Original Paper

The Mechanism of Action of Zingerone in the Pacemaker Potentials of Interstitial Cells of Cajal Isolated from Murine Small Intestine

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Key Words

Zingerone • Interstitial Cells of Cajal • Pacemaker Potentials • Gastrointestinal Motility

Abstract

Background/Aims: Zingerone, a major component found in ginger root, is clinically effective for the treatment of various diseases. Interstitial cells of Cajal (ICCs) are the pacemaker cells responsible for slow waves in the gastrointestinal (GI) tract. We investigated the effects of zingerone on the pacemaker potentials of ICCs to assess its mechanisms of action and its potential as a treatment for GI tract motility disorder. **Methods:** We isolated ICCs from small intestines, and the whole-cell patch-clamp configuration was used to record the pacemaker potentials in cultured ICCs. **Results:** Under the current clamping mode, zingerone inhibited pacemaker potentials of ICCs concentration-dependently. These effects were blocked not by capsazepine, a transient receptor potential vanilloid 1 (TRPV1) channel blocker, but by glibenclamide, a specific ATP-sensitive K⁺ channel blocker. Pretreatment with SQ-22536 (an adenylate cyclase inhibitor), LY294002 (a phosphoinositide 3-kinase inhibitor), and calphostin C (a protein kinase C (PKC) inhibitor) did not block the effects of zingerone on the pacemaker potentials relative to treatment with zingerone alone. However, zingerone-induced pacemaker potential inhibition was blocked by 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ; a guanylate cyclase inhibitor), KT5823 (a protein kinase G (PKG) inhibitor), and L-NAME (a non-selective nitric oxide synthase (NOS) inhibitor). In addition, zingerone stimulated cyclic guanosine monophosphate (cGMP) production in ICCs. Finally, pretreatment with PD98059 (a p42/44 mitogen-activated protein kinase (MAPK) inhibitor), SB203580 (a p38 MAPK inhibitor), and SP600125 (c-Jun N-terminal kinases (JNK)-specific inhibitor) blocked the zingerone-

induced pacemaker potential inhibition. **Conclusion:** These results suggest that zingerone concentration-dependently inhibits pacemaker potentials of ICCs via NO/cGMP-dependent ATP-sensitive K⁺ channels through MAPK-dependent pathways. Taken together, this study shows that zingerone may have the potential for development as a GI regulation agent.

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Introduction

Ginger (*Zingiber officinale* Roscoe, family: Zingiberaceae) originated in South-East Asia and is the most commonly used spice worldwide. It is a pungent, aromatic spice, which adds a special flavor and zest to our food [1, 2]. Ginger contains numerous active compounds, which vary significantly between plant varieties and regions in which it is grown. More than 60 active constituents are known to be present in ginger [1, 2]. Among active constituents, zingerone is produced during drying of ginger directly and is present in a significant amount of approximately 9.25% in ginger. Zingerone is a member of the Methoxyphenol family and its related derivatives [3]. Ginger is an herbal medicine for the treatment of gastrointestinal (GI) disorders, including constipation and diarrhea, and zingerone is a likely active constituent responsible for the antidiarrheal activity of ginger [4, 5]. Ginger has suppressive effects on gut motility and inhibits rat ileal motility via inhibition of enteric neural excitatory transmission and smooth muscle mechanical activity *in vitro* [6]. In addition, ginger extracts inhibit colonic motility in rats [7]. Ghayur and Gilani [8] reported inhibitory effects of ginger crude extract on high K⁺-induced contractions in isolated guinea pig colon. Zingerone inhibits enterotoxin-induced fluid secretion in the ileum of mice [9]. Zingerone can inhibit colonic motility via direct action on smooth muscles and can attenuate colonic motility *in vivo* without affecting blood pressure and heart rate [10]. Zingerone also significantly reduces colonic transit, fecal output, neutrophil infiltration, and lipid peroxide formation in stress-induced irritable bowel disorder, producing marked improvement [11].

The GI tract shows spontaneous contraction that is evoked by periodic membrane depolarizations called slow waves. There has been increasing evidence that interstitial cells of Cajal (ICCs) are pacemaker cells that generate slow waves [12–14]. ICCs produce spontaneous electrical activity (pacemaker activity) that is transmitted to smooth muscle cells through gap junctions [15, 16]. Damage to ICCs or reduction in ICC numbers has been described in many GI motility disorders [17, 18]. Therefore, it is important to identify the mechanisms underlying pacemaker activity of ICCs to understand GI function. However, no scientific studies conducted to date have presented evidence of any relationship between zingerone and ICCs. Therefore, in the present study, we investigated the effects of zingerone on the pacemaker potentials of ICCs.

Materials and Methods

Preparation of ICCs and ICC clusters

Institute of Cancer Research (ICR) mice (Samtako Bio Korea Co., Ltd., Osan, Republic of Korea; 2–5 days old; weighing 2.0–2.3 g) of either sex were used to isolate and culture ICCs and we examine the effects of zingerone on pacemaker potentials of ICCs. Animal care and experiments were conducted in accordance with the guidelines issued by the Institutional Animal Care and Use Committee (IACUC) at Pusan National University (Busan, Republic of Korea; Approval no. PNU-2016-1370) and those issued by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Small intestines were removed and opened along the mesenteric border. Luminal contents were removed using Krebs-Ringer bicarbonate solution. Mucosae were then removed by sharp dissection, and small tissue strips of intestinal muscle were then equilibrated for 30 min in Ca²⁺-free Hank's solution (containing (in mM): KCl 5.36, NaCl 125, NaOH 0.34, Na₂HCO₃ 0.44, glucose 10, sucrose 2.9, and HEPES 11; pH 7.4). Cells were then dispersed in an enzyme solution containing 1.8 mg ml⁻¹ collagenase (Worthington Biochemical, Lakewood, NJ, USA), 2.4 mg ml⁻¹

bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA), 2.5 mg ml⁻¹ trypsin inhibitor (Sigma-Aldrich), and 1.00 mg ml⁻¹ ATP (Sigma-Aldrich). Next, cells were cultured at 37°C in a 95% O₂-5% CO₂ incubator in smooth muscle growth medium (SMGM) (Clonetics, San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and 5 ng ml⁻¹ of murine stem cell factor (SCF) (Sigma-Aldrich). At first, the ICCs were cultured for <12 h, and we verified their identity immunologically using an anti-c-kit antibody (eBioscience, San Diego, CA, USA) at a dilution of 1:50 for 20 min [19]. However, after this treatment, the ICCs were adversely affected, and we could not subject them to patch-clamp methods. Therefore, because ICCs differed morphologically from other cell types in culture, they were identified under phase contrast microscopy. The morphology of ICCs was spindle-shaped, with several branches emanating from a central cell body, connecting with neighboring cells and showing networks. Spontaneous rhythmicity was routinely recorded from cultured ICCs under current-clamp conditions and ICCs within networks had a more robust electrical rhythmicity; tissue-like spontaneous slow waves were recorded from these cells [20].

Patch-clamp experiments

We used whole-cell patch clamp methods to record the effects of zingerone on the pacemaker potentials of ICCs. The physiological salt solution used to bathe cultured ICCs contained (in mM): KCl 5, NaCl 135, CaCl₂ 2, glucose 10, MgCl₂ 1.2, and HEPES 10 (adjusted to pH 7.4 with NaOH). The pipette solution used to examine pacemaker potentials contained (in mM): KCl 140, MgCl₂ 5, K₂ATP 2.7, NaGTP 0.1, creatine phosphate disodium 2.5, HEPES 5, and EGTA 0.1 (adjusted to pH 7.2 with KOH). Patch-clamp techniques were conducted using Axopatch I-D and Axopatch 200B amplifiers (Axon Instruments, Foster, CA, USA). Command pulses were applied using an IBM-compatible personal computer and pClamp software (version 6.1 and version 10.0; Axon Instruments). Data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor, and/or on a pen recorder (Gould 2200) (Gould, Valley View, OH, USA). Results were analyzed using pClamp and Origin software (version 6.0) (Microcal, Northampton, MA, USA). All experiments were performed at 30°C–31°C.

Cyclic guanosine monophosphate (cGMP) assay

ICCs were preincubated with 100 mM 3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich) for 30 min at 37°C to inhibit cGMP degradation, and were then incubated with zingerone for 10 min. Following homogenization in a buffer containing 4 mM ethylenediaminetetraacetic acid (EDTA) to prevent enzymatic degradation of cGMP, homogenates were heated for 5 min in a boiling water bath to denature proteins, and were then centrifuged at 3,950 × g for 5 min at 4°C. The supernatants subsequently obtained were transferred to fresh tubes and were stored at 4°C. Samples were assayed for cGMP using cGMP enzyme-linked immunosorbent assay kits (ELISA, Enzo Life Sciences, Inc., Farmingdale, NY, USA). These assays were conducted according to the manufacturer's protocol.

Drugs

PD98059, SB203580, SP600125, and LY294002 were purchased from Calbiochem (San Diego, CA, USA). All other drugs were obtained from Sigma-Aldrich. For stock solutions, all drugs were dissolved in distilled water (DW) or dimethyl sulfoxide (DMSO) and stored at -20°C. Zingerone was first dissolved in DW at 1,000 times the concentration to be used at room temperature and then stored at -20°C. The stock solution was diluted to the desired concentration in bath solution immediately before use. The bath solution was warmed just prior to entering the experimental bath, and the bath volume (0.1 ml) was exchanged at a rate of 0.11 ml s⁻¹. The final concentration of DMSO in the bath solution was always <0.1% and we confirmed that this concentration did not affect the results. Furthermore, the addition of these chemicals did not alter the pH of the bath solution.

Statistical analysis

Results are expressed as the means ± SEMs. N values refer to the numbers of cells used in the experiments. For multiple comparison analysis, we used one-way ANOVA with Bonferroni's *post hoc* tests. For statistical analyses, we used Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA) and Origin version 8.0 (OriginLab Corporation, Northampton, MA, USA). P values of <0.05 were considered statistically significant.

Results

Effects of zingerone on the pacemaker potentials in ICCs of murine small intestine

Using the whole-cell patch clamp technique, we investigated the characteristics of cultured ICC clusters. ICC clusters formed network-like structures after culture for <12 h and were viable as demonstrated by the generation of spontaneous rhythmic contractions. Under the current clamp mode ($I = 0$), ICCs generated pacemaker potentials (Fig. 1) with a mean resting membrane potential of -57.8 ± 1.0 mV, mean frequency of 17.9 ± 1.0 cycles/min, and mean amplitude of 24.8 ± 1.3 mV. Zingerone (100–1000 μ M) suppressed the pacemaker potentials and decreased their frequencies in a concentration-dependent manner, but had no effect on the resting membrane potentials (Fig. 1A–1F). In addition, the amplitude was inhibited only at 1000 μ M zingerone (Fig. 1G). In the presence of zingerone, the mean resting membrane potentials were -58.0 ± 1.2 mV at 100 μ M, -58.1 ± 1.1 mV at 300 μ M, -58.3 ± 0.9 mV at 500 μ M, and -57.9 ± 1.4 mV at 1000 μ M (Fig. 1E; $n = 20$); the mean frequencies were 17.7 ± 1.1 cycles/min at 100 μ M, 12.0 ± 0.8 cycles/min at 300 μ M, 5.2 ± 0.9 cycles/min at 500 μ M, and 0.8 ± 0.2 cycles/min at 1000 μ M (Fig. 1F; $n = 20$); and the mean amplitudes were 24.1 ± 1.3 mV at 100 μ M, 24.5 ± 0.9 mV at 300 μ M, 24.4 ± 1.5 mV at 500 μ M, and 2.0 ± 0.6 mV at 1000 μ M (Fig. 1G; $n = 20$). These results suggest that zingerone suppresses the pacemaker potentials of ICCs concentration-dependently.

Involvement of ATP-sensitive K^+ channels in zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine

To determine the involvement of ATP-sensitive K^+ channels in zingerone-induced pacemaker potential inhibition of ICCs, we used the ATP-sensitive K^+ channel blocker, glibenclamide. In a previous study, we found that pinacidil (an ATP sensitive K^+ channel opener) decreased the frequencies and amplitudes of pacemaker potentials, and these pinacidil-

Fig. 1. Effects of zingerone on the pacemaker potentials of ICCs in murine small intestine. (A–D) The change in pacemaker potentials of ICCs by zingerone (100–1000 μ M) in current-clamp mode ($I = 0$). Zingerone concentration-dependently inhibited pacemaker potentials and suppressed pacemaker potential frequencies and amplitudes. Responses to zingerone regarding resting membrane potential (RMP), frequency, and amplitude are summarized in (E), (F), and (G). Bars represent means $\pm$ SEMs. ** $P < 0.001$: significantly different from the control. CTRL: Control.

induced effects were reversed by glibenclamide treatment [21, 22]. Therefore, we believe that ATP sensitive K^+ channels may be involved in the regulation of pacemaker potentials in ICCs. The zingerone-induced inhibition of the frequency and amplitude of pacemaker potentials in ICCs was blocked by glibenclamide treatment (10 μ M) (Fig. 2A). In addition, pretreatment with glibenclamide blocked the zingerone-induced pacemaker potential inhibition (Fig. 2B). The frequencies of pacemaker potentials before and after the co-treatment of glibenclamide and zingerone were 0.8 ± 0.2 and 17.5 ± 1.0 cycles/min, respectively, with corresponding amplitudes of 2.0 ± 0.6 and 24.1 ± 1.2 mV ($n = 8$; Fig. 2C-2D). These results suggest that zingerone suppresses the pacemaker potentials via ATP sensitive K^+ channels in ICCs.

No involvement of transient receptor potential vanilloid 1 (TRPV1) channels in zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine

Many experiments on different cells have shown that zingerone activates TRPV1 channels [23, 24]. Therefore, we investigated whether TRPV1 channels are required for zingerone-induced pacemaker potential inhibition. We incubated the ICCs with the TRPV1 receptor antagonist, capsazepine (10 μ M), prior to the treatment with zingerone. Pretreatment with capsazepine did not block the zingerone-induced pacemaker potential inhibition (Fig. 3A). The frequencies of pacemaker potentials before and after the co-treatment of zingerone and capsazepine were 17.8 ± 1.1 and 0.9 ± 0.5

Fig. 2. Effects of glibenclamide (GBC) on zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine. (A) Zingerone-induced pacemaker potential inhibition was prevented by GBC (10 μ M). (B) Treatment with GBC alone did not affect pacemaker potentials, and following GBC pretreatment, zingerone did not inhibit pacemaker potentials. Responses to zingerone and GBC are summarized in (C) and (D). Bars represent means $\pm$ SEMs. ** $P < 0.001$: significantly different from non-treated controls. CTRL: Control.

Fig. 3. Effects of capsazepine (CPZ) on zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine. (A) Treatment with CPZ alone did not affect pacemaker potentials, but following CPZ pretreatment, zingerone inhibited pacemaker potentials. Responses to zingerone and CPZ are summarized in (B) and (C). Bars represent means $\pm$ SEMs. ** $P < 0.001$: significantly different from non-treated controls. CTRL: Control.

cycles/min, respectively, with corresponding amplitudes of 24.0 ± 1.6 and 1.1 ± 0.6 mV ($n = 6$, Fig. 3B-3C). These results suggest that TRPV1 channels are not involved in zingerone-induced pacemaker potential inhibition in ICCs.

No involvement of adenylate cyclase, phosphoinositide 3-kinase (PI3K), and protein kinase C (PKC) pathways in zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine

To determine the mechanisms of zingerone inhibition of the pacemaker potentials of ICCs, ICCs were pretreated with 100 μ M SQ-22536 (an adenylate cyclase inhibitor), 10 μ M LY294002 (a PI3K inhibitor), or 10 μ M calphostin C (a PKC inhibitor). We found that these inhibitors did not block the zingerone-induced pacemaker potential inhibition in ICCs ($n = 4$, Fig. 4A, 4B, and 4C). The summarized values and bar graph of the effects of zingerone,

Fig. 4. Effects of SQ-22536 (an adenylate cyclase inhibitor), LY294002 (a PI3K inhibitor), and calphostin C (a PKC inhibitor) on zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine. (A) SQ-22536 (100 μ M) had no effects on zingerone-induced pacemaker potential inhibition. (B) LY294002 (10 μ M) had no effects on zingerone-induced pacemaker potential inhibitions. (C) Calphostin C (10 μ M) also had no effects on zingerone-induced pacemaker potential inhibition. Responses to zingerone, SQ-22536, LY294002, and calphostin C are summarized in (D) and (E). Bars represent means $\pm$ SEMs. $^{**}P < 0.001$: significantly different from non-treated controls. N.S.: Not Significant. CTRL: Control. SQ.: SQ-22536. LY.: LY294002. Cal.: Calphostin C.

Fig. 5. Effects of OEQ (a guanylate cyclase inhibitor), KT5823 (a PKG inhibitor), and L-NAME (a non-selective NOS inhibitor) on zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine. (A-C) OEQ (100 μ M), KT5823 (1 μ M), and L-NAME (10 μ M) blocked zingerone-induced pacemaker potential inhibition. Responses to zingerone, OEQ, KT5823 and L-NAME are summarized in (D) and (E). Bars represent means $\pm$ SEMs. $^{**}P < 0.001$: significantly different from non-treated controls. CTRL: Control. OEQ: OEQ. KT5823: KT5823. L-NAME: L-NAME.

SQ-22536, LY294002, and calphostin C on pacemaker potentials are shown in Fig. 4D and 4E. These results suggest that cAMP, PI3K, and PKC pathways are not involved in zingerone-induced pacemaker potential inhibition in ICCs.

Involvement of guanylate cyclase, protein kinase G (PKG), and nitric oxide (NO) pathways in zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine

To determine whether zingerone-induced pacemaker potential inhibition is mediated by the guanylate cyclase pathway, a guanylate cyclase inhibitor (1H-[1, 2,4] oxadiazolo [4, 3-a] quinoxalin-1-one; ODQ) was used to treat the ICCs. Preincubation with ODQ (100 μ M) alone for 5 min had no effect on pacemaker potentials, but 100 μ M ODQ suppressed zingerone-induced pacemaker potential inhibition ($n = 5$; Fig. 5A). The effects of a PKG inhibitor (KT5823) were also investigated. Preincubation with KT5823 alone had no effect on pacemaker potentials, but 1 μ M KT5823 suppressed zingerone-induced pacemaker potential inhibition ($n = 5$; Fig. 5B). In addition, NO, which is generated from L-arginine by the action of NO synthases (NOS), activates soluble guanylate cyclase, resulting in the formation of cGMP and activation of PKG [25]. To investigate whether the inhibitory effect of zingerone on pacemaker potential is mediated by NO, we added L-NAME (10 μ M), a non-selective NOS inhibitor. Preincubation with L-NAME alone had no effect on pacemaker potentials, but 10 μ M L-NAME suppressed zingerone-induced pacemaker potential inhibition ($n = 5$; Fig. 5C). The summarized values and bar graph of the effects of zingerone, ODQ, KT5823, and L-NAME on pacemaker potentials are shown in Fig. 5D and 5E. Finally, intracellular cGMP content was measured under basal and zingerone-stimulated conditions, and zingerone was revealed to stimulate cGMP production [Fig. 6; control (12.2 ± 0.5 pmol/mg protein) vs. zingerone (13.9 ± 0.9 pmol/mg protein)]. These results suggest that NO, cGMP, and PKG-dependent pathways play a vital role in zingerone-induced pacemaker potential inhibition in ICCs.

Fig. 6. Association of cyclic guanosine monophosphate (cGMP) production with zingerone-induced pacemaker potential inhibition in ICCs. Preincubation of ICCs with zingerone significantly stimulated cGMP production. Bars represent means $\pm$ SEMs. * $P < 0.05$: significantly different from non-treated controls. CTRL: Control.

Fig. 7. Effects of PD98059 (a p42/44 MAPK inhibitor), SB203580 (a p38 MAPK inhibitor), and SP600125 (a c-Jun N-terminal kinases (JNK)-specific inhibitor) on zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine. (A–C) PD98059 (10 μ M), SB203580 (10 μ M), and SP600125 (10 μ M) blocked the zingerone-induced pacemaker potential inhibition. Responses to zingerone, PD98059, SB203580, and SP600125 are summarized in (D) and (E). Bars represent means $\pm$ SEMs. ** $P < 0.001$: significantly different from non-treated controls. CTRL: Control. PD.: PD98059. SB.: SB203580. SP.: SP600125.

Involvement of mitogen-activated protein kinase (MAPK) pathways in zingerone-induced pacemaker potential inhibition in ICCs of murine small intestine

To investigate the involvement of MAPKs in zingerone-induced pacemaker potential inhibition, we used PD98059 (a p42/44 MAPK inhibitor), SB203580 (a p38 MAPK inhibitor), and SP600125 (a c-Jun N-terminal kinase [JNK]-specific inhibitor). It has been reported that zingerone activity occurs through MAPK pathways in many cell types [26, 27]. In the presence of PD98059 (10 μ M), zingerone did not inhibit pacemaker potentials ($n = 5$; Fig. 7A), which indicates that p 42/44 plays a role in zingerone-induced pacemaker potential inhibition. In addition, both SB203580 and SP600125 blocked zingerone-induced pacemaker potential inhibition ($n = 5$; Fig. 7B and 7C). The summarized values and bar graph of the effects of zingerone, PD98059, SB203580, and SP600125 on pacemaker potentials are shown in Fig. 7D and 7E. These results suggest that MAPK-dependent pathways are involved in zingerone-induced pacemaker potential inhibition in ICCs.

Discussion

Ginger has been used for thousands of years as a spice and for medicinal purposes for the treatment of various diseases [2, 26, 28]. Ginger contains several active ingredients such as gingerols, shogaols, paradols, gingerdiols, and zingerone [29]. Among them, zingerone is a major component of several spice plants, but is mainly found in ginger root [26, 30]. Zingerone is vanillylacetone, which, chemically, is a member of the phenolic alkanone group; its pharmacological properties are diverse, including possessing antioxidant [31], anti-inflammatory [26], anticancer [32], and antimicrobial activities [33]. Zingerone has the ability to degrade free radicals generated by radiolysis of various food products and suppresses ferric ascorbate-induced lipid peroxidation in rat brain [34]. Zingerone has been reported to protect *in vitro* DNA against stannous chloride-induced reactive oxygen species (ROS) oxidative damage [31]. Therefore, zingerone is a potent antioxidant. Zingerone also has anti-inflammatory and anticancer effects. Zingerone suppresses activity of both peroxisome proliferator-activated receptor (PPAR) and nuclear factor (NF)- κ B, and interferes with MAPK signaling pathways [35, 36]; it has been shown to inhibit colitis in rats by downregulating NF- κ B activity and the IL-1 β signaling pathway [37]. Zingerone induces a significant decrease in tumor incidence, aberrant crypt foci formation, and levels of tissue lipid peroxidation [32]. In addition, zingerone is a remedy for GI disorders such as diarrhea [32–34, 38]. Zingerone exerts an inhibitory effect on colonic motility by directly acting on the smooth muscles of the colon [10], is responsible for antidiarrheal activity of ginger, and modifies bacterial as well as host cell metabolism [9]. In addition, zingerone significantly reduces colonic transit, fecal output, neutrophil infiltration, and lipid peroxide formation and produces marked improvement in stress-induced irritable bowel disorder [38]. However, the cellular and molecular mechanisms underlying the GI motility effects of zingerone are not well understood.

The GI tract shows spontaneous mechanical contractions mediated by the periodic generation of electrical pacemaker potentials [39]. ICCs are the pacemaker cells in the GI tract. They are involved in physiological GI motility, and therefore have clinical importance in many bowel disorders, such as GI motility-related diseases [40]. Because many GI motility disorders have been associated with ICC abnormalities, it is extremely important to understand the mechanisms underlying pacemaker activity and ICC excitability [18].

In the present study, we found that zingerone concentration-dependently inhibits pacemaker potentials of ICCs via NO/cGMP-dependent ATP-sensitive K⁺ channels through MAPK-dependent pathways. Zingerone-induced pacemaker potential inhibition was blocked not by capsazepine (Fig. 3), but by glibenclamide (Fig. 2). SQ-22536, LY294002, and calphostin C did not block the effects of zingerone on the pacemaker potentials relative to treatment with zingerone alone (Fig. 4). However, zingerone-induced pacemaker potential inhibition was blocked by ODQ, KT5823, or L-NAME (Fig. 5). In addition, zingerone stimulated

cGMP production in ICCs (Fig. 6). Finally, PD98059, SB203580, and SP600125 blocked the zingerone-induced pacemaker potential inhibition (Fig. 7). Therefore, zingerone shows the potential for development as a GI regulating agent.

Some natural pungent compounds such as capsaicin activate a nonselective cation channel (NSCC) termed TRPV1 [41]. Zingerone has been demonstrated to evoke opening of TRPV1 in cultured TG neurons [42]. However, in this study, TRPV1 was not involved in zingerone-induced pacemaker potential inhibition (Fig. 3). It is known that the pacemaker activities of ICCs in the murine small intestine are mainly due to periodic activations of NSCCs [13, 43] or Cl⁻ channels [44]. Kim et al [13]. suggested that transient receptor potential melastatin (TRPM) 7 channel is required for pacemaker activity, and a Ca²⁺-activated Cl⁻ channel (CaCC) is involved in the slow waves generated by ICCs [44]. Therefore, we think that zingerone might regulate NSCCs (TRPM7) or Cl⁻ channels (CaCC), but not TRPV1 channels, and we will investigate these possibilities in future studies.

In summary, the results of the present study show that (1) Zingerone concentration-dependently inhibits the pacemaker potentials of ICCs; (2) Zingerone affects pacemaker potentials through ATP-sensitive K⁺ channels, not TRPV1 channels; (3) Adenylate cyclase, PI3K, and PKC pathways are not involved in zingerone-induced pacemaker potential inhibition; (4) Zingerone-induced pacemaker potential inhibition is dependent on guanylate cyclase, PKG, and NO pathways; and (5) Zingerone-induced pacemaker potential inhibition is dependent on MAPK pathways. Collectively, these results indicate that zingerone has the potential for development as a GI regulation agent for use in GI motility disorders.

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Kim BJ designed the research; Kim JN, Kim HJ and Kim I performed the experiments; Kim JN, Kim YT and Kim BJ analyzed the data; and Kim BJ wrote the paper.

Disclosure Statement

The authors declare to have no potential conflict of interests.

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