Distribution of transient receptor potential vanilloid types 2 and 1 in the distal colon in a trinitrobenzene sulfonicacid-induced rat colitis model with visceral hypersensitivity

Kenjiro Matsumoto (kenjiro@mb.kyoto-phu.ac.jp)
Kyoto Pharmaceutical University
Fumika Sugimoto
Kyoto Pharmaceutical University
Toshiki Mizuno
Kyoto Pharmaceutical University
Taisei Hayashi
Kyoto Pharmaceutical University
Ririka Okamura
Kyoto Pharmaceutical University
Takuya Nishioka
Kyoto Pharmaceutical University
Hiroyuki Yasuda
Kyoto Pharmaceutical University
Syunji Horie
Josai International University
Mizuho A. Kido
Saga University
Shinichi Kato
Kyoto Pharmaceutical University

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Abstract

Transient receptor potential vanilloid type 2 (TRPV2) and type 1 (TRPV1) are originally identified as heat-sensitive TRP channels. We compared the expression patterns of TRPV2 and TRPV1 in the rat distal colon and extrinsic primary afferent neurons, and investigated their roles in visceral hypersensitivity in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis rats. Both TRPV2 and TRPV1 expressions in the colon, dorsal root ganglion (DRG), and nodose ganglion (NG) were significantly upregulated in the TNBS-induced colitis model. TRPV2 cell bodies co-localized with the intrinsic primary afferent marker NeuN and the inhibitory motor neuronal marker nNOS in the myenteric plexus. TRPV2 expressions were further detected in the resident macrophage marker ED2 in the mucosa. In contrast, no TRPV1-expressing cell bodies were detected in the myenteric plexus. Both TRPV2- and TRPV1-positive cell bodies in the DRG and NG were double-labeled with the neuronal retrograde tracer fluorescent fluorogold. Large- and medium-sized TRPV2-positive neurons were labeled with the A-fiber marker NF200, calcitonin gene-related peptide (CGRP), and substance P (SP) in the DRG while small-sized TRPV1-positive neurons were labeled with the C-fiber markers IB4, CGRP, and substance P. TRPV2-and TRPV1-positive NG neurons were labeled with NF200 and IB4. Although TNBS treatment did not affect the TRPV1 and TRPV2 subpopulations in the DRG and NG, increased p-ERK1/2-positiveneurons, which co-localized with TRPV1, and TRPV2. Both TRPV2 and TRPV1 antagonists significantly alleviated visceral hypersensitivity in TNBS-induced colitis model rats. These findings suggest that sensitization and upregulation of intrinsic/extrinsic TRPV2- and extrinsic TRPV1-neurons may contribute to visceral hypersensitivity.

1. Introduction

Transient receptor potential (TRP) channels are non-selective cation channels that are activated by various chemical and physical stimuli, such as temperature, oxidative stress, and osmotic pressure. Eleven TRP channels in mammalian cells have been identified as thermosensitive TRP channels (Uchida et al. 2017). Among them, TRP vanilloid type 2 (TRPV2) and type 1 (TRPV1) are activated by high temperatures, ≥ 52℃ and ≥ 43℃, respectively. Human and rat TRPV2 were cloned based on their homology to TRPV1 and accordingly named vanilloid-receptor-like protein 1 (Caterina et al. 1999). Although TRPV2 has structural homology with TRPV1, their cellular distributions and physiological roles differ. In the gastrointestinal tract, TRPV2 is expressed in primary afferent neurons, inhibitory motor neurons, and several innate and adaptive immune cells and regulates phagocytosis and smooth muscle relaxation (Issa et al. 2014; Mihara et al. 2010, 2013). TRPV1 is predominantly expressed in primary afferent neurons and contributes to visceral sensing and smooth muscle contraction (Abdullah N et al. 2020; Matsumoto et al. 2009).

Gastrointestinal nociceptors constitute a specialized population of primary afferent neurons that detect noxious stimuli in the enteric nervous system (ENS) and relay this information to the central nervous system (Abdullah et al. 2020). Afferent neurons of the gastrointestinal tract are broadly classified into intrinsic primary afferent neurons, whose cell bodies are located in the myenteric, submucosal plexus,

and extrinsic primary afferent neurons, whose cell bodies are located in the dorsal root ganglia (DRG), nodose ganglia (NG), and jugular ganglia.

Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease, is an emerging health problem with worldwide prevalence. Abdominal pain, a frequent symptom of IBD, lowers patients' quality of life. TRP has received increasing attention as a target for visceral hypersensitivity in IBD (Balemans et al. 2017). During gastrointestinal inflammation, both sensitization and upregulation of TRPV1 contribute to visceral hypersensitivity in experimental colitis model and patients with IBD (Holzer 2004 and 2011). A high percentage of extrinsic primary afferent neurons possess TRPV1 in the NG and DRG (Lai et al. 2017). Activation of TRPV1 at the nerve terminals of primary afferent neurons leads to the release of neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), in the mucosa, which contributes to inflammatory responses by modulating leukocyte migration and plasma extravasation, leading to visceral hypersensitivity (Lapointe et al. 2015). However, the proportion of intrinsic and extrinsic primary afferent neurons expressing TRPV2, and the physiological and pathological roles of TRPV2 in visceral sensing remain unclear. In the present study, we compared the expression patterns of TRPV2 in comparison with TRPV1 in the rat ENS and extrinsic primary afferent neurons, and investigated the roles of these channels in visceral sensitivity in TNBS-induced colitis in rats.

2. Materials And Methods

2.1. Animals and ethics statement

Male Sprague–Dawley rats (Japan SLC Inc., Shizuoka, Japan), aged 8or 9 weeks were used in this study. Animals were housed in a temperature-controlled room at 24°C with lights on from 07:00 to 19:00, with free access to food and water under specific pathogen-free conditions. All experiments involving animals were performed in accordance with the ARRIVE guidelines (McGrath and Lilley 2015). The protocols were approved by the Committee on the Ethics of Animal Research of Kyoto Pharmaceutical University, Japan (permit number: 19 – 009). The number of rats used was kept to the minimum necessary for meaningful interpretation of data, and animal discomfort was kept to a minimum.

2.2. Induction of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis

Colitis was induced as described previously (Morris et. al 1989). Briefly, after being deprived of food for 12 h with free access to water, the rats were randomly divided into normal and TNBS-treated groups. They were anesthetized with 2.0% isoflurane, and a flexible catheter was carefully inserted into the colon (5 cm proximal to the anus). TNBS (0.6 mL of a 30 mg/mL solution in 30% ethanol; Sigma-Aldrich, St. Louis, MO, USA) was administered transanally to the rats. After injection, the tail of the rat was lifted and inverted for 30 s. The animals were allowed to recover for 5 days before they were sacrificed for experimental procedures.

2.3. Immunohistochemistry

The rats were sacrificed by spinal dislocation, and then the distal colon was removed, washed with cold phosphate-buffered saline (PBS), and immersed in 4% paraformaldehyde for 3 h at 4°C. The animals were deeply anesthetized with medetomidine–midazolam–butorphanol and perfused through the ascending aorta with PBS, followed by 4% paraformaldehyde. After the perfusion, the lumbar (L5–6) DRG, sacral (S1) DRG, and NG were removed and post-fixed in the same fixative for 3 h at 4°C. Immunohistochemical examination was performed as previously described by Matsumoto et al. (2020). The sources of all primary and secondary antibodies, as well as the optimized dilutions, are listed in Supplementary Table 1. TRPV2 immunoreactivity was detected using fluorescein-conjugated tyramide amplification. Other molecules were detected by indirect staining with specific antibodies. No specific immunostaining was observed in control experiments.

2.4. Retrograde labeling

Neurons in the DRG or NG projecting to the rat distal colon were determined by retrograde tracing using fluorescent fluorogold dye (Fluorochrome, Denver, Colorado, USA), as reported previously (Matsumoto et al. 2016). Approximately 5 µL of 4% fluorogold solution dissolved in sterile water was injected circumferentially into the distal colon at five sites under 2% isoflurane anesthesia. Tissue recovery occurred 1 week after fluorogold injection.

2.5. Microscopy and image analysis

The sections were viewed using a confocal microscope (A1R^{+}; Nikon, Tokyo, Japan). Confocal images in Z-stacks were projected onto a single plane and reconstructed using the NIS-Elements AR 4.20.00 software. Sections were viewed at 200× magnification. For quantitative analysis of TRPV2- or TRPV1-immunopositive cell bodies in the myenteric plexus, DRG and NG were counted and normalized to the same area (10^4 µm^2). Data were labelled with date and sample identifiers and analyzed by two independent observers blinded to the animal group assignment.

2.6. Assessment of the visceromotor response (VMR) to colorectal distention (CRD)

Assessment of VMR to CRD was performed 4 days after TNBS treatment, as previously reported (Matsumoto et al. 2016). As a visceral stimulus, mechanical distensions of the distal colon were performed by pressure-controlled air inflation of a flexible polyethylene balloon connected to an electronic distension device (Distender Series II barostat, G&J Electronics, Willowdale, ON, Canada). The balloon was lubricated, inserted intra-anally, and positioned 2 cm proximal to the anus. When the balloon was inflated, it mainly touched the distal colon area, which corresponded to the area used for the immunohistochemical study. The VMR to CRD was quantified by electromyographic (EMG) recordings of abdominal wall muscle activity. Mice were challenged with distending pressures of 20, 40, 60, and 80 mmHg, with two 10-second trials at each pressure and a 2-minute recovery period between the distensions. Data were imported into an eight-channel analyzer software (Starmedical, Tokyo, Japan) for

analysis. Representative raw electromyographic recordings are presented in millivolts. The EMG baseline activity during the 10 s before stimulation was subtracted from the 10 s of each reflex response.

2.7. Reagents

N-(4-tertiary butylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carboxamide (BCTC) was purchased from Sigma-Aldrich (St. Louis, MO). Tranilast was purchased from the Tokyo Chemical Industry (Tokyo, Japan).

2.8. Statistical analyses

Data are presented as mean ± SEM. Statistical analyses were performed using the GraphPad Prism 9.2.0 (GraphPad Software, La Jolla, CA, USA). Multiple groups were compared using a two-way analysis of variance (ANOVA). If the ANOVAs revealed a significant main effect or interaction between the factors, a post-hoc Holm–Sidak test was performed. The Student's t-test was used to compare the two sets of data. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Expression of TRPV2 and TRPV1 in the rat distal colon

First, we investigated the TRPV2 and TRPV1 expressions in the distal colon of normal rats (Fig. 1a). TRPV2 immunoreactivities were identified not only within nerve fibers in the mucosa and muscle layers, but also cell-like structures in the mucosa of the distal colon. Numerous TRPV1-immunoreactive nerve fibers were observed in the mucosa and muscle layers of the distal colon. Next, we performed double-labeling experiments with PGP 9.5, a pan-axonal marker, to identify nerve fibers. TRPV2 and TRPV1-immunoreactive neuron-like structures co-localized with PGP9.5 (Fig. 1a). The subsequent quantitative analysis indicated that TRPV2 and TRPV1 immunoreactive areas were significantly increased in TNBS-induced colitis rats compared with normal rats (Fig. 1b).

3.2. Characterization of TRPV2 and TRPV1-immunoreactiviies in horizontal sections of the rat distal colon

To gain a better understanding of the distribution of TRPV2 and TRPV1 in the distal colon, serial sections were cut along the horizontal plane (Fig. 2). We identified TRPV2-immunoreactive cell bodies in the myenteric plexus of the normal and TNBS-treated colitis models (Fig. 2a). No significant difference was observed in the number of TRPV2 cell bodies between the normal and TNBS-treated groups (Fig. 2a). To characterize intrinsic TRPV2 neurons, we performed double-labeling experiments using nNOS as a marker for inhibitory motor neurons and NeuN as a marker for intrinsic primary afferent neurons in the myenteric plexus (Fig. 2b). Intrinsic TRPV2 neurons co-localized with nNOS and NeuN in the myenteric plexus. No significant difference in the percentage of co-localization was observed between the normal and TNBS-treated groups. Meanwhile, TRPV1 staining of cell bodies was not detected in myenteric plexuses of the

normal and TNBS-treated colitis models (Fig. 2c). Furthermore, TRPV2-immunopositive cell-like structures, which co-localized with the resident macrophage marker ED2, were observed in the mucosa of the normal and TNBS-treated colitis models (Fig. 2d).

3.2. Origin of the afferent innervation of the distal colon using the retrograde fluorescent tracer

We next investigated the origin of afferent innervation of the distal colon using the retrograde fluorescent tracer fluorogold dye in normal rats (Fig. 3). TRPV2- and TRPV1-immunoreactive cell bodies in the lumbar 6 (L6) and sacral 1 (S1) DRG were double-labeled with fluorogold that was retrogradely transported from the distal colon. Additionally, the majority of TRPV2- and TRPV1-immunoreactive cell bodies in the NG were double-labeled with fluorogold.

3.3. Localization and characterization of TRPV2 and TRPV1 immunoreactivity in rat DRGs

Figure 4 showed the alterations of TRPV2- and TRPV1-positive neurons in the DRG of the normal and TNBS-treated rats. The number of TRPV2- (Fig. 4a) and TRPV1- (Fig. 4c) immunoreactive cell bodies in the TNBS-treated group was significantly higher than those in the normal group. We investigated the co-localization of TRPV2 (Fig. 4b) and TRPV1 (Fig. 4d) with NF200 and IB4 in the rat L6/S1 DRGs neurons. Almost all TRPV2 cells were positive for NF200, a marker of myelinated A-fibres while TRPV1 cells were positive for IB4, a marker of myelinated C-fibres. We then investigated the co-localization of TRPV2 and TRPV1 with the neuropeptides CGRP and SP in the DRG of normal and TNBS-treated rats (Fig. 5). TRPV1 cells were highly co-localized with CGRP in the DRG of normal and TNBS-treated rats (Fig. 5a). Meanwhile, approximately 50% of TRPV2-positive cells co-localized with CGRP in in normal and TNBS-treated rats (Fig. 5a). Both TRPV2 and TRPV1 were highly co-localized with SP in the DRGs of normal and TNBS-treated rats (Fig. 5b). The co-localization percentage of TRPV2 and TRPV1 with each neuropeptide was not changed between the normal and TNBS-treated groups.

3.4. Localization and characterization of TRPV2 and TRPV1 immunoreactivity in rat NG

We next investigated the alterations of TRPV2 and TRPV1 neurons in normal and TNBS-treated NG rats (Fig. 6). The number of TRPV2- (Fig. 6a) and TRPV1- (Fig. 6c) immunoreactive cell bodies in the TNBS-treated group was significantly higher than that in the normal group. Regarding the co-localization of TRPV2 (Fig. 6b) and TRPV1 (Fig. 6d) with NF200 and IB4 in the rat NG, TRPV2 was expressed in NF200- and IB4-positive neurons. TRPV1 expression was higher in IB4-positive neurons than NF200-positive neurons.

3.5. ERK1/2 activation in TRPV2 and TRPV1-immunopositive neurons in rat DRGs and NG induced by TNBS

To confirm the activation of TRPV2 and TRPV1 neurons in DRG and NG, we examined p-ERK1/2 labeling in normal and TNBS-treated rats (Fig. 7). The percentage of p-ERK1/2-immunoreactive neurons, which co-localized with TRPV2 and TRPV1, in the DRG was significantly increased after TNBS treatment compared with normal rats (Fig. 7a) and NG (Fig. 7b).

3.6. Effects of TRPV2 and TRPV1 inhibition on TNBS-induced visceral hypersensitivity in rats

We investigated the effect of TRPV2 and TRPV1 inhibitions on noxious mechanical stimulation-induced visceral hypersensitivity in TNBS-treated rats. To investigate the involvement of TRPV2 and TRPV1 in physiological and pathological visceral hypersensitivity, we treated rats with the TRPV2 inhibitor tranilast and TRPV1 antagonist BCTC (Fig. 8). The VMR to CRD ratio was significantly increased in rats treated with TNBS-vehicle compared with that in the normal-vehicle group, which is indicative of the development of visceral hyperalgesia. In TNBS-induced visceral hypersensitivity, Treatment with tranilast and BCTC reversed TNBS-induced visceral hypersensitivity to normal levels at all tested CRD pressures (Fig. 7a, b).

4. Discussion

The gastrointestinal tract is innervated by intrinsic and extrinsic visceral afferent and visceral motor afferent neurons (Niu et al. 2020). Visceral afferent neurons convey gastrointestinal information to the brain through the vagal and spinal afferents, with their cell bodies located within the NG and DRG, respectively. To the best of our knowledge, this is the first report to compare the expression patterns of the thermosensitive TRPV2 and TRPV1 in the rat distal colon, DRG, and NG in normal and TNBS-induced colitis model.

We previously reported that TRPV2 was expressed in intrinsic primary afferent neurons and inhibitory motor neurons in the normal myenteric plexus of the rat esophagus (Matsumoto et al. 2021). Mihara et al. have demonstrated that TRPV2 was expressed in CGRP-positive intrinsic/extrinsic sensory and nNOS-positive intrinsic inhibitory motor neurons in murine small intestines and stomachs (Mihara et al. 2010 and 2013). In this study, we clearly identified subpopulations of intrinsic TRPV2 neurons, approximately half of the TRPV2-positive neurons were intrinsic primary afferent neurons and half were inhibitory motor neurons in the myenteric plexus. Although several studies have demonstrated that TRPV1 is limited to extrinsic primary afferent neurons, some studies have argued for intrinsic expression of TRPV1 in the enteric nervous system (Buckinx et al. 2013). In this study, TRPV1-imuunoreactive cell bodies were not detected in the myenteric plexus of normal and colitis model, suggesting that TRPV1-positive nerve fibers are not of intrinsic origin in the rat distal colon.

TRPV2 is expressed in both neuronal and non-neuronal cells such as mast cells, aortic smooth muscle cells, and monocytes/macrophages (Perálvarez et al. 2013). TRPV2 expression in macrophages has mainly been demonstrated in resident populations in peripheral organs, such as the liver, skin, lung, and testis (Eubler et al. 2021). In the gastrointestinal tract, non-neuronal TRPV2 is predominantly expressed in macrophages in the esophagus and oral cavity (Matsumoto et al. 2021; Shimohira et al. 2009). We

confirmed that TRPV2-immunoreactive cells were mainly co-localized with ED2-positive resident macrophages in the mucosa of normal and colitis rats. Non-neuronal TRPV2 regulates innate and adaptive immune responses (Santoni et al. 2013). TRPV2 is essential for phagocytosis and chemotaxis in macrophages (Nagasawa et al. 2007; Link et al. 2010). Issa et al. have reported less severe intestinal inflammation in TRPV2-deficient mice than wild-type mice in a murine colitis model because of the reduced infiltration of macrophages, suggesting that the TRPV2 pathway plays a key role in the development of colitis (Issa et al. 2014). Further studies are required to elucidate the role of TRPV2 in the progression of intestinal inflammation in macrophages.

Spinal afferents innervating the distal colon originate from the lumbosacral region and play a major role in visceral hypersensitivity following colitis (Abdullah et al. 2020; Robinson et al. 2004). Vagal afferents may play a complex role in visceral pain processes (Chen et al. 2008). As previously reported, TRPV1 is expressed in the spinal and vagal afferents innervating the distal colon in retrograde labeling and/or functional experiments (Hong et al. 2011; Christianson et al. 2006). However, the origin of TRPV2 positive primary afferent innervation in the rat distal colon has not been analyzed in detail. Consistent with these previous reports, the retrograde tracer-labeled colonic neurons were immunopositive for TRPV1, with approximately 40% and 46% of TRPV1 neurons in the DRG and NG innervating the distal colon, respectively. Meanwhile, we observed that approximately 23% and 65% of TRPV2 neurons in the DRG and NG innervate the distal colon, respectively. The immunohistochemical results of the myenteric plexus and extrinsic primary afferent neurons suggest that TRPV2 and TRPV1 immunopositive nerve fibers in the distal colon are exogenous primary afferent neuronal projections from the DRG and NG.

TRPV1 immunopositive cells have small cell bodies, and highly co-localized with the C-fiber marker IB4 but not the A-fiber marker NF200 in normal rat DRG (Tonelotto et al. 2019). As previously reported, almost all middle- and large-sized TRPV2 neurons labelled with NF200 in the normal rat DRG (Lewinter et al. 2004). We confirmed that TRPV2 immunopositive cells had large- and middle-sized cell bodies and highly co-localized with NF200 but not with IB4 in normal and TNBS-treated rat DRG. Meanwhile, TRPV1 positive cells were small and highly co-localized with IB4 in normal and TNBS-treated rat DRG. Hence, TRPV2 and TRPV1 are expressed in the Aδ-/Aβ- and C-fibres of spinal afferent neurons, respectively.

Primary sensory neurons contain neuropeptides including CGRP and SP, which contribute to pain transduction. TRPV1 can promote the release of CGRP and SP from spinal afferent nerve terminals of DRG neurons under TNBS-induced visceral hypersensitivity conditions (Patapoutian A et al. 2009). Here, we confirmed that TRPV1 neurons were highly co-localized with SP and CGRP in normal and TNBS-treated rat DRG. As previously reported, approximately 40% of TRPV2 neurons co-localized with CGRP in the normal mouse DRG (Hsieh et al. 2012). Middle-sized TRPV2 neurons highly co-localized with SP (90%), but partially with CGRP (50%). SP and CGRP are the principal peptides released mainly from Aδ- and C-fiber axons associated with the DRG (Feng et al. 2012). These results suggest that both TRPV2-positive Aδ- and TRPV1-positive C-fibers are peptidergic neurons that contribute to the release of CGRP and SP during intestinal inflammation.

It has been reported that vagal afferent neuron, mainly consist of NG, is associated with visceral hypersensitivity in rats (Gschossmann et al. 2002; Chen et al. 2008; Kupari et al. 2019). Low-intensity electrical vagal stimulation of Aδ-fibers reduced the VMR to CRD, but high-intensity electrical vagal stimulation of C-fibers had no effect in normal rats (Chen et al. 2008). TRPV1 neurons co-localized with IB4 and NF200 in rat NG (Sun et al. 2009). However, TRPV2-neurons in the NG remain uncharacterized with respect to distribution and co-localization with IB4 and NF200. Double immunofluorescence analysis has suggested that co-expression of TRPV2 and TRPV1 is abundant in the NG, but not in the DRG (Ichikawa and Sugimoto 2003). However, we could not conduct co-expression experiments of TRPV2 and TRPV1 because the primary antibodies of TRPV2 and TRPV1 were raised in the same host species. We identified that both TRPV2 and TRPV1 co-localized with IB4-positve unmyelinated C-fibers and NF200-positive myelinated A-fibers in normal and TNBS-treated rats. By contrast, TRPV2 and TRPV1 expressed neurons were clearly different in the normal and TNBS-treated rat DRG. Thus, TRPV2 and TRPV1 have demonstrated different properties in spinal afferent neurons, but similar properties in vagal afferent neurons.

Peripheral inflammation has been reported to increase TRPV2 and TRPV1 expression in the rat DRG (Shimosato et al. 2005; De Schepper et al. 2008; Miranda et al. 2007). Here, TNBS-induced intestinal inflammation caused a significant increase in TRPV2 and TRPV1 expression in DRG and NG. However, the subpopulations of TRPV2 and TRPV1 expressed neurons did not change. These results suggest that TNBS-induced intestinal inflammation affects the number of TRPV2 and TRPV1 neurons, but not their characteristics. ERK1/2 activation in afferent neurons has been suggested to be involved in peripheral sensitization in TNBS-induced experimental colitis. ERK1/2 activation also mediates upregulation of TRP expression (Van den Eynde C et al. 2021). We examined the phosphorylation of ERK1/2 in the DRG and NG five days after intracolonic TNBS treatment. The number of p-ERK1/2-immunoreactive neurons that co-localized with TRPV2 and TRPV1 in TNBS-treated rats was significantly higher than that in normal rats in the DRG and NG. Therefore, DRG and NG neurons innervating the rat distal colon probably respond to various noxious stimuli in their peripheral nerve endings through TRPV1 and TRPV2 activation, and transduce nociceptive information to the central nervous system.

TRPV1 has attracted international attention as a therapeutic target for visceral hypersensitivity because of its high expression in C-fibers, which play an important role in visceral sensation (Balemans et al. 2017); a TRPV1 antagonist attenuates visceral hypersensitivity in TNBS-treated rats (Miranda et al. 2007). Consistent with previous findings, the TRPV1 selective antagonist BCTC significantly attenuated visceral hypersensitivity in TNBS-treated rats, indicating a role of TRPV1 in exacerbating visceral hypersensitivity in pathological conditions. However, no reports have been conducted on the role of TRPV2 in visceral sensing in humans and rodents. TRPV2-expressing afferents convey nociceptive mechanical information to the spinal cord and participate in the development of mechanical hyperalgesia and allodynia in rats (Petitjean et al. 2014). TRPV2 on primary afferent neurons has also been reported to contribute to mechanosensitive mechanisms in TRPV2-defiecient mice (Katanosaka et al. 2018). Myelinated Aδ-fibers that respond to nociceptive mechanical and thermal stimuli also transmit visceral sensations to the center, although the role of Aβ-fibers that detect low-threshold mechanical stimuli

remains unclear. Thus, we used tranilast to inhibit TRPV2-expressing Aδ-/Aβ-fibers of the spinal primary afferents/intrinsic primary afferents and evaluated their effects on TNBS-induced visceral hypersensitivity. We observed that tranilast attenuated TNBS-induced visceral hypersensitivity compared to the control (normal vehicle-treated group) at all pressures.

This study had several limitations. First, TRPV2 is expressed not only in spinal/vagal primary afferent neurons but also in macrophages, intrinsic primary afferent neurons, and inhibitory motor neurons. However, we could not clearly determine the contribution of each TRPV2-expressed site to TNBS-induced visceral hypersensitivity. Second, further mechanistic studies including TRPV2 knockout mice might be needed to determine the involvement of TRPV2 in visceral hypersensitivity because selective TRPV2 antagonists are still not commercially available. Third, we did not identify a difference between TRPV2 and TRPV1 in vagal afferent neurons in the development of visceral hypersensitivity.

Our findings reveal that TRPV2 is expressed in resident macrophages, intrinsic primary afferent neurons, inhibitory motor neurons, and Aδ-/Aβ-fibers of spinal afferent and vagal afferent neurons innervating the distal colon. In contrast, TRPV1 is expressed in the C-fibers of spinal afferent and vagal primary afferent neurons innervating the distal colon but not intrinsic neurons. Sensitization and upregulation of TRPV2 and TRPV1 contributes to visceral hypersensitivity in an experimental colitis model.

Declarations

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Conflict of interest statement

The authors declare no conflict of interest.

Author contributions

KM, SH, and SK planned and designed the experiments. FS, TM, TH, RO, and TN performed the experiments. KM, FS, TM and SK analysed the data. KM, and SK wrote the manuscript. KM, HY, MAK, SK reviewed and discussed the data. All authors read and approved the final manuscript.

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Figures

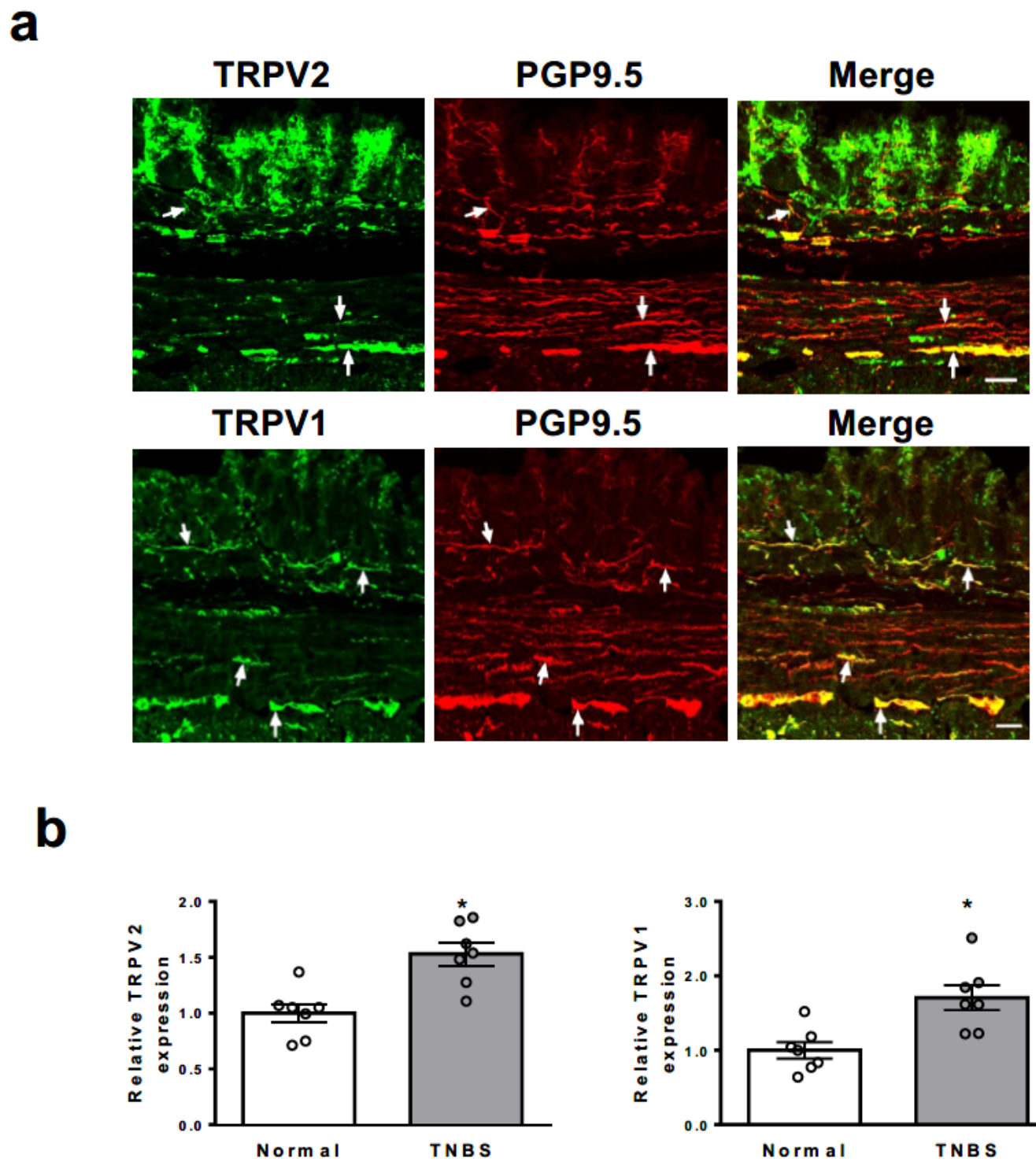
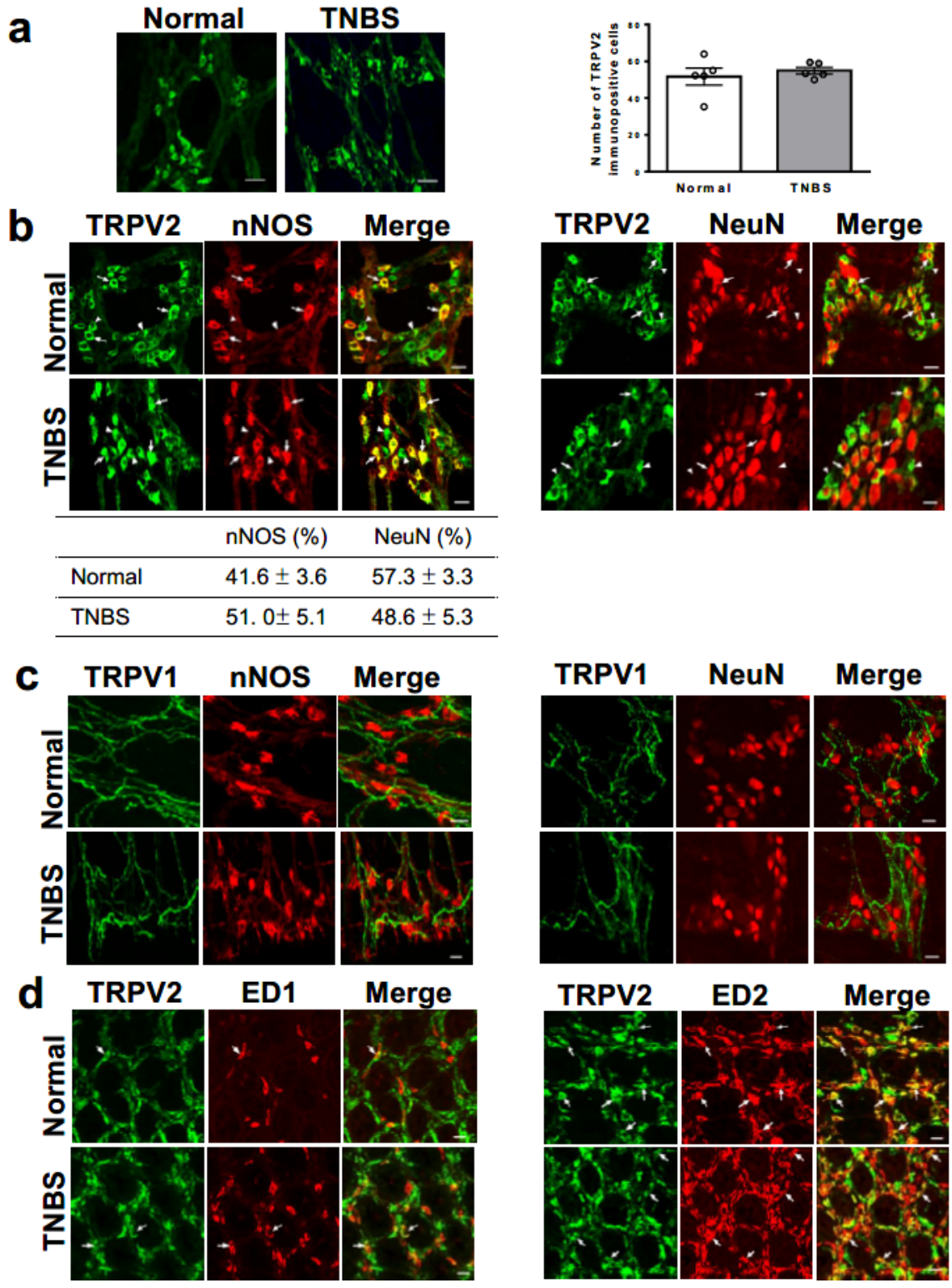


Figure 1

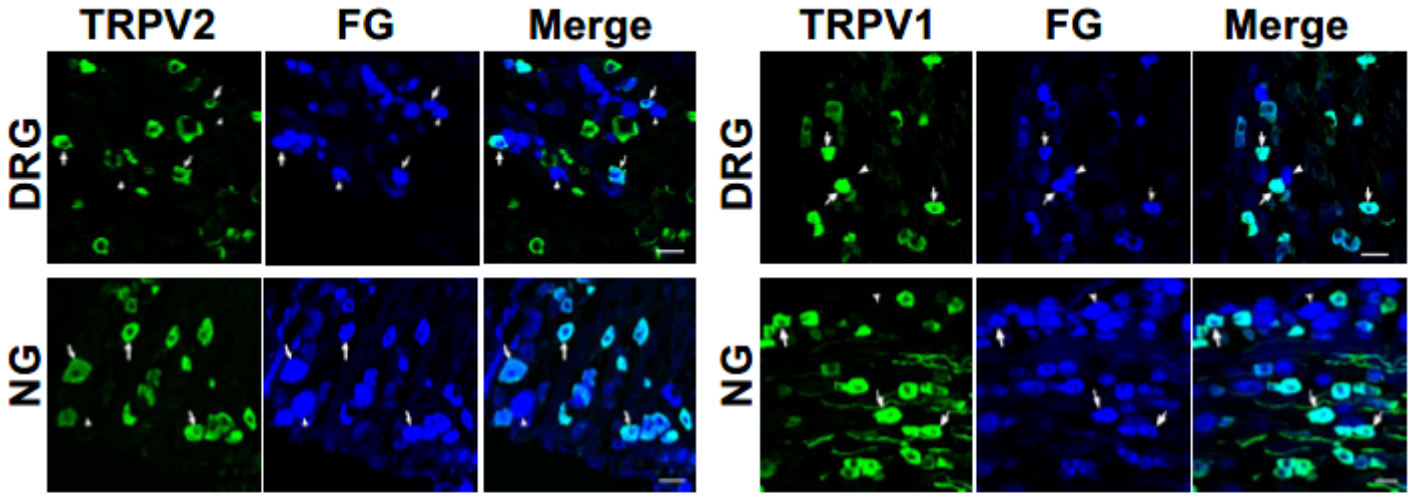
TNBS treatment upregulated TRPV2 and TRPV1 expressions in the distal colon of rats. Double labeling of TRPV2 or TRPV1 with the pan-neuronal marker PGP9.5 in transverse sections of the normal rat distal colon (A). The arrows indicate co-localization of TRPV2-or TRPV1-immunopositive neurons with PGP9.5. The scale bars are 50 μm. Quantitative analysis of TRPV2 and TRPV1 expressions in the rat distal colon in normal and TNBS-induced colitis models. Data are presented as mean ± SEM (n = 7). *p < 0.05, compared to the normal group.



	nNOS (%)	NeuN (%)
Normal	41.6 ± 3.6	57.3 ± 3.3
TNBS	51. 0± 5.1	48.6 ± 5.3

Figure 2

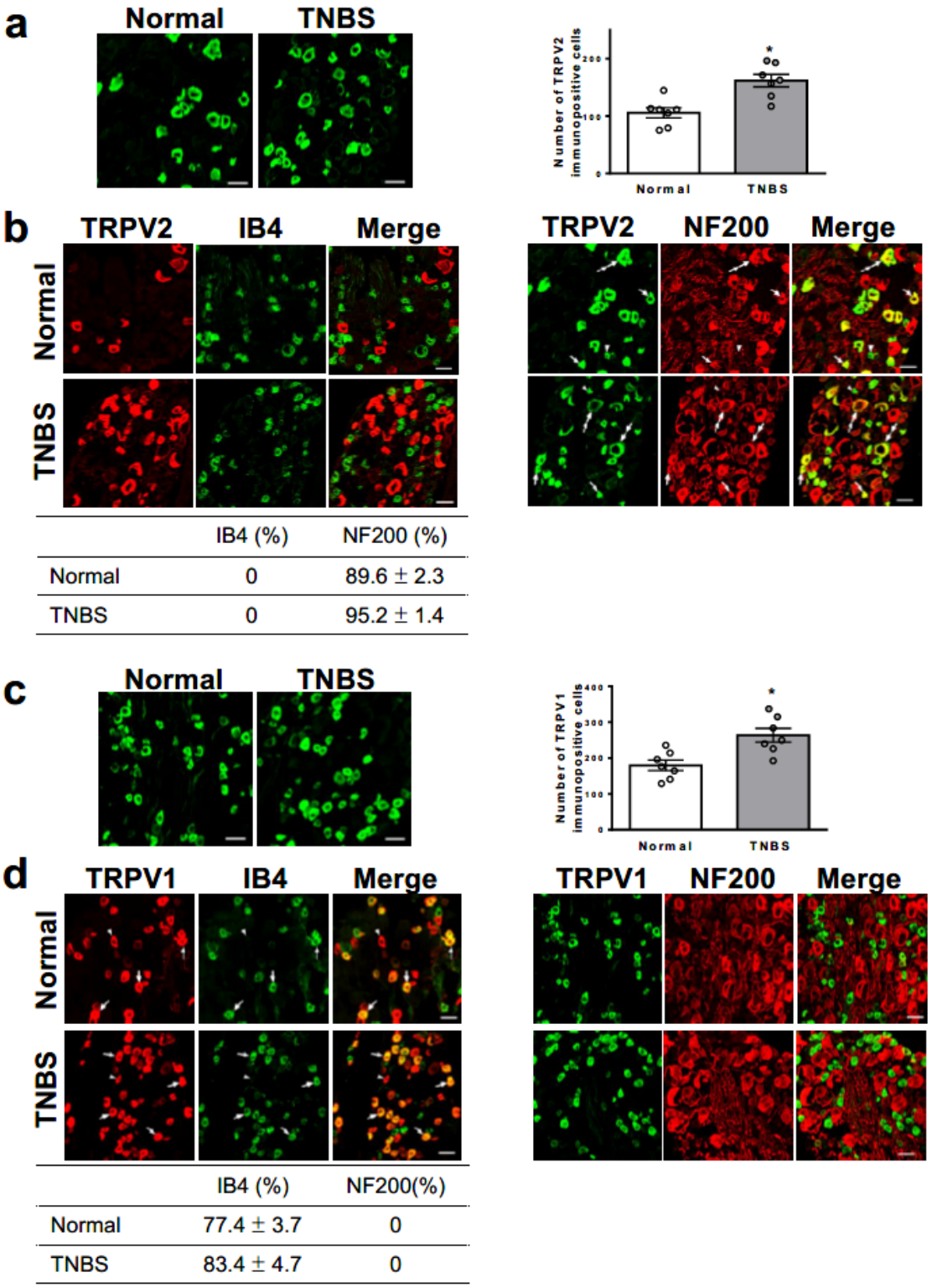
TRPV2 expressed in resident macrophages, inhibitory motor neurons, and intrinsic primary afferent neurons in the rat distal colon, but TRPV1 was not expressed in the intrinsic neurons. Quantitative analysis of TRPV2-immunopostive neuron in horizontal sections of the myenteric plexus in the normal and TNBS-induced colitis model (A). Double-labeling of TRPV2 (B) or TRPV1 (C) with nNOS and NeuN in horizontal sections of myenteric plexus in the normal and TNBS-induced colitis model. Co-localization percentage of TRPV2 immunopositive neurons with nNOS or NeuN immunoreactivity in the myenteric plexus of the normal and TNBS-induced colitis model rats. Data are presented as the mean ± SEM (n = 5). Double labeling of TRPV2 with ED1and ED2 in horizontal sections of the mucosa (D). The arrows indicate co-localization of TRPV2-immunopositive neurons with each marker. The arrowheads indicate TRPV2 neurons that do not co-localize each marker. The scale bars are 20 μm.



	TRPV2 (%)	TRPV1 (%)
DRG	23.4 ± 3.3	39.6 ± 11.9
NG	65.1 ± 4.0	45.7 ± 4.7

Figure 3

TRPV2 and TRPV1 expressed in spinal and vagal afferent neurons innervated at the rat distal colon. Double labeling of TRPV2 and TRPV1 with fluorogold (FG) in L6/S1 dorsal root ganglion (DRG) and nodose ganglion (NG). The arrows indicate co-localization of TRPV2 or TRPV1 neurons with FG immunoreactive neurons. The arrowheads indicate TRPV2 or TRPV1 neurons that do not co-localize FG. The scale bars are 20 μm. Co-localization percentage of TRPV2 or TRPV1 immunopositive neurons with FG immunoreactivity in the DRG and NG of normal rats. Data are presented as the mean ± SEM (n = 7).



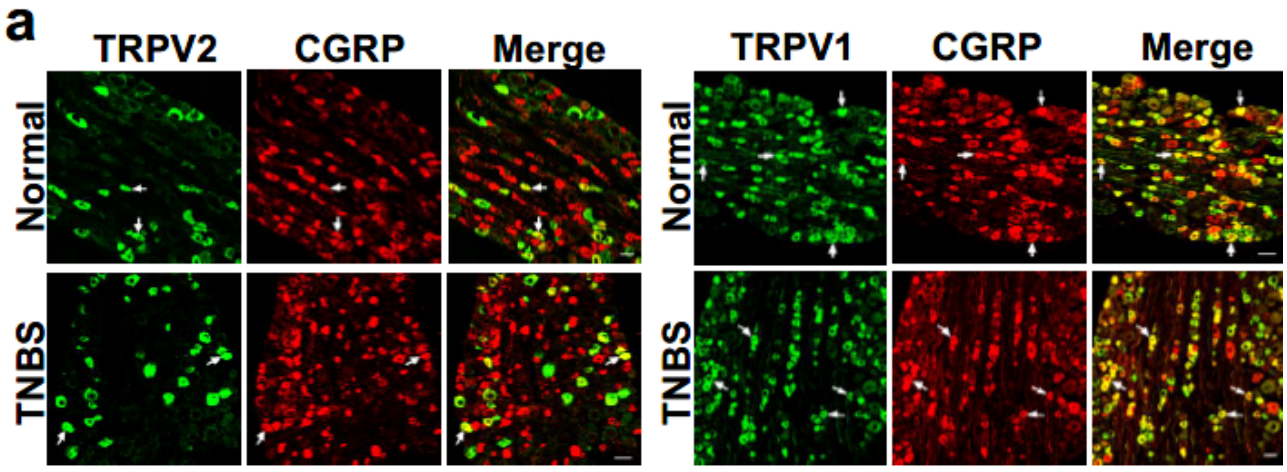
	IB4 (%)	NF200 (%)
Normal	0	89.6 ± 2.3
TNBS	0	95.2 ± 1.4

	IB4 (%)	NF200(%)
Normal	77.4 ± 3.7	0
TNBS	83.4 ± 4.7	0

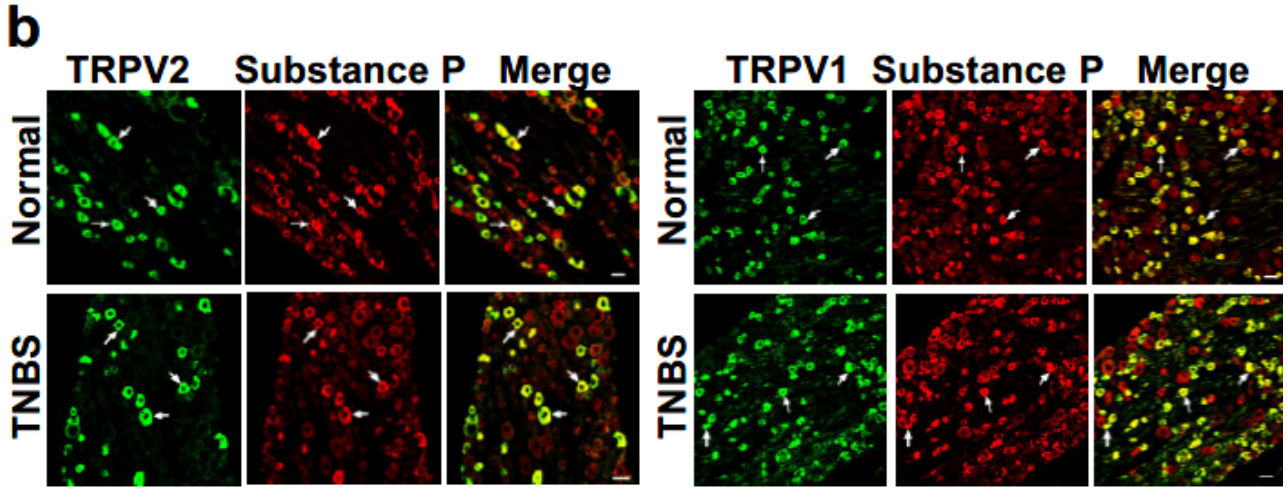
Figure 4

The TNBS treatment increased the TRPV2 and TRPV1 expressions but did not affect the distribution in L6/S1 DRG. Quantitative analysis of TRPV2- (A) and TRPV1-immunopostive neuron (C) in normal and TNBS-induced colitis rats L6/S1 DRG. *p < 0.05 compared with the normal group. Double labeling of TRPV2 (B) or TRPV1 (D) with IB4 and NF200 in the normal and TNBS-induced colitis rats L6/S1 DRG. The arrows indicate co-localization of TRPV2- or TRPV1-immunopositive neurons with each marker. The

arrowheads indicate TRPV2- or TRPV1-immunopositive neurons that do not co-localize a marker. Data are presented as the mean ± SEM (n = 7). The scale bars are 20 μm.



	TRPV2 (%)	TRPV1 (%)
Normal	52.4 ± 2.3	87.5 ± 1.0
TNBS	47.5 ± 5.6	82.9 ± 4.7

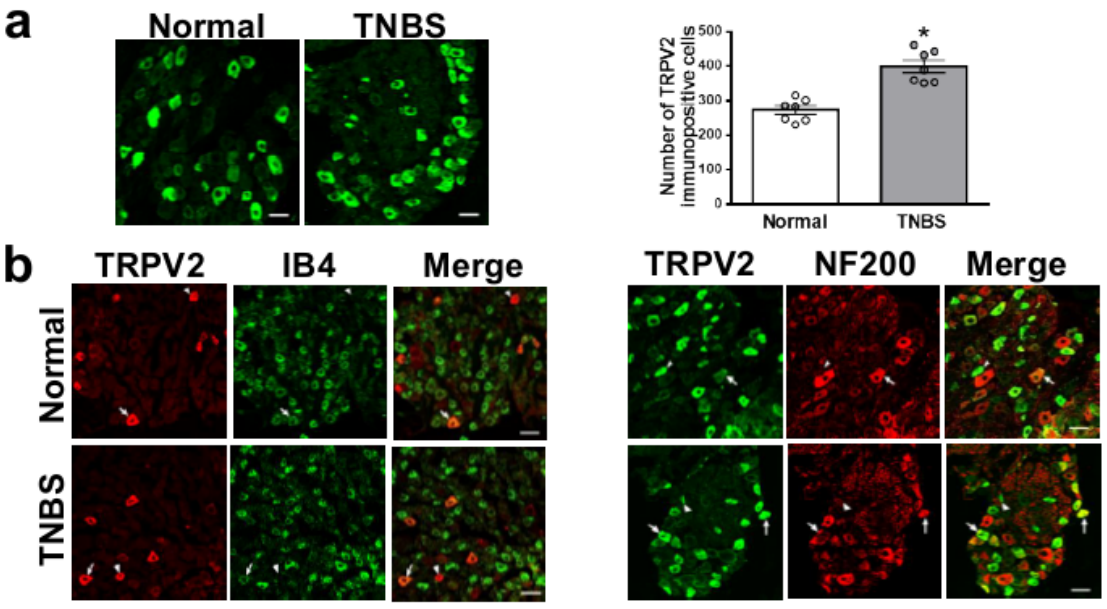


	TRPV2 (%)	TRPV1 (%)
Normal	93.0 ± 4.6	93.5 ± 1.3
TNBS	87.7 ± 3.2	95.1 ± 0.9

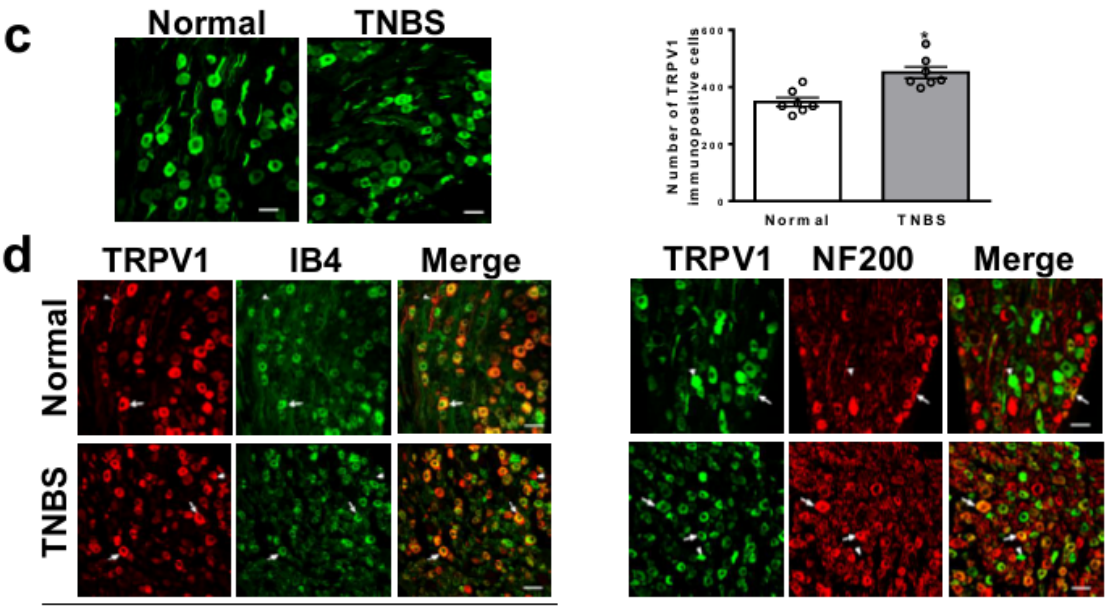
Figure 5

TNBS treatment increased the TRPV2 and TRPV1 expressions but did not affect the distribution in the nodose ganglion. Quantitative analysis of TRPV2- (A) and TRPV1-immunopostive neurons (C) in the

normal and TNBS-induced colitis rats NG. * p< 0.05 compared with the normal group. Double labeling of TRPV2 (B) or TRPV1 (D) with IB4 and NF200 in the normal and TNBS-induced colitis rats' nodose ganglion. The arrows indicate co-localization of TRPV2- or TRPV1-immunopositive neurons with each marker. The arrowheads indicate TRPV2- or TRPV1-immunopositive neurons that do not co-localize a marker. Data are presented as the mean ± SEM (n = 7). The scale bars are 20 μm.



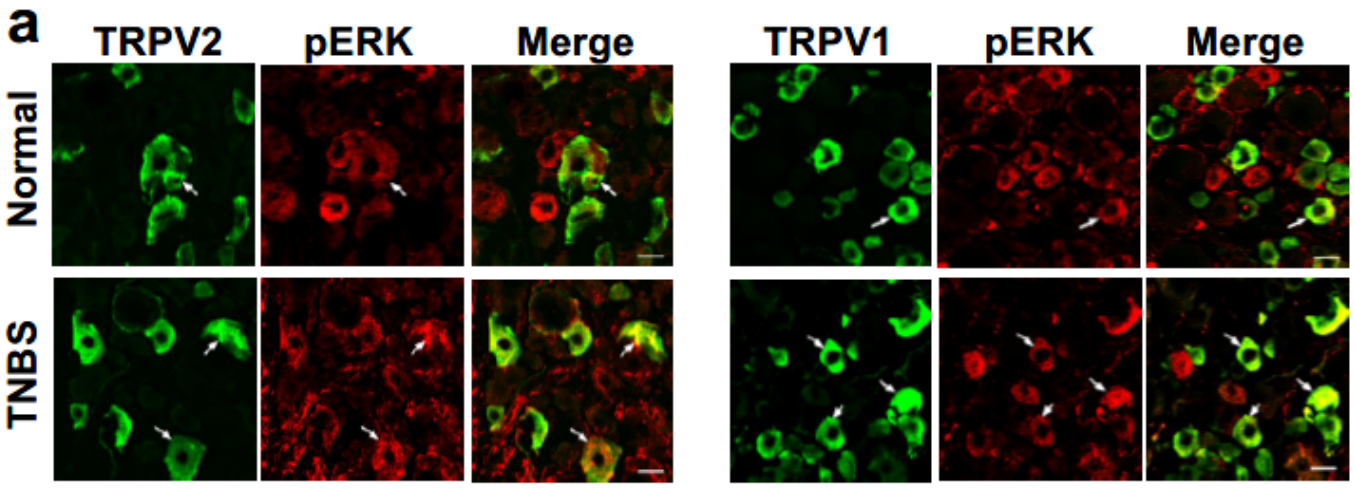
	IB4 (%)	NF200 (%)
Normal	31.2 ± 4.3	29.3 ± 2.6
TNBS	48.1 ± 6.1	39.0 ± 4.2



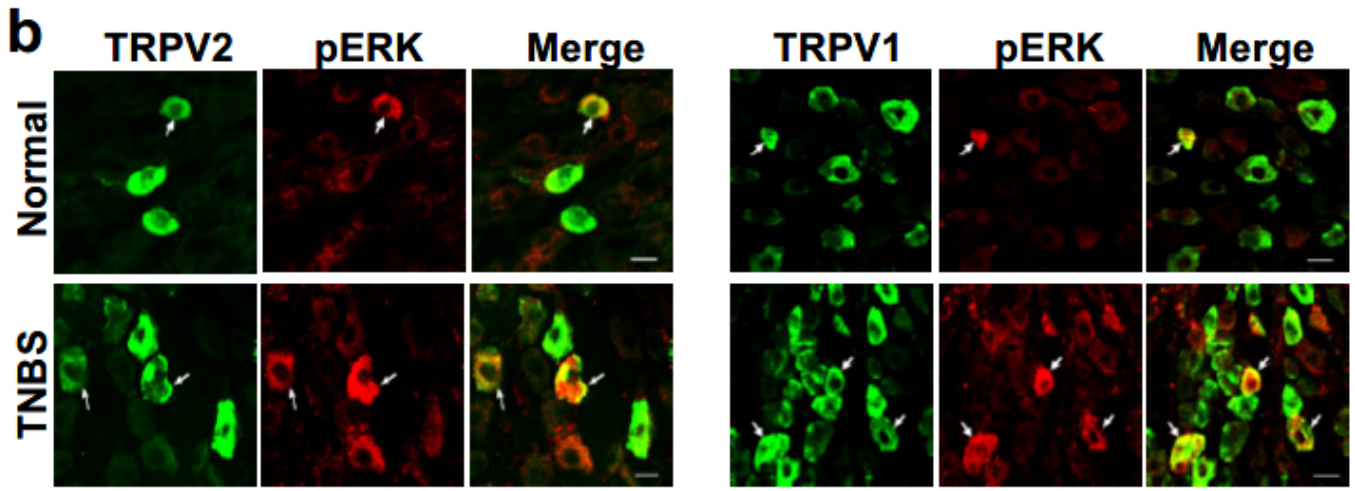
	IB4 (%)	NF200(%)
Normal	60.6 ± 2.5	19.9 ± 2.4
TNBS	71.3 ± 3.1	19.8 ± 1.7

Figure 6

p-ERK1/2 neurons co-localized with TRPV2 and TRPV1 was increased in the DRG and NG after TNBS treatment. Double labeling of TRPV2 and TRPV1 with pERK1/2 in L6/S1 DRG (A) and NG (B). The arrows indicate co-localization of TRPV2- or TRPV1-immunopositive neurons with pERK1/2. Data are presented as the mean ± SEM (n = 5). The scale bars are 20 μm.



	TRPV2 (%)	TRPV1 (%)
Normal	16.9 ± 2.0	19.1 ± 5.7
TNBS	52.1 ± 7.4*	63.7 ± 2.9*



	TRPV2 (%)	TRPV1 (%)
Normal	21.1 ± 2.3	25.3 ± 1.3
TNBS	47.5 ± 3.9*	50.4 ± 1.3*

Figure 7

Pre-treatment with TRPV2 inhibitor tranilast (200 mg/kg, i.p.) and TRPV1 antagonist BCTC (10 mg/kg, i.p.) significantly attenuated TNBS-induced visceral hyperalgesia. Abdominal electromyographic response to colorectal distension (CRD) in mice treated with TNBS for 5 days. Under this experimental condition (see Materials and Methods), the distension mainly stimulates the distal colon area corresponding to the area used for immunohistochemical study. (A) Representative electromyographic recordings of normal-vehicle, TNBS-vehicle, TNBS-tranilast, and TNBS-BCTC rats. (B) Visceromotor response (VMR) to in CRD normal-vehicle, TNBS-vehicle, TNBS-tranilast and TNBS-BCTC rats. Data are presented as means ± SEM, n = 6-8 rats per group. *P <0.05 for the comparison with the TNBS-vehicle group. $^{\#}$P < 0.05 for the comparison with the normal-vehicle group.

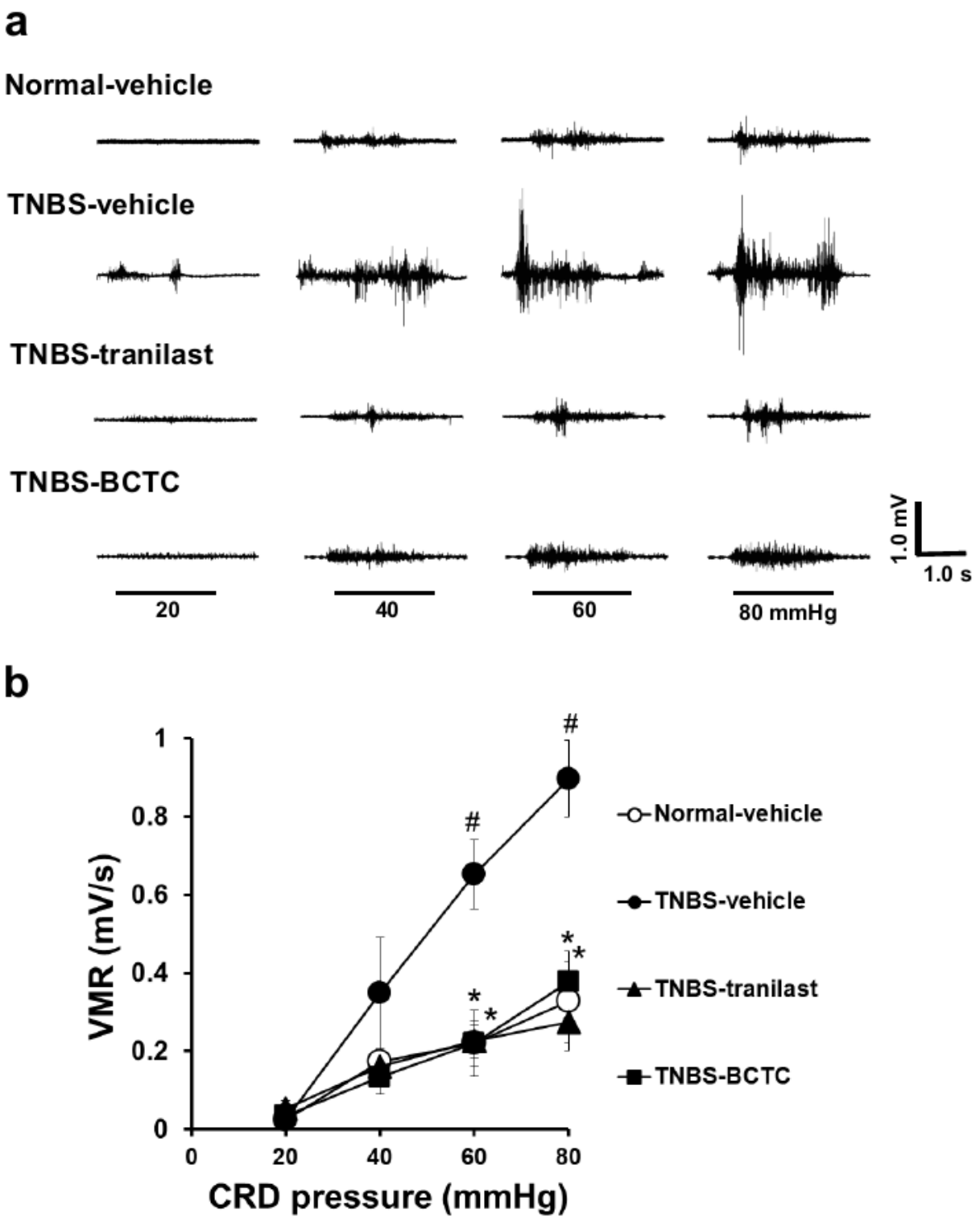


Figure 8

Legend not included with this version

Supplementary Files

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