생쥐 소장의 interstitial cells of Cajal에서 기록된 향도잡이 전류에 대한 Prostaglandins의 효과

The Effects of Prostaglandins on Pacemaker Currents in Cultured Interstitial Cells of Cajal Isolated from Murine Small Intestine

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朝鮮大學校 大學院

醫 學 科

許 光 培

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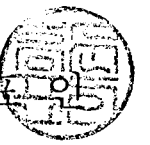
委員長　조선 大學校 敎授　홍순표 

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생쥐 소장의 interstitial cells of Cajal에서 기록된 향도잡이 전류에 대한 prostaglandins의 효과

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Interstitial cells of Cajal (ICC)은 위장관의 향도잡이 세포로, 위장관 근육에 전기적 활동을 발생하는 기능을 가지고 있으며, 신경전달물질 또는 호르몬과 같은 내인성 물질에 의해 부단히 조절 지배를 받고 Prostaglandins (PG)이 위장관에서의 다양한 기능을 가진다는 많은 연구보고를 통해 세포막전압 고정법을 이용하여 생쥐 소장으로부터 배양된 ICC에서 발생하는 향도잡이 전류의 활동도 및 전도도에 대한 PG의 효과를 이 실험에서 관찰하였다.

ICC는 전압고정상태에서 자발적인 향도잡이 전류를 보여준다. 평균 전류크기는 -452 ± 39 pA이고 평균 빈도는 분당 18 ± 2 회이다(n = 6). 정상 상태에서 PGE_2와 PGI_2 (1 μM) 처리시 평균 전류크기와 빈도 모두를 억제하였고, 휴식상태의 전류는 밖으로 향하는 방향으로 증가시켰다. $PGF_{2\alpha}$와 안정된 thromboxane A_2인

U-46619 그리고 PGD_2 1 μM처리시 두 약물 모두 향도잡이 전류를 억제 시켰지만, PGE_2와는 달리 휴식상태의 전류는 안으로 향하는 방향으로 감소 시켰다. ICC에서 나오는 향도잡이 전류에 대한 PGE_2 억제효과에 관여하는 EP 수용체 아형을 확인하기 위해 EP 수용체 길항제를 사용하였다. EP2 수용체 길항제인 butaprost를 정상상태의 pacemaker 전류에 1 μM 처리시 전류 크기, 빈도 모두를 억제하였다. 그러나, EP1과 EP3 수용체 길항제인 sulprostone 1 μM은 큰 영향을 주지 못했다. 일반적인 PG의 효과는 cyclic nucleotide 의존적인 신호전달체계를 이용한다는 기존 보고를 통해, 이 실험에서도 adenylate cyclase 억제제인 SQ-22536를 100 μM을 사용한 결과 ICC의 pacemaker 전류에 큰 영향을 보여주진 못하였다.

이상의 실험결과를 종합해보면 PG는 직접적으로 ICC에서 발생하는 pacemaker 전류를 조절하였다. 특히 향도잡이 전류를 억제하는 PGE_2 효과는 EP2 수용체를 통하여 이루어지며, cyclic AMP생성과는 무관하게 이루어지는 것으로 사료된다.

중심단어 : Interstitial cells of Cajal, PG, EP2 수용체

Introduction

Prostaglandins (PG) are widely distributed throughout the gastrointestinal tract and play a significant role in the physiology and pathophysiology[1,37 and 40]. There is many report that PG affect water and electrolyte transport, mucous secretion and blood flow etc[28]. Especially, PG act as local regulatory agents controlling smooth muscle contractile activity at different levels of the digestive tract, in particular the small intestine[4, 30, 35]. This action is extremely variable, depending on the concentration, the organ, the species and even the muscle layer studied[11, 12, 35]. In generally, PGE_2 is well known to contract the longitudinal muscle and to relax the circular one in human and various animal species[12, 29].

The relatively large number of naturally occurring prostanoids, their high potencies, and the variety of the responses elicited by them in different cells throughout the mammalian body made this an ideal area in which to study receptor subtypes. The classification of receptors into DP, EP, FP, IP and TP recognised the fact that receptors exist that are specific for each of the five naturally occurring prostanoids, PG D_2, E_2, $F_{2\alpha}$, I_2 and TXA_2, respectively; it is certainly not true of catecholamines, tachykinins, or leukotrienes. Evidence

arose for a subdivision within the the EP receptor family. There is now subdivision within the EP receptor family. There is now evidence for the existence of four subtypes of EP receptors, termed arbitrarily EP1, EP2, EP3 and EP4. The recent cloning and expression of receptors for the prostnoids has not only confirmed the existence of at least four of the five classes of prostanoid receptor, EP, FP, IP and TP, but has also supported the subdivision of EP receptors into at least three subtypes, corresponding to EP1, EP2 (or EP4) and EP3. The current classification and nomenclature of prostanoid receptors is summarized in table 1.

Many regions of the tunica muscularis of the gastrointestinal tract display spontaneous contraction and these spontaneous contractions are mediated by the periodic generation of electrical slow waves[36]. Recent studies have been shown that the interstitial cells of Cajal (ICC) act as pacemakers and conductors of electrical slow waves in gastrointestinal smooth muscles[16, 25, 27, 32, 39]. Although the exact mechanisms for these events still remain unclear, several reports suggest that endogenous agents such as neurotransmitter, hormones and paracrine substances can modulate gastrointestinal tract motility by influencing the interstitial cells of Cajal (ICC).

Previous studies have shown that PG have function in gastrointestinal tract[4,

[30, 35]. Therefore, in this study, I investigated the possibility that PG may have effects on electrical properties of cultured ICC cells and also EP receptor subtypes involved these effects were characterized.

Materials and Methods

Material

SC-19220, butaprost and sulprostone were purchased from Cayman Chemicals. Prostaglandin $F_{2\alpha}$, U-46619, Prostglandin D_2 and Prostglandin I_2 were purchased from Calbiochem Co. and prostaglandin E_2 was from the Sigma Chemical Co. To prepare stock solutions, all drugs were dissolved into DW or DMSO and stored at -20 °C.

Preparation of cells and tissues

Balb/C (8-13 days old) of either sex were anethetized 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 tissue stripes of intestinal muscle (both circular and longitudinal muscles are contained) were equilibrated in Ca^{2+}-free Hanks solution containing 5.36 mM KCl, 125 mM NaCl, 0.34 mM NaOH, 0.44 mM Na_2HCO_3, 10 mM glucose, 2.9 mM sucrose and 11 mM

HEPES for 30 min. And then 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 dish. 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 (ICCs) were indentified immunologically with a monoclonal antibody for Kit protein (ACK2) labelled with Alexa Fluor 488 (molecular prove, Eugene, OR, USA). The morphologies of ICCs were distinct from other cell types in the culture, so it was possible to identify the cells with phase contrast microscopy once the cells were verified with ACK2-Alexa Fluor 488 labeling.

Patch clamp experiments

The whole-cell configuration of the patch-clamp technique was used to record membrane currents (voltage clamp) and potentials (current clamp)

from cultured ICCs. Axopatch 1-D (Axon Instruments, Foster, CA, USA) amplified membrane currents and potentials. Command pulse was applied using a 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). The cells were bathed in a solution containing 5 mM KCl, 135 mM NaCl, 2 mM $CaCl_2$, 10 mM glucose, 1.2 mM $MgCl_2$ and 10 mM HEPES adjusted to pH 7.2 with tris. The pipette solution contained 140 mM KCl, 5 mM $MgCl_2$, 2.7 mM K_2ATP, 0.1 mM Na_2GTP, 2.5 mM creatine phosphate disodium, 5 mM HEPES, 0.1 mM EGTA adjusted to pH 7.2 with tris.

Results were analyzed using pClamp and Graph Pad Prism (version 2.01) software. All experiments were performed at 30 °C.

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.

Results

Spontaneous inward currents and depolarizations in ICC

Under a voltage clamp at a holding potential of -70 mV, ICC showed spontaneous inward currents, which is referred to as pacemaker current (Fig. 1A). The frequency of the pacemaker currents was 14 ± 1.6 cycles/min and the amplitude and resting current level were -420 ± 57 pA and -22 ± 18 pA, respectively (bar graph not shown). Converting the amplifier to current clamp mode, spontaneous depolarization was generated in ICC (Fig. 1B). In the remainder of the experiments, I used a constant holding potential of -70 mV.

Effect of PGE_2, $PGF_{2\alpha}$ and TXA_2 on pacemaker currents in cultured ICC

Previous reports suggested that naturally occurring prostaglandins (PGs) exist PGs D_2, E_2, $F_{2\alpha}$, I_2 and TXA_2[20, 10]. Under control conditions at a holding potential of -70 mV, the frequency, the amplitude and resting current level were 15 ± 1.8 cycles/min, -418 ± 39 pA and -24 ± 12 pA. When applied to PGE_2 (1 μM) in ICC, pacemaker currents was decreased both the frequency and the amplitude of pacemaker currents and increased the resting currents in the outward direction under voltage-clamp conditions (Fig. 2A). In the

presence of PGE_2 (1 μM), the resting currents were −10 ± 15 pA. Also, the corresponding frequencies and amplitude were 3.2 ± 0.8 cycles/min and −26.4 ± 28 pA (Bar graph not shown; n = 9). In presence of $PGF_{2\alpha}$ (1 μM) under voltage-clamp control condition, pacemaker currents also were inhibited (Fig. 2B). $PGF_{2\alpha}$ decreased the frequency and the amplitude of pacemaker currents in ICC. But, in case of resting currents, $PGF_{2\alpha}$ decreased in the inward direction of pacemaker currents. I also tested the effects of U-46619 (1 μM), a stable thromboxane A_2, on pacemaker currents under voltage-clamp mode. In presence of U-46619, the frequency, the amplitude and the resting current level of pacemaker currents had changed as same the actions of $PGF_{2\alpha}$ (Fig. 2C). These results suggested that PGE_2 and $PGF_{2\alpha}$, TXA_2 inhibited the frequency and amplitude of pacemaker currents. But the inhibitory effects of PGE_2 and $PGF_{2\alpha}$, TXA_2 on pacemaker currents in ICC may have each different signal pathway or mediate each other channels. Therefore, in naturally occurring prostaglandins (PGs), I found that PGE_2, $PGF_{2\alpha}$ and TXA_2 inhibited the pacemaker currents in cultured ICCs, but the actions of PGE_2 on resting current differed from that of $PGF_{2\alpha}$ and TXA_2.

Effect of PGI_2 and PGD_2 on pacemaker currents in cultured ICCs

Under control conditions at a holding potential of -70 mV, the frequency, the amplitude and resting current level were 15 ± 1.8 cycles/min, -418 ± 39 pA and -24 ± 12 pA. When applied to PGI_2 (1 μM) in ICC, pacemaker currents was decreased both the frequency and the amplitude of pacemaker currents and increased the resting currents in the outward direction under voltage-clamp conditions (Fig. 3A). In the presence of PGI_2 (1 μM), the resting currents were -7 ± 12 pA. Also, the corresponding frequencies and amplitude were 2.8 ± 0.6 cycles/min and -19.5 ± 8 pA (Bar graph not shown; n = 9). In presence of PGD_2 (1 μM) under voltage-clamp control condition, pacemaker currents also were inhibited (Fig. 3B). PGD_2 decreased the frequency and the amplitude of pacemaker currents in ICC. But, in case of resting currents, PGD_2 decreased in the inward direction of pacemaker currents. Therefore, in naturally occurring prostaglandins (PGs), taken together with figure 2, results suggested that PGE_2, PGF_{2a}, TXA_2, PGI_2 and PGD_2 inhibited the pacemaker currents in cultured ICCs, but the actions of PGE_2 and PGI_2 on resting current differed from that of PGF_{2a}, TXA_2, PGD_2.

Dose-dependency of PGE_2 actions on pacemaker currents in cultured ICC

In previous results, I found that PGE_2 have inhibitory effects on

pacemakercurrents in cultured ICC. At present, I tested that PGE_2 have whether dose-dependent or not inhibitory effects on pacemaker currents in cultured ICC. Under a voltage clamp at a holding potential of -70 mV, ICC generated spontaneous inward currents. The frequency of the pacemaker currents was 13 ± 1.4 cycles/min and the amplitude and resting current level were -360 ± 42 pA and -27 ±9 pA, respectively (n = 6). In dose-dependent experiments with PGE_2, the addition of 10 and 100 nM PGE_2 slightly decreased the amplitude and the frequency of pacemaker currents in ICC. Also, 10 and 100 nM PGE_2 a little increased resting currents in the outward direction (Fig. 4A and B). In the treatment of 10 and 100 nM PGE_2, the frequency were 10 ± 2.6 cycles/min at 10 nM and 8.3 ±3.2 cycles/min at 100 nM and the resting currents and amplitudes were -24 ± 7 pA and -196 ± 32 pA at 10 nM and -20 ± 8 pA and -127 ± 26 pA at 100 nM (n = 7; Fig. 5, 6 and 7). In presence of 1 and 10 μM PGE_2 under voltage-clamp condition, pacemaker currents were largely inhibited by 1 and 10 μM PGE_2 and also increased the resting currents in outward direction (Fig. 4C and D). The inhibitory frequency and amplitudes by PGE_2 were 2.1 ± 1.8 cycles/min and -20.9 ± 16 pA at 1 μM PGE_2 and 1.8 ± 1.4 cycles/min and -16 ± 19 pA at 10 μM PGE_2. The resting current level were -6 ± 2.9 pA at 1 μM PGE_2 and -4 ±

3.6 pA at 10 μM PGE_2 (n = 7; Fig. 5, 6 and 7). These results suggested that PGE_2 inhibited pacemaker currents in dose-dependent manner in cultured ICC.

Characterization of EP receptor subtypes involving the effects of PGE_2 on pacemaker currents in cultured ICC

There is now evidence for the existence of four subtypes of EP receptor, termed arbitrarily EP1, EP2, EP3 and EP4. In this study, I checked what of EP receptor subtypes mediate the inhibitory actions of PGE_2 on pacemaker currents in cultured ICC. First, I examined the effects of butaprost, a selective agonist for the EP2 receptor subtype, on pacemaker currents in cultured ICC. In addition of butaprost (1 μM) on spontaneous pacemaker currents in control conditions, butaprost caused a reduction in spontaneous inward currents frequency and amplitude in cultured ICC (Fig. 8A) and also increased the resting currents in the outward direction (n = 5; Fig. 9A, B and C) (Control : Butaprost; The resting currents = -51 ± 12 pA : -49 ± 24 pA; The amplitude = -378 ± 39 pA : -68 ± 29 pA; The frequency = 17 ± 1.9 cycles/min : 6 ± 1.4 cycles/min). These results were similar that of PGE_2 treatments in previous results. For examining other EP receptor subtypes,

sulprostone, an EP3 and EP1 receptor agonist, was used. In presence of sulprostone (1 μM), sulprostone had no effects of the frequency and the amplitude on pacemaker currents in ICC. In resting currents on pacemaker currents, sulprostone also had no effects in cultured ICC (data not shown). For the reason that sulprostone have the affinity both on EP3 and EP1 receptor subtypes, examining for this, SC-19220, an EP1 receptor antagonist, was used in this studies. Under control condition, ICC generated spontaneous pacemaker currents. Then pretreatment of SC-19220 (1 μM) did not have any influence and co-treatment of SC-19220 (1 μM) and sulprostone (1 μM) also did not have the effects on pacemaker currents (Fig. 8B). These results suggest that PGE_2 inhibited the pacemaker currents in ICC by stimulating of EP2 subtype receptors.

PGE_2-induced pacemaker currents inhibition not mediating adenylate cyclase pathway

For investigating inhibitory effects of PG on pacemaker currents whether or not by the cyclic nucleotide-dependent pathway, SQ-22536, an inhibitor of adenylate cyclase were used. Preincubation of SQ-22536 (100 μM) for 10 min had not effects on control states of pacemaker currents and then

co-treatment of SQ-22536 (100 μM) and PGE_2 (0.1 μM) still inhibited the pacemaker currents (n = 5; Fig. 10A). That is, SQ-22536 had no influence on PGE_2-induced inhibition of pacemaker currents. Also, the treatment of PGI_2 by preincubation of SQ-22536 (100 μM) for 10 min PGI_2 (0.1 μM) still inhibited the pacemaker currents (n = 5; Fig. 10B). These results suggest that SQ-22536 itself had no effect on pacemaker currents and cyclic AMP is not mediate the inhibition of pacemaker currents by PGE_2 and PGI_2.

Table 1

Classification and nomenclature of prostanoid receptors with selective agonists and system of response transduction

Receptor/subtype	Selective agonists	Transduction system
DP	BW 245C, RS93520	cAMP via G protein
EP		
EP1	Iloprost, PGE_2	Intracellular Ca^{2+}
EP2	Butaprost, Misoprostol	cAMP via G protein
EP3	Enprostil, Sulprostone	cAMP via G protein
EP4	None	cAMP via G protein
FP	Fluprostenol, Prostalene	PI turnover
IP	Cicaprost, Iloprost	cAMP via G protein
TP	U46619, SQ 26655	PI turnover

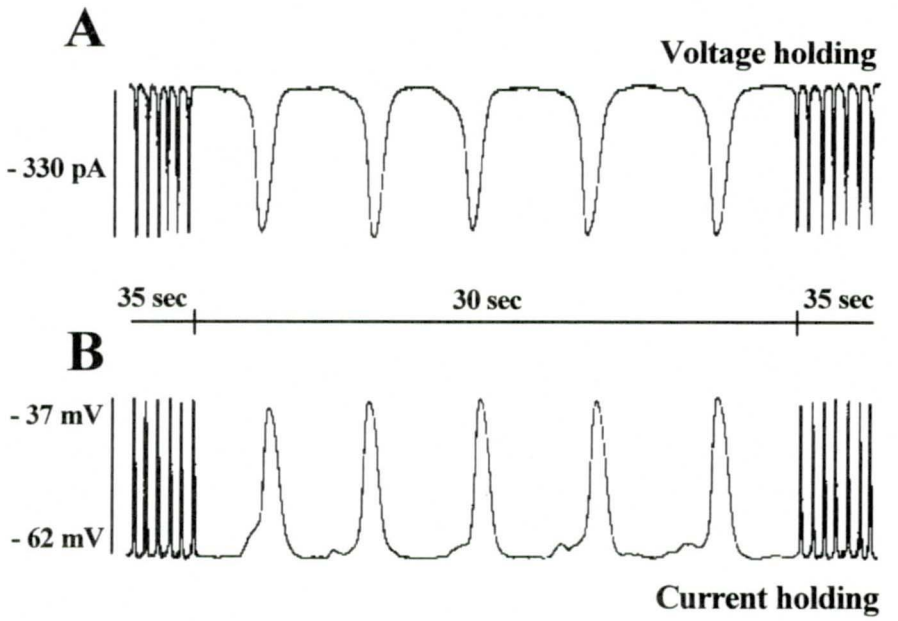

Fig. 1. Spontaneous inward currents and depolarizations in cultured ICCs of the murine small intestine. (A) Under a voltage clamp at a holding potential of −70 mV, ICCs showed spontaneous inward currents oscillations, called pacemaker currents. (B) Under a currents clamp mode, spontaneous depolarization was generated from the same cell.

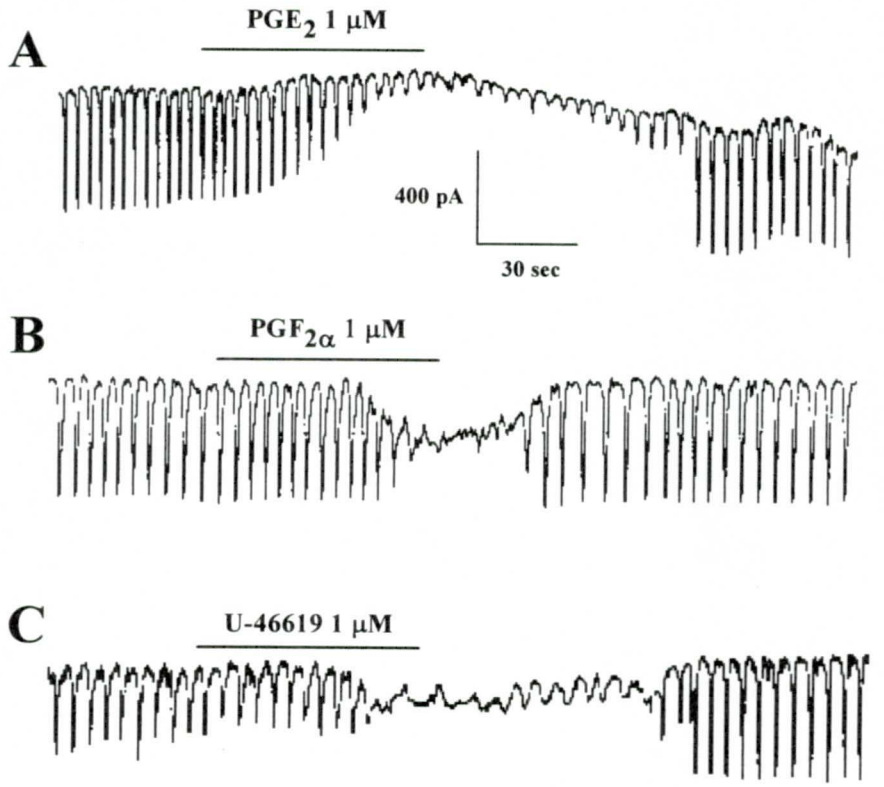

Fig. 2. Effects of prostaglandins (PGs) on pacemaker currents. Under control conditions at a holding potential of -70 mV, (A) PGE_2 (1 μM) inhibited the amplitude and the frequency of pacemaker currents and increased the resting currents in the outward direction in ICCs. (B) and (C) $PGF_{2\alpha}$ and U-46619, a TP receptor agonists, inhibited the amplitude and the frequency of pacemaker currents but decreased the resting currents in the inward direction in ICCs.

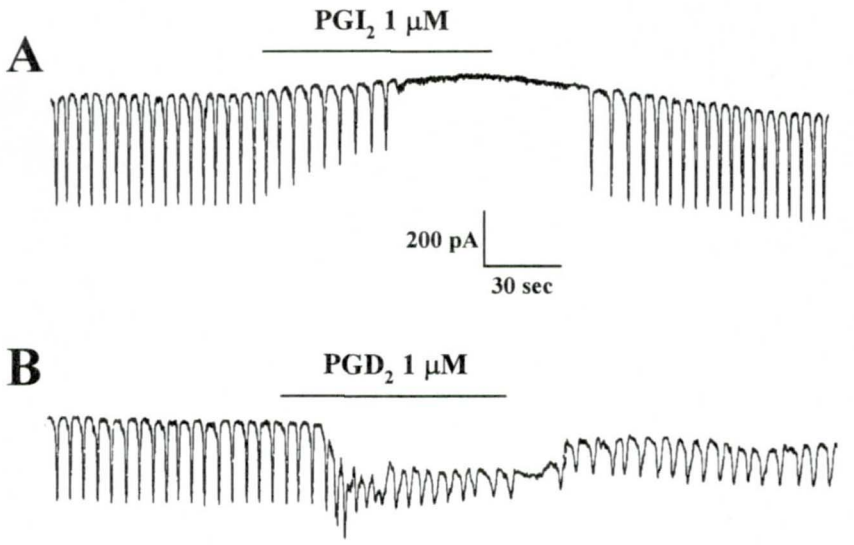

Fig. 3. Effects of prostaglandins (PGs) on pacemaker currents. Under control conditions at a holding potential of -70 mV, (A) PGI_2 (1 μM) inhibited the amplitude and the frequency of pacemaker currents and increased the resting currents in the outward direction in ICCs. (B) PGD_2 (1 μM) inhibited the amplitude and the frequency of pacemaker currents but decreased the resting currents in the inward direction in ICCs.

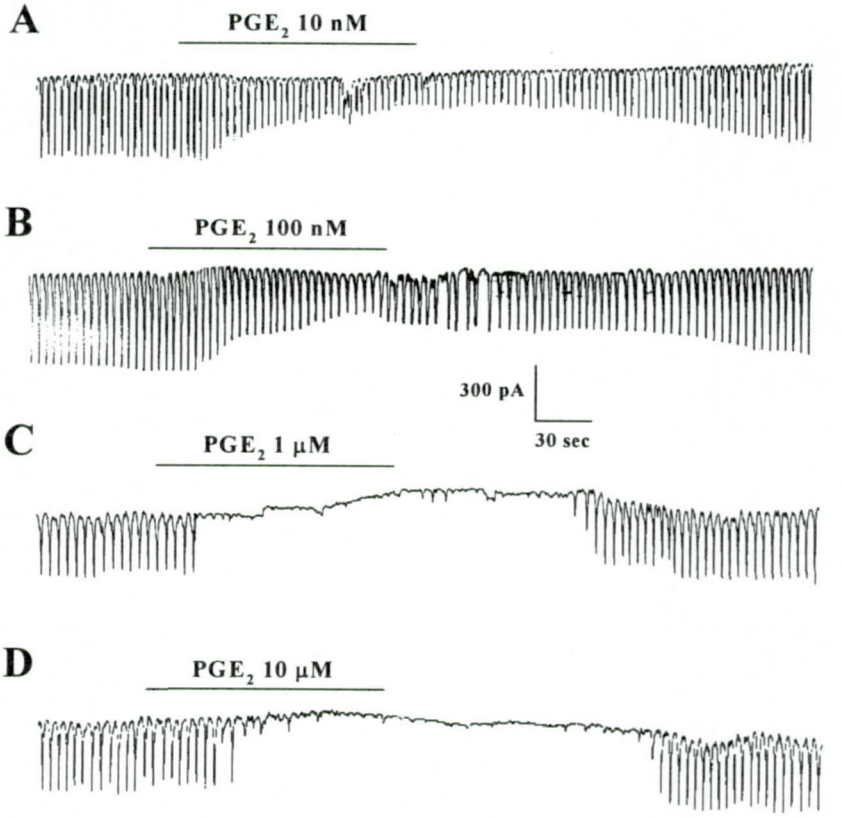

Fig. 4. Dose-dependent effects of PGE_2 on pacemaker currents in cultured ICCs of the murine small intestine. (A), (B), (C) and (D) show the pacemaker currents of ICCs exposed to PGE_2 (0.01, 0.1, 1 and 10 μM) at a holding potential of -70 mV.

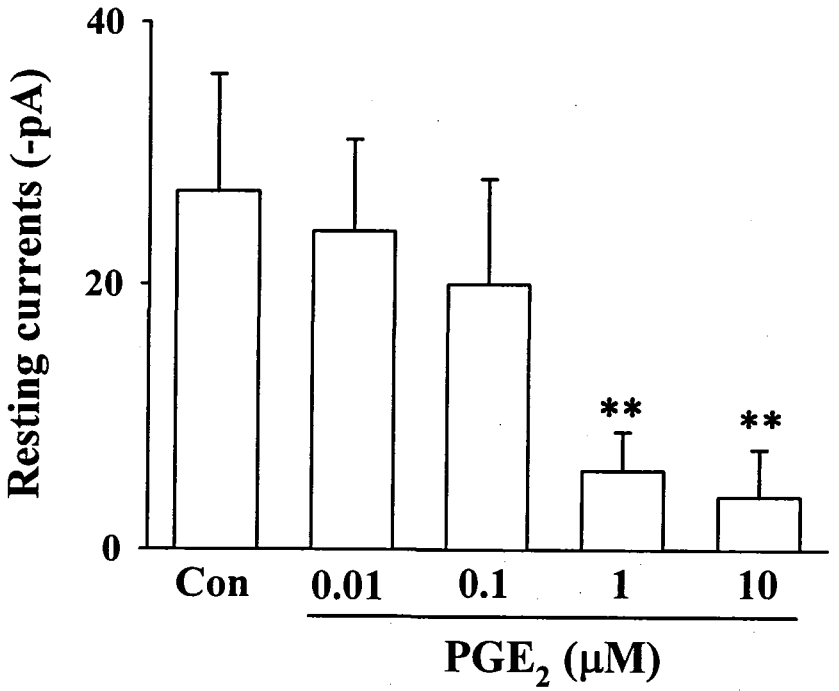

Fig. 5. Dose-dependent effects of PGE_2 on the resting currents of pacemaker currents in cultured ICCs of the murine small intestine. Figure shows the summarized resting currents of ICCs exposed to PGE_2 (0.01, 0.1, 1 and 10 μM) at a holding potential of -70 mV. Those noted with * were significantly different from the controls ($p < 0.05$).

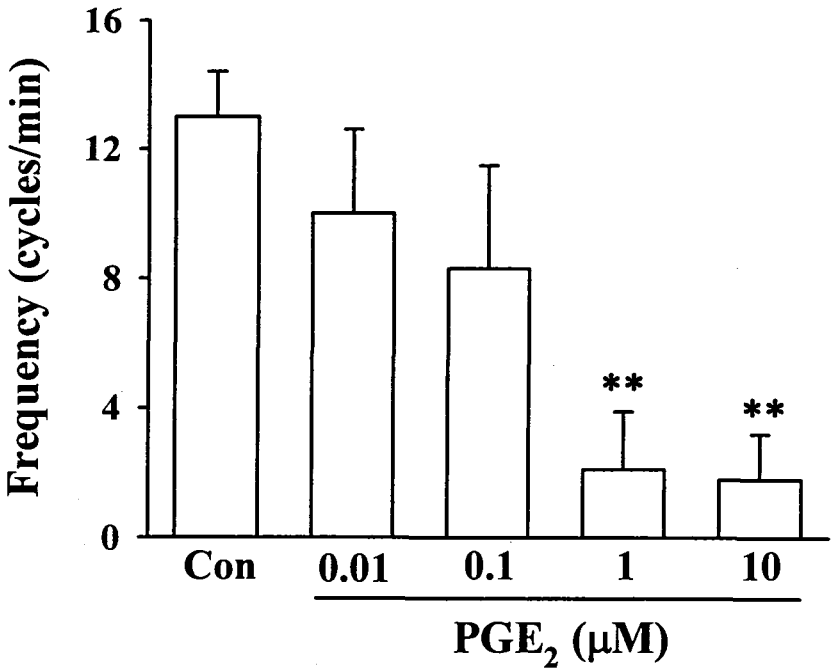

Fig. 6. Dose-dependent effects of PGE_2 on the frequency of pacemaker currents in cultured ICCs of the murine small intestine. Figure shows the summarized frequency of ICCs exposed to PGE_2 (0.01, 0.1, 1 and 10 μM) at a holding potential of -70 mV. Those noted with * were significantly different from the controls ($p < 0.05$).

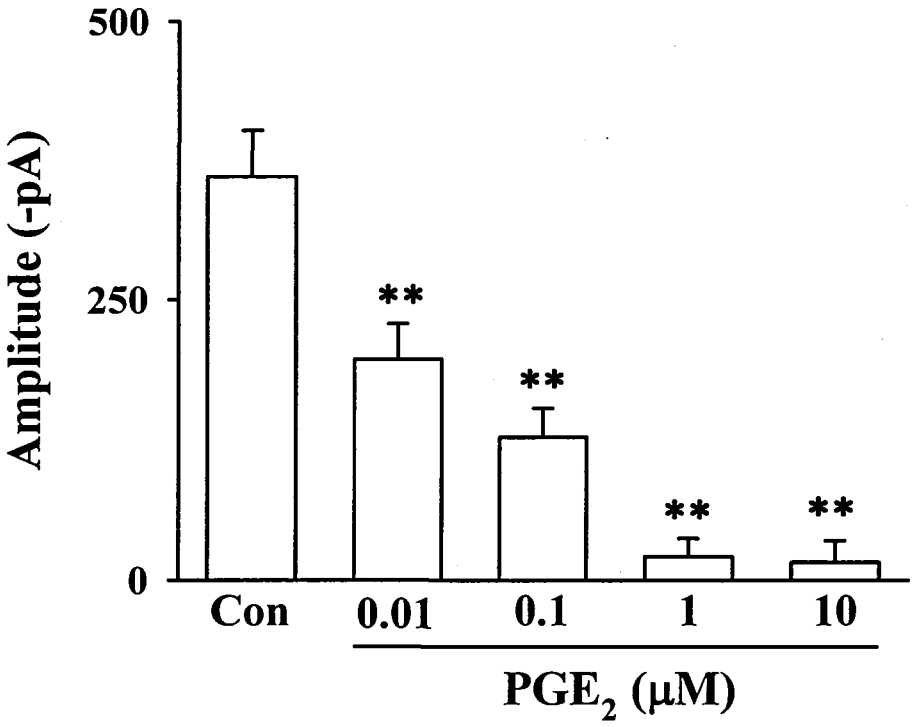

Fig. 7. Dose-dependent effects of PGE_2 on the amplitude of pacemaker currents in cultured ICCs of the murine small intestine. Figure shows the summarized amplitude of ICCs exposed to PGE_2 (0.01, 0.1, 1 and 10 μM) at a holding potential of -70 mV. Those noted with * were significantly different from the controls ($p < 0.05$).

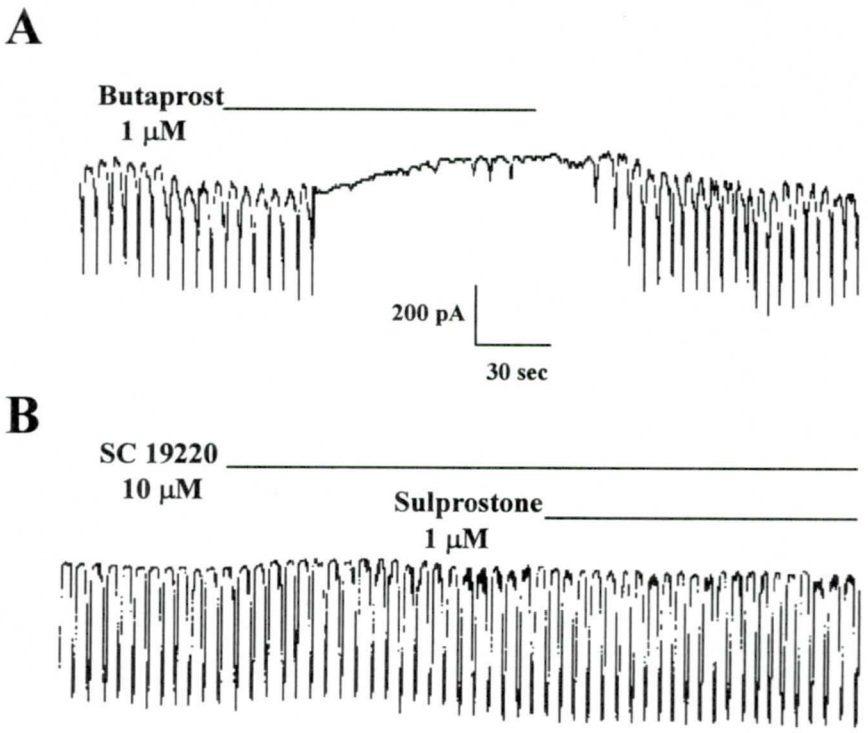

Fig. 8. Effects of EP2 (butaprost) and EP3 (sulprostone) receptor agonists on spontaneous inward currents from cultured ICCs. (A) Butaprost (1 μM) caused decreased the frequency and the amplitude in spontaneous inward current and increased the resting currents in outward directions. (B) In pretreatment with an EP1 antagonists (SC 19220, 10 μM), sulprostone (an EP3 and EP1 agonists, 1 μM) had no effects on pacemaker currents.

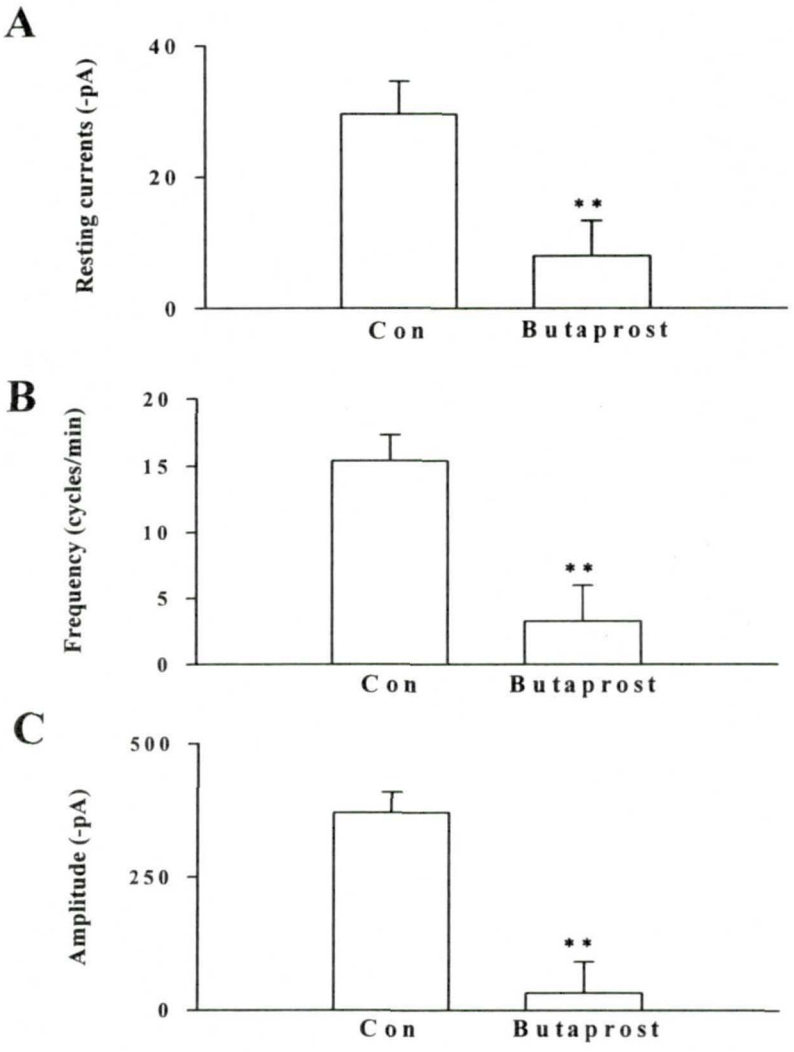

Fig. 9. (A) The effects of Butaprost (1 μM) on resting currents of pacemaker currents in ICC. (B) The effects of Butaprost (1 μM) on frequency of pacemaker currents in ICC. (C) The effects of Butaprost (1 μM) on amplitude of pacemaker currents in ICC. Each bar represents the mean ±SE. (n = 5/group). Those noted with * were significantly different from the controls ($p < 0.05$).

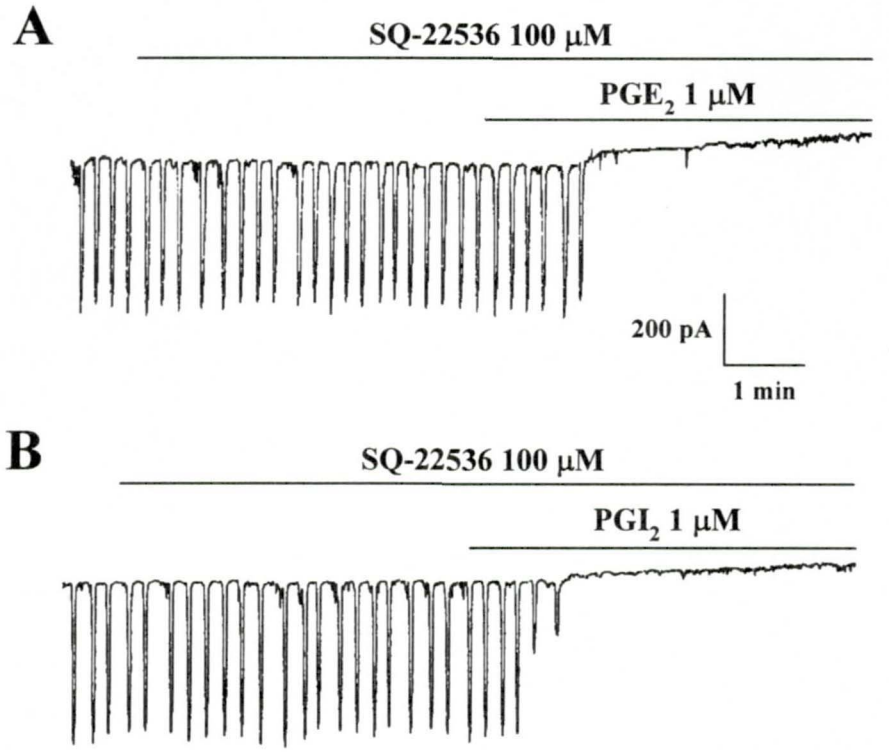

Fig. 10. Effects of SQ-22536, an inhibitor of adenylate cyclase on pacemaker currents in cultured ICCs. (A) Pretreatment of SQ-22536 (100 μM) had no effects on the inhibitory effects of PGE_2 (1 μM) on spontaneous inward currents (B) Pretreatment of SQ-22536 (100 μM) also had no effects on the inhibitory effects of PGI_2 (1 μM) on pacemaker currents in cultured ICCs.

Discussion

Prostaglandins (PG) act as local regulatory agents controlling smooth muscle contractile activity and PG of the E_2 type have been shown to contract intestinal longitudinalsmooth muscle and relax circular smooth muscle[12, 29]. These means that PG may be regulate gastrointestinal motility. Also, because interstitial cells of Cajal generated electrical slow waves that are basic determinant of gastrointestinal motility, PGE_2 may have the effects on slow waves in ICC for controlling of gastrointestinal motility. Here, I demonstrate that PG have the inhibitory effects on pacemaker currents in ICC. In addition to, PGE_2 receptor subtypes that involve the inhibitory effects of PGE_2 on pacemaker currents were characterized.

The enzyme fatty acid cyclooxygenase is distributed in the gastrointestinal tract and converts eicosatetraenoic acid (arachidonic acid) primarily to prostacyclin (prostaglandin I_2) and, to a lesser extent, to PGE_2, $PGF_{2\alpha}$ and thromboxane A_2[19, 18, 26, 28]. In previous functional studies on motility of gastrointestinal tract, generally PGE_2 contract the longitudinal smooth muscle layer of the small intestine and relax the circular layer[3, 38]. In contrast, $PGF_{2\alpha}$ contract both smooth muscle layers[2]. That is PGE_2 and $PGF_{2\alpha}$ have function

on motility but may have different actions in gastrointestinal tract. In cultured ICC, Fig. 2A and B showed that PGE_2 and $PGF_{2\alpha}$ inhibited the frequency and the amplitude on pacemaker currents. But, in the case of resting currents on pacemaker currents, PGE_2 and $PGF_{2\alpha}$ showed reverse actions. PGE_2 increased the resting currents in the outward direction but $PGF_{2\alpha}$ in the inward direction. Therefore these results suggest that, in ICC, PGE_2 and $PGF_{2\alpha}$ have the inhibitory effects on pacemaker currents but may modulate different signal pathway. In taken together action of PGs, PGE_2 and PGI_2 inhibited the pacemaker currents and increased the resting currents in the outward direction but $PGF_{2\alpha}$, TXA_2 and PGD_2 decreased the resting currents in the inward direction.

In case of concentration, many reports suggest that PGE_2 have dual effects. PGE_2 have been shown to suppressive effects in low concentration but activated in high concentration on colonic motility of rabbit in vivo and vitro studies and on stomach mechanical activity of guinea-pig[11]. In this study, PGE_2 showed only the inhibitory effects on pacemaker currents in dose-dependent manner and, at 1 nM and moreover a less 100 pM, PGE_2 showed slightly the inhibitory effects or no effects on pacemaker currents (data not shown). Despite of many reports about PGE_2 dual actions, PGE_2

have only the inhibitory effects on pacemaker currents in this study and the effect was dose-dependent manner in cultured ICC.

The recent cloning and expression of receptors for the prostaglandins (PG) has not only confirmed the existence of at least four of the five classes of prostagland receptor, IP (for PGI_2 binding), FP (for PGF_2 binding), EP (for PGE_2 binding) and TP(for TXA_2 binding), but has also supported the subdivision of EP receptors into at least three subtypes, corresponding to EP1, EP2 (or EP4) and EP3. There is reported a selective agonist and antagonist for each EP receptor. To date, butaprost appears to be the most selective agonist for the EP2 receptor subtype[13]. Sulprostone is active at the EP1 and EP3 receptor[30], while currently no selective agonists for the EP4 receptor subtype. SC19920 is an antagonist known to block the EP1 receptor[33], but as yet antagonists for EP2 and EP3 receptors have not been described. In this study, butaprost showed the inhibitory effects on pacemaker currents in cultured ICC and the aspect of butaprost effect is similar that of PGE_2 effect. But, in case of sulprostone, there was no effect on pacemaker currents (data not shown) and before the addition of sulprostone, pretreatment of SC19920 for blocking of EP1 receptor also showed that sulprostone had not effects on spontaneous inward currents in

cultured ICC. This fact indicates that PGE_2 have influence on pacemaker currents in ICC by stimulating the EP2 receptor subtypes.

Almost all of the studies of prostanoids and second messengers until the late 1980s were concerned with cyclic nucleotides, particularly cAMP. Butcher and colleagues were the first to demonstrate an association between PGs and cAMP[5], and although their observation made little initial impact, it became increaseingly accepted that E-series PGs at least were capable of stimulating adenylyl cyclase to cause increases in intracellular cAMP[24]. Several reports suggested the participation of cAMP on PGs actions, especially the EP2 receptor. The results of Simon et al.[26] provide indirect evidence for positive coupling of an EP receptor to adenylate cyclase, but more direct evidence has been provided by Hardcastle et al.[14], in their demonstration of an association between EP2 receptors and cAMP generation in enterocytes. Similarly, Jumblatt and Peterson[17] found an association between EP2 receptor stimulation and cAMP generation in corneal endothelial cells. Furthermore, in cells expressing the recombinant murine EP2 receptor, PGE_2 increased the intracellular cAMP level without any change in inositol phosphate content[15]. These several reports predict that, on pacemaker currents in ICC, PGE_2 may have the actions of cAMP signaling pathway.

Namely, in ICC, the generation of pacemaker currents may involve the cAMP signaling. But interestingly, in preparation study (data not shown), the treatment of 8-bromo-cAMP (cell-permeable cAMP analog) on control pacemaker currents not showed any effects. Also pretreatment of SQ-22536, an inhibitor of adenylate cyclase, did not show any influence of PGE_2 and PGI_2 actions on pacemaker currents. In putting various reports and results, PGE_2 and PGI_2 have function in diverse cells and tissues by modulating of the cyclic AMP-dependent pathway but in ICC, PGE_2 and PGI_2 have inhibitory actions on pacemaker currents by not-mediating the cyclic AMP-dependent pathway. In ICCs, further experiments of PGE_2 and PGI_2 actions must be needed, especially on second messenger.

In summary, the results of the present study indicate that PG alter directly the pacemaker currents in ICC. The involved PGE_2 receptor subtypes are the EP2 receptor and the effects of PGE_2 and PGI_2 on pacemaker currents are not mediated via cyclic AMP- pathway.

Summary

The interstitial cells of Cajal (ICC) are the pacemaker cells in gastrointestinal tract and generate electrical rhythmicity in gastrointestinal muscles. Therefore, ICC may be modulated by endogenous agents such as neurotransmitter, hormones etc. Because of many previous reports about the actions of prostaglandins (PG) on gastrointestinal tract, here I investigated the effects of prostaglandins on pacemaker currents in cultured interstitial cells of Cajal (ICC) from murine small intestine by using whole-cell patch clamp techniques.

ICC generated spontaneous slow waves under voltage-clamp conditions and showed a mean amplitude of -452 ±39 pA and frequency of 18 ± 2 cycles/min (n = 6). Treatments of PGE_2 (1 μM) decreased both the frequency and amplitude of the pacemaker currents, and increased the resting currents in the outward direction. In case of $PGF_{2\alpha}$ (1 μM) and U-46619 (1 μM, a stable thromboxane A_2), they had the inhibitory effects on pacemaker currents, but decreased the resting currents in the inward direction. Also, treatments of PGI_2 decreased both the frequency and amplitude of the pacemaker currents and increased the resting currents in the outward

direction. In case of PGD_2 had the inhibitory effects on pacemaker currents but decreased the resting currents in the inward direction. For characterization of EP receptor subtypes involving the effects of PGE_2 on pacemaker currents in ICC, EP receptor agonists were used. Butaprost (1 μ M), EP2 receptor agonist, caused a reduction in the spontaneous inward current frequency and amplitude in cultured ICC (n=5). But sulprostone (1 μ M), a mixed EP1 and EP3 agonist, had no effects on the frequency, amplitude and resting currents of pacemaker currents (n=5). To investigate possible regulation of pacemaker currents by the cyclic nucleotide-dependent pathway in PGE_2 and PGI_2 treated cells, SQ-22536 (an inhibitor of adenylate cyclase) were used. In cultured ICC, SQ-22536 (100 μM) had no effects on PGE_2 and PGI_2 actions of pacemaker currents.

These observations indicate that PG alter directly the pacemaker currents in ICC. The involved PGE_2 receptor subtypes are the EP2 receptor and the effects of PG on pacemaker currents are not mediated via cyclic AMP-dependent pathway.

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