August 2021

Master's Degree Thesis

Dual modulation of intracellular Ca^{2+} oscillation by cyclic nucleotides in interstitial cells of Cajal from mouse colonn

Graduate School of Chosun University

Department of Medicine

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마우스 대장의 카할 사이질세포에서
뉴클레오타이드에 의한 세포내
칼슘변화 조절

27th August 2021

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A thesis submitted to the Graduate School of Chosun University in partial fulfillment of the requirements of the degree of Master of Medicine

April 2021

Graduate School of Chosun University

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The thesis prepared by

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in partial fulfillment of the requirements for the degree of

MASTER OF MEDICINE

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May 2021

Graduate School of Chosun University

CONTENTS

LIST OF TABLE

LIST OF FIGURES

ABBREVIATIONS

SMP	Submuscular plexus
SMC	Smooth muscle cells
SCF	Stem cell factor
NO	Nitric oxide
K_{ATP}	ATP sensitive potassium channel
IP_3	Inositol 1,4,5-trisphosphate
IM	Intramuscular
ICC	Interstitial cells of Cajal
HCN	Hyperpolarization-activated cyclic nucleotide-gated
GI	Gastrointestinal tract
ER	Endoplasmic reticulum
DMP	Deep muscular plexus
cGMP	3',5'-cyclic guanosine monophosphate
cAMP	3',5'-cyclic adenosine monophosphate
Ca^{2+}	Calcium
$[Ca^{2+}]_i$	Intracellular calcium

국 문 초록

마우스 대장의 카할사이질세포에서 뉴클레오타이드에 의한 세포내 칼슘변화 조절

Zhang Jing Wei

지도교수: 전제열

조선대학교 대학원 의과학과

카할 사이질 세포는 위장관 평활근에서 발생되는 서파 (slow waves)를 생산하는 향도잡이 활동도를 가진 특수 세포이다. 더불어 cAMP 또는 cGMP는 세포내 중요한 이차신호전달계로 다양한 기능을 수행한다. 기존 연구에서 cAMP 또는 cGMP에 대한 카할사이질세포의 향도잡이 기능에 연구는 수행되어 왔지만 아직까지 향도잡이 발생에 가장 중요한 시작인 세포내 칼슘에 대한 연구는 아직 미비한 실정이다. 따라서 본 연구자는 세포내 칼슘 염색약을 이용해 cAMP 또는 cGMP의 세포내 칼슘 역할을 확인하였고 더불어 이와 관련이 깊은 이온통로에 대한 효과를 확인해 보았다.

연구 결과 도출을 위해 본 연구자는 세포내 칼슘 분석 방법을 실시하였다.

정상상태에서 카할사이질세포에 칼슘 염색약인 fluo-4AM을 처리시 비디오 상에서 자발적인 규칙적인 칼슘 농도 변화를 보여주었다. 8-bromo cAMP 그리고 rolipram 처리시 자발적인 칼슘 농도 변화의 빈도가 증가되었고 adenylyl cyclase 억제제인 SQ22536과 dedeoxyadenosine은 규칙적인 칼슘 농도 변화를 억제하였다. 다음 실험으로 8-bromo cGMP와 nitric oxide donor인 SNAP은 칼슘 농도 변화의 빈도를 강하게 억제하였고 zapranast 역시 억제하였다. 더불어 guanylyl cyclase 억제제인 ODQ는 칼슘 농도 변화의 빈도를 증가시켰다.

cAMP 또는 cGMP와 관련된 그리고 카할사이질세포의 향도잡이 활동도와 관련된 이온통로에 관한 연구에서 ATP 민감성 칼륨 통로와 HCN 이온 통로가 카할사이질세포의 칼슘 농도변화의 빈도에 영향을 주는 것을 발견하였다.

본 연구는 대장 카할사이질세포의 칼슘 활동도에 대한 cAMP 또는 cGMP의 효과

를 확인하였다. 카할사이질세포의 향도잡이 활동도에 대한 효과와 유사한 결과를 보여 주었고 ATP 민감성 칼륨 통로와 HCN 이온 통로가 카할사이질세포의 칼슘 농도 변화를 효과를 보여주었다. 이러한 결과는 카할사이질세포의 세포내 칼슘의 중요성과 더불어 향도잡이 활동도에 대한 다양한 이온통로가 세포내 칼슘 조절을 통해 영향을 줄 수 있다는 점을 시사한다.

1. INTRODUCTION

1.1. Gastrointestinal Tract

The gastrointestinal tract (GI) is a long muscular canal that runs from the mouth to the anus, divided into several functional compartments. According to the anatomical order, it is generally divided into esophagus, stomach, intestine and rectum from top to bottom (Fig. 1). The GI tract absorbs nutrients and water into our body with elimination of waste products. For this, GI tract has four physiological process : digestion, secretion, absorption and motility.

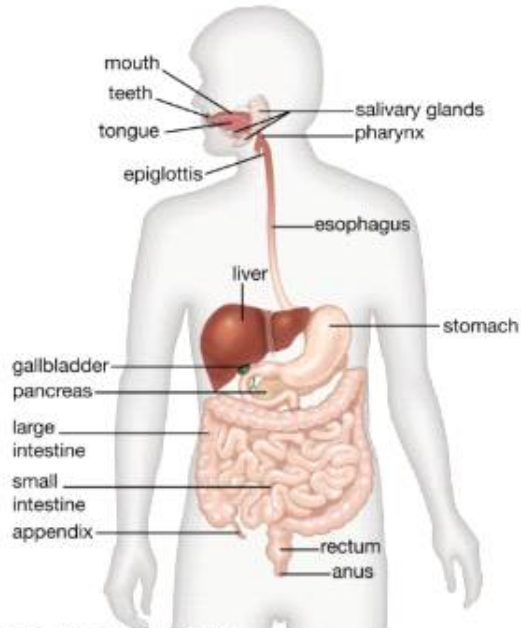

Fig. 1 The human digestive system

1.2. Motility in GI tract

GI motility is defined by the movements of the digestive system, and the transit of the contents within it. When nerves or muscles in any portion of the digestive tract do not function with their normal strength and coordination, a person develops symptoms related to motility problems.

1.3. Histology of the GI tract

Histology of the gastrointestinal tract helps us understand the underlying mechanisms responsible for GI motility. Although there may be some histophisological variations, the general histology of the GI tract consists of the following layers (innermost to outermost)(Fig. 2)

a. The mucosa: it provides a surface for absorption and acts as a barrier to noxious substances via epithelial cells.
b. The submucosa: the submucosal neural plexus, blood vessels, lymphatics, and a thin smooth muscle are all found in this supportive connective tissue layer.
c. The muscularis externa: the mynteric neural plexus is sandwiched between the circular and longitudinal smooth muscle layers in the muscularis externa, which provides muscular contraction
d. The serosa: a protective layer of mesothelial cells covers the outside of the tube.

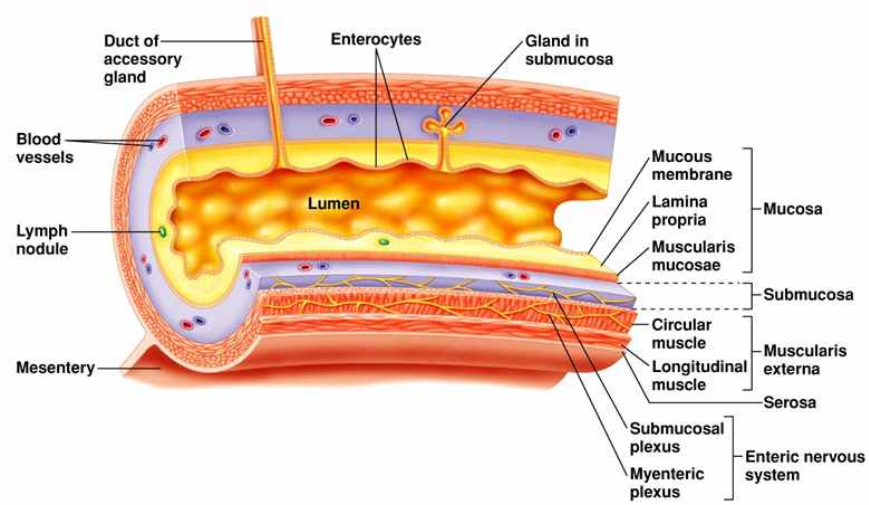

Fig. 2. Different Layers of Cells in the GI Tract

(Benjamin Cummings, 2008)

1.4. Smooth Muscle Cell and Slow Wave

The contractions of smooth muscle in GI tract cause motility. SMC (smooth muscle cell) shows spontaneous and rhythmic depolarizations which was termed slow wave (Fig. 3). Slow wave is a intrinsic property of smooth muscle but do not elicit smooth muscle contractions by themselves. When the membrane potential of slow wave passes over the Ca^{2+} channel open threshold, voltage-dependent Ca^{2+} channels activated, resulting in Ca^{2+} enter the smooth muscle cells and triggers smooth a muscle cells to contract. The generally accepted mechanism for slow wave generation based on the key point that peacemaking activity was generated in ICC （Interstitial Cells of Cajal） (Thuneberg, 1982; Huizinga et al., 1995).

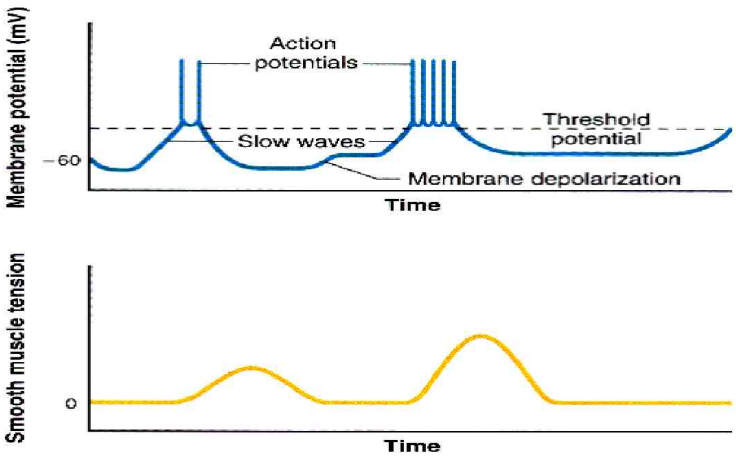

Fig.3. In the gastrointestinal tract, slow wave potential is a rhythmic electrophysiological event.

1.5. Interstitial Cells of Cajal (ICC)

ICC were first identified by Spanish anatomist Santiago Ramon Y Cajal (Cajal, 1911). He described a network of cells in GI tissues and proposed that these cells are nervous system accessory components that modulate smooth muscle contraction. ICC are pacemaker cells that generate slow waves in GI smooth muscle, and are thereby implicated in regulating smooth muscle activity (Ward et al., 1994). ICC are connected to each other and smooth muscles through gap junctions, and form networks within the GI tract. Therefore, spontaneous pacemaker potentials produced by ICC are directly transmitted to smooth muscle and recorded as slow waves, and are followed by spontaneous contractions (Hanani et al., 2005).

1.6. Importance of Intracellular Calcium for Pacemaker Activity in ICC

Recent studies have suggested that pacemaker activity depends on a link between Ca^{2+} release from cellular stores, oxidative metabolism, and the pacemaker conductance in the plasma membrane (Ward et al., 2000). Especially, I noted the inositol 1,4,5-triphosphate receptor plays a role in generating spontaneous electrical activity in GI pacemaker cells and previous suggestion that periodic Ca^{2+} release from intracellular Ca^{2+}($[Ca^{2+}]_i$) stores produces $[Ca^{2+}]_i$ oscillations in ICC, using cell cluster preparations isolated from mouse ileum and these actions seen in ICC are considered to be the primary pacemaker activity in GI tract (Aoyama et al., 2004).

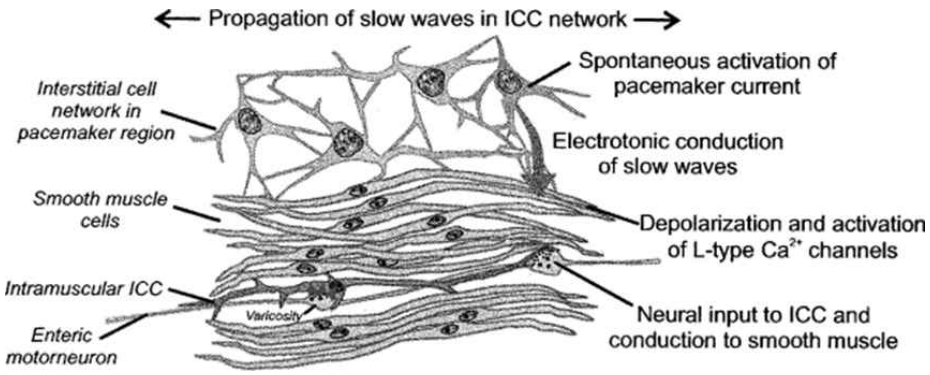

Fig.4. Slow Waves Generated by ICC. (Neurogastroenterology & Motility, 2002)

1.7. The Involvement of ATP-sensitive K^+ Channel and Hyperpolarizing Cyclic Nucleotide-gate Channel on Pacemaker Activity in ICC

ATP-senstivie K^+ (K_{ATP}) channel stabilize the resting membrane potential, and are basally activated in mouse colonic smooth muscle cells (Koh et al., 1998). Inhibition of these channels depolarizes the membrane potential and increase cell excitability. However, activation of these channels hyperpolarizes the membrane potential and decrease cell excitability. Recent report have found that Pinicidil, an K_{ATP} channel opener, hyperpolarize the membrane potential and reduce the frequency and amplitude of pacemaker currents in cultured mouse intestinal ICC (Jun et al., 2005). These effects were blocked by treatment with glibenclamide, an K_{ATP} channel blocker, which suggests the existence of K_{ATP} channel in ICC (Jun et al., 2004).
Hyperpolarizing cyclic nucleotide-gated (HCN) channel is a class of non-selective cation channel that is activated by hyperpolarization of membrane potential and can bind intracellular cAMP directly. This channel was found in variety of spontaneously active cells such as cardiocytes and act as pacemaker channel to regulate cell excitability (DiFrancesco, 1991). Recently, it has been suggested that HCN may be a pacemaker channel in the mouse colon (Shahi et al., 2014). The generation of pacemaker potentials was abolished by adenylate cyclase inhibitors in colonic ICC and HCN channel inhibitors blocked the pacemaker potentials.

1.8. Purpose of This Study

The pacemaker is activated by the release of $[Ca^{2+}]_i$ from the endoplasmic reticulum in ICC. Although many reports have suggested the action of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) on pacemaker activity in ICC and there are some reports that the influence of K_{ATP} channel or HCN channel on pacemaker activity in ICC, there is no study to $[Ca^{2+}]_i$ for these. To understand this, I tried the experiment to find out how cyclic nucleotides modulate Ca^{2+} oscillations directionally in mouse colon cells in ICC.

2. MATERIALS AND METHODS

2.1. Ethical Approval

ICR mice (4–7days old) male and female used for these studies that had a standard mouse milk diet until the day of experimentation. Diethyl ether was used to anesthetize the mice, and they were sacrificed by cervical dislocation. All animals were treated ethically in accordance with the approved guiding principles for animal care and use in the field of physiological sciences approved by the Institutional Animal Use and Care Committee at Chosun University College of Medicine.

2.2. Tissue Isolation and ICC Culture

The animals were ether-anesthetized and cervical dislocated to sacrifice. An abdominal incision was made immediately after the cervical dislocation, and the entire colon was removed and placed in an autoclaved Ca^{2+} free solution. The colon was uncoil using microdissection. The luminal contents were washed away with a Ca^{2+}-free solution after they were opened along the mesenteric border. The tissue mucosa was isolated and pinned to the underside of a Sylgard dish. After sharp dissection, the submucosa was peeled away and only the tunica muscularis of the colon was used. After that, the tissue was equilibrated with a Ca^{2+}-free solution. The tissues were placed in a collagenase-containing enzyme solution and incubated for 15 minutes at 37°C without stirring. Tissues were washed repeatedly (3-5 times) with Ca^{2+}-free solution after incubation in the enzyme solution. To create a cell suspension, the tissues were triturated with small-diameter blunt pipettes, and incubated at 37°C in a 95% O_2 and 5% CO_2 incubator in smooth muscle Medium 231 (Gibco, Grand Island, NY, USA) supplemented with 2% antibiotics/antimycotics and murine stem cell factor(SCF) on sterile glass coverslips coated with poly-L lysine in 35mm culture dishes. After 24 hours, the media is replaced with one that does not contain SCF, and the cells are allowed to grow for another 24 hours before the Ca^{2+} imagine is performed.

2.3. Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured using 24-30 hour cultured ICC. The media of culture dish was removed, and the cells were washed twice with the extracellular solution before being incubated at 37°C for 15 minutes. The cells were then loaded with Fluo-4/AM (Gibco, Grand Island, NY, USA) at a final concentration of 1 μM and incubated for a further 15 minutes at 37°C in the dark. Fluo-4/AM The dye was removed from the cell by washing it twice with extracellular solution before placing it in the perfusion chamber. The cells were scanned no delay with Nikon Eclipse TI-HUBC/A inverted microscope equipped with a Nikon TI-PS100W Confocal Scanner and a Hamamatsu CAMERA CONTROLLER (×200; Hamamatsu Instrument, Hamamatsu, Shizuoka, Japan). The fluorescence was excited at 488 nm, and the light emitted was measured at 515 nm. The temperature of the perfusion chamber containing the cultured ICC was kept at 30°C during the Ca^{2+} imaging scanning. The intensity variations of Ca^{2+} fluorescence emission were expressed as Fl/F0, where F0 is the intensity of the first imaging. The data were displayed on computer monitor.

Results were analyzed using the NIS-Elements BR (version 4.30.00) and GraphPad Prism software (version 2.01, GraphPad Software Inc., San Diego, CA, USA) and Coreldraw (version 9).

2.4. Solutions and Drugs

2.4.1. Ca^{2+}-free Solution (mM) :

135 NaC1, 5 KCl, 1 $MgCl_2$, 10 Glucose, 10 HEPES. Tris was used to adjust the pH to 7.4 (Sigma provides all drugs).

2.4.2. Enzymatic Solution :

1 μg/ml collagenase (Worthington Biochemical Co., Lakewood, NJ, USA), 2 μg/ml bovine serum albumin (Sigma, St. Louis, MO, USA), 2 μg/mL trypsin inhibitor (Sigma, St. Louis, MO, USA) with Ca^{2+}-free solution.

2.4.3. Extracellular Solution(mM) :

135 NaC1, 5 KCl, 1 $MgCl_2$, 10 Glucose, 10 HEPES and 1.8 $CaCl_2$. Tris was used to adjust the pH to 7.4 (Sigma provides all drugs).

2.5. Drugs and Chemicals

Summarized informations about drugs and chemicals used in this study were showed as follows:

Drug Names	Companies	Catalog No.
8-bromo-cAMP	sigma	B5386
SQ22536(SQ)	tocris	1435
2',5'-Dideoxyadenisine（DDX）	cayman	D7408
Rolipram	sigma	R6520
8-bromo-cGMP	sigma	138
(±)-S-nitroso-Nacetylpenicillamine (SNAP)	sigma	487910-M
1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one(ODQ)	sigma	O3636
Zaprinast	sigma	Z0878
Zatebradine Hydrocholoride	cayman	Z0127
Ivabradine Hydrochloride	sigma	SML0281
Pinacidil	sigma	P154
Glibanclamide (GBC)	sigma	G0639

Table. 1. Drugs and Chemicals

2.6. Statistical Analysis

Data are expressed as means±standard errors (SEM). Student's t-test was used for statistical comparison. P values < 0.05 indicated statistically significant. The n values stand for the number of cells used in Ca^{2+} imagine.

3. RESULTS

3.1. The Spontaneous of $[Ca^{2+}]_i$ Oscillations Generated from ICC in Colon

Periodic release and uptake of Ca^{2+} from endoplasmic reticulum is a primary pacemaker mechanism of ICC. To explore this, I tested Ca^{2+} analysis with fluo-4/AM under control condition. $[Ca^{2+}]_i$ oscillations were observed after identifying the colonic ICC under the microscope and setting up the ICC in a culture dish loaded with fluo-4/AM. $[Ca^{2+}]_i$ triggered spontaneous transients that manifested as either highly localized events.

3.2. Action of cAMP and Rolipram on $[Ca^{2+}]_i$ Oscillations

The action of cyclic nucleotides was investigated in $[Ca^{2+}]_i$ oscillations caused by colonic ICC. Previous research found that 8-bromo-cAMP increased pacemaker activity in the colon. First, I investigated the effect of 8-bromo-cAMP at the concentration of 100 μM on $[Ca^{2+}]_i$ oscillations of colonic ICC. The amplitude was not significantly different from the control condition, but the number of frequencies generated was significantly increased (Fig. 5A). After the treatment of 8-bromo-cAMP at the concentration of 100 μM, the frequeney changed from 11 ± 1 cycles/5min under control condition to 17 ± 1 cycles/5min.

The phosphodiesterase (PDE) enzyme is responsible for breaking the phosphodiester bond. Inhibitors of PDE4 can prolong or improve the effects of physiological processes mediated by cAMP by inhibiting PDE4. Therefore, I selected cAMP-specific phosphodiesterase (PDE4) inhibitor, Rolipram to understand this. In (Fig. 5B), I could find increase the frequency of $[Ca^{2+}]_i$ oscillations in ICC by exposure Rolipram (10 μM). The summarized data about control, 8-bromo-cAMP or Rolipram showed in (Fig. 5C) (n = 8).

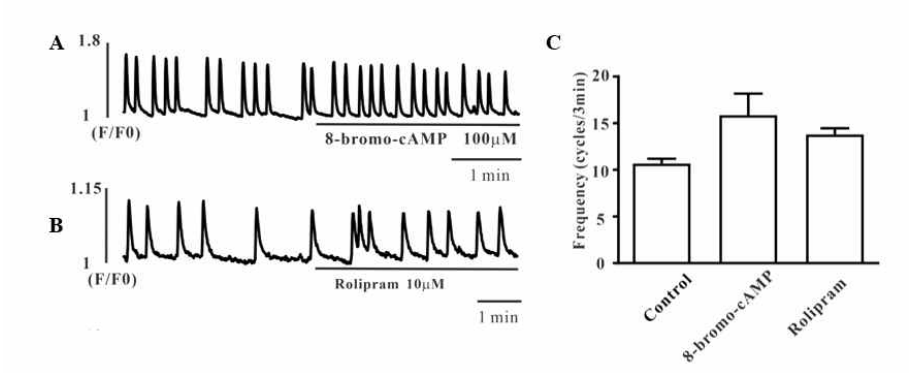

Figure 5. Effect of cyclic AMP on the intracellular Ca^{2+} oscillations. Addition of 100μM 8-bromo-cAMP inereased the frequency of the intracellular Ca^{2+} oscillations (Fig. 5A). Effect of the drugs Rolipram 10 μM to block the phosphodiesterase IV enzyme activity also increased the frequency of the intracellular Ca^{2+} oscillations(Fig. 5B). Changes in the frequency were represented in (Fig. 5C), the bar graphic representation of changes in frequency respectively after the treatment of 8-bromo-cAMP and Rolipram. After treatment with 100 μM of cell permeable analogue of cAMP and Rolipram, the Ca^{2+} wave frequency increased significantly.

3.3. Action of Adenylyl Cyclase Inhibitors on $[Ca^{2+}]_i$ Oscillations from ICC

SQ22536 or 2',5'-Dideoxyadenisine（DDX）inhibits adenylyl cyclase and may play a key role in inhition of cAMP transduction. To understand the role of cAMP on $[Ca^{2+}]_i$ oscillations in ICC, I treated these. The $[Ca^{2+}]_i$ oscillations significantly decreased after treating SQ22536 (100 μM) (Fig. 6A). Also, 2',5'-Dideoxyadenisine 100 μM inhibite the spontaneous $[Ca^{2+}]_i$ oscillations (Fig. 6B). The summarized data about control, SQ22536 or 2',5'-Dideoxyadenisine showed in (Fig. 6C) (n = 7).

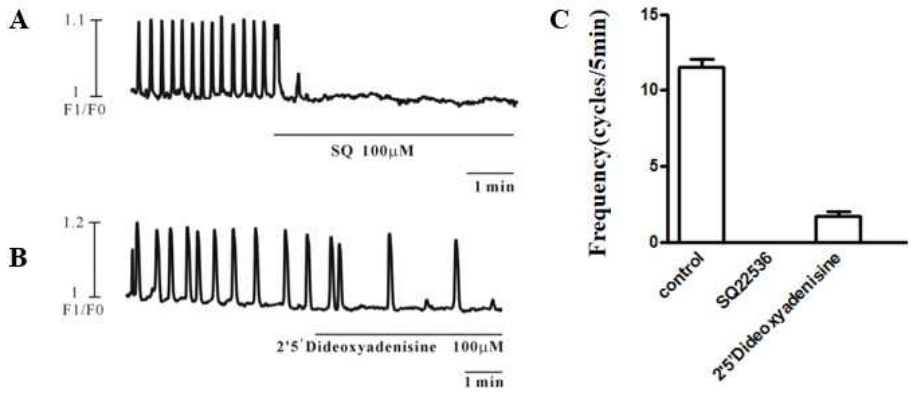

Figure 6. Measurement of $[Ca^{2+}]_i$ in the presence of adenylyl cyclase inhibitor. The periodic Ca^{2+} spikes which caused by a typical intracellular calcium oscillations are abolished in the presence of SQ22536 (100 μM)(Fig. 6A), 2',5'-Dideoxyadenisine(Fig. 6B) was treated to the cells at the concentration of 100 μM, inhibited the frequency of the intracellular Ca^{2+} oscillations. (Fig. 6A) showed the Ca^{2+} wave frequency decreased significantly.

3.4. Action of 8-bromo-cGMP, a Nitric Oxide Donor, cGMP-specific phosphodiesterases on $[Ca^{2+}]_i$ Oscillations from ICC

Nitric oxide (NO) is major inhibitory neurotransmitter in GI tract. Exposure to NO reduced frequency and a decreased amplitude of slow waves in GI smooth muscles that leads to relaxation (Ward et al., 1992). The main signal action of NO is mediated by an increase of cGMP stimulation after guanylate cyclase activation in a variety of cells. Therefore, I tested whether cGMP or NO can influence on $[Ca^{2+}]_i$ oscillations in ICC. Because 8-bromo-cGMP is a cell-permeable cGMP analog of cGMP, I treated this in ICC. The $[Ca^{2+}]_i$ oscillations of ICC significantly decreased after treating 8-bromo-cGMP (100 μM) (Fig. 7Ⓐ). Also, SNAP, a nitric oxide donor, inhibited the $[Ca^{2+}]_i$ oscillations of ICC (Fig. 7Ⓑ). These results suggest that the action of NO on ICC may involve the regulation of spontaneous $[Ca^{2+}]_i$ oscillations by 8-bromo -cGMP. Zaprinast prolong the cGMP-mediated activation of cGMP-dependent protein kinase. In this study, I could find Zaprinast (100 μM) also inhibits the $[Ca^{2+}]_i$ oscillations of ICC (Fig. 7C).The summarized data about these showed in (Fig. 7D)(n = 7).

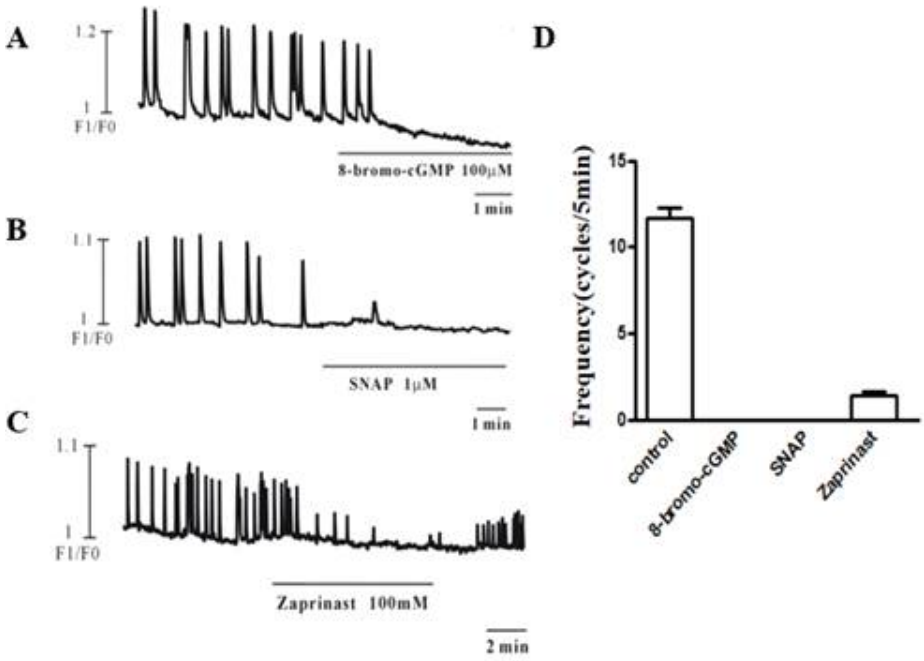

Figure 7. The effect of cyclic GMP on the intracellular Ca^{2+} oscillations. (Fig. 7A) Addition of 100 μM 8-bromo-cGMP abolished the frequency of the intracellular Ca^{2+} oscillations. (Fig. 7B) Effect of the drugs (±)-S-nitroso-Nacetylpenicillamine(SNAP) 1 μM also abolished the frequency of the intracellular Ca^{2+} oscillations. Zaprinast prolong the cGMP-mediated activation of cGMP-dependent protein kinase. In this study, I could find Zaprinast (100 μM) also inhibits the $[Ca^{2+}]_i$ oscillations of ICC (Fig. 7C). Under wash out, the frequency was returned to the fast state. (Fig. 7D) is the bar graphic representation of changes in frequency respectively after the treatment of 8-bromo-cGMP SNAP and Zaprinast.

3.5. Action of Guanylyl Cyclase Inhibitors on $[Ca^{2+}]_i$ Oscillations from ICC

ODQ is a highly selective inhibitor of guanylyl cyclase. To more understand cGMP action, ICC was exposed with ODQ with Ca^{2+} analysis. In (Fig. 7E), spontaneous $[Ca^{2+}]_i$ oscillations of ICC could find under normal condition. The spontaneous $[Ca^{2+}]_i$ oscillations of ICC were increased by presence of ODQ (10 μM). The summarized data about this showed in (Fig. 7F) (n = 8).

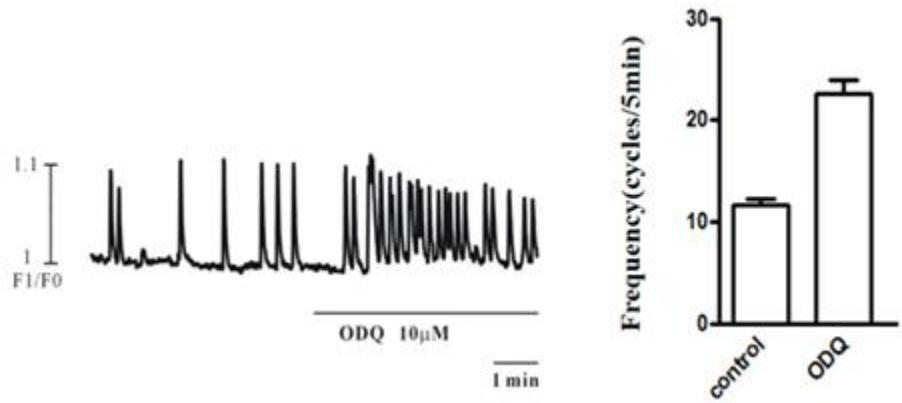

Figure 7. The effect of cyclic GMP on the intracellular Ca^{2+} oscillations. Addition of 10 μM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) inereased the frequency of the intracellular Ca^{2+} oscillations(Fig. 7E). The bar graphic representation of changes in frequency respectively after the treatment of ODQ(Fig. 7F).

3.6. The Role of K_{ATP} Channel on $[Ca^{2+}]_i$ Oscillations from ICC

For evaluation of whether K_{ATP} channel is involved in the regulation of spontaneous $[Ca^{2+}]_i$ oscillations of ICC, the action of pinacidil, the synthetic K_{ATP} channel opener, was examined. In presence of Pinacidil, spontaneous $[Ca^{2+}]_i$ oscillations of ICC were strongly inhibited (Fig. 8A). For further verification, I used glibenclamide, an K_{ATP} channel blocker. Interestinly, glibenclamide showed increasing of frequency of $[Ca^{2+}]_i$ oscillations of ICC (Fig. 8B). The summarized data about this showed in (Fig. 8C) (n = 8). These findings suggest that K_{ATP} channel may be involved in the regulation of pacemaker activity of colonic ICC by acting $[Ca^{2+}]_i$.

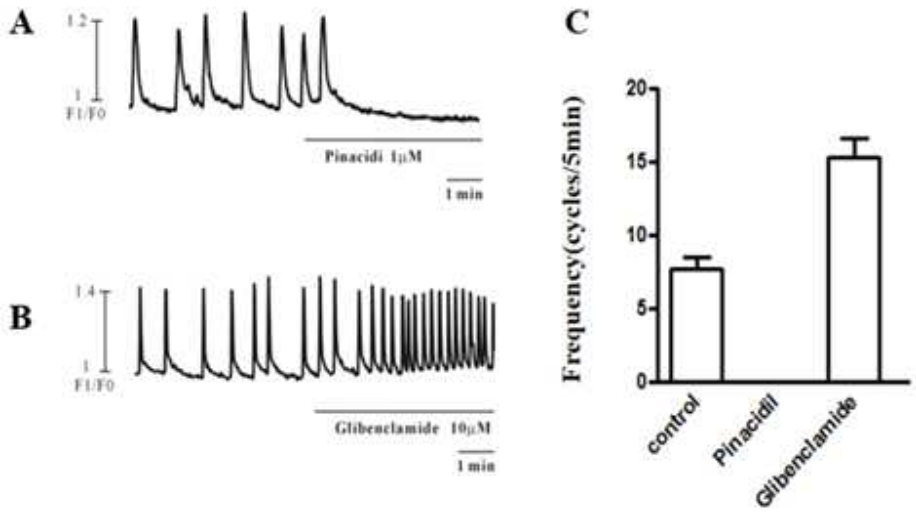

Figure 8. The effect of ATP-sensitive potassium channel on the intracellular Ca^{2+} oscillations. Addition of 1 μM Pinacidil abolished the frequency of the intracellular Ca^{2+} oscillations(Fig. 8A). Effect of the drugs Glibenclamide 10 μM increased the frequency of the intracellular Ca^{2+} oscillations(Fig. 8B). Changes in the frequency were represented in (Fig. 8C) bar graphic.

3.7. The Role of HCN Channel on $[Ca^{2+}]_i$ Oscillations from ICC

Recently, it was suggested that HCN channel may regulate the pacemaker activity of mouse colonic ICC. This means that HCN can modulate pacemaker activity by acting $[Ca^{2+}]_i$ oscillations in ICC. Thus I investigated whether HCN blocker. In (Fig. 9A-B), Zatebradine decreased the frequency of the intracellular Ca^{2+} oscillations. Or Ivabradine, also inhibits the $[Ca^{2+}]_i$ oscillations of ICC. The summarized data about this showed in (Fig. 9C) (n = 8). This is suggesting that HCN channel can be important channel for pacemaker activity in ICC and the regulation of $[Ca^{2+}]_i$ can be main mechanism.

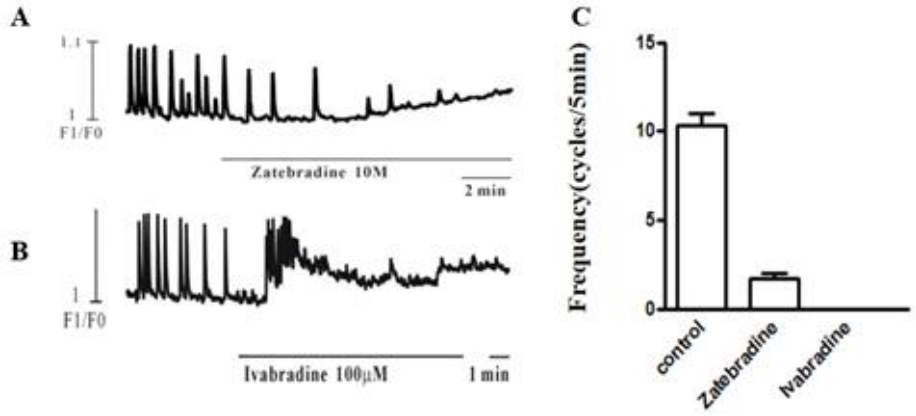

Figure 9. The effect of HCN channel on the intracellular Ca^{2+} oscillations. Addition of 10 μM Zatebradine hydrocholoride(Fig. 9A) decreased the frequency of the intracellular Ca^{2+} oscillations. and Ivabradine(Fig. 9B) inhibits the $[Ca^{2+}]_i$ oscillations of ICC. (Fig. 9C) is the bar graphic representation of changes in frequency respectively after the treatment of Zatebradine hydrocholoride and Ivabradine hydrocholoride.

4. DISCUSSION

This study investigated the dual modulation of cAMP and cGMP on spontaneous $[Ca^{2+}]_i$ oscillations in colonic ICC. For understanding this, I cultured ICC from mouse colon tissue and using the Ca^{2+} imagining technique, investigated whether cAMP or cGMP can modulate spontaneous $[Ca^{2+}]_i$ oscillations in colonic ICC. The main findings are discussed in more details in below. The spontaneous pacemaker activity of interstitial ICC is dependent on release of $[Ca^{2+}]_i$ from the endoplasmic reticulum. Particularly, IP_3-dependent $[Ca^{2+}]_i$ oscillations are the primay mechanism for generating pacemaker activity, which is well-matched with the periodic activation of pacemaker channels. In previous studies, patch clamp technology found that pacemaker current in the intestinal ICC may be stimulated by cAMP, while it may be inhibited by cGMP. The purpose of this study was to know cAMP or cGMP act on $[Ca^{2+}]_i$ oscillations in the colon.

Pacemaker currents are activated by the periodic release of Ca^{2+} from IP3 receptors. Ca^{2+} uptake by mitochondria is triggered by Ca^{2+} release from IP_3 receptors, possibly by gating the uniporter rapid uptake mode (Sparagna et al,1995; Litsky&Pfeiffer,1997). The main oscillatory process responsible for GI autorhythmicity appears to be the uptake and periodic release of Ca^{2+} from IP_3 receptor-operated stores. As a result, the pacemaker mechanism is a complex process that necessitates physical proximity and coordination between ion channels and transport proteins in the ER, mitochondria, and plasma membrane (Ward et al, 2000). Therefore, the understanding of spontaneous $[Ca^{2+}]_i$ oscillations can be the evidence how pacemaker activity is regulated.

The recent study showed HCN channel exists in colonic ICC and cAMP regulation in pacemaker activity(Shahi et al., 2014). This means intracellular cAMP can influence on generation of pacemaker activity by acting $[Ca^{2+}]_i$ oscillations because $[Ca^{2+}]_i$ oscillations is primary event for generation of pacemaker activity. In this study, I found cAMP itself showed increasing frequency of $[Ca^{2+}]_i$ oscillations. When Rolipram was treated, it had the

same effect as when cAMP was treated externally. This implies that cAMP plays a role for regulation of pacemaker activity by acting $[Ca^{2+}]_i$ oscillations in colonic ICC. Furthermore, I treated SQ22536, which reduced intracellular cAMP production. The spontaneous $[Ca^{2+}]_i$ oscillations generated by ICC was completely eliminated as a result of this action. This phenomenon was confirmed by using 2',5'-Dideoxyadenisine, a different type of adenylyl cyclase inhibitor. A similar potential suppression was observed, confirming the role of cAMP for regulation of pacemaker activity by acting $[Ca^{2+}]_i$ oscillations.

NO is a major inhibitory nurotransmitter in GI tract. NO produce a membrane hyperpolarization, the reduced frequency and a decrease of amplitudes of slow waves in GI smooth muscles that leads to relaxation. The main signal action of NO is mediated by the increase cGMP stimulation after guanylate cyclase activation of a variety cells. In turn cGMP activates protein kinase G. Recently, it suggestes NO inhibits pacemaker activity by the activation of K_{ATP} channels. This was showing cGMP-independent mechanisms are involved for NO signal action. To understanding this, I tested directly and found cGMP or SNAP itself inhibited the frequency of $[Ca^{2+}]_i$ oscillations. Although there is the report that This suggests that cGMP-dependent or independent mechanisms can be worked in colonic ICC.

The opening of K_{ATP} channels produces membrane potential hyperpolarization leading to a decrease in cell excitability. By contrat, the closing of K_{ATP} channel produces membrane potential depolarization leading to increase in cell excitability. Clinically, K_{ATP} channel is the targets for several drugs. Sulfonylureas such as glibenclamide and tolbutamide are used to treat diabetes and K_{ATP} openers like pinacidil and diazoxide are used to treat angian and hypertension. And previous study, Pinacidil inhibited pacemaker activity. Pacemaker potentials showed membrane hyperpolarization with pinacidil in ICC and this blocked by glibenclamide indicating the activity of K_{ATP} channel in ICC may have function on in regulating the pacemaker activity. In this study, I could find pinacidil inhibited the frequency of spontaneous $[Ca^{2+}]_i$ oscillations of ICC and

glibenclamde itself increased the frequency of $[Ca^{2+}]_i$ oscillations. This suggests that K_{ATP} channel is an important channel for regulating of pacemaker activity of ICC and this is via $[Ca^{2+}]_i$ regulation.

Furthermore, recent paper strongly suggested that periodically activated HCN channels by basal intracellular cAMP production are present in colonic ICC and they regulate pacemaker activity. To confirm this, I tested this and found or can be suggested HCN channel may be an effective therapeutic target for abnormal colonic motility disorders because this result showed HCN channel can influence on $[Ca^{2+}]_i$ oscillations. And this effect can modulate pacemaker activity of colonic ICC.

In conclusion, this results indicate that cAMP or cGMP can modulate the frequency of $[Ca^{2+}]_i$ oscillations directly and this provides the evidence how pacemaker activity can be regulated by various components. Especially, K_{ATP} or HCN channel can be a crucial channel for regulation of pacemaker activity and the mechanism is by acting $[Ca^{2+}]_i$ of colonic ICC.

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6. ABSTRACT

BACKGROUND: Interstitial cells of Cajal (ICC) generate spontaneous pacemaker acitivity responsible for the production of slow waves in gastrointestinal (GI) smooth muscle. yclic nucleotides are important second messenger molecules in signal transduction. Cyclic AMP (cAMP) particularly activates protein kinases that are involved in numerous signal transduction and metabolic pathways. Cyclic GMP (cGMP) mainly activates the cGMP-dependent protein kinase G and is involved in physiological processes such as eye vision, relaxation of smooth musculature and regulation of the Insuline level. Dual modulation of intracellular Ca^{2+} oscillations by cyclic nucleotides in interstitial cells of Cajal from mouse colon, thereby affecting the smooth muscle movement of the colon.

METHODS: To understand the role of cyclic nucleotides on intracellular Ca^{2+} oscillations in colonic ICC, I performed Ca^{2+} imaging techniques with fluo-4/AM under control condition.

RESULTS: Cyclic nucleotides has shown a dual effect on pacemaker potentials in Ca^{2+} imaging techniques with fluo-4/AM under control condition. 8-bromo-cAMP inereased the frequency of the intracellular Ca^{2+} oscillations. Meanwhile, the effect induced Rolipram (cAMP-specific phosphodiesterase (PDE4) inhibitor) and ODQ was mimic by the 8-bromo-cAMP. SQ22536 or dideoxyadenosine inhibits adenylyl cyclase. cGMP, SNAP (a nitric oxide donor) and Zaprinast(selective inhibitor of cGMP-specific phosphodiesterase), Pinacidil (Ca^{2+}-ATPase agonist, K^+ channel openner) inhibited intracellular Ca^{2+} oscillations. In recordings of Ca^{2+} imaging, glibenclamide(K_{ATP} channel blocker) increased intracellular Ca^{2+} oscillations. HCN can regulate the activity of the pacemaker by acting on $[Ca^{2+}]_i$ oscillations of ICC. HCN channel blocker Zatebradine decrease the frequency of the intracellular Ca^{2+} oscillations. Or Ivabradine, to inhibit the $[Ca^{2+}]_i$ oscillations of ICC.

CONCLUSION: cAMP or cGMP can modulate the frequency of $[Ca^{2+}]_i$ oscillations, The periodic release of Ca^{2+} from IP_3 receptors activated pacemaker currents. Furthermore, K_{ATP} or HCN channel can be a crucial channel for regulation of pacemaker activity and the mechanism is by acting $[Ca^{2+}]_i$ of colonic ICC.

7. ACKNOWLEDGEMENTS

This study was accomplished in the department of Physiology, School of Medicine at Chosun University.

This master's degree thesis could not be considered complete before I have the possibility to thank the people who carried out me across this intense and half two years long vovage.

First of all, I would like to express gratitude to my dissertation supervisor and mentor Professor Jae Yeoul Jun without his mentorship and unremitting guidance, this work would not have been possible. I would like to thank him for allowing me the freedom to work in my own way and also sharing his ideas .

Secondly I want to thank Professor Seok Choi, who has been teaching me and helping me in many aspects of my study during the course of my M. M. whenever required. I would like to thank him for always been there to help me when I needed it. Professor Chansik Hong gave me so many good advises which helping me a lot in my research.

Thanks so much to my supervising committee members.

I would like to take the opportunity to sincerely thanks to all of my lab members: Jiao Hanyi, Wu Wenhao and Huang Xingyou, Choi Seohua, Jeong Byeongseok, Jeon Dongju. I would like to thank all the individuals who have passed through the department during the course of my M. M.

I would also like to thank my family and friends for their inspiration, love and support through this process. Without them, I would not have been able to accomplish my goals.

Last, but not least, thanks to my husband Zhixiong Jiao for his understanding and love during the past years. His support and encouragement was in the end what made this dissertation possible.

저작물 이용 허락서

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	영문 Dual modulation of intracellular Ca^{2+} oscillation by cyclic nucleotides in interstitial cellsof Cajal from mouse colon				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집과 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물 이용의 허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속 대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 반대( )

2021 년 08 월 일

저작자: 장 정 미 (인) 장 정 미

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