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Molecules and Cells

Effects of Transient Receptor Potential Channel Blockers on Pacemaker Activity in Interstitial Cells of Cajal from Mouse Small Intestine

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The interstitial cells of Cajal (ICCs) are pacemakers in the gastrointestinal tract and transient receptor potential melastatin type 7 (TRPM7) is a candidate for pacemaker channels. The effect of the 5-lipoxygenase (5-LOX) inhibitors NDGA, AA861, MK886 and zileuton on pacemaking activity of ICCs was examined using the whole cell patch clamp technique. NDGA and AA861 decreased the amplitude of pacemaker potentials in ICC clusters, but the resting membrane potentials displayed little change, respectively. Also, perfusing NDGA and AA861 into the bath reduced both inward current and outward current in TRPM7-like current in single ICC, respectively. But, they had no effects on Ca^{2+} activated Cl^- currents. The 5-LOX inhibitors MK886 and zileuton were, however, ineffective in pacemaker potentials in ICC clusters and in TRPM7-like current in single ICC, respectively. A specific TRPC3 inhibitor, pyrazole compound (Pyr3), and a specific TRPM4 inhibitor, 9-phenanthrol, had no effects in pacemaker potentials in ICC clusters and in TRPM7-like current in single ICC. These results suggest that, among the tested 5-LOX inhibitors, NDGA and AA861 modulate the pacemaker activities of the ICCs, and that the TRPM7 channel can affect intestinal motility.

INTRODUCTION

Interstitial cells of Cajal (ICCs) are the pacemaking cells in the gastrointestinal (GI) muscles that generate the rhythmic oscillations in the membrane potential known as slow waves (Huizinga et al., 1995; Sanders, 1996; Ward et al., 1994). Slow waves propagate within ICC networks, conduct into smooth muscle cells *via* gap junctions, and initiate phasic contractions by activating Ca^{2+} entry through L-type Ca^{2+} channels. The pacemaker activity in the murine small intestine is due mainly to periodic activation of nonselective cation channels (NSCCs) (Koh et al., 2002) or Cl^- channels (Huizinga et al., 2002; Zhu et al., 2009). ICCs also mediate or transduce inputs from the enteric nervous system. Because of the central role of ICCs in GI motility, loss of these cells would be extremely detrimental. Research into the biology of ICCs provides exciting new opportunities to understand the etiology of diseases that have long eluded comprehension. We have suggested that, as a primary molecular candidate for NSCC responsible for pacemaking activity in ICCs, transient receptor potential (TRP) melastatin 7 (TRPM7) is required for pacemaking activity of murine small intestine (Kim et al., 2005). Therefore, TRPM7 is a potentially promising new target for pharmacological treatment of GI motility disorders.

TRP channels were first cloned from *Drosophila* species and constitute a superfamily of proteins that encode a diverse group of Ca^{2+}-permeable NSCCs (Clapham, 2003). The TRP family is divided into seven subfamilies: classical TRPs (TRPC), which display the greatest similarity to Drosophila TRP; vanilloid TRPs (TRPVs); TRPMs; mucolipin TRPs; polycystin TRPs; NOMPC (no mechanoreceptor potential C) TRP; and ankyrin 1 TRPs. TRPM7, a member of TRPM ion channel subfamily, is a widely expressed bifunctional protein with both ion channel and α-kinase domains (Nadler et al., 2001; Runnels et al., 2001).

Recently, Chen et al. (2010) suggested that the 5-lipoxygenase (5-LOX) inhibitors, NDGA and AA861 were potent blockers of the TRPM7 channel capable of attenuating TRPM7's function, and making them effective tools for the biophysical characterization and suppression of TRPM7 channel conductance. However, the effect of the 5-LOX inhibitors on pacemaking activity of ICCs has not been investigated. Therefore, we undertook to investigate the effect of the 5-LOX inhibitors and other specific TRP channel blockers in the pacemaking activity of ICCs.

MATERIALS AND METHODS

Preparation of cells and cell cultures

The mice used were treated ethically according to the Guidelines for the Care and Use of Animals approved by Pusan National University. Balb/c mice (8-13-days-old) of either sex were anaesthetized with ether and killed by cervical dislocation. The small intestines from 1 cm below the pyloric ring to the caecum

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were removed and opened along the mesenteric border. The luminal contents were washed out with Krebs-Ringer bicarbonate solution. The tissues were pinned to the base of a Sylgard dish and the mucosa was removed by sharp dissection. *Sma*ll strips of intestinal muscle (consisting of both circular and longitudinal muscles) were equilibrated in Ca^{2+} free Hank's solution containing 5.36 mM KCl, 125 mM NaCl, 0.34 mM NaOH, 0.44 mM Na_2HCO_3, 10 mM glucose, 2.9 mM sucrose and 11 mM *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid (HEPES) for 30 min, and the cells were then dispersed with a solution containing 1.3 mg/ml collagenase (Worthington Biochemical, USA), 2 mg/ml bovine serum albumin (Sigma-Aldrich, USA), 2 mg/ml trypsin inhibitor (Sigma-Aldrich) and 0.27 mg/ml ATP. The dispersed cells were plated onto sterile glass coverslips coated with 2.5 mg/ml murine collagen (Falcon/BD, USA) in 35 mm-diameter culture dishes and cultured at 37°C in a 95% O_2-5% CO_2 incubator in smooth muscle growth medium (SMGM; Clonetics, USA) supplemented with 2% antibiotics/antimycotics (Gibco, USA) and 5 ng/ml murine stem cell factor (SCF; Sigma-Aldrich). ICCs were identified immunologically by incubation with anti-*c-kit* antibody [phycoerythrin (PE)-conjugated rat anti-mouse *c-kit* monoclonal antibody; eBioscience, USA] at a dilution of 1:50 for 20 min. Since the morphology of the ICCs differed from those of other cell types in the culture, it was possible to identify them by phase contrast microscopy after incubation with anti-*c-kit* antibody.

Patch-clamp experiments

The physiological salt solution used to bathe cells (Na^+-Tyrode) contained 5 mM KCl, 135 mM NaCl, 2 mM $CaCl_2$, 10 mM glucose, 1.2 mM $MgCl_2$ and 10 mM HEPES, adjusted to pH 7.4 with NaOH. The pipette solution contained 140 mM KCl, 5 mM $MgCl_2$, 2.7 mM K_2ATP, 0.1 mM NaGTP, 2.5 mM creatine phosphate disodium, 5 mM HEPES and 0.1 mM ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), adjusted to pH 7.2 with KOH. In case of single ICC, the cells were bathed in a solution containing 2.8 mmol/L KCl, 145 mmol/L NaCl, 2 mmol/L $CaCl_2$, 10 mmol/L glucose, 1.2 mmol/L $MgCl_2$ and 10 mol/L HEPES, adjusted to pH 7.4 with NaOH. The pipette solution contained 145 mmol/L Cs-glutamate, 8 mmol/L NaCl, 10 mmol/L Cs-2-bis(2-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid, and 10 mmol/L HEPES-CsOH, adjusted to pH 7.2 with CsOH. The whole-cell configuration of the patch-clamp technique was used to record membrane potentials (current clamp) of the cultured ICC, and an Axopatch I-D (Axon Instruments, UK) was used to amplify the membrane currents and potentials. Command pulses were applied using an IBM-compatible personal computer and pClamp software (version 6.1; Axon Instruments). The data were filtered at 5 kHz, displayed on an oscilloscope, computer monitor and with a Gould 2200 pen recorder (Gould, USA), and analyzed with pClamp and Origin (version) software. All experiments were performed at 30°C.

Ca^{2+} activated Cl^- channel expression in human embryonic kidney-293 cells

Human embryonic kidney (HEK)-293 cells transfected with pEGFP-N1-mANO1 construct were grown on glass coverslips with Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. Whole-cell patch-clamp experiments were performed at 21-25°C 24 h after induction by using cells grown on glass coverslips. The bath solution contained 146 mmol/L HCl, 10 mmol/L HEPES, 10 mmol/L glucose, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$ and 150 mmol/L N-methyl-D-glucamine (NMDG), adjusted to pH 7.4. The pipette solution contained 134 mmol/L HCl, 5 mmol/L HEPES, 10 mmol/L glucose, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$ and 150 mmol/L NMDG, adjusted to pH 7.2.

Drugs

Nordihydroguaiaretic acid (NDGA) was purchased from Calbiochem. AA861 and zileuton were purchased from Biomol (USA). The other drugs were purchased from Sigma-Aldrich. For stock solutions, all drugs were dissolved in distilled water or dimethylsulfoxide (DMSO), and stored at -20°C. The final concentration of DMSO in the bath solution was always < 0.1%, and did not affect the recorded traces.

Statistics

All data are expressed as mean ± S.E. Student's *t*-test for unpaired data was used to compare control and experimental groups. A p value < 0.05 was considered statistically significant.

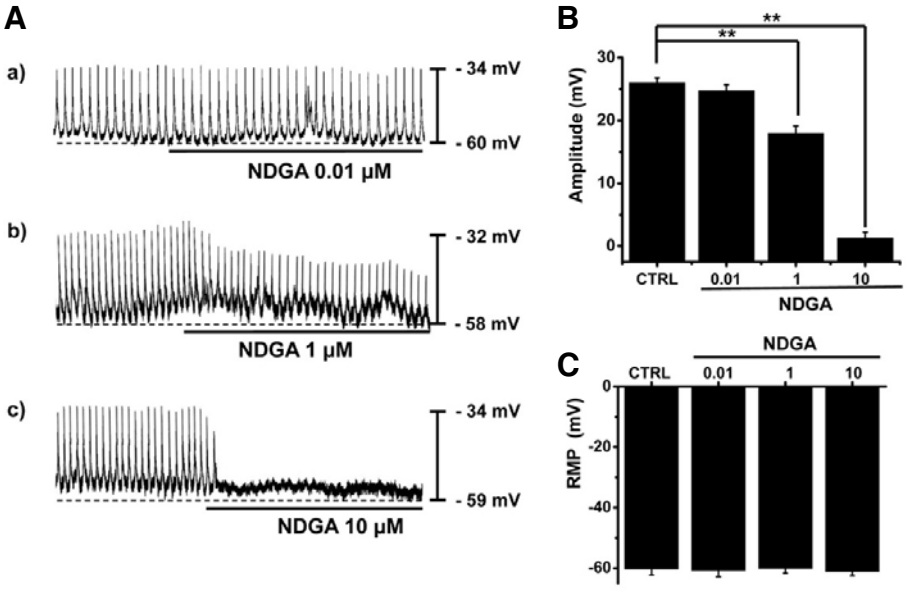

Fig. 1. NDGA decreases the amplitude of pacemaker potentials in ICC clusters. (A) In current clamp mode (I = 0), the addition of NDGA (0.01-10 μM) decreased the amplitude of pacemaker potentials. (B) The histograms summarize the decrease of amplitude with NDGA concentration. (C) The histograms summarize the change of resting membrane potentials with NDGA concentration.

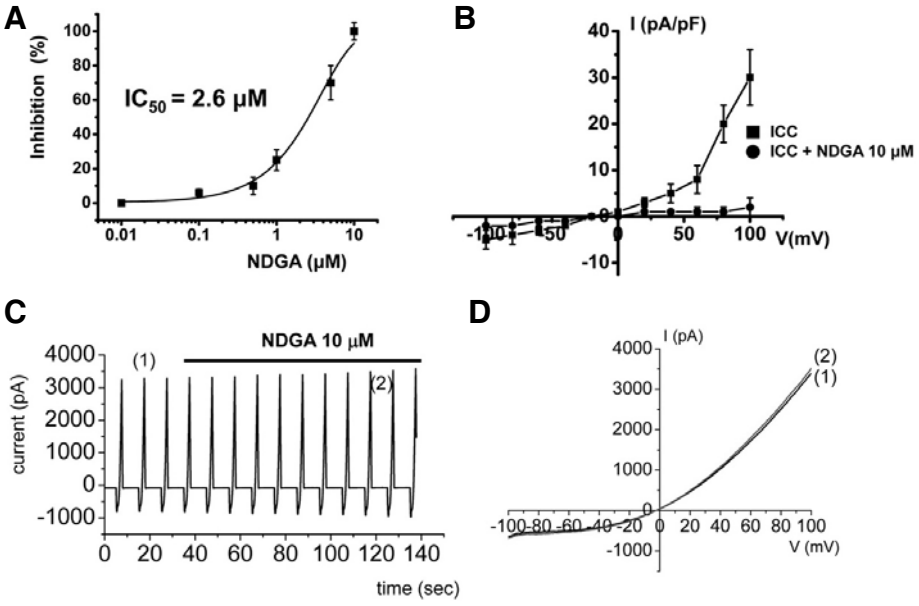

Fig. 2. Effects of NDGA on pacemaker potentials in ICC clusters, on TRPM7-like current in single ICCs, and on overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. (A) Concentration-dependent inhibition of pacemaker potentials in ICC clusters. The estimated median inhibitory concentration value for NDGA was 2.6 μM. (B) Representative TRPM7-like currents in single ICCs at NDGA 10 μM. C. Representative trace of the effect of NDGA on Ca^{2+}-activated Cl^- currents in HEK293 cells. NDGA had no effect on the currents. (D) Representative I-V relationships of (C). A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

RESULTS

NDGA decreases the amplitude of pacemaker potentials in ICC clusters

To understand the relationship between 5-LOX inhibitors and modulation of pacemaker activity in ICCs, we examined the effects of NDGA on pacemaker potentials in ICC clusters. In current clamp mode ($I = 0$), ICC had a mean resting membrane potential of -60.1 ± 1.3 mV and produced electrical pacemaker potentials ($n = 34$). The frequency of this pacemaker potential was 18 ± 2 cycles/min with an amplitude of 25.3 ± 1.5 mV ($n = 34$; Fig. 1A). The addition of NDGA (0.01-10 μM) decreased the amplitude of the pacemaker potentials, but the resting membrane potentials exhibited little change (Fig. 1). The amplitudes were 24.6 ± 0.9 mV at 0.01 μM NDGA ($n = 4$), 18.1 ± 1.1 mV at 1 μM ($n = 3$), and 1.2 ± 0.9 mV at 10 μM ($n = 4$) (Fig. 1B). The resting membrane potentials were -60.7 ± 2.0 mV at 0.01 μM, -60.1 ± 1.5 mV at 1 μM, and -61.1 ± 1.3 mV at 10 μM (Fig. 1C). NDGA, however, did not have any obvious effects on their frequency (Fig. 1A). NDGA from 0.01-10 μM effectively inhibited the amplitude of pacemaker potentials of ICCs. Concentrations of NDGA >10 μM produced no further inhibition. The IC_{50} of NDGA on pacemaker activity in ICC clusters was 2.6 μM (Fig. 2A). To investigate the effect of NDGA in pacemaking activity, we performed whole-cell voltage-clamp recordings in cultured single ICC. Single ICC was identified with phycoerythrin-bound anti- c-kit antibody. A voltage ramp from +100 mV to -100 mV evoked an outward-rectifying cation current at positive potentials with standard bath solution and with pipette solutions lacking magnesium adenosine triphosphate (Fig. 2B). These features are very similar to those associated with the recently cloned TRPM7 channel (Nadler et al., 2001). Perfusing NDGA (10 μM) into the bath reduced both inward current and outward current in TRPM7-like current in single ICC ($n = 5$) (Fig. 2B). Recently, Zhu et al. (2009) suggested that Ca^{2+}-activated Cl^- conductance is involved in slow wave current in ICC and that ANO1 participates in pacemaker activity. Therefore, we investigated the effect of NDGA in Ca^{2+}-activated Cl^- conductance. We overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. Whole cell currents were recorded using patch-clamp techniques. To determine the current-voltage (I-V) relationship, we applied a ramp pulse from -100 mV to +100 mV for 2 s. However, NDGA had no effect on Ca^{2+}-activated Cl^- conductance (Figs. 2C and 2D).

AA861 decreases the amplitude of pacemaker potentials in ICC clusters

To understand the relationship between AA861 and modulation of pacemaker activity in ICCs, we examined the effects of AA861 on pacemaker potentials in ICC clusters. The addition of AA861 (0.01-10 μM) decreased the amplitude of the pacemaker potentials, but the resting membrane potentials exhibited little change (Fig. 3). The amplitudes were 24.8 ± 1.0 mV at 0.01 μM AA861 ($n = 4$), 13.8 ± 0.9 mV at 1 μM ($n = 4$), and 1.3 ± 0.9 mV at 10 μM ($n = 4$) (Fig. 3B). The resting membrane potentials were -60.5 ± 1.0 mV at 0.01 μM, -60.3 ± 1.1 mV at 1 μM, and -61.0 ± 1.5 mV at 10 μM (Fig. 3C). AA861, however, did not have any obvious effects on their frequency (Fig. 3A). AA861 from 0.01-10 μM effectively inhibited the amplitude of pacemaker potentials of ICCs. Concentrations of NDGA >10 μM produced no further inhibition. The IC_{50} of NDGA on pacemaker activity in ICC clusters was 3.2 μM (Fig. 4A). To investigate the effect of AA861 in pacemaking activity, we performed whole-cell voltage-clamp recordings in cultured single ICC. Single ICC was identified with phycoerythrin-bound anti- c-kit antibody. A voltage ramp from +100 mV to -100 mV evoked an outward-rectifying cation current at positive potentials with standard bath solution and with pipette solutions lacking magnesium adenosine triphosphate (Fig. 4B). Perfusing AA861 (10 μM) into the bath reduced both inward current and outward current in TRPM7-like current in single ICC ($n = 4$) (Fig. 4B). Next, we investigated the effect of AA861 in Ca^{2+}-activated Cl^- conductance. We overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. Whole cell currents were recorded using patch-clamp techniques. To determine the I-V relationship, we applied a ramp pulse from -100 mV to +100 mV for 2 s. Similar to the results obtained with NDGA, AA861 had no effect on Ca^{2+}-activated Cl^- conductance (Figs. 4C and 4D). However, another 5-LOX inhibitor, MK886, had no effect on pacemaker activity in ICCs (Fig. 5A). Also, perfusing zileuton into the bath did not inhibit inward current and outward current in TRPM7-like current in single ICC (Fig. 6A). But, MK886 inhibited Ca^{2+}-activated

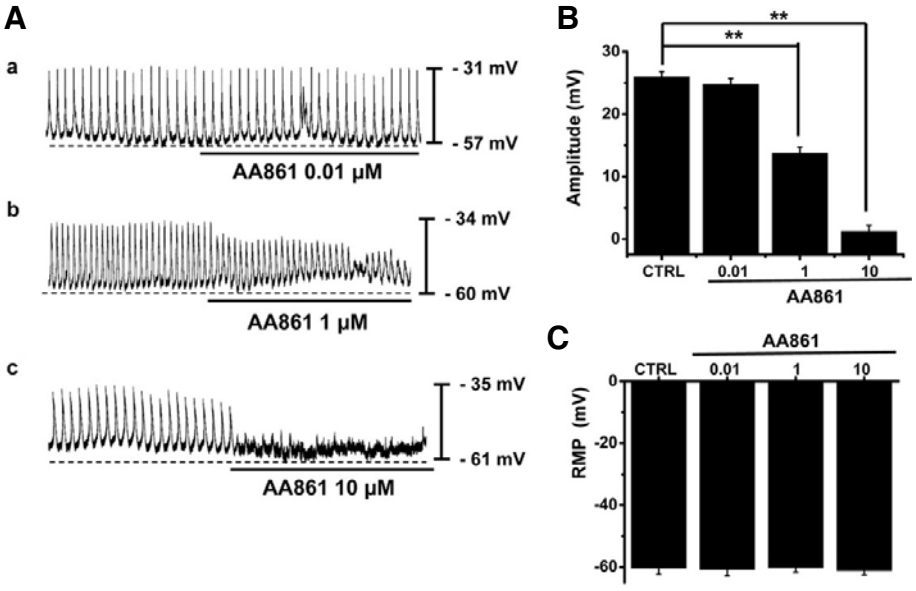

Fig. 3. AA861 decreases the amplitude of pacemaker potentials in ICC clusters. (A) In current clamp mode (I = 0), the addition of AA861 (0.01-10 μM) decreased the amplitude of pacemaker potentials. (B) The histograms summarize the decrease of amplitude with AA861 concentration. (C) The histograms summarize the change of resting membrane potentials with AA861 concentration.

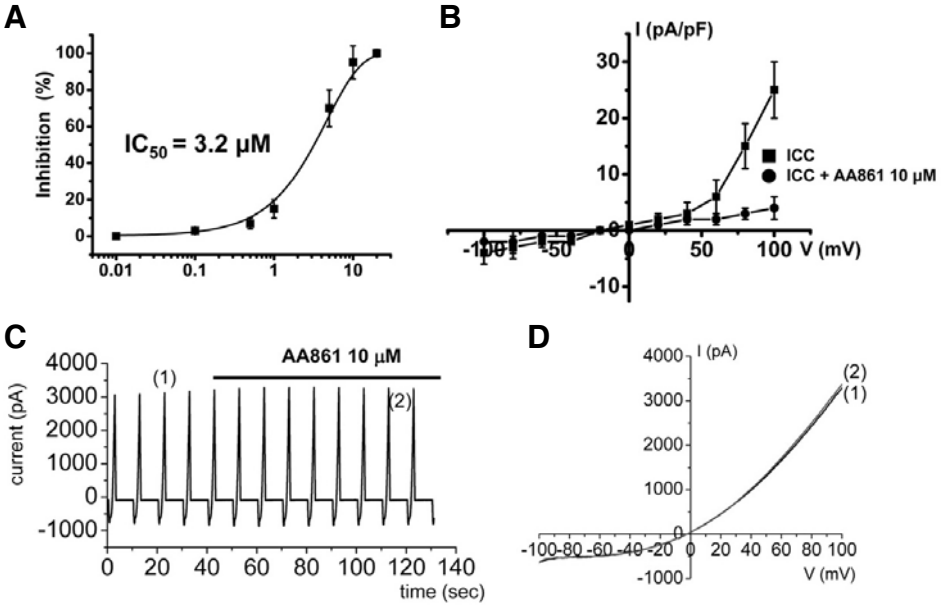

Fig. 4. Effects of AA861 on pacemaker potentials in ICC clusters, on TRPM7-like current in single ICCs, and on overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. (A) Concentration-dependent inhibition of pacemaker potentials in ICC clusters. The estimated median inhibitory concentration value for AA861 was 3.2 μM. (B) Representative TRPM7-like currents in single ICCs at AA861 10 μM. (C) Representative trace of the effect of AA861 on Ca^{2+}-activated Cl^- currents in HEK293 cell. AA861 had no effect on the currents. (D) Representative I-V relationships of (C). A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

Cl^- conductance (Figs. 6B and 6C). Chen et al. (2010) suggested that, among the 5-LOX inhibitors, zileuton is ineffective in suppressing TRPM7 channel activity. In the present experimental system, zileuton also had no effect on pacemaker activity in ICCs (Fig. 7A). Perfusing zileuton into the bath did not inhibit both inward current and outward current in TRPM7-like current in single ICC (Fig. 8A). Also, zileuton had no effects on Ca^{2+}-activated Cl^- conductance (Figs. 8B and 8C). Taken together, our data suggest that NDGA and AA861 play an important role in the modulation of pacemaker activity through TRPM7 channels in ICCs.

Effects of specific TRPC3 and TRPM4 inhibitors on pacemaker activity in ICCs

To reinforce the effects of TRPM7 channels on pacemaker activity in ICCs, we applied another specific TRP channel blockers. As shown in Fig. 9, we used a pyrazole compound (Pyr3), which selectively inhibits TRPC3 channels (Kiyonaka et al., 2009). Addition of 1, 2, and 5 μM Pyr3 in the culture medium did not modulate the pacemaker activity in ICC clusters (n = 5; Fig. 9A). Also, Pyr3 had no effects on TRPM7-like currents in single ICC (Fig. 9B). As shown in Fig. 10, we used a 9-phenanthrol, which is useful for studying the functional significance of TRPM4 (Gonzales et al., 2010). Addition of 10, 30, and 50 μM 9-phenanthrol in the culture medium did not modulate the pacemaker activity in ICC clusters (n = 5; Fig. 10A). Also, 9-phenanthrol had no effects on TRPM7-like currents in single ICC (Fig. 10B). Taken together, the data indicate that TRPC3 and TRPM4 are not involved in the modulation of pacemaker activity of ICCs.

DISCUSSION

GI smooth muscles are spontaneously active, and generate

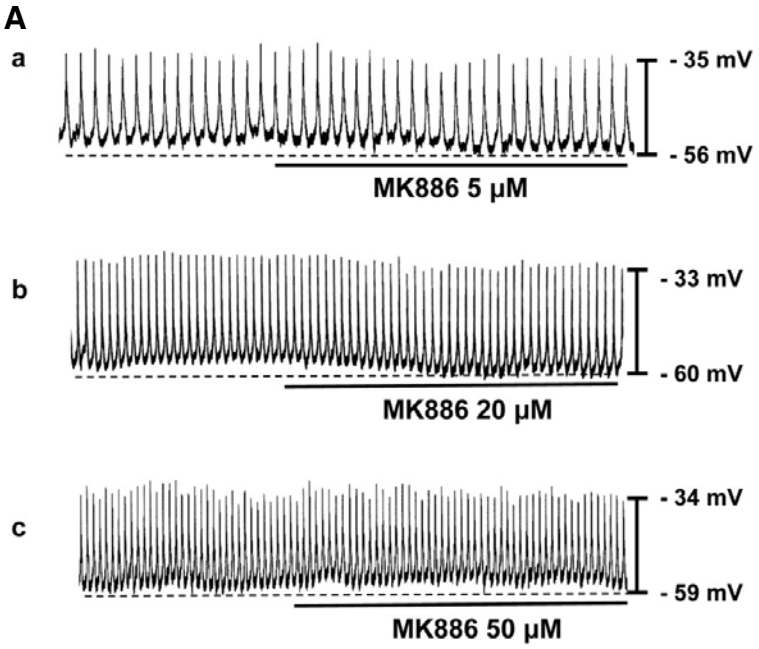

Fig. 5. MK886 has no effects on pacemaker potentials in ICC clusters. (A) In current clamp mode (I = 0), the addition of MK886 (5-50 μM) did not affect the pacemaker potentials.

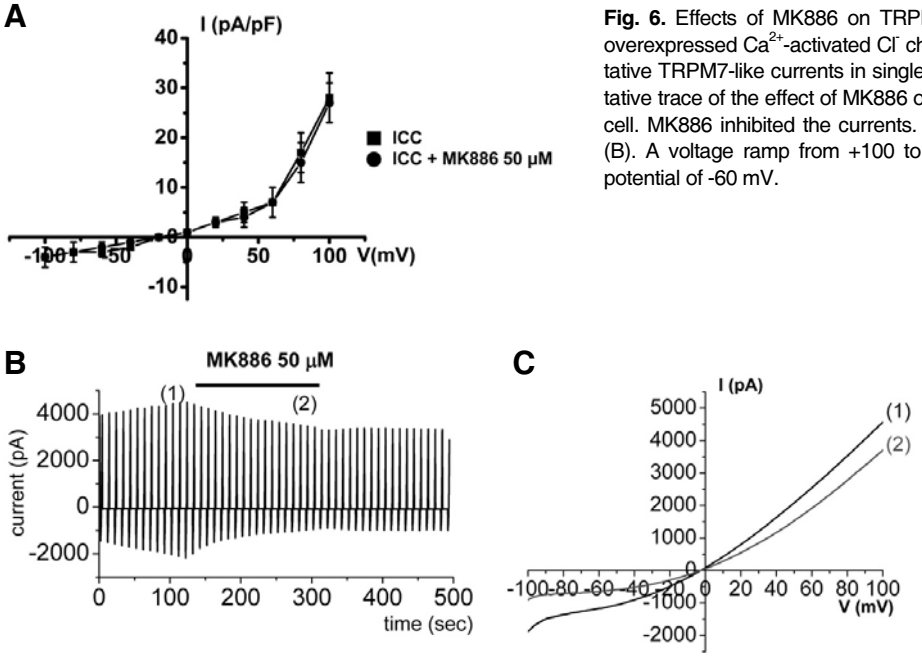

Fig. 6. Effects of MK886 on TRPM7-like current in single ICCs and on overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. (A) Representative TRPM7-like currents in single ICCs at MK886 50 μM. (B) Representative trace of the effect of MK886 on Ca^{2+}-activated Cl^- currents in HEK293 cell. MK886 inhibited the currents. (C) Representative I-V relationships of (B). A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

rhythmic slow electrical waves (Tomita, 1981). The slow waves originate in the ICCs distributed in the GI tract (Dickens et al., 1999; Huizinga et al., 1995; Langton et al., 1989; Torihashi et al., 1995; Ordog et al., 1999; Ward et al., 1994). ICCs express c-kit immunoreactivity and form gap junctional connections with ICCs and with smooth muscle cells (Huizinga et al., 1997; Komuro et al., 1992; Sanders, 1996). Many types of ICC with different immunohistochemical and electrical properties, such as myenteric ICC (ICCMY), intramuscular ICC, deep muscular plexus ICC and submucosal ICC, are distributed in the GI tract (Sanders et al., 1999). In animal models lacking ICC-MY, the slow waves in the small intestine are strongly attenuated, indicating that these cells are indeed essential for pacemaking activity in the GI tract (Huizinga et al., 1995; Maeda et al., 1992). ICCs are involved in physiological GI motility, and therefore have clinical importance in many bowel disorders, including inflammatory bowel disease, chronic idiopathic intestinal pseudo-obstruction, intestinal obstruction with hypertrophy, achalasia, Hirschsprung disease, juvenile pyloric stenosis, juvenile intestinal obstruction, and anorectal malformation (Sanders et al., 1999). Discovering the molecules involved in the generation of pacemaker activity in ICCs may lead to dramatic new therapies for chronic GI diseases that result in lifelong suffering. The pacemaker activity in the murine small intestine is due mainly to periodic activation of nonselective cation channels (NSCCs) (Koh et al., 2002) and we suggested that the electrophysiologi-

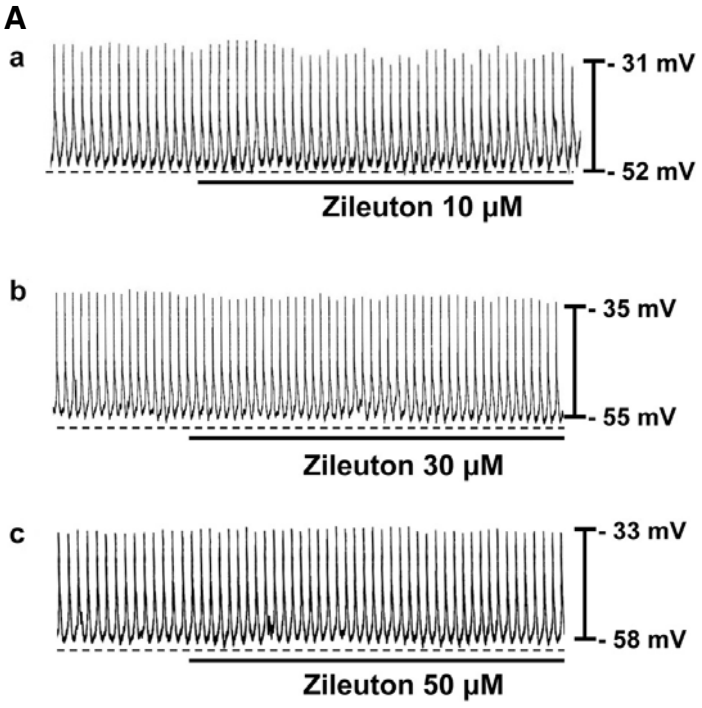

Fig. 7. Zileuton has no effects on pacemaker potentials in ICC clusters. (A) In current clamp mode (I = 0), the addition of zileuton (10-50 μM) did not affect the pacemaker potentials.

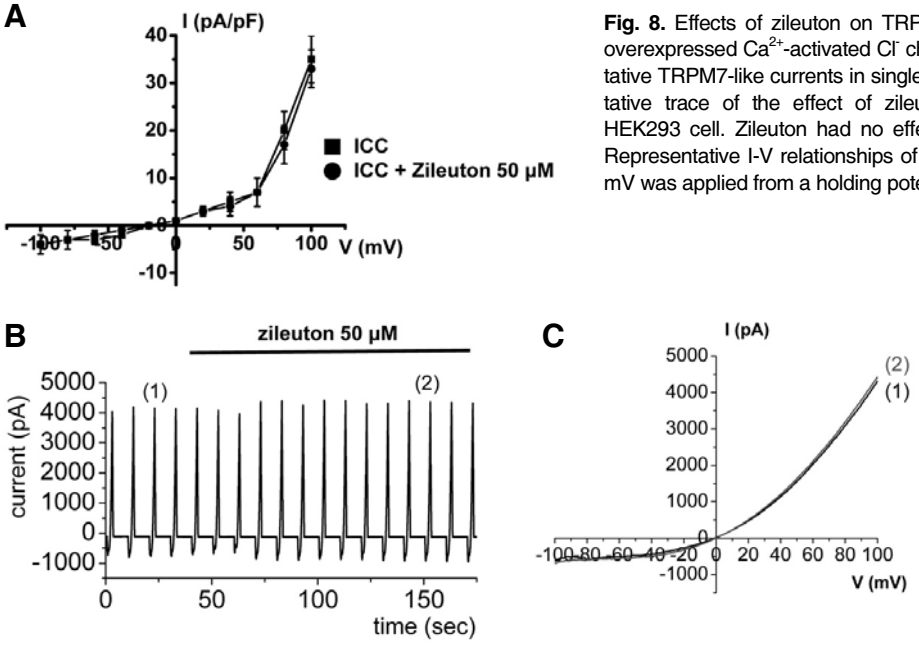

Fig. 8. Effects of zileuton on TRPM7-like current in single ICCs and on overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. (A) Representative TRPM7-like currents in single ICCs at zileuton 50 μM. (B) Representative trace of the effect of zileuton on Ca^{2+}-activated Cl^- currents in HEK293 cell. Zileuton had no effects on Ca^{2+}-activated Cl^- currents. (C) Representative I-V relationships of (B). A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

cal and pharmacological properties of TRPM7 and the NSCCs in ICCs were the same and, therefore, the TRPM7 protein is an essential molecular component of NSCC in ICCs (Kim et al., 2005). Also, Zhu et al. (2009) suggested that Ca^{2+}-activated Cl^- conductance is also involved in slow wave current in ICC and that ANO1 participates in pacemaker activity.

Recently, Chen et al. (2010) suggested that the 5-LOX inhibitors, NDGA, AA861 and MK886 reduced the TRPM7 channel activity independent of their effect on 5-LOX activity. Also, application of AA861 and NDGA reduced cell death for cells overexpressing TRPM7 cultured in low extracellular divalent cations. In this study, we found that NDGA and AA861 decreased the amplitude of pacemaker potentials in cultured ICCs, but the resting membrane potentials had little change, respectively (Figs. 1 and 3). Also, perfusing NDGA and AA861 into the bath reduced both inward current and outward current in TRPM7-like current in single ICC, respectively (Figs. 2 and 4). However, surprisingly, MK886 did not affect the amplitude of pacemaker potentials in cultured ICCs and the resting membrane potentials had little changes. Also, in TRPM7-like current in single ICC, MK886 had no effect (Figs. 5 and 6). Another 5-LOX inhibitor, zileuton, also had no effects on pacemaker activity in ICCs (Fig.

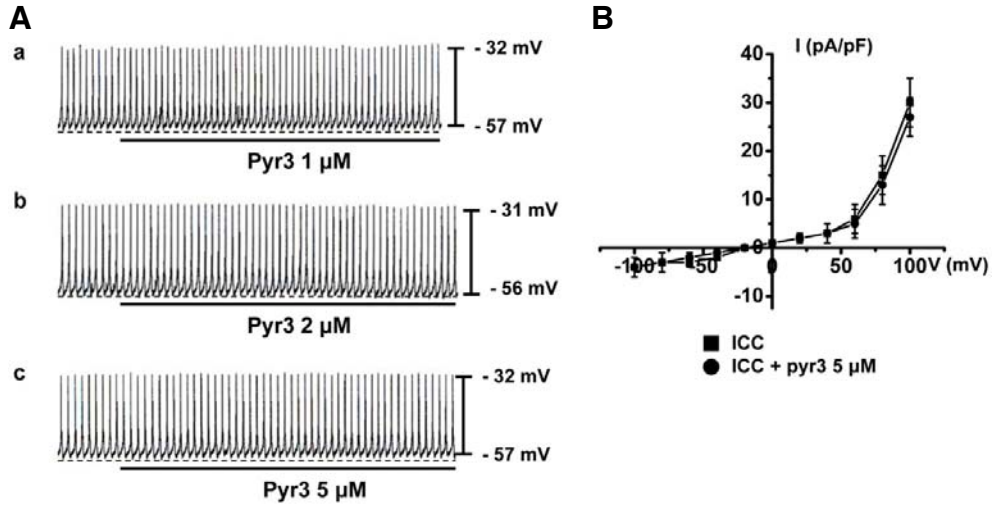

Fig. 9. Effects of pyr3 on pacemaker potentials in ICC clusters and TRPM7-like current in single ICCs. (A) In current clamp mode (I = 0), the addition of pyr3 (1-5 μM) did not affect the amplitude of pacemaker potentials. (B) Representative TRPM7-like currents in single ICCs at 5 μM pyr3. A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

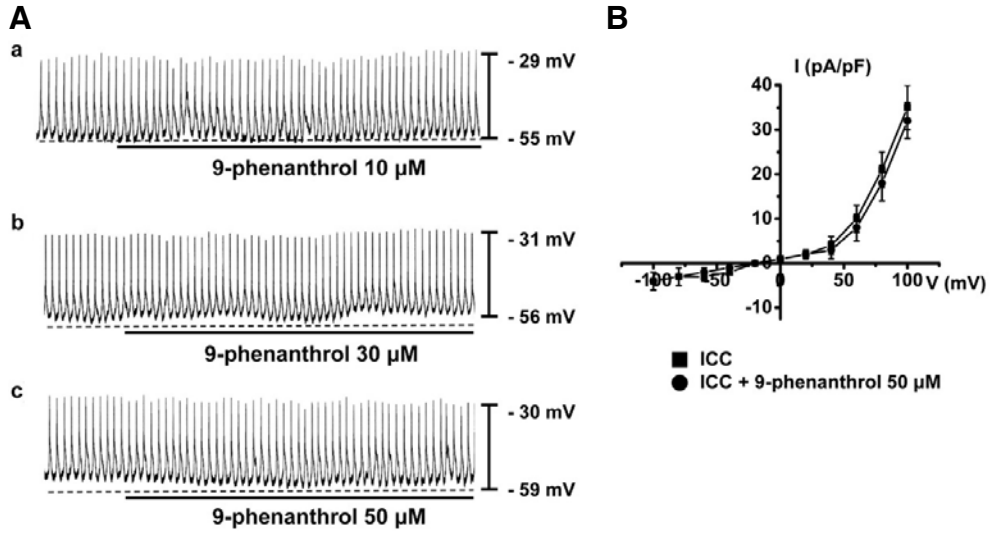

Fig. 10. Effects of 9-phenanthrol on pacemaker potentials in ICC clusters and TRPM7-like current in single ICCs. (A) In current clamp mode (I = 0), the addition of 9-phenanthrol (10-50 μM) has no effects on the amplitude of pacemaker potentials. (B) Representative TRPM7-like currents in single ICCs at50 μM 9-phenanthrol. A voltage ramp from +100 to -100 mV was applied from a holding potential of -60 mV.

7). Although NDGA, AA861, MK886 and zileuton are all 5-LOX inhibitors, there were some differences in their characteristics. For example, NDGA and AA861 reduce cell death for cells overexpressing TRPM7, but MK866 is toxic to cells (Chen et al., 2010). NDGA is a lipophilic reducing agent that blocks catalysis by reducing the active site iron in 5-LOX, whereas AA861 competes with binding of arachadonic acid to the enzyme (Werz, 2002; Yoo et al., 2009; Yoshimoto et al., 1982). The structurally unrelated indole-containing MK886 is also lipophilic, blocking 5-LOX activity by binding to 5-LOX-activating protein (FLAP), a membrane protein that facilitates 5-LOX enzymatic activity by enhancing the delivery of arachidonic acid to 5-LOX (Peters-Golden et al., 2003). Also, zileuton is an oral drug for asthma (Chen et al., 2010). Like this, there are structurally differences in 5-LOX inhibitors. Therefore, there might be the differences of the effect of various 5-LOX inhibitors on ICCs. However, the exact action mechanisms need to be investigated.

TRPM7 or Ca^{2+}-activated Cl^- conductance is involved in pacemaking activity in ICCs (Kim et al., 2005; Zhu et al., 2009). To investigate the effects of 5-LOX inhibitors on Ca^{2+}-activated Cl^- conductance, we overexpressed Ca^{2+}-activated Cl^- channels in HEK293 cells. However, NDGA, AA861 and zileuton had no effects on Ca^{2+}-activated Cl^- conductances and MK886 inhibited Ca^{2+}-activated Cl^- conductances. These results are different from those of effects 5-LOX inhibitors in ICCs. However, the effects of 5-LOX inhibitors on TRPM7 channels are similar with those of effects in ICCs. Therefore, we think that TRPM7 is involved in pacemaking activity in ICCs.

Until now, the exact functions of TRPM7 and Ca^{2+}-activated Cl^- conductances remain unclear. Mazzone et al. (2011) suggested that Changes in Ca^{2+}-activated Cl^- conductances expression in ICCs may directly contribute to human diabetic gastroparesis. However, Kim et al. (2008) suggested TRPM7 has an important role in gastric cancer cell death. The exact functions of TRPM7 and Ca^{2+}-activated Cl^- conductances in GI tract need to be investigated in future.

A specific TRPC3 inhibitor, Pyr3, and a specific TRPM4 inhibitor, 9-phenanthrol, had no effects in pacemaker activity of ICCs and in TRPM7-like current in single ICC. Therefore, we think that TRPM7 has an important role in the modulation of pacemaker activity of ICCs.

TRPM7 has been suggested to have a central role in cellular Mg^{2+} homeostasis (Schmitz et al., 2003), in central nervous system ischemic injury (Aarts et al., 2003), in skeletogenesis in

zebrafish (Elizondo et al., 2005), in the defecation rhythm in *Caenorhabditis elegans* (Vriens et al., 2004), in cholinergic vesicle fusion with the plasma membrane (Brauchi et al., 2008), in phosphoinositide-3-kinase signaling in lymphocytes (Sahni et al., 2008), in cell death in gastric cancer (Kim et al., 2008), in osteoblast proliferation (Abed et al., 2009) and in breast cancer cell proliferation (Guilbert et al., 2009). But, until now, the effect of the 5-LOX inhibitors, NDGA, AA861 and MK886 on these TRPM7 several physiological functions have not yet been investigated. Therefore, these effects should be explored.

In conclusion, TRPM7 channel modulates intestinal motility and is a likely potential drug for pharmacological treatment of motor disorders of the gut.

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REFERENCES

Aarts, M., Iihara, K., Wei, W.L., Xiong, Z.G., Arundine, M., Cerwinski, W., MacDonald, J.F., and Tymianski, M. (2003). A key role for TRPM7 channels in anoxic neuronal death. Cell *115*, 863-877.

Abed, E., and Moreau, R. (2009). Importance of melastatin-like transient receptor potential 7 and magnesium in the stimulation of osteoblast proliferation and migration by platelet derived growth factor. Am. J. Physiol. Cell. Physiol. *297*, C360-C368.

Brauchi, S., Krapivinsky, G., Krapivinsky, L., and Clapham, D.E. (2008). TRPM7 facilitates cholinergic vesicle fusion with the plasma membrane. Proc. Natl. Acad. Sci. USA *105*, 8304-8308.

Chen, H.C., Xie, J., Zhang, Z., Su, L.T., Yue, L., and Runnels, L.W. (2010). Blockade of TRPM7 channel activity and cell death by inhibitors of 5-lipoxygenase. PLoS One *5*, e11161.

Clapham, D.E. (2003). TRP channels as cellular sensors. Nature *426*, 517-524.

Dickens, E.J., Hirst, G.D., and Tomita, T. (1999). Identification of rhythmically active cells in guinea-pig stomach. J. Physiol. *514*, 515-531.

Elizondo, M.R., Arduini, B.L., Paulsen, J., MacDonald, E.L., Sabel, J.L., Henion, P.D., Cornell, R.A., and Parichy, D.M. (2005). Defective skeletogenesis with kidney stone formation in dwarf zebrafish mutant for trpm7. Curr. Biol. *15*, 667-671.

Guilbert, A., Gautier, M., Dhennin-Duthille, I., Haren, N., Sevestre, H., and Ouadid-Ahidouch, H. (2009). Evidence that TRPM7 is required for breast cancer cell proliferation. Am. J. Physiol. Cell. Physiol. *297*, C493-C502.

Huizinga, J.D., Thuneberg, L., Kluppel, M., Malysz, J., Mikkelsen, H.B., and Bernstein, A. (1995). W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature *373*, 347-349.

Huizinga, J.D., Thuneberg, L., Vanderwinden, J.M., and Rumessen, J.J. (1997). Interstitial cells of Cajal as targets for pharmacological intervention in gastrointestinal motor disorders. Trends Pharmacol. Sci. *18*, 393-403.

Huizinga, J.D., Zhu, Y., Ye, J., and Molleman, A. (2002). High-conductance chloride channels generate pacemaker currents in interstitial cells of Cajal. Gastroenterology *123*, 1627-1636.

Kim, B.J., Lim, H.H., Yang, D.K., Jun, J.Y., Chang, I.Y., Park, C.S., So, I., Stanfield, P.R., and Kim, K.W. (2005). Melastatin-type transient receptor potential channel 7 is required for intestinal pacemaking activity. Gastroenterology *129*, 1504-1517.

Kim, B.J., Park, E.J., Lee, J.H., Jeon, J.H., Kim, S.J., and So, I. (2008). Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer. Cancer Sci. *99*, 2502-2509.

Koh, S.D., Jun, J.Y., Kim, T.W., and Sanders, K.M. (2002). A Ca^{2+}-inhibited nonselective cation conductance contributes to pacemaker currents in mouse interstitial cell of Cajal. J. Physiol. *540*, 803-814.

Komuro, T., Seki, K., and Horiguchi, K. (1999). Ultrastructural characterization of the interstitial cells of Cajal. Arch. Histol. Cytol. *62*, 295-316.

Langton, P., Ward, S.M., Carl, A., Norell, M.A., and Sanders, K.M. (1989). Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon. Proc. Natl. Acad. Sci. USA *86*, 7280-7284.

Maeda, H., Yamagata, A., Nishikawa, S., Yoshinaga, K., Kobayashi, S., Nishi, K., and Nishikawa, S. (1992). Requirement of c-kit for development of intestinal pacemaker system. Development *116*, 369-375.

Mazzone, A., Bernard, C.E., Strege, P.R., Beyder, A., Galietta, L.J., Pasricha, P.J., Rae, J.L., Parkman, H.P., Linden, D.R., Szurszewski, J.H., et al. (2011). Altered expression of ano1 variants in human diabetic gastroparesis. J. Biol. Chem. *286*, 13393-13403.

Nadler, M.J.S., Hermosura, M.C., Inabe, K., Perraud, A.L., Zhu, Q., Stokes, A.J., Kurosaki, T., Kinet, J.P., Penner, R., Scharenberg, A.M., et al. (2001). LTRPC7 is a MgNATP-regulated divalent cation channel required for cell viability. Nature *411*, 590-595.

Ordog, T., Ward, S.M., and Sanders, K.M. (1999). Interstitial cells of cajal generate electrical slow waves in the murine stomach. J. Physiol. *518*, 257-269.

Peters-Golden, M., and Brock, T.G. (2003). 5-lipoxygenase and FLAP. Prostaglandins Leukot. Essent. Fatty Acids *69*, 99-109.

Runnels, L.W., Yue, L., and Clapham, D.E. (2001). TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science *291*, 1043-1047.

Sahni, J., and Scharenberg, A.M. (2008). TRPM7 ion channels are required for sustained phosphoinositide 3-kinase signaling in lymphocytes. Cell. Metab. *8*, 84-93.

Sanders, K.M. (1996). A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology *111*, 492-515.

Sanders, K.M., Ordog, T., Koh, S.D., Torihashi, S., and Ward, S.M. (1999). Development and plasticity of interstitial cells of Cajal. Neurogastroenterol. Motil. *11*, 311-338.

Schmitz, C., Perraud, A.L., Johnson, C.O., Inabe, K., Smith, M.K., Penner, R., Kurosaki, T., Fleig, A., and Scharenberg, A.M. (2003). Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. Cell *114*, 191-200.

Tomita, T. (1981). Electrical activity (spikes and slow wave) in gastrointestinal smooth muscles. E. Bulbring, A.F. Brading, A.W. Jones, and T. Tomita, eds. (London, UK: Edward Arnold), pp. 127-156.

Torihashi, S., Ward, S.M., Nishikawa, S., Nishi, K., Kobayashi, S., and Sanders, K.M. (1995). c-kit-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. Cell Tissue Res. *280*, 97-111.

Vriens, J., Owsianik, G., Voets, T., Droogmans, G., and Nilius, B. (2004). Invertebrate TRP proteins as functional models for mammalian channels. Pflugers Arch. *449*, 213-226.

Ward, S.M., Burns, A.J., Torihashi, S., and Sanders. K.M. (1994). Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J. Physiol. *480*, 91-97.

Werz, O. (2002). 5-lipoxygenase: cellular biology and molecular pharmacology. Curr. Drug. Targets Inflamm. Allergy *1*, 23-44.

Yoo, S., Han, S., Park, Y.S., Lee, J.H., Oh, U., and Hwang, S.W. (2009). Lipoxygenase inhibitors suppressed carrageenan-induced Fos-expression and inflammatory pain responses in the rat. Mol. Cells *27*, 417-422.

Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S., and Shiraishi, M. (1982). 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow reacting substance of anaphylaxis. Biochim. Biophys. Acta *713*, 470-473.

Zhu, M.H., Kim, T.W., Ro, S., Yan, W., Ward, S.M., Koh, S.D., and Sanders, K.M. (2009). A Ca^{2+}-activated Cl^- conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. J. Physiol. *587*, 4905-4918.