Original Article

The Transient Receptor Potential Vanilloid Receptor 1, TRPV1 (VR1) Inhibits Peristalsis in the Mouse Jejunum

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Abstract

Background: The transient receptor potential vanilloid receptor 1, TRPV1 [previously termed the capsaicin or vanilloid receptor 1 (VR1)] is a nonselective cation channel that has been cloned and is expressed predominantly in sensory neurons. TRPV1 is activated by protons as well as capsaicin. Despite extensive research, the physiological function of TRPV1 in the gastrointestinal tract and other tissues remains elusive. We have examined the effect of the selective TRPV1-receptor ligand, capsaicin, on intestinal peristalsis by studying migrating motor complexes (MMCs).

Methods: We performed experiments on Knockout mice (KO) in which the TRPV1 gene was disrupted using standard gene targeting techniques and their wildtype (WT) littermates. Jejunal contractile activity was recorded from in vitro segments of the jejunum, 4 – 5cm in length. When distended to 2 – 3 cm with H2O, the segments generated regular MMCs that were recorded as changes in intraluminal pressure.

Results: Capsaicin (1 – 100 nM) caused a dose-dependent inhibition of motility manifested as an increase in the interval between motor complexes (MCs) in the WT animal only, a response abolished by pre-treatment with TRPV1 antagonist capsazepine (Capz), ruthenium red (RR), and L-NAME. At higher doses of capsaicin (1 – 100 μM), periodic MCs were replaced by tonic increases in pressure upon which were superimposed continuous phasic contractions. This stimulation occurred in both KO and WT mice and was unaffected by pre-treatment with Capz, RR, and L-NAME.

Conclusion: These data demonstrate the potential role of TRPV1 receptors in organized peristalsis in the mouse jejunum. These findings also suggest that inhibition of contractions in mouse jejunum by TRPV1-receptor activation does involve a nitric oxide synthetase (NOS) pathway.

Keywords: Jejunum, migrating motor complexes (MMCs), mouse, TRPV1

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Introduction

Isolated segments of mouse jejunum develop a complex pattern of contractile activity consisting of periods of phasic contractions that migrate in an aboral direction interspersed by periods of quiescence. The gastrointestinal tract is innervated by enteric and extrinsic neurons. Enteric and extrinsic sensory mechanisms provide input to the reflex circuits that determine these motor complexes (MCs).

The transient receptor potential vanilloid 1 (TRPV1) receptor, previously termed the capsaicin or vanilloid receptor 1 (VR1), belongs to a large family of related receptors called transient receptor potential (TRP) channels.[1] TRPV1 is predominantly expressed on extrinsic sensory neurons in both DRG and nodose ganglia although there are reports of expression on enteric neurons and extra-neuronal structures.[2,3] Both vagal and spinal afferents supplying the gastrointestinal tract can be activated by capsaicin, an agonist at the TRPV1 receptor and this has been used as a tool to probe the role of extrinsic afferents in the control of reflex gut function.[4,5]

TRPV1 is a multimodal receptor that can be activated by heat and protons, as well as vanilloids such as capsaicin. In this respect, the sensitivity to extracellular acid may have physiological and pathophysiological relevance for gut sensory signalling. Thus, patients with functional gastrointestinal disorders such as irritable bowel syndrome often exhibit abnormal motility.[6] Recently, a possible link between TRPV1 and small intestinal hypersensitivity has been suggested by the demonstration that TRPV1 might be upregulated in inflamed bowels in humans.[7,8] The aim of this study is to characterize the role of TRPV1 in distension-evoked MCs in order to understand the role of TRPV1 in enteric reflex control of intestinal motility.

Materials and Methods

Experiments were performed on mice in which the TRPV1 gene was disrupted using standard gene targeting techniques[9] and on wild-type (WT) littermates. TRPV1 WT and TRPV1 knockout (KO) mice that had a genetic background of C57/BL6 were generated at GlaxoSmithKline (Harlow, UK). Briefly, transmembrane domains 2 – 4 of the TRPV1 coding sequence were eliminated by using the homologous recombination technique in embryonic stem cells. In this technique the target domains of the mouse TRPV1 gene (DNA encoding amino acids 460 – 555) were replaced by a cassette that comprised a neomycin selectable marker and a *lacZ* reporter gene. Chimeras were produced by blastocyst injection and then mated to C57BL/6 mice to achieve germ line transmission. No TRPV1 receptor expression was detectable in TRPV –/– mice when examined by reverse transcription polymerase chain reac-

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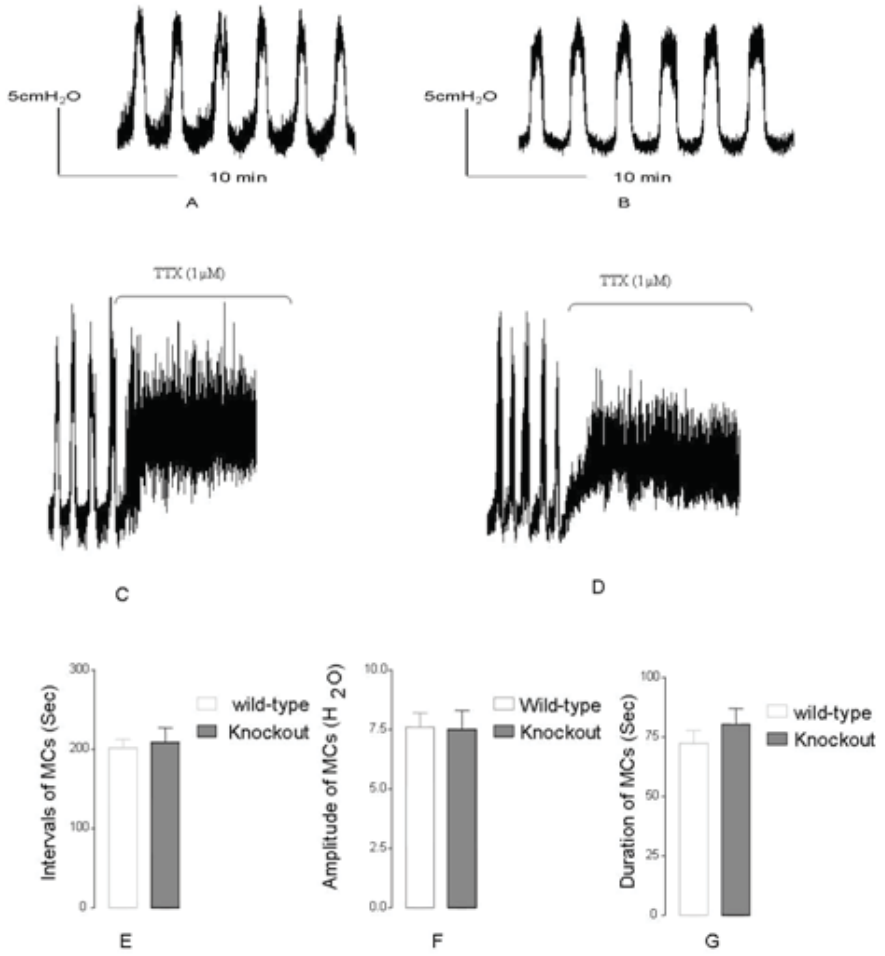

Figure 1. Regular pattern of periodic motor complexes (MCs) were initiated following distention of small intestine to 2 – 3 c m with H_2O in both WT and TRPV1 KO mice (A, B). MMCs were completely abolished (C, D) by a toxin called tetrodoxin (TTX1μM, n = 3). This pattern of activity consisted of periodic increases in intraluminal pressure interspersed by periods of relative quiescence (A, B). There is no statistical significance among both groups (E, F, G).

tion (RT-PCR). The mutant was backcrossed onto C57BL/6 for 3 generations. Heterozygous inter-crossing was then employed to generate the WT and TRPV1-receptor homozygous KO animals that were examined in the current study. Mating pairs of TRPV1 –/– and TRPV1 +/+ littermates were obtained in order to generate separate colonies of WT and KO mice at the University of Sheffield. All the procedures were carried out in accordance with the Principles of Laboratory Animal Care and Home Office Guidelines [Animals (Scientific Procedures) Act 1986].

Adult male mice that weighed 20 – 30 g were killed by lethal injections of pentobarbitone sodium anaesthetic (80 mg/kg, i.p.). Jejunal segments were mounted horizontally in a Trendelenburg type preparation. The isolated segments were continuously perfused with 95% O_2 , 5% CO_2, and warmed 37°C. Krebs bicarbonate buffer solution (composition in mM: 117 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2 NaH_2PO_4, 1.2 H_2O, and 11 D-glucose) via a Minipuls 3 perfusion pump (World Precision Instruments, USA) at a flow rate of 4 mL/min. Jejunal contractile activity was recorded from in vitro segments of jejunum, 4 – 5cm in length. When distended to 2 – 3 cm with H_2O, the segments generated regular migrating motor complexes (MMCs) recorded as changes in intraluminal pressure. The oral and aboral ends were secured, with nylon thread to 2 metal cannula at either end of the chamber, which were adjusted to maintain the segment at its resting length. The oral end was connected to a perfusion pump for intrajejunal infusion of isotonic saline or acidified saline as described below. The aboral end was attached to a pressure transducer (Elcomatic EM 760, Elcomatic Ltd., Glasgow, UK) that continuously recorded the amplitude and frequency of propagated contractions, measured as changes in intraluminal pressure under isovolumetric conditions. The baseline intraluminal pressure was adjusted using the perfusion pump connected to the proximal cannula. The distal (aboral) cannula was connected via a 3-way tap to a drain that allowed the lumen to be flushed, during which the cannula was diverted to waste. The output from the pressure transducer was relayed to a data-acquisition system (CED 1401+, Cambridge Electronic Design, Cambridge, UK) and from there to a computer running Spike 2 software (CED), which displayed the pressure recordings

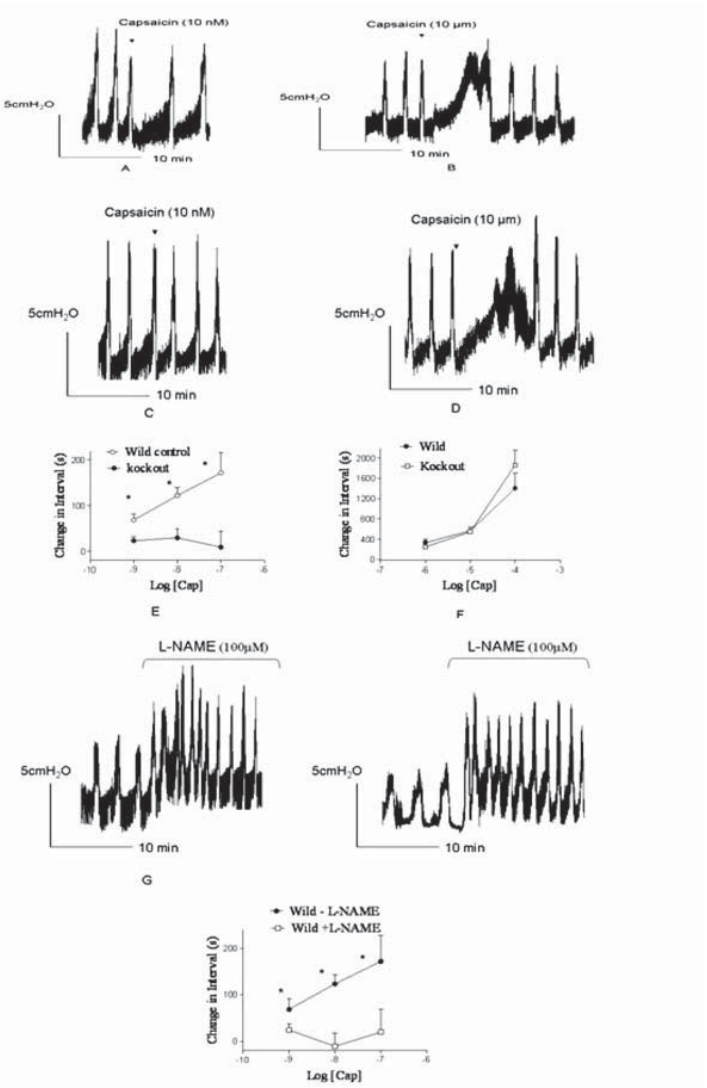

Figure 2. Representative traces showing the effects of low and high doses of TRPV1 agonist, capsaicin at 10 nM (A) and 10 µM (B) on typical MCs evoked by distension in Wild-type (WT) mice jejunum. Representative traces showing effects of low and high doses of TRPV1 agonist, capsaicin at 10 nM (C) and 10 µM (D) on typical MCs evoked by distension in the knockout (KO) mice jejunum. Concentration response curves to effects of capsaicin on Interval MMCs in WT and KO mice (E, F). Effects of nitric oxide (NO) inhibitor, L-NAME (100 µM) on distension evoked MCs (G). L-NAME augmented amplitude, basal tone, and frequency of MMCs in WT and KO (G) mice. L-NAME produced a decrease in the intervals and duration of MCs in both WT and KO mice jejunum. Comparison of capsaicin effects in the absence and presence of L-NAME in WT mice (H). L-NAME at 100 µM inhibited capsaicin effects at low doses (1-100 nM) in WT mice. Data expressed as mean ± SD.(n=5, *P*<0.05).

online and stored data for subsequent offline analysis. Capsaicin (8-methy-N-vanillyl-6-nonenamide, TRPV1 agonist) and capsazepine (a TRPV1 receptor antagonist, Capz)[10] were purchased from Tocris Cookson, Avonmouth, UK. N_e-nitro-L-arginine methyl ester (L-NAME) was acquired from Sigma Chemical Co. Freshly diluted aliquots were used in all experiments. Stock solutions were made by dissolving drugs in distilled water, except for Capz which was dissolved in DMSO and capsaicin which was dissolved in a solution of 80% DMSO plus 20% Tween80.

Data were reported as mean ± SD and analyzed by the t-test and paired t-test as appropriate.

Results

A regular pattern of periodic MCs were initiated following distension of the small intestine to 2 – 3 cm with H_2O in both WT and TRPV1 KO mice (Figures 1A, B). MMCs were completely abolished (Figures 1C, D) by a toxin called tetrodoxin (TTX, 1 µM, n = 3). This pattern of activity consisted of periodic increases in intraluminal pressure interspersed by periods of relative quiescence (Figures 1E, F, G).

Effects of capsaicin

Capsaicin at concentrations of 1 nM – 100 µM had a marked effect on the pattern of MCs. In the WT preparations, low concentrations of capsaicin (1 – 100 nM, n = 5) did not affect the duration and amplitude of MCs but evoked a concentration–dependent increase in the MC interval with a more than doubling of the interval at 100 nM ($P < 0.05$, n = 5, Figure 2A). In contrast, capsaicin at low concentrations had no effect on the amplitude, duration, or interval of MCs in the TRPV1 KO preparations (n = 5, Figures 2C, D). At higher concentrations (1 – 100 µM), capsaicin disrupted the typi-

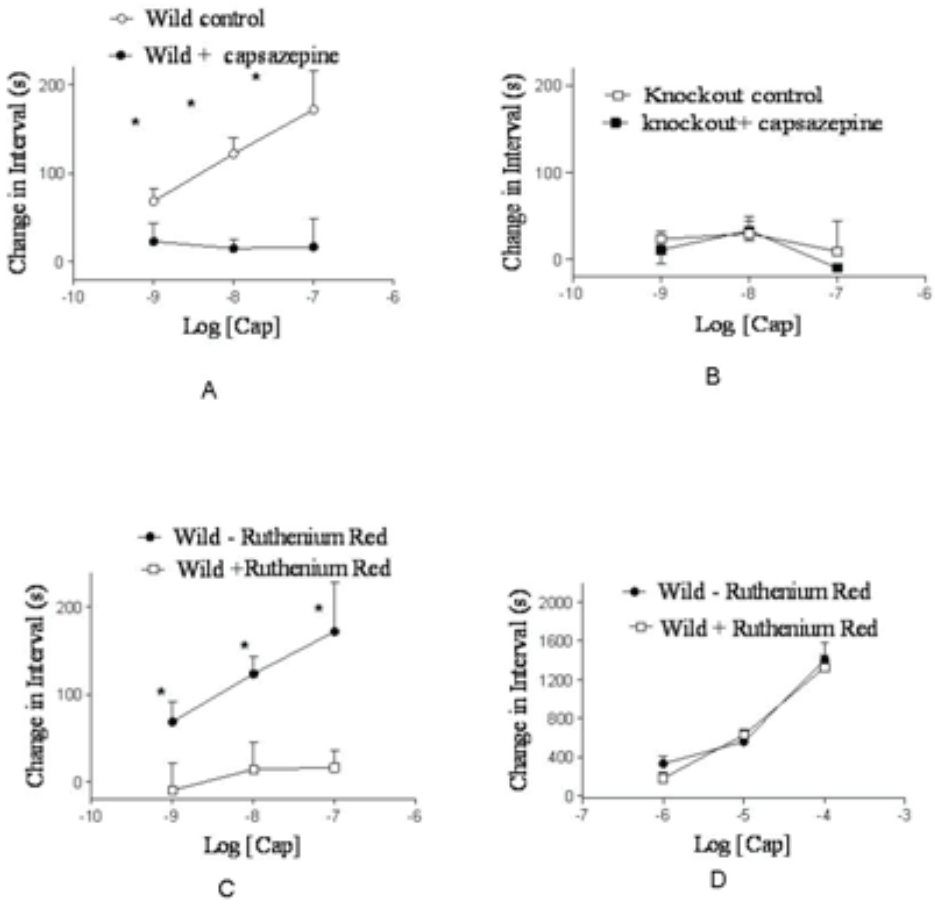

Figure 3. Concentration response curves to effects of TRPV1 receptor agonist, capsaicin (Cap) on interval MCs in WT mice (A). Data obtained in the absence or presence of the TRPV1 antagonist, capsazepine (**Capz** ,3μM). Capsaicin responses are expressed as change in intervals. Each point represents the mean±SD of values. Concentration response curves to effects of capsaicin on interval MMCs in the KO mouse jejunum with **Capz** treatment (3 μM) and without **Capz** (B). C and D show effects of capsaicin (1 nM-100μM) on MC intervals in the WT mouse jejunum with and without ruthenium red (RR) treatment (3 μM). (n=5, *P*<0.05).

cal pattern of MCs by causing sustained contractions, as evidenced by the elevated basal tone in both WT and KO mice (Figures 2B, D). The effects of capsaicin were reversible and MCs gradually returned to pre-treatment levels after washout. Capsaicin (1 – 100 nM) caused a dose-dependent inhibition of motility manifested as an increase in the interval between MMCs in the WT animal only (Figure 2). At higher doses of capsaicin (1 – 100 μM), the periodic MMCs were replaced by tonic increases in pressure upon which were superimposed continuous phasic contractions. This stimulation occurred in both KO and WT mice (Figures 2E, F).

As nitric oxide (NO) is an important inhibitory neurotransmitter in the gut we examined the role of NO release using the NO-synthase blocker, L-NAME. L-NAME increased MCs amplitude and frequency. The concentration of 100 μM L-NAME produced a decrease in the MMC interval, an effect that developed within 1 min of its application and was sustained in its continued presence for up to 25 min (Figure 2G). Capsaicin (1 – 100 nM, n = 5) had no effect on MMCs in the presence of L-NAME (Figure 2H).

Effects of TRPV1 antagonists

To determine whether capsaicin interacts with native TRPV1 receptors, the effect of Capz, a competitive TRPV1 antagonist,[10] on the inhibitory responses to capsaicin was examined. Capz (3 μM) did not affect spontaneous contractile activity in WT and TRPV1 KO preparations (data not shown) but inhibited the effect of low dose capsaicin on MMCs interval in WT (Figure 3A). Capz (3 μM) had no effect on spontaneous motor activity in TRPV1 KO in the presence of capsaicin (Figure 4B). Similarly, pre-treatment with ruthenium red (RR, 3 μM), a non-competitive antagonist,[10] alone had no effect on spontaneous motor activity in WT and TRPV1 KO preparations (n = 5). However, RR prevented the inhibitory effects of 1 – 100 nM capsaicin on MMCs in the WT preparations but failed to impact motor responses to higher concentrations of capsaicin (1 – 100 μM) in WT or TRPV1 KO preparations (Figures 3C, D).

Discussion

Spontaneous motor activity was similar in WT and KO, which indicated that the VR1 receptor did not contribute to the generation of MCs. It seemed that the pattern and pharmacological properties of motor activity we reported in TRPV1 WT and TRPV1 KO

(TRPV1$^{-/-}$) mice were equivalent to MMCs in other species that have been described.[11–13] MMCs were completely abolished by a toxin called tetrodoxin (TTX) , an inhibitor of neurotransmission in the jejunum of WT and KO mice. In contrast, the nitric oxide synthase (NOS) inhibitor L-NAME produced an increase in both MMC frequency and amplitude.

Capsaicin (1 – 100 nM), a ligand for the TRPV1 receptor, caused a dose-dependent inhibition of motility manifested as an increase in the interval between MMCs only in the WT animal. The relaxant effect of capsaicin was strongly inhibited in jejunum preparations from TRPV1 KO mice when compared to their WT controls. In addition, the relaxant effect of capsaicin was abolished following treatment with both Capz and RR which have been reported to block TRPV1 channels. This has indicated that capsaicin acts via activation of TRPV1 receptors possibly located on the peripheral terminals of afferent nerves in the mice jejunum. This observation is consistent with the findings that the TRPV1 receptor has been identified on both intrinsic and extrinsic neurons of the gastrointestinal tract. Both vagal and spinal afferents of the gastrointestinal tract can be activated by capsaicin.[14–17] The results of the present study are consistent with other investigators[18–20] who have suggested that capsaicin-sensitive primary afferent neurons participate in the regulation of gastrointestinal motility. Other investigators have reported that TRPV1-immunoreactivity was detected on both intrinsic and extrinsic neurons in the guinea-pig ileum and colon, and rat ileum.[21]

At higher doses of capsaicin (1 – 100 μM), periodic MCs were replaced by tonic increases in pressure upon which were superimposed continuous phasic contractions. This stimulation occurred in both KO and WT mice. It can be suggested that capsaicin possibly acts by sensitizing sensory neurons through an unknown site which may be a subtype of an as yet unknown transient receptor potential (TRP) receptors. However, the relative lack of knowledge about the properties of other TRP receptor family members make it difficult to make inferences regarding the role of these receptors in the high-dose capsaicin effects we have described. Due to the lack of specific TRP channel blockers only a limited pharmacological characterization has been conducted to date. It has been established that some members of the TRP channel family respond not only to an exclusive range of temperatures but also to natural plant-derived products that include mustard oil and menthol, which are similar to capsaicin.[22] It has been reported that TRPA1 is present in a sub-population of primary sensory neurons that express TRPV1 but not TRPM8.[23] In the isolated mouse intestine it has been shown that TRPA1, but not TRPM8, is functionally expressed in the enteric sensory neurons that may also be co-expressed with TRPV1 throughout the mouse intestinal tract; however, the contractile responses to TRPA1 activation differ according to the location of the segments in the mouse intestine.[24] Therefore, it has been suggested that TRPA1 is involved in regulating gastrointestinal motility through capsaicin-sensitive sensory neurons.

L-NAME blocked the inhibitory effect of the low dose TRPV1 agonist capsaicin in the WT only. It has been concluded that nitric oxide (NO), possibly of sensory origin, is involved in the relaxant action of capsaicin in the mouse jejunum. Thus, TRPV1 may contribute to intestinal motility through NO production. TRPV1 is a promising target for controlling intestinal movement. The results of the present study have made it possible to identify the distribution of TRPV1-expressing nerves and nitregic neurons in the small intestine of the mouse.

According to reports, capsaicin induces NO-mediated relaxation in circular muscle-oriented preparations isolated from the human and mouse colon, which agree with the present findings.[25] Retrograde labeling and immunofluorescence studies in dorsal root ganglia (DRG) have also shown high degrees of colocalization between TRPV1, calcitonin-gene-related peptide (CGRP), substance P (SP) and NOS in the spinal sensory neurons that supply the mouse jejunum.[26] TRPV1s are expressed in both sensory neurons and non-neuronal tissues.[17,19,27–31] Although there has been no direct evidence that functional TRPV1 receptor is expressed in non-neural tissues in the gut, some results have suggested that capsaicin-induced relaxation is associated with direct inhibitory action intracellularly on the voltage-operated Ca++ channels.[28] Studies of the effect of capsaicin on smooth muscle contractions are controversial and have shown both contraction and relaxation in various preparations.[18,19,25,32–34]

The stimulatory effect of high dose capsaicin observed in both WT and KO animals was unaffected by L-NAME. Therefore, capsaicin effects in high doses are likely to be non-TRPV1. Capsaicin may act on other channels (other TRPs)[24,29] or may directly release transmitters (ATP, NO, ACh)[35–38] and other factors (tachykinins , calcitonin-gene-related peptide, substance P)[20,39] that can act on afferent nerves or smooth muscle to modulate their responses to different stimuli.

On the basis of these results, there appears to be a fundamental difference between the mechanism of capsaicin at low and high doses, which suggests that additional mechanisms besides TRPV1 are involved in the isolated mouse jejunum.

These findings suggest that inhibition of contractions in mouse jejunum by TRPV1-receptor activation does involve an NOS pathway and that NO is an endogenous regulator which plays a key role in the regulation of small intestinal motility. These data demonstrate the potential role of TRPV1 in organized peristalsis in the mouse jejunum.

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References

1. Gunthorpe MJ, Benham CD, Randall A, Davis JB. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci.* 2002; **23:** 183 – 191.
2. Anavi-Goffer S, Coutts AA. Cellular distribution of vanilloid VR1 receptor immunoreactivity in the guinea-pig myenteric plexus. *Eur J Pharmacol.* 2003; **458:** 61 – 71.
3. Ichikawa H, Sugimoto T. The co-expression of VR1 and VRL-1 in the rat vagal sensory ganglia. *Brain Res*. 2003; **980:** 293 – 296.
4. Su X, Wachtel RE, Gebhart GF. Capsaicin sensitivity and voltage-gated sodium currents in colon sensory neurons from rat dorsal root ganglia. *Am J Physiol*. 1999; **277:** 1180 – 1188.
5. Blackshaw LA, Page AJ, Partosoedarso ER. Acute effects of capsaicin on gastrointestinal vagal afferents. *Neuroscience.* 2000; **96:** 407 – 416.
6. Camilleri M, Coulie B, Tack JF. Visceral hypersensitivity: Facts, speculations, and challenges. *Gut.* 2001; **48:** 125 – 131.
7. Yiangou Y, Facer P, Dyer NH, Chan CL, Knowles C, Williams NS, et al. Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet*. 2001; **357:** 1338 – 1339.
8. Chan CL, Facer P, Davis JB, Smith GD, Egerton J, Bountra C, et al.

Sensory fibers expressing capsaicin receptor TRPV1 in patients with rectal hypersensitivity and faecal urgency. *Lancet.* 2003; **361:** 385 – 391.
9. Davis JB, Gray J, Gunthorpe, MJ, Hatcher JP, Davey PT, Overend P, et al. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature*. 2000; **405:** 183 – 187.
10. Messeguer A, Planells-Cases R, Ferrer-Montiel A. Physiology and pharmacology of the vanilloid receptor. *Curr Neuropharmacol*. 2006; **4:** 1 – 15.
11. Mule F, D'Angelo S, Tabacchi G, Amato A, Serio R. Mechanical activity of small and large intestine in normal and mdx mice: A comparative analysis. *Neurogastroenterol Motil*. 1999; **11:** 133 – 139.
12. Bercik P, Bouley L, Dutoit P, Blum AL, Kucera P. Quantitative analysis of intestinal motor patterns: Spatiotemporal organization of nonneural pacemaker sites in the rat ileum. *Gastroenterology.* 2000; **119:** 386 – 394.
13. Abdu F, Hicks GA, Hennig G, Allen JP, Grundy D. Somatostatin sst (2) receptors inhibit peristalsis in the rat and mouse jejunum. *Am J Physiol Gastrointest Liver Physiol*. 2002; **282:** 624 – 633.
14. MacNaughton WK, Van Sickle MD, Keenan CM, Cushing K, Mackie K, Sharkey KA. Distribution and function of the cannabinoid-1 receptor in the modulation of ion transport in the guinea pig ileum: Relationship to capsaicin-sensitive nerves. *Am J Physiol Gastrointest Liver Physiol*. 2004; **286(5):** 863 – 871.
15. Ward SM, Bayguinov J, Won KJ, Grundy D, Berthoud HR. Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. *J Comp Neurol*. 2003; **465(1):** 121 – 135.
16. Ichikawa H, Sugimoto T. The co-expression of VR1 and VRL-1 in the rat vagal sensory ganglia. *Brain Res*. 2003; **980(2):** 293 – 296.
17. Knotkova H, Pappagallo MA. Capsaicin (TRPV1 agonist) therapy for pain relief: Farewell or revival? *Clin J Pain*. 2008; **24 (2):** 142 – 154.
18. Bartho L, Benko R, Patacchini R, Petho G, Holzer-Petsche U, Holzer P, et al. Effects of capsaicin on visceral smooth muscle: A valuable tool for sensory neurotransmitter identification. *Eur J Pharmacol.* 2004; **500:** 143 – 157.
19. Matsumoto KE, Kurosawa E, Terui H, Hosoya T, Tashima K, Murayama T, et al. Localization of TRPV1 and contractile effect of capsaicin in mouse large intestine: High abundance and sensitivity in rectum and distal colon. *Am J Physiol Gastrointest Liver Physiol*. 2009; **297:** 348 – 360.
20. Matsumoto K, Hosoya T, Tashima K, Namiki K, Murayama T, Horie S. Distribution of transient receptor potential vanilloid 1 channel-expressing nerve fibers in mouse rectal and colonic enteric nervous system: Relationship to peptidergic and nitrergic neurons. *Neuroscience.* 2011; **172:** 518 – 534.
21. Anavi-Goffer S, Coutts AA. Cellular distribution of vanilloid VR1 receptor immunoreactivity in the guinea-pig myenteric plexus. *Eur J Pharmacol.* 2003; **458:** 61 – 71.
22. Calixto JB, Kassuya CA, Andre E, Ferreira J. Contribution of natural products to the discovery of the transient receptor potential (TRP) channels family and their functions. *Pharmacol Ther.* 2005; **106:** 179 – 208.
23. Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, et al. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. *J Comp Neurol.* 2005**; 493:** 596 – 606.
24. Penuelas A, Tashima K, Tsuchiya S, Matsumoto K, Nakamura T, Horie S, et al. Contractile effect of TRPA1 receptor agonists in the isolated mouse intestine. *Eur J Pharmacol.* 2007; **576(1-3):** 143 – 150.
25. Benko R, Lazar Z, Undi S, Illenyi L, Antal A, Horvath OP, et al. Inhibition of nitric oxide synthesis blocks the inhibitory response to capsaicin in intestinal circular muscle preparations from different species. *Life Sci.*. 2005; **76:** 2773 – 2782.
26. 26. Tan LL, Bornstein JC, Anderson CR. Distinct chemical classes of medium-sized transient receptor potential channel vanilloid 1-immunoreactive dorsal root ganglion neurons innervate the adult mouse jejunum and colon. *Neuroscience.* 2008; **156:** 334 – 343.
27. Poli-Neto OB, Filho AA, Rosa e Silva JC, Barbosa Hde F, Candido Dos Reis FJ. Increased capsaicin receptor TRPV1 in the peritoneum of women with chronic pelvic pain. *Clin J Pain.* 2009; **25(3):** 218 – 222.
28. Sim JH, Kim YC, Kim SJ, Lee SJ, Suh SH, Jun JY, et al. Capsaicin inhibits the voltage-operated calcium channels intracellularly in the antral circular myocytes of guinea-pig stomach. *Life Sci.* 2001; **68(21):** 2347 – 2360.
29. Kullmann FA, Shah MA, Birder LA, de Groat WC. Functional TRP and ASIC-like channels in cultured urothelial cells from the rat. *Am J Physiol Renal Physiol*. 2009; **296(4):** 892 – 901.
30. Yamada T, Ugawa S, Ueda T, Ishida Y, Kajita K, Shimada S. Differential localizations of the transient receptor potential channels TRPV4 and TRPV1 in the mouse urinary bladder. .*J Histochem Cytochem.* 2009; **57(3):** 277 – 287.
31. Spencer NJ, Kerrin A, Singer CA, Hennig GW, Gerthoffer WT, McDonnell O. Identification of capsaicin-sensitive rectal mechanoreceptors activated by rectal distension in mice. *Neuroscience.* 2008; **153(2):** 518 – 534.
32. Barthou L, Lenard L Jr, Patacchini R, Halmai V, Wilhelm M, Holzer P, et al. Tachykinin receptors are involved in the ''local efferent'' motor response to capsaicin in the guinea-pig small intestine and esophagus. *Neuroscience.* 1999; **90:** 221 – 228.
33. Saitoh C, Kitada C, Uchida W, Chancellor MB, de Groat WC, Yoshimura N. The differential contractile responses to capsaicin and anandamide in muscle strips isolated from the rat urinary bladder. *Eur J Pharmacol*. 2007; **570:** 182 – 187.
34. Fujimoto S, Mori M, Tsushima H, Kunimatsu M. Capsaicin-induced, capsazepine-insensitive relaxation of the guinea-pig ileum. *Eur J Pharmacol.* 2006; **530:** 144 – 151.
35. Birder LA**,** de Groat WC. Mechanisms of disease: Involvement of the urothelium in bladder dysfunction. *Nat Clin Pract Urol.* 2007; **4:** 46 – 54.
36. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature.* 2000; **407:** 1011 – 1015
37. Kullmann FA**,** Artim DE, Birder LA, de Groat WC. Activation of muscarinic receptors in rat bladder sensory pathways alters reflex bladder activity. *J Neurosci.* 2008; **28:** 1977 – 1987.
38. Vlaskovska M**,** Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, et al. P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. *J Neurosci.* 2001; **21:** 5670 – 5677.
39. Hernandez JM, Cox G, Janssen LJ. Involvement of the neurokinin-2 receptor in airway smooth muscle stretch-activated contractions assessed in perfused intact bovine bronchial segments. *J Pharmacol Exp Ther.* 2008; **327(2):** 503 – 510.