TRPV1 Function in Mouse Colon Sensory Neurons Is Enhanced by Metabotropic 5-Hydroxytryptamine Receptor Activation

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Using whole-cell patch-clamp methods, we examined the hypothesis that serotonin [5-hydroxytryptamine (5-HT)] receptor activation enhances TRPV1 function in mouse colon sensory neurons in lumbosacral dorsal root ganglia, which were identified by retrograde labeling with Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine methanesulfonate) injected into multiple sites in the wall of the descending colon. 5-HT increased membrane excitability at a temperature below body temperature in response to thermal ramp stimuli in colon sensory neurons from wild-type mice, but not from TRPV1 knock-out mice. 5-HT significantly enhanced capsaicin-, heat-, and proton-evoked currents with an EC50 value of 2.2 μM. 5-HT (1 μM) significantly increased capsaicin-evoked (100 nM) and proton-evoked (pH 5.5) currents 1.6- and 4.7-fold, respectively, and significantly decreased the threshold temperature for heat current activation from 42°C to 38°C. The enhancement of TRPV1 by 5-HT was significantly attenuated by selective 5-HT2 and 5-HT4 receptor antagonists, but not by a 5-HT1 receptor antagonist. In support, 5-HT2 and 5-HT4 receptor agonists mimicked the facilitating effects of 5-HT on TRPV1 function. Downstream signaling required G-protein activation and phosphorylation as intracellularly administered GDP-β-S [guanosine 5′-O-(2-thiodiphosphate), protein kinase A inhibitors, and an A-kinase anchoring protein inhibitor significantly blocked serotonergic facilitation of TRPV1 function; 5-HT2 receptor-mediated facilitation was also inhibited by a PKC inhibitor. We conclude that the facilitation of TRPV1 by metabotropic 5-HT receptor activation may contribute to hypersensitivity of primary afferent neurons in irritable bowel syndrome patients.

Key words: visceral pain; thermal hyperalgesia; DRG; serotonin receptor; capsaicin receptor; Dil

Introduction

Irritable bowel syndrome (IBS) is a chronic disorder characterized by abdominal pain and discomfort and changes in bowel habits in the absence of structural or biochemical abnormalities. IBS is prevalent, with symptoms at least transiently affecting up to one in five Americans each year, and is the second leading cause of workplace absenteeism in the United States after the common cold, resulting in estimated direct and indirect costs exceeding $30 billion annually (Sandler et al., 2002). The mechanisms leading to the development of functional gastrointestinal disorders such as IBS are poorly understood. However, mounting evidence suggests that changes in visceral sensation (i.e., visceral hypersensitivity) play an important role in the pathogenesis of these disorders. Sensitization of peripheral sensory pathways and altered central processing (central sensitization) both contribute to visceral hypersensitivity. Although changes in ion channel expression during inflammation increase the excitability of primary afferent neurons, it remains unclear how peripheral mechanisms contribute to altered sensations that arise in the absence of pathology.

The gastrointestinal tract is the richest source of serotonin [5-hydroxytryptamine (5-HT)] in the body, and many studies have focused on 5-HT as a mediator of visceral sensory neuron sensitization (Gershon, 1999). Most intestinal 5-HT is stored in enteroendocrine cells, where it is released locally by mechanical or chemical stimuli and can activate intrinsic and extrinsic sensory neurons, which express a variety of 5-HT receptors. Interestingly, several recent studies have reported an increased number of enteroendocrine cells in IBS patients (Bearcroft et al., 1998; Spiller et al., 2000; Houghton et al., 2003). Furthermore, the peripherally acting 5-HT3 receptor antagonist alosetron blunts visceral sensation in animal models and humans and has been used clinically in the treatment of IBS (Kozlowski et al., 2000; Camilleri et al., 2001). Although circumstantial in nature, these data raise the question whether and how 5-HT affects visceral sensation and contributes to the development of visceral hypersensitivity.

Serotonin excites mechanosensitive afferent fibers innervating the colon through effects on the ionotropic 5-HT3, as well as metabotropic 5-HT receptors (Hicks et al., 2002). Although sev-
eral studies have demonstrated effects of metabotropic 5-HT receptors on voltage-dependent ion channels in neurons (Brahaj et al., 1993; Torres et al., 1995; Cardenas et al., 2001), the mechanisms by which G-protein-coupled 5-HT receptors trigger action potentials are not known (Christian et al., 1989; Cardenas et al., 1997a,b). An attractive target is the TRPV1 channel, a proton and heat-gated channel that is also activated by endogenous lipid mediators and the pungent vanilloid capsaicin. TRPV1 is present in visceral afferent neurons and is increased in inflammatory diseases of the gastrointestinal tract (Yiangou et al., 2001) and in patients with rectal hypersensitivity (Chan et al., 2003). To test the hypothesis that 5-HT receptor activation enhances TRPV1 function, we performed whole-cell patch-clamp experiments in mouse colon sensory neurons.

Materials and Methods

Male CB57BL/6 mice (20–30 gm; Harlan, Indianapolis, IN) and TRPV1 knockout mice (TRPV1−/−; 20–30 gm; The Jackson Laboratory, Bar Harbor, ME) were used for all experiments. Mice were fed a standard laboratory diet and maintained on a 12 hr light/dark cycle (lights on 6:00 A.M. to 6:00 P.M.). All experimental procedures were approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Labeling of colon sensory neurons. Mice were anesthetized with a mixture of ketamine (17.5 mg/ml) and xylazine (2.5 mg/ml; 5 µl/gm, i.p.), and the descending colon was exposed through a paramedian incision. One microliter of the dicarbocyanine dye 1,1'-dioctadecyl-3,3',3',tetramethindocarbocyanine methanesulfonate [DiI; 100 mg in 2 ml of dimethylsulfoxide (DMSO)] was injected into five sites in the wall of the descending colon using a Hamilton microliter syringe with a 30 guage needle. Thirty seconds after the injection, the needle was carefully re-removed, avoiding leakage of dye into the peritoneal cavity. The abdomen was then closed, and mice were allowed to recover. DiI injection sites were confirmed after harvesting dorsal root ganglia (DRGs), and DiI was located in sites ~10–25 mm from the anus.

Cell preparation. Mice were anesthetized with ketamine/xylazine (as above), killed by cervical dislocation, and the L6, S1, and S2 DRGs were quickly removed bilaterally under a dissection microscope. DRGs were located in sites (accuracy, ±0.1°C). Colon sensory neurons were identified by their red-orange color under Hoffman Contrast Optics (400×) in fluorescent light with a rhodamine filter (excitation wavelength, 546 nm; barrier filter, 580 nm). Only DiI-labeled cells were studied.

Whole-cell voltage-clamp and current-clamp recordings were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Recordings were filtered at 5 kHz and digitized at 1 kHz using a Digidata 1320A interface (Axon Instruments). For current-clamp experiments, only neurons with a stable resting membrane potential of at least −40 mV were included in the study. Unless stated otherwise, all experiments were performed at room temperature.

Immunohistochemistry. Acutely dissociated DRG cells (see above) were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 10 min. After washing with 0.01 M PBS, the specimens were incubated in 3% normal goat serum (NGS; Sigma, St. Louis, MO) for 30 min at room temperature to reduce nonspecific binding and then with an N-terminal specific polyclonal TRPV1 antibody (rabbit anti-capsaicin receptor at a dilution of 1 µg/ml; Calbiochem, La Jolla, CA) in 3% NGS at 4°C overnight. Specimens were then washed with 0.01 M PBS and incubated with goat anti-rabbit IgG (1:200; Alexa Fluoro 488; Molecular Probes, Eugene, OR) in 3% NGS for 2 hr at room temperature. After washing, specimens were mounted with Fluoromount G (Electron Microscopy Sciences, Washington, PA) and coverslipped. Control experiments were performed by incubating without primary antibody, or by preincubating primary antibody with the antigenic peptide (capsaicin receptor control peptide; Calbiochem) for 4 hr at 4°C. Slides were examined with a fluorescence microscope (Optiphot; Nikon, Tokyo, Japan) equipped with separate filters. Images were captured with a model 2.3.1 SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI).

Chemicals. 5-HT, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminophenol hydrochloride (DOI), 5-methoxytryptamine (5-MeOT), (1-butyl-4-piperidinyl)methyl-8-amino-7-chloro-1,4-benzodioxane hydrochloride (SB204070), guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S), N-[2-(p-bromocinnamylamino)ethyl]-5-isouquinolinesulfonamide dihydrochloride (H-89), N-amidino-3, 5-diamino-6-chloropyrazinecarboxamide dihydrochloride (amlodine), N-[2-[4-(2-chlorophenyl)ethylamino]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carboxoamide (capsazepine), and 8-methyl-N-vanilin-trans-6-nonenamide (capsacin) were purchased from Sigma. 3-[2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl]-2,4[1H,3H]-quinoxalinedione tartrate (ketanserin), α-methyl-5-hydroxytriptamine maleate (α-Me-5-HT), troparyl 3,5-dichlorobenzoate (MDL72222), (±)-4-amino-5-chloro-N-[1-(3′,4′-AS)-3-(4-fluorophenyl)propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide (cisapride), 1-methyl-1H-indole-3-carboxylic acid, and [1-{[(3-methylsulfonyl)amin o]ethyl}-4-piperidinyl]methyl ester (GR133808) were purchased from Tocris (Ellisville, MO). The protein kinase A (PKA) inhibitor 6-22 amide, AKAP inhibitor peptide (St-Ht31), and calphostin C were purchased from Calbiochem. All chemicals were dissolved in distilled water or DMSO and applied in a final concentration of <0.1%.

Data analysis. Data are expressed as means ± SEM. The software package pCLAMP9.0 (Axon Instruments) and SigmaPlot 8.02 and SigmaStat 2.03 (SPSS, Chicago, IL) were used for data acquisition and analysis. Concentration–response curves were fitted by the Hill equation, \( I = I_{\text{max}} + \frac{(I_{\text{max}} - I_{\text{min}}) \times [X]^n}{[EC_{50}]^n + [X]^n} \), where \( I \) is the current amplitude ratio triggered by pH 5.5 before and after a given serotonin concentration, [X]; 5-HT, or the current density at different proton concentrations, \( [X] \); \( I_{\text{max}} \), or \( I_{\text{min}} \) is the maximum or minimum response of the cell; \( n \) is the Hill slope; and EC_{50} is the concentration eliciting a half-maximal response.

Data were compared using Student’s two-tailed unpaired or paired t tests or repeated-measures, one-way or two-way ANOVA as appropriate. Statistical significance was determined at p < 0.05.

Results

Temperature-dependent activation of colon sensory neurons

In wild-type mice, temperature ramps depolarized all colon sensory neurons tested, triggering bursts of action potentials near 42°C (Fig. 1a,b). When we tested the capsaicin sensitivity of wild-type neurons after temperature ramps, all of the 13 cells tested...
depolarized in response to 10 μM capsaicin. Although capsaicin did not trigger action potential in all cells when given after the initial heat stimulus, it elicited spikes when given to untreated cells. In TRPV1−/− mice, temperatures below 50°C typically generated few action potentials, presumably through activation of TRPV2 or other temperature-sensitive currents (Fig. 1c), and impulse frequency at 45°C was significantly lower in TRPV1−/− mice than in C57BL/6 mice (t = 3.49; p < 0.01) (Fig. 1d).

**Characterization of TRPV1 function in colon sensory neurons**

Inward current in colon DRG neurons developed at ~42°C with an extremely steep temperature dependency (Fig. 2a), consistent with our current-clamp data and previous reports in DRG neurons (Sugiura et al., 2002). The mean threshold temperature for activation (defined as the intersection where two lines approximating the stable baseline current and clearly increasing temperature-dependent current) was 41.8 ± 0.7°C. Similar to results obtained with current clamp, colon DRG neurons also responded to subsequent challenge with capsaicin. The amplitude of the capsaicin-evoked current was smaller than that of the heat-evoked current because of desensitization of TRPV1 activation. When given to naive cells, the peak current density evoked by capsaicin (409.8 ± 33.0 pA/pF; n = 3) was greater than that of heat (249.1 ± 60.8 pA/pF; n = 6). The capsaicin-evoked current showed a lasting desensitization; when applied a second time 1 min after the first application of capsaicin (1 μM), the response was 77 ± 4% (n = 6) of the first response to capsaicin (Fig. 2b) (F = 50.11; p < 0.001). Only 6 of 42 colon sensory neurons tested did not respond to capsaicin. In two of these capsaicin-insensitive neurons, we tested the response to temperature and only saw inward currents when the bath temperature exceeded 50°C (data not shown). None of the neurons from TRPV1−/− mice responded to capsaicin (10 μM; n = 4) (Fig. 2f).

Consistent with previous reports, all capsaicin-sensitive colon sensory neurons tested also responded to protons with slowly activating and sustained inward currents (Fig. 2b,c,f) (Caterina et al., 1997). This acid-evoked current did not desensitize significantly when neurons were repeatedly stimulated in the absence of extracellular calcium (F = 0.57; p = 0.7). However, in the presence of extracellular calcium, repetitive proton applications triggered current responses that desensitized significantly (F = 2.96; p < 0.05) (Fig. 2b). The TRPV1 blocker capsazepine (10 μM) significantly attenuated the sustained acid-evoked current (Fig. 2c, middle; d). In contrast, amiloride (200 μM) did not significantly affect the acid-evoked inward current (Fig. 2c, right; e), suggesting that proton-sensitive currents in colon sensory neurons are primarily carried through TRPV1 channels. We therefore examined acid-evoked currents in colon sensory neurons from TRPV1−/− animals. Consistent with the pharmacological data, cells from knock-out mice did not show significant sustained-type current responses to protons (Fig. 2f). To further characterize the properties of acid-evoked currents, we examined the pH dependence of these currents using solutions with pH between 7.0 and 3.5. We allowed 1 min between each 5 sec application to prevent desensitization. As shown in the left trace of Figure 2f, the current is activated at pH 5.5 with half-activation at pH 4.57 ± 0.07 (Fig. 2f, right) (n = 8).

To obtain additional evidence that colon sensory neurons express TRPV1, we examined immunoreactivity (IR) for TRPV1 in acutely dissociated DRG neurons. Figure 3a shows double staining of retrograde tracer that was injected into the colon wall and TRPV1-like IR in a DRG neuron. In control experiments performed by omitting the primary antibody (Fig. 3c) or preincubating the primary antibody with blocking peptide (Fig. 3d), immunohistochemical staining of DRG cells for TRPV1-like IR was abolished.

**5-HT enhances excitability of colon sensory neurons through TRPV1 activation**

To test the working hypothesis, we next examined the effect of 5-HT (1 μM; 2 min; bath application) on heat-evoked depolarization and action potential firing in colon sensory neurons. Although neither vehicle nor 5-HT altered resting membrane potential (−49.4 ± 1.8 mV and −46.6 ± 1.5 mV in vehicle- and
5-HT-treated neurons, respectively; n = 8/group), responses to heat were significantly enhanced in the presence of 5-HT in DRG neurons from C57BL/6 mice (Fig. 4a,c). Notably, action potentials were generated by heat at temperatures below core body temperature (Fig. 4c, broken vertical line) in the presence of 5-HT. In contrast, there was no significant temperature dependency of responses in TRPV1−/− mice or effect of 5-HT within the temperature range tested (Fig. 4b,d).

In voltage-clamp experiments, neither vehicle nor 1 μM 5-HT triggered current responses. In contrast to the desensitization seen with repeated capsaicin application (Fig. 2b), pretreatment with 5-HT (1 μM; 50 sec) between the first and second application of capsaicin not only prevented the desensitization but significantly enhanced the response to the second application of capsaicin more than twofold relative to the typically desensitized response to a second application of capsaicin (Fig. 5a). Similarly, 5-HT enhanced responses to heat. Defining the threshold temperature (Tth) as the intersection where two lines approximating the stable baseline current and the clearly increasing temperature-dependent current cross (Sugiura et al., 2002), 5-HT (1 μM; 2 min; bath application) pretreatment significantly reduced the mean Tth to 38.1 ± 1.2°C (n = 7; t = -2.50; p < 0.05) from a control 41.8 ± 0.7°C (n = 6) (Fig. 4b). Likewise, the acid-activated current, pH 5.5, was also significantly enhanced by pretreatment with 5-HT (1 μM; 50 sec) (Fig. 5c). Consistent with results obtained in neurons from TRPV1−/− mice, the TRPV1 receptor antagonist capsazepine (10 μM) significantly attenuated enhancement of the acid-activated current by 5-HT (to 19.6 ± 3.8%; n = 3) relative to pH 5.5 application in the absence of the antagonist (100.1 ± 6.8%; n = 8; t = 6.86; p < 0.001) (Fig. 5c).

to determine the concentration dependency of the enhancing effect of 5-HT, we examined the acid-activated current, pH 5.5, after exposure of cells to various 5-HT concentrations between 1 nM and 10 μM, testing each cell only once to prevent desensitization to 5-HT. Greater concentrations of 5-HT were given in the presence of MDL72222 (3 μM), a selective 5-HT3 receptor antagonist, to block activation of 5-HT3 receptors, which caused a decrease in serotoninergic enhancement of acid-evoked currents (30 and 100 μM; data not shown). Within the range between 1 nM and 10 μM, the effect of 5-HT was concentration dependent, with an EC50 value of 2.2 μM and Hill coefficient of 1.1 (Fig. 5d). Figure 5e shows the magnitude and duration of 5-HT (1 μM) enhancement of the acid-activated current, pH 5.5; 5-HT was applied once for 50 sec after the first application of acidic solution. The

to the effect of the single application of 5-HT lasted at least 7 min (n = 7; p < 0.05; vs vehicle, n = 7). When tested at a greater concentration in the same protocol, 5-HT (3 μM) enhanced the pH-activated current for at least 20 min (10 min: 2.11 ± 0.60-fold, n = 6; 20 min: 1.97 ± 0.48-fold, n = 3). As shown in Figure 5f, 5-HT (1 μM) significantly shifted the pH of half-activation from 4.57 ± 0.07 (n = 8) to 5.14 ± 0.09 (n = 7; t = 5.06; p < 0.001) and Hill coefficient of 1.69 ± 0.35 and 3.86 ± 0.45, respectively.
5-HT evoked a slowly activating current in seven of eight colon sensory neurons, which increased in amplitude and showed a more rapid activation and desensitization at higher concentrations of 5-HT, consistent with the expression of the ligand-gated 5-HT_{3} receptor (Fig. 6a). To confirm this, we used the selective 5-HT_{3} receptor antagonist MDL72222 (0.6 μM), which completely and reversibly blocked the inward current produced by 10 μM 5-HT in colon neurons (8.4 ± 3.6% of control; n = 6; t = 25.3; p < 0.001) (Fig. 6b). MDL72222 (0.6 μM), however, did not affect serotonergic enhancement of the acid-activated inward current in colon sensory neurons (Fig. 6c), revealing that metabotropic 5-HT receptors mediate this effect.

Six families of G-protein-coupled 5-HT receptors with several members have been characterized (Barnes and Sharp, 1999). Of those, 5-HT_{1}, 5-HT_{2}, and 5-HT_{4} receptors are linked with either G_{q/11}, which is known to stimulate phospholipase C (PLC) and result in activation of PKC, or G_{o}, which is known to stimulate adenylyl cyclase and result in activation of PKA, respectively, we examined the involvement of protein kinase pathways by adding selective PKA or PKC inhibitors to the internal pipette solution. The PKA inhibitors H-89 and PKI_{6-22} blocked the 5-HT-induced enhancement of TRPV1 function (Fig. 8a,d). Similarly, the PKC inhibitor calphostin C reduced serotonergic enhancement of TRPV1 function (Fig. 8a,d).

To examine the contribution of PKA and PKC in 5-HT-mediated facilitation of TRPV1 function in more detail, we tested selective 5-HT_{2} or 5-HT_{4} agonist-mediated facilitation. Consistent with the finding linking the 5-HT_{3} receptor with PLC and PKC, the selective 5-HT_{3} receptor agonist DOI-mediated enhancement of TRPV1 function was attenuated by calphostin C.
(Fig. 8b,e). Interestingly, PKI₆₋₂₂ and A-kinase anchoring protein inhibitor St-Ht-31 also significantly attenuated DOI-mediated enhancement of TRPV1 function (Fig. 8b,e). Similarly, enhancement of TRPV1 by the selective 5-HT₄ receptor agonist 5-MeOT was blocked by PKI₆₋₂₂ and AKAP inhibitor peptide, but not by calphostin C, consistent with the known coupling between the 5-HT₄ receptor and adenylyl cyclase (Fig. 8c,f).

**Mouse core temperature**

Considering the shift in temperature-dependent action potential firing of colon sensory neurons by 5-HT, we measured colon temperature in C57BL/6 mice. The rectally obtained core temperature of 37.8 ± 0.3°C (n = 5) falls within the range of a significant increase in spontaneous firing because of facilitation of TRPV1 function.

**Discussion**

The extrinsic, afferent innervation of the gastrointestinal tract conveys information to the CNS that gives rise to the sensations of pain and discomfort. Most afferent input from the visera, however, is not consciously perceived under normal circumstances. However, patients with functional disorders such as IBS, in which pain and discomfort are common, experience these sensations in the absence of noxious intensities of stimulation or apparent pathology, suggesting the presence of hypersensitivity. In whole-cell patch-clamp recordings from identified colon sensory neurons, we demonstrated a significant leftward shift in the temperature sensitivity of TRPV1 by 5-HT to include activation of TRPV1 at normal body temperature. This suggests that 5-HT release from enteroendocrine cells by normal mechanical or chemical stimuli could play a role in development of the altered sensations that arise in IBS patients.

Although we did not use heterologous expression systems, the present results are interpreted in the context of TRPV1 (proton, heat, and capsaicin activation) and supported by use of TRPV1⁻/⁻ mice, previously reported to exhibit attenuated thermal responsiveness (Caterina et al., 2000; Davis et al., 2000) and immunocytochemistry. Although there are many receptors that transduce thermal energy (Patapoutian, 2003), enhancement of responses to temperature, protons, and capsaicin by 5-HT was absent in TRPV1⁻/⁻ mice, pointing at an important role of this channel in modulating colon sensory neuron excitability.

Consistent with documentation of TRPV1-like IR in mouse colon neurons (Robinson et al., 2004), >80% of the colon DRG neurons tested here responded to capsaicin or generated slowly sustained currents in response to acid that were blocked by capsaizpeine. This differs from results obtained in unidentified DRG neurons, in which <50% of cells responded to capsaicin (Dirajal et al., 2003), consistent with studies showing mRNA or IR for TRPV1 in <50% of DRG neurons (Caterina et al., 1997; Michael and Priestley 1999; Robinson et al., 2004). Similarly, the response to acid appears to be distinct between identified visceral DRG neurons and unidentified DRG neurons. In colon sensory neurons from C57BL/6 mice, protons almost exclusively activated...
sustained currents that were blocked by capazepine, but not by amiloride, and thus likely are attributable to activation of TRPV1. A previous study of unidentified DRG neurons reported a mixture of transient and sustained acid-sensitive currents, both of which were significantly inhibited by amiloride (Dirajlal et al., 2003), suggesting a significant contribution of ASICs. In addition, the majority of the mouse colon DRG neurons in the present study responded to heat, whereas in a previous study only about half of unidentified DRG neurons were heat sensitive (Nagy and Rang, 1999). Because the fraction of capsaicin-sensitive neurons innervating the rat colon is lower (Su et al., 1999), direct comparisons between identified somatic and visceral neurons in the same species are necessary to determine whether colon sensory neurons actually have properties distinct from other, somatic DRG neurons.

The present results point at a potential role of TRPV1 as a modulator of neuron excitability. Changes in TRPV1 function through phosphorylation or release from inhibition through lipid mediators have previously been reported in response to bradykinin, ATP, nerve growth factor, and prostaglandin (Chuang et al., 2001; Tominaga et al., 2001; Bhave et al., 2003). Although these mediators play an important role in changes attributable to injury and inflammation, the effects of 5-HT on colon sensory neurons provides a mechanism by which physiological stimuli can trigger enhanced responses in normal tissue. Mucosal stimuli trigger 5-HT release from enteroendocrine cells, which in turn activates intrinsic and extrinsic primary afferent neurons within the gut wall (Pan and Gershon, 2000; Hicks et al., 2002). Given the apparent expression of TRPV1 in nearly all C57BL/6 colon DRG neurons, and the generation of spontaneous action potentials at normal body temperature after exposure to 5-HT, the activation of peripheral afferents may contribute to sensation and even conscious perception of visceral stimuli in the uninflamed gut.

Serotonin enhanced responses to TRPV1 activation at con-
The proton-binding kinetics to TRPV1 in colon DRG neurons.

Several signaling pathways converge on TRPV1 to modulate its activity and, as shown in this and previous studies, alter neuron excitability (Bhave et al., 2003; Carr et al., 2003; Moriyama et al., 2003; Dai et al., 2004; Ferreira et al., 2004; Liu et al., 2004; Premkumar et al., 2004; Puntambekar et al., 2004). The potential role of TRPV1 in integrating different physical, chemical, and inflammatory signals and the comparatively high number of capsaicin-responsive colon sensory neurons support the relevance of this channel in sensation and visceral nociception. Consistent with these considerations, luminal application of capsaicin or capsaicin injection into the gut wall lowers sensory threshold or triggers pain in humans (Drewes et al., 2003; Schmulson et al., 2003; Lee et al., 2004). Interestingly, prolonged administration of red pepper, which contains capsaicin, improved symptoms in patients with functional dyspepsia (Bortolotti et al., 2002), likely because of neurotoxic effects of persistent TRPV1 stimulation that leads to transient analgesia in experimental models of neuropathic or inflammatory pain (Karai et al., 2004).

Previous studies demonstrated the presence of metabotropic 5-HT receptors in intestinal afferent neurons and suggest that only a subgroup express 5-HT3 receptors (Hillsley and Grundy,
The peripherally acting 5-HT3 receptor antagonist alosetron and the blood brain barrier may exert both peripheral and central effects. The multiplicity of 5-HT effects (Cooke et al., 1997; Jin et al., 1999; Michel et al., 1998) of 5-HT4 receptors and their expression in gastrointestinal smooth muscle cells and intrinsic neurons complicates the interpretation of 5-HT effects (Cooke et al., 1997; Jin et al., 1999; Michel et al., 1998). Spinal 5-HT receptors also play important roles in descending modulation of pain, including visceral pain (Urban and Gebhart, 1999), and drugs that cross the blood brain barrier may exert both peripheral and central effects.

The peripherally acting 5-HT3 receptor antagonist alosetron blunts responses to colorectal distension in experimental animals and improves discomfort and pain in a subgroup of patients with functional bowel disorders (Camilleri et al., 1999; Kozlowski et al., 2000). Interestingly, other 5-HT3 receptor antagonists do not consistently affect visceral pain in experimental animals or humans (Langlois et al., 1996; Kim and Camilleri, 2000). Less is known about the effects of agonists and antagonists of metabotropic 5-HT receptors on visceral sensation. The recently introduced 5-HT4 receptor agonist tegaserod accelerates colonic transit but does not consistently affect sensory function in humans (Coffin et al., 2003; Lacy and Yu, 2002). Although a peripherally acting 5-HT4 receptor antagonist has been reported to blunt somatic and visceral pain in mice, sensory thresholds to colorectal distension were not affected by inhibition of the 5-HT3 receptor in humans (Espejo and Gil, 1998; Bharucha et al., 2000).

The present findings provide new information about a potential peripheral mechanism that contributes to visceral hypersensitivity. Additional studies in vitro and in vivo are needed to determine whether inhibition of TRPV1 channels or their modulation through 5-HT and/or other signaling pathways may be useful therapeutic targets in the treatment of visceral pain syndromes.

References

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