapride (100 nmol/L) increased the rate of propulsive motility, and this effect was blocked by the 5-HT_4R antagonist SB204070 (10 nmol/L) (**P* < .05 vs vehicle; n = 5). (*B*) No change in pellet propulsion was detected when naronapride was added to the bathing solution (n = 6–19).

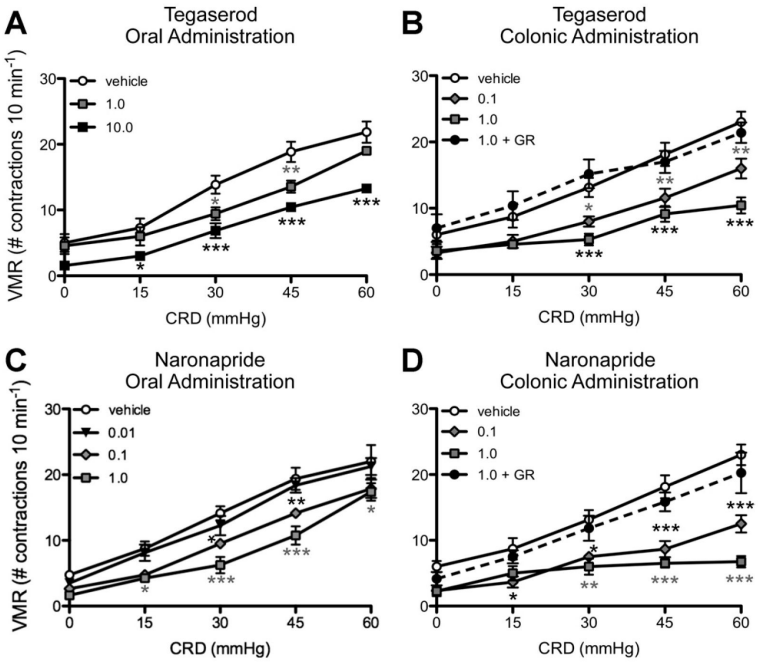

Figure 7.

(*A*) Oral doses of 10 mg kg^{-1} and 1.0 mg kg^{-1} tegaserod significantly reduced the VMR at distension pressures of 30 mm Hg and greater (**P* < .05 vs control; ***P* < .01 vs control; ****P* < .001 vs vehicle; n = 6–7). (*C*) Oral naronapride decreased the VMR at doses of 0.1 mg kg^{-1} and higher, with a maximal effect at 1 mg kg^{-1}. Intracolonic administration of (*B*) tegaserod and (*D*) naronapride decreased the VMR to CRD at doses of 0.1 and 1.0 mg kg^{-1} (n = 5–6). This effect was inhibited by the 5-HT_4R antagonist, GR113808 (1.0 mg kg^{-1}), administered intracolonically (*dashed lines*; n = 5–6).