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Ginseng Saponins Enhance Maxi Ca^{2+}-activated K^+ Currents of the Rabbit Coronary Artery Smooth Muscle Cells

Induk Chung# and Nak-Doo Kim*

Department of Physiology, Hanyang University College of Medicine and
**Department of Pharmacy, Seoul National University*
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Abstract : Potassium channels play an important role in regulating vascular smooth muscle tone. Four types of K^+ channels areknown to be expressed in vascular smooth muscle cells, and maxi Ca^{2+}-activated K^+ channel (BK_{Ca}) is a dominant type of K^+ channels in these cells. Because total ginseng saponins and ginsenoside Rg_3 cause vasodilation with unclear mechanisms, we hypothesized that total ginseng saponins and ginsenoside Rg_3 induce vasodilation via activation of maxi Ca^{2+}-activated K^+ channels. Whole-cell BK_{Ca} currents were voltage-dependent with half maximum activation at -14 mV, and the currents were sensitive to nanomolar ChTX and millimolar TEA. External application of total ginseng saponins increased the amplitude of the whole-cell BK_{Ca} current in a concentration-dependent manner. Single-channel analysis indicates that total ginseng saponins caused the channel opening for a longer period of time. Ginsenoside Rg_3 increased the amplitude of whole-cell K_{Ca} currents without affecting voltage dependence of the currents and increased single-channel open time. Hence, the results suggest that ginseng saponin-induced vasodilation may be due to activation of K_{Ca}.

Key words : Ca^{2+}-activated K^+ channel (Kca), whole-cell Kca currents, delayed rectifier, steady-state K^+ currents, single-channel Kca currents

Introduction

The roots of *Panax ginseng* have long been used in traditional Chinese medicine as an anti-inflammatory, an analgesic, and as a tonic aid for rapid recovery from chronic illness. Besides proteins and carbohydrates which are usually present in most herbs, ginseng also contains saponins known as ginsenosides. The ginseng saponins extracted from Panax ginseng are known to decrease heart rate in healthy volunteers, lower serum cholesterol levels, cause vasodilation of cerebral and coronary vessels, inhibit progression of liver and ovarian cancer, improve concentration and intelligence, and prevent memory loss in old rats.[9] A recent report indicates that in rat aorta ginsenosides cause vascular relaxation when the endothelium is present[7]. In addition, certain pure ginsenosides have more potent effect for vascular relaxation than the other. For instance, protopanaxadiol (PPD) and protopanaxatriol (PPT) are the major form of ginsenosides.[6] Ginsenosides from PPD do not affect vascular tone or production of cGMP in the rat aorta, while ginsenosides from PPT cause vascular relaxation with formation of cGMP.[6,7] Since tetraethylammonium (TEA), a K^+ channel blocker, prevented ginsenoside Rg_3-evoked relaxation in endothelium-denuded aortic rings constricted with high KCl, Kim and colleagues have concluded that ginsenoside Rg_3 may cause vascular relaxation through activation of K^+ channels.[8]

Activation of K^+ channels in vascular smooth muscle hyperpolarizes the cell membrane, which subsequently closes voltage-dependent Ca^{2+} channels. This results in a decrease in intracellular Ca^{2+} with vascular relaxation.[3] Thus, K^+ channels are currently known as a major regulator of vascular tone. Four types of K^+ channels have been described in vascular smooth muscle cells so far, and they are the voltage-dependent K^+ channels (K_V), maxi Ca^{2+}-activated K^+ channels (BK_{Ca}), ATP-sensitive K^+ channels (K_{ATP}), and inward rectifier (K_{IR}).[3,4] Because BK_{Ca} is expressed in large

#To whom correspondence should be addressed.
(Tel)/(Fax) 02-2290-0613
E-mail: indukchun@netscape.net

numbers in vascular smooth muscle cells and directly regulated by nitric oxide (NO),[1,10,11] BK_{Ca} is regarded as a major type of K^+ channels of vascular smooth muscle cells. In the present study we hypothesize that total ginseng saponins and ginsenoside Rg_3 relax vascular smooth muscle cells by increasing the BK_{Ca} activity. Using the whole-cell and single-channel patch-clamp technique we directly measured the BK_{Ca} activity in single smooth muscle cells isolated from rabbit coronary artery.

Materials and Methods

1. Cells

The rabbit coronary artery smooth muscle cells were prepared from healthy 1.5~2 Kg male NZ rabbits. Coronary arteries were separated from the heart under a dissecting microscope bathed with tyrode working solution containing (in mM) 135 NaCl, 5 KCl, 10 HEPES, 0.1 EGTA, 10 Glucose, 1 $MgCl_2$ and 2 $CaCl_2$. The connective tissues attached to the arteries were easily removed through a simple peeling procedure. The clean arteries were cut into smaller pieces, which were then incubated for 5 minutes in calcium-free tyrode working solution at 37°C in a water bath, 5 minutes in enzyme I solution containing papain (0.5 mg/m*l*), bovine serum albumin (2.5 mg/ml), and DL-dithiothreitol (1 mg/m*l*), and 25 minutes in enzyme II solution containing collagenase (4 mg/ml), hyaluronidase (1.3 mg/m*l*), bovine serum albumin (2 mg/m*l*), and DL-Dithiothreitol (1.5 mg/m*l*). After the series of incubation, single smooth muscle cells were collected into the KB solution consisting of (in mM) 50 L-Glutamic acid, 40 KCl, 20 Taurine, 20 KH_2PO_4, 3 $MgCl_2$, 10 Glucose, 10 HEPES and 0.5 EGTA for patch-clamp recording.

2. Electrophysiological recordings

All recordings were made in the whole-cell and inside-out patch-clamp configurations using an Axopatch 1D patch-clamp amplifier.[5] pClamp software (ver. 6.0.3) and Labmaster hardware were used to control voltage and to acquire and analyze data. Pipettes were fabricated of borosilicate glass capillary tubing with resistances of about 3~6 MΩ with the pipette solutions used. The uncompensated series resistance was about 10 MΩ during whole-cell recordings, so the voltage error was less than 5 mV for current amplitude of 500 pA. With the pipette in the bath, the zero-current potential was measured, then remeasured after a seal was formed. This new junction potential was then subtracted using the Axopatch internal circuit during whole-cell recordings. Recordings of whole-cell K^+ currents began about 10 min after the onset of the whole-cell configuration. Currents were filtered at 5 kHz with the 8-pole Bessel filter in the Axopatch amplifier. Exchange of the bathing medium was performed by continuous perfusion. All recordings were made at room temperature (22~24°C). Where appropriate, results are presented as mean± S.E.M. (n, number of cells).

3. Solutions

For whole-cell recordings, the bathing medium was NaCl saline consisting of (in mM) 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, adjusted to pH 7.4, and the pipette solution contained KCl saline consisting of (in mM) 130 KCl, 0.095 EGTA, 0.1 $CaCl_2$, 1 $MgCl_2$, 2 ATP, and 10 HEPES, adjusted to pH 7.4. For single-channel recordings, pipette contained the NaCl saline and the bath contained the KCl saline. Intracellular free Ca^{2+} was buffered to 1 μM to activate BK_{Ca} currents using a computer program written by Dr. J. Kleinschmidt (New York University, NY).

Results

1. Biophysical and pharmacological properties of BK_{Ca}

The predominant currents seen in whole-cell recordings from single cells of rabbit coronary artery are a voltage- and time-dependent delayed rectifier (Fig. 1A). The BK_{Ca} current began to activate at depolarizing potentials above -40 mV from a holding potential of -60 mV, but it did not inactivate. The current vs. voltage relations for the steady-state current shows outward rectification (Fig. 1B). From the conductance vs. voltage relations the half-maximal voltage for activation was -14 mV (Fig. 1C). The currents were sensitive to TEA and ChTX at all voltage range.

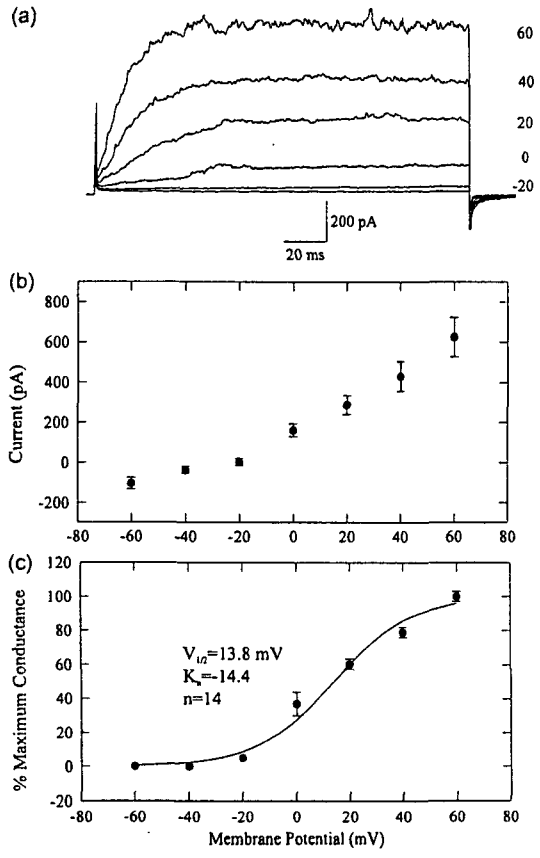

Fig. 1. Families of whole-cell Ca^{2+}-activated K^+ currents. A: Whole-cell K_{Ca} currents were recorded from a holding potential of -60 mV at voltage steps from -40 mV to 60 mV applied every 3 sec in 20 mV increments. B: Current vs. voltage relation for steady-state K^+ currents. Steady-state K^+ currents at 150 ms were plotted at each command potential. Data represent mean±S.E.M of 14 cells. C: Conductance vs. voltage relation for activation. Steady-state K^+ conductance at each voltage step was normalized to the maximum K^+ conductance obtained at a command potential of 60 mV. The fitted activation curve was then obtained by non-linear least squares fit of $g(V)=g_{max}/1+\exp[(V_{1/2}-V)/K_n]$, where $V_{1/2}$ is the voltage producing half-maximum K^+ conductance, K_n is the slope factor corresponding with the voltage sensitivity of activation, and n is the number of cells.

When the steady-state currents in the presence of TEA or ChTX were normalized to the control currents, 500 nM ChTX inhibited the whole-cell K_{Ca} current by 67±7% (n=4) and 10 mM and 20 mM TEA caused 57±5 % and 82±8% (n=3, each) reduction in the current, respectively (Fig. 2).

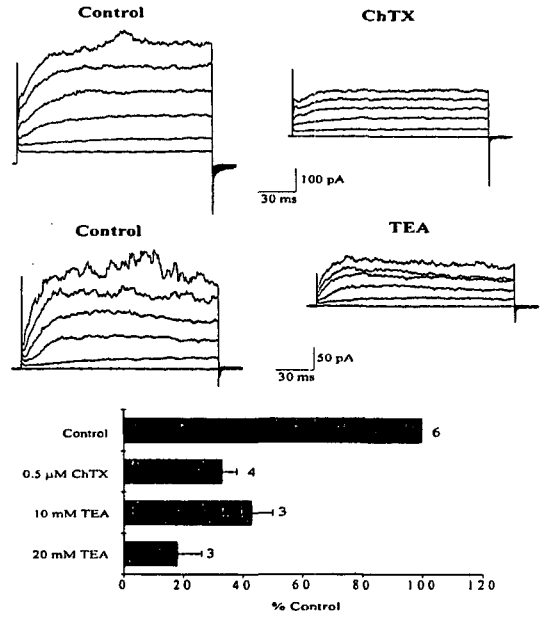

Fig. 2. Pharmacology of K_{Ca} currents A, B: Whole-cell K_{Ca} currents were recorded at voltage steps (-60 mV to 40 mV for A, -60 mV to 20 mV for B) in 20 mV increments from a holding potential of -60 mV. Then 20 mM TEA or 500 nM ChTX was perfused into bathing medium and the K^+ currents were recorded. C: BK_{Ca} currents in the presence of TEA or ChTX were normalized to the control K^+ current at 40 mV (*TEA*) or 70 mV (*ChTX*). Data indicate mean±S.E.M and number of cells.

2. Effects of total ginseng saponins and ginsenoside Rg_3 on K_{Ca}

We tested whether total ginseng saponins and ginsenoside Rg_3 affect the BK_{Ca} activity. Perfusion of total ginseng saponins increased the channel activity; 0.5 and 1 mg/ml total ginseng saponins increased the current amplitudes by 49±5% and 108±10% (n=5, each), respectively (Fig. 3A). When single-channel BK_{Ca} current was recorded in inside-out patch configuration, diffusion of 50 μg/m*l* total ginseng saponins caused more and longer opening of 200 pS BK_{Ca} (Fig. 3B). Ginsenoside Rg_3 also increased whole-cell BK_{Ca} current (Fig. 4A), and 100 μg/m*l* Rg_3 increased the current by 96±8% (n=6, Fig. 4B). Five hundred nanomolar ChTX inhibited the currents increased by Rg_3, confirming that ginsenoside Rg_3 activates ChTX-sensitive BK_{Ca}. Single-channel BK_{Ca} currents were recorded in inside-out patch configuration. As ginsenoside Rg_3 was diffused into membrane patch, 200 pS BK_{Ca} was

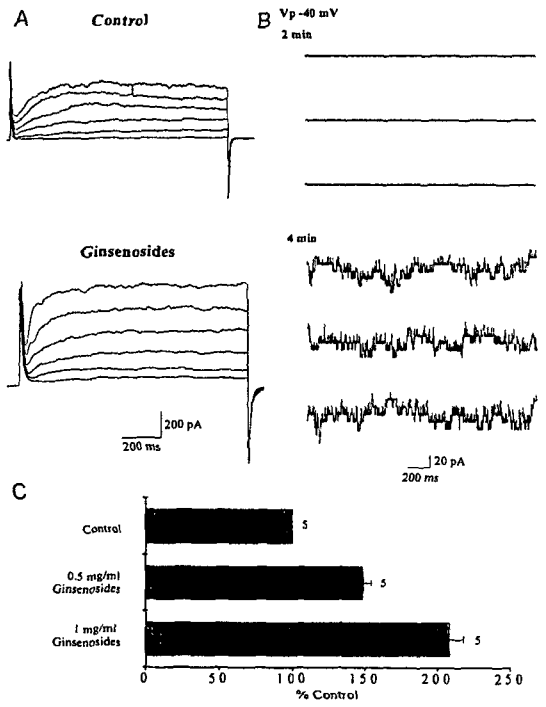

Fig. 3. Activation of whole-cell and single-channel K_{Ca} currents by total ginseng saponins. A: Whole-cell K_{Ca} currents were elicited from a holding potential of -60 mV at voltage steps from -60 mV to 60 mV in 20 mV increments (*Control*). Total ginseng saponins (0.5 and 1 mg/ml) were then perfused into bathing medium and the K_{Ca} currents were recorded (*Ginsenosides*). B. Single-channel KCa currents in inside-out configuration. Total ginseng saponins (50 μg/ml) were back filled into pipette solution. 200 pS BK_{Ca} currents were elicited from a pipette potential (Vp) of -40 mV at 2 and 4 min after establishing inside-out patch mode. C: Whole-cell K_{Ca} currents in the presence of 0.5 or 1 mg/ml total ginseng saponins (Ginsenosides) were normalized to the control K_{Ca} current at -60 mV (Control).

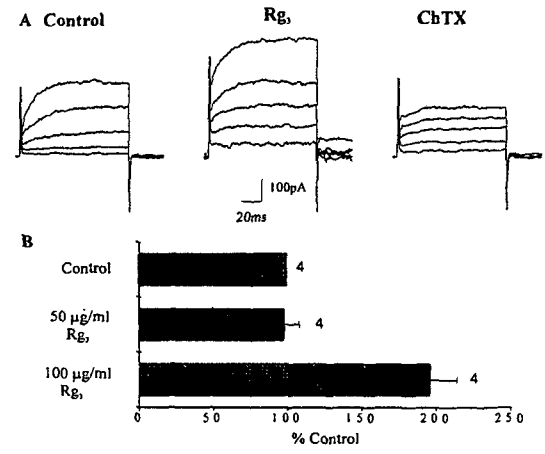

Fig. 4. Activation of whole-cell K_{Ca} currents by Rg_3. A: Whole-cell K_{Ca} currents were recorded from a holding potential of -60 mV at command potentials from -60 mV to 40 mV under control condition (Control) and after perfusion with 100 μg/ml Rg_3 (Rg_3) or 500 nM ChTX (*ChTX*). B: Whole-cell K_{Ca} currents in the presence of 50 and 100 μg/ml Rg_3 were normalized to the control K^+ current at 40 mV. Data indicate mean±S.E.M. and number of cells.

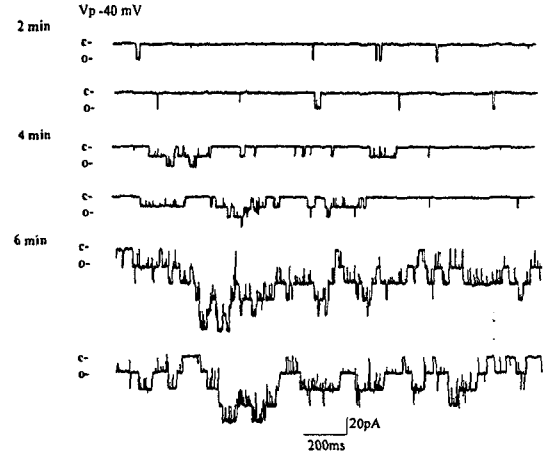

Fig. 5. Activation of single-channel K_{Ca} current by Rg_3. Single-channel K_{Ca} currents were elicited in inside-out patch configuration from a pipette potential of -40 mV at 2, 4, and 6 min after establishment of gigaohm seal. Pipette contained the standard pipette solution plus10 μg/ml Rg_3 and 200 nM ChTX. Ten times lower Rg_3 was used to record stable single-channel currents, and ChTX was included to prevent maximum activation of K_{Ca} by Rg_3.

activated at a pipette potential of -40 mV. At approximately 6 min after establishing inside-out patch mode, most of channels were open at all times (Fig. 5).

Discussion

The purpose of this study was to examine whether ginseng saponins cause vascular relaxation via activation of BK_{Ca} of rabbit coronary artery. We demonstrated that both total ginseng saponin and ginsenoside Rg_3 increased the whole-cell K_{Ca} current amplitude without affecting voltage-dependence of the current in the coronary artery smooth muscle cells. Single-channel analyses have shown that most BK_{Ca} remained open in the presence of total ginseng

saponins and ginsenoside Rg_3.[11] Electrophysiological and pharmacological parameters of BK_{Ca} from this study are similar to those described in other vascular smooth muscle cells.[3,4,11] Total ginseng saponins and ginsenoside Rg_3 increased the activity of TEA- and ChTX-sensitive BK_{Ca}. Furthermore, the increase of the current by ginseng saponins was inhibited by ChTX, indicating that ginseng saponins specifically activated BK_{Ca}. Ginseng saponins appear to increase other types of K^+ channels. We observed activity of voltage-gated K^+ channels (Chung, unpublished data) and ATP-sensitive K^+ channels was increased.[2] Hence, we propose that an increase in BK_{Ca} conductance contributes to maintain resting membrane potential, resulting in relaxation of vascular tone.

요 약

혈관의 평활근 세포막에 존재하는 포타슘채널은 근세포의 막전압을 조절하여 근수축 및 이완을 조절한다. 네가지 유형의 포타슘채널이 근세포막에 존재하며 이중 전도도가 큰 칼슘의존성-포타슘채널(BK_{Ca})은 평활근 막전압 조절에 중요한 기능을 담당하는 채널로 알려져 있다. 현재 홍삼 복합사포닌이 혈관 평활근의 이완을 증진시켜 혈압강하를 촉진시킨다고는 알려져 있으나 어떤 분자적 기전이나 전기생리학 기전으로 작용하는지 정확히 알려져 있지 않다. 본 연구는 홍삼 복합사포닌 및 사포닌 Rg_3 성분이 토끼 관상동맥 평활근 세포의 BK_{Ca}채널의 활성을 증진시켜 막전압을 과분극시키고 곧 평활근 이완을 촉진한다는 가설을 테스트하였다. 관상동맥 평활근세포의 BKCa 채널은 막전압 의존성, 외향정류(outward rectification) 특성을 보였고 단일채널의 전도도는 200 pS으로 측정되었으며 charybdotoxin 및 tetraethylammonium에 억제되는 약리학적 특성을 보였다. Whole-cell BK_{Ca}활성은 홍삼 복합사포닌에 의해서 농도 의존적으로 증가되었으나 막전압 의존성은 변화되지 않았으며, 단일채널이 열리는 시간은 증가되었다. 홍삼 사포닌 Rg_3성분도 막전압 의존성에는 영향을 주지 않으면서BK_{Ca}의 활성을 증가시켰으며 단일채널이 열리는 시간도 증가시켰다. 따라서 홍삼 복합사포닌 및 사포닌 Rg_3성분은 BK_{Ca}의 활성을 증가시켜 막전압을 과분극시켜 평활근의 이완을 촉진한다고 여겨진다.

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