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Research Paper

Role of transient receptor potential melastatin 2 (TRPM2) channels in visceral nociception and hypersensitivity

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ABSTRACT

Transient receptor potential melastatin 2 (TRPM2) is a thermosensitive, Ca^{2+}-permeable cation channel. TRPM2 contributes to the pathogenesis of inflammatory bowel disease, and inflammatory and neuropathic pain. We hypothesized that TRPM2 is important for visceral nociception and the development of visceral hypersensitivity. Therefore, we investigated the expression of TRPM2 channels and their involvement in visceral nociception in normal physiology and under pathological conditions that cause visceral hypersensitivity in rats. TRPM2 immunoreactivities were detected in the mucosa and muscle layer of the rat gastrointestinal tract. TRPM2 immunopositive cell bodies were almost completely co-localized with calretinin- and NeuN-positive cells in the myenteric plexus. We found that the majority of the TRPM2-immunoreactive cells were double-labeled with the retrograde marker fluorogold in lumbar 6/sacral 1 dorsal root ganglia (DRG), indicating that TRPM2 is expressed in spinal primary afferents innervating the distal colon. Subtypes of TRPM2-immunopositive DRG neurons were labeled by the A-fiber marker NF200, the C-fiber marker IB_4, substance P, calcitonin gene-related peptide, or P2X3 receptor. We found that oral administration of the TRPM2 inhibitor econazole (30 mg/kg) reduced the visceromotor response (VMR) to noxious colorectal distention (CRD) at 80 mm Hg in control rats. Expression of TRPM2 in the mucosa of the distal colon was increased in a trinitrobenzene sulfonic acid-induced colitis model. The VMR to CRD significantly increased in colitis model rats compared with control rats at 40, 60, and 80 mm Hg. Econazole restored visceral hypersensitivity to the control level. Furthermore, TRPM2-deficient mice showed significantly attenuated trinitrobenzene sulfonic acid induced visceral hypersensitivity compared with wild-type mice. In conclusion, TRPM2 channels contribute to visceral nociception in response to noxious stimuli under normal conditions and visceral hypersensitivity in pathological conditions.

1. Introduction

Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease, is an emerging health problem with rising prevalence worldwide. Abdominal pain and bloody diarrhea are the most common symptoms of IBD. In particular, abdominal pain lowers the quality of life in patients with IBD. As previously reported, multiple transient receptor potential (TRP) channels such as TRPV1, TRPA1, TRPV4, and TRPM8 are expressed in sensory neurons and contribute to visceral nociception in normal physiology and visceral hypersensitivity associated with intestinal inflammation (Akbar et al., 2008; Brierley et al., 2008, 2009; Hosoya et al., 2014).

TRP channels are nonselective cation channels activated by a variety of chemical and physical stimuli such as temperature, oxidative stress, and osmotic pressure. Transient receptor potential melastatin 2 (TRPM2) is a thermosensitive and Ca^{2+}-permeable cation channel that belongs to the melastatin subgroup of the TRP channel superfamily. TRPM2 channels are activated by intracellular ADP-ribose and extracellular stimuli such as reactive oxygen species (Perraud et al., 2001; Hara et al., 2002; Wehage et al., 2002). However, the physiological roles of TRPM2 are not well understood. It has been reported that TRPM2 is involved in insulin secretion in mice (Uchida et al., 2011; Uchida and Tominaga, 2014). Recently, TRPM2 has attracted interest as a potential therapeutic target in pathogenic processes related to oxidative stress

Abbreviations: CGRP, calcitonin gene-related peptide; CRD, colorectal distention; DRG, dorsal root ganglion; EMG, electromyographic; FG, fluorogold; IBD, inflammatory bowel disease; L6, lumbar 6; S1, sacral 1; PGP, protein gene product; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TRPM2, transient receptor potential melastatin 2; TRP, transient receptor potential; VMR, visceromotor response.

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(Kashio et al., 2012). For example, TRPM2 activity has been less linked to the pathology of several disorders including inflammatory and neuropathic pain, Alzheimer's dementia, and ischemic cell death (Haraguchi et al., 2012; Alim et al., 2013; Park et al., 2014).

In the gastrointestinal tract, TRPM2 channels are expressed in mucosal macrophages and mast cells, and have been shown to contribute to the progression of experimental colitis and food allergy, respectively, in mice (Yamamoto et al., 2008; Oda et al., 2013). Moreover, TRPM2 is involved in the production of the CXCL2 chemokine, which in turn results in neutrophilic influx in the colon (Yamamoto et al., 2008). TRPM2 deficiency suppresses exacerbation of inflammation in a dextran sodium sulfate-induced colitis mouse model (Yamamoto et al., 2008). However, the proportion of sensory neuron expressing TRPM2 and the physiological and pathological roles of TRPM2 in the GI tract are not well understood (Sousa-Valente et al., 2014). We hypothesized that TRPM2 plays an important role in visceral nociception and the development of hypersensitivity because several lines of evidence indicate that TRPM2 channels are likely expressed in neurons (Olah et al., 2009). In the present study, we characterized the expression pattern of TRPM2 in the rat enteric nervous system and dorsal root ganglia (DRGs), and investigated the roles of TRPM2 channels in visceral sensitivity under normal and pathological conditions in rats.

2. Materials and methods

2.1. Animals

We used male Sprague-Dawley rats (Japan SLC, Yokohama, Japan) aged 8–10 weeks. TRPM2-deficient mice (TRPM2 −/−) were generated in a C57BL/6J background as described previously (Yamamoto et al., 2008). Wild-type (TRPM2 +/+) and TRPM2 −/− mice were derived from heterozygous mating. The mice were 8–10 weeks old at the time of the experiments. 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. This study was carried out in strict accordance with ARRIVE guidelines for reporting experiments involving animals. The protocols were approved by the committee on the Ethics of Animal Research of Josai International University and Kyoto Pharmaceutical University. The number of animals used was kept to the minimum necessary for a meaningful interpretation of the data, and animal discomfort was kept to the minimum.

2.2. Immunohistochemistry

Tissue preparation and immunohistochemical procedures were performed as described previously (Matsumoto et al., 2009, 2011). Tissues were frozen in optimal cutting temperature (Sakura Finetek, Toyko, Japan) mounting medium, and sectioned on a cryostat (Leica Instruments, Nussloch, Germany) at a thickness of 30 μm for gastrointestinal tract samples and 6 μm for DRGs. Sources of all primary and secondary antibodies, as well as the optimized dilutions, are listed in Tables 1 and 2. TRPM2 immunoreactivity and other molecules were detected by indirect staining with specific antibodies. No specific immunostaining could be observed in control experiments. Specificities of the antibodies are described in our previous studies (Matsumoto et al., 2011, 2012). The specificities of TRPM2 channels were shown by loss of immunostaining when the primary antibody was pre-adsorbed with the corresponding antigen peptide: Peptide (C)HTFQGKEWDPKKHVQE, corresponding to amino acid residues 105–120 of mouse TRPM2 (Accession Q91YD4), intracellular, N-terminus.

Table 1
Primary antibodies.

Antigen	Host	Dilution	Source
TRPM2	Rabbit	1:2000 (distal colon)	Almone labs
TRPM2	Rabbit	1:500 (DRG)	Almone labs
NeuN	Mouse	1:1000	Millipore
CGRP	Sheep	1:2000	BIOMOL
Substance P	Guinea pig	1:4000	Abcam
CD163 (ED2)	Mouse	1:1000	AbD Serotec
NF200	Mouse	1:40,000	Sigma
IB4	FITC Conjugate	1:5000	Sigma
$P2X_3$	Guinea-pig	1:1000	Abcam
PGP9.5	Rabbit	1:40,000	Biogenesis

Table 2
Secondary antibodies.

Secondary antibody	Conjugate probe	Dilution	Source
Donkey anti-rabbit IgG	Biotin-SP	1:400	Jackson
Donkey anti-rabbit IgG	TRITC	1:400	Jackson
Donkey anti-guinea-pig IgG	TRITC	1:400	Jackson
Donkey anti-mouse IgG	FITC	1:400	Jackson
Donkey anti-sheep IgG	TRITC	1:400	Jackson

2.3. Microscopy and image analysis

Sections were viewed using a confocal microscope (FV-1000, Olympus, Tokyo, Japan) and images were captured using Olympus Fluoview version 1.7a software with an excitation wavelength appropriate for fluorescein isothiocyanate (488 nm) or tetramethyl rhodamine isothiocyanate (543 nm). Immunofluorescence was observed using a confocal microscope. Multiple images in Z-stacks were projected onto a single plane and reconstructed using Fluoview version 1.7a software (Olympus). For quantitative analysis, sections were viewed at 200× magnification for the distal colon and at 600× magnification for DRGs using a confocal microscope. The number of immunopositive cells in horizontal sections of the myenteric plexus and DRGs were counted under a confocal microscope in 100-μm squares at 200× magnification and in 300-μm squares at 600× magnification, respectively. The numbers of TRPM2 and protein gene product (PGP9.5)-immunopositive nerve fibers were counted and normalized to the area of the mucosa (1 mm^2). All counting was conducted by two investigators who were blinded to the experimental groups.

2.4. Retrograde labeling

The origin of the primary afferent innervation of the rat distal colon was determined by retrograde tracing using fluorescent fluorogold dye (FG; Fluorochrome, Denver, Colorado, USA). Approximately 5 μL of 4% FG was percutaneously injected circumferentially into the external anal sphincter under 2% isoflurane anesthesia. Tissue recovery occurred 1 week after the FG injection.

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

TNBS (0.2 mL of a 30 mg/mL solution in 50% ethanol; Sigma-Aldrich, St. Louis, MO) was administered transanally via a 22-gauge feeding needle in rats. In the mice, 0.1 mL of TNBS solution was administered transanally. While anesthetized, the rats and mice were held inverted for 5 and 1 min, respectively, to prevent leakage. The animals were allowed to recover for 7 days before they were sacrificed for experimental procedures.

2.6. Assessment of the visceromotor response to colorectal distention

As a visceral stimulus, mechanical distensions of the rectum were performed by pressure-controlled air inflation of a 2-cm (rat) or 1.5-cm (mouse) flexible polyethylene balloon connected to an electronic distension device (Distender Series II barostat, G&J Electronics, Willowdale, Ont., Canada). The balloon was lubricated, inserted intra-anally and positioned 3 cm (rat) or 0.5 cm (mouse) proximal to the