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Ginsenoside Rg_3 Increases the ATP-sensitive K^+ Channel Activity in the Smooth Muscle of the Rabbit Coronary Artery

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Abstract : ATP-sensitive K^+ channels (K_{ATP}) are expressed in vascular smooth muscle cells, skeletal muscle cells, pancreatic β cells, neurons and epithelial cells. K_{ATP} contributes to regulate membrane potential to control vascular tone, to protect myocardial ischemia, and to regulate insulin secretion in pancreatic β cells. We previously demonstrated that ginseng saponins and ginsenoside Rg_3 activated maxi Ca^{2+}-activated K^+ channel, and this might cause vasodilation. Because K_{ATP} plays an important roles to regulate the resting membrane potential in vascular smooth muscle cells, we investigated whether ginsenoside Rg_3 produces vasodilation by activating K_{ATP} We showed in this study that K_{ATP} is expressed in rabbit coronary artery smooth muscle cells. K_{ATP} was inwardly rectifying and was inhibited by internal application of ATP. Micromolar minoxidil activated, but glyburide inhibited the activity of K_{ATP} Ginsenoside Rg_3 relieved inactivaiton of whole-cell K_{ATP} current without affecting the peak amplitude of K_{ATP} currents presumably due to more opening of the channels.

Key words : ATP-sensitive K^+ channel, single-channel K^+ currents, activated K_{ATP} current, whole-cell K^+ currents, inward rectification

Introduction

K^+ channels that are inhibited by intracellular ATP, called ATP-sensitive K^+ channel (K_{ATP}), are expressed in a wide variety of tissues including in the cardiac myocytes,[10] arterial smooth muscle cells,[13] and pancreatic β cells.[4] K_{ATP} maintains the membrane potential during ischemia in the cardiac myocytes, thereby-preventing further deterioration from a series of Ca^{2+}-dependent physiological events.[12] It also maintains the membrane potential of vascular smooth muscle cells and enhances relaxation of the muscle.[3,5,10] In the pancreatic β cells its inhibition facilitates glucose-stimulated insulin secretion.[1] Hence, K_{ATP} is regarded as an important therapeutic target for hypertension and diabetes.

The ginseng saponins extracted from *Panax ginseng* have been shown to relax aortic rings via production of cGMP and/or activation of TEA-sensitive K^+ channels[7,8,9] and maxi Ca^{2+}-activated K^+ channels.[2] It also appears that ginsenoside Rg_3 might cause vascular relaxation by activating K_{ATP}, because micromolar glibenclamide, a specific blocker of K_{ATP} significantly decreased Rb^+ efflux from rat aortic rings.[9] In this present study we investigated whether ginsenoside Rg_3 causes vascular relaxation via an increase in the K_{ATP} 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 as previously described.[2] Briefly, coronary arteries of healthy 1.5~2 kg male NZ rabbits were separated in tyrode working solution (in mM, 135 NaCl, 5 KCl, 10 HEPES, 0.1 EGTA, 10 Glucose, 1 $MgCl_2$ and 2 $CaCl_2$). After removal of the connective tissues, clean arteries were incubated in calcium-free tyrode solution at 37°C, enzyme I solution (0.5 mg/ml papain, 2.5 mg/ml bovine serum albumin, and 1 mg/ml DL-dithiothreito) for 5 min each, and

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then for 25 min in enzyme II solution (4 mg/ml collagenase, 1.3 mg/ml hyaluronidase, 2 mg/ml bovine serum albumin, and 1.5 mg/ml DL-Dithiothreitol). After the series of incubation, single smooth muscle cells were collected into the KB solution (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).

2. Electrophysiological recordings

Whole-cell and single-channel recordings were made according to Hamill *et al.*[6] An Axopatch 1D patch-clamp amplifier and 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 (resistances 3~6 MΩ) with the pipette solutions used. 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), and results are presented as mean ± S.E.M. (*n*, number of cells).

3. Solutions

For whole-cell recordings, the bathing medium was normal KCl saline containing (in mM) 140 KCl, 1 $MgCl_2$, 10 glucose and 10 HEPES, adjusted to pH 7.4, and the pipette solution was low Ca^{2+}-KCl saline consisting of (in mM) 140 KCl, 10 EGTA, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, adjusted to pH 7.4. For single-channel recordings, the bathing medium was low Ca^{2+}-KCl saline and the pipette solution was normal KCl saline. ATP was not included to activate K_{ATP} current. Intracellular free Ca^{2+} was buffered to <10 nM to inhibit Ca^{2+}-dependent currents according to a computer program written by Dr. J. Kleinschmidt (New York University, NY).

Results and Discussion

1. Electrophysiological and pharmacological properties of K_{ATP} currents

Whole-cell K^+ currents were elicited in symmetrical KCl saline from a holding potential of 0 mV. It became activated at more hyperpolarizing potentials. The whole-cell current vs. voltage relation for the peak current showed strong inward rectification at potentials more negative than -100 mV. The K^+ currents were reversed at 0 mV, indicating that this current is highly selective to K^+ (Fig. 1A). Single-channel K^+ currents were recorded at a pipette potential of 60 mV (Fig. 1B). In inside-out patch configuration, perfusion of the bathing medium with 2 mM ATP completely inhibited the current. When ATP was washed out, the current was recovered, indicating that the channels were sensitive to intracellular ATP. Phamacological properties of the current were further examined. The peak amplitude of the inward whole-

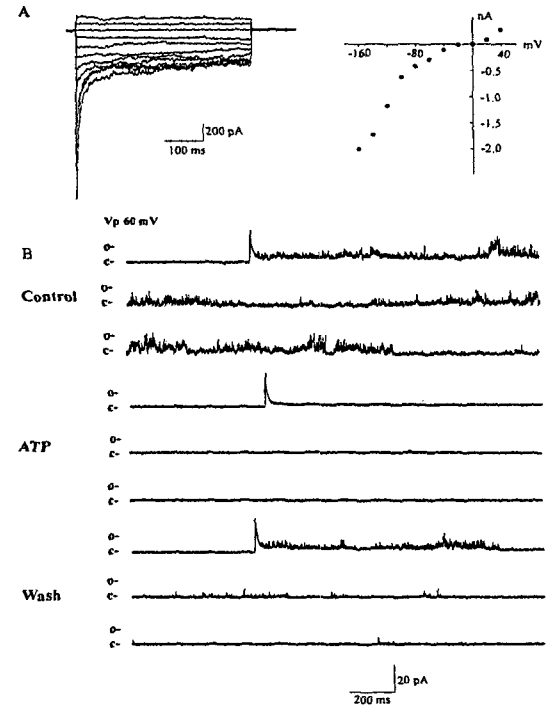

Fig. 1. Whole-cell and single-channel K_{ATP} currents. A: Bathing medium and pipette solution contained symmetrical KCl saline. Whole-cell K_{ATP} currents were recorded from a holding potential of 0 mV at voltage steps from 160 mV to 40 mV applied every 3 sec in 20 mV increments (*left*). The peak current vs. voltage relation exhibits inward rectification (*right*). B: Single-channel K_{ATP} currents were reversed in inside-out patch configuration at a pipette potential of 60 mV (*Control*) and after 2 mM ATP was perfused (*ATP*) or the bath was washed out (*Wash*).

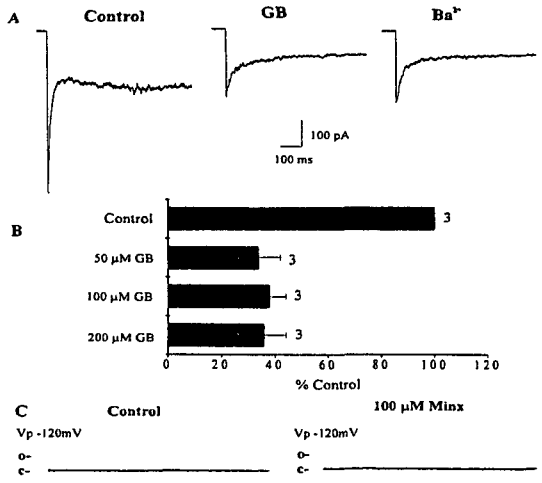

Fig. 2. Pharmacology of K_{ATP} currents. A: Whole-cell K_{ATP} currents were elicited at 120 mV from a holding potential of 0 mV (*Control*). Then 50 μM glyburide (*GB*) or 500 μM Ba^{2+} (*Ba^{2+}*)was perfused into bathing medium. B: K_{ATP} currents in the presence of glyburide were normalized to the control K^+ current at 120 mV. 50, 100, and 200 μM glyburide inhibited the current by 64±8%, 62±6% and 66±8% (S.E.M., n=3 each), respectively. C: Single-channel K_{ATP} currents were recorded in inside-out pach mode at a pipette potential of 120 mV. Perfusion of 100 or 200 μM minoxidil increased the currents.

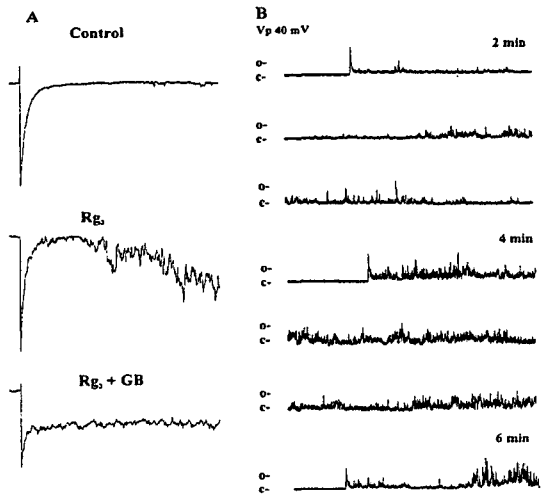

Fig. 3. Activation of K_{ATP} currents by Rg_3. A: Whole-cell K_{ATP} currents were evoked from a holding potential of 0 mV at a command voltage of 120 mV (*Control*). Then 100 μg/ml Rg_3 was perfused into bathing medium (*Rg_3*). The current increased by Rg_3 was inhibited by 20 μM glyburide (*Rg_3+GB*) in the presence of Rg_3. B: Single-channel K_{ATP} currents was elicited at pipette potential of 40 mV. Pipette contained 200 nM ChTX to prevent activation of BK_{Ca} and 10 μg/ml Rg_3. The concentration of Rg_3 used was reduced by ten times compared with that employed for whole-cell recordings to prevent excessive activation of K_{ATP} The currents were recorded at 2, 4 and 6 min after establishment of inside-out patch configuration.

cell K^+ currens was inhibited by micromolar glyburide; 50, 100, and 200 μM glyburide caused 64±8%, 62±6%, 66±8% inhibition (n=3 each), respectively (Fig. 2A, B). However, bath perfusion of 500 μM Ba^{2+} did not inhibit the inward K^+ currents, indicating that K_{ATP} currents recorded were not contaminated with a component of Ba^{2+}-sensitive inward rectifier (Fig. 2B). We also used minoxidil, an activator of K_{ATP} Two hundred micromolar minoxidil activated the current. Thus, electrophysiological and pharmacological characteristics of the current clearly indicate that it is the ATP-sensitive K^+ current (Fig. 2C).

2. Effects of Rg_3 on K_{ATP} current

We then examined effects of ginsenoside Rg_3 on whole-cell K_{ATP} current. Perfusion of Rg_3 removed relaxation of the K_{ATP} current, which was subsequently relaxed by the following perfusion of micromolar glyburide. Washout of Rg_3 and glyburide slightly recovered the relaxation induced by ginsenoside Rg_3 and glyburide (Fig. 3A). Effects of Rg_3 on single-channel K_{ATP} current were further investigated (Fig. 3B). We reduced the concentration of Rg_3 by ten times recordings to establish stable single-channel recordings and included charybdotoxin for to prevent activation of BK_{Ca}. In inside-out patch configuration Rg_3 increased the activity of K_{ATP} with time, suggesting that the K_{ATP} current is activated by ginsenoside Rg_3

3. Functional implication of K_{ATP}

ATP-sensitive K^+ channels control vascular smooth muscle tone by maintaining resting membrane potential of vascular smooth muscle cells.[3,5,10] Consistently, Kim *et al.*[9] have recently shown that ginsenoside Rg_3 caused vasorelaxation of aortic rings in the absence of the endothelium. Since glybenclamide, a potent inhibitor of K_{ATP}, inhibited Rg_3-induced Rb^+ efflux, it was

concluded that ginsenoside Rg_3-induced vasorelaxation of aortic rings was due to activation of K_{ATP}[9] The present study is also consistent with this idea that ginsenoside Rg_3 may cause vasorelaxation via activation of K_{ATP} because the whole-cell K_{ATP} current activated by ginsenoside Rg_3 was inhibited by application of glyburide. In overall, this study suggests a pharmacological action of ginsenoside Rg_3 on K_{ATP} in vascular smooth muscle cells.

요 약

K_{ATP}채널은 세포내 ATP에 의해서 억제되는 포타슘 채널로서 혈관평활근, 골격근 및 췌장의 β세포 막에 존재하여, 근세포의 막전압 조절을 통하여 근수축 및 이완을 조절할 뿐만 아니라 췌장의 β세포로부터 인슐린분비를 조절한다. 홍삼 복합사포닌 및 사포닌 Rg_3성분은 토끼 관상동맥 평활근세포의 칼슘의존성-포타슘 채널(BK_{Ca})의 활성을 증가시켜 막전압의 과분극을 유발하여 혈관평활근을 이완시킨다. 사포닌 Rg_3성분은 홍삼의 복합사포닌 성분보다 BK_{Ca}에 더 높은 활성을 보이기 때문에 본 연구는 사포닌 Rg_3성분이 토끼 관상동맥 단일 평활근세포의 K_{ATP}채널의 활성도를 조절하는지를 팻치클램프 방법으로 기록하였다. 막전압 의존성과 함께 내향전류(inward rectification)특성을 보이는 K_{ATP}채널의 활성을 토끼 관상동맥 평활근 세포로부터 기록하였다. 이 K_{ATP}채널은 ATP와 glyburide에 의해서 억제되었으며 minoxidil에 의해서 활성이 증가되었다. 홍삼 사포닌 Rg_3성분은 K_{ATP}채널의 전류극대치에는 영향을 주지 않고 전류의 inactivation을 억제시켜 결과적으로 K_{ATP}채널의 활성을 증가시켰으며, 단일 K_{ATP}채널이 열리는 시간도 증가시켰다. 따라서 본 실험 결과는 사포닌 Rg_3성분이 K_{ATP}채널의 활성을 증가시켜 막전압을 조절하여 관상동맥 평활근의 이완을 촉진한다고 여겨진다.

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