Supplementary Materials and Methods

Strains and Sources of Animals

Vermont PCR, amperometry, goblet cell cavitation, and motility studies: Hartley guinea pigs (250–350 g; Charles River, Montreal, Quebec, Canada); Balb/cJ mice (10–14 weeks, male; Jackson Labs, Bar Harbor, ME); Swiss Webster (CFW) mice (12–14 weeks, male; Charles River); and CD1 mice (12 weeks, male; Charles River). Calgary Ussing chamber studies: CD1 mice (5–6 weeks, male; Charles River), Swiss Webster mice (12–14 weeks, male; Charles River), Hartley guinea pigs (250–350 g; Charles River). Oklahoma studies: male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 250–350 g were used in VMR studies.

Immunohistochemistry/BAC Transgenic Mice

The eGFP signal in 5-HT_4(BAC)-eGFP mice was amplified by immunostaining with a chicken anti-GFP (1:1000; #ab13970; Abcam, Cambridge, MA) and donkey anti-chicken fluorescein isothiocyanate antisera (1:100; #703-095-155; Jackson ImmunoResearch Laboratories, Inc, West Grove, PA). The fluorescent staining using this antibody had a similar pattern, but was more intense than the fluorescence emitted by eGFP alone. In some cases, sections were double-stained with goat anti–5-HT (1:800; #20079; ImmunoStar, Inc, Hudson, WI) or goat anti-mucin 2 (1:50; #13312; Santa Cruz Biotechnology, Inc, Santa Cruz, CA) and donkey anti-goat CY3 antisera (1:500; #705-165-147; Jackson ImmunoResearch Laboratories, Inc). Sections were examined on an Olympus AX70 fluorescence microscope (Olympus America, Inc) equipped with an Optronics MagnaFire digital camera (Optronics). Images of microscopic fields were captured using identical exposure settings with a 20× objective.

Physiological Solutions

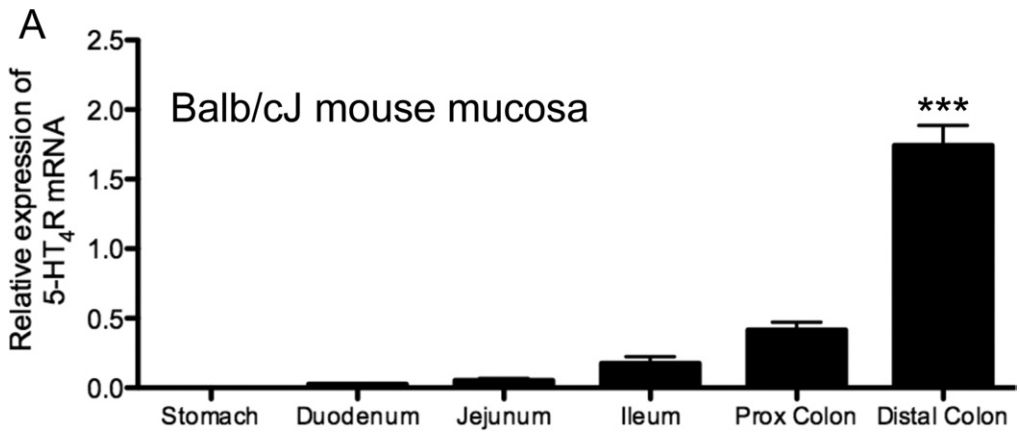
Ussing chamber studies. Standard Krebs solution: NaCl, 117 mmol/L; KCl, 4.8 mmol/L; $CaCl_2$, 2.5 mmol/L; $MgCl_2$, 1.2 mmol/L; $NaHCO_3$, 25 mmol/L; NaH_2PO_4, 1.2 mmol/L; and glucose, 11 mmol/L; pH 7.4 (Sigma-Aldrich, St. Louis, MO). Experiments on colonic preparations used Krebs solution containing 11 mmol/L D-glucose, whereas small intestinal preparations were bathed in Krebs solution containing 11 mmol/L D-glucose serosally, and an equimolar concentration of mannitol mucosally.

The involvement of Cl^- ions was assessed using chloride-free Krebs solution (D-gluconic acid, sodium salt, 117 mmol/L; D-gluconic acid, potassium salt, 4.8 mmol/L; $NaHCO_3$, 25 mmol/L; NaH_2PO_4, 1.2 mmol/L; D-gluconic acid, hemimagnesium salt, 1.2 mmol/L; D-gluconic acid, calcium salt, 2.5 mmol/L; and D-glucose, 11 mmol/L; Sigma-Aldrich).

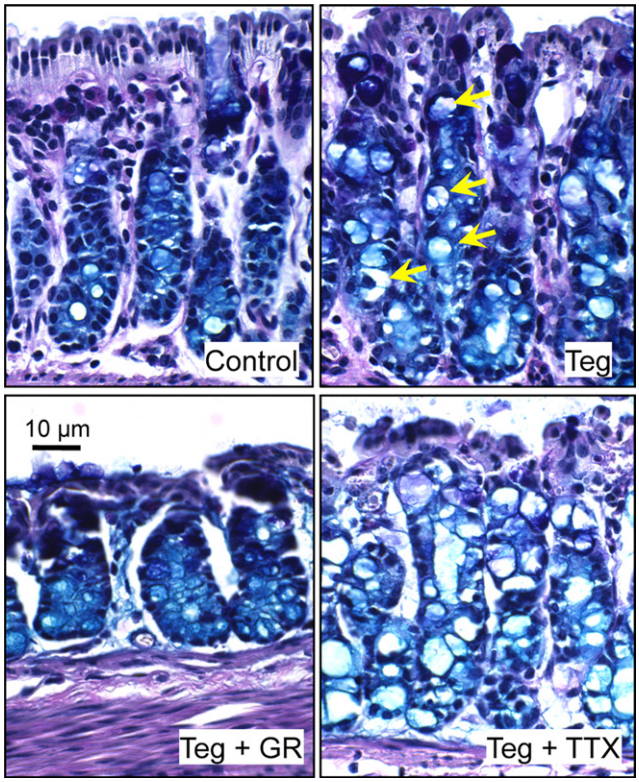
Motility and mucus secretion studies. Standard Krebs solution: (NaCl, 121 mmol/L; KCl, 5.9 mmol/L; $CaCl_2$, 2.5 mmol/L; $MgCl_2$, 1.2 mmol/L; $NaHCO_3$, 25 mmol/L; NaH_2PO_4, 1.2 mmol/L; and glucose, 8 mmol/L; Sigma-Aldrich).

Reagents

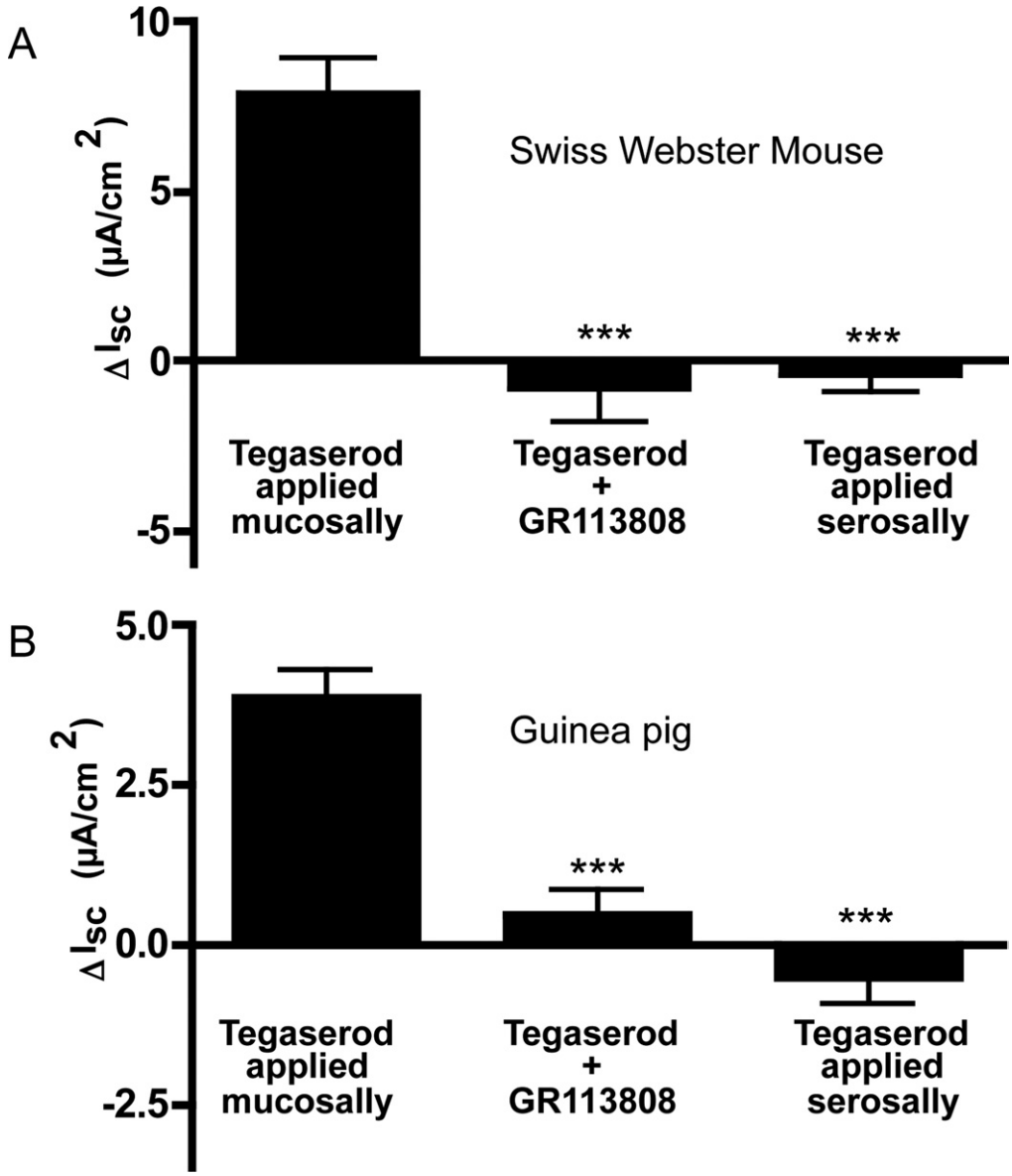
Tegaserod and cisapride were acquired as a gift from Drs John McRorie and Russell Spruell of Proctor and Gamble (Cincinnati, OH). Tegaserod was used in all assays except for the amperometry assay because it oxidizes at the same voltage as 5-HT, and the motility studies because Grider et al previously showed its efficacy in the guinea pig propulsive motility assay.[8] The 5-HT_4R agonist, naronapride (ATI-7505), was provided by Monica Palme of ARYx Therapeutics (Fremont, CA), and because of limited availability, was only used for the motility and visceral sensitivity assays. The 5-HT_4R antagonist, GR113808, and TTX were purchased from Tocris Bioscience (Ellisville, MO). GR113808 was used in all assays except for the motility assay, in which SB204070 was used because we had previous experience with this compound in that assay. SB204070, atropine, and forskolin were purchased from Sigma-Aldrich. SB204070 was also used in the guinea pig amperometry studies, and it inhibited the cisapride-induced increase in oxidation current to a similar extent as GR113808 in mouse (Figure 3*D* and *E*). In most studies, the earlier-described compounds except for TTX and atropine initially were dissolved in dimethyl sulfoxide and used at final concentrations of 0.05% dimethyl sulfoxide or less. TTX and atropine were dissolved in water. In the amperometry studies, the stock solutions were diluted to their final concentrations in a 1:1 solution of glycerol and Krebs, and applied directly to the mucosal surface.



Supplementary Figure 1. As was detected in Swiss Webster mice (Figure 1*A*), mucosal 5-HT_4R transcript levels detected in the distal colon of Balb/cJ mice 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 ($P < .01$). ***$P < .001$ as compared with other regions; n = 5–6.



Supplementary Figure 2. Application of the 5-HT$_4$R agonist, tegaserod (Teg; 1 μmol/L), to the mucosal surface of the guinea pig 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$_4$R antagonist, GR113808 (1 μmol/L), but not by TTX (0.3 μmol/L). n = 5 per group.



Supplementary Figure 3. Mucosal application of the 5-HT_4R agonist, tegaserod (1 μmol/L), elicited an increase in I_{SC} in distal colon preparations of (*A*) Swiss Webster mice and (*B*) guinea pigs. In both species, the response to mucosally applied tegaserod was inhibited by the 5-HT_4R antagonist, GR113808 (1 μmol/L). Serosal application of tegaserod failed to induce a change in I_{SC}. n = 4 per group. ***$P < .001$.

Supplementary Table 1. Composite of Data Collected From the Different Species Studied Regarding Mucosal 5-HT_4 Receptors in the Colon

	Human	Guinea pig	Rat	Mouse (strain)
PCR	*		*	*(SW, CD1 and Balb/cJ)
Morphologic localization				*(SW)
5-HT release		*		*(SW)
Mucus secretion		*		*(SW)
ISC		*		*(SW, CD1)
Motility		*		
Sensation			*	

NOTE. Asterisk indicates the species that were used for each type of experiment in this study. In the mouse column, the strains that were tested are shown in parentheses.
SW, Swiss Webster.