February 2024

Ph.D (M.D). Thesis

The Effect and Mechanism of Prokinetic Agents on Pacemaker Activity in Digestive System

Graduate School of Chosun University

Department of Biomedical Sciences

HUANG XINGYOU

The Effect and Mechanism of Prokinetic Agents on Pacemaker Activity in Digestive System

위, 소장, 대장의 카할 간질세포에서 발생되는 향도잡이 활동도에 대한 위장운동촉진제의 비교 연구

23th February 2024

Graduate School of Chosun University

Department of Biomedical Sciences

HUANG XINGYOU

The Effect and Mechanism of Prokinetic Agents on Pacemaker Activity in Digestive System

Advisor: Prof. Seok Choi

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

October 2023

Graduate School of Chosun University

Department of Biomedical Sciences

HUANG XINGYOU

The thesis prepared by

HUANG XINGYOU

in partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

is certified by

Committee Chairman : Jae Yeoul Jun (인)

Committee : Jong Seong Park (인)

Committee : Jun Hyeong Lee (인)

Committee : Chansik Hong (인)

Committee : Seok Choi (인)

January 2024

Graduate School of Chosun University

CONTENTS

LIST OF TABLE

LIST OF FIGURES

ABBREVIATIONS

GI - Gastrointestinal

SMC - Smooth Muscle Cells

ICC - Interstitial Cell of Cajal

Ca^{2+} - Calcium

ER - Endoplasmic Reticulum

RyR - Ryanodine Receptors

IP3R - Inositol 1,4,5-trisphosphate Receptors

ANO1 Channel - Anoctamin1 Channel

CACC – Ca^{2+}-Activated Cl^- Channel

HCN - Hyperpolarization-activated Cyclic Nucleotide-gated Channels

TRP - Transient Receptor Potential Channels

CICR - Calcium-induced Calcium Release

STICs - Spontaneous Transient Inward Currents

STDs - Spontaneous Membrane Potentials

초 록

위, 소장, 대장의 카할 간질세포에서 발생되는 향도잡이 활동도에 대한 위장관촉진제 비교 연구

HUANG XINGYOU
지도교수: 최석
조선대학교 대학원 의과학과

서론 : 카할 간질세포는 위장관의 향도잡이 세포로 평활근 수축을 조절하는 중요한 기능을 담당하고 있다. 위장관촉진제는 위장관에 소화를 도와주는 역할로 널리 알려져 있으나, 향도잡이 세포인 카할 간질세포에 대한 기능은 전무한 실정이다. 그러므로, 본 연구는 위, 소장, 대장의 카할 간질세포에서 발생되는 향도잡이 전압 활동도에 대한 위장관촉진제의 효과를 관찰하였고 관련 기전에 대한 연구를 진행하였다.

연구방법 : 위장관촉진제의 기능을 확인하고자 본 연구자는 마우스의 위, 소장 그리고 대장에서 카할 간질세포를 배양하였고 배양된 세포를 이용하여 whole cell patch clamp 방법을 실시하였다.

결과 : 위, 소장 그리고 대장의 카할 간질세포에서 기록된 정상 향도잡이 활동도는 소장에서 규칙적이고 빠른 빈도의 향도잡이 활동도를 보여주었고 위 카할 간질세포에서는 규칙적이지만 소장보다 느린 빈도의 향도잡이 활동도를 확인하였다. 대장의 카할 간질세포에서는 비규칙적이고 느린 빈도의 향도잡이 활동도를 관찰 할 수 있었다. 위장관촉진제 중 cisapride 처리는 위, 소장 그리고 대장의 카할 간질세포에서 탈분극 현상을 관찰되었고 대장의 카할 간질세포에서만 향도잡이 활동도의 빈도를 증가시켰다. Mosapride의 투여는 위 그리고 소장 카할 간질세포의 작은 탈분극을 확인할 수 있었지만, 대장 카할 간질세포의 향도잡

이 활동도를 억제하는 것을 확인하였다. Metoclopramide는 위와 대장 카할 간질세포의 탈분극을 유발하였고 특히 대장 카할 간질세포의 향도잡이 활동도의 빈도를 증가하는 효과가 관찰되었다. 하지만 소장에 대한 효과는 나타나지 않았다. Pruclaopride의 경우 metoclopramide와 같이 위와 대장 카할 간질세포의 탈분극을 유발하였고 역시 대장 카할 간질세포의 향도잡이 활동도의 빈도를 증가시켰다. 마지막으로 domperidone은 위, 대장 그리고 소장의 카할 간질세포의 탈분극을 유발하였고 특히 대장에서 흥분성 신경전달물질과 비슷한 강한 탈분극 및 빈도의 증가가 관찰되었다. 위장관촉진제에 대한 5-HT_4 수용체의 기능을 확인하고자 5-HT_4 수용체 억제제와 위장관촉진제를 동시에 투여한 결과 mosapride에 의해 발생되는 위 그리고 소장에 탈분극이 억제됨을 확인할 수 있었다.

결론 : 위장관촉진제는 실제 임상에 많이 사용되는 약물로 중요한 역할을 담당하고 있다. 본 연구를 통해 위장관촉진제의 위, 소장 그리고 대장 카할 간질세포의 효과에 대한 효과를 확인하였다. 이와 같은 결과는 일반적으로 사용되는 위장관촉진제가 아닌 특정 소화관에 선택적인 처치가 가능한 기초적인 자료를 제공할 것으로 사료된다.

1. INTRODUCTION

1.1 Gastrointestinal tract and its functions

Gastrointestinal (GI) tract constitutes a vital component of the human body, encompassing all major organs of the digestive system. It comprises the mouth, esophagus, stomach, pylorus, duodenum, small intestine, cecum, colon, and rectum. This anatomical entity is demarcated by the duodenal ligament into two distinct regions: the upper gastrointestinal tract and the lower gastrointestinal tract. The gastrointestinal tract comprises four distinct layers, arranged from innermost to outermost: the mucosa, submucosa, muscular layer, and serosa. The journey of food through this tract commences with entry through the mouth, followed by processing within the stomach, subsequent nutrient and water extraction within the small and large intestines, culminating in excretion in the form of feces. The gastrointestinal tract's functionality is executed through four key physiological processes: motility, secretion, digestion, and absorption. The motility process further divides into peristalsis and segmentation (Sensoy I et al., 2021).

1.2 Slow waves and its mechanism

Contraction of smooth muscle in GI tract plays a pivotal role in facilitating GI motility. Previous research has demonstrated that GI smooth muscle cells (SMCs) possess the intrinsic capability to present regular electrical activity, known as slow waves. Slow waves encompass two distinct phases: depolarization and repolarization (References).

Previous studies have demonstrated the presence of various types of slow waves in the stomach, small intestine and colon (fig. 1). In the stomach, these occur at a rate of 3 cycles/minute and this is beneficial for food mixing and stirring (Du P et al., 2013). Conversely, in the small intestine, the frequency of slow waves can reach 12-20 cycles/minute. This rapid and frequent peristalsis serves to expedite the efficient absorption of nutrients from ingested food, whereas in the colon, this frequency is considerably lower, ranging from 3-8 cycles/minute. The reduced frequency of slow

waves within the colon aids in the reabsorption of water and electrolytes, culminating in the formation of solid stool for eventual elimination (Reference).

Slow waves represent a fundamental characteristic of smooth muscle. It is crucial to emphasize that the initiation of smooth muscle contraction is contingent upon the slow wave's membrane potential reaching a certain threshold. Only when this threshold is attained, the voltage-dependent Ca^{2+} channels open, permitting the influx of Ca^{2+} into the SMCs, subsequently inducing smooth muscle cell contraction. In the absence of reaching this threshold, slow waves in isolation are incapable of eliciting a response in smooth muscle (Huizinga et al., 1995).

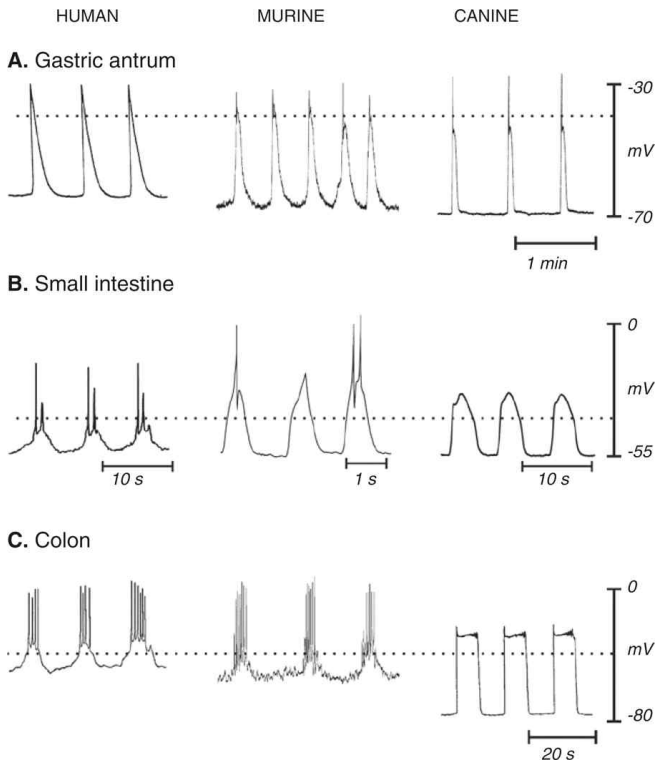

Fig. 1. Diverse slow wave patterns observed in the stomach, small intestine and colon. These recordings were conducted through the utilization of intracellular microelectrodes, focusing on the circular muscle layers of isolated muscle strips from the antrum, ileum, jejunum, and proximal colon (Sanders et al., 2014).

1.3 Interstitial cell of Cajal

Since the late 19th century when Spanish neuroanatomist Santiago Ramón y Cajal discovered and christened the interstitial cell of Cajal (ICC), extensive research endeavors have been dedicated to the exploration of this distinctive cell type (Cajal, 1911). Ramón y Cajal provided a comprehensive description of the cell's morphology and distinctive features, bestowing upon it the name it carries to this day. ICC constitute an indispensable element within the GI tract's pacemaker system. ICC are responsible for generating pacemaker currents that form the foundation for the slow-wave activity observed in the gastrointestinal tract's muscular layers. Over the past two decades, substantial research efforts have solidified the multifaceted roles of ICC. These roles encompass their function as pacemaker cells, facilitators of active electrical slow wave propagation, sites of innervation for peripheral

motor neurons, and mechanical sensory entities (Sanders et al., 2006).

1.3.1 Morphology and identification of ICC

Across various organs within individual species (such as mouse, guinea pig, rat, and dog), ICC exhibit varying shapes, distributions, and ultrastructural characteristics along the digestive tract at distinct anatomical sites (Komuro, 1999). Researchers have delineated ICC based on their ultrastructural features, aiding in their identification. The presence of ICC encompasses: 1) a fragmented basal lamina; 2) an abundance of mitochondria and caveolae; 3) numerous intermediate filaments; 4) a highly developed Golgi apparatus, limited ribosomes, and both rough and smooth endoplasmic reticulum; 5) intimate associations and gap junctions with nerves and SMC, enabling the formation of a network spanning the intestinal wall and connections with SMC (Al-Shboul, 2013).

Traditionally, ICC development was perceived to rely on the expression of c-Kit, a proto-oncogene that encodes the receptor tyrosine kinase Kit. However, c-Kit is not unique in this regard, as mast cells and melanocytes are also known to express it (Torihashi et al., 1999). Recent revelations highlighting that the transmembrane protein 16A (TMEM16A) encodes anactomin1 (ANO1) hold profound significance. This discovery enhances the specificity of ICC cell identification, as ANO1 exhibits superior expression for ICC (Gomes-Pinilla et al., 2009).

1.3.2 Distribution characteristics of ICC

The study of electrical rhythms in the muscles of the GI tract has been a longstanding area of investigation. Slow waves emanate from precise regions within tract, and these regions are characterized by the presence of ICC networks, which establish gap junction connections with smooth muscle cells (Sanders et al., 1996). ICC are distributed uniformly throughout the entire GI tract, spanning from the esophagus to the internal anal sphincter (Hagger R et al., 1998; Hanani M et al., 2005). Densely populated ICC networks are prominently present in the gastric body, antrum, and pylorus. Furthermore, the myometrial plexus represents a branching network of cells situated between the longitudinal and circumferential sublayers of the muscularis. ICC located within this region is referred to as myenteric ICC (ICC-MY) (Ward et al., 2000). Within the small intestine, three distinct types of ICC are identified: ICC-MY, ICC-IM, and ICC-DMP (with full name). Various

categories of ICC play crucial roles in both pacemaking and neurotransmission (fig. 2). ICC possess specific ionic conductances that render them distinctive in their capacity to initiate and transmit slow waves within GI muscles, as well as to convert neural input (Sanders et al., 1999). Distinctly, there exist at least three autonomous functional classifications of ICC. In the majority of GI regions, the ICC network resides within the intermuscular expanse, situated between the circular and longitudinal muscle layers, particularly at the myenteric plexus level, often referred to as ICC-MY. Notably, ICC-MY serve as pacemaker cells in the stomach and small intestine, instigating the onset of slow waves within the sarcolemma. Intramuscular interstitial cells (ICC-IM) are situated within the smooth muscle layer of the stomach. They predominantly exhibit a spindle-shaped morphology, with bipolar processes aligned parallel to the long axis of adjacent smooth muscle cells. ICC-IM also establish gap junction connections with neighboring smooth muscle cells, suggesting their role in mediating intestinal motor neurotransmission (Kito et al., 2011).

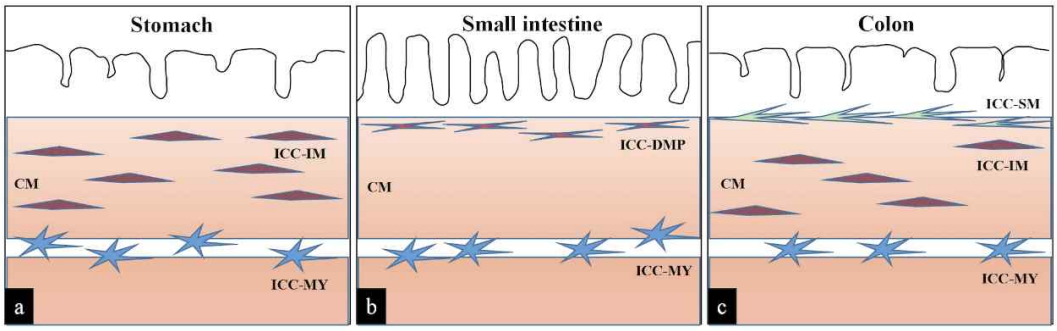

Fig. 2. Distribution of ICC in stomach, small intestine and colon. Please provide full name of CM, ICC-IM, ICC-MY and so on

1.3.3 Functions of ICC

The coordination of GI tract movement is an intricate process that involves the collaborative regulation of ICC cells, smooth muscle cells, and enteric nerves. ICC holding a important role in this orchestration.

(a) Firstly, ICC function as the pacemaker cells of the gastrointestinal tract, capable of

autonomously generating pacemaker activity. Prior research has revealed that specific types of ICC fail to develop in animals with mutations in c-Kit or stem cell factor, a ligand for the c-Kit receptor. The removal of ICC results in the disappearance of slow waves (Sanders et al., 1996; Ördög et al., 1999).

(b) Additionally, ICC appear to facilitate motor nerve transmission within the smooth muscle. Among these, ICC-IM serves as the principal target for enteromotor innervation of gastrointestinal muscles (Beckett et al., 2005). Furthermore, certain studies have indicated the presence of mechanosensitive Na^+ channel currents in human intestinal ICC, which seem to play a role in controlling digestive tract movement (Strege et al., 2003). It is possible that ICC have additional functions yet to be discovered, and further research is warranted to explore their full range of roles.

1.4 Prokinetic agents

Prokinetic agent, also commonly known as gastroprokinetic agent, are utilized to alleviate and treat a variety of GI symptoms, including bloating, constipation, nausea, vomiting, and other related discomforts (Reference). Furthermore, they found application in managing specific diseases such as irritable bowel syndrome, gastroparesis and gastritis. These GI agents function by stimulating smooth muscle contractions, which in turn enhance gastric emptying and transit within both the small and large intestines (Acosta et al., 2015). Prokinetic agents also play a pivotal role in coordinating contractions across different segments of the gastrointestinal tract, facilitating the smoother passage of intestinal contents (Hiyama et al., 2009). It's worth noting that research has revealed their effectiveness beyond the gastrointestinal realm, extending to conditions affecting the central nervous system, respiratory system, urinary system, and metabolic organs (Karamanolis et al., 2006).

1.4.1 Classification of prokinetic agent

Prokinetic agent are categorized based on their pharmacological properties, falling into distinct groups: (1) Serotonin receptor agonists ($5\text{-}HT_4$) (2) Dopamine receptor antagonists (D_2) (3) Motilin receptor agonists (4) Cholinesterase inhibitors (5) Ghrelin Receptor Agonist. However, it's important to note that many drugs exhibit multiple mechanisms of action, which can complicate their clinical application in the context of

prokinetic agent. While studies have shown that prokinetic drugs can be effective in treating specific GI diseases, like gastroparesis, it's worth mentioning that the quality of their treatment outcomes may not always meet the standards established in countries such as the United States and Europe (Grover et al., 2019; Pittayanon et al., 2019).

1.4.2 The function of prokinetic agents in GI tract

The following summarizes some of the functions and targets for prokinetic agents within GI tract:

(a) Serotonin receptor agonists (5-HT_4)

5-HT_4 receptors are recognized as pivotal components in the pathophysiology and physiology of GI motility (Gershon et al., 2007). Agonists targeting 5-HT_4 receptors are well-established as effective prokinetic agents, acting on serotonin receptors within the intestines. They promote GI motility, hasten gastric emptying, and alleviate symptoms associated with esophageal reflux. The 5-HT_4 receptor has emerged as a potential therapeutic target for disorders linked to GI motility, including chronic constipation. Notably, both cisapride and mosapride exhibit affinity for the 5-HT_4 receptor. Mosapride functions as a selective 5-HT_4 agonist. Moreover, its metabolite, M1, can serve as a 5-HT_3 antagonist. Mosapride is employed to expedite gastric emptying and is used in the treatment of functional dyspepsia, irritable bowel syndrome, gastritis, and gastroesophageal reflux disease (Tack et al., 2012; Odaka et al., 2006). Prucalopride represents a novel serotonin (5-HT_4) receptor agonist characterized by its high selectivity and affinity. It stimulates colonic mass movement, providing effective impetus for fecal expulsion. Prucalopride stands out for its absence of cardiac side effects. It induces relief from gastroparesis symptoms and accelerates gastric emptying more safely than some other medications (Bouras et al., 1999).

(b) Dopamine receptor antagonists

Domperidone is a dopamine antagonist medication employed in the treatment of nausea, vomiting, and specific GI issues. It enhances the movement of food through the stomach by elevating GI motility, making it a valuable therapy for gastroparesis (Reddymasu et al., 2007 ; Barone et al., 1999). Metoclopramide, another D_2 receptor antagonist, possesses additional properties, including a degree of 5-HT_4 receptor agonism. It exhibits prokinetic and antiemetic effects. Research has demonstrated that the actions of the endogenous neurotransmitter dopamine can impede the release of acetylcholine (ACh), leading to reduced

motility in the gastric and proximal small intestine. This effect underscores the significance of dopamine antagonists in managing GI motility disorders like gastroparesis, with metoclopramide standing as the sole medication accessible for this purpose in the United States (Tonini et al., 2004).

(c) Motilin receptor agonists

Macrolide antibiotics, specifically erythromycin, are commonly employed as motilin receptor agonists. They stimulate motilin receptors within GI tract, thereby enhancing contractions in the gastric antrum and fundus. Concurrently, erythromycin inhibits pyloric contractions, This mechanism enables the enhancement of gastric emptying (Catnach et al., 1992; Parkman et al., 1995).

(d) Cholinesterase inhibitors

Cholinesterase inhibitors exert prokinetic effects throughout the entire GI tract. They have proven to be highly effective in managing a spectrum of intestinal motility disorders, which includes postoperative ileus, as well as chronic and constipation-related ileus (Stanghellini et al., 2010).

(e) Ghrelin Receptor Agonist

Ghrelin is predominantly found within the stomach. This 28-amino acid hormone is known for its appetite-stimulating properties. In adequate concentrations, it has the capacity to enhance proximal gastric tone through both central and peripheral mechanisms. Furthermore, research has indicated its ability to expedite gastric emptying in individuals suffering from gastroparesis (Peeters et al., 2003; Camilleri et al., 2009).

1.5 Thesis purpose

Above mentioned, it is well known that prokinetic agents are useful for GI motility disorders. However, although there is no ague ICC is important for modulating GI motility by regulating slow waves of SMC, there is no report prokinetic agents on ICC. Hence, the present study seeks to compare the effects and elucidate the underlying mechanisms of prokinetic agents on pacemaker activity in stomach, colon and small intestinal ICC.

2. MATERIALS AND METHODS

2.1 Laboratory animals and ethic guidelines

The utilization and treatment of animals in this study adhered rigorously to the "Guidelines for the Care and Use of Laboratory Animals." Furthermore, all procedures were carried out only after obtaining identification and clearance from the Ethics Committee of Chosun University.

2.2 ICC preparation from stomach, colon and small intestine Ⅰ

In this experiment, ICR mice were employed as the experimental subjects. These mice received a daily diet and drinking water consistent with the established feeding standards for experimental mice. Typically, mice aged 5-8 days were utilized for cell culture purposes. For humane euthanasia, the mice were anesthetized using ether, followed by cervical vertebra dislocation. This method ensured a painless and swift process. Subsequently, a midline incision was made along the abdomen's midline, allowing for full exposure of all abdominal organs.

In this experiment, cells will be cultured from the stomach, small intestine, and colon separately. To prepare the small intestine and colon for cell culture, they were longitudinally incised along the mesentery to expose the intestinal lumen. Subsequently, they were placed in a Sylgard dish filled with Ca^{2+}-free Hank's solution and maintained at a temperature of -4°C. Following the removal of the pylorus and fundus, the stomach was dissected open along the lesser curvature and secured in the dish using the same procedure. Subsequently, the contents within the intestinal cavity were thoroughly cleaned, and the mucosal and submucosal layers were carefully peeled off. The separation process was completed within a 30-minute timeframe. Subsequently, the isolated tissue was immersed in an enzyme solution. It's worth noting that the enzyme solution's composition varied across different sections. Typically, for the small intestine and colon, a solution containing 2 mg/ml collagenase (Worthington Biochemical, USA), 2 mg/ml bovine serum albumin (Sigma), and 1mg/mL trypsin inhibitor (Sigma) was prepared by dissolving these components in 2 ml of liquid. The tissue samples were then incubated in a water bath at 37 °C for 14 and 16 minutes, respectively. For gastric tissue, a specific enzyme solution was prepared. This solution contained 1.5 mg/ml collagenase, 2 mg/mL bovine serum albumin, and 2 mg/ml trypsin

inhibitor, which were dissolved in 1 ml of liquid. The gastric tissue samples were then subjected to incubation for a duration of 17 minutes. Subsequently, the tissue samples were washed three times with Ca^{2+}-free Hank's solution. Afterward, they were finely chopped into smaller pieces using a 1mL pipette. The resulting cells were then transferred to a 35 mm culture dish containing sterile glass coverslips and allowed to stand for a period of 25 minutes. To enhance cell adhesion, the coverslips were treated with 200 μl of poly-L-lysine (Sigma). Subsequently, medium 231 (Gibco) was gently added along the walls of the culture dish. Additionally, 2 % antibiotic-antimycotic (Gibco) and stem cell factor (SCF) at a concentration of 5 ng/ml (Sigma) were incorporated. The cell culture was then transferred to an incubator and maintained for a duration of 2 days under conditions of 37 °C and 5 % CO_2.

2.3 Current-clamp Recording

Following 48 hours of cell culture in the incubator (with medium replacement after 24 hours), the cell solution was substituted with extracellular solution, and the flow rate was maintained at 1-2 ml/minute. A temperature control instrument was utilized to regulate the solution's temperature to 30 °C. After a 20 minute equilibration period, recording of the pacing potential of ICC from various tissues was initiated under the microscope. The pipette was filled to approximately 1/3-1/2 of its capacity with intracellular solution. Subsequently, the pipette was carefully positioned to make gentle contact with the cell membrane, forming a secure seal. Pacing potentials were amplified and recorded utilizing the Axopatch 200B system (Axon Instruments, Foster, CA, USA) in conjunction with Digidata 1322A. Simultaneously, data analysis was conducted using pClamp 9.2 software.

2.4 Solutions

2.4.1 Enzymatic Solution:

Collagenase (Worthington Biochemical), bovine serum albumin (Sigma), trypsin inhibitor (Sigma). The specific ratios of enzyme solutions are detailed in the "Isolation and Cell Preparation from Various Tissues" section.

2.4.2 Ca^{2+}-free Hank's Solution

135 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES, 10 mM Glucose; adjust the pH to 7.4 with Trisma-base.

2.4.3 Extracellular solution

135 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES, 10 mM Glucoseand 1.8 mM $CaCl_2$; adjust the pH to 7.4 with Trisma-base.

2.4.4 Intracellular solution

Intracellular solution was loaded into the micropipette for patch clamp manipulation. The intracellular solution consisted of the following components: 140 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM EGTA, 2.7 mM MgATP, 0.1 mM Na_2GTP, 2.5 mM Creatine Phosphate Disodium, and 5 mM HEPES. The pH of the solution was adjusted to 7.2 using Tris-base.

2.5 Drugs and Chemicals

The chemicals utilized in this study are listed in the table below:

Drug names	Companies	Catalog No.
cisapride	sigma	C4740
mosapride	sigma	M2946
metoclopramide	sigma	M0763
prucalopride	sigma	SML1371
domperidone	sigma	D122
serotonin (5-HT)	sigma	H7752
RS23597	sigma	SML1909
Collagenase	wothington biochemical	LS004176
Bovine Serum Albumin	sigma	A6003
Trypsin Inhibitor	sigma	T9128

Table 1. Drugs and Chemicals

2.6 Statistical analysis

The results are displayed as means ± standard errors. Paired data were subjected to Student's t-test to assess differences. A p-value less than 0.05 was deemed statistically meaningful. The 'n' values cited in the text indicate the quantity of cells employed in the recording.

3. RESULTS

3.1 Recording pacemaker activity generated by ICC in the stomach, colon and small intestine

As mentioned in the introduction section, previous studies have revealed significant variations in slow waves across different segments of the digestive tract, yet the pacemaker activity of ICC in distinct anatomical regions remains unexplored. Therefore, in this study, ICC were cultured from diverse tissue sources, and patch clamp technology was employed to record the pacemaker activity of ICC in the stomach, colon, and small intestine under microscopic observation (Fig. 3).

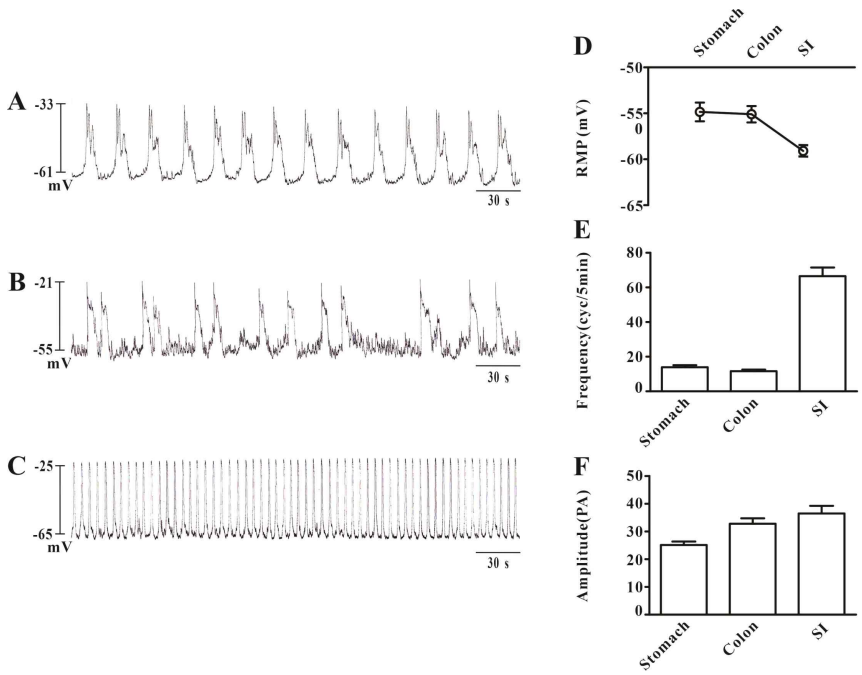

Fig. 3. Three characteristic pacemaker activities observed in cultured ICC isolated from the stomach, colon, and small intestine of mice. (A) Pacemaker activities within cultured ICC derived from the stomach of mice (B) Pacemaker activities within cultured ICC derived from the colon (C) Pacemaker activities within cultured ICC derived from the small intestine (D-F). Alterations in the resting membrane potentials, summarized information regarding frequency and amplitude. SI : Small intestine, S : Second.

In this study, the colon exhibited a resting membrane potential of approximately −56 mV ± 1.2 mV, characterized by a frequency of 13 cycles/5 minutes and an amplitude of 31 mV. In contrast to the stomach and small intestine, the pacemaker activity in the colon appeared to be notably more erratic (n = 15). Through the recording of ICC pacemaker activity in the stomach, it was observed that the membrane resting potential in the stomach closely resembled that of the colon, approximately −55 mV. However, the stomach exhibited a slightly higher frequency, approximately 16 cycles/5 minutes, characterized by a more consistent and rhythmic pattern. An intriguing finding was the significantly longer duration of the stomach's pacemaker activity in comparison to that of the colon and small intestine (n = 15). This observation may be attributed to their distinct roles in the digestive process. Upon analyzing the recorded pacemaker activity in the small intestine, it became evident that this region exhibits remarkably stable electrical activity, characterized by an exceptionally high frequency of 66 cycles/5 minutes. Additionally, in contrast to the stomach and colon, the small intestine maintains a lower resting potential of −58 ± 0.7 mV (n = 15).

3.2 Effects of Cisapride on ICC activity in the stomach, colon and small intestine

Cisapride, formerly employed as a prokinetic agent to enhance gastrointestinal motility, is associated with a plethora of significant adverse effects (Madisch A et al., 2017). Following a period of sustained and stable electrical activity recorded in the stomach, which persisted for 5 minutes, cells were subjected to treatment with 10 μM cisapride. This intervention led to a modest alteration in the gastric membrane potential, resulting in an increase from −56 mV to −51 mV. Simultaneously, the frequency of the observed electrical activity decreased from 16 cycles/5 minutes to 13 cycles/5 minutes (fig. 4E). The identical experimental approach was employed in the colon and small intestine, revealing distinct responses of cells in different anatomical regions to cisapride. Following cisapride treatment, there was a noteworthy average increase in the membrane potential of the colon by approximately 15 mV. Specifically, the resting membrane potential elevated from −58 mV to −43 mV. Additionally, the frequency of electrical activity in the colon displayed a corresponding increase, rising from 13 cycles/5 minutes to 20 cycles/5 minutes. Remarkably,

in the small intestine, the introduction of cisapride led to a transient rise followed by a subsequent decline in the resting potential (fig. 4C). Simultaneously, there was a notable reduction in frequency during this period, decreasing from 75 cycles/5 minutes to 40 cycles/5 minutes. These findings indicate that the prokinetic effect of cisapride was more pronounced in the colon compared to the stomach and small intestine.

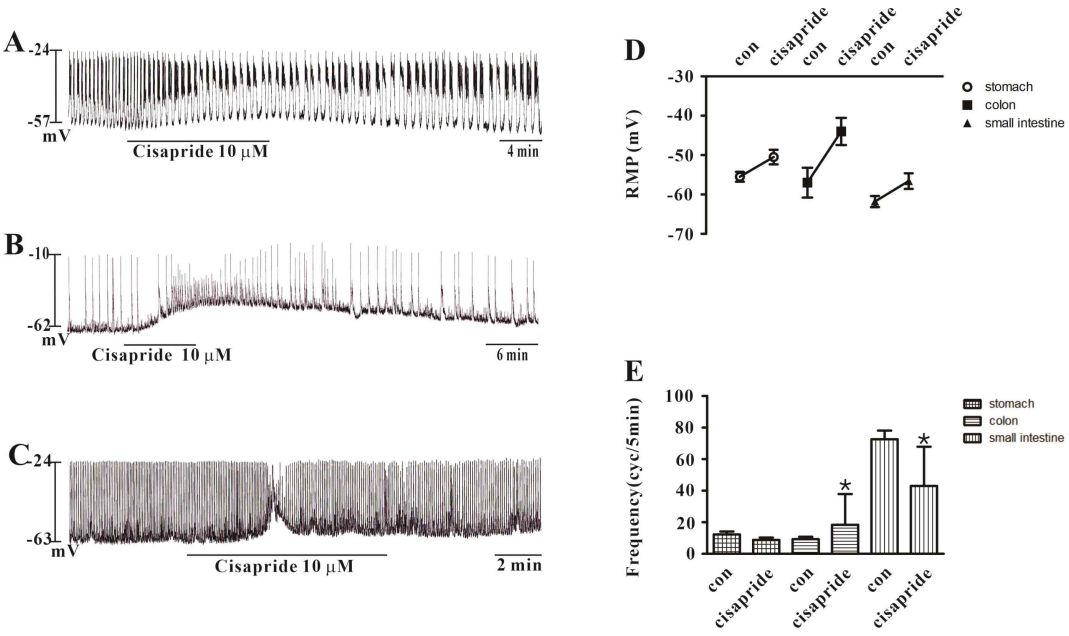

Fig. 4. (A-C) Effects of Cisapride (10 μM) on ICC activity in the stomach, colon and small intestine. (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.3 Effects of Mosapride on ICC activity in the stomach, colon and small intestine

Similar to cisapride, mosapride also functions as a 5-HT_4 agonist and is employed to ameliorate certain conditions associated with gastric insufficiency. In line with this, an experiment was conducted using mosapride at a concentration of 10 μM, and its effects were compared across the stomach, colon, and small intestine. Following a stabilization period, it was observed that mosapride induced only a slight depolarization in the gastric membrane potential, shifting it from −57 mV to −54 mV. Furthermore, the frequency exhibited only a marginal reduction, ranging from 1 to 2 cycles/5 minutes. A similar scenario was encountered in the small intestine, where the membrane potential increased from −59 mV to −56 mV upon mosapride treatment. And there is no significant alterations in frequency were noted in this context (fig. 5D and fig. 5E). Particular attention should be given to the distinctive response elicited by mosapride in the colon. Intriguingly, mosapride not only failed to augment the pacemaker activity of ICC but also resulted in a reduction in frequency, diminishing from 15 cycles/5 minutes to 3 cycles/5 minutes. Furthermore, in contrast to other prokinetic agent, mosapride did not induce depolarization of the cell membrane's resting potential in this context (fig. 5B).

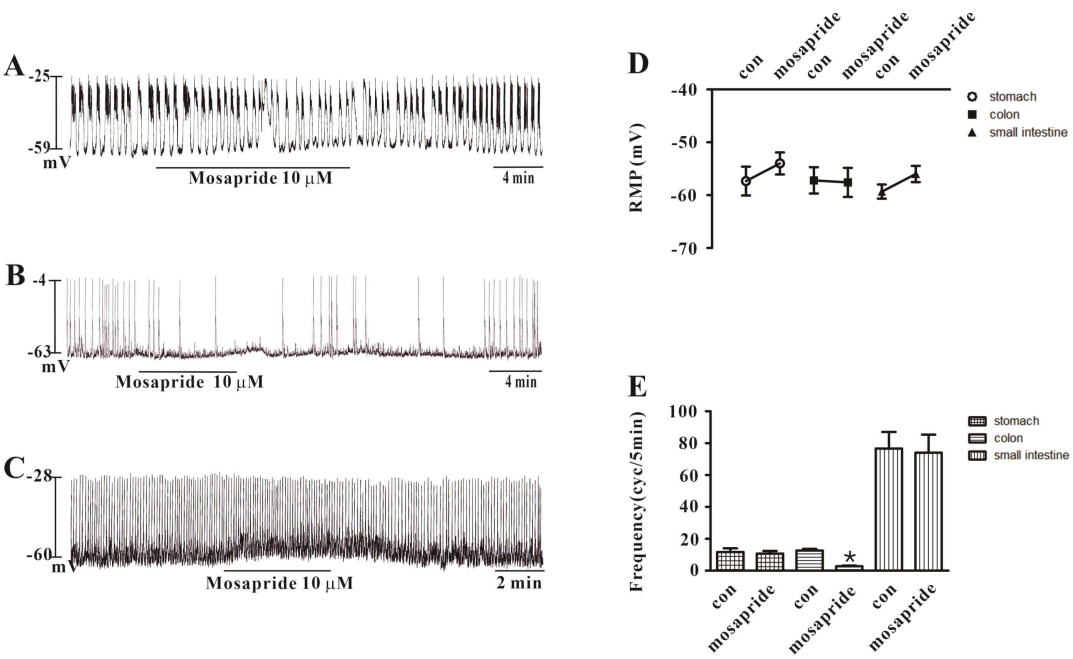

Fig. 5. (A-C) Effects of Mosapride 10 μM on ICC activity in the stomach, colon and small intestine which were cultured from murine. (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.4 Effects of Metoclopramide on ICC activity in the stomach, colon and small intestine

To investigate the potential influence of metoclopramide on pacemaker potential alterations, pacemaker activity was recorded following pretreatment with metoclopramide. The findings revealed that metoclopramide induced a modest degree of membrane potential depolarization, albeit statistically insignificant, in both the stomach and colon, shifting from −53 mV to −49 mV and from −56 mV to −52 mV, respectively. However, no discernible changes were observed in the small intestine. It is worth emphasizing that a drug concentration of 30 μM was employed in the colon, as the 10 μM concentration failed to induce any alterations. Following the increase in concentration, depolarization and an elevation in frequency were evident, with the frequency transitioning from 12 cycles/5 minutes to 16 cycles/5 minutes (fig. 6B). Additionally, it is noteworthy that metoclopramide did not exert any influence on the pacing frequency of ICC in the stomach and small intestine.

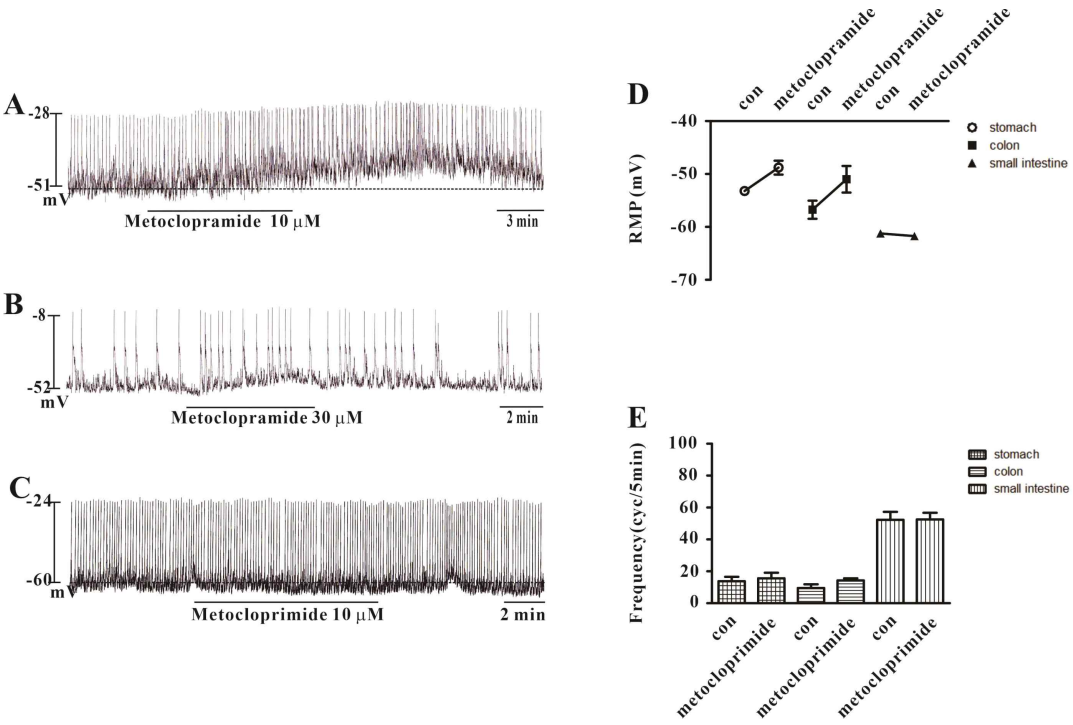

Fig. 6. (A-C) Effects of Metoclopramide 10 μM or 30 μM on ICC activity in the stomach, colon and small intestine which were cultured from ICR mice. (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.5 Effects of Prucalopride on ICC activity in the stomach, colon and small intestine

Prucalopride, a drug characterized by higher affinity and selectivity for the 5-HT_4 receptor, was likewise subjected to testing in this experiment. The findings indicate that gastric ICC experienced noteworthy depolarization, although the frequency remained unaltered. In contrast, the impact of prucalopride in the small intestine was found to be negligible. Through concentration gradient testing (specific results not yet disclosed), it was ascertained that when the drug concentration reached 30 μM, Prucalopride plays a minor role in the

colon, resulting in only a slight increase in the cell membrane potential and an increment of approximately 3 cycles/5 minutes in frequency. These findings suggest that the highly selective prucalopride's effect on the pacemaker activity of ICC may not be particularly pronounced.

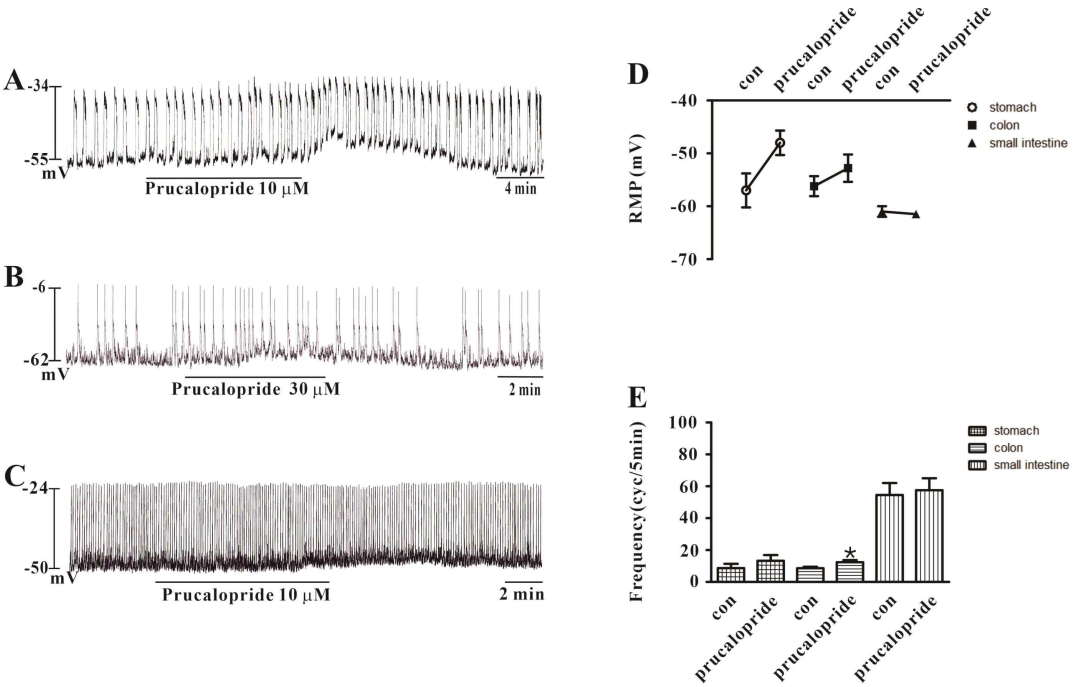

Fig. 7. (A-C) Effects of Prucalopride 10 μM or 30 μM on pacemaker activity in the stomach, colon and small intestine which were recorded with whole-cell patch clamp machine (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.6 Effects of Domperidone on ICC activity in the stomach, colon and small intestine

As widely recognized, the administration of dopamine receptor antagonists can augment the release of ACh, consequently expediting gastrointestinal motility. In light of this, domperidone was employed in this study. The outcomes demonstrated that domperidone exhibited its most pronounced effectiveness in the colon. The amplitude of the membrane potential increase was notably substantial, showcasing an impressive difference of 20 mV. Furthermore, a significant alteration in frequency was also apparent, transitioning from the

typical 8 cycles/5 minutes to approximately 40 cycles/5 minutes in the colon. Conversely, the changes in frequency were less conspicuous in both the stomach and small intestine. Notably, the membrane potential exhibited a substantially greater increase in the stomach compared to the small intestine (fig. 8D and fig. 8E). When compared to metoclopramide, another dopamine receptor antagonist, domperidone appears to exert a more potent effect.

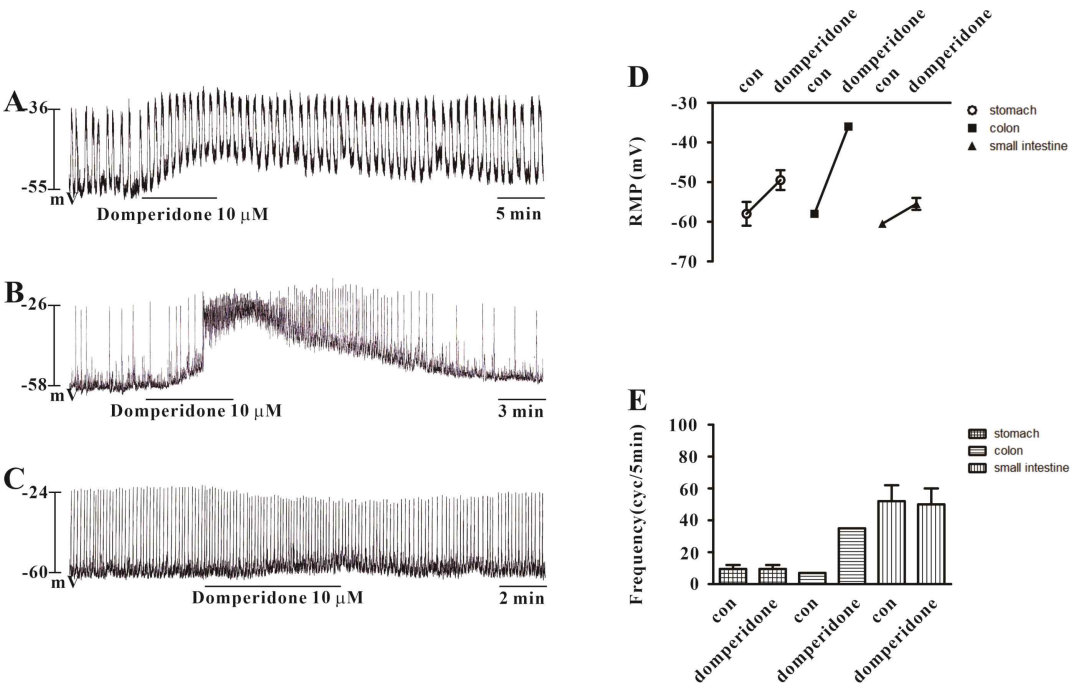

Fig. 8. (A-C) Effects of Domperidone (10 μM) on pacemaker activity in the stomach, colon and small intestine which were recorded with patch clamp technology (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.7 Effects of cisapride on ICC activity in the stomach, colon and small intestine after pretreatment with RS 23597

RS 23597 is a 5-HT_4 receptor antagonist. To investigate whether the effect of cisapride on ICC in the stomach, colon, and small intestine is mediated through 5-HT_4 receptors, cells were pre-treated with RS 23597 before the addition of cisapride. The findings reveal that in

the stomach, 10 μM RS23597 alone induced a depolarization of approximately 4 mV without affecting the frequency. Subsequently, when cisapride was add in solution, the cell membrane potential continued to increase on the foundation of the effect initiated by RS23597 itself (fig. 9A). In a parallel experimental setup, under the same conditions, it was observed that in the colon, 10 μM RS 23597 not only increased the pacing frequency but also induced depolarization of the cell membrane. In addition, when cisapride was used, its effect remained conspicuous, leading to a significant elevation in both frequency and membrane potential (fig. 9B). Varied results were noted in the small intestine. RS 23597 alone did not influence the pacemaker activity of small intestine ICC. Furthermore, when the cells were subjected to 10 μM cisapride, neither the membrane potential nor the frequency of the cells displayed any alterations (fig. 9C).

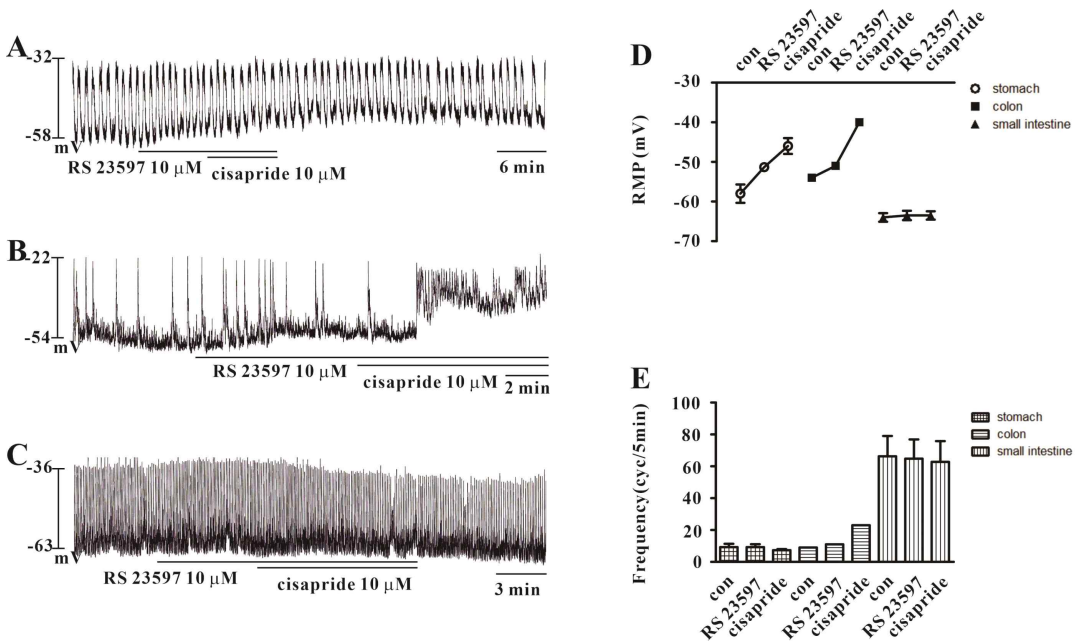

Fig. 9. (A-C) The effect of Cisapride (10 μM) on the pacemaker activity of the stomach, colon, and small intestine after pre-treatment with RS 23597 (10 μM). (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

3.8 Effects of mosapride on ICC activity in the stomach, colon and small intestine after pretreatment with RS 23597

In a manner akin to cisapride, Investigate the potential relationship between mosapride and 5-HT_4 receptors in the ICC mechanism. Subsequent to pre-administering RS 23597 in gastric ICC, it was observed that RS 23597 was ineffective in impeding the efficacy of mosapride, mosapride continued to elicit an approximately 2 mV increase in cell membrane potential (fig. 10A). In the context of the colon, Mosapride similarly demonstrated inhibitory effects (fig. 10B). In the small intestine, mosapride maintained its functionality, resulting in an elevation of membrane potential (fig. 10C).

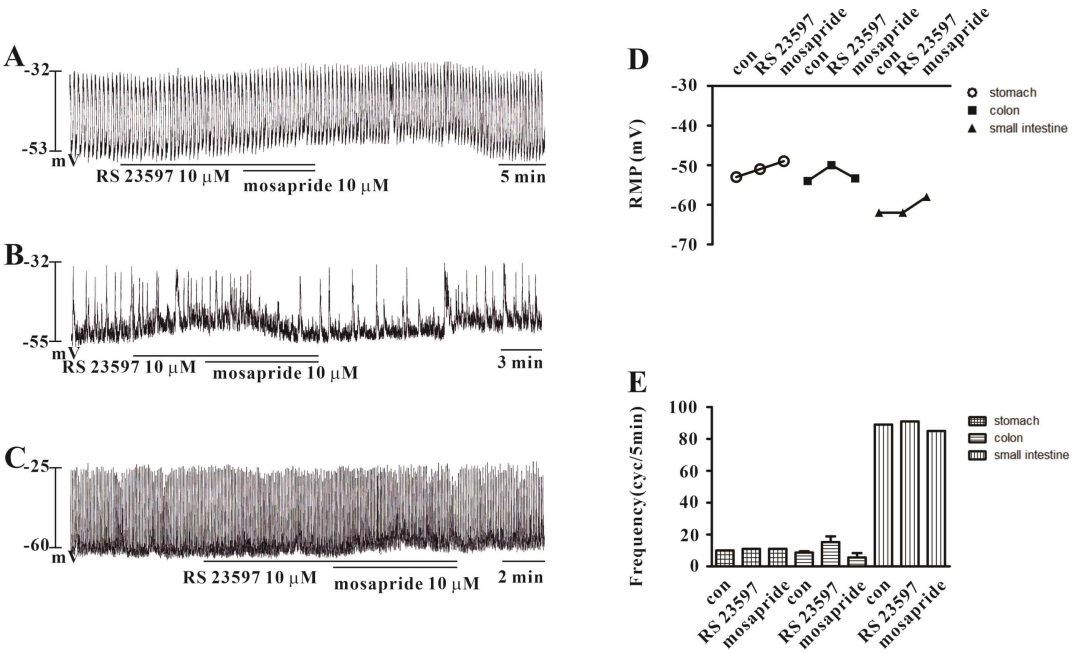

Fig. 10. (A-C) The effect of Mosapride (10 μM) on the pacemaker activity of the stomach, colon, and small intestine after pre-treatment with 5-HT_4 blocker (10 μM). (D-E) Bar graph illustrating a comparison of resting membrane potential and frequency. The bars depict mean values ± standard error (SE). *Asterisks indicate statistical significance compared to the control ($p < 0.05$).

4. DISCUSSION

This study reveals that the pacemaker activity initiated by ICC exhibits distinct characteristics in the stomach, colon and small intestine, encompassing variations in resting membrane potential, frequency, and waveform. Specifically, ICC in stomach exhibits a more extended plateau phase and in small intestine demonstrates a higher frequency of pacemaker activity, while ICC in colon displays a less regular pattern. These observations imply that the pacemaker activity mechanisms of ICC may vary in different anatomical regions, potentially attributed to differences in their respective slow waves (Sanders et al., 2014). Furthermore, concerning prokinetic agents, which are a class of drugs known to enhance the motility of digestive organs, their efficacy may exhibit variations across different anatomical locations within the body. This study discovered that, among the drugs examined, virtually all prokinetic agents exerted a stimulatory influence on the pacemaker activity induced by ICC. However, it is noteworthy that in the tests conducted on the large intestine, only mosapride exhibited a contrary outcome. The present findings suggest that prokinetic agents display distinct pharmacological profiles within the stomach, colon and small intestine. In this experiment, a range of drugs, including cisapride, mosapride, metoclopramide, prucalopride and domperidone, were employed to investigate their effects and potential mechanisms in these three anatomical regions.

It was reported that cisapride may enhance GI motility and transit (De et al., 1993) and cisapride has the ability to stimulate peristaltic motility in the smooth muscles of the esophagus, stomach, colon, and small intestine, both in vitro and in vivo (Barone et al., 1994). Furthermore, in experiments involving isolated guinea pig gastric smooth muscle, cisapride is shown to depolarize the membrane while simultaneously reducing the frequency of slow waves (Ohno et al., 1995). These finding is consistent with the results obtained in our experiment, where cisapride depolarize ICC-induced pacemaker activity in the stomach. Conversely, there was a slight decrease in the frequency of electrical activity, which may directly account for the observed reduction in slow wave frequency in the smooth muscle experiments. This phenomenon may directly account for the observed reduction in slow wave frequency in the smooth muscle experiments.

Mosapride significantly augmented both the contraction wave and its amplitude in the guinea pig stomach induced by electrical stimulation (Ji et al., 2003). Furthermore, it has demonstrated the capacity to ameliorate symptoms and enhance gastric motility in patients afflicted with IFN-induced gastroparesis (Kawamura et al., 2012). Likewise, in intragastric ICC, mosapride only induces depolarization of the resting potential. When this depolarization propagate to the SMC, it has the capacity to upregulate the slow wave, facilitating its attainment of the threshold and consequently intensifying contractions. It has been reported that metoclopramide, in concentrations ranging from 10 to 100 μM, can enhance electric field stimulation (EFS)-induced contractions in the murine forestomach (Bassil et al, 2005). Similarly, prucalopride, at concentrations from 0.1 to 30 μM, has shown the capacity to stimulate EFS-induced contractions in the human esophagus and gastric fundus (Broad et al., 2014). In isolated human stomach tissues, low concentrations of metoclopramide may augment electrically induced cholinergic activity by elevating neuronal acetylcholine release, whereas domperidone does not operate through this pathway, despite both agents' ability to accelerate gastric emptying (Sanger et al., 1985). Regarding the study of this experiment, it was observed that the pacemaker activity generated by ICC in the stomach underwent varying degrees of depolarization when exposed to these three drugs. However, further experiments are needed to elucidate their specific mechanisms of action.

The pacemaker activity generated by ICC in the colon exhibits a lower frequency and greater irregularity when compared to that in the stomach and small intestine. Consequently, the prokinetic effect of prokinetic agents on gastric motility is expected to be more pronounced. The result show that cisapride demonstrated a potent effect on colonic ICC, leading to a significantly increased frequency and more prominent depolarization compared to other prokinetic agents. This observation aligns with previous experimental findings in the colon, such as cisapride's ability to induce contraction in the ascending colon of guinea pigs through activation of 5-HT_4 receptors (Briejer et al., 1993). Several studies have consistently reported that mosapride selectively augments the motility of the upper gastrointestinal tract, while exerting no influence on the lower gastrointestinal tract. Conversely, other 5-HT_4 receptor agonists have been demonstrated to enhance colon contractions (Ji et al., 2003; Mine et al., 1997). Additionally,

experimental evidence has shown that mosapride expedites transport by increasing contractions in the proximal colon (Amano et al., 2015). However, the results obtained in this particular experiment differ significantly from the typical effects of mosapride. In this experiment, mosapride exhibited inhibitory effects in the colon, characterized by a reduction in frequency and hyperpolarization of the membrane potential. These findings raise the possibility that mosapride may enhance gastrointestinal motility through direct interactions with cell types other than ICC. Limited reports exist regarding experiments involving prucalopride and metocalopride on the colon. In rabbit models, intracolonic administration of prucalopride elicits the activation of 5-HT_4 receptors, resulting in heightened propulsive motor activity (Shokrollahi et al., 2019). However, it is noteworthy that lower concentrations (10 μM) of prucalopride and metocalopride yielded no discernible impact in colon experiments. When the concentration is elevated to 30 μ M, a modest increase in frequency is observed. This suggests that the propulsive effects of these two drugs on the colon may not be as pronounced when compared to other prokinetic agents. Numerous studies have consistently reported that domperidone exhibits limited to negligible influence on colon motility (Nieto et al., 2013; Longo et al., 1993). Nevertheless, the remarkable efficacy of domperidone in this particular experiment underscores its potential as a future therapeutic option for enhancing colon motility.

Cisapride-induced clustered and irregular spikes in the small intestine of rats have been previously documented (Lördal et al., 1988). In my experiments, when cisapride was applied to cultured ICC, it produced a comparable effect, inducing a brief irregular spike in pacemaker activity before returning to the baseline state. It has been observed that mosapride enhances ileal contractions in guinea pigs by promoting the release of acetylcholine from cholinergic neurons within the gastrointestinal tract (Ji SW et al, 2003). However, in my experiment, mosapride solely impacted the resting potential of the cell membrane, resulting in an increase of approximately 3 mV, potentially linked to acetylcholine release. Indeed, In fact, there is an article that asserts the reduced efficacy of many prokinetic agents in the small intestine compared to their effects in the stomach (Soffer EE et al, 1998). This observation aligns with the findings from my experiments, which indicated that prucalopride and metocalopride had no impact on the

small intestine. Furthermore, the influence of domperidone on the small intestine was significantly weaker in comparison to its effects on the colon. Collectively, these results imply that prokinetic agents may not sufficiently address motility dysfunction in the small intestine.

The 5-HT_4 receptors are believed to play a pivotal role in gastrointestinal motility physiology and pathophysiology. They have been identified as potential therapeutic targets for gastrointestinal motility disorders such as chronic constipation. Selective serotonin reuptake inhibitors (SSRI) have demonstrated prokinetic effects on the small intestine. In this study, Presently, a number of prokinetic agents are associated with the activation of 5-HT_4 receptors, including cisapride and mosapride. As a result, 5-HT_4 receptor blockers were employed in this experiment. This finding suggests that the alterations induced by cisapride in ICC of the small intestine are mediated by 5-HT_4 receptors, whereas the effects of mosapride do not appear to be connected to 5-HT_4 receptors. Furthermore, it underscores that the mechanism of action of prokinetic agents varies across different anatomical regions, emphasizing the need for further experiments to elucidate their specific mechanisms of action. Furthermore, this also underscores the variability in the mechanism of action of prokinetic agents across different anatomical regions. To establish the specific mechanisms of action, further experiments are warranted.

In conclusion, all the drugs examined exhibited prokinetic effects in the ICC of the stomach, colon, and small intestine, with the exception of mosapride, which demonstrated an inhibitory effect specifically in the colon. These prokinetic agent-induced effects bear similarities to those induced by 5-HT. It appears that cisapride's action in the small intestine is mediated through 5-HT_4 receptors, whereas the gastro-motility effect of mosapride does not seem to be connected to 5-HT_4 receptors. Future research should explore the relationship between other prokinetic agents and 5-HT_4 receptors.

5. REFERNCES

Acosta A, Camilleri M. Prokinetics in gastroparesis. Gastroenterol Clin North Am. (2015) 44(1); 97-111

Al-Shboul OA. The importance of interstitial cells of cajal in the gastrointestinal tract. Saudi J Gastroenterol. (2013) 19(1); 3

Amano T, Ariga H, Kurematsu A, Yamato S, Morioka S, Masaka A, Kanazawa M, Fukudo S. Effect of 5-hydroxytryptamine receptor 4 agonist mosapride on human gastric accommodation. Neurogastroenterol Motil. (2015) 27(9); 1303-1309

Cajal SR. Histologie du syste me nerveux de I'Homme et des verte be s. Maloine (Paris). (1911) 2; 891–942

Barone JA, Jessen LM, Colaizzi JL, Bierman RH. Cisapride: a gastrointestinal prokinetic drug. Ann Pharmacother. (1994) 28(4); 488-500

Barone JA. Domperidone: a peripherally acting dopamine2-receptor antagonist. Ann Pharmacother. (1999) 33(4); 429-440

Bassil AK, Dass NB, Murray CD, Muir A, Sanger GJ. Prokineticin-2, motilin, ghrelin and metoclopramide: prokinetic utility in mouse stomach and colon. Eur J Pharmacol. (2005) 524(1-3); 138-144

Blair PJ, Rhee PL, Sanders KM, Ward SM. The significance of interstitial cells in neurogastroenterology. Journal of Neurogastroenterology and Motility. (2014) 20(3); 294–317

Boedtkjer DMB, Kim S, Jensen AB, Matchkov VM, Andersson KE. New selective inhibitors of calcium-activated chloride channels - T16A (inh) -A01, CACC (inh) -A01

and MONNA - what do they inhibit? Br J Pharmacol. (2015) 172(16); 4158

Bouras EP, Camilleri M, Burton DD, McKinzie S. Selective stimulation of colonic transit by the benzofuran $5HT_4$ agonist, prucalopride, in healthy humans. Gut. (1999) 44(5); 682-686

Briejer MR, Akkermans LM, Meulemans AL, Lefebvre RA, Schuurkes JA. Cisapride and a structural analogue, R 76,186, are 5-hydroxytryptamine4 ($5\text{-}HT_4$) receptor agonists on the guinea-pig colon ascendens. Naunyn Schmiedebergs Arch Pharmacol. (1993) 347(5); 464-470

Broad J, Hughes F, Chin-Aleong J, Sifrim D, Sanger GJ. Regionally dependent neuromuscular functions of motilin and $5\text{-}HT_4$ receptors in human isolated esophageal body and gastric fundus. Neurogastroenterol Motil. (2014) 26(9); 1311-1322

Cajal SR. Histologie du syste me nerveux de I'Homme et des verte be s. Maloine (Paris). (1911) 2; 891–942

Camilleri M, Papathanasopoulos A, Odunsi ST. Actions and therapeutic pathways of ghrelin for gastrointestinal disorders. Nat Rev Gastroenterol Hepatol. (2009) 6(6); 343-352

Catnach SM, Fairclough PD. Erythromycin and the gut. Gut. (1992) 33(3); 397-401

Choi S, Seo H, Lee K, Shin DH, Wu MJ, Wu W, Huang X, Zhang J, Hong C, Jun JY. Hyperpolarization-activated cyclic nucleotide-gated channels working as pacemaker channels in colonic interstitial cells of Cajal. J Cell Mol Med. (2022) 26(2); 364

De Ridder WJ, Schuurkes JA. Cisapride and 5-hydroxytryptamine enhance motility in the canine antrum via separate pathways, not involving 5-hydroxytryptamine1,2,3,4 receptors. J Pharmacol Exp Ther. (1993) 264(1); 79-88

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

Du P, O'Grady G, Gao J, Sathar S, Cheng LK. Toward the virtual stomach: progress in multiscale modeling of gastric electrophysiology and motility. Wiley Interdiscip Rev Syst Biol Med. (2013) 5(4); 481-493

Drumm BT, Hennig GW, Battersby MJ, Cunningham EK, Sung TS, Ward SM, Sanders KM, Baker SA. Clustering of Ca^{2+} transients in interstitial cells of Cajal defines slow wave duration. J Gen Physiol. (2017) 149(7); 703

Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology. (2007) 132(1); 397-414

Gomez-Pinilla PJ, Gibbons SJ, Bardsley MR, Lorincz A, Pozo MJ, Pasricha PJ, Van de Rijn M, West RB, Sarr MG, Kendrick ML, Cima RR, Dozois EJ, Larson DW, Ordog T, Farrugia G. ANO1 is a selective marker of interstitial cells of Cajal in the human and mouse gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol. (2009) 296(6); G1370

Grover M, Farrugia G, Stanghellini V. Gastroparesis: a turning point in understanding and treatment. Gut. (2019) 68(12); 2238-2250

Hagger R, Gharaie S, Finlayson C, Kumar D. Distribution of the interstitial cells of Cajal in the human anorectum. J Auton Nerv Syst. (1998) 73(2-3); 75-79

Hanani M, Farrugia G, Komuro T. Intercellular coupling of interstitial cells of Cajal in the digestive tract. Int Rev Cytol. (2005) 73; 249-282

Hiyama T, Yoshihara M, Tanaka S, Haruma K, Chayama K. Effectiveness of prokinetic

agents against diseases external to the gastrointestinal tract. J Gastroenterol Hepatol. (2009) 24(4); 537-546

Huizinga JD, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature. (1995) 373(6512); 347

Huizinga JD, Zhu Y, Ye J, Molleman A. High-conductance chloride channels generate pacemaker currents in interstitial cells of Cajal. Gastroenterology. (2002) 123(5); 1627

Ji SW, Park HJ, Cho JS, Lim JH, Lee SI. Investigation into the effects of mosapride on motility of Guinea pig stomach, ileum, and colon. Yonsei Med J. (2003) 44(4); 653-664

Karamanolis G, Tack J. Promotility medications--now and in the future. Dig Dis. (2006) 24(3-4); 297-307

Kaupp UB, Seifert R. Molecular diversity of pacemaker ion channels. Annu Rev Physiol. (2001) 63; 235-257

Kawamura E, Enomoto M, Kotani K, Hagihara A, Fujii H, Kobayashi S, Iwai S, Morikawa H, Kawabe J, Tominaga K, Tamori A, Shiomi S, Kawada N. Effect of mosapride citrate on gastric emptying in interferon-induced gastroparesis. Dig Dis Sci. (2012) 57(6); 1510-1516

Komuro T. Comparative morphology of interstitial cells of Cajal: ultrastructural characterization. Microsc Res Tech. (1999) 47(4); 267

Kito Y. The functional role of intramuscular interstitial cells of Cajal in the stomach. J Smooth Muscle Res. (2011) 47(2); 47-53

Longo WE, Vernava AM 3rd. Prokinetic agents for lower gastrointestinal motility disorders. Dis Colon Rectum. (1993) 36(7); 696-708

Lördal M, Hellström PM, Johansson C. Cisapride induces clustered spikes and irregular spiking of the small intestine of the rat. Scand J Gastroenterol Suppl. (1988) 152; 48-53

Madisch A, Vinson BR, Abdel-Aziz H, Kelber O, Nieber K, Kraft K, Storr M. Modulation of gastrointestinal motility beyond metoclopramide and domperidone : Pharmacological and clinical evidence for phytotherapy in functional gastrointestinal disorders. Wien Med Wochenschr. (2017) 167(7-8); 160-168

Mine Y, Yoshikawa T, Oku S, Nagai R, Yoshida N, Hosoki K. Comparison of effect of mosapride citrate and existing 5-HT_4 receptor agonists on gastrointestinal motility in vivo and in vitro. J Pharmacol Exp Ther. (1997) 283(3); 1000-1008

Nieto JE, Maher O, Stanley SD, Larson R, Snyder JR. In vivo and in vitro evaluation of the effects of domperidone on the gastrointestinal tract of healthy horses. Am J Vet Res. (2013) 74(8); 1103-1110

Notomi T, Shigemoto R. Immunohistochemical localization of Ih channel subunits, HCN1-4, in the rat brain. J Comp Neurol. (2004) 471(3); 241-276

Odaka T, Suzuki T, Seza A, Yamaguchi T, Saisho H. [Serotonin 5- HT_4 receptor agonist (mosapride citrate)]. Nihon Rinsho. (2006) 64(8); 1491-1494

Ohno N, Zhang G, Xue L, Yamamoto Y, Itoh M, Suzuki H. Excitation and inhibition by cisapride of spontaneous and evoked electrical responses in smooth muscles of guinea-pig stomach. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. (1995) 112(2); 145-152

Ordög T, Ward SM, Sanders KM. Interstitial cells of cajal generate electrical slow waves in the murine stomach. J Physiol. (1999) 518(Pt 1); 257

Parkman HP, Pagano AP, Vozzelli MA, Ryan JP. Gastrokinetic effects of erythromycin: myogenic and neurogenic mechanisms of action in rabbit stomach. Am J Physiol. (1995) 269(3 Pt 1); G418-426

Pittayanon R, Yuan Y, Bollegala NP, Khanna R, Lacy BE, Andrews CN, Leontiadis GI, Moayyedi P. Prokinetics for Functional Dyspepsia: A Systematic Review and Meta-Analysis of Randomized Control Trials. Am J Gastroenterol. (2019) 114(2); 233-243

Reddymasu SC, Soykan I, McCallum RW. Domperidone: review of pharmacology and clinical applications in gastroenterology. Am J Gastroenterol. (2007) 102(9); 2036-2045

Sanders KM. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology. (1996) 111(2); 492-515

Sanders KM, Ordög T, Koh SD, Torihashi S, Ward SM. Development and plasticity of interstitial cells of Cajal. Neurogastroenterol Motil. (1999) 11(5); 311-338

Sanders KM, Ward SM, Koh SD. Interstitial cells: regulators of smooth muscle function. Physiol Rev. (2014) 94(3); 859-907

Sanders KM, Koh SD, Ward SM. Interstitial cells of Cajal as pacemakers in the gastrointestinal tract. Annu Rev Physiol. (2006) 68; 307–343

Sanger GJ. Effects of metoclopramide and domperidone on cholinergically mediated contractions of human isolated stomach muscle. J Pharm Pharmacol. (1985) 37(9); 661-664

Sensoy I. A review on the food digestion in the digestive tract and the used in vitro models. Curr Res Food Sci. (2021) 4; 308-319

Shahi PK, Choi S, Zuo DC, Kim MY, Park CG, Kim YD, et al. The possible roles of hyperpolarization-activated cyclic nucleotide channels in regulating pacemaker activity in colonic interstitial cells of Cajal. Journal of Gastroenterology. (2014) 49(6); 1001–1010

Shokrollahi M, Chen JH, Huizinga JD. Intraluminal prucalopride increases propulsive motor activities via luminal 5-HT_4 receptors in the rabbit colon. Neurogastroenterol Motil. (2019) 31(10); e13598

Soffer EE. Small Bowel Dysmotility. Curr Treat Options Gastroenterol. (1998) 1(1); 8-14

Stanghellini V, Cogliandro RF, De Giorgio R, Barbara G, Cremon C, Antonucci A, Fronzoni L, Cogliandro L, Naponelli V, Serra M, Corinaldesi R. Natural history of intestinal failure induced by chronic idiopathic intestinal pseudo-obstruction. Transplant Proc. (2010) 42(1); 15-18

Strege PR, Ou Y, Sha L, Rich A, Gibbons SJ, Szurszewski JH, Sarr MG, Farrugia G. Sodium current in human intestinal interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol. (2003) 285(6); G1111

Peeters TL. Central and peripheral mechanisms by which ghrelin regulates gut motility. J Physiol Pharmacol. (2003) 4; 95-103

Tack J, Camilleri M, Chang L, Chey WD, Galligan JJ, Lacy BE, Müller-Lissner S, Quigley EM, Schuurkes J, De Maeyer JH, Stanghellini V. Systematic review: cardiovascular safety profile of 5-HT(4) agonists developed for gastrointestinal disorders. Aliment Pharmacol Ther. (2012) 35(7); 745-767

Torihashi S, Horisawa M, Watanabe Y. c-Kit immunoreactive interstitial cells in the human gastrointestinal tract. J Auton Nerv Syst. (1999) 75(1); 38-50

Tonini M, Cipollina L, Poluzzi E, Crema F, Corazza GR, De Ponti F. Review article: clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics. Aliment Pharmacol Ther. (2004) 19(4); 379-390

Ward SM, Beckett EA, Wang X, Baker F, Khoyi M, Sanders KM. Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. J Neurosci. (2000) 20; 1393-1403

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. The Journal of Physiology. (2009) 587(20); 4905–4918

6. ABSTRACT

Background: The importance of prokinetic agents on GI motility is well understood. However, there is no report the effects of prokinetic agents on ICC that is pacemaker cell in GI tract and produces slow waves of SMC. Therefore, I studied the effects of prokinetic agents on pacemaker activity in stomach, colon and small intestine from mouse.

Method: In order to draw results from this research, we cultured ICC from stomach, colon and small intestine of ICR mice and performed whole cell patch clamp to record pacemaker potentials of ICC and compare the difference between them.

Results: Under control condition, ICC of stomach and small intestine showed spontaneous consistent and rhythmic pattern of pacemaker activity. Although ICC of colon generated irregular and slow pacemaker activity, the pacemaker activity of small intestinal ICC was regular and more faster than colonic ICC. The pattern of pacemaker activity in stomach is very similar with colonic ICC. The exposure of cisapride in small, colonic and stomach ICC generated depolarization of membrane potentials of ICC and only frequency of pacemaker activity in colonic ICC was increased. Interestingly, mosapride showed inhibitory action in colonic ICC. The action of metoclopramide, prucalopride or domperidone was similar with cisapride. Furthermore, only mosapride action on pacemaker activity was inhibited by 5-HT_4 receptor antagonist.

Conclusion: Prokinetic agents are important for treatment of patients for improving GI symtoms. These results provide critical background how prokinetic agents act on pacemaker activity of ICC.

7. ACKNOWLEDGEMENT

This research was conducted at the Department of Physiology, Chosun University.

First and foremost, I extend my sincere gratitude to my supervisor, Professor Seok Choi, for his invaluable guidance and unwavering support throughout my doctoral journey, which ultimately led to the successful completion of my Ph.D. I am especially thankful for his enthusiastic and insightful supervision during the execution of my doctoral project.

Secondly, I wish to express my appreciation to Jae Yeoul Jun, the department manager. His expert advice and prompt solutions have been instrumental in overcoming various challenges encountered in my research. I'd also like to acknowledge Professor Hong, who, although not directly affiliated with my research group, has consistently shown a caring interest in both my experiments and personal well-being.

Furthermore, I am deeply indebted to all the members of our laboratory. Their collective efforts and knowledge-sharing have significantly enriched my research and personal growth. I consider myself fortunate to have had the privilege of working alongside such dedicated and supportive colleagues.

Lastly, I would like to extend my heartfelt thanks to my parents, friends, and everyone who has provided unwavering support and encouragement throughout this academic journey. Your belief in me has been a source of inspiration and motivation.

Thank you wholeheartedly!