Effects of prostaglandin F$_{2\alpha}$ on small intestinal interstitial cells of Cajal

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Abstract

AIM: To explore the role of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) on pacemaker activity in interstitial cells of Cajal (ICC) from mouse small intestine.

METHODS: In this study, effects of PGF$_{2\alpha}$ in the cultured ICC cells were investigated with patch clamp technology combined with Ca$^{2+}$ image analysis.

RESULTS: Externally applied PGF$_{2\alpha}$ (10 μmol/L) produced membrane depolarization in current-clamp mode and increased tonic inward pacemaker currents in voltage-clamp mode. The application of flufenamic acid (a non-selective cation channel inhibitor) or niflumic acid (a Cl$^{-}$ channel inhibitor) abolished the generation of pacemaker currents but only flufenamic acid inhibited the PGF$_{2\alpha}$-induced tonic inward currents. In addition, the tonic inward currents induced by PGF$_{2\alpha}$ were not inhibited by intracellular application of 5'-[S]-diphosphate trilithium salt. Pretreatment with Ca$^{2+}$-free solution, U-73122, an active phospholipase C inhibitor, and thapsigargin, a Ca$^{2+}$-ATPase inhibitor in endoplasmic reticulum, abolished the generation of pacemaker currents and suppressed the PGF$_{2\alpha}$-induced tonic inward currents. However, chelerythrine or calphostin C, protein kinase C inhibitors, did not block the PGF$_{2\alpha}$-induced effects on pacemaker currents. When recording intracellular Ca$^{2+}$ ([Ca$^{2+}$]$_i$) concentration using fluo-3/AM, PGF$_{2\alpha}$ broadly increased the spontaneous [Ca$^{2+}$]$_i$ oscillations.

CONCLUSION: These results suggest that PGF$_{2\alpha}$ can modulate pacemaker activity of ICC by acting non-selective action channels through phospholipase C-dependent pathway via [Ca$^{2+}$]$_i$ regulation

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Key words: Prostaglandin F$_{2\alpha}$; Interstitial cells of Cajal; Tonic inward currents; Intestinal motility

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INTRODUCTION

Prostaglandins (PGs) of the E, F, and I series are widely...
distributed in all body tissues, including the gastrointestinal (GI) tract and have been shown to affect water and electrolyte transport, mucous secretion and blood flow\cite{1,2,3}. Also, there is much evidence that PGs may be involved in the control of the contractions of intestinal smooth muscle. Inhibition of endogenous PG synthesis by indomethacin appears to enhance intestinal motility by inducing a fed-like pattern\cite{4,6}. These reports suggest that endogenous PGs may play an important role in the modulation of intestinal motility.

In general, PGE$_2$ is known to contract longitudinal muscle and to relax circular muscle, whereas PGF$_{2\alpha}$ induces contractions of both muscular layers\cite{5,6,9}. PGF$_{2\alpha}$ causes contractions of both colonic muscle layers in the guinea pig and in humans and rats\cite{10,11}. Endogenous PGF$_{2\alpha}$ is suggested to modify contractile activity of antral smooth muscle and intestinal muscle\cite{12,13}. Therefore, exogenous PGF$_{2\alpha}$ and PGF$_{2\beta}$ synthesized in the intestinal smooth muscle cells may affect motor activities.

The interstitial cells of Cajal (ICC) have functions of pacemaker cells and neuromediator cells in the tunica muscularis of the GI tract\cite{14}. The ICC generate the rhythmic oscillations in membrane potential known as slow waves and this generation of slow waves is due to spontaneous inward currents called pacemaker currents\cite{15,16,17}. Although the exact mechanisms regarding these events are still unclear, many reports have suggested that the activation of non-selective cation channels, Cl channels, and spontaneous intracellular Ca$^{2+}$ ([Ca$^{2+}]$) activities in ICC are involved in the production of pacemaker activity\cite{18,19}. Also, it is well known that many endogenous agents such as neurotransmitters, hormones and paracrine substances modulate GI tract motility by influencing ICC.

There are many reports that PGF$_{2\alpha}$ has a role in GI motility by acting on smooth muscles but no studies have been performed to determine the effects of PGF$_{2\alpha}$ on electrical events in ICC. Therefore, the purpose of our study was to investigate the signal transduction effects of PGF$_{2\alpha}$ on pacemaker activity in cultured ICC.

**MATERIALS AND METHODS**

**Ethics**

All experiments were carried out according to the guiding principles for the care and use of animals approved by the ethics committee of Chosun University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize both the number of animals used and their suffering.

**Solutions and drugs**

The cells were bathed in a solution containing: 5 mmol/L KCl, 135 mmol/L NaCl, 2 mmol/L CaCl$_2$, 10 mmol/L glucose, 1.2 mmol/L MgCl$_2$, and 10 mmol/L HEPES, adjusted to pH 7.2 with Tris. The pipette solution contained 140 mmol/L KCl, 5 mmol/L MgCl$_2$, 2.7 mmol/L K$_2$ATP, 0.1 mmol/L Na$_2$GTP, 2.5 mmol/L creatine phosphate disodium, 5 mmol/L HEPES, 0.1 mmol/L EGTA, adjusted to pH 7.2 with Tris.

Drugs used were: PGF$_{2\alpha}$, guanosine 5’-[thio]diphosphate trilithium salt (GDP-$\beta$-S), U-73122, Calphostin C, Chelethrin, and Thapsigargin. All drugs were purchased from the Sigma Chemical Co (St. Louis, MO, USA). Flufenamic acid and niflumic acid were purchased from Calbiochem (San Diego, CA, USA).

All drugs were dissolved in DW or DMSO to prepare stock solutions (10 or 100 mmol/L), and were either added to the bath solution or applied to the whole-cell preparations by superfusion. The final concentration of DMSO was less than 0.05%.

**Preparation of cells and tissues**

Balb/c mice (3- to 7-d-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. The luminal contents were washed away with Krebs-Ringer bicarbonate solution. The tissues were pinned to the base of a Sylgard dish and the mucosa removed by sharp dissection. Small strips of intestinal muscle were equilibrated in Ca$^{2+}$-free Hank’s solution for 30 min and the cells were dispersed 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 plated onto sterile glass coverslips coated with murine collagen (2.5 μg/mL, Falcon/BD) in 35 mm culture dishes. The cells were then cultured at 37°C in a 95% O$_2$:5% CO$_2$ incubator in smooth muscle growth medium (SMGM, Clonetics Co., 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 (ACK2) labeled 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 use of an 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, Valley view, OH, USA).

Results were analyzed using pClamp and Sigma plots (version 9.0) software. All experiments were performed at 30°C.

**Measurement of the [Ca$^{2+}$]i concentration**

Changes in the [Ca$^{2+}$]i concentration were monitored by using fluo-3/AM, which was initially dissolved in dimethyl
sulfoxide and stored at -20°C. The cultured ICC on coverslips (25 mm) were rinsed twice with a bath solution (5 mmol/L KCl, 135 mmol/L NaCl, 2 mmol/L CaCl₂, 10 mmol/L glucose, 1.2 mmol/L MgCl₂ and 10 mmol/L HEPES, adjusted to pH 7.4 with Tris). The coverslips were then incubated in the bath solution containing 5 μmol/L fluo-3 with 5% CO₂ at 37°C for 5 min, rinsed two more times with the bath solution, mounted on a perfusion chamber, and scanned every 0.4 s with a Nikon Eclipse TE200 inverted microscope equipped with a Perkin-Elmer Ultraviolet confocal scanner and a Hamamatsu Orca ER 12-bit CCD camera (× 400). Fluorescence was determined with an excitation wavelength of 488 nm, and emitted light was observed at 515 nm. During scanning of the Ca²⁺ imaging, the temperature of the perfusion chamber containing the cultured ICC was kept at 30°C. The variations of [Ca²⁺]i fluorescence emission intensity were expressed as F1/F0 where F0 is the intensity of the first imaging.

Statistical analysis
Data are expressed as the mean ± SE. Differences in the data were evaluated by Students’ t-test. P < 0.05 was taken as a statistically significant difference. The n values reported in the text refer to the number of cells used in the patch-clamp experiments.

RESULTS
Effect of PGF₂α on pacemaker potentials and currents in cultured ICC
ICC, identified by Kit immunofluorescence, had a distinctive morphology that was easily recognized in cultures. We thus performed the electrophysiological recording from cultured ICC under current (I = 0) and voltage clamp mode. Under current clamp mode, ICC showed spontaneous pacemaker potentials. The resting membrane potential was -53 ± 4 mV and amplitude was 27 ± 2 mV. In the presence of PGF₂α (10 μmol/L), 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, Figure 1A, bar graph not shown). These results are in agreement with previous studies showing that ICC have spontaneous pacemaker activity and we also found PGF₂α to have action on this electrical activity of ICC. Under a voltage clamp at a holding potential of -70 mV, the ICC generated spontaneous inward currents. Treatment with various concentrations of PGF₂α in cultured ICC produced tonic inward currents and decreased the frequency and the amplitude of pacemaker currents in a dose-dependent manner (Figure 1B-D). As shown in Figure 1E-G, the values of frequency, amplitude and resting currents with regard to pacemaker currents under control conditions were significantly different from those obtained in the presence of PGF₂α.

Effects of non-selective cation channel blocker or Cl⁻ channel blocker on PGF₂α-induced responses in cultured ICC
In order to characterize the tonic inward currents induced by PGF₂α, we used flufenamic acid, a non-selective cation channel blocker, or niflumic acid, a CI channel blocker. Figure 2A shows that treatment with flufenamic acid (10 μmol/L) abolished the generation of pacemaker currents and blocked the PGF₂α-induced tonic currents in ICC. The summarized bar graph (Figure 2B) indicates that the resting currents produced by PGF₂α were -21 ± 9 pA in the presence of flufenamic acid and that this value was significantly different when compared with control values obtained in the absence of flufenamic acid (n = 4). In the presence of niflumic acid (10 μmol/L), the pacemaker currents were abolished. Under this condition, PGF₂α still produced the tonic inward currents (Figure 2C). In the presence of niflumic acid, the resting currents produced by PGF₂α were -98 ± 12 pA; this value was not significantly different when compared with control values obtained in the absence of niflumic acid (n = 5, Figure 2D).

No involvement of G proteins in the PGF₂α-induced tonic inward currents in cultured ICC
The effects of GDP-β-S, 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 PGF₂α in ICC. When GDP-β-S (1 mmol/L) was in the pipette, PGF₂α (10 μmol/L) still showed the tonic inward currents (Figure 3A). In the presence of GDP-β-S in the pipette, the resting currents in the control were -23 ± 9 pA. The resting currents during treatment with PGF₂α in the presence of GDP-β-S were -139.9 ± 36 pA (n = 4, Figure 3B), which were not significantly different from those obtained by treatment with PGF₂α in the absence of GDP-β-S.

External Ca²⁺-free solution and Ca²⁺-ATPase inhibitor of endoplasmic reticulum suppress PGF₂α effects in cultured ICC
To investigate the role of external Ca²⁺ or internal Ca²⁺, PGF₂α was tested under external Ca²⁺-free conditions and in the presence of thapsigargin, a Ca²⁺-ATPase inhibitor of endoplasmic reticulum. The application of external Ca²⁺-free solution completely inhibited the pacemaker currents in voltage clamp mode at a holding potential of -70 mV and in this condition, PGF₂α (10 μmol/L)-induced effects on pacemaker currents were blocked (n = 5, Figure 4A). The value of resting currents with PGF₂α (10 μmol/L) in Ca²⁺-free solution was significantly different when compared with control values obtained in normal solution (Figure 4B). In addition, the treatment with thapsigargin (5 μmol/L) inhibited the pacemaker currents in ICC and blocked the PGF₂α-induced tonic inward currents (Figure 4C). In the presence of thapsigargin, the value of resting currents during treatment with PGF₂α was significantly different from those obtained by treatment with PGF₂α in the absence of thapsigargin (n = 6, Figure 4D).

Effects of phospholipase C inhibitor on the PGF₂α-induced tonic inward currents in cultured ICC
To investigate whether the PGF₂α-induced effects on
pacemaker currents are mediated by the activation of phospholipase C (PLC), we treated the ICC with U-73122, a PLC inhibitor. U-73122 (5 μmol/L) abolished the generation of pacemaker currents and blocked the PGF₂α-induced tonic inward currents (Figure 5A). In the presence of U-73122, the tonic inward currents produced by PGF₂α (10 μmol/L) were -21 ± 11 pA. The value of resting currents induced by PGF₂α was significantly different from those obtained by treatment with PGF₂α in the absence of U-73122 (n = 5, Figure 5B). These results suggest that the PGF₂α-induced tonic inward currents may be a PLC-dependent mechanism.

**Effects of protein kinase C inhibitor on PGF₂α-induced responses in cultured ICC**

We tested the effects of chelerythrine or calphostin C, inhibitors of protein kinase C (PKC), to investigate whether PGF₂α-induced responses to pacemaker currents are mediated by the activation of PKC. Chelerythrine (1 μmol/L) or calphostin C (10 μmol/L) did not have an effect on tonic inward currents induced by PGF₂α (10 μmol/L) (Figure 6A and C) and the value also was not significantly different when compared with the tonic inward currents induced by PGF₂α obtained in the absence of chelerythrine or calphostin C (n = 5, Figure 6B and D).

**Increasing of [Ca²⁺]i intensity by PGF₂α**

Because many reports have suggested that [Ca²⁺] oscillations in ICC could be considered to be the primary mechanism for the pacemaker activity in GI activity, we examined the effect of PGF₂α on [Ca²⁺] oscillations in ICC. In this study, we measured spontaneous [Ca²⁺] oscillations of ICC which are connected with cell clusters. Spontaneous [Ca²⁺] oscillations observed in ICC which were loaded with fluo3-AM and the time series data show the spontaneous regular [Ca²⁺] oscillations. In the presence of PGF₂α (10 μmol/L), the basal points of [Ca²⁺] oscillations were increased but the peak points of [Ca²⁺] oscillations were slightly decreased (Figure 7A). The data of the time series are summarized in Figure 7B. These results suggest

**Figure 1** The effects of Prostaglandin F₂α on pacemaker potentials and pacemaker currents recorded in cultured interstitial cells of Cajal from mouse small intestine. A: Pacemaker potentials of interstitial cells of Cajal (ICC) exposed to Prostaglandin F₂α (PGF₂α) (10 μmol/L) in the current-clamping mode (I = 0). Vertical solid line scales denote amplitude of pacemaker potential and horizontal solid line scales denote duration of recording (s) pacemaker potentials; B-D: Pacemaker currents of ICC recorded at a holding potential of -70 mV exposed to various concentrations of PGF₂α (1, 5, and 10 μmol/L). The dotted lines indicate zero current levels. Vertical solid line scales denote amplitude of pacemaker current and horizontal solid line scales denote duration of recording (s) pacemaker currents. The responses to PGF₂α are summarized in E-G. The bars represent mean ± SE. *P < 0.01 vs the untreated control.
that the action of PGF$_{2\alpha}$ on ICC may involve the regulation of spontaneous [Ca$^{2+}$]$_i$ oscillations.

**DISCUSSION**

Although the actions of PGF$_{2\alpha}$ have been demonstrated with regard to GI motility in tissue and smooth muscle cells, this is the first study in ICC in which an attempt has been made to determine the effects of PGF$_{2\alpha}$ on electrical activity in the small intestine.

The results of the present study demonstrate that PGF$_{2\alpha}$ regulates intestinal motility by modulating the pacemaker currents of ICC and that this modulation is mediated via the action on non-selective cation channels and [Ca$^{2+}$]$_i$ mobilization in a PKC-independent manner.

Most regions of the GI tract generate spontaneous elec-
trical and mechanical activity in the absence of 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.[18] ICC generate spontaneous pacemaker inward currents that depolarize membrane, this spreads to smooth muscle via gap junctions resulting in depolarization of membrane in smooth muscle leading to contraction by generating acting potentials through voltage-dependent Ca\(^{2+}\) channel activation[19]. From previous studies, many reports suggested that PGF\(_{2\alpha}\) usually showed contractile actions in vivo and in vitro studies[19-21]. These reports indicate the possibility that PGF\(_{2\alpha}\) may have stimulatory functions on the electrical activity of ICC. In the present study we found that ICC produced spontaneous pacemaker inward currents under voltage clamp mode and that the application of PGF\(_{2\alpha}\) evoked the tonic inward currents of pacemaker currents. This result offers the new suggestion that the

Figure 4 The effects of an external Ca\(^{2+}\)-free solution or thapsigargin on the prostaglandin F\(_{2\alpha}\)-induced response in pacemaker currents in cultured interstitial cells of Cajal from mouse small intestine. A: External Ca\(^{2+}\)-free solution abolished the generation of pacemaker currents. Under these conditions, the prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) (10 \(\mu\)mol/L)-induced tonic inward currents were blocked; C: Thapsigargin (5 \(\mu\)mol/L) abolished the generation of pacemaker currents. Thapsigargin also blocked the PGF\(_{2\alpha}\) (10 \(\mu\)mol/L)-induced tonic inward currents. The dotted lines indicate the zero current levels. Responses to the PGF\(_{2\alpha}\) in the external Ca\(^{2+}\)-free solution and in the presence of thapsigargin are summarized in B and D. Vertical solid line scales denote amplitude of pacemaker current and horizontal solid line scales denote duration of recording (s) pacemaker current. The bars represent mean ± SE. \(b\) \(P < 0.01\) vs the untreated control.

Figure 5 The effects of U-73122 on the prostaglandin F\(_{2\alpha}\)-induced response in pacemaker currents of cultured interstitial cells of Cajal from mouse small intestine. A: U-73122 (5 \(\mu\)mol/L) abolished the generation of pacemaker currents. U-73122 also blocked the Prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) (10 \(\mu\)mol/L)-induced tonic inward currents. The dotted lines indicate the zero current levels. Responses to the PGF\(_{2\alpha}\) are summarized in B. Vertical solid line scales denote amplitude of pacemaker current and horizontal solid line scales denote duration of recording (s) pacemaker current. The bars represent mean ± SE. The effect of U-73122 on PGF\(_{2\alpha}\)-induced pacemaker currents was significantly different from the PGF\(_{2\alpha}\)-induced pacemaker currents in the absence of U-73122 (\(b\) \(P < 0.01\)).
regulation of electrical activity in ICC may be involved in the contractile effects of PGF$_{2\alpha}$ in the GI tract.

Until now, the exact mechanism of pacemaker activity generation has not been fully understood in ICC.
Two suggestions exist: that pacemaker currents of ICC are mediated by the activation of voltage-independent non-selective cation channels and that inwardly rectifying Cl channels can be generated by the rhythmic inward currents. For checking of these suggestions, we used the blockers of non-selective cation and inwardly rectifying Cl channels and we could find that iflunamic acid, a non-selective cation blocker, abolished pacemaker current generation and that PGFα-induced tonic inward currents were blocked by iflunamic acid. However, although iflunamic acid, a Cl channel blocker, also abolished pacemaker generation, niifumic acid did not block the PGFα-induced tonic inward currents. Furthermore, we have already found that substance P and bradykinin may modulate intestinal motility acting on ICC through the activation of non-selective cation channels. Therefore, these data strongly provide support for the suggestion that, and it is likely that, both Cl channels and non-selective cation channels are essentially needed for the generation of the spontaneous pacemaker currents in ICC. However, the agent-induced tonic inward currents in ICC may modulate the pacemaker currents by regulating non-selective cation channels in ICC.

The effects of PGs are mediated by specific receptors, classified into basic types (DP, EP, FP, IP, and TP) according to the PG ligand that each binds with the greatest affinity. They have different cell- and tissue-specific functions, as determined by selective coupling to G proteins and by the expression of splicing isoforms. In the case of PGFα, PGFα induces inositol (1,4,5) trisphosphate (IP3) production and increases Ca2+ levels via FP receptors, which are coupled to G proteins for functional action in various tissues. For example, PGFα induces phosphoinositol (PI) turnover in isolated luteal cells and the effect of PGFα on Ca2+ mobilization in mouse fibroblasts occurs in conjunction with formation of IP3, and is pertussis toxin-insensitive. These findings suggest that FP receptors can interact with a member of the G protein family to activate phospholipase C (PLC), leading ultimately to an IP3-mediated mobilization of Ca2+ from intracellular pools. However, there also appears to be a secondary phase of Ca2+ release by PGFα-treated mouse fibroblasts that is likely due to extracellular Ca2+ entry. In this present study, when GDP-β-S was present in the pipette, the tonic inward pacemaker currents induced by PGFα were still present. This means that the effects of PGFα on electrical activity in ICC may be not related to G proteins. Additionally, since many proposals have been made that PGFα may have biological activity through mobilization of [Ca2+]i and PLC activation, we used thapsigargin, a potent endoplasmic reticulum Ca2+-ATPase inhibitor and U-73122, an active PLC inhibitor, and found that these inhibitors suppressed the PGFα-induced tonic inward currents. These results strongly support the proposition that the release of Ca2+ from internal storage and PLC activation by PGFα are essential to produce tonic inward currents. Also, these findings have correlations with previous suggestions. In addition, it is well known that the generation of pacemaker currents is dependent upon [Ca2+]i oscillation and that the periodic release of Ca2+ from endoplasmic reticulum is essential for generating pacemaker currents. We believe that our experiments using thapsigargin and U-73122 underscore this information.

In many tissues, it is well known that the binding of PGFα to its receptor results in not only the activation of PLC (and PLC is the initial step of the PI cascade that generates diacylglycerol (DAG), IP3, and Ca2+ release) but also in the activation of a PKC-dependent pathway. The increase in [Ca2+]i not only promotes translocation of some PKC isoforms to the plasma membrane, but, in concert with DAG, is essential in activating the conventional isoforms of PKC. However, in the present study, chelerythrine or calphostin C, specific and potent PKC inhibitors, did not block PGFα-induced effects, suggesting that PKC is not involved in the actions of PGFα in ICC.

The periodic pacemaker activity of ICC is dependent on [Ca2+]i oscillations. The pacemaker mechanism is initiated by release of Ca2+ from the endoplasmic reticulum and is followed by uptake of Ca2+ into the mitochondria. In our results, we found spontaneous [Ca2+]i oscillations in ICC and this means that the spontaneous pacemaker activity of ICC is closely involved with [Ca2+]i oscillations in this experiment; the treatment with PGFα in ICC increased the basal point of [Ca2+]i oscillation and decreased the peak point. However, the [Ca2+]i intensity was broadly increased as the action of tonic inward currents reversed. Our previous report suggested that PGFα inhibited [Ca2+]i oscillations by ATP-sensitive K+ channel activation in cultured ICC. The observed actions of PGFα on [Ca2+]i oscillation in ICC support the suggestion that [Ca2+]i oscillations are important actions of pacemaker activity. Namely, PGFα and PGFβ have opposing actions in ICC. However, both PGFα and PGFβ have the same target for modulating the pacemaker activity of ICC; that is, the [Ca2+]i in ICC. Therefore, these results suggest that the spontaneous oscillation of [Ca2+]i is essential for pacemaker activity of ICC and that the [Ca2+]i can be the main regulatory target for various endogenous agents or neurotransmitters in ICC.

In the present study, externally applied PGFα depolarized ICC membranes and formed spike potentials which would result in muscle contraction. As the ICC are electrically coupled with nearby smooth muscles through the gap junctions, the resulting contraction propagates around and along the gut in a coordinated manner and ultimately regulates GI motility.

In conclusion, this study describes the effects of PGFα on ICC in the mouse small intestine. PGFα depolarized the membrane with increased tonic inward currents, which were activated by non-selective cation channels via external Ca2+ influx, PLC, and internal Ca2+ mobilization, in a PKC-independent manner. Thus, PGFα may play a very important role in regulating the rhythm and contraction of small intestinal smooth muscles by acting on ICC.
Background

Prostaglandins (PGs) of the E, F, and I series are widely distributed in all body tissues, including the gastrointestinal (GI) tract. Many reports have suggested that PGFsα has an important role in the modulation of intestinal motility.

Research frontiers

There are many reports that PGFsα has a function in GI motility by acting on smooth muscles but no studies have been performed to determine the effects of PGFsα on electrical events in interstitial cells of Cajal (ICC). Therefore, the purpose of our study was to investigate the signal transduction effects of PGFsα on pacemaker activity in cultured ICC.

Innovations and breakthroughs

This study showed the actions of PGFsα on ICC in the mouse small intestine. PGFsα depolarized the membrane with increased inward currents, which were activated by non-selective cation channels via external Ca2+ influx, phospholipase C, and internal Ca2+ mobilization, in a protein kinase C-independent manner.

Applications

The role of PGFsα in ICC may be one theory for understanding the excitatory action of PGFsα in GI motility.

Terminology

ICC have functions of pacemaker cells and neuromediator cells in the tunica muscularis of the GI tract. The ICC generate rhythmic oscillations in membrane potential known as slow waves and this generation of slow waves is due to spontaneous inward currents called pacemaker currents.

Peer review

It is an interesting paper dealing with a demanding subject. They found that PGFsα can regulate intestinal motility through the modulation of ICC pacemaker activities.

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