TRPV1 receptor signaling mediates afferent nerve sensitization during colitis-induced motility disorders in rats

H. U. De Schepper,1 J. G. De Man,1 N. E. Ruysers,1 A. Deiteren,1 L. Van Nassauw,2 J.-P. Timmermans,2 W. Martinet,3 A. G. Herman,3 P. A. Pelekmans,1 and B. Y. De Winter1

1Division of Gastroenterology, Faculty of Medicine, 2Research Group Cell Biology and Histology, Department of Veterinary Sciences, and 3Laboratory of Pharmacology, Faculty of Pharmacy, University of Antwerp, Antwerp, Belgium

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De Schepper HU, De Man JG, Ruysers NE, Deiteren A, Van Nassauw L, Timmermans J-P, Martinet W, Herman AG, Pelekmans PA, De Winter BY. TRPV1 receptor signaling mediates afferent nerve sensitization during colitis-induced motility disorders in rats. Am J Physiol Gastrointest Liver Physiol 294: G245–G253, 2008. First published November 8, 2007; doi:10.1152/ajpgi.00351.2007.—Rats with experimental colitis suffer from impaired gastric emptying (GE). We previously showed that this phenomenon involves afferent neurons within the pelvic nerve. In this study, we aimed to identify the mediators involved in this afferent hyperactivation. Colitis was induced by trinitrobenzene sulfate (TNBS) instillation. We determined GE, distal front, and geometric center (GC) of intestinal transit 30 min after intragastric administration of a semifluid Evans blue solution. We evaluated the effects of the transient receptor potential vanilloid type 1 (TRPV1) antagonists capsazepine (5–10 mg/kg) and N-(4-tetrahydropyrindin-2-yl)tetracydroazepine-1(2H)carboxamide (BCTC; 1–10 mg/kg) and the calcitonin gene-related peptide (CGRP) receptor antagonist CGRP-(8-37) (150 μg/kg). To determine TRPV1 receptor antagonist sensitivity, we examined their effect on capsazepine-induced relaxations of isolated gastric fundus muscle strips. Immunocytochemical staining of TRPV1 and RT-PCR analysis of TRPV1 mRNA were performed in dorsal root ganglion (DRG) L6–S1. TNBS-induced colitis reduced GE but had no effect on intestinal motility. Capsazepine reduced GE in controls but had no effect in rats with colitis. At doses that had no effects in controls, BCTC and CGRP-(8-37) significantly improved colitis-induced gastroparesis. Capsazepine inhibited capsazepine-induced relaxations by 35% whereas BCTC completely abolished them. TNBS-induced colitis increased TRPV1-like immunoreactivity and TRPV1 mRNA content in pelvic afferent neuronal cell bodies in DRG L6–S1. In conclusion, distal colitis in rats impairs GE via sensitized pelvic afferent neurons. We provided pharmacological, immunocytochemical, and molecular biological evidence that this sensitization is mediated by TRPV1 receptors and involves CGRP release.

gastric emptying; sensory nerve; pelvic nerve; CGRP

PATIENTS WITH INFLAMMATORY bowel disease (IBD) often suffer from disorders of gastrointestinal motility and sensitivity, imposing a significant load on the patient’s quality of life (20). These alterations are known to appear both during inflammatory episodes and in periods of remission and can occur either at the site of inflammation or at a distance from this site (43). Especially concerning the latter situation, little is known about the underlying pathophysiological mechanisms. There have been several studies documenting the effects of isolated experimental colitis on small intestinal neuromuscular function, but the in vivo consequences on gut transit were inconclusive (3, 5, 29). McHugh et al. (37) reported that rats with trinitrobenzene sulfate (TNBS)-induced colitis suffer from a reduction of gastric emptying, but did not investigate the underlying mechanisms. We recently confirmed that rats with experimental acute colitis suffer from impaired gastric emptying in the absence of local gastric inflammatory changes (13), a phenomenon that has also been portrayed in human IBD patients with colonic involvement (2, 22). The colitis-induced gastroparesis in rats was neurally mediated and disappeared after section of the pelvic nerve, suggesting the involvement of an extrinsic reflex pathway activated by colonic inflammation (13). Because colorectal distension in healthy rats also impairs gastric emptying (i.e., cologastric inhibitory reflex) (26), the colitis-induced gastroparesis can be interpreted as a sensitized state of this physiological reflex. This hypothesis was supported by f-Fluorescence studies showing heightened activity in the pelvic nerve dorsal root ganglion (DRG) S1 in the absence of a distension stimulus (15).

A large number of molecular targets have already been identified as possible mediators of extrinsic afferent nerve sensitization (23). One of the most attractive candidates is the transient receptor potential vanilloid type 1 (TRPV1) receptor, which occurs predominantly on extrinsic afferent nerve fibers in the rat gut (55). This polymodal cation channel receptor is best known for its responsiveness to the spicy pungent capsaicin and has been shown to act as an integrator of inflammatory stimuli and as a key player in the pathophysiology of nerve sensitization. Importantly, the TRPV1 receptor has already been implicated in colonic mechanosensitivity and its modulation by inflammatory agents (30). In addition, TRPV1 receptor immunoreactivity was found to be increased in patients with IBD (58). These data make TRPV1 a very likely mediator for colitis-induced changes of neuronal function.

The aim of this study was to determine whether peripheral TRPV1 receptors are involved in the colitis-induced sensitization of the afferent pelvic nerve fibers leading to impaired gastric emptying in the rat model of TNBS-induced colitis. We also investigated the effect of an antagonist of the CGRP receptor on colitis-induced gastroparesis, because this neuropeptide is released by afferent nerves at the spinal dorsal horn synapse. Moreover, it was shown to be involved in the generation of colonic hypersensitivity to distension (16).

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Address for reprint requests and other correspondence: B. Y. De Winter, Laboratory of Gastroenterology, Faculty of Medicine, Univ. of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium) (e-mail: benedicte.dewinter@ua.ac.be).

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MATERIALS AND METHODS

Animal model. Distal colitis was induced in male Wistar rats (200–225 g) according to published methods (13, 41). Rats were fasted for 24 h. After pentobarbital anesthesia (45 mg/kg ip) an enema of 0.5 ml of saline (controls) or trinitrobenzene sulfate (7.5 mg TNBS dissolved in 50% ethanol) was administered in the colorectum. All further experiments were performed 72 h after induction of colitis. All procedures were approved by the Committee for Medical Ethics and the Use of Experimental Animals at the University of Antwerp.

Evaluation of colitis. After each experiment, the colorectum was removed and macroscopic mucosal damage was assessed by a standardized scoring system ranging from 0 to 10 (13, 40). Rats were included if the score was ≥5, indicating major signs of damage. Colonic tissue samples were harvested for measurement of myeloperoxidase (MPO) content, a biochemical marker for the extent of inflammation. MPO was quantified according to published methods (40).

In vivo measurements of gastrointestinal motility. A protocol was adapted from De Winter et al. (14) as previously described (13). Briefly, rats were fasted for 48 h with free access to tap water containing 5% glucose. This prolonged fasting was necessary to assure that the stomach was completely free of contents before administration of Evans blue. On the day of experiment, 1 ml of a semiliquid nonnutrient dye (Evans blue 50 mg/ml dissolved in 0.5% methylcellulose) was administered intragastrically. Thirty minutes later, rats were anesthetized and euthanized. The stomach and small intestine were carefully removed. Intestinal transit was measured from the pylorus to the most distal point of migration and expressed as a percentage of the total length of the small intestine. The small intestine was then divided into 10 segments of equal length. The stomach (segment 1) and the intestinal segments (segments 2–11) were put in 25 ml of 0.1 N NaOH, minced, and placed in an ultrasonic bath for 1 h (Bransonics 2000). The resulting suspension was left at room temperature for 1 h, and 5 ml of the supernatant was then centrifuged at 1,356 g for 20 min at 4°C. Samples were further diluted (1:5 for intestinal specimens and 1:50 for the stomach), and absorbance (A) was read at wavelength 565 nm. Gastric emptying (GE) was calculated as %GE = [Asmall intestine]/[Asmall intestine + Asmall intestine] × 100. The geometric center (GC) of intestinal transit (38) was calculated both including (GCs-1) and omitting (GCs) the stomach segment, because the latter method gives a better idea about small intestinal peristaltic function independent of gastric emptying efficacy. These values were calculated as GC = Σ[%A(segment) × (number of segment)]/100.

Protocols. Experiments were performed 72 h after induction of colitis. To study the involvement of TRPV1 receptors, we treated rats with the most used TRPV1 receptor antagonist capsazepine (5, 10 mg/kg ip, 1 h before instillation of Evans blue) (4) or its vehicle (78% saline, 20% DMSO, 1% ethanol, 1% Tween80). Alternatively, rats were treated with the novel, more specific TRPV1 antagonist N-(4-tertiarybutylphenyl)-4-(3-chlorophenyl-2-yl)tetrahydropyrazine-1(2H)carboxamide (BCTC 1, 5, 10 mg/kg ip, 1 h before instillation of Evans blue) (53) or its vehicle (25% hydroxypropyl-β-cyclodextrin). The role of CGRP-mediated neurotransmission was assessed by treating the rats with the specific receptor antagonist CGRP-(8-37) (150 µg/kg ip, 1 h before instillation of Evans blue) or its vehicle (saline). The doses of capsazepine, BCTC, and CGRP-(8-37) were used as previously shown to reduce somatic or visceral hyperalgesia in rats (8, 16, 53). CGRP-(8-37) is a frequently used and well-validated antagonist for the CGRP receptor and has been shown to abolish CGRP-induced responses both in vitro (19) and in vivo in rats (36). Capsazepine and BCTC are specific TRPV1 receptor antagonists that potently inhibit capsaicin-induced responses in rats. Since capsazepine has documented aspecific effects on ion channel dynamics, and since BCTC is a relatively novel compound, the effect of these antagonists was tested on capsaicin-induced relaxations of isolated rat gastric fundus muscle strips to prove their efficacy in rats (31).

The effects of each antagonist and vehicle on gastrointestinal motility and contractility, on the macroscopic score of inflammation, and on the MPO content of the colon were determined. The inflammatory indexes were determined to exclude an acute modulation of the local inflammatory environment by the antagonists used.

In vitro measurements of gastrointestinal contractility. Rats were fasted for 24 h with free access to water, anesthetized with diethyl ether, and exsanguinated by cardiac puncture. After laparotomy, the stomach was removed and immersed in ice-cold Krebs-Ringer solution (118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 25 mM NaHCO3, 0.026 mM CaEDTA, and 11.1 mM glucose). Muscle strips were prepared as previously described (11, 12). After removal of the mucosa, gastric fundus muscle strips were prepared in the longitudinal direction and mounted in an organ bath (volume 5 ml) filled with Krebs-Ringer solution (37°C, continuously aerated with a mixture of 95% O2–5% CO2). One end of the strip was fixed and the other end was attached to a strain gauge transducer (Stain Transducers, Amersham, France) for continuous recording of isometric tension. After an initial equilibration period of 30 min during which the strips were washed every 10 min, the muscle strips were contracted with 100 nM carbachol. After washout the strips were stretched, and when the baseline tension of the preparations was stabilized 100 nM carbachol was added again. This procedure was repeated until the carbachol-induced contraction was maximal and an optimal length-tension relationship was achieved (±0.5 g) (11, 12). Experiments were started after an additional equilibration period of 60 min, during which the strips were washed with fresh Krebs-Ringer solution every 15 min. We investigated the relaxant effect of capsaicin (1 µM) on a precontraction induced by carbachol (100 nM), in the presence of capsazepine (1, 5 µM) or BCTC (10, 100 nM), or their respective vehicles. Relaxations were expressed as a percentage of the precontraction to carbachol (100 nM).

Tracing and immunocytochemistry. Rats were anesthetized with pentobarbital (60 mg/kg ip). A midline laparotomy was performed and the colorectum was exposed over 2–3 cm. The tracer dye Fast blue (2% in 10% DMSO) was injected in the colonic wall at eight sites (four injections at two levels) under aseptic conditions. The animals were allowed to recover for 14 days before induction of TNBS colitis. Three days later the rats were anesthetized, a lumbar laminectomy was performed, and the DRG L6 and S1 were harvested. The specimens were immediately immersed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.0) at room temperature. After a 10-min fixation period, the DRG were processed for cryosectioning according to published methods (9). To assess the presence of TRPV1 in spinal neurons, cryostat sections were immunocytochemically stained with a rabbit antibody directed against TRPV1 (AB5370; CHEMICON International, Temecula, CA; diluted 1:400) and a Cy3-conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA; diluted 1:4,000). Briefly, all incubations were performed at room temperature. Sections were immersed for 30 min in 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 0.05% thimerosal (PBS*), 5% normal horse serum (NHS; Jackson Immunoresearch Laboratories), and 1% Triton X-100, prior to incubation for 18 h with the primary antibody diluted in PBS* containing 5% NHS and 0.1% Triton X-100. After being rinsed in 0.01 M PBS, they were incubated for 1 h with the secondary antibody diluted in PBS* containing 1% NHS. After washing, the cryosections were mounted in Citifluor. For negative controls, the primary antiserum was omitted. For quantification, the number of neurons expressing TRPV1 immunoreactivity and/or Fast blue tracing was counted on 10 slides per ganglion, and results were expressed as a percentage of the total numbers of neurons identified or as the percentage of Fast blue positive neurons expressing TRPV1 immunoreactivity.

Real-time PCR. Rats were anesthetized with pentobarbital (60 mg/kg ip) and a lumbar laminectomy was performed. For each rat, the DRG L6 and S1 were harvested bilaterally, embedded in optimal cutting temperature compound (OCT), and stored at –80°C. The DRG were then sliced into 50–60 frozen sections (20 µm) that were immediately immersed in lysis buffer. Total RNA was isolated using the Absolutely RNA micro prep kit (Stratagene; La Jolla, CA). A TaqMan gene expression assay was performed for...
TRPV1 (assay ID Rn00676880_m1; Applied Biosystems, Foster City, CA) on an ABI Prism 7300 sequence detector system (Applied Biosystems) in 25 μl reaction volumes containing One-step Universal PCR Master Mix (Applied Biosystems). The parameters for polymerase chain reaction (PCR) amplification were 48°C for 30 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All data were controlled for quantity of cDNA input by performing measurements on the endogenous reference gene β-actin (assay ID Rn00667869_m1; Applied Biosystems) and calculation of comparative cycle thresholds \( \Delta C_T = C_T(\text{TRPV1}) - C_T(\text{β-actin}) \). Relative expression of mRNA species was then calculated as \( 2^{-\Delta \Delta C_T} = \frac{\Delta C_T}{\text{TRPV1}} - \Delta C_T(\text{control}) \).

Solutions and drugs. Carbachol, Evans blue, hexadecyltrimethylammonium bromide, o-dianisidine dihydrochloride, and Tween 80 were all purchased from Sigma-Aldrich, St. Louis, MO; BCTC was purchased from Ascent Scientific, Weston-Super-Mare, UK; capsazepine was purchased from Biomol International, Exeter, UK; CGRP-(8-37) was obtained from Sigma-Aldrich; hydrogen peroxide and diethyl ether were purchased from Merck, Darmstadt, Germany; capsaicin and TNBS were obtained from Fluka, Neu Ulm, Germany; pentobarbital (Nembutal) was purchased from Ceva, Brussels, Belgium; and Fast blue was obtained from Polysciences Europe, Eppelheim, Germany.

Presentation of results and statistical analysis. Parametric values are shown as means ± SE for \( n \) indicating the number of rats used. For statistical analysis, unpaired Student’s \( t \)-test or two-way ANOVA was performed. Post hoc testing was carried out by Student-Newman-Keuls analysis or Student’s \( t \)-test when appropriate. For nonparametric data, results are presented as median with 25th and 75th percentile. Nonparametric analysis was performed via a Mann-Whitney \( U \)-test. \( P < 0.05 \) was considered statistically significant. Data were analyzed via SPSS 11.5 software (SPSS, Chicago, IL) and GraphPad Prism 4.00 (GraphPad Software, San Diego, CA).

RESULTS

Effect of TNBS-induced colitis on gastrointestinal motility. Instillation of TNBS in the distal colon resulted in a high-grade inflammatory response characterized macroscopically by mucosal thickening, ulceration, and necrosis. This was accompanied by a pronounced increase in colonic MPO content from \( 1.8 \pm 0.9 \) U/g in controls to \( 41.5 \pm 5.8 \) U/g in rats with TNBS colitis \( (P < 0.001, n = 6) \). Distal experimental colitis significantly reduced gastric emptying and the GCS\(_{5+1} \) in all vehicle-treated rats (Fig. 1, A and B) but had no effect on the front of intestinal transit or on the GCI (Fig. 1, C and D).

Effect of capsazepine on colitis-induced motility changes. Capsazepine caused a significant but dose-independent decrease of gastric emptying and the GCS\(_{5+1} \) in controls \( (P < 0.05, \) Fig. 1, A and B). This inhibition was absent in rats with TNBS-induced colitis [not significant (NS), Fig. 1, A and B]. Capsazepine had no significant effect on the GCI or the distal front of intestinal transit in control or TNBS rats (Fig. 1, C and D). Capsazepine treatment did not modulate the magnitude of the inflammatory response, as evidenced by the macroscopic score of inflammation and the MPO-content of the distal colon (Table 1).

Effect of BCTC on colitis-induced motility changes. In controls, BCTC caused a reduction of gastric emptying and the GCS\(_{5+1} \) that was significant at 10 mg/kg \( (P < 0.05, \) Fig. 2, A and B). In rats with TNBS-induced colitis, BCTC 5 mg/kg significantly improved gastric emptying and the GCS\(_{5+1} \) compared with vehicle treatment \( (P < 0.05, \) Fig. 2, A and B). A lower dose had no effect whereas the effect of 10 mg/kg was comparable to the effect of 5 mg/kg. BCTC had no effect on small intestinal motility (Fig. 2, C and D). BCTC did not influence the macroscopic score of inflammation or the MPO content of the distal colon (Table 1).

Effect of CGRP-(8-37) on colitis-induced motility changes. The CGRP antagonist CGRP-(8-37) did not significantly modulate gastric emptying and the GCS\(_{5+1} \) in controls (NS, Fig. 3, A and B). Likewise, CGRP-(8-37) had no effect on the GCI or the front of intestinal transit (Fig. 3, C and D). In rats with TNBS-induced colitis, the antagonist significantly improved gastric emptying \( (P < 0.05, \) Fig. 3A). This effect was reflected in the GCS\(_{5+1} \) (Fig. 3B). CGRP-(8-37) had no effect on the GCI or the front of intestinal transit (Fig. 3, C and D). BCTC did not influence the macroscopic score of inflammation or the MPO content of the distal colon (Table 1).

Fig. 1. Effects of capsazepine 5–10 mg/kg (CZP) on gastric emptying (GE; A), the geometric center of transit including the stomach (GCS\(_{5+1} \), B), the distal front of intestinal transit (C), and the geometric center of transit omitting the stomach (GCI, D). Results are expressed as percentage gastric emptying (%GE), percentage intestinal transit (%transit), and segment number (GCS\(_{5+1} \) and GCI) and are shown as means ± SE for \( n = 10 \). TNBS, trinitrobenzene sulfone. *\( P < 0.05 \), significantly different from vehicle-treated controls, 2-way ANOVA with Student-Newman-Keuls post hoc analysis.
additional effect on the GCI nor on the front of intestinal transit in rats with TNBS colitis (Fig. 3, C and D).

CGRP-(8-37) did not modulate the inflammatory response since controls and TNBS rats showed similar scores for macroscopic inflammation and colonic MPO content (Table 1).

Effect of TRPV1 receptor antagonists on capsaicin-induced relaxations. In gastric fundus muscle strips precontracted with carbachol (100 nM), capsaicin (1 μM) induced a sharp relaxation that gradually recovered (Fig. 4A). This relaxation showed the tachyphylaxis typical for capsaicin-induced phenomena: a muscle strip that relaxed to capsaicin did not respond a second time to the TRPV1 agonist. Capsazepine significantly decreased the relaxatory response to capsaicin at the concentration of 5 μM (Fig. 4, B and D). BCTC dose dependently inhibited the relaxation to capsaicin and, at 100 nM, almost completely abolished the relaxation to capsaicin (Fig. 4, C and E).

Effect of experimental colitis on TRPV1 receptor expression in the DRG L6–S1. Two weeks after injection of Fast blue in the colonic wall, 8.9 ± 1.1% of neuronal cell bodies in DRG L6–S1 stained positive for Fast blue in controls and 10.1 ± 0.9% stained positive in rats with TNBS colitis (n = 5, NS). TRPV1 expression of these colon-derived DRG neurons was significantly higher in rats with TNBS-induced colitis (53.4 ± 3.5%) compared with control rats (40.6 ± 4.1%) (P < 0.05, Fig. 5).

Effect of experimental colitis on TRPV1 receptor mRNA levels in the DRG L6–S1. TNBS-induced colitis caused a significant upregulation of TRPV1 receptor mRNA in the pelvic nerve DRG L6–S1, as evidenced by an increase compared with controls in its expression relative to the housekeeping gene β-actin (P < 0.05, Fig. 6).

DISCUSSION

Patients with IBD such as Crohn’s disease have been shown to suffer from gastroparesis or reduced gastric emptying, leading to symptoms like nausea, early satiety, and abdominal discomfort (2, 22).

We previously reported on the occurrence of gastric motor inhibition in rats with TNBS-induced colitis (13). We provided proof that the afferent branch of this reflex pathway is contained within the pelvic nerve, because its section resulted in a restoration of gastric motor function in TNBS-treated rats. We hypothesized that this colitis-induced gastroparesis actually represents the sensitized state of an extrinsic reflex pathway

Table 1. Effect of CZP, BCTC, and CGRP-(8-37) or their respective vehicles on the MPO content and the macroscopic score of inflammation of the distal colon in rats with TNBS-induced colitis

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>MPO</th>
<th>Macro</th>
<th>Vehicle</th>
<th>MPO</th>
<th>Macro</th>
<th>Vehicle</th>
<th>MPO</th>
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<th>Vehicle</th>
<th>MPO</th>
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<tr>
<td>CZP</td>
<td>47.3 ± 5.6</td>
<td>8.5 (7.5–10)</td>
<td>40.5 ± 5.9</td>
<td>8 (6–10)</td>
<td>8 (6–9)</td>
<td>37.3 ± 5.9</td>
<td>8 (6–8)</td>
<td>8 (6–9)</td>
<td>41.5 ± 4.7</td>
<td></td>
<td></td>
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<tr>
<td>BCTC</td>
<td>53.7 ± 7.2</td>
<td>9 (8–10)</td>
<td>53.7 ± 7.2</td>
<td>8 (6–8)</td>
<td>8 (6–9)</td>
<td>37.3 ± 5.9</td>
<td>8 (6–10)</td>
<td>8 (6–9)</td>
<td>41.5 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGRP-(8-37)</td>
<td>38.7 ± 6.3</td>
<td>8 (6–8)</td>
<td>37.3 ± 5.9</td>
<td>8 (6–8)</td>
<td>8 (6–9)</td>
<td>41.5 ± 4.7</td>
<td>8 (6–9)</td>
<td>8 (6–9)</td>
<td>41.5 ± 4.7</td>
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MPO results are expressed as U/g tissue and presented as mean ± SE. Macroscopic score (Macro) ranges from 0 to 10 and is presented as median with 25th and 75th percentile. CZP, capsazepine 10 mg/kg; BCTC, N-(4-tertiarybutylphenyl)-4-(3-chlorophyridin-2-yl)tetrahydropyrazine-1(2H)carboxamide 5 mg/kg; CGRP-(8-37), calcitonin gene-related peptide receptor antagonist 150 mg/kg; TNBS, trinitrobenzene sulfate; NS, not significantly different from vehicle treatment, unpaired Student’s t-test for MPO analysis or Mann-Whitney U-test for analysis of macroscopic score.
known as cologastric inhibition. Indeed, physiological inhibition of gastric emptying in response to innocuous colorectal distension occurs both in rats (26) and in humans (59) and probably plays a role in the homeostasis of whole gut motor coordination. In this study, we showed that sensitization of pelvic afferent neurons is indeed involved in colitis-induced gastroparesis and involves TRPV1 and CGRP.

We designed the present study to identify the neuropharmacological mediators responsible for the sensitization process leading to colitis-induced gastroparesis. Of the key players involved in sensory nerve sensitization, the vanilloid TRPV1 receptor currently represents the most attractive target. This polymodal cation channel receptor is present on the majority of sensory nerve fibers in the rat colon (55) and is activated and/or sensitized by heat, protons, mechanical stimuli, and a number of lipid derivatives such as LTB4 and 12-HPETE (21). A “true” specific endogenous agonist, however, remains unidentified. Likewise, the physiological role of TRPV1-mediated signaling is largely unknown, even though our immunocytochemical results clearly show that TRPV1 receptors are abundantly present in 40% of normal colonic afferent cell bodies in DRG L6–S1, comparable to other findings (7).

The lack of knowledge concerning the physiological role of TRPV1 signaling is largely due to the lack of a TRPV1

\[ \text{Fig. 3. Effects of CGRP-(8-37) on gastric emptying} \]
\[ \text{(A), the geometric center of transit including the stomach} \]
\[ \text{(B), the distal front of intestinal transit} \]
\[ \text{(C), and the geometric center of transit omitting the} \]
\[ \text{stomach (D). Results are expressed as percentage} \]
\[ \text{gastric emptying, percentage intestinal transit, and} \]
\[ \text{segment number and are shown as means ± SE for} \]
\[ n = 10. *P < 0.05, \text{significantly different from} \]
\[ \text{vehicle-treated controls, #P < 0.05, significantly} \]
\[ \text{different from vehicle-treated TNBS rats, 2-way} \]
\[ \text{ANOVA with Student-Newman-Keuls post hoc} \]
\[ \text{analysis.} \]

\[ \text{Fig. 4. Top: typical tracings of isolated muscle strips from the rat gastric fundus showing} \]
\[ \text{relaxations to 1 \text{\textmu}M capsaicin in strips treated with saline} \]
\[ \text{(A), capsaicin (CZP, 5 \text{\textmu}M; B) and BCTC (100 nM; C). Arrow depicts addition of capsaicin in the organ bath. W, wash-out of drugs from the organ bath. Bottom: mean effect of capsaicinine (1–5 \text{\textmu}M, n = 7–9; D) and BCTC (10–100 nM, n = 7–9; E) on the relaxation to 1 \text{\textmu}M capsaicin. Results are shown as means ± SE. *P ≤ 0.05, unpaired Student’s} \]
\[ \text{t-test.} \]
receptor antagonist with a good specificity profile and with pharmacokinetic properties that are optimized for in vivo studies. The most commonly used competitive antagonist for in vivo use in rats is the synthetic capsaicin analog capsazepine (4).

Capsazepine significantly inhibited gastric emptying and the geometric center of gastrointestinal transit in controls, without affecting intestinal transit or the GCI. In rats with TNBS colitis, however, it no longer significantly affected gastric emptying. This differential effect implies a modulation of vanilloid signaling under the influence of an acute and local inflammatory process. The inhibition of gastric emptying by capsazepine in controls could be due to a physiological role of TRPV1 in the regulation of gastric emptying, or it may be due to the aspecific actions attributed to this compound. Indeed, it is well known that capsazepine does not only inhibit TRPV1 receptor activation but equally affects neuronal calcium currents (17) and nicotinic acetylcholine receptors (33). Our in vitro data show that even at a concentration of 5 μM capsazepine did not completely inhibit capsaicin-induced relaxations in the gastric fundus, raising additional questions about its sensitivity. Therefore, we also tested the effects of the competitive TRPV1 receptor antagonist BCTC on colitis-induced motility disorders. BCTC has an optimized pharmacokinetic profile, combining favorable bioavailability with a relatively long half-life and improved specificity (46). Compared with capsazepine, it has the additional advantage of blocking low-pH-induced TRPV1 activation (53). This is very important, because local acidosis is a key phenomenon in tissue inflammation in general and a powerful stimulus of TRPV1-mediated sensitization of afferent nerve endings (27, 49). At the dose of 100 nM, BCTC completely inhibited capsaicin-induced relaxations of isolated...
muscle strips of the gastric fundus, confirming its sensitivity and affinity as a TRPV1 receptor antagonist. At a dose that did not affect gastric emptying in controls, BCTC significantly improved gastroparesis in rats with TNBS-induced colitis. This observation clearly highlights the modulatory role of TRPV1 in sensitization related to colitis-induced gastroparesis. The highest dose tested in this study significantly decreased gastric emptying in controls and likewise no longer improved it in TNBS rats. The mechanism by which high-dose BCTC selectively inhibits gastric emptying whereas it does not modulate intestinal motility was beyond the scope of this study and not investigated in further detail.

TRPV1 expression and function are heavily influenced by a variety of inflammatory mediators such as bradykinin (50), nerve growth factor (1), PGE2 (34), 5-HT (48), and ATP (51). The latter act through upregulation of protein kinases resulting in phosphorylation of TRPV1 and sensitization of the receptor. Recently, Jones et al. (30) demonstrated that colonic afferent fibers become resistant to the sensitizing effects of an inflammatory soup in TRPV1 knockout mice, emphasizing the pivotal role of vanilloid signaling in the immunomodulation of visceral sensory functions. Inflammation not only induces sensitization of receptor function but is also known to upregulate TRPV1 receptor expression (57). Using immunocytochemical staining of traced pelvic afferent neuronal cell bodies (DRG L6–S1) we found that in our model colitis indeed caused a significant 1.3-fold increase of TRPV1 receptor expression, which is comparable with other reports (39). This upregulation was also found when studying TRPV1 mRNA by RT-PCR.

Although the effects of immunomodulation on TRPV1 function have been studied intensively, the in vivo consequences of visceral TRPV1 sensitization are less clear and largely derived from somatic pain research (54), even though it was shown by Christianson et al. that TRPV1-positive colonic afferents are more numerous than TRPV1-positive somatic afferents (6). Our combination of functional, immunocytochemical, and molecular data clearly emphasizes the crucial role of TRPV1 in the in vivo model of colitis-induced gastroparesis. Future studies will have to show whether BCTC effectively reduces electrophysiological primary afferent neuron sensitization.

Administration of TRPV1 antagonists may modulate gut inflammation (28). Still, in our study inflammatory indexes were not altered by capsazepine or BCTC 1 h after administration. It remains to be determined whether the participation of TRPV1 in the inflammatory process may complicate the clinical use of TRPV1 antagonists to treat inflammation-induced motility and sensitivity disorders.

Capsazepine and BCTC did not fully restore gastric emptying in colitis rats to control values. It is very likely that TRPV1 is an important but not the only integrator of sensitizing stimuli on afferent nerve terminals. Indeed, Wynn et al. (56) showed that TNBS colitis-induced sensitization of pelvic afferent nerve activity in an isolated colorectum setup was mediated by P2X3 receptors. Future studies will have to elucidate whether purinergic signaling is another important cofactor in colitis-induced gastroparesis, next to vanilloid signaling.

After peripheral stimulation of primary afferent neurons, the sensory signal is carried to the laminae I, II, V, and X of the spinal dorsal horn where the synapse with the second-order neuron occurs and the information is either discarded or gated to peripheral (autonomic) or central (thalamic) relay centers (24). It has been shown that CGRP plays an important modulatory role as a neurotransmitter at this synapse. Indeed, nociceptive visceral and somatic stimulation increase both its synthesis (18) and release (25). CGRP will then increase protein kinase activity in the postsynaptic neuron, thereby increasing neuronal excitability and NK1 receptor expression (47). This results in windup of the second-order neuron, accounting for a more sustained central sensitization. Plourde et al. (44) reported that intrathecal administration of a CGRP antagonist reduced acetic acid-induced colonic visceral hypersensitivity. Systemic CGRP inhibition in our model had no significant effect on gastric emptying in controls, in line with previous reports (36, 45). However, the CGRP antagonist significantly restored gastric emptying to normal levels in rats with TNBS colitis, suggesting that spinal sensitization of afferent nerve fibers also accounts for colitis-induced gastroparesis. We cannot completely exclude a local gastric or colonic effect of the antagonist, since CGRP is also released from peripheral terminals of stimulated nerve fibers and may thus play a modulatory role in peripheral excitation of colonic afferents or in gastric motility. Still, centrally administered CGRP induces a hyperalgesic state and intracisternal or intravenous application of CGRP inhibit gastric emptying in a CGRP-(8-37)-sensitive manner in rats, supporting our hypothesis (32, 35, 36).

An alternative explanation for colitis-induced gastroparesis could be stress, which may have been present in our study as the rats were fasted and which is known to inhibit gut motility (42). However, the pronounced effect of pelvic nerve section (13) and the beneficial effect of TRPV1 antagonists suggest that a peripheral neural pathway involving afferent sensitization accounted for the gastroparesis in our study.

The motility alterations at distance of an inflammatory region we reported here bear resemblance to the panenteric field effect as described for postoperative and septic ileus and may possibly involve similar pathophysiological mechanisms (10, 15, 52).

In conclusion, we showed that TRPV1 and CGRP receptors, biological mediators of sensitization, are involved in the pathophysiology of gastroparesis induced by TNBS colitis. Previous studies revealed that the afferent arm of this pathological reflex phenomenon is contained within the pelvic nerve (13). In this study we provide evidence that during colitis, both TRPV1 receptor production and expression in the DRG of the afferent pelvic nerve are increased. This peripheral sensitization triggers a reflex pathway involving CGRP, leading to overt inhibi-
bition of gastric emptying. Our data reveal that in colitis-induced gastroparesis we may have identified a novel in vivo model for TRPV1-mediated visceral afferent nerve sensitization as an alternative for visceral hyperalgesia. In addition, our results indicate that TRPV1 and CGRP modulation may be beneficial for IBD-associated motility disorders at a distance from the inflammatory site.

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