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The relationship between inflammation-induced neuronal excitability and disrupted motor activity in the guinea pig distal colon

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Abstract

Background *Colitis is associated with increased excitability of afterhyperpolarization neurons (AH neurons) and facilitated synaptic transmission in the myenteric plexus. These changes are accompanied by disrupted propulsive motility, particularly in ulcerated regions. This study examined the relationship between myenteric AH neuronal hyperexcitability and disrupted propulsive motility.* ***Methods*** *The voltage-activated Na^+ channel opener veratridine, the intermediate conductance Ca^{2+}-activated K^+ channel inhibitor TRAM-34 and the 5-HT_4 receptor agonist tegaserod were used to evaluate the effects of neuronal hyperexcitability and synaptic facilitation on propulsive motility in normal guinea pig distal colon. Because trinitrobenzene sulfonic acid (TNBS)-colitis-induced hyperexcitability of myenteric afferent neurons involves increases in hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel activity, the HCN channel inhibitors Cs^+ and ZD7288 were used to suppress AH neuronal activity in TNBS-colitis.* ***Key Results*** *In non-inflamed preparations, veratridine halted propulsive motility ($P < 0.001$). The rate of propulsive motor activity was significantly reduced following addition of TRAM-34 and tegaserod ($P < 0.001$). In TNBS-inflamed preparations, in which motility was temporarily halted or obstructed at sites of ulceration, both Cs^+ and ZD7288 normalized motility through the inflamed regions. Immunohistochemistry studies demonstrated that the proportion of AH neurons in the myenteric plexus was unchanged in ulcerated regions, but there was a 10% reduction in total number of neurons per ganglion.* ***Conclusions and Inferences*** *These findings support the concept that inflammation-induced neuroplasticity in myenteric neurons, involving changes in ion channel activity that lead to enhanced AH neuronal excitability, can contribute to impaired propulsive colonic motility.*

Keywords *colitis, gastrointestinal motility, myenteric neurons, neuronal hyperexcitability, peristaltic reflex.*

Abbreviations: AH neuron, afterhyperpolarization neuron; AHP, afterhyperpolarization; GIMM, gastrointestinal motility monitor; HCN, hyperpolarization-activated, cyclic nucleotide gated, non-selective cation channels; ICC, interstitial cells of Cajal; I_h, hyperpolarization activated current; IK_{Ca}, intermediate-conductance calcium-activated potassium channel; S neuron, synaptic neuron; TNBS, trinitrobenzene sulfonic acid.

INTRODUCTION

Over the past 2 decades, the components of gut neural circuitry, and many of the ionic conductances that underlie enteric neurotransmission have been identified.[1] One neuron at the afferent arm of myenteric circuits regulating motility is the afterhyperpolarization neuron (AH neurons). Afterhyperpolarization neurons project to the mucosa and respond to changes in the chemical nature of lumenal contents and physical forces acting on the intestinal wall by initiating motor responses.[2–4] Two aspects of AH neuron physiology influence their capacity to respond to such stimuli. Firstly, AH neurons are synaptically coupled, forming reciprocal networks that communicate via slow

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excitatory postsynaptic potentials. Secondly, AH neurons exhibit a characteristic, prolonged afterhyperpolarization (AHP) following the action potential that temporarily limits their excitability. This AHP is mediated by an intermediate-conductance calcium-activated potassium channel (IK_{Ca}).[5,6] The resulting hyperpolarization opens hyperpolarization-activated, cyclic-nucleotide gated (HCN), non-specific cation channels to produce a hyperpolarization-activated current (I_h) that helps restore resting membrane potential.[7,8] Thus, AH neuronal excitability follows a cyclic pattern with potential to synchronize firing within the network. Furness and colleagues have demonstrated that *in vivo* administration of the IK_{Ca} channel blocker, TRAM-34 leads to disrupted motor patterns in the small intestine.[9] They suggested that the AHP determines the synchrony of action potential firing amongst AH neurons, and that this coordination, in turn, influences motor neuron activity and resulting patterns of muscle contractions. Furthermore, using a computer modeling paradigm, Bornstein and colleagues have proposed that the prolonged AHP of AH neurons contributes to the stability of the neural network.[10]

A number of studies of intestinal inflammation have demonstrated that AH neurons are particularly vulnerable to changes leading to hyperexcitability, including a reduction in the magnitude of the AHP.[11] In 2,4,6-trinitrobenzene sulfonic acid (TNBS)-colitis, myenteric AH neurons exhibit a smaller AHP and slower accommodation and have increased spontaneous activity and anodal break action potentials. These changes are due, at least in part, to an increase in I_h.[12] The myenteric synaptic (S) neuron category includes neurons that serve as interneurons and motor neurons, and some S neurons are mechanosensitive. In TNBS-colitis, synaptic inputs to S neurons are augmented.[12,13] Propulsive motility is decreased[14] particularly in ulcerated regions,[15] and it is possible that enhanced myenteric neurotransmission and disrupted motor activity are linked, but this relationship has not been previously investigated.

The aim of this study was to determine whether enhanced myenteric neurotransmission contributes to inflammation-induced dysmotility. We tested whether pharmacological manipulations that mimic the effects of inflammation on myenteric neurons disrupt colonic motility, and whether suppression of AH neuronal excitability could restore motility following inflammation. As AH neuronal excitability is governed largely by the AHP, we tested the effects of the IK_{Ca} channel blocker, TRAM-34 on propulsive motility in the normal colon and the effects of the HCN channel blockers, Cs^+ and ZD7288 on motility in TNBS-colitis. We also evaluated the integrity of myenteric ganglia in ulcerated regions to determine whether disruption of the myenteric plexus could contribute to disrupted motility. The results of this study support the concept that increased neuronal activity, in the form of AH neuronal hyperexcitability and synaptic facilitation, can disrupt the peristaltic reflex activity in a given region, and therefore impair propulsive motility.

MATERIALS AND METHODS

Animals

Guinea pigs weighing 250–350 grams (Charles River, Montreal, QC, Canada) were housed in the University of Vermont Animal Resources Center, given *ad libitum* access to food and water, and maintained on a 12:12 h light–dark cycle with an ambient temperature of 20–26 °C. Animals were euthanized by isoflurane overdose and exsanguination, and the distal colon removed. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

TNBS administration

Colitis was induced by injection of 0.3 mL TNBS (25 mg mL^{-1} in 30% ethanol; Fluka, Milwaukee, WI, USA) through an intracolonic catheter inserted 7 cm into the rectum under isoflurane anesthesia (4% induction, 2% maintenance in O_2). Animals were euthanized on day 6 post-TNBS, and severity of colitis was assessed by weight loss and determination of a damage score.[14,16,17] Briefly, this score is determined by wall thickness (in mm) and presence of hyperemia, adhesions and/or diarrhea, as well as the presence and extent of ulceration. Trinitrobenzene sulfonic acid-inflamed colons in the present study had an average score of 7.4 (range 4.3–9.4), which is comparable with previous reports.[14,17] As has been described previously, we defined ulcerations as disruptions of the mucosal surface visible macroscopically or with the aid of a dissection microscope.[15]

Motility analysis

The guinea pig distal colon allows for evaluation of propulsive motility *ex vivo*. For these studies, the Gastrointestinal Motility Monitor (GIMM; Catamount Research and Development, Inc., St. Albans, VT, USA) was used to record propulsion velocities from colons of naïve guinea pigs and inflamed colons 6 days post-TNBS. Segments (approximately 5 cm) were pinned at both ends in a Sylgard-lined organ bath perfused with recirculating warmed and oxygenated (95% O_2, 5% CO_2) Krebs solution at a flow rate of 10 mL min^{-1} (mmol L^{-1}: NaCL, 121; KCL, 5.9; $CaCL_2$, 2.5; $MgCL_2$, 1.2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 8; Sigma, St. Louis, MO, USA). An epoxy-coated pellet was inserted into the oral end to initiate peristalsis and the motility pattern was tracked with a digital camera coupled to GIMM analysis software. At least three trials, lasting 5 min each, were acquired after a 30 min equilibration period, with 5 min between each trial.

Intracellular recording

The mucosal and submucosal layers were removed to allow for recordings from circular muscle cells to assess the effects of compounds on smooth muscle function, and for neuronal record-

ings, the circular muscle was also removed. Dissected preparations were bathed in 37 °C oxygenated Krebs solution circulating at a flow rate of 10 mL min^{-1}. Glass microelectrodes with resistances ranging from 80 to 100 MΩ were filled with 2.0 mol L^{-1} KCl. Smooth muscle bundles and myenteric ganglia were visualized through an inverted microscope (Nikon Diaphot, Melville, NY, USA) and randomly impaled. Transmembrane potential was measured with an Axoclamp-2A amplifier (Axon Instruments, Union City, CA, USA), and electrical signals were acquired and analyzed using PowerLab Chart (version 5.01; ADInstruments, Castle Hill, NSW, Australia). Afterhyperpolarization neuron were identified by a characteristic shoulder on the repolarizing phase of the action potential, which persists in TNBS-treated preparations, and by the prolonged AHP following one or more action potentials.

Immunohistochemistry

In some preparations, regions where dysmotility was observed were fixed overnight at 4 °C in 2% paraformaldehyde and 0.2% picric acid in phosphate buffered saline (PBS, pH 7.4). The mucosal, submucosal and circular muscle layers were dissected to reveal the myenteric plexus and incubated for 2 h in blocking solution containing 0.5% Triton X-100 and 4% normal goat serum. Sections were incubated overnight with a 1:200 dilution of mouse anti-HuC/D (Molecular Probes, Carlsbad, CA, USA) and 1:1000 rabbit anti-calbindin (Swant, Bellinoza, Switzerland). Following three 15 min washes in PBS, sections were incubated for 2 h in a 1:500 dilution of CY3-conjugated goat anti-rabbit and 1:100 FITC-conjugated goat anti-mouse secondary antiserum (Jackson ImmunoResearch, West Grove, PA, USA). Sections were rinsed three times for 15 min in PBS, followed by a final rinse in dH_2O, and then mounted on FisherBrand glass slides with Citifluor anti-fade mounting medium (Electron Microscopy Services, Washington, PA, USA).

Image analyses

HuC/D-positive neurons (representing all neurons) and calbindin-positive neurons (representing AH neurons) were counted in myenteric ganglia using an Olympus AX70 fluorescence microscope (Olympus America, Inc., Melville, NY, USA) with an Optronics MagnaFire digital camera (Optronics, Goleta, CA, USA) with filter sets for CY3 (excitation 510–550 nm; emission 590 nm) and FITC (excitation 470 nm; emission 525 nm). Counts were averaged from 30 ganglia each from untreated colons, and ulcerated and non-ulcerated regions from TNBS-inflamed preparations. The non-ulcerated samples were at least 1 cm away from the nearest ulcer. Images were saved to an Apple computer (Apple, Cupertino, CA, USA) and cropped using Adobe Photoshop (Version 7.0; Adobe Systems, San Jose, CA, USA).

Experimental compounds

Veratridine and TRAM-34 were purchased from Sigma-Aldrich (St. Louis, MO, USA), ZD7288 was purchased from Tocris Bioscience (Ellisville, MO, USA) and tegaserod was acquired as a gift from Dr. John McRorie of Proctor and Gamble. Veratridine, TRAM-34 and tegaserod were initially dissolved in DMSO and used at final concentrations of ≤0.1% DMSO. ZD7288 was dissolved in water.

Data analysis

Statistical analyses were performed using GraphPad Prism (Version 5.0a; GraphPad Software, La Jolla, CA, USA). Differences between control and inflamed groups were determined by unpaired Student's *t*-test or a one-way ANOVA with Bonferroni multiple comparisons test. A P value < 0.05 was considered to be statistically significant. Data presented are means ± SEM for n animals.

RESULTS

Effects of drugs that target enteric neurons on propulsive motility in the normal colon

We hypothesized that neuronal hyperexcitability in the inflamed colon results in a discoordination of peristaltic reflex activity and subsequent decrease in the rate of propulsive motility. This hypothesis was initially tested with veratridine (1.0 μmol L^{-1}) an activator of voltage activated Na^+ channels that causes repetitive firing of myenteric neurons.[18] When veratridine was added to the bathing solution, spontaneous contractions in the muscle were still observed (Supplemental data Movie S1), yet propulsion was completely inhibited (Fig. 1; P < 0.0001; n = 6).

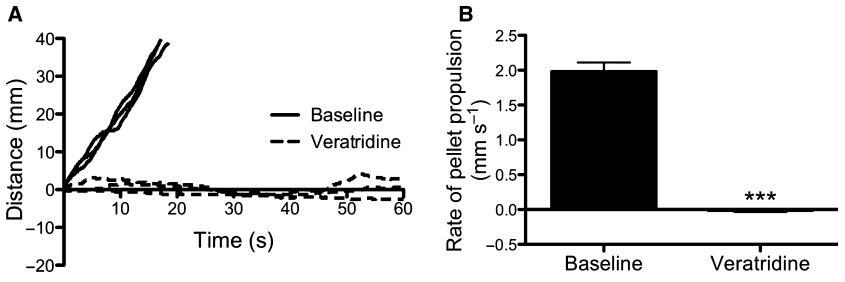

Figure 1 Enhanced neuronal excitability inhibits colonic pellet propulsion. (A) Time–distance plots representing the movement of a fecal pellet along a segment of distal colon in individual trials before (solid line) and after (dashed line) bath application of the voltage-gated Na^+ channel opener, veratridine (1 μmol L^{-1}) in a naïve guinea pig distal colon. Each line represents a single trial within the experiment. (B) Graph of the rate of fecal pellet propulsion demonstrating that application of veratridine eliminates propulsive motility (n = 6). Graph represents mean velocity ± SEM. *** indicates P < 0.0001.

In an effort to reproduce the neurophysiological changes observed in TNBS-colitis, we evaluated the effects of increasing AH neuronal excitability in naïve preparations. The IK_{Ca} channel blocker TRAM-34 depolarizes and reduces the AHP of myenteric AH neurons, leading to an associated increase in excitability.[19,20] Therefore, inhibition of these channels should mimic the AH neuron hyperexcitability previously described in experimental models of inflammation.[12,17,21–23] When TRAM-34 (10 μmol L^{-1}) was added to the bath, there was a significant decrease in the rate of propulsion (Figs 2A,B; control, 2.2 ± 0.1 mm s^{-1}; TRAM-34, 1.86 ± 0.1 mm s^{-1}; $P < 0.01$; one-way ANOVA, $n = 6$). As IK_{Ca} channel expression has been reported on epithelial cells,[24,25] we tested the effects of intralumenal TRAM-34, applied from the oral end via an intralumenal cannula, and found no effect on motility (Fig. 2C). Furthermore, we found that TRAM-34 did not affect the membrane potential or slow wave frequency in circular muscle cells (Fig. 2D; $n = 4$ cells).

As increased synaptic transmission also occurs in the myenteric S neurons of the TNBS-inflamed colon,[12,13] we evaluated whether facilitation of synaptic transmission with the 5-HT_4 agonist tegaserod would lead to a further disruption of motility. Agonists of the 5-HT_4 receptor have been shown to enhance fast synaptic transmission in S neurons via a presynaptic facilatatory mechanism.[26,27] When tegaserod (1.0 μmol L^{-1}) was added with TRAM-34, we observed a decrease in propulsion to a rate of 1.5 ± 0.1 mm s^{-1}, which was significantly slower than measured with TRAM-34 alone (Figs 2A,B; $P < 0.001$ *vs* control and *vs* TRAM-34 alone; one-way ANOVA, $n = 6$). These results suggest that increased excitability of myenteric neurons and synaptic facilitation can act together to decrease propulsive motility in TNBS-colitis.

Altered motility along the TNBS-inflamed distal colon

In the normal *ex vivo* guinea pig distal colon, propulsive motility of fecal pellets is fairly linear and velocity in the range of 1.8–2.3 mm s^{-1} (14, 15, 17, 21, current study). In some control preparations, brief pauses, lasting <5 s were observed. In TNBS-colitis ($n = 11$), we observed two patterns of disrupted motility that were similar to those previously described.[15] One pattern involved temporary halting of the fecal pellet at an ulcer, typically lasting 10–15 s, and the other pattern involved obstruction at the ulcerated site. In

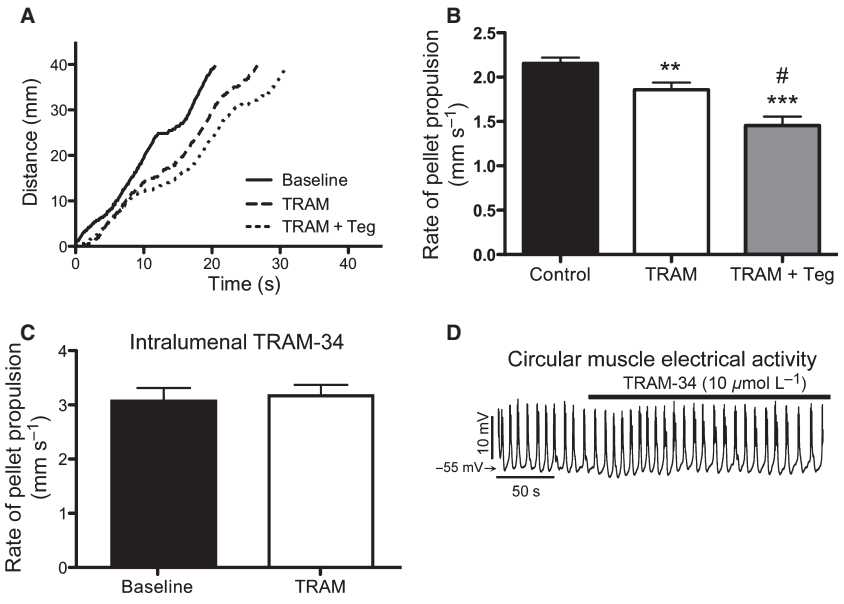

Figure 2 Application of the IK inhibitor, TRAM-34, and the 5-HT_4 receptor agonist, tegaserod, decrease the rate of colonic propulsive motility in naïve tissue. (A) Time–distance plot demonstrating the movement of a fecal pellet along a segment of distal colon in normal Krebs' solution (solid line), following addition of 10 μmol L^{-1} TRAM-34 (dashed line), and in the presence of TRAM-34 plus 1 μmol L^{-1} tegaserod (dotted line). (B) Graph demonstrating that addition of TRAM-34 to the circulating Krebs' solution causes a significant decrease in the rate of propulsive motility, and a further decrease in the rate of motility occurs when tegaserod is also added to the bathing solution (**indicates $P < 0.01$ *vs* control, ***indicates $P < 0.001$ *vs* control; # indicates $P < 0.001$ *vs* TRAM-34 alone; $n = 6$). (C) Graph demonstrating that intralumenal administration of TRAM-34 did not alter the rate of propulsive motility ($P = 0.47$; $n = 6$). (D) Representative intracellular recording from a circular smooth muscle cell demonstrating that TRAM-34 did not alter the resting membrane potential or slow wave activity in colonic smooth muscle ($n = 4$).

the case of obstructed motility, the investigator had to facilitate the movement of the pellet through the disrupted region by infusing a bolus of Krebs solution into the lumen.

Effects of HCN channel inhibition on propulsive motility in the inflamed colon

We have previously reported that myenteric AH neuronal hyperexcitability in TNBS-colitis involves an increase in the I_h.[12] Suppression of the I_h with Cs^+ in inflamed preparations restores the magnitude of the AHP and reduces AH neuron excitability. Therefore, in the current study, we evaluated the effects of I_h inhibition on propulsive motility in TNBS-colitis.

In normal preparations, which typically exhibit a linear time-distance relationship, bath application of the I_h inhibitor, ZD7288 (10 μmol L^{-1}) did not affect the rate or pattern of propulsive motility (Figs 3A,B). When ZD7288 was applied to TNBS-inflamed colons, it largely restored propulsive motility, resulting in relatively smooth and rapid transit of the fecal pellet through the regions of previously disrupted motility (n = 6). In preparations exhibiting complete obstruction of motility (Supplemental data Movie S2) (Figs 3C,D) or temporarily halted motility (Fig. 3D), the pellet traversed the ulcerated region in a linear trajectory following the addition of ZD7288 to the bathing solution. To ensure that the restorative effects of ZD7288 on propulsive motility were not due to a non-specific action of the compound, we also examined the actions of 2 mmol L^{-1} CsCl on propulsive motility in TNBS-colitis. As was observed with ZD7288, addition of CsCl to the bathing solution improved the passage of the fecal pellet through halted or obstructed regions (n = 5; Figs 3E,F).

Previous studies have detected I_h in AH neurons, but not S neurons,[7,8] and I_h has not been reported in smooth muscle cells or interstitial cells of Cajal (ICC). However, to confirm the site of action of I_h inhibition, we examined the effects of ZD7288 on smooth muscle cells, AH neurons and S neurons. ZD7288 (10 μmol L^{-1}) did not affect the membrane potential, slow wave activity or spontaneous junction potentials

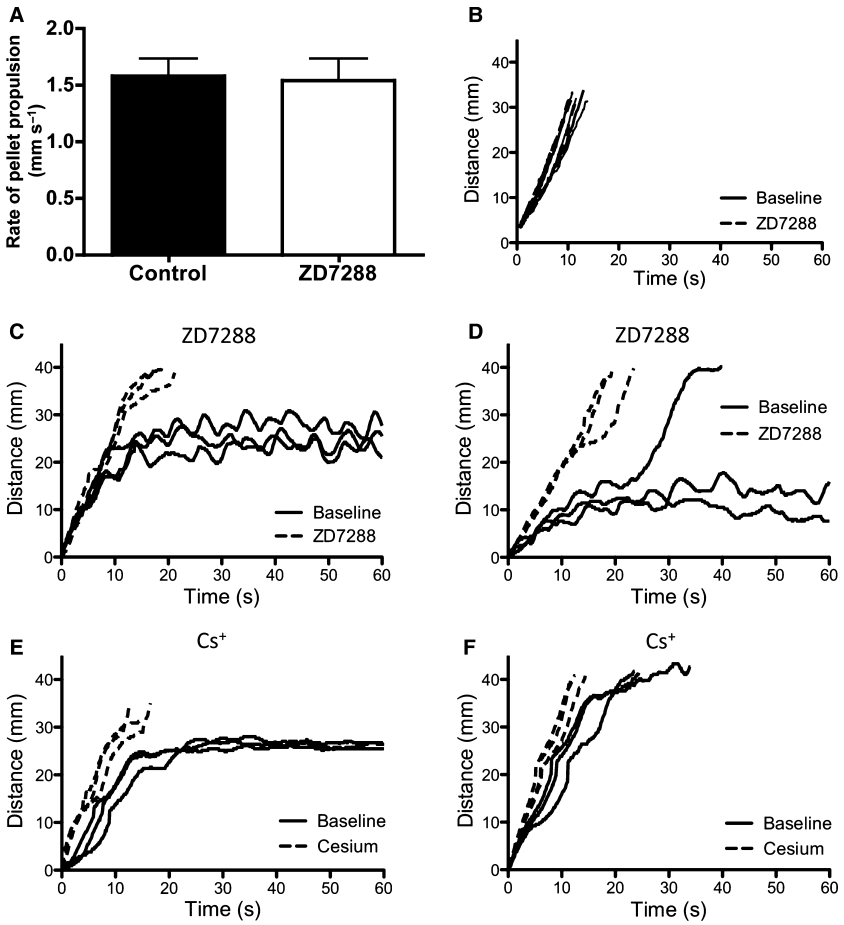

Figure 3 Disrupted peristalsis observed in the TNBS-inflamed colon can be normalized by addition of ZD7288 to the bathing solution. (A) Graph demonstrating there is no effect of intralumenal application of 10 μmol L^{-1} ZD7288 on the rate of propulsive motility in the naïve distal colon (P = 0.9; n = 5). (B) Time-distance plot demonstrating the pattern of a fecal pellet along a segment of distal colon in normal Krebs' solution (solid line) is unchanged following addition of 10 μM ZD7288 (dashed line). (C–F) Sixty second time–distance plots from the colons of four different TNBS-treated colons demonstrating that propulsive motility is obstructed or temporarily halted in normal Krebs' solution (solid lines). These areas of disrupted motility typically occur in ulcerated regions. Upon application of 10 μmol L^{-1} ZD7288 or 2 μmol L^{-1} CsCl (dashed lines) to the bathing solution, propulsive motility is improved and the fecal pellet proceeds through the regions of disrupted motility.

in circular muscle (Figs 4A,B; n = 4,12). This suggests that at the concentration used in this study, ZD7288 has little or no effect on basal smooth muscle or ICC function, or spontaneous neuromuscular transmission. Intracellular recordings were obtained from 1 AH neuron and 6 S neurons from 3 TNBS-inflamed preparations. Similar to the effect of Cs^+,[12] ZD7288 decreased the number of action potentials generated during a depolarizing current pulse, and reduced the sag on the electrotonic potential, which is a feature of I_h, during a hyperpolarizing current pulse (Figs 4C,D). On the other hand, ZD7288 had no apparent effect on the passive or active electrical properties of the 6 S neurons tested. This is consistent with previous studies in which I_h has been studied in AH neurons, but was not detected in S neurons.[7,8] Taken together, these findings suggest that a primary site of action of ZD7288 and Cs^+ is on myenteric AH neurons, and that attenuating I_h activity in the inflamed colon promotes propulsive motility through ulcerated regions.

Evaluation of the integrity of the myenteric plexus in ulcerated regions of the TNBS-inflamed colon

The results described above indicate that inflammation-induced changes in the neurophysiological properties of the myenteric plexus likely contribute to disrupted motility in ulcerated regions. Another possible factor is a loss of myenteric ganglia in the ulcerated regions, where motility is most extensively disrupted. We have previously evaluated the effects of TNBS-colitis on neuronal survival in the guinea pig and detected a loss of roughly 20% of the neurons that is indiscriminate with regard to neuronal subtype.[28] However, in that study, inflamed preparations including both ulcerated regions and adjacent non-ulcerated inflamed sections of colon were quantified. A study of the myenteric plexus in rat TNBS-colitis revealed an elimination of myenteric ganglia in ulcerated regions as well as regions immediately adjacent to ulcers.[29] Therefore, in the current study, we confirmed that ganglia were intact in the ulcerated region, and we counted neurons in ulcerated and adjacent non-ulcerated regions and compared to normal controls.

In whole-mount longitudinal muscle – myenteric plexus preparations from guinea pigs 6 days post-TNBS, the myenteric plexus was largely intact (Fig. 5A). The number of neurons per ganglion in both the ulcerated and non-ulcerated regions of TNBS-inflamed preparations was approximately 10% less than in ganglia of control preparations (Fig. 5B) (control, 98.6 ± 2.0 neurons/ganglion; ulcerated, 87.9 ± 2.8 neurons/ganglion, $P < 0.05$ *vs* control; non-ulcerated, 85.9 ± 2.6; n = 6, $P < 0.01$ *vs* control; n = 5–6). However, there was no change in the proportion of calbindin-immunoreactive (AH) neurons per ganglion in the ulcerated regions or non-ulcerated regions of colons from animals 6 days post-TNBS as compared to controls (Fig. 5C) (control, 5.2 ± 0.8% calbindin + neurons/ganglia; ulcerated, 5.0 ± 0.19% calbindin +

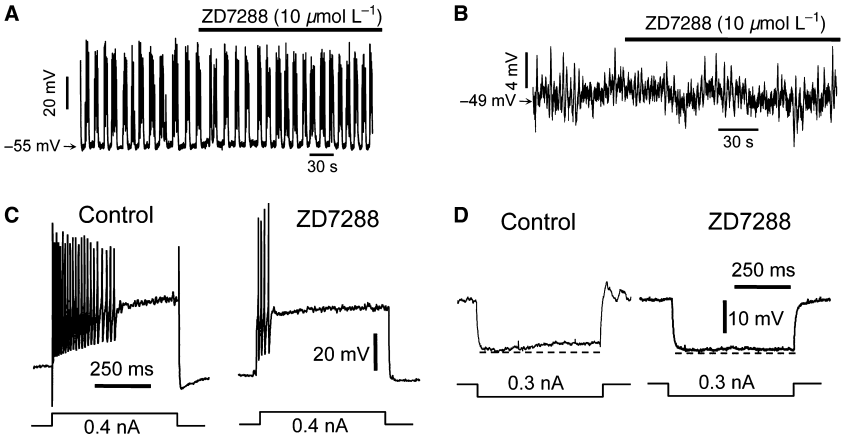

Figure 4 The hyperpolarization-activated current (I_h) inhibitor ZD7288 does not alter smooth muscle activity in normal tissue, but attenuates AH neuron hyperexcitability in trinitrobenzene sulfonic acid (TNBS) colitis. (A, B). Representative intracellular recordings of circular muscle cells, in naïve preparations, before and during bath application of ZD7288 demonstrating that 10 μmol L^{-1} ZD7288 did not alter the resting membrane potential, slow wave activity, or spontaneous junction potentials in colonic smooth muscle (n = 4–12). (C, D) Intracellular recordings from an AH neuron in a TNBS-inflamed preparation. The number of action potentials in response to a depolarizing current pulse was decreased (C) and the sag on the electrotonic potential during a hyperpolarizing current pulse, that is due to the I_h current (area above dashed lines in D), was reduced following application of 10 μmol L^{-1} ZD7288.

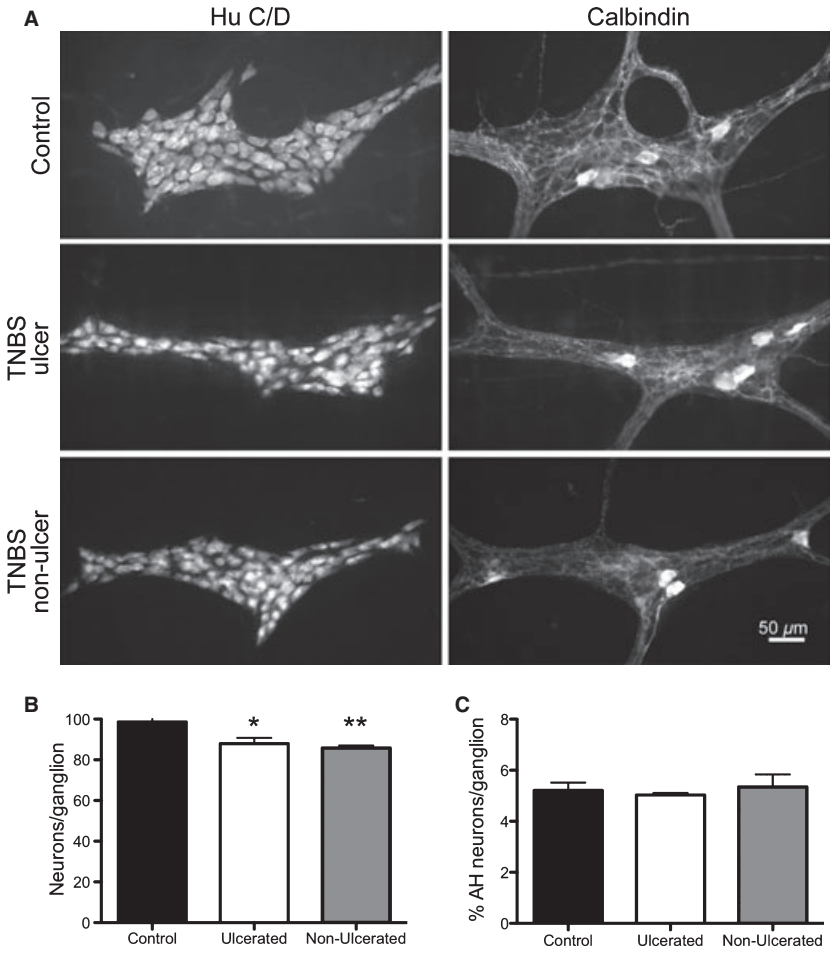

Figure 5 The proportion of myenteric Afterhyperpolarization neurons is unchanged in ulcerated regions of trinitrobenzene sulfonic acid (TNBS) colitis. (A) High power photomicrographs of myenteric ganglia immunostained for HuC/D, a pan-neuronal marker that labels all neurons, and calbindin D28, which labels AH neurons. (B) Graph demonstrating the mean number of neurons per ganglion that was HuC/D-immunoreactive in control colons and ulcerated or non-ulcerated regions of TNBS-inflamed preparations (*indicates $P < 0.05$; **indicates $P < 0.01$ compared with control; ANOVA, $n = 5–6$). (C) Graph showing the proportion of calbindin-positive neurons per ganglion in control colons and ulcerated or non-ulcerated regions of TNBS-inflamed preparations ($P > 0.05$; $n = 5–6$).

neurons/ganglion; non-ulcerated, 5.4 ± 1.09% calbindin + neurons/ganglion; $n = 6$, $P > 0.05$ *vs* control; $n = 5–6$). These findings indicate that the proportion of AH neurons within the myenteric plexus is maintained in guinea pig TNBS-colitis.

DISCUSSION

Intestinal inflammation causes alterations in myenteric neural function, including AH neuronal hyperexcitability and facilitated synaptic transmission in animal models of colitis.[12,13] We have previously shown these changes are associated with disrupted propulsive motility in the guinea pig distal colon, and that they persist for weeks beyond the recovery of inflammation,[14,17] yet the relationship between altered neurotransmission and colonic motility is not clear. This investigation was conducted to test the hypothesis that altered function in myenteric AH neurons contributes to colonic dysmotility. Our findings indicate that altering ion channel functions to increase neuronal activity in all myenteric neurons, or just AH neurons, leads to disrupted propulsive motility. Furthermore, attenuation of AH neuronal hyperexcitability improved motility in TNBS-colitis. Collectively, these results support the concept that inflammation-induced neuroplasticity of electrical properties of myenteric AH neurons, which lead to enhanced excitability, contribute to disrupted propulsive motility in colitis.

Drugs that enhance neuronal excitability decrease colonic motility

The myenteric plexus is a ganglionated network, comprised of intrinsic afferent neurons, interneurons and motor neurons, that generates propulsive and mixing motor patterns, including peristalsis. We hypothesized that a disruption in the precision of neural signaling, via widespread increases in neuronal excitability and synaptic activity, would result in altered propulsive motility. We first tested the effects

of causing a nonselective increase in myenteric neuronal activity by bath application of veratridine, which causes sustained, repetitive action potential generation in myenteric neurons.[18] Veratridine inhibited propulsive motility such that progress of the fecal pellet was completely impeded. We suggest that this occurs because random, overactive firing of enteric neurons supersedes the coordinated reflex activity necessary to generate an effective pressure gradient.

Mimicking inflammation-induced neuroplasticity in the normal colon

Aterhyperpolarization neurons serve a critical role in intestinal peristalsis as they function at the afferent end of the peristaltic reflex circuit.[2–4] Calcium-activated potassium channels are responsible for the hyperpolarization phase of the prolonged AHP, and the magnitude of the IK_{Ca} can therefore govern the excitability of AH neurons and resulting reflex activity.[5,6] As inflammation-induced AH neuronal hyperexcitability is associated with decreased magnitude of the AHP in TNBS-induced colitis,[12,30] TNBS-induced ileitis,[19] and *Trichinella spiralis*-induced ileitis,[22,23] we tested the effects of suppressing the AHP on peristalsis with the IK_{Ca} inhibitor TRAM-34.[19,20] In computer modeling studies of the role of the AHP and slow EPSPs in enteric neural network activity, reduction of the AHP destabilizes network activity.[10] Furthermore, previous studies reported altered motility following TRAM-34 administration in rat *ex vivo* small [31] and large [20] intestine motility assays and rat small intestinal transit *in vivo*,[9] and in the current study, TRAM-34 reduced the rate of colonic propulsive motility. These effects are likely due to direct action on AH neurons as TRAM-34 has no effect on S neurons,[20] or on the electrical properties of smooth muscle (current study). Furthermore, while the IK_{Ca} channel is expressed on non-neuronal cell types, including enterocytes in the colonic mucosa,[25] intralumenal application of TRAM-34 did not alter the rate of propulsive motility.

To more extensively mimic colitis-induced neuroplasticity, we also tested the effects of co-administration of TRAM-34 with the 5-HT_4 receptor agonist tegaserod, which facilitates synaptic transmission through augmented acetylcholine release.[32] A further decrease in propulsive motility was observed in the presence of TRAM-34 plus tegaserod. Enhanced propulsive motility has been reported in response to administration of 5-HT_4 agonists, however, the agonists were administered to the lumen rather than by bath application.[33,34] Because of the complex nature of the neuroplastic changes caused by inflammation on propulsive motility, hyperexcitability and synaptic facilitation are likely mediated by multiple mechanisms. The finding that motility is altered by increasing excitability in AH neurons, representing only 5% of the total neurons in the colonic myenteric plexus, supports the notion that the prolonged AHP is an important feature of these cells, and of the neurophysiology of peristalsis. Furthermore, these results are consistent with the concept that increased neural activity, resulting from inflammation, can contribute to dysmotility.

Reversing inflammation-induced neuroplasticity in the inflamed colon

Previous studies of motility in the TNBS-inflamed colon have concluded that the rate of propulsion is decreased by measuring the time that elapsed as a pellet traveled a given distance along an isolated segment of colon.[14] More recent assessments using the GIMM system, which allows for the continuous assessment of propulsive motility, have revealed a more complex pattern during inflammation that involves the pellet stopping or pausing in ulcerated regions.[15] In the current study, propulsive motility was typically obstructed in regions of ulceration. In these cases, the pellet did not traverse a given region of colon even though contractile activity is clearly observed in the preparation. The other pattern observed in some trials from two of the preparations was transiently halted motility; the pellet was propelled in the aboral direction, and then paused before resuming transit along the colon. As in obstructed motility, the area of halted motility corresponded to the location of an ulcer.

We hypothesized that if hyperexcitability of AH neurons contributes to dysmotility in the inflamed colon, we should be able to improve colonic motor function by dampening the activity of these cells. The late hyperpolarization phase of the AHP in AH neurons activates an I_h current that counters the IK_{Ca} current, and attenuates the amplitude and duration of the AHP.[7] Cs^+ blocks the I_h current and increases the magnitude of the AHP.[7,8] In TNBS-colitis, the decrease in AHP magnitude involves an increase in I_h, and the AHP is restored, and hyperexcitability reduced, by blocking I_h with Cs^+.[12] In the current study, Cs^+ and ZD7288 were used to test the effects of restoring the AH neuron AHP on colonic motility in TNBS-colitis. Prior to the addition of I_h inhibitors, propulsive motility was temporarily or completely impeded in the ulcerated regions, but in the presence of the I_h

blocker, propulsive motility appeared normal. This is likely due to an action of Cs^+ and ZD7288 on AH neurons because the I_h is not detected in S neurons.[7,8] In the current study we found that ZD7288 decreased AH neuron excitability in a TNBS inflamed preparation, and this is consistent with the decrease in AH neuron excitability that we have previously reported in TNBS inflamed preparations treated with Cs.[12] However, it should be noted that ZD7288 was only tested on a single AH neuron, and additional and confirming data will required before a generalization can be made.

Other factors that may alter motility in the inflamed colon

While the effects of intestinal inflammation on intestinal structure and function are complex, this study provides evidence supporting the concept that inflammation-induced alterations in myenteric neuronal function contribute to dysmotility in TNBS-colitis. Another factor that likely contributes to inflammation-induced dysmotility is altered mucosal serotonin signaling at ulcerated regions. For example, enterochromaffin cell numbers and decreased expression of the serotonin reuptake transporter have been previously described in guinea pig [14] and mouse TNBS-colitis,[35] and human inflammatory bowel disease (IBD).[36]

Despite the fact that the effects observed following pharmacological induction of AH neuron hyperexcitability and increased synaptic transmission are comparable with those observed following intestinal inflammation, there are important differences to consider. In particular, in TNBS-colitis, we find that forward motion of the fecal pellet becomes temporarily or completely impeded at ulcerated regions.[15] In the current study, propulsive motility was slowed, but was impeded in a subset of trials in the presence of TRAM-34 alone or TRAM-34 and tegaserod. Therefore, while hyperexcitability and synaptic activity likely contribute to disrupted motility in colitis, changes in mucosal integrity, 5-HT signaling, smooth muscle function and scar tissue formation are also likely to affect motility in the inflamed and ulcerated colon. On the other hand, propulsive motility was dramatically improved in the inflamed colon by the addition of Cs^+ or ZD7288, which, to the best of our knowledge, are only acting on the AH neurons, thus supporting the notion that AH neuron hyperexcitability is an important contributing factor to dysmotility.

Concluding remarks

In summary, the results of this study indicate that enhancement of the activity of myenteric neurons can disrupt propulsive motility. Furthermore, these data suggest that dampening inflammation-induced hyperexcitability of AH neurons can restore colonic motor function in inflamed preparations. As the effects of our model of inflammation are consistent with clinical observations, these results also provide a novel mechanism that could contribute to altered gut function in IBD.

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AUTHOR CONTRIBUTION

JH, NM and GM performed the experiments, and all authors contributed to the analysis and interpretation of the data; JH, KS and GM conceived the study and designed the experiments; JH and GM generated the original draft of the manuscript; and all authors contributed to critical revisions and ultimately approved the final version. The experiments were carried out at the University of Vermont.

CONFLICTS OF INTERESTS

The authors have no competing interests to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Movie S1. Demonstration of propulsive motility in a segment of normal guinea pig distal colon before and after the addition of 1.0 μmol L^{-1} veratridine. In normal Kreb's solution, the fecal pellet progresses through the segment of colon with a linear time–distance relationship. Following addition of veratridine, the fecal pellet does not make forward progress, indicating the neural elements of the preparation are not capable of generating a pressure gradient to propel the pellet in an oral to anal direction. In the presence of veratridine, contractile activity is still detectable, confirming the viability of the preparation. The movie is shown at four times normal speed.

Movie S2. Demonstration of propulsive motility in a segment of TNBS-inflamed guinea pig distal colon before and after the addition of 10 μmol L^{-1} ZD7288. In this segment of colon, the fecal pellet is propelled in an aboral direction, but its progress is obstructed in the region of an ulcer. Following addition of ZD7288 to the bathing solution, the fecal pellet progresses through the entire segment of colon, including the ulcerated region, with a fairly linear time–distance relationship. The movie is shown at four times normal speed.

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