2008 年 2 月
博士學位論文

생쥐 소장에서 분리된 Interstitial Cells of Cajal에 대한 Prostaglandin $F_{2\alpha}$의 작용 기전

朝鮮大學校 大學院
醫 學 科
金 相 勳

생쥐 소장에서 분리된 Interstitial Cells of Cajal에 대한 Prostaglandin $F_{2\alpha}$의 작용 기전

Effects of Prostaglandin $F_{2\alpha}$ on Pacemaker Activity in Interstitial Cells of Cajal from Murine Small Intestine

2008 年 2 月

朝鮮大學校 大學院

醫 學 科

金 相 勳

생쥐 소장에서 분리된 Interstitial Cells of Cajal에 대한 Prostaglandin $F_{2\alpha}$의 작용 기전

指導敎授 소 금 영

이 論文을 醫學博士學位 論文으로 提出함

2007 年 10 月

朝鮮大學校 大學院

醫 學 科

金 相 勳

金 相 勳의 博士學位論文을 認准함

委員長　朝鮮大學校　　敎授　　전 제 열

委　員　朝鮮大學校　　敎授　　정 종 달

委　員　朝鮮大學校　　敎授　　조 남 수

委　員　朝鮮大學校　　敎授　　장 인 엽

委　員　朝鮮大學校　　敎授　　소 금 영

2008 年　　2 月

朝鮮大學校 大學院

목 차

국 문 초 록

생쥐 소장에서 분리된 Interstitial Cells of Cajal에 대한 Prostaglandin $F_{2\alpha}$의 작용 기전

김 상 훈
지도교수 : 소 금 영
조선대학교 대학원 의학과

위장관 운동성에 대한 prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)의 기능은 지금까지 평활근 수준으로 많이 연구가 이루어져 있지만, 위장관의 향도잡이 세포의 역할을 담당하는 Interstitial cells of Cajal (ICC)에 대한 $PGF_{2\alpha}$의 연구는 아직까지 이루어지지 않고 있다. 따라서 본 연구는 세포막 고정법을 이용하여 ICC의 향도잡이 활동도에 대한 $PGF_{2\alpha}$의 영향에 대하여 연구하고자 하였다. 전류고정 상태에서 $PGF_{2\alpha}$는 세포막의 탈분극을 야기시키고, 전압고정 상태에서 긴장성 내향성 전류를 발생시켰다. ICC에 Flufenamic acid 또는 niflumic acid 처리시 ICC에서 발생하는 향도잡이 전류는 소멸되었고, flufenamic acid만이 $PGF_{2\alpha}$에 의해 유도되는 긴장성 내향성 전류를 억제시켰다. 또한 세포내 GDPβS 처리한 결과 역시 $PGF_{2\alpha}$에 의해 유도되는 긴장성 내향성 전류에 큰 영향을 주지 못하였다. ICC에 Ca^{2+}-free 용액, U-73122, thapsigargin을 전처리시 향도잡이 전류가 사라지는 것을 알 수 있었고, 이 상태에 $PGF_{2\alpha}$를 동시 투여하면 $PGF_{2\alpha}$에 의해 유도되는 긴장성 내향성 전류가 억제되는 것을

보여주었다. 그러나 chelerythrine과 calphostin C는 $PGF_{2\alpha}$에 의해 유도되는 긴장성 내향성 전류에 큰 영향을 주지 못하였다. Ca^{2+} 이온 indicator인 Fluo-3를 이용한 세포내 칼슘이온에 대한 실험에서는 $PGF_{2\alpha}$에 의해 유도되는 긴장성 내향성 전류가 세포내의 칼슘이온의 증가와 밀접한 관계가 있는 것으로 밝혀졌다.

이러한 결과들은 $PGF_{2\alpha}$가 ICC에서 발생되는 향도잡이 활동도를 조절할 수 있다는 것을 제시해주며, ICC는 $PGF_{2\alpha}$의 작용을 위한 하나의 표적이 될 수 있다는 것을 제시해준다. 또한 ICC와 $PGF_{2\alpha}$의 상호작용은 장관 운동성에 영향을 미칠 수 있다는 것을 시사한다.

I. Introduction

Prostaglandins (PGs) of the E, F, and I series are widely distributed in all over body tissues, including the gastrointestinal (GI) tract, and have been shown to affect water and electrolyte transport, mucous secretion, blood flow in GI tract (Bennett et al., 1981; Fulgratt et al., 1974; Robert, 1981; Sanders and Northrup, 1983). Also, there are many evidences that PGs may be involved in the control of the contraction of intestinal smooth muscle. Inhibition of endogenous PGs synthesis by PGs inhibitor, indomethacin, appears to enhance intestinal motility by inducing a fedlike pattern (Karim et al., 1967; Karim et al., 1968). This study suggests that endogenous prostaglandins may play a role in the modulating of intestinal motility (Bennett and Flescher, 1970; Waller, 1973). In generally, PGE_2 is known to contract intestinal longitudinal muscle and to relax circular muscle and $PGF_{2\alpha}$ increases contractility of circular and longitudinal muscle in various animal species by studying in vitro. In addition to, vivo studies have shown that $PGF_{2\alpha}$ increases contractility of the canine small intestine (Hawley and Rampton, 1985; Pierce et al., 1971).

The interstitial cells of Cajal (ICC) have functions as pacemaking

and neuromediator in the tunica muscularis of the GI tract (Sanders, 1996). The ICC generate the rhythmic oscillations in membrane potential known as slow wave potentials and this generation of slow wave potentials is due to spontaneous inward currents called pacemaker currents (Ward et al., 1994; Ward et al., 1995). Although the exact mechanisms about these events still unclear, many reports suggested that the activation of non-selective K^+ channels, Cl^- channel, and spontaneous intracellular Ca^{2+} activities in ICC involve the producing of pacemaker activity (Koh et al., 1998; Thomsen et al., 1998). Morethan above, many endogenous agents such as neurotransmitters, hormones, and paracrine substances play a role in modulating GI tract motility by way of influencing ICC.

No studies have been performed to determine the effects of $PGE_{2\alpha}$ on electricalevents in ICC. Therefore, the purpose of our study was to investigate the actions of $PGE_{2\alpha}$ on pacemaker currents in cultured ICC.

II. Materials and Methods

Preparation of cells

Balb/C mice (8-13 days old) of either sex were anesthetized with ether and sacrificed by cervical dislocation. The small intestines from 1 cm below the pyloric ring to the cecum were removed and opened along the mesenteric border. Luminal contents were washed away with Krebs-Ringer bicarbonate solution. The tissues were pinned to the base of Sylgard dish and the mucosa removed by sharp dissection. Small stripes of intestinal muscle were equilibrated in Ca^{2+}-free Hank's solution containing (in mM) KCl, 5.36; NaCl, 125; NaOH, 0.336; Na_2HCO_3, 0.44; glucose, 10; sucrose, 2.9 and HEPES, 11 adjusted to pH 7.4 with tris for 30 min and after incubation for 15 min at 37 C with an enzyme solution containing collagenase (Worthington Biochemical Co, Lakewood, NJ, USA), 1.3 mg/ml, bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA), 2 mg/ml, trypsin inhibitor (Sigma), 2 mg/ml and ATP, 0.27 mg/ml, cells were dispersed. Cells were plated onto sterile glass coverslips coated with murine collagen (2.5 g/ml, Falcon/BD) in 35 mm culture dishes. The cells were then cultured at 37 C in a 95 % O_2-5 % CO_2 incubator in SMGM (smooth muscle growth medium, Clonetics Corp., San Diego, CA, USA) supplemented with 2 % antibiotics/antimycotics

(Gibco, Grand Island, NY, USA) and murine stem cell factor (SCF, 5 ng/ml, Sigma). Interstitial cells of Cajal (ICC) were identified immunologically with a monoclonal antibody for Kit protein (ACK_2) labelled with Alexa Fluor 488 (Molecular Probes, Eugene, OR, USA).

Patch clamp experiments

The whole-cell configuration of the patch-clamp technique was used to record membrane currents (voltage clamp) and membrane potentials (current clamp) from cultured ICC. Currents or potentials were amplified by Axopatch 1-D (Axon Instruments, Foster, CA, USA). Command pulse was applied using an IBM-compatible personal computer and pClamp software (version 6.1; Axon Instruments). The data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor, and a pen recorder (Gould 2200, Gould, Vally view, OF, USA). Results were analyzedusing pClamp and Graph Pad Prism (version 2.01) software. All experiments were performed at 30 C.

Measurement of intracellular Ca^{2+} concentration

Changes intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were monitored by using fluo-3/AM, which was initially dissolved in dimethyl sulfoxide and stored at -20 °C. The cultured ICC on coverslip (25 mm) were

rinsed twice with a bath solution [in mM: KCl 5, NaCl 135, $CaCl_2$ 2, glucose 10, $MgCl_2$ 1.2 and HEPES 10, adjusted to pH 7.4 with tris], incubated in the bath solution containing 5 μM fluo-3/AM with 5% CO_2-95% O_2 at 37 C for 5 min, rinsed two more times with the bath solution, mounted on a perfusion chamber, and scanned every 0.4 second with a confocal microscope (200× fluoviews 300, Olympus). Fluorescence was excited at 488 nm, and emitted light was observed at 515 nm. During scanning of Ca^{2+} imaging, the temperature of the perfusion chamber containing the cultured ICC was kept at 30 °C. The variations of intracellular Ca^{2+} fluorescence emission intensity were expressed as F1/F0 that F0 means the intensity of first imaging.

Solutions and drugs

The cells were bathed in a solution containing (mM) : KCl, 5; NaCl, 135; $CaCl_2$, 2; glucose, 10; $MgCl_2$, 1.2 and HEPES, 10 adjusted to pH 7.2 with tris. The pipette solution contained (mM): K-aspartate, 120; KCl, 20; $MgCl_2$, 5; K_2ATP, 2.7; Na_2GTP, 0.1; creatine phosphate disodium, 2.5; HEPES, 5; EGTA, 0.1 adjusted to pH 7.2 with tris.

Drugs used were: Prostaglandin $F_{2\alpha}$, Guanosine 5'-[-thio]diphosphate trilithium salt (GDPβS), U-73122, calphostin C, chelethrine, and thapsigargin. All drugs were purchased from the

sigma chemical co. Flufenamic acid and niflumic acid were purchased from the Calbiochem.

Statistical analysis

Data were expressed as means standard errors. Differences in the data were evaluated by Student's t test. A P values less than 0.05 were taken as a statistically significant difference. The n values reported in the text refer to the number of cells used in patch-clamp experiments.

III. Results

Spontaneous activity in interstitial cells of Cajal

First, ICC cultured from the murine small intestine were identified with the Kit immunofluroescence. Because cells with Kit positive had a distinctive morphology, ICC was easily selected out of the murien cultured cells (Jun et al., 2005). Recording from cultured ICC under current clamp mode ($I=0$) showed spontaneously pacemaker potentials. The resting membrane potential was - 53 ± 3 mV and amplitude was 23 ± 5 mV (n = 5, Fig. 1A). Converting the amplifier to voltage clamp mode at a holding potential -70 mV, ICC generated spontaneous inward currents called 'pacemaker currents'. The average frequency of the currents was 14 ±2 cycles/min and the amplitude averaged - 436 ±62 pA (n = 8, Fig. 1B).

Effect of $PGF_{2\alpha}$ on pacemaker potentials and currents in ICC

I performed the electrophysiolgical recording from cultured kit labelled ICC under current ($I=0$) and voltage clamp mode. Under current clamp mode, ICC showed spontaneously pacemaker potentials. The resting membrane potential was -53 ±4 mV and amplitude was 27 ±2 mV. In presence of $PGF_{2\alpha}$ (10 μM), membrane potentials were depolarized to -29

± 3.4 mV and the amplitude of pacemaker potentials was decreased to 3.9 ± 1.6 mV (n=5, Fig. 2A, bar graph not shown). Under a voltage clamp mode at a holding potential -70 mV, spontaneous pacemaker currents was generated. Adding $PGF_{2\alpha}$ (10 μM) produced tonic inward currents and decreased the frequency and amplitude of pacemaker currents (Fig. 2B). These results are in agreement with the previous study that ICC have the spontaneous pacemaker activity. We could find the effect of $PGF_{2\alpha}$ have action on electrical activity of ICC. In addition, we examined the effects of $PGF_{2\alpha}$ on pacemaker currents with various concentrations of $PGF_{2\alpha}$, and found that $PGF_{2\alpha}$ generated the tonic inward currents in a dose dependent manner (Fig. 2B-D).

Effect of non-selective cation channel blocker or Cl^- channel blocker in $PGF_{2\alpha}$-induced responses in cultured ICC

For characterizing of the tonic inward currents by $PGF_{2\alpha}$, we used flufenamic acid, a non-selective cation channel blocker and niflumic acid, a Cl^- channel blocker. Figure 3A shows that pacemaker currents were stopped by the treatment of flufenamic acid (10 μM) and then $PGF_{2\alpha}$-induced action on pacemaker currents was disappeared by flufenamic acid. The summarized bar graph (Fig. 3C) also indicates that the resting currents produced by $PGF_{2\alpha}$ were -21 ±9 pA in presence

of flufenamic acid, which were significantly different when comparing with omitting flufenamic acid (n=4). In the presence of the application of niflumic acid (10 μM), the pacemaker currents also were abolished. In this condition, $PGF_{2\alpha}$ still produced tonic inward currents (Fig. 3B). In the presence of niflumic acid, the resting currents produced by $PGF_{2\alpha}$ were -98 ± 12 pA, this value was not significantly different when compared with the control values obtained in the absence of niflumic acid (n=4, Fig. 3D).

No involovement of G proteins in $PGF_{2\alpha}$- induced tonic inward currents in cultured ICC

The effects of GDPβS, a nonhydrolysable guanosine 5'-diphosphate analogue, which permanently inactivates GTP binding proteins, were examined to determine whether the G-protein is involved in the effects of $PGF_{2\alpha}$ in ICC. When GDPβS (1 mM) was in the pipette, $PGF_{2\alpha}$ (10 μM) still showed the tonic inward currents (Fig. 4A). In the presence of GDPβS in the pipette, the resting currents were -23 ± 9 pA, respectively. In application of $PGF_{2\alpha}$, the resting currents produced by $PGF_{2\alpha}$ were -159.9 ±36 pA, respectively (n = 4, Fig. 4B).

External Ca^{2+}-free solution and Ca^{2+}-ATPase inhibitor of endoplasmic reticulum suppress $PGF_{2\alpha}$ action in cultured ICC

To investigate the role of external Ca^{2+} or internal Ca^{2+}, $PGF_{2\alpha}$ was tested under external Ca^{2+}-free conditions and in the presence of thapsigargin, a Ca^{2+}-ATPase inhibitor of endoplasmic reticulum. The application of external Ca^{2+}-free solution completely inhibited the pacemaker currents in voltage clamp mode at a holding potential of -70 mV and in this condition, $PGF_{2\alpha}$ (10 μM)-induced action on pacemaker currents were blocked (n = 5, Fig. 5A). The value of resting currents with $PGF_{2\alpha}$ (10 μM) in Ca^{2+}-free solution was significantly different when compared in normal solution (Fig. 5C). Also, the treatment of thapsigargin (5 μM) inhibited the pacemaker currents and $PGF_{2\alpha}$ action in ICC (Fig. 5B). In the presence of thapsigargin, the value of resting currents with $PGF_{2\alpha}$ was significantly different when compared in the absence of thapsigargin (n=4, Fig. 5D).

Effects of phospholipase C inhibitor on $PGF_{2\alpha}$ -induced tonic inward currents in cultured ICC

Since the tonic inward currents by $PGF_{2\alpha}$ was related with intracellular Ca^{2+} mobilization, it is likely that the production of $PGF_{2\alpha}$ effects on pacemaker currents require phospholipase C (PLC)

activation. To test this possibility, $PGF_{2\alpha}$-induced tonic inward currents were measured in the absence and presence of the U-73122, an active PLC inhibitor. Pacemaker currents recorded at a holding potential of -70 mV were completely abolished by application of U-73122 (5 μM) and $PGF_{2\alpha}$ (10 μM)-induced tonic currents were suppressed (n=4, Fig. 6A). The value of resting currents by $PGF_{2\alpha}$ was significantly different when compared with $PGF_{2\alpha}$ in the absence of U-73122 (n=5, Fig. 6B). These results show that PLC inhibitor blocked the $PGF_{2\alpha}$-induced tonic inward currents in ICC.

Effects of protein kinase C inhibitor in $PGF_{2\alpha}$-induced responses in cultured ICC

I tested the effects of chelerythrine or calphostin C, an inhibitor of protein kinase C, to investigate whether $PGF_{2\alpha}$-induced pacemaker currents response are mediated by the activation of protein kinase C. Chelerythrine (1 μM) or calphostin C (10 μM), did not have an effect on tonic inward currents by $PGF_{2\alpha}$ (10 μM) (Fig. 7A and B) and the value also was not significantly different when compared with tonic inward currents by $PGF_{2\alpha}$ obtained in the absence of chelerythrine or calphostin C (n=5, Fig. 7C and D).

Increasing of $[Ca^{2+}]_i$ intensity by $PGF_{2\alpha}$

Because many reports suggested $[Ca^{2+}]_i$ oscillations in ICC are considered to be the primary mechanism for the pacemaker activity in GI activity, I examined the effect of $PGF_{2\alpha}$ on $[Ca^{2+}]_i$ oscillations in ICC. In this study, I measured spontaneous $[Ca^{2+}]_i$ oscillations of ICC which are connected with cell clusters. Spontaneous $[Ca^{2+}]_i$ oscillations observed in many ICC (low magnification not shown) under the loading with fluo3-AM (Fig. 8A). And the data of time series are showed the spontaneous regular $[Ca^{2+}]_i$ oscillations (Fig. 8B). And in the presence of $PGF_{2\alpha}$ (10 μM), the basal points of $[Ca^{2+}]_i$ oscillations were increased but the peak points of $[Ca^{2+}]_i$ oscillations were slightly decreased (Fig. 9A). The data of time series are summarized in Fig. 9B. These results suggest that the action of $PGF_{2\alpha}$ on ICC may involve the regulation of spontaneous $[Ca^{2+}]_i$ oscillations.

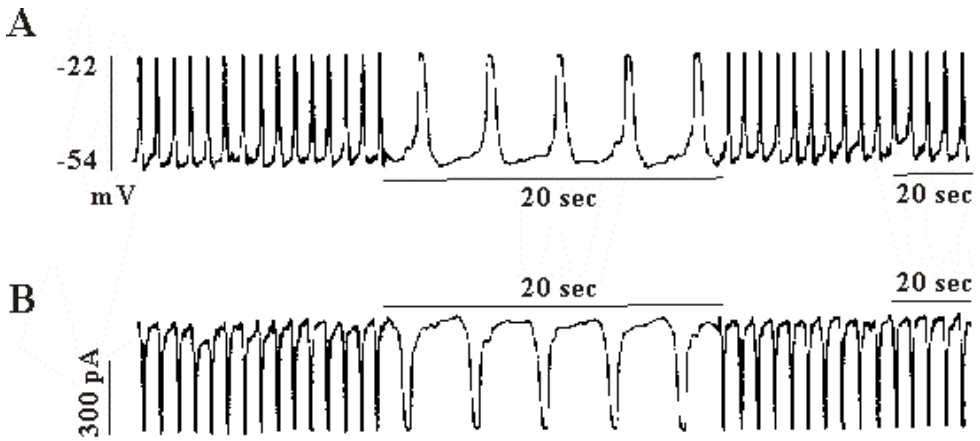

Fig 1. Typical trace of pacemaker potentials in current clamping mode (A) spontaneous pacemaker currents in voltage clamping mode recorded at a holding potential of -70 mV (B) in cultured ICC from murine small intestine.

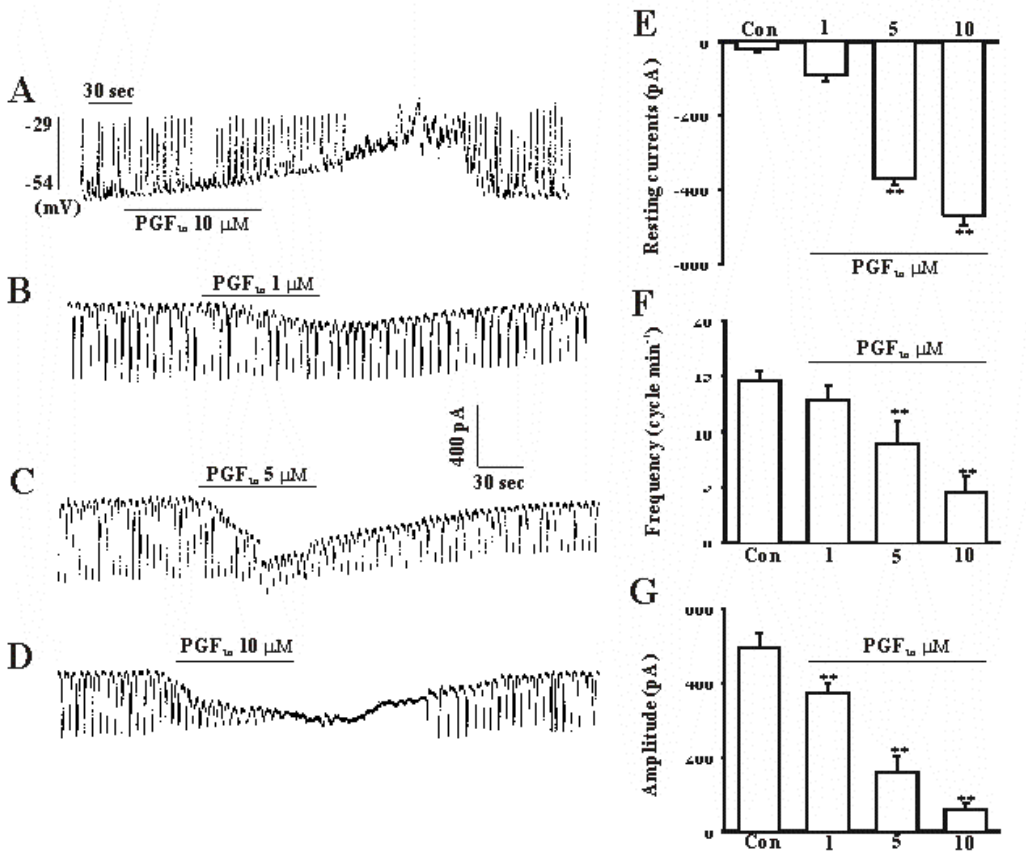

Fig 2. Effects of $PGF_{2\alpha}$ on pacemaker potentials and pacemaker currents recorded of the cultured ICC from the murine small intestine. (A)shows the pacemaker potentials of ICC exposed to $PGF_{2\alpha}$ (10 μM) in the current clamping mode (/=0). (B), (C), and (D) show the pacemaker currents of ICC recorded at a holding potential of -70 mV exposed to various concentrations of $PGF_{2\alpha}$ (1, 5, and 10 μM). The responses to $PGF_{2\alpha}$ are summarized in (E), (F) and (G). The bars represent mean values ± SE. **(P<0.01) significantly different from the untreated control. Con: control.

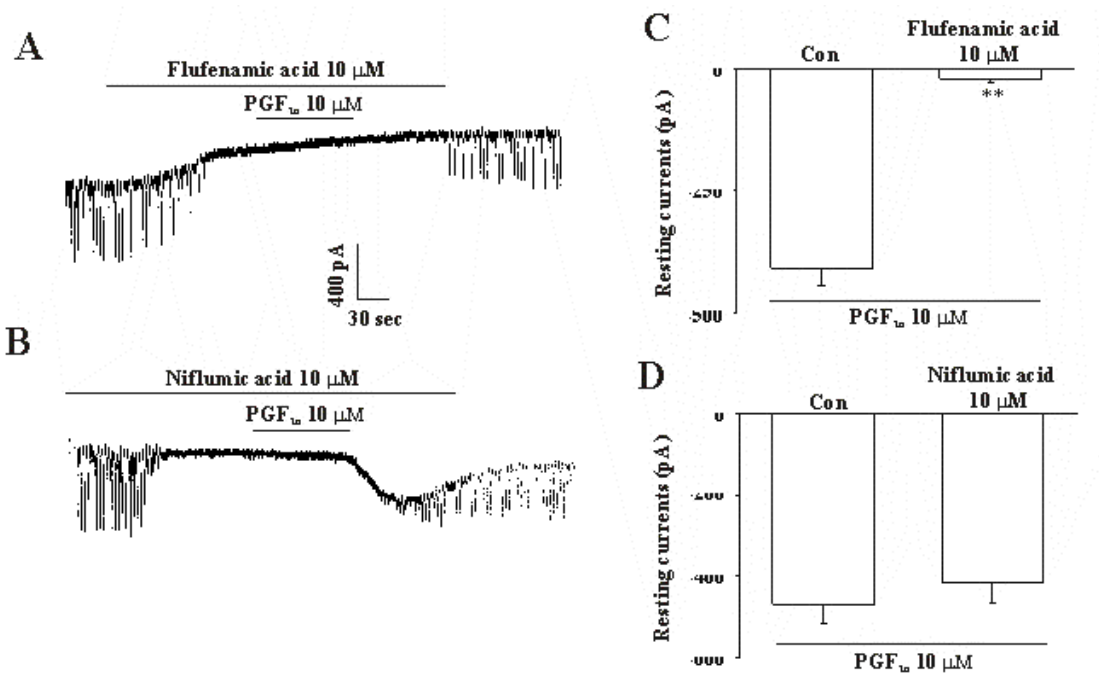

Fig 3. Effects of flufenamic acid, a nonselective cation channel blocker, or niflumic acid, a Cl^- channel blocker, on $PGF_{2\alpha}$-induced responses on pacemaker currents of the cultured ICC of the murine small intestine. (A) The application of flufenamic acid (10 μM) abolished the generation of the pacemaker currents. Under these conditions, $PGF_{2\alpha}$ (10 μM) did not produce tonic inward currents. (B) Niflumic acid (10 μM) also abolished the generation of the pacemaker currents. However, niflumic acid did not block the $PGF_{2\alpha}$ (10 μM)-induced tonic inward currents. Responses to $PGF_{2\alpha}$ in the presence of flufenamic acid or niflumic acid are summarized in (C) and (D). The bars represent mean values ± SE. **(P<0.01) significantly different from the untreated control. Con: control.

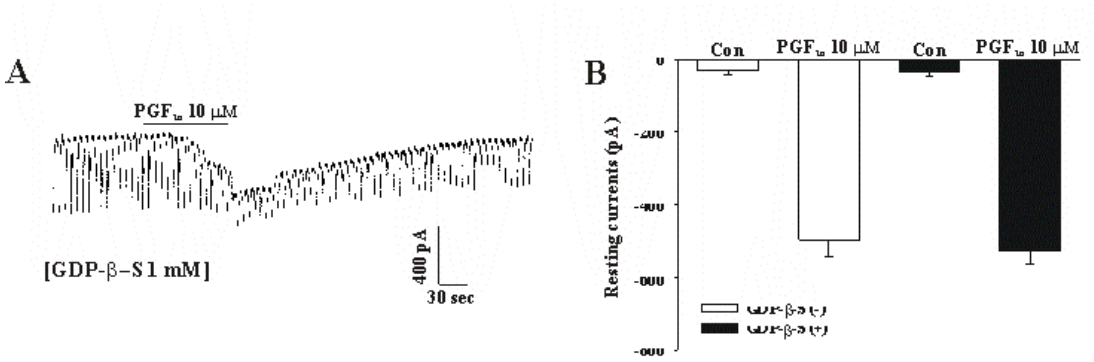

Fig 4. Effects of GDPβS on the response to $PGF_{2\alpha}$. (A) Pacemaker currents of ICC exposed to $PGF_{2\alpha}$ (10 μM) in presence of GDPβS (1 mM) in the pipette. The effects of $PGF_{2\alpha}$ in the presence of GDPβS are summarized in (B). The bars represent mean values ± SE. **(P<0.01) significantly different from the untreated control. Con: control.

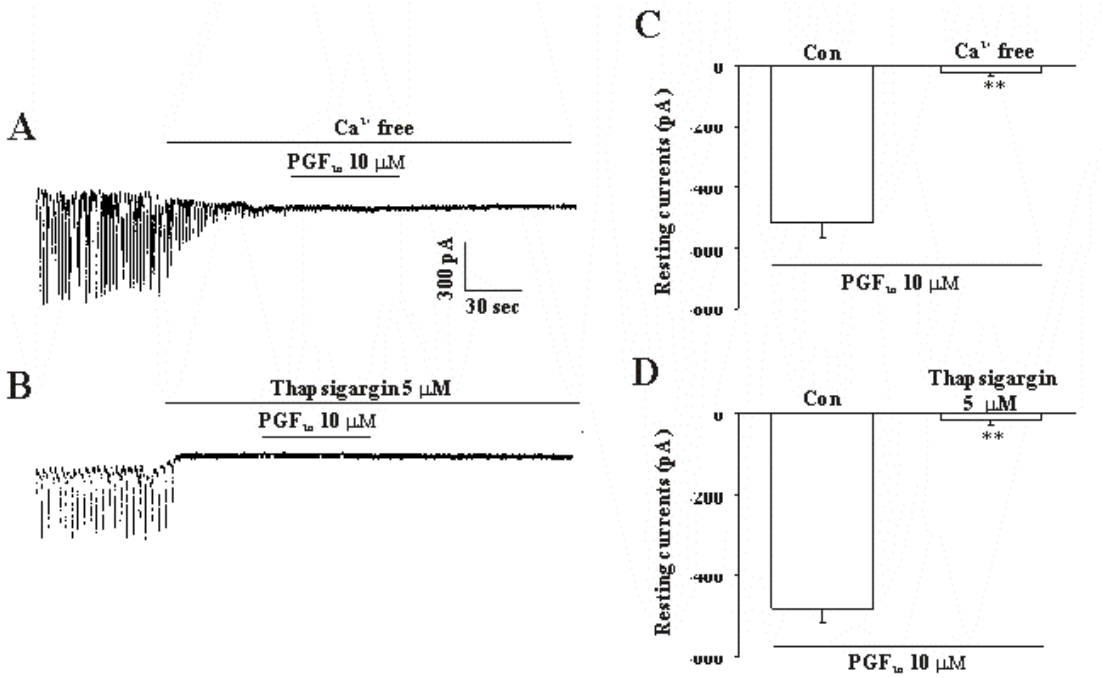

Fig 5. Effects of an external Ca^{2+}-free solution or thapsigargin, a Ca^{2+}-ATPase inhibitor of endoplasmic reticulum, on $PGF_{2\alpha}$-induced response on pacemaker currents of the cultured ICC of the murine small intestine. (A) The external Ca^{2+}-free solution abolished the generation of pacemaker currents. Under this condition, $PGF_{2\alpha}$ (10 μM)-induced tonic inward currents were blocked. (B) Thapsigargin (5 μM) abolished the generation of pacemaker currents. Thapsigargin also blocked $PGF_{2\alpha}$ (10 μM)-induced tonic inward currents. Responses to $PGF_{2\alpha}$ in the external Ca^{2+}- free solution and in the presence of thapsigargin are summarized in (C) and (D). The bars represent mean values ± SE. **($P<0.01$) significantly different from the untreated control. Con: control.

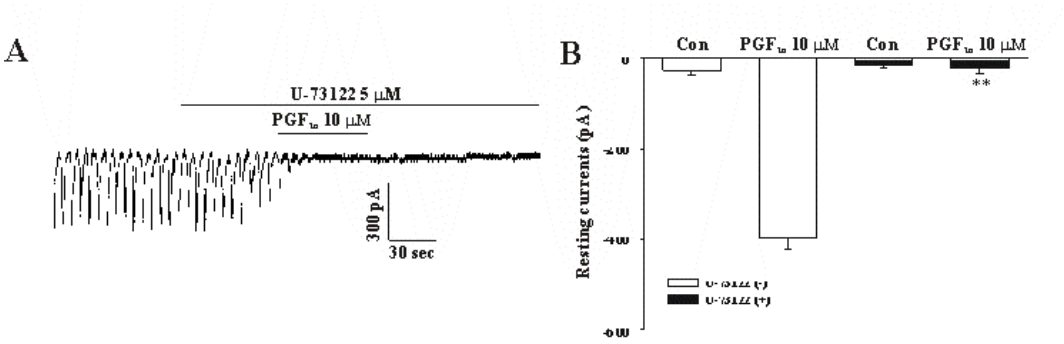

Fig 6. Effects of U-73122, an active phospholipase C inhibitor, on $PGF_{2\alpha}$-induced response on pacemaker currents of the cultured ICC of the murine small intestine. (A) U-73122 (5 μM) abolished the generation of pacemaker currents. U-73122 also blocked $PGF_{2\alpha}$ (10 μM)-induced tonic inward currents. Responses to $PGF_{2\alpha}$ in the presence of $PGF_{2\alpha}$ are summarized in (B). The bars represent mean values ± SE. **(P<0.01) significantly different from the untreated control. Con: control.

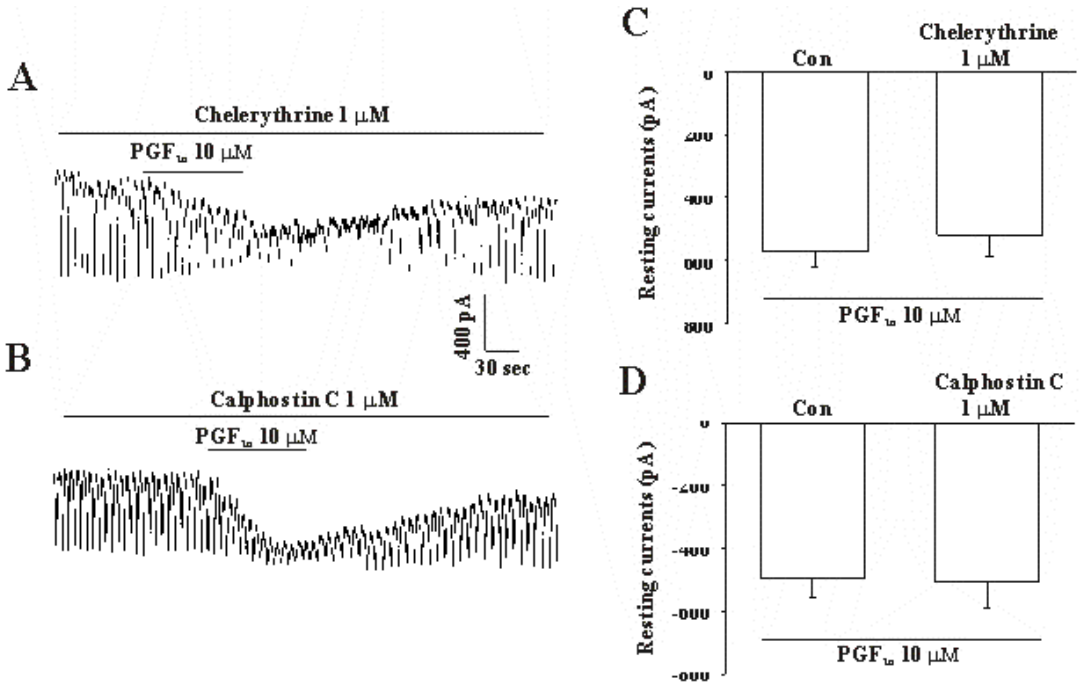

Fig 7. Effects of chelerythrine or calphostin C, inhibitors of protein kinase C, on $PGF_{2\alpha}$-induced response on pacemaker currents of the cultured ICC of the murine small intestine. (A), (B) Pacemaker currents of ICC exposed to $PGF_{2\alpha}$ (10 μM) in the presence of chelerythrine (1 μM) or calphostin C (10 μM). In this condition, $PGF_{2\alpha}$ caused tonic inward currents. Responses to $PGF_{2\alpha}$ in the presence of chelerythrine or calphostin C are summarized in (C) and (D). The bars represent mean values ± SE. **(P<0.01) significantly different from the untreated control. Con: control.

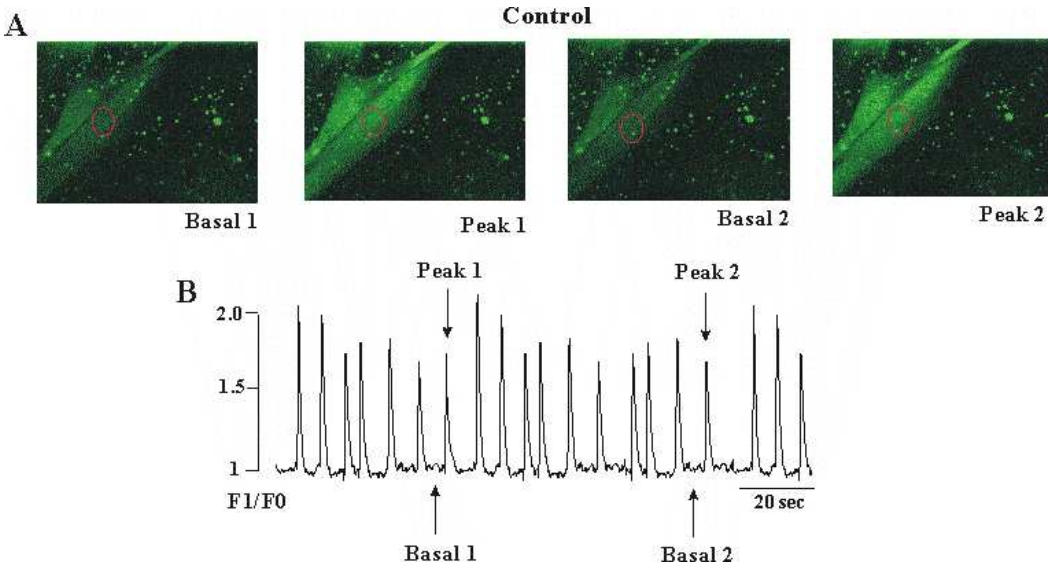

Fig 8. Spontaneous intracellular Ca^{2+} oscillation in cultured ICC from murine small intestine. (A) Sequential fluorescence intensity images of fluo-3-loaded cultured ICC in normal condition. The images in (A) acquired at basal (1 and 2) and peak (1 and 2) points of Ca^{2+} oscillations in control condition. The image of basal (1 and 2) and peak (1 and 2) points in (A) acquired from indicators in (B). (B) Fluorescence intensity change plotted in (A) red marker.

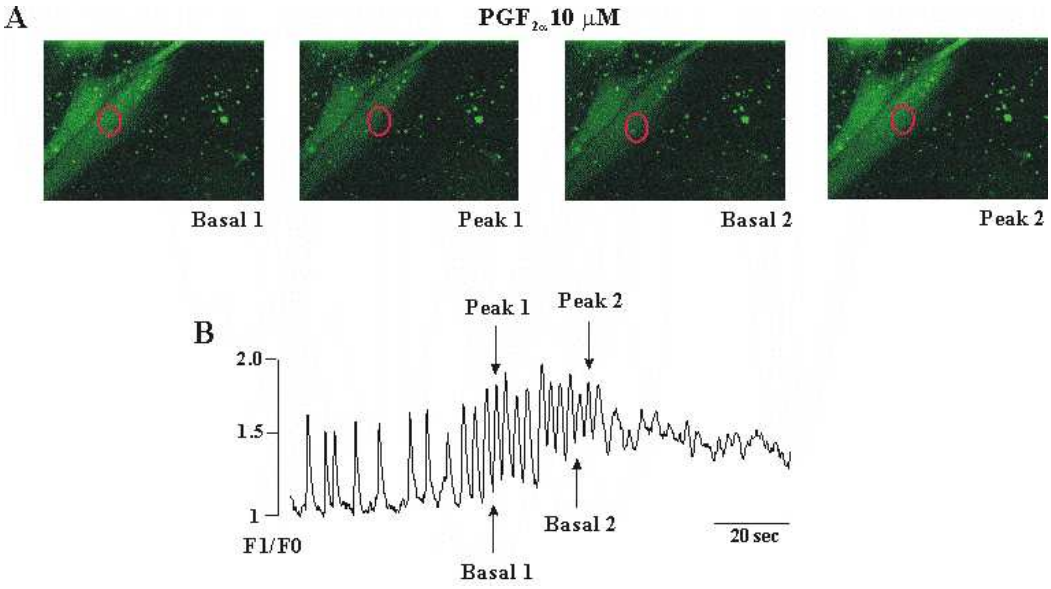

Fig 9. The effects of $PGF_{2\alpha}$ on intracellular Ca^{2+} oscillation in cultured ICC from murine small intestine. (A) Sequential fluorescence intensity images of fluo-3-loaded cultured ICC in presence of $PGF_{2\alpha}$. The images in (A) acquired at basal (1 and 2) and peak (1 and 2) points of Ca^{2+} oscillations in presence of $PGF_{2\alpha}$. The image of basal (1 and 2) and peak (1 and 2) points in (A) acquired from indicators in (B). (B) Fluorescence intensity change plotted in (A) red marker.

IV. Disucussion

Although the actions of $PGF_{2\alpha}$ has been demonstrated about the gastrointestinal (GI) motiliy in smooth muscle cells, this is the first study in interstitial cells of Cajal (ICC) in which an attempt has been made to determine the effects of $PGF_{2\alpha}$ on electrical activity in small intestine.

The results of the present study demonstrate that $PGF_{2\alpha}$ regulates intestinal motility by modulating the pacemaker currents of the in ICC, and this modulation is mediated via acting on non-selective cation channels and intracellular Ca^{2+} mobilization in a protein kinase C-independent manner.

Most regions of the GI tract generate spontaneous electrical and mechanical activity without stimulation. When electrical recordings are made from smooth muscle cells lying in the GI tract, a regular discharge of long lasting waves of depolarization, called slow waves, is detected. It has recently become apparent that slow waves are generated by a specialized population of smooth muscle cells, known as ICC (Szurszewski 1987). ICC generate spontaneous pacemaker inward currents that depolarize membrane, this spread to smooth muscle via gap junctions resulting in depolarization of membrane in smooth muscle

leads to contraction by generating acting potential through voltage dependent Ca^{2+} channel activation (Farrugia, 1999). In previous studies, many reports suggested that $PGF_{2\alpha}$ usually showed contractile actions in vivo and vitro studies (Walus et al., 1981). These reports indicate the possibility that $PGF_{2\alpha}$ may have stimulatory functions on electrical activity of ICC. Actually, I demonstrated find that ICC produced spontaneous pacemaker inward currents under voltage clamp mode and the application of $PGF_{2\alpha}$ evoked the tonic inward currents of pacemaker currents. These results offer the new suggestion that the regulation of electrical activity in ICC may be involved in the contractile effects of $PGF_{2\alpha}$ in GI tract.

Until now, the exact mechanism of pacemaker activity generation is still not yet fully understood in ICC. Two suggestions that pacemaker currents of ICC are mediated by the activation of voltage-independent non-selective cation channels and inwardly rectifying Cl^- currents can be generated the rhythmic inward currents, are existed (Koh et al., 1998; Thomsen et al., 1998; Huizinga et al., 2002). For checking of these suggestions, we used the blockers of non-selective cation and inwardly rectifying Cl^- channels, so I could find that flufenamic acid abolished pacemaker currents generation and $PGF_{2\alpha}$-induced tonic inward currents were blocked by flufenamic acid. Although niflumic acid, a Cl^-

channel blocker, abolished pacemaker generation, but did not block the $PGF_{2\alpha}$-induced tonic inward currents. Therefore, these data strongly provide support for the suggestion above described, and it is likely that both Cl^- channels and non-selective cation channels contribute to the generation of the spontaneous pacemaker currents in ICC but $PGF_{2\alpha}$ only have function on regulation of pacemaker currents by regulating of nonselective cation channels in ICC.

The effects of prostaglandins are mediated by specific receptors, classified into basic types (DP, EP, FP, IP, and TP) according to the for prostaglandin ligand binding affinity (LeDuc and Needleman, 1979; Waller, 1973). They have different cell- and tissue-specific functions, as determined by selective coupling to G proteins and by the expression of splicing isoforms. In case of $PGF_{2\alpha}$, $PGF_{2\alpha}$ induce inositol (1,4,5) trisphosphate (IP_3) production and increases in Ca^{2+} levels via FP receptors, which are coupled to G proteins for functional actions in various tissue. For example, $PGF_{2\alpha}$ induces PI turnover in isolated luteal cells and the effect of $PGF_{2\alpha}$ to induce Ca^{2+} mobilization in murine fibroblasts occurs in conjunction with formation of IP_3 and is pertussis toxin insensitive (Athari and Jungermann, 1989; Harks et al., 2003). These findings suggest that FP receptors can interact with a member of the G protein family to

activate phospholipase C, leading ultimately to an IP_3 mediated mobilization of Ca^{2+} from intracellular pools. However, there also appears to be a secondary phase of Ca^{2+} release by $PGF_{2\alpha}$ treated murine fibroblasts that are likely due to extracellular Ca^{2+} entry. In this study, when GDPβS was present in the pipette, the tonic inward currents of pacemaker currents by $PGF_{2\alpha}$ still were present. This means that the effects of $PGF_{2\alpha}$ on electrical activity of ICC may be not related with G proteins. Because many suggestions have been mentioned that $PGF_{2\alpha}$ may have biological activity through mobilization of intracellular Ca^{2+} and PLC activation, we used thapsigargin, a potent endoplasmic reticulum Ca^{2+}-ATPase inhibitor and U-73122, an active PLC inhibitor, and found that those inhibitors suppress the $PGF_{2\alpha}$-induced tonic inward currents. These results suggest that both the release of Ca^{2+} from internal storage and the PLC activation by $PGF_{2\alpha}$ are essential to produce tonic inward currents. Also, these findings have correspondence with previous suggestions. It is well known that the generation of pacemaker currents was dependent upon intracellular Ca^{2+} oscillation and the periodic release of Ca^{2+} from endoplasmic reticulum is essential for generating pacemaker currents. Namely, we thought that our experiments using thapsigargin and U-73122 establish the fact of these suggestions.

Generally, it is well known that the binding of $PGF_{2\alpha}$ to its receptor results in the activation of PLC and activation of PLC is the initial step of the phosphoinositide cascade that generates the diacylglycerol, IP_3, and Ca^{2+} release (Hunt et al., 1975; Ishizawa and Miyazaki, 1975). The increase in intracellular Ca^{2+} not only promotes translocation of some protein kinase C (PKC) isozymes to the plasma membrane, but in concert with DAG, is essential in activating the conventional isoforms of PKC. However, in present study, chelerythrine or calphostin C, protein kinase C inhibitors, did not block $PGF_{2\alpha}$ -induced effects, suggesting that protein kinase C is not involved on the actions of $PGF_{2\alpha}$ in ICC.

The periodic pacemaker activity of ICC is dependent on intracellular Ca^{2+} oscillations. The pacemaker mechanism is initiated by release of Ca^{2+} from the endoplasmic reticulum and is followed by reuptake of Ca^{2+} into the mitochondria (Ward et al., 2000). In my results, I found spontaneous $[Ca^{2+}]_i$ oscillations in ICC and this means that the spontaneous pacemaker activity of ICC is closely involved with intracellular Ca^{2+} oscillations in this experiment. And the treatment with $PGF_{2\alpha}$ in ICC increased the basal point of Ca^{2+} oscillation and decreased the peak point. However, the intracellular Ca^{2+} intensity was broadly increased like as the action of tonic inward

currents reversely. Previous report suggested that PGE_2 inhibited intracellular Ca^{2+} oscillations by ATP-sensitive K^+ channels activation in cultured ICC. The observed actions of PGE_2 on $[Ca^{2+}]_i$ oscillations in ICC support the suggestion that $[Ca^{2+}]_i$ oscillations are important actions of pacemaker activity. Namely, PGE_2 and $PGF_{2\alpha}$ has the reverse action on ICC. However, both PGE_2 and $PGF_{2\alpha}$ have the same target, intracellular Ca^{2+},for modulating the pacemaker activity of ICC. So, these results suggested that the spontaneous oscillation of intracellular Ca^{2+} is essential for pacemaker activity of ICC and the intracellular Ca^{2+} can be the main target for various endogeneous agents, neurotransmitters *etc* in ICC.

In conclusion, this study describes the effects of $PGF_{2\alpha}$ on ICC in the murine small intestine. $PGF_{2\alpha}$ depolarized the membrane with increased tonic inward currents, which was activated by non-selective cation channels via external Ca^{2+} influx, phospholipase C activation, and Ca^{2+} release from internal storage in a protein kinase C-independent manner. Thus, the excitatory action of $PGF_{2\alpha}$ in GI motility may be through by the regulation of electrical activity on ICC.

V. Conclusion

In conclusion, this study describes the effects of $PGF_{2\alpha}$ on ICC in the murine small intestine. $PGF_{2\alpha}$ depolarized the membrane with increased tonic inward currents, which was activated by non-selective cation channels via external Ca^{2+} influx, phospholipase C activation, and Ca^{2+} release from internal storage in a protein kinase C-independent manner. Thus, the excitatory action of $PGF_{2\alpha}$ in GI motility may be through by the regulation of electrical activity on ICC.

VI. References

Athari A, Jungermann K (1989) Direct ctivation by prostaglandin F_2alpha but not thromboxane A2 of glycogenolysis via an increase in inositol 1,4,5-triphosphate in rat liver. Biochem Biophys Res Commun 163: 1235-1242.

Bennett A, Flescher B (1970) Prostaglandins and the gastrointestinal tract. Gastroenterology 59: 790-800.

Bennett A, Hensby CN, Sanger GJ, Stanford IF (1981) Metabolites of arachidonic acid formed by human gastrointestinal tissues and their actions on the muscle layers. Br J Pharmacol 74: 435-444.

Fulgratt G, Bradenbusch G, Heintze K (1974) Dose response relation of the renal effect of PGA, PGE_2, and $PGF_{2\alpha}$ in dogs. Prostaglandins 8:21-30.

Harks EG, Scheenen WJ, Peters PH, van Zoelen EJ, Theuvenet AP (2003) Prostaglandin F2alpha induces unsynchronized intracellular calcium oscillations in monolayers of gap junctionally couple NRK fibroblasts. Pflugers Arch 447: 78-86.

Hawley CJ, Rampton DS (1985) Prostaglandins and the gastrointestinal mucosa: are they important in its function, disease, or treatment?. Gastroenterology 89: 1162-1188.

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

Hunt RH, Dilawari JB, Misiewicz JJ (1975) The effect of intravenous prostaglandin F_2 and E_2 on the motility of the sigmoid colon. Gut 16: 47-49.

Ishizawa M, Miyazaki E (1975) Effect of prostaglandin F_2 on propulsive acitivity of the isolated segmental colon of the guinea pig. Prostaglandins 10:759-768.

Jun JY, Choi S, Chang IY, Yoon CK, Jeong HG, Kong ID, So I, Kim KW, You HJ (2005) Deoxycholic acid inhibits pacemaker currents by activating ATP-dependent K^+ channels through prostaglandin E_2 in interstitial cells of Cajal from murine small intestine. Br J Pharmacol 144: 242-251.

Karim SMM, Sandler M, Williams ED (1967) Distribution of prostaglandins in human tissues. Br J Pharmacol 31: 340-344.

Karim SMM, Hillier K, Devlin J (1968) Distribution of prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ in some animal tissues. J Pharm Pharmacol 20: 749-753.

Koh SD, Bradley KK, Rac MG, Keef KD, Horowitz B, Sanders KM (1998) Basal activation of ATP-sensitive potassium channels in murine colonic smooth muscle cells. Biophys J 75: 1793-1800.

LeDuc LE, Needleman P (1979) Regional localization of prostacyclin and thromboxane synthesis in dog stomach and intestinal tract. J Pharmacol Exp Ther 211: 181-188.

Pierce NF, Carpentier CCJ, Elliott HJ, Greenough B (1971) Effects of prostaglandins, theophylline, and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology

60: 22-23.

Robert A (1981) Prostaglandins and the gastrointestinal tract. In: Physiology of the Gastrointestinal Tract, edited by LR Johnson. New York: Raven, pp 1407-1434.

Sanders KM and Northrup TE (1983) Prostaglandin synthesis by microsomes of the circular and longitudinal gastrointestinal muscles. AM J Physiol 244:G442-G448.

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

Szurszewski JH (1987) Electrical basis for gastrointestinal motility. In Physiology of the gstrointestinal tract, 2nd edn, ed. Johnson LR, pp.383-422, Raven Press, New York.

Thomsen L, Robinson TL, Lee JC, Farraway LA, Hughes MJ, Andrews DW, Huizinga JD (1998) Interstitial cells of Cajal generate a rhythmic pacemaker current. Nat Med 4: 848-851.

Ward SM, Burns AJ, Torihashi S, Sanders KM (1994) Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J Physiol 480: 91-99.

Ward SM, Burns AJ, Torihashi S, Harney SC, Sanders KM (1995) Impaired development of interstitial cells and intestinal electrical rhythmicity in steel mutants. Am J Physiol 269: 1577-1615.

Ward SM, Ordog T, Koh SD, Baker A, Jun JY, Amberg G, Monaghan K, Sanders KM (2000) Pacemaking in interstitial cells of Cajal

depends upon calcium handling by endoplasmic reticulum and mitochondria. J Physiol 525: 355-361.

Waller SL (1973) Prostaglandins and the gastrointestinal tract. Gut 14: 402-417.

Walus KM, Jacobson ED (1981) Relation between small intestinal motility and circulation. Am J Physiol 241: G1-G15.

저작물 이용 허락서

학 과	의 학 과	학 번	20067349	과 정	박 사
성 명	한글 김 상 훈	한문 金 相 勳		영문 Kim Sang Hun	
주 소	광주광역시 동구 학동 삼익세라믹 1-1208				
연 락 처	E-mail : drshkim4187@yahoo.co.kr				
논문제목	한글 생쥐 소장에서 분리된 Interstitial Cells of Cajal에 대한 Prostaglandin $F_{2\alpha}$의 작용 기전				
	영문 Effects of Prostaglandin $F_{2\alpha}$ on Pacemaker Activity in Interstitial Cells of Cajal from Murine Small Intestine				

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(0) 반대( )

2007 년 10 월 17 일

저작자: **김 상 훈** (인)

조선대학교 총장 귀하