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博士學位 論文

Effects of Protein Kinases on the Spontaneous Activity in Gastrointestinal Tract

朝鮮大學校 大學院

醫學科

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李 美 貞의 博士學位論文을 認准함

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이 미 정

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Protein kinases는 세포내 조절인자로서 다양한 생리 및 병태생리적 세포내 반응들을 초래하며 위장관내에서도 평활근의 수축과정에 참여하여 운동성을 조절하는데 깊이 관여하고 있다. 카할 사이질 세포 (Interstitial Cells of Cajal; ICCs)는 위장관 평활근에서 자발적 수축을 초래하는 향도잡이 세포로서 위장관 운동 조절에 중요한 역할을 하고 있다. 본 연구는 위장관 카할 사이질 세포에서 발생되는 자발적 향도잡이 전압 (pacemaking potentials)에

대한 protein kinases의 작용을 연구한 내용으로 다음과 같은 실험 결과들을 얻었다.

1. Tyrosine kinase 억제제인 genistein과 herbymicin A는 대장 카할 사이질 세포에서 발생되는 자발적인 향도잡이 전압 발생을 차단하였다.
2. Mitogen activating protein kinase (MAPK) 억제제들에서 PD 98059 (p42/p44 MAPK 억제제)는 대장 카할 사이질 세포의 자발적인 향도잡이 발생에 대해 아무런 영향을 보이지 않은 반면, SB203580 (p38 MAPK 억제제)과 JNK inhibitor II (c-jun NH_2-terminal kinase 억제제)들은 자발적인 향도잡이 발생을 차단하였다.
3. Protein kinase A 억제제인 KT5720, protein kinase G 차단제인 KT5823 과 protein kinase C 억제제인 chelerythrine과 bisindolylmaleimide 들은 대장 카할 사이질 세포의 자발적인 향도잡이 발생에 대해 아무런 영향을 보이지 않았다.

4. 소장 카할 사이질 세포에서 발생되는 자발적인 향도잡이 발생에 대해 tyrosine kinase 억제제, MAPK 억제제, protein kinase A 억제제, protein kinase G 억제제 및 protein kinase C 억제제 모두 자발적인 향도잡이 발생에 대해 아무런 영향을 보이지 않았다.

이상의 실험결과들로부터 내인성 tyrosine kinase와 MAPK가 대장 카할 사이질 세포에서 자발적 향도잡이 전압 발생에 기초적으로 작용하여 관여하고 있는 반면 소장 카할 사이질 세포의 자발적 향도잡이 전압 발생에는 관여하고 있지 않는 것으로 생각되며, 소장과 대장의 자발적 향도잡이 전압 발생에 있어 그 기전이 다를 수 있음을 알 수 있었다.

핵심단어: 카할 사이질 세포, 향도잡이 전압, tyrosine kinase, MAPK, 대장, 소장

INTRODUCTION

The smooth muscles of gastrointestinal (GI) tract represent spontaneous mechanical contractions by electrical cyclic depolarization of the membrane, which are termed as slow waves. Slow waves control the frequency and timing of smooth muscle contraction[1]. The cause of slow waves are due to interstitial cells of Cajal (ICCs) located within the GI wall. ICCs generate spontaneous pacemaker potentials and transmitted into smooth muscles via gap junctions, thereby recorded with slow waves in smooth muscle cells[2]. ICCs are connected with each other through gap junctions and formed network in GI tract[3, 4]. When pacemaker potentials were reached at the threshold in smooth muscle cells, external Ca^{2+} entered cells through the opened voltage-dependent Ca^{2+} channels and triggers a muscle to contract[5]. Together with ICCs are closely associated with varicosities of the enteric nerves, which mediate inhibitory and excitatory nerve signal to smooth muscles[6, 7]. ICCs also serve as stretch receptor[8]. These mean that ICCs play an important role as a basic determinant to regulating GI motility. Many articles reported that GI motility disorders are closely related with the disruptions of ICCs[9, 10].

Protein kinases are important regulators of cell function. Diverse

substances represent their cellular actions through protein kinas A (PKA), protein kinase G (PKG), and protein kinase C (PKC) that are activated by intracellular cAMP, cGMP, and diacylglycerol second messenger, respectively in GI tract[11]. Especially tyrosine kinase and/ or mitogen-activated protein kinase (MAPK) pathways are intracellular signaling cascade that play an important role in the mediation of diverse physiological and pathological cellular responses including gastrointestinal (GI) smooth muscle contractions[12-16]. Protein tyrosine kinases are classified into two groups: receptor tyrosine kinases, such as epidermal growth factor receptor or platelet-derived growth factor receptor (PDGFR), and non-receptor tyrosine kinases, such as the Src family kinases[17, 18]. The MAPK family consists of three main forms in cells: p42/p44 MAPK, p38 MAPK, and c-Jun N-terminal kinase (JNK)[19]. The tyrosine kinase and MAPK pathways are activated by various extracellular and intracellular stimuli, such as G-protein coupled receptor, cytokines, growth factors, lipopolysaccharides, and intracellular another protein kinases[20-22]. In addition, the MAPK pathways are activated by down-stream regulators of receptor tyrosine kinase and non-receptor tyrosine kinase[23]. Tyrosine kinase and/or MAPK signaling pathways play an important role in the contractile response not only of normal intestinal

smooth muscle but also of inflamed intestinal smooth muscle. Thus, recently it has been suggested the possibility that MAPKs signaling pathways represent ideal targets for generation of novel therapeutics for patients with GI motility disorders[24]. The pacemaking mechanism of ICCs is closely associated with between intracellular Ca^{2+} and pacemaking ion channels. Transient receptor potential (TRP) channels or Ca^{2+}-activated Cl^- channels are candidate as pacemaker channels[25, 26]. Both channels are also influenced by tyrosine kinase and MAPKs in several cells[27, 28]. Recently, it was reported hyperpolarization-activated cyclic nucleotide gated (HCN) channels were participated in generating pacemaker potentials in colonic ICCs and suggested as a possible pacemaker channels in colonic ICCs[29]. However, there is no report on the role of tyrosine kinase and MAPK in modulation of pacemaker activity in ICCs until now. Thus, in the present study, I studied the role of MAPK and tyrosine kinases in generating of pacemaker potentials of colonic ICCs using MAPK inhibitors and tyrosine kinase inhibitors and compared the effects to small intestinal ICCs.

MATERIALS AND METHODS

Preparation of cells

The protocols and animal care used in these experiments were in accordance with the guiding principles approved by the ethics committee of the Chosun University and the National Institutes of Health Guide, South Korea for the Care and Use of Laboratory Animals. Mice had free access to water and a standard mouse diet until the day of experimentation. Balb/C mice (5–8 days old) of either sex were anesthetized with ether and killed by cervical dislocation. The colon from below the cecum to the rectum was removed, and the middle portion of the colon was used. The small intestine from ileum to jejunem was used. The colon and small intestine were opened along the mesenteric border. The luminal contents were washed with Krebs–Ringer bicarbonate solution. The tissues were pinned to the base of a Sylgard dish and the mucosa was removed by sharp dissection. Small strips of the colonic and small intestinal muscle were equilibrated in Ca^{2+}-free Hank's solution for 30 min, and the cells were dispersed with an enzyme solution containing 1.3 mg/mL collagenase (Worthington Biochemical Co, Lakewood, NJ,

USA), 2 mg/mL bovine serum albumin (Sigma, St. Louis, MO, USA), 2 mg/mL trypsin inhibitor (Sigma), and 0.27 mg/mL ATP. Cells were plated onto sterile glass coverslips coated with murine collagen (2.5 μg/mL Falcon/BD) in 35-mm culture dishes. The cells were then cultured at 37°C in a 95% O_2/5% CO_2 incubator in smooth muscle growth medium (SMGM; Clonetics Corp., San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and 5 ng/mL urine stem cell factor (SCF, Sigma).

Patch-clamp experiments

The whole-cell configuration of the patch-clamp technique was used to record pacemaker potentials in ICCs that showed the network-like structures in culture (2–3 days). Pacemaker potentials were amplified using Axopatch 200B (Axon Instruments, Foster, CA, USA). The data were filtered at 5 kHz and displayed on a computer monitor. The results were analyzed using pClamp and GraphPad Prism software (version 5.0, GraphPad Software Inc., San Diego, CA, USA). All experiments were performed at 30°C.

Reagents

The cells were bathed in a solution containing 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 7.2 using Tris. The pipette solution contained 20 mM potassium aspartate, 120 mM KCl, 5 mM $MgCl_2$, 2.7 mM K_2ATP, 0.1 mM Na_2GTP, 2.5 mM creatine phosphate disodium, 5 mM HEPES, and 0.1 mM EGTA, adjusted to pH 7.2 using Tris.

The drugs used were PD98059, SB203580, JNK inhibitor II, genistein, herbimycin A, daidzein, KT 5720, KT 5823, chelerythrine, and bisindolylmaleimide. All drugs were purchased from Sigma Chemical.

Statistical analysis

Data are expressed as the means ± standard errors. Differences in the data were evaluated using the Student's *t* test. A P-value < 0.05 was considered to indicate a statistically significant difference. The *n* values reported in the text refer to the number of cells used in the patch-clamp experiments.

RESULTS

Pacemaker Potentials in Colonic and small intestinal ICCs

The patch clamp technique was tested with ICCs that had network-like structures in culture (2-3 days). Under a current clamp, ICCs generated pacemaker potentials in colonic and small intestinal ICCs (Figure 1 A and B). In colonic ICCs, the resting membrane potential, amplitude, and frequency were –47.2 ± 3.8 mV, 38.2 ± 6.8 mV, and 11.3 ± 2.4 cycles/5min, respectively (n=24). In small intestinal ICCs, the resting membrane potential, amplitude, and frequency were –51.4 ± 2.8 mV, 28.2 ± 2.4 mV, and 14 ± 2 cycles/min, respectively (n=18) (Figure 2A, B, and C).

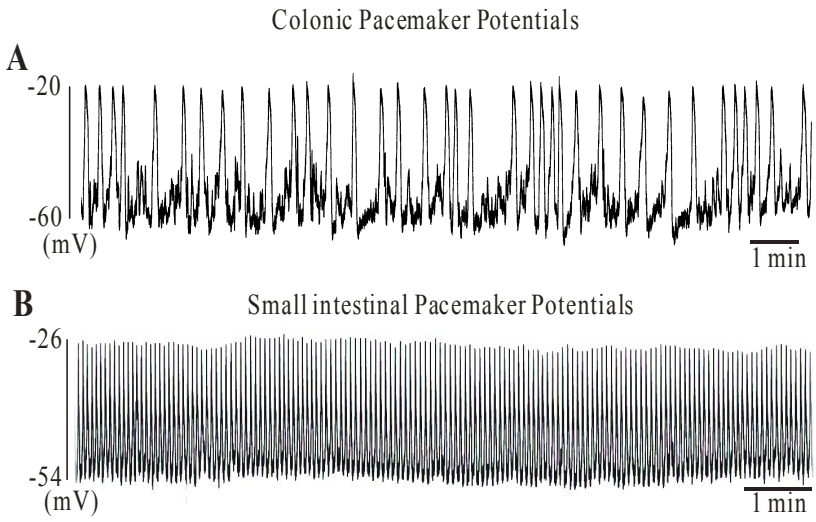

Figure 1. Typical traces of pacemaker potentials in current clamping mode in colonic ICCs (A) and small intestinal ICCs (B) from mouse.

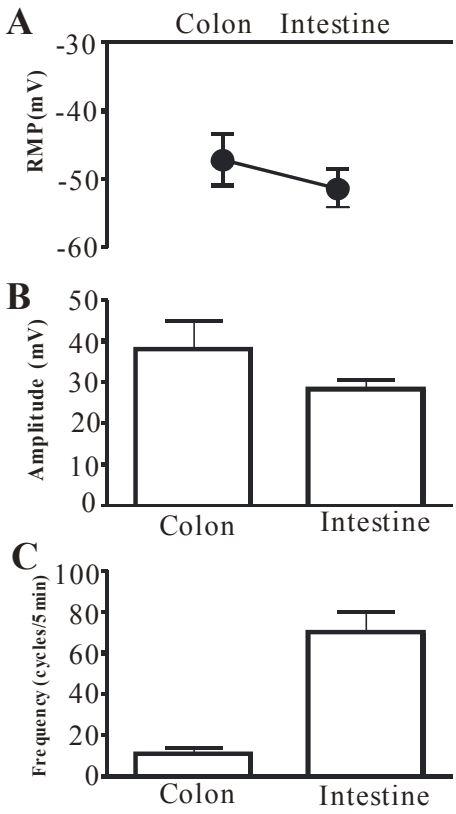

Figure 2. Summarized data of the control pacemaker potentials in colonic and small intestinal ICCs. The values in the resting membrane potential are summarized in (A). (B) and (C) are the summarized data of amplitude and frequency, respectively in control colonic and small intestinal ICCs.

Effects of tyrosine kinase inhibitors on pacemaker potentials in colonic ICCs

To investigate whether tyrosine kinase involves in generating pacemaker potentials or not, genistein and herbimycin A, tyrosine kinase inhibitors, were tested. Both genistein (100 μM) and herbimycin A (10 μM) decreased the pacemaker potential frequency of colonic ICCs and hyperpolarized the membrane (Figure 3A and B). However, daidzein (10 μM, n=5), an inactive form of genistein, had no effects on pacemaker potentials (Figure 3C). The values of the pacemaker potential frequency induced by genistein (n=7) and herbimycin A (n=6) were significantly different from control values. However, the values of the resting membrane potential induced by genistein and herbimycin A were not significantly different from control values even though they hyperpolarized the membrane (Figure 4A and B). These results suggest that endogenous tyrosine kinase involves in generating pacemaker potential in colonic ICCs.

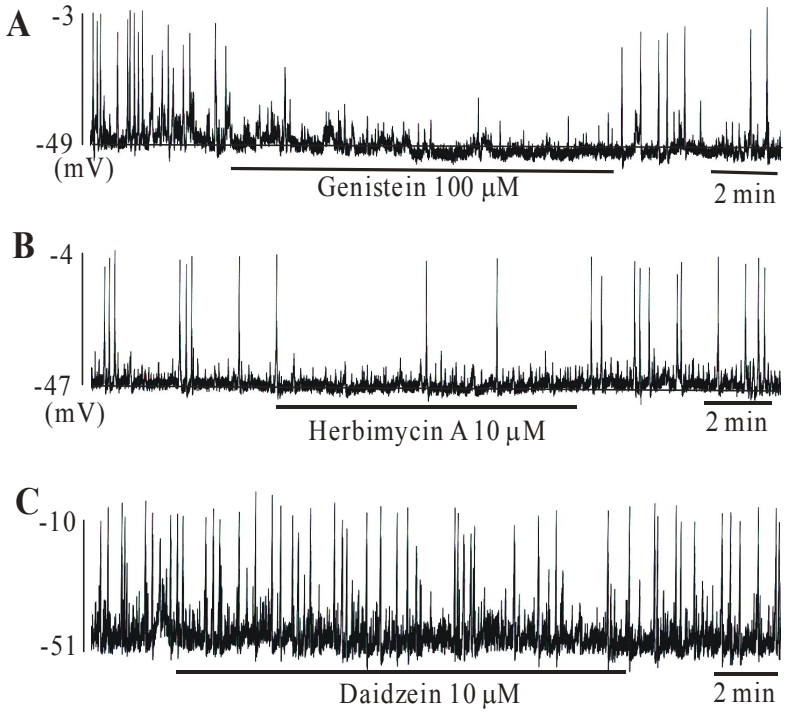

Figure 3. Effects of tyrosine kinase inhibitors on the spontaneous pacemaker potentials in colonic ICCs. Under current clamping mode, addition of 100 μM genistein (A) and 10 μM herbimycin A (B) decreased the frequency of the pacemaker potentials. However, 10 μM daidzein, an inactive form of genistein, had no influence on the pacemaker potentials (C).

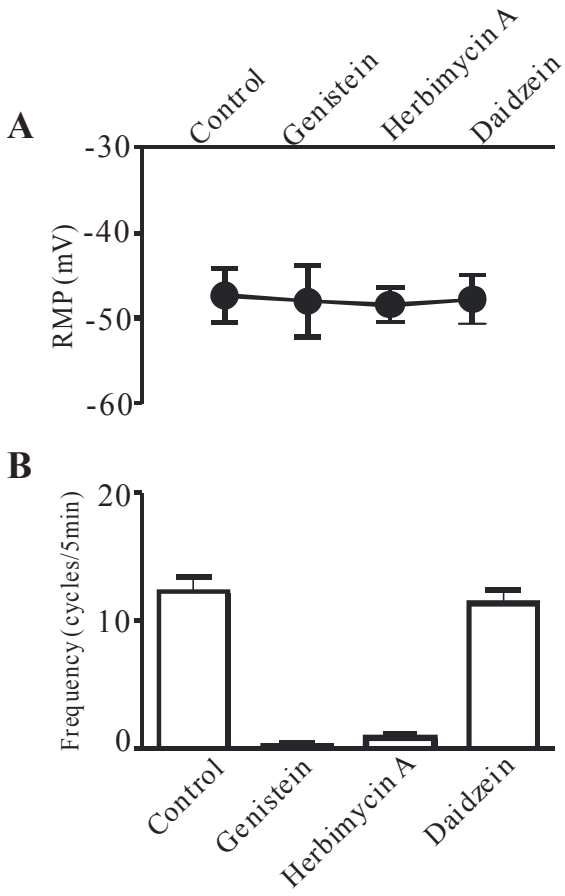

Figure 4. Summarized data of the tyrosine kinase inhibitors on pacemaker potentials in colonic ICCs. The values in the resting membrane potential by 100 μM genistein, 10 μM herbimycin A, and 10 μM daidzein are summarized in (A). (B) is the summarized data of frequency by genistein, herbimycin A, and daidzein.

Effects of MAPK inhibitors on pacemaker potentials in colonic ICCs

To investigate whether MAPK involves in generating pacemaker potentials or not, specific MAPK inhibitors were tested. When the pretreatments of ICCs with PD 98059 (a selective p42/44 inhibitor), SB 203580 (a selective p38 inhibitor) or JNK inhibitor II (a selective JNK inhibitor), the generation of spontaneous pacemaker potential was blocked and decreased the pacemaker potential frequency by SB 203580 (10 μM) and JNK inhibitor II (10 μM) but not by PD 98059 (10 μM, n=8) (Figure 5A, B and C). The values of the pacemaker potential frequency induced by SB 203580 (n=8) and JNK inhibitor II (n=9) were significantly different from control values. However, the resting membrane potential induced by SB 203580 and JNK inhibitor II was not changed (Figure 6A and B). These results suggest that endogenous p38 and JNK MAPKs involve in generating pacemaker potential in colonic ICCs.

Effects of PKA and PKG inhibitors on pacemaker potentials in colonic ICCs

To investigate whether protein kinase A (PKA) or protein kinase G (PKG) involves in generating pacemaker potentials or not, specific PKA and PKG inhibitors were tested. When the pretreatments of ICCs with KT5720 (a selective PKA inhibitor) and KT5823 (a selective PKG inhibitor), both KT5720 (10 μM) and KT5823 (10 μM) did not show any influence on spontaneous pacemaker potentials (Figure 7A and B). The values of the resting membrane potential and the pacemaker potential frequency and induced by KT5720 (n=8) and KT5823 (n=9) were not significantly different from control values (Figure 8A and B).

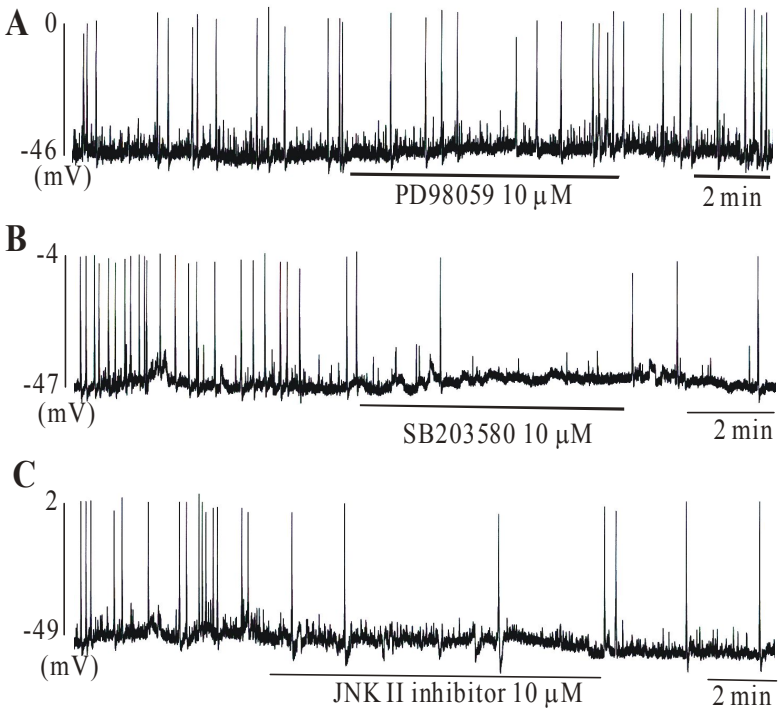

Figure 5. Effects of MAPK inhibitors on the spontaneous pacemaker potentials in colonic ICCs. Under current clamping mode, addition of 10 μM PD98059 (A) had no influence on the pacemaker potentials. However, addition of 10 μM SB203580 (B) and 10 μM JNK inhibitor II (C) decreased the frequency of the pacemaker potentials.

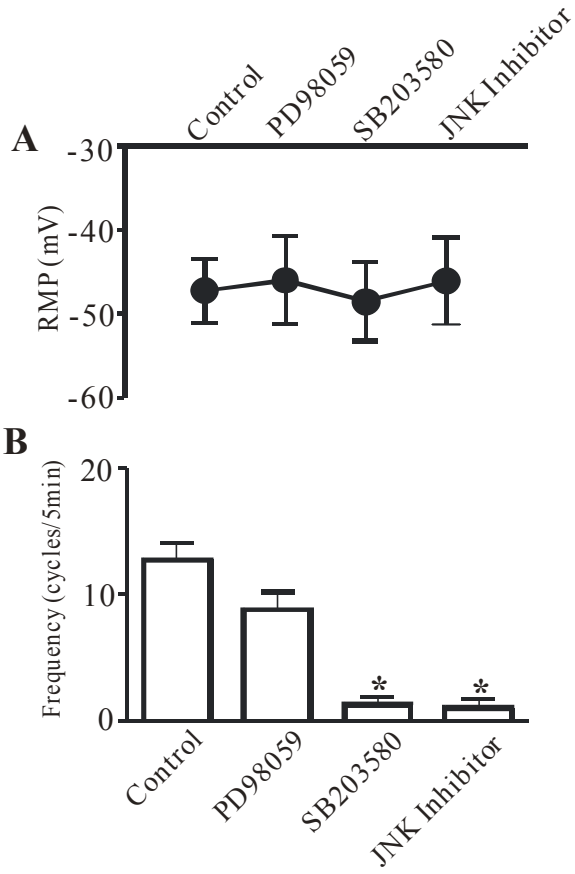

Figure 6. Summarized data of the MAPK kinase inhibitors on pacemaker potentials in colonic ICCs. The values in the resting membrane potential by 10 μM PD98059 (A), 10 μM SB203580 (B), and 10 μM JNK inhibitor II are summarized in (A). (B) is the summarized data of frequency by PD98059, SB203580, and JNK inhibitor II.

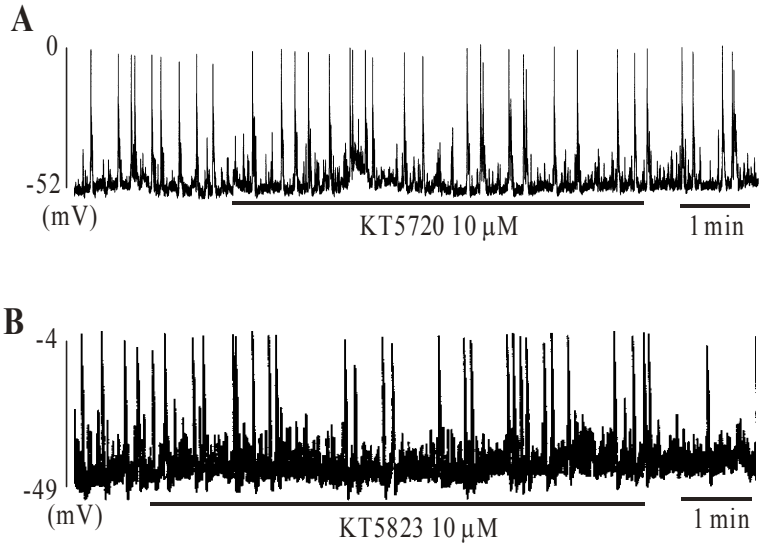

Figure 7. Effects of PKA and PKG inhibitors on the spontaneous pacemaker potentials in colonic ICCs. Under current clamping mode, addition of 10 μM KT5720 (A) and 10 μM KT5823 (B) had no influence on the pacemaker potentials.

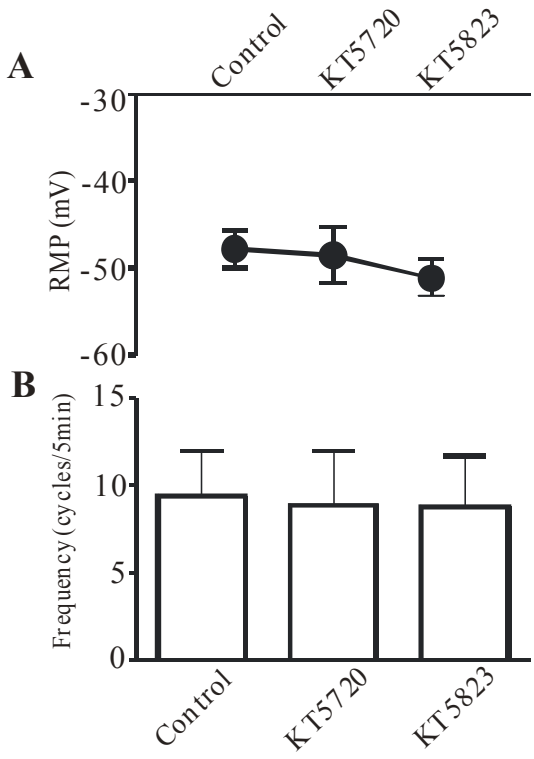

Figure 8. Summarized data of the PKA and PKG inhibitors on pacemaker potentials in colonic ICCs. The values in the resting membrane potential by 10 μM KT5720 and 10 μM KT5823 (B) are summarized in (A). (B) is the summarized data of frequency by KT5720 and KT5823.

Effects of PKC inhibitors on pacemaker potentials in colonic ICCs

To investigate whether protein kinase C (PKC) involves in generating pacemaker potentials or not, specific PKC inhibitors were tested. When the pretreatments of ICCs with chelerythrine and bisindolylmaleimide (PKC inhibitors), both chelerythrine (1 μM) and bisindolylmaleimide (1 μM) did not show any influence on spontaneous pacemaker potentials (Figure 9A and B). The values of the resting membrane potential and the pacemaker potential frequency and induced by chelerythrine (n=7) and bisindolylmaleimide (n=6) were not significantly different from control values (Figure 10A and B).

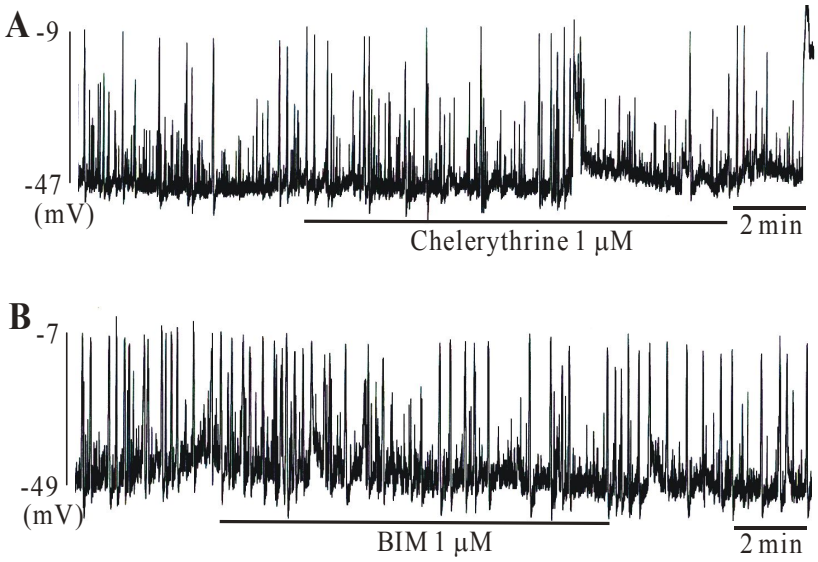

Figure 9. Effects of PKC inhibitors on the spontaneous pacemaker potentials in colonic ICCs. Under current clamping mode, addition of 1 μM chelerythrine (A) and 1 μM bisindolylmaleimide (B) had no influence on the pacemaker potentials. BIM: bisindolylmaleimide.

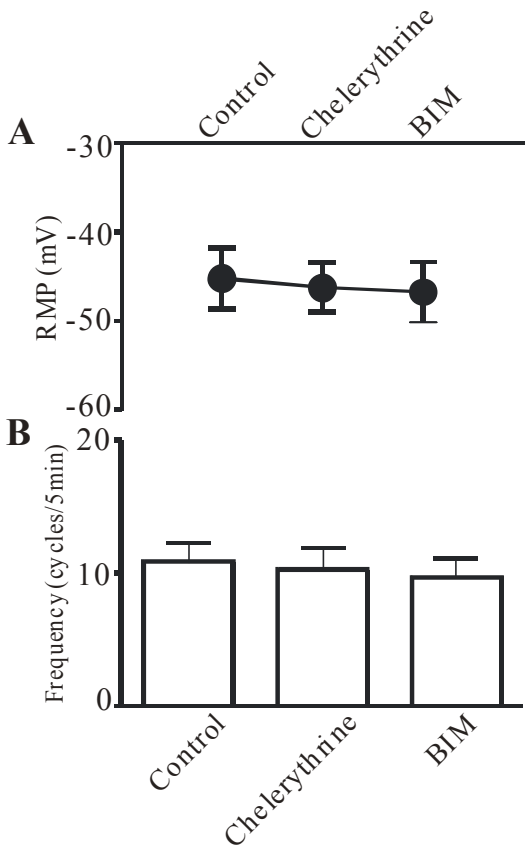

Figure 10. Summarized data of the PKC inhibitors on pacemaker potentials in colonic ICCs. The values in the resting membrane potential by 1 μM chelerythrine and 1 μM bisindolylmaleimide (B) are summarized in (A). (B) is the summarized data of frequency by chelerythrine and bisindolylmaleimide. BIM: bisindolylmaleimide.

Effects of tyrosine kinase inhibitors in small intestinal ICCs

To investigate whether endogenous tyrosine kinase also involves in generating pacemaker potentials of small intestinal ICCs, genistein and herbimycin A were treated. However, in contrast with colonic ICCs, genistein (100 μM, n=6) and herbimycin A (10 μM, n=7) had no effects on spontaneous pacemaker potentials of small intestinal ICCs (Figure 11 A and B).

Effects of MAPK inhibitors on pacemaker potentials in small intestinal ICCs

To investigate whether endogenous MAPK also involves in generating pacemaker potentials of small intestinal ICCs, PD 98059 (10 μM), SB 203580 (10 μM) and JNK inhibitor II (10 μM) were treated. However, all drugs had no effects on spontaneous pacemaker potentials of small intestinal ICCs (Figure 12A, B and C). These results suggest that endogenous MAPK do not involve in generating pacemaker potentials in small intestinal ICCs.

Effects of PKA, PKG and PKC inhibitors on pacemaker potentials in small intestinal ICCs

To investigate whether PKA or PKG or PKC involves in generating pacemaker potentials of small intestinal ICCs, KT 5720 (10 μM, n=7), KT 5823 (10 μM, n=6), chelerythrine (1 μM, n=6) and bisindolylmaleimide (1 μM, n=7) were treated. However, all drugs also had no effects on spontaneous pacemaker potentials of small intestinal ICCs (Figure 13A, B, C and D).

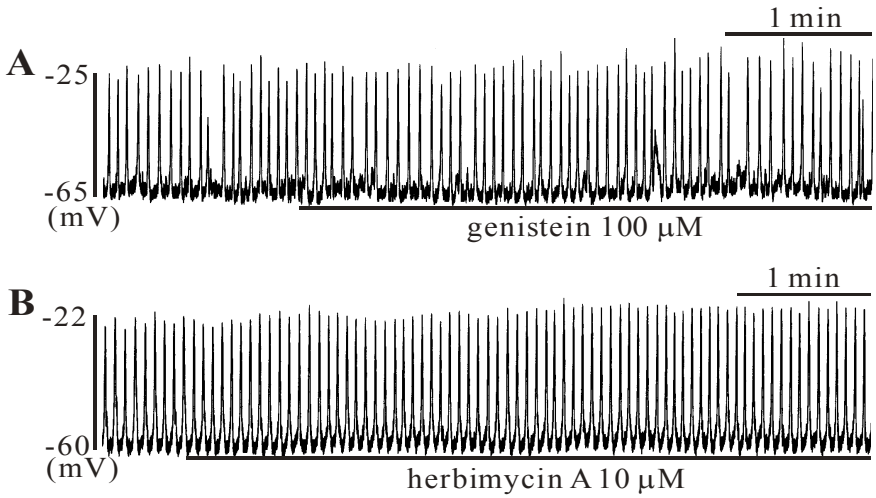

Figure 11. Effects of tyrosine kinase inhibitors on the spontaneous pacemaker potentials in small intestinal ICCs. Under current clamping mode, addition of 100 μM genistein (A) and 10 μM herbimycin A (B) as well as 10 μM daidzein had no influence on the pacemaker potentials.

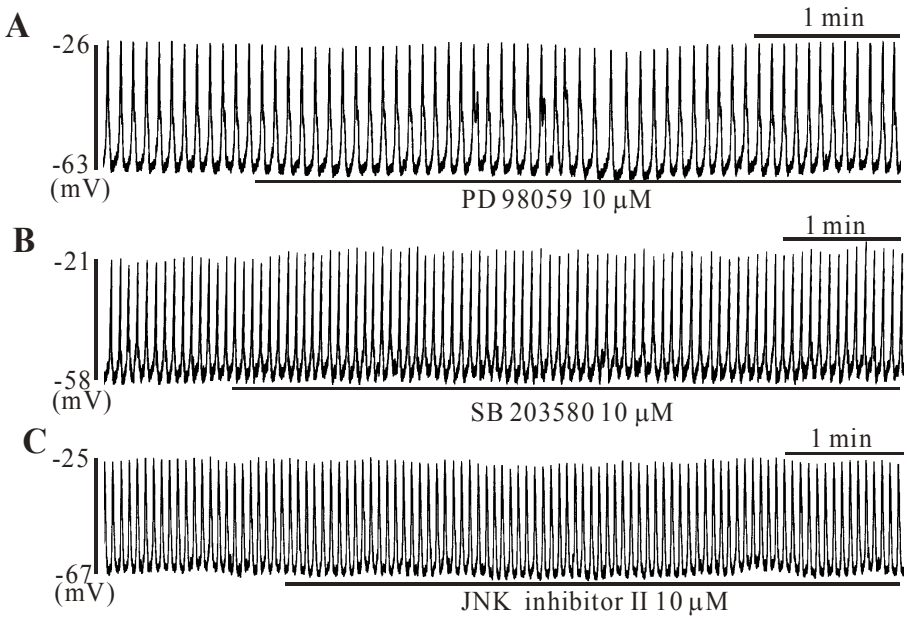

Figure 12. Effects of MAPK inhibitors on the spontaneous pacemaker potentials in small intestinal ICC. Under current clamping mode, addition of 10 μM PD98059 (A), 10 μM SB203580 (B), and 10 μM JNK inhibitor II (C) had no influence on the pacemaker potentials.

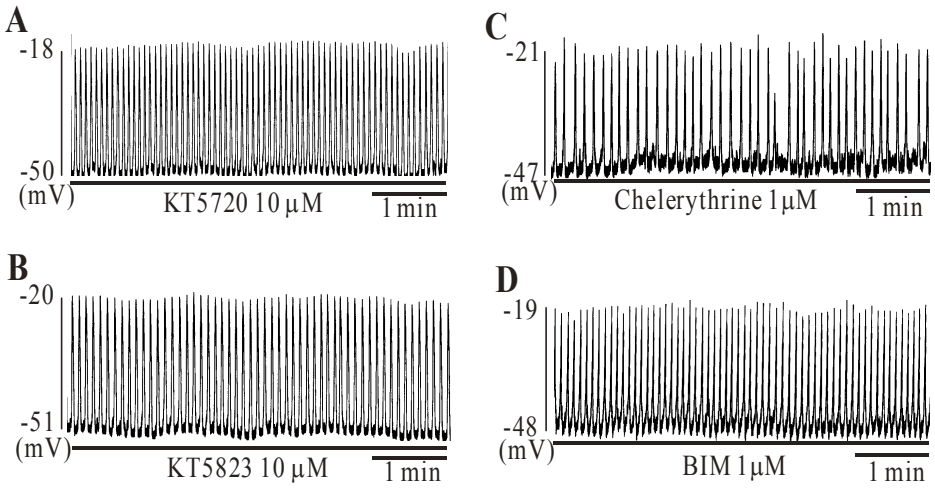

Figure 13. Effects of PKA, PKG, and PKC inhibitors on the spontaneous pacemaker potentials in colonic ICCs. Under current clamping mode, addition of 10 μM KT5720 (A), 10 μM KT5823 (B), 1 μM chelerythrine and 1 μM bisindolylmaleimide had no influence on the pacemaker potentials. BIM: bisindolylmaleimide.

DISCUSSION

The peristaltic GI motility is done by smooth muscle contraction and relaxation. The spontaneous contractions of GI smooth muscle are coupled to periodic activation of pacemaker channels in interstitial cells of Cajal (ICCs). In the present study, I found that endogenous MAPK and tyrosine kinase participate in generating pacemaker potentials in colonic ICCs but not in small intestinal ICCs.

MAPKs and tyrosine kinases are important modulators of GI smooth muscle contractions. Muscarinic activation was coupled to Src tyrosine kinase and subsequent activation of p42/p44 MAPK in cultured canine colonic smooth muscle cells[30]. Bombesin contracted cat esophageal circular smooth muscle cells via tyrosine kinase pathway or p42/p44 MAPK pathway[31]. Both p42/p44 and p38 MAPK pathways contribute to hypercontractility in experimental colitis[16]. LPS influence intestinal contractility via JNK MAPK pathway[32]. In small intestinal ICCs, H_2O_2 inhibited the generation of pacemaker activity by activating ATP-sensitive K^+ channels through p38 MAPK, JNK MAPK, and tyrosine kinase and sphingosine-1-phospate depolarized the pacemaker potentials through JNK MAPK-dependent mechanism[33, 34]. These results suggested

that MAPK- and tyrosine kinase pathways are modulate the pacemaker activity of ICCs indirectly. The inhibitory mediation of JNK MAPK by H_2O_2 and the excitatory mediation of JNK MAPK by sphingosin-1-phosphate are suggesting that JNK MAPK mediate cellular actions acting on cellular target indirectly rather than direct modulation of cellular target. In this study, PD 98058 did not influence on pacemaker potentials of colonic ICCs, but SB 20356 and JNK inhibitor II suppressed the generation of pacemaker potentials and decreased the pacemaker potential frequency in colonic ICCs. In addition, pacemaker potentials were suppressed by genistein and herbimycin A but not by daidzein in colonic ICCs. However, tyrosine kinase inhibitors and MAPK inhibitors did not affect the generation of pacemaker potentials in small intestinal ICCs. Based on these results mean that endogenous p38 MAPK, JNK MAPK, and Src-family tyrosine kinase are participating in generation of pacemaking potentials in colonic ICCs. Whereas MAPKs and tyrosine kinase are working when external agents are acting only in small intestinal ICCs. However, from this study, I could not know the cause of different effects of tyrosine kinase inhibitor and MAPK inhibitors on pacemaker potentials between colonic ICCs and small intestinal ICCs. Even though, I think that the one possible cause may be the different

generating mechanism of pacemaker potentials between colonic ICCs and small intestinal ICCs. The pacemaking mechanism of ICCs is closely associated with intracellular Ca^{2+} oscillations. IP_3-dependent Ca^{2+} release from endoplasmic reticulum and reuptake Ca^{2+} into mitochondria is linked to activate pacemaker channels[35]. Until now transient receptor potential channels and Ca^{2+}-activated Cl^- channels have been candidate as pacemaker channels in ICCs[25, 26]. However, recently, it was reported that hyperpolarization activated cyclic nucleotide-gated (HCN) channels participated in generating spontaneous pacemaker potential in colonic ICCs but not small intestinal ICCs from mouse[29]. HCN channels are non-selective cation channels that maintain or determine the cell excitability in spontaneous active cells like as neuronal cells and cardiac cells[36, 37]. HCN channels activated by membrane hyperpolarization or directly by intracellular cAMP. Many intracellular molecules and protein kinases modulate HCN channel activities[38, 39]. It has been reported that p38-MAPK modulate HCN channels via the direct phosphorylation of channel proteins or the indirect alteration of intracellular second messengers, such as membrane acidic lipids, cAMP and proton or transactivation of other protein kinases[40, 41]. Also, genistein suppressed HCN channels, thus it was suggested that tyrosine kinase might modulate

HCN channels through the direct phosphorylation of channel proteins[42]. Therefore, I think that modulation of HCN channels by endogenous actions of MAPKs and tyrosine kinases in colonic ICCs may possible different mechanism between colon and small intestine. However, further studies are required to clarify the relationship of MAPKs, tyrosine kinases and HCN channels. cAMP and cGMP are second messengers that act as inhibitory mediators in GI tract. Intracellular cAMP and cGMP activate PKA and PKG, respectively and followed cellular actions. Also, PKC is activated by diacylglycerol that synthesized by phospholipase C[11]. Besides, PKA, PKG, and PKC had been expressed in ICCs[43-45], suggesting that these protein kinases can modulate pacemaker activity of ICCs. However, in this study, KT 5766 and KT 5788, inhibitors of PKA and PKG, as well as chelerythrine and BIM, inhibitors of PKC, had no effects on pacemaker potentials in colonic and small intestinal ICCs. These results suggest that PKA, PKG, and PKC are not involved in generating pacemaker potential in ICCs.

In conclusion, endogenous tyrosine kinases and p38 and JNK MAPKs participate in the generation of pacemaker potentials in colonic ICCs but not small intestinal ICCs. The target of MAPK and tyrosine kinase is therapeutic strategy for treatment of colonic motility disorders.

REFERENCES

1. Szurszewski JH. Electrical basis for gastrointestinal motility. In: Physiology of the Gastrointestinal Tract, 2nd edn, ed. Johnson LR, 1987, pp. 383-422. Raven Press, New York.
2. Thomsen L, Robinson TL, Lee JC, Farraway LA, Hughes MJ, Andrews DW, Huizinga JD. Interstitial cells of Cajal generate a rhythmic pacemaker current. Nature Med. 1998;4:848-51.
3. Sanders KM, Ordög T, Koh SD, Torihashi S, Ward SM. Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil. 1999;11:311-38.
4. Hanani M, Freund HR.Interstitial cells of Cajal--their role in pacing and signal transmission in the digestive system. Acta Physiol Scand. 2000;170:177-90.
5. Sanders KM, Koh SD, Ward SM. Interstitial cells of cajal as pacemakers in the gastrointestinal tract. Annu Rev Physiol. 2006;68:307-43.
6. Iino S, Horiguchi K. Interstitial cells of cajal are involved in neurotransmission in the gastrointestinal tract. Acta Histochem

Cytochem. 2006;39:145-53.

7. Ward SM, Sanders KM. Involvement of intramuscular interstitial cells of Cajal in neuroeffector transmission in the gastrointestinal tract. J Physiol. 2006;576:675-82
8. Won KJ, Sanders KM, Ward SM. Interstitial cells of Cajal mediate mechanosensitive responses in the stomach. Proc Natl Acad Sci U S A. 2005;102:14913-8.
9. Jain D, Khalid M, Manish T, Joan CM, Deborah DP. Role of interstitial cells of Cajal in motility disorders of the bowel. Am J Gastroenterol. 2003;98:618-24.
10. Farrugia G. Interstitial cells of Cajal in health and disease. Neurogastroenterol Motil. 2008;20:54-63.
11. Sanders KM. G protein-coupled receptors in gastrointestinal physiology IV. Neural regulation of gastrointestinal smooth muscle. Am J Physiol. 1998;275:G1-G7.
12. Puri RN, Fan YP, Rattan S. Role of pp60(c-src) and p(44/42) MAPK in ANG II-induced contraction of rat tonic gastrointestinal smooth muscles. Am J Physiol Gastrointest Liver Physiol. 2002;283:G390-9.
13. Cao W, Sohn UD, Bitar KN, Behar J, Biancani P, Harnett KM.

MAPK mediates PKC-dependent contraction of cat esophageal and lower esophageal sphincter circular smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2003;285:G86-95.

14. Xiao ZL, Rho AK, Biancani P, Behar J. Effects of bile acids on the muscle functions of guinea pig gallbladder. Am J Physiol Gastrointest Liver Physiol. 2002;283:G87-94.
15. Ihara E, Moffat L, Ostrander J, Walsh MP, MacDonald JA. Characterization of protein kinase pathways responsible for Ca^{2+} sensitization in rat ileal longitudinal smooth muscle. Am J Physiol Gastrointest Liver Physiol. 2007;293:G699-710.
16. Ihara E, Beck PL, Chappellaz M, Wong J, Medlicott SA, MacDonald JA. Mitogen-activated protein kinase pathways contribute to hypercontractility and increased Ca^{2+} sensitization in murine experimental colitis. Mol Pharmacol. 2009;75:1031-41.
17. Schlessinger J, Ullrich A. Growth factor signalling by receptor tyrosine kinases. Neuron. 1992;9:383–91.
18. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol. 1997; 13:513–609.
19. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase

pathways: regulation and physiological functions. Endocr Rev. 2001 Apr;22(2):153-83.

20. Wan Y, Kurosaki T, Huang XY. Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. Nature. 1996;380:541-4.
21. Barona I, Fagundes DS, Gonzalo S, Grasa L, Arruebo MP, Plaza MÁ, Murillo MD. Role of TLR4 and MAPK in the local effect of LPS on intestinal contractility. J Pharm Pharmacol. 2011;63:657-62.
22. Korhonen R, Moilanen E. Mitogen-activated protein kinase phosphatase 1 as an inflammatory factor and drug target. Basic Clin Pharmacol Toxicol. 2014;114:24-36.
23. Sakai H, Watanabe Y, Honda M, Tsuiki R, Ueda Y, Nagai Y, Narita M, Misawa M, Chiba Y. Involvement of the Tyr kinase/JNK pathway in carbachol-induced bronchial smooth muscle contraction in the rat. Anesthesiology. 2013;118:1076-85.
24. Ihara E, Akiho H, Nakamura K, Turner SR, Macdonald JA. MAPKs represent novel therapeutic targets for gastrointestinal motility disorders. World J Gastrointest Pathophysiol. 2011;2:19-25.

25. Kim BJ, Lim HH, Yang DK, Jun JY, Chang IY, Park CS, So I, Stanfield PR, Kim KW. Melastatin-type transient receptor potential channel 7 is required for intestinal pacemaking activity. Gastroenterology. 2005;129:1504-17.

26. Zhu MH, Kim TW, Ro S, Yan W, Ward SM, Koh SD, Sanders KM. A Ca^{2+}-activated Cl^- conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. J Physiol. 2009;587:4905-18.

27. Crépel V, Panenka W, Kelly ME, MacVicar BA. Mitogen-activated protein and tyrosine kinases in the activation of astrocyte volume-activated chloride current. J Neurosci. 1998;18:1196-206.

28. Auger R, Motta I, Benihoud K, Ojcius DM, Kanellopoulos JM. A role for mitogen-activated protein kinase(Erk1/2) activation and non-selective pore formation in P2X7 receptor-mediated thymocyte death. J Biol Chem. 2005;280:28142-51.

29. Shahi PK, Choi S, Zuo DC, Kim MY, Park CG, Kim YD, Lee J, Park KJ, So I, Jun JY. The possible roles of hyperpolarization-activated cyclic nucleotide channels in regulating pacemaker activity in colonic interstitial cells of Cajal. J Gastroenterol.

2014;49:1001-10.

30. Singer CA, Vang S, Gerthoffer WT. Coupling of M_2 muscarinic receptors to Src activation in cultured canine colonic smooth muscle cells. Am J Physiol Gastrointest Liver Physiol. 2002;282:G61-8.
31. Park SU, Shin CY, Ryu JS, La HO, Park SY, Song HJ, Min YS, Kim DS, Sohn UD. Signal transduction of bombesin-induced circular smooth muscle cell contraction in cat esophagus. World J Gastroenterol. 2006;12:2259-63.
32. Barona I, Fagundes DS, Gonzalo S, Grasa L, Arruebo MP, Plaza MÁ, Murillo MD. Role of TLR4 and MAPK in the local effect of LPS on intestinal contractility. J Pharm Pharmacol. 2011;63:657-62.
33. Choi S, Yeum CH, Kim YD, Park CG, Kim MY, Park JS, Jeong HS, Kim BJ, So I, Kim KW. Receptor tyrosine and MAP kinase are involved in effects of H_2O_2 on interstitial cells of Cajal in murine intestine. J Cell Mol Med. 2010;14:257-66.
34. Kim YD, Han KT, Lee J, Park CG, Kim MY, Shahi PK, Zuo DC, Choi S, Jun JY. Effects of sphingosine-1-phosphate on pacemaker activity of interstitial cells of Cajal from mouse small intestine.

Mol Cells. 2013;35:79-86.

35. Ward SM, Ordog T, Koh SD, Baker SA, Jun JY, Amberg G, Monaghan K, Sanders KM. Pacemaking in interstitial cells of Cajal depends upon calcium handling by endoplasmic reticulum and mitochondria. J Physiol. 2000;525:355-61.
36. DiFrancesco D. The contribution of the 'pacemaker' current (if) to generation of spontaneous activity in rabbit sino-atrial node myocytes. J Physiol. 1991; 434:23-40.
37. Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu Rev Physiol. 1996; 58:299-327.
38. Baruscotti M, Bucchi A, Difrancesco D. Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacol Ther. 2005; 107:59-79.
39. Biel M, Wahl-Schott C, Michalakis S, Zong X. Hyperpolarization-activated cation channels: from genes to function. Physiol Rev. 2009; 89:847-85.
40. Fogle KJ, Lyashchenko AK, Turbendian HK, Tibbs GR. HCN pacemaker channel activation is controlled by acidic lipids downstream of diacylglycerol kinase and phospholipase A2. J

Neurosci. 2007;27:2802-14.

41. He C, Chen F, Li B, Hu Z. Neurophysiology of HCN channels: from cellular functions to multiple regulations. Prog Neurobiol. 2014;112:1-23.
42. Zong X, Eckert C, Yuan H, Wahl-Schott C, Abicht H, Fang L, Li R, Mistrik P, Gerstner A, Much B, Baumann L, Michalakis S, Zeng R, Chen Z, Biel M. A novel mechanism of modulation of hyperpolarization-activated cyclic nucleotide-gated channels by Src kinase. J Biol Chem. 2005;280:34224-32.
43. Poole DP, Van Nguyen T, Kawai M, Furness JB. Protein kinases expressed by interstitial cells of Cajal. Poole DP, Van Nguyen T, Kawai M, Furness JB. Histochem Cell Biol. 2004;121:21-30.
44. Iino S, Horiguchi K, Nojyo Y, Ward SM, Sanders KM. Interstitial cells of Cajal contain signalling molecules for transduction of nitrergic stimulation in guinea pig caecum. Neurogastroenterol Motil. 2009;21:542-50.
45. Furness JB, Hind AJ, Ngui K, Robbins HL, Clerc N, Merrot T, Tjandra JJ, Poole DP. The distribution of PKC isoforms in enteric neurons, muscle and interstitial cells of the human intestine. Histochem Cell Biol. 2006;126:537-48.