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2008년 8월

博士學位論文

Phentolamine inhibits intestinal pacemaker activity of murine interstitial cells of Cajal by activating ATP-sensitive K^+ channel

朝鮮大學校 大學院

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이 논문을 의학 박사학위 신청 논문으로 제출함

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安 城 煥의 博士學位 論文을 認准함

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소장 카할 사이질 세포에서 Phentolamine의 ATP-민감성 칼륨통로 활성화를 통한 향도잡이 활동도 억제작용

안 성 환

지도교수: 김 진 호

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위장관내 존재하는 Cajal 사이질 세포에서 발생하는 전기적 현상에 대한 phentolamine의 효과와 작용기전을 규명하고자 생쥐 작은 창자에서 배양된 Cajal 세포에서 세포막 전압 고정법을 시행하였다. Cajal세포는 서파와 자발적인 내향성 전류(향도잡이 전류)를 발생하였다. 아드레날린 수용체 길항제인 phentolamine은 막전압의 과분극을 초래하였으며 서파의 크기를 감소시켰다. 또한 향도잡이 전류의 크기 및 발생빈도를 억제하였으며 동시에 외향성 전류를 발생시켰다. 그런 반면 다른 아드레날린 수용체 길항제인 prazocine, phenoxybenzamine, clonidine은 향도잡이 전류에 대해 아무런 작용을 나타내지 않았다. Phentolamine의 작용은 ATP-민감성 K^+ 통로 차단제인 glibenclamide에 의해서 억제되었다. 그러나 막전압-의존성 K^+ 통로와 Ca^{2+}-의존성 K^+ 통로 차단제인 TEA, 4-AP와 apamin 및 NO synthase 억제제인 L-NAME는 phentolamine의 작용을 차단하지 못 하였다. ATP-민감성 K^+ 통로 개방제인 pinacidil은 향도잡이 전류에 대해 phentolamine과 동일한 효과를 보였으며 glibenclamide에 의해서 차단되었다. 세포내 Ca^{2+} 이미지 실험에서 phentolamine은 Ca^{2+} 농도를 저하시켰다. 이상의 실험결과들로부터

phentolamin은 Cajal세포에 작용하여 위장관 운동성을 조절할 수 있음을 알 수 있으며 그 작용은 아드레날린 수용체와는 무관하고 ATP-민감성 K^+ 통로의 활성화를 통하여 향도잡이 활동도를 조절하여 이루어지는 것으로 생각된다.

I. Introduction

Phentolamine is a competitive nonselective antagonist acting on the α1- and α2-adrenoceptor that have imidazoline compound (Hoffman and Lefkowitz, 1991). Phentolamine also has nonadrenergic-dependent physiological functions in several tissues. Phentolamine increases insulin secretion from pancreatic β-cells (Shepherd et al., 1996; Jonas et al., 1992; Dunne, 1991) and relaxes human corpus cavernosum (Silva et al., 2005) and prevents ischemia related arrhythmias in heart (Wilde et al., 1994) and increase spontaneous myogenic activity in the rat portal vein (Schwietert et al., 1992), which are not mediated by α-adrenoceptor mechanism. The main action mechanism of phentolamine in pancreatic β-cells, corpus cavernosum and cardiac ventricular cells is the modulation of ATP-sensitive K^+ channels. However, the effects of phentolamine on ATP-sensitive K^+ channels are different each other. In pancreatic β-cells (Proks and Ashcroft, 1997), cardiac ventricular cells, and portal vein phentolamine inhibits ATP-sensitive K^+ channels, whereas phentolamine activates ATP-sensitive K^+ channels in corpus cavernosum. Together it has been reported that phentolamine inhibited exocytosis of glucagons in rat pancreatic α-cells independently of the modulation of ATP-sensitive K^+ channels (Hoy et al., 2001). Also clonidine (a selective α_2-adrenergic agonists) and yohimbin (a α_2-adrenergic antagonist) inhibits ATP-sensitive K^+ channels in pancreatic β-cells by α-adrenoceptor independent mechanism (Plant and Henquin, 1990; Plant, 1991).

Interstitial cells of Cajal (ICC) are playing an important role in regulating gastrointestinal motility. ICC are pacemaker cells that generate electrical slow waves in gastrointestinal tracts (Ward et al., 1994; Huizinga et al., 1995). ICC are connected each

other and with smooth muscles through gap junction. Besides ICC are innervated by enteric nervous system, which mediates enteric signals to smooth muscle (Sanders et al., 2006). Therefore ICC are targets of neurotransmitters, paracrine substances, and drugs. ICC are expressed or identified several receptors in cell membranes (Epperson et al., 2000; Ward and Sanders 2006). It was previously reported that β-adrenoceptor activation modulated pacemaker activity in cultured murine intestinal ICC (Jun et al., 2004). In the present experiment, i also report phentolamine can modulate pacemaker activity of intestinal interstitial cells of Cajal (ICC) by adrenoceptor independent mechanism.

II. Methods

1. Preparation of cells and tissues

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 tissue stripes of intestinal muscle (both circular and longitudinal muscles are contained) were equilibrated in Ca^{2+}-free Hank's solution containing 5.36 mM KCl, 125 mM NaCl, 0.34 mM NaOH, 0.44 mM Na_2HCO_3, 10mM 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^{-1}, bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) 2 mg ml^{-1}, trypsin inhibitor (Sigma) 2 mg ml^{-1} and ATP 0.27 mg ml^{-1}. Cells were plated onto sterile glass coverslips coated with murine collagen (2.5 μg ml^{-1}, 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^{-1}, Sigma). ICC were identified immunologically with a monoclonal antibody for Kit protein (ACK2) labelled with Alexa Fluor 488 (molecular prove, Eugene, OR, USA) (Jun et al, 2005). The morphologies of ICC 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.

2. 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 ICC. Axopatch 1-D (Axon Instruments, Foster, CA, USA) amplified membrane currents and potentials. 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 analyzed using pClamp and Graph Pad Prism (version 2.01) software. All experiments were performed at 30 °C.

3. Solutions and drugs

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.

Drugs used were: phentolamine, prazosine, yohimbine hydrochloride, clonidine,

tetraethylammonium chloride, apamin, glibenclamide, 4-aminopyridine, ODQ (1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one) and L-NAME. ODQ was purchased from Calbiochem Co., and the others were purchased from the Sigma Chemical Co.

All drugs were dissolved into DW or DMSO to prepare stock solutions (10 mM or 100 mM) and were added to the bath solution in experiments and all drugs were applied to the whole cell preparations by superfusion. The final concentration of DMSO was less than 0.05 %.

4. Measurement of the intracellular Ca^{2+} concentration

Changes in the 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 coverslips (25 mm) were rinsed twice with a bath solution (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 7.4 with Tris). The coverslips were then, incubated in the bath solution containing 5 μM fluo-3/AM with 5% CO_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 seconds with a confocal microscope (×200; fluoviews 300, Olympus). Fluorescence was excited at a wavelength of 488 nm, and emitted light was observed at 515 nm. During scanning of the 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 where F0 is the intensity of the first imaging.

5. 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

1. Phentolamine inhibits pacemaker currents in a dose-dependent manner in ICC

Under a voltage clamp at a holding potential of –70 mV, ICC generated spontaneous inward currents, which is referred to as 'pacemaker currents'. The frequency of the pacemaker currents was 16 ± 1.4 cycles min^{-1} and the amplitude and resting current level were –410 ± 57 pA and –22 ± 18 pA, respectively (n= 12). The addition of phentolamnie (0.1 to 10 μM) decreased both the frequency and the amplitude of the pacemaker currents, and increased resting currents in the outward direction in a concentration dependent manner (Fig. 1A, B, C and D). In the presence of phentolamine, the resting currents were –18 ± 11 pA at 0.1 μM (n=8), -9 ± 2.0 pA at 1 μM (n=6), –5 ± 3.04 pA at 5 μM (n=5), and -2 ± 3.04 pA at 10 μM (n=6) (Fig. 1E) and the corresponding frequencies and amplitudes were 12 ± 2.6 cycles min^{-1}, 2.7 ± 0.9 cycles min^{-1}, 1.2 ± 0.2 cycles min^{-1}, and 1.2 ± 0.2 cycles min^{-1} and –260 ± 52 pA, -20.8 ± 18 pA, –17 ± 19 pA, and –10 ± 15 pA (Fig. 1F and G). These values at 1, 5 and 10 μM have significantly different from control values. These results suggest that phentolamine inhibits pacemaker currents in dose-dependent manner in ICC.

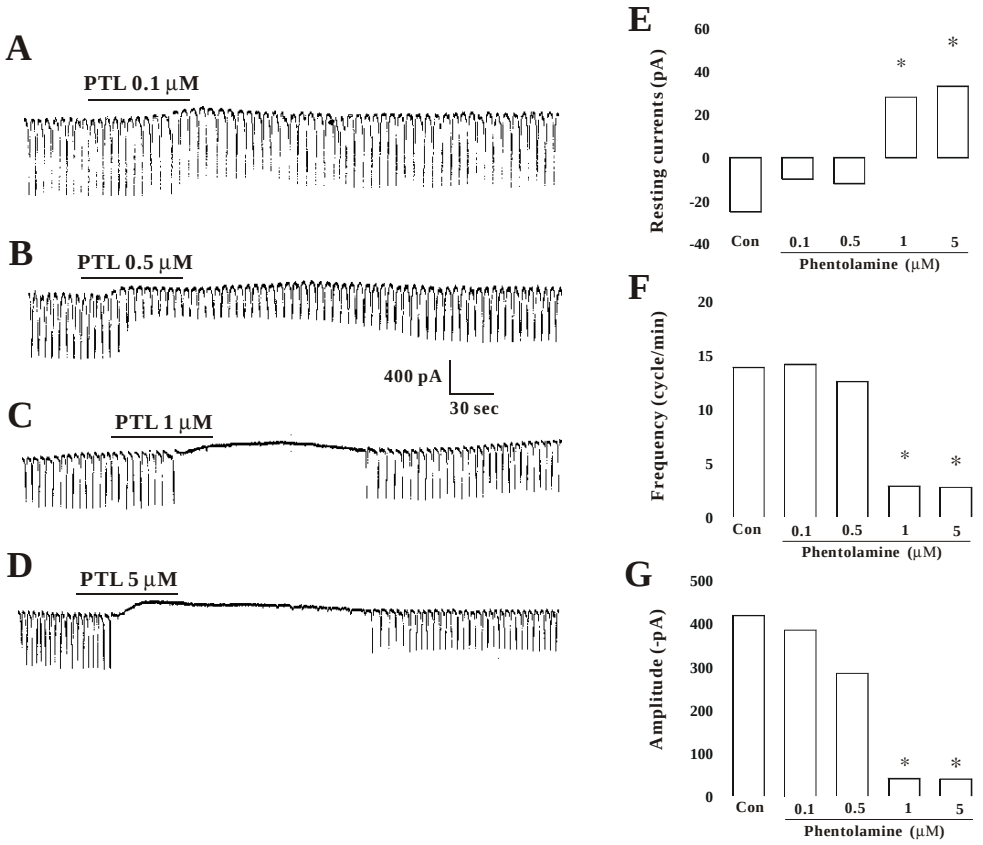

Fig. 1. The dose-dependent effects of phentolamine on pacemaker currents in cultured ICC of the murine small intestine. (A), (B), (C), and (D) show pacemaker currents of ICC exposed to phentolamine (0.1, 0.5, 1, or 5 μM) at a holding potential of -70 mV. (E), (F), and (G) summarize the inhibitory effects of phentolamine on pacemaker currents in ICC. Bars represents means ± SE (n = 6-7/group). *Asterisks mean significantly different from the controls ($p < 0.05$). PTL : phentolamine, Con : control.

2. Phentolamine activates ATP-sensitive K^+ channels in cultured ICC

Whether K^+ channel is involves or not in phentolamine-induced inhibition of pacemaker currents K^+ blockers were pretreated. TEA (tetraethylammonium; 2 or 10 mM), 4-aminopyridine (5 mM), and apamin (1 μM) were pretreated for 5 min before application of phentolamine. Phentolamine still inhibited pacemaker currents in the presence of TEA, 4-aminopyridine, or apamin at a -70 mV of holding potential (Fig. 2A, B, C and D). However, glibenclamide, an ATP-sensitive K^+ channel blocker, inhibited the phentolamine-induced responses. When phentolamine (10 μM) was applied in ICC, both the frequency and the amplitude of pacemaker currents were decreased, and the resting currents were increased in the outward directions. The increased outward current was returned to basal level by glibenclamide (10 μM) (Fig. 3A). In addition phentolamine-induced responses were completely inhibited in the presence of glibenclamide (Fig. 3B).

In a current clamp mode, ICC generated pacemaker potentials. I examined the effects of phentolamine on pacemaker potentials. Phentolamine produced membrane hyperpolarization and decreased the amplitude of pacemaker potentials (n=12) (Fig. 4A). Under control conditions at *I*=0, the resting membrane potential was –61 ± 1.4 mV, and the amplitude of pacemaker potentials was 23 ± 2 mV. In the presence of phentolamine (1 μM), the membrane was hyperpolarized to –69.8 ± 1.1 mV (Fig. 4B) and the amplitude of pacemaker potentials decreased to 2.9 ± 1.1 mV (Fig. 4C). This phentolamine-induced membrane hyperpolarization was blocked by the glibenclamide, an inhibitor of ATP-sensitive K^+ channels (Fig. 4A). The decreased amplitude of pacemaker potentials by phentolamine was returned to the basal level (i.e. 21.7 ± 1.7

mV) by glibenclamide (10 μM) (Fig. 4C). The membrane hyperpolarization by phentolamine was also returned to control level (i.e. –61 ± 1.6 mV) by glibenclamide (10 μM) (Fig. 4B). Taken together, these results suggest that phentolamine may activate ATP-sensitive K^+ channels in ICC.

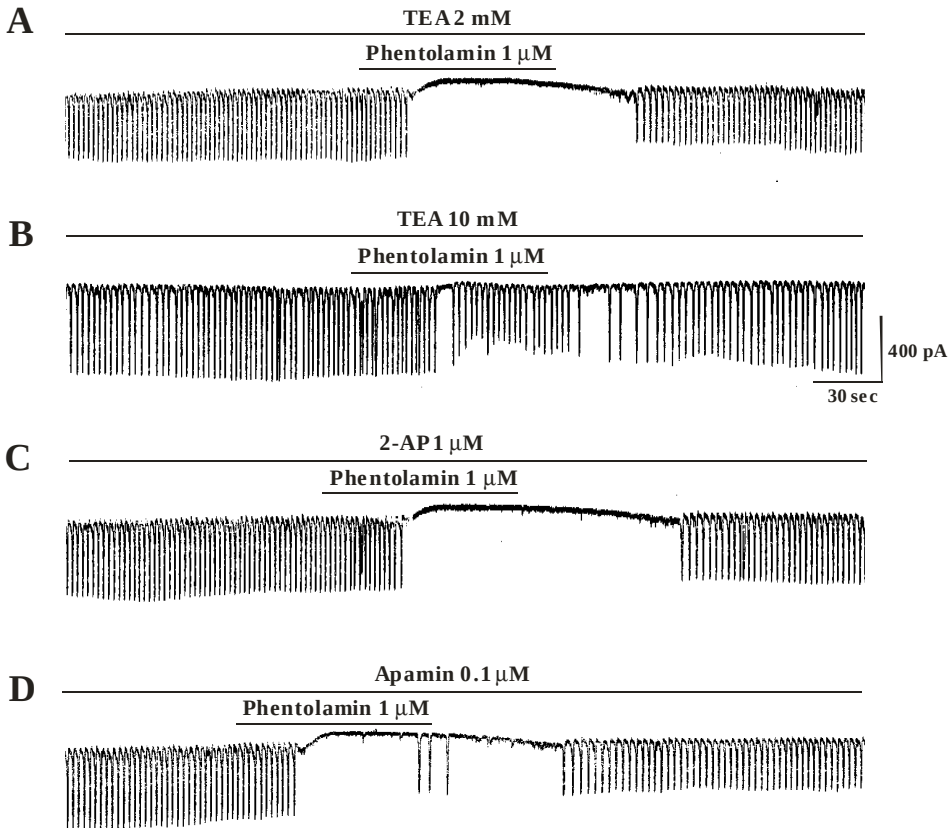

Fig. 2. Effects of various K^+ channels blockers on phentolamine-induced action in cultured ICC (A) and (B) The effects of 2 mM and 10 mM TEA on phentolamine action on pacemaker currents. (C) 2-AP (1 μM) pretreating had no effects on phentolamine action on pacemaker currents. (D) Apamin (0.1 μM) pretreating had no effects on phentolamine action on pacemaker currents.

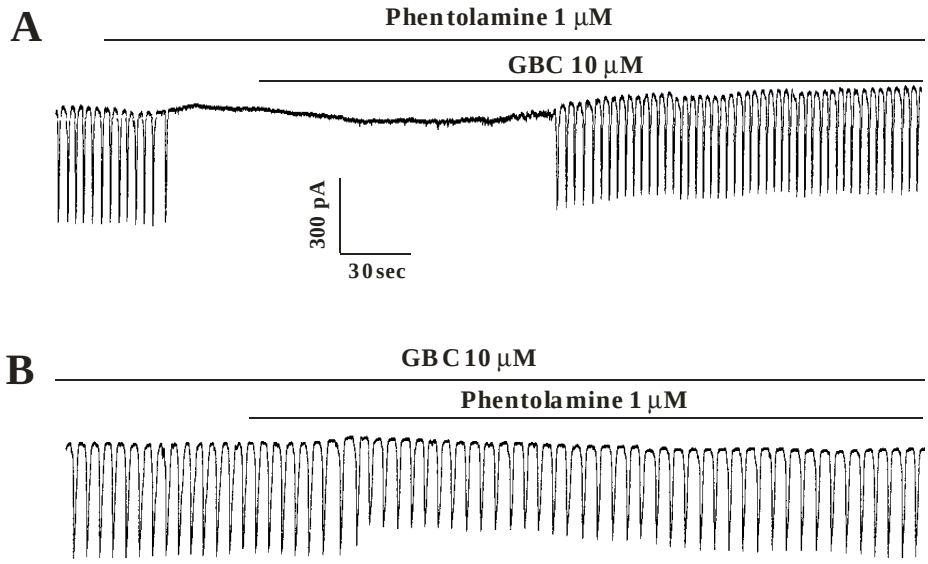

Fig. 3. Effects of glibenclamide, ATP-sensitive K^+ channel blocker, on phentolamine action on pacemaker currents in cultured ICC of mouse small intestine. (A) Pacemaker currents exposed to phentolamine (1 μM) at a holding potential of -70 mV. Phentolamine decreased the frequency and amplitude of the pacemaker currents, and increased the basal outward currents. These effects were reversed by adding glibenclamide (10 μM). (B) Phentolamine effects on pacemaker currents in the pretreatment of glibenclamide. GBC: glibenclamide.

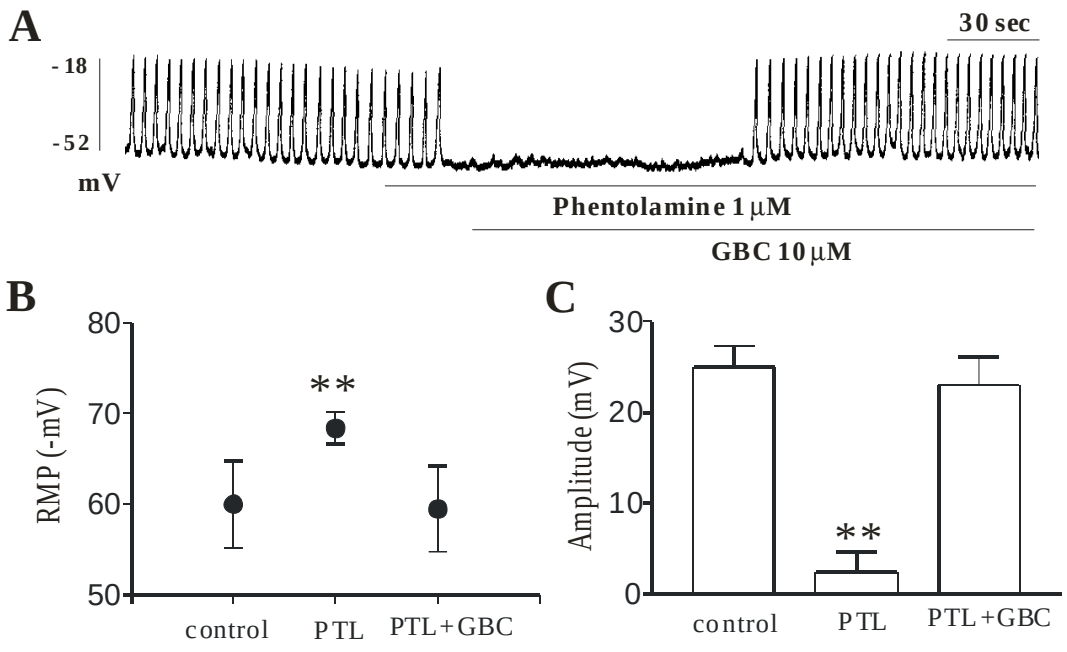

Fig. 4. Effects of phentolamine on pacemaker potentials in cultured ICC of mouse small intestine. (A) Pacemaker potentials of ICC exposed to phentolamine (1 μM) in the current clamping mode (*I*=0). The phentolamine produced membrane hyperpolarization and the decreased amplitude of pacemaker potentials were reversed by glibenclamide (10 μM). Response to phentolamine are summarized in (B) and (C). Bars represent mean vales±s.e. *(*P*<0.05) Significantly different from the untreated control. PTL: phentolamine, GBC: glibenclamide.

3. Effects of other α-adrenoceptor drugs on pacemaker currents.

To rule out whether phentolamine-induced effect is related with α-adrenoceptor activity or not, i treated several α-adrenoceptor antagonists and agonist in pacemaker currents. Prazosin (10 μM, a selective α_1-adrenergic antagonist), yohimbin(10 μM, a selective α_2 antagonist), and clonidine (10 μM, a selective α_2-adrenergic agonists) were treated on pacemaker currents, respectively. However, all of these drugs had no effects on pacemaker currents (Fig. 5A, B and C), suggesting phentolamine-induced effect is mediated by α- adrenoceptor independent action.

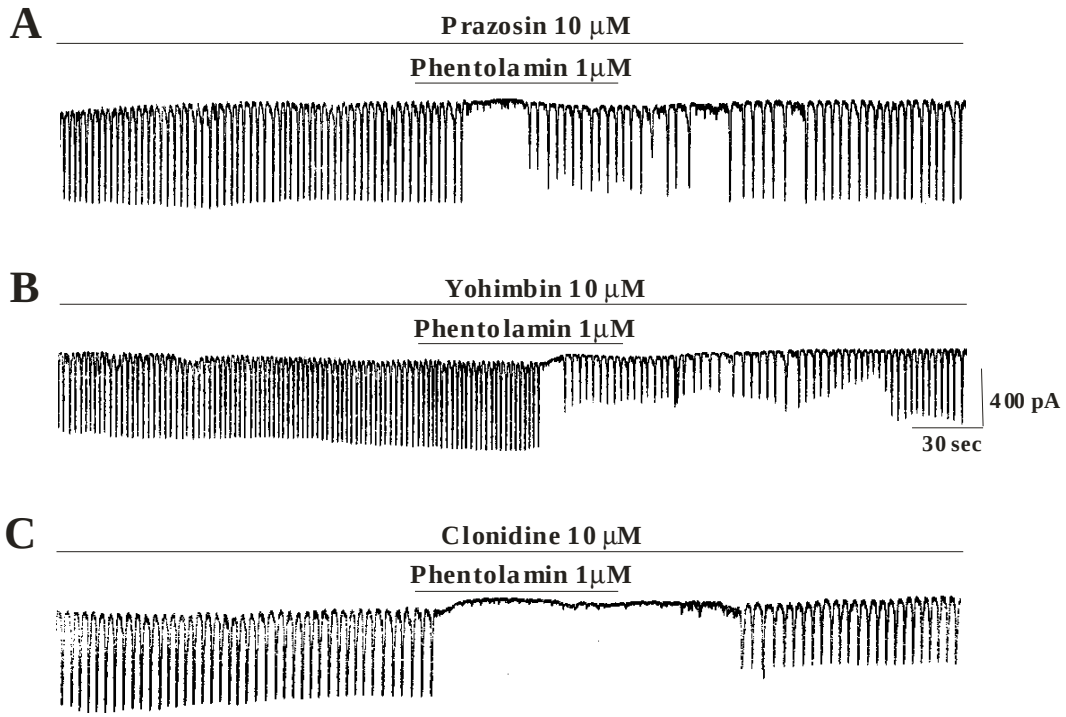

Fig. 5. Effects of several α-adrenoceptor antagonists on phentolamine action on pacemaker currents in cultured ICC of mouse small intestine. (A) Phentolamine (1 μM) action exposed to prazosin (10 μM), a selective α_1-adrenergic antagonist, at a holding potential of -70 mV. (B) Phentolamine (1 μM) action exposed to yohimbin (10 μM), a selective α_2 antagonist, at a holding potential of -70 mV. (C) Phentolamine (1 μM) action exposed to clonidine (10 μM), a selective α_2 antagonist, at a holding potential of -70 mV.

4. Effects of L-NAME or ODQ on the phentolamine-induced effects inhibition of pacemaker currents.

The effects of L-NAME (an inhibitor of nitric oxide synthase) or ODQ (an inhibitor of guanylate cyclase) were examined to investigate possible regulation of pacemaker currents by nitric oxide or cGMP-dependent pathway in the phentolamine-induced effects. L-NAME (10 μM) or ODQ was pretreated for 10 min before the application of phentolamine. In the presence of L-NAME or ODQ, the effects of phentolamine (1 μM) on pacemaker currents was not inhibited (Fig. 6A and B). And L-NAME or ODQ itself had no effect on pacemaker currents. These findings suggest that nitric oxide or cGMP may not mediate the phentolamine-induced effects in ICC.

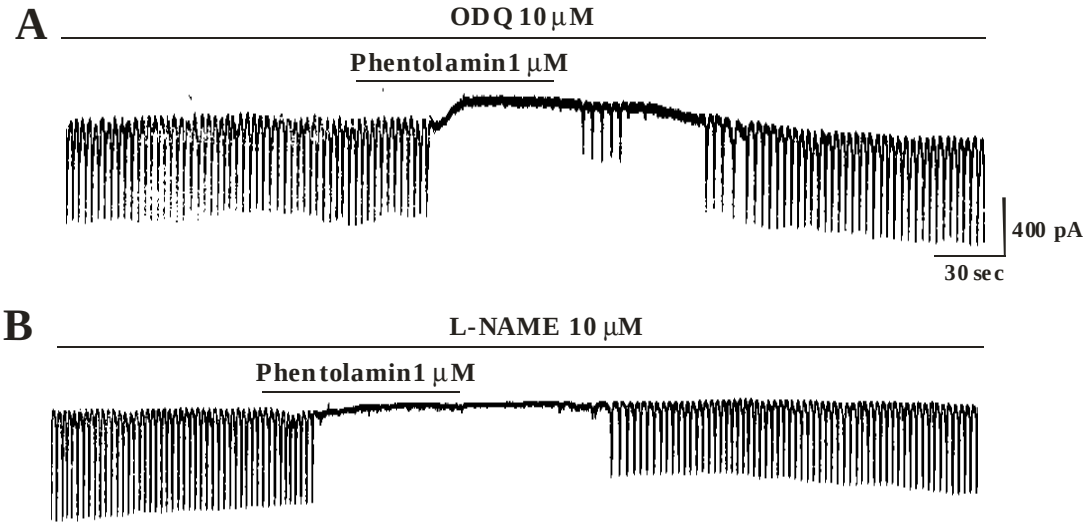

Fig. 6. Effects of L-NAME or ODQ on phentolamine action on pacemaker currents in cultured ICC of mouse small intestine. (A) Phentolamine (1 μM) action exposed to ODQ 10 μM (an inhibitor of guanylate cyclase) at a holding potential of -70 mV. (B) Phentolamine (1 μM) action exposed to L-NAME 10 μM (an inhibitor of nitric oxide synthase) at a holding potential of -70 mV. (C) Phentolamine (1 μM) action exposed to clonidine (10 μM), a selective α_2 antagonist, at a holding potential of -70 mV.

5. Effects of phentolamine on intracellular Ca^{2+}

Because many reports suggested $[Ca^{2+}]_i$ oscillations in ICC are considered to be the primary mechanism for the pacemaker activity in gastrointestinal activity, i examined the effect of phentolamine 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 (Fig. 7A) which was loaded with fluo3-AM. And in the presence of phentolamine (1 μM), $[Ca^{2+}]_i$ oscillations in ICC rapidly was declined (Fig. 7B). Also, spontaneous $[Ca^{2+}]_i$ oscillations inhibited by phentolamine (1 μM) in ICC was recovered by co-treatment of 10 μM glibenclamide (Fig. 7C). The data of time series are summarized in Fig. 7D. These results suggest that the action of phentolamine on ICC may involve the regulation of spontaneous $[Ca^{2+}]_i$ oscillations.

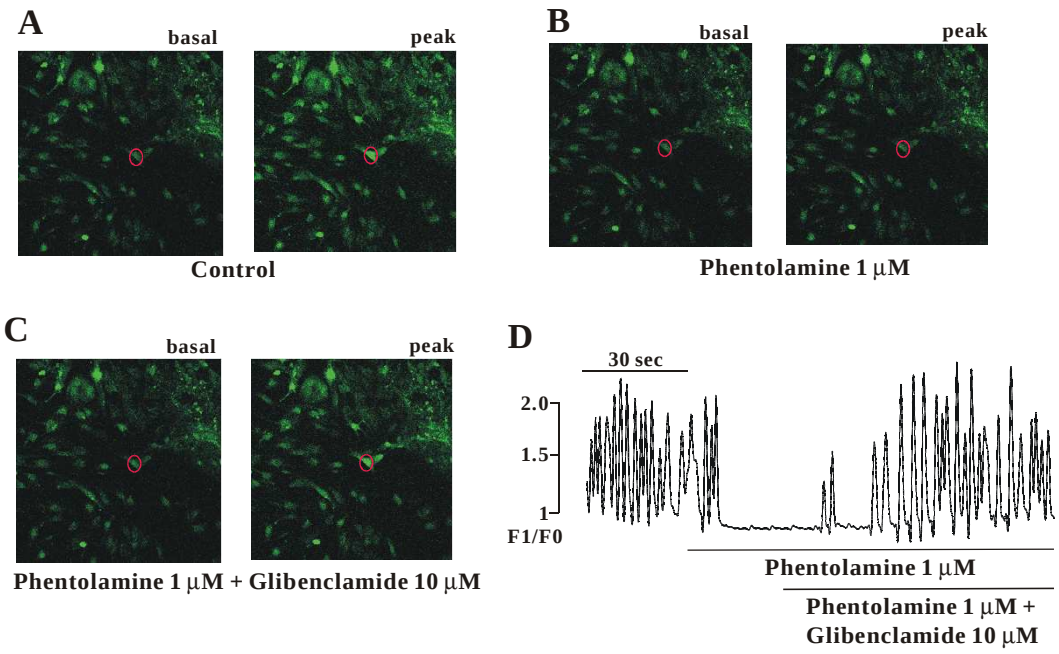

Fig. 7. Effects of phentolamine on intracellular Ca^{2+} oscillation in cultured ICC from mouse small intestine. (A) Sequential fluorescence intensity images of fluo-3-loaded cultured ICC in normal condition. The interval of representative frame was 1 second and the exposure time of each frame was 500 ms. (B) Sequential fluorescence intensity images of fluo-3-loaded cultured ICC in presence of phentolamine 1 μM. (C) Sequential fluorescence intensity images of fluo-3-loaded cultured ICC in presence of phentolamine 1 μM and glibenclamide 10 μM. (D) Fluorescence intensity change plotted in (A), (B) and (C) red marker.

V. Discussion

This study shows that phentolamine directly activates ATP-sensitive K^+ channels in intestinal ICC by non-adrenergic dependent mechanism. ATP-sensitive K^+ channels are regulates the membrane electrical potential, thus they determined cell excitability. ATP-sensitive K^+ channels are opened by pinacidil, diazoxide and are closed by sulfonylurea drugs such as glibenclamide or tolbutamide. Activation of ATP-sensitive K^+ channels produces hyperpolarization of cell membrane, thus decrease Ca^{2+} influx and inhibit cell excitability (Thorneloe and Nelson, 2005; Rodrigo and Standen, 2005). Several neurotransmitters participate in regulating gastrointestinal motility acting on ATP-sensitive K^+ channels of smooth muscle (Zhang et al., 1994; Quayle et al., 1997; Jun et al., 2001). It was also reported that ATP-sensitive K^+ channels existed in murine intestinal ICC. ICC show electrical pacemaker activities that responsible to gastrointestinal motor function. Pinacidil, deoxycholic acid and PGE_2 inhibited intestinal pacemaker activities by the activation of ATP-sensitive K^+ channels (Jun et al., 2005; Choi et al, 2006). In this study phentolamine showed the mimicked action with pinacidil. Phentolamine produced hyperpolarization of pacemaker potential and inhibited frequency and amplitude of pacemaker currents with the increase outward currents in ICC. These phentolamine effects are antagonized by glibenclamide but are not TEA, apamin, and 4-aminopyridine. It suggests phentolamine inhibits pacemaker activities of intestinal ICC by activation of ATP-sensitive K^+ channels. This result of phentolamine on ICC was consistent with the observation that application of phentolamine to human and rabbit relaxed corpus cavernosum by activation of ATP-sensitive K^+ channels (Vemulapalli and Kurowski, 2001; Silva et al., 2005). On the

contrary this effect of phentolamine on ICC was inconsistent with the observation that application of phentolamine to pancreatic β-cells, cardiac ventricular cells and vascular smooth muscle inhibited ATP-sensitive K^+ channels (Rustenbeck et al., 1995; Wilde e t al., 1994; Schwietert et al., 1992). I do not know this difference of phentolamine effects between intestinal ICC and pancreatic β-cells, ventricular cells and vascular smooth muscle. ATP-sensitive K^+ channels are comprised Kir (Kir 6.1 or Kir 6.2) subunits that form the K^+-selective ion channel pore and SUR (SUR1 or SUR2A or SUR2B) subunits, which are receptors of sulfonylureas. In pancreatic β-cell, cardiac muscle and vascular smooth muscle ATP-sensitive K^+ channels are composed with Kir6.2 and SUR1, Kir 6.2 and SUR 2A, and Kir 6.1 and SUR2B, respectively. In smooth muscle, ATP-sensitive K^+ channels are composed with Kir 6.2 and SUR 2B (Mannhold, 2003). In murine cultured intestinal ICC, ATP-sensitive K^+ channels are composed with Kir 6.2 and SUR 2B. Together, there are regional differences of electrical activities between tissues to tissues. Therefore, i think that subunit combination and different tissue type are possible explanation the difference of phentolamine effects between intestinal ICC and pancreatic β-cells, ventricular cells, and vascular smooth muscle cells.

ATP-sensitive K^+ channels are regulated by the second messengers. CGRP activates ATP-sensitive K^+ channels after production of cAMP in vascular and gallbladder smooth muscle (Zhang et al., 1994). Angiotensin II or acetylcholine inhibits ATP-sensitive K^+ channels by activation of protein kinase C in vascular or gastrointestinal smooth muscle, respectively (Quayle et al., 1994; Jun et al., 2001). Together, it has been reported that NO/cGMP involves in activation ATP-sensitive K^+ channels in vascular smooth muscle and bladder smooth muscles (Murphy and Brayden 1995; Deka and Brading 2004). I found that cAMP system did not involve in modulation of ATP-

sensitive K^+ channels, whereas external NO inhibited intestinal pacemaker activities by cGMP-dependent activation of ATP-sensitive K^+ channels in intestinal ICC (Park et al., 2007). In rabbit corpus cavernosum, L-NAME (a NO synthase inhibitor) significantly attenuated electrical filed stimulation produced relaxations to phentolamine (Vemulapalli and Kurowski, 2001). Together, L-NAME and ODQ (a guanylate cyclase inhibitor) significantly inhibited the phentolamine-induced relaxation in human corpus cavernosum strips precontracted with K^+ 40 mM (Silva et al., 2005). It suggests that NO/cGMP pathway may be able to mediate the phentolamine-induced effects in intestinal ICC, too. However, in this study, L-NAME and ODQ did not block the phentolamine-induced effects; suggest phentolamine may directly activate ATP-sensitive K^+ channels in intestinal ICC.

The activation of ATP-sensitive K^+ channels decreases intracellular Ca^{2+} levels in some tissues through inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels or the inhibition of Ca^{2+} release from intracellular stores (Brayden 2002; Small *et al*. 1992). Phentolamine increase intracellular Ca^{2+} of pancreatic β-cells which contribute to insulin release (Rustenbeck et al., 1995). 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 (Sanders et al., 2006). In my results, i found spontaneous $[Ca^{2+}]_i$ oscillations in ICC and treatment with phentolamine inhibited the intracellular Ca^{2+} oscillations.

Phentolamine effects on intestinal ICC was not mediated by α-adrenoceptor dependent mechanism. Because the other nonselective α-adrenoceptor agonist and antagonists had no effects on intestinal pacemaker activities. It was already reported that the inhibition

of pacemaker activities by noradrenaline is mediated by β-adrenoceptor activation, which is not involve ATP-sensitive K^+ channels (Jun et al., 2004).

In conclusion, phentolamine can regulate intestinal motility through directly activation of ATP-sensitive K^+ channels in ICC by non-adrenergic receptor dependent mechanism. ATP-sensitive K^+ channels activation involves intracellular Ca^{2+} kinetics.

V. Summary

The actions of phentolamine on pacemaker activities were investigated using a whole-cell patch-clamp technique at 30℃ in cultured interstitial cells of Cajal (ICC) from murine small intestine. ICC generated spontaneous pacemaker currents at a holding potential of -70 mV. The treatment of ICC with phentolamine resulted in a decrease in the frequency and amplitude of pacemaker currents and increases in resting outward currents. Also, under current clamping *(I=0)*, phentolamine produced the hyperpolarization of membrane potential and decreased the amplitude of the pacemaker potentials. Prozosin, yohimbin, phenoxybenzamine and clonidine which are α-adrenergic drugs were no effects on intestinal pacemaker activities. Phentolamine-induced effects on pacemaker currents and pacemaker potentials were significantly inhibited by glibenclamide, an ATP-sensitive K^+ channel blocker and not by TEA, apamin, and 4-aminopyridine. L-NAME, a NO synthase inhibitor, and ODQ, a guanylate cyclase inhibitor, did not inhibit the phentolamine-induced effects. These results show that phentolamine activates ATP-sensitive K^+ channels by non-adrenergic receptor mechanism in intestinal ICC. This observation suggests phentolamine can regulate gastrointestinal motility through changing pacemaker activities of ICC.

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저작물 이용 허락서

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논문제목	한글: **소장 카할 사이질세포에서 phentolamine 의 ATP-민감성 칼륨통로 활성화를 통한 향도잡이 활동도 억제작용**				
	영문: **Phentolamine inhibits intestinal pacemaker activity of murine interstitial cells of Cajal by activating ATP-sensitive K^+ channel**				

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

- 다 음 -

1. 저작물의 DB 구축 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 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1 개월 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( )

2008 년 7 월 일

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조선대학교 총장 귀하