Stereospecific Effects of Ginsenoside Rg₃ Epimers on Swine Coronary Artery Contractions

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Previous reports have shown that ginseng saponins, the active ingredients of *Panax ginseng*, induce relaxation of hormone- or high K⁺-induced blood vessel contraction. We recently demonstrated that 20(R)- and 20(S)-ginsenoside Rg₃ epimers regulate ion channel activities in a stereospecific manner. Here, we examined whether ginsenoside Rg₃ epimers also exhibit differential effects on swine coronary artery contractions induced by high K⁺ or 5-HT. We found that treatment with 20(S)- but not 20(R)-ginsenoside Rg₃ caused a potent concentration-dependent, endothelium-independent relaxation of coronary artery contraction induced by 25 mM KCl. However, treatment with both 20(S)- and 20(R)-ginsenoside Rg₃ induced a significant, concentration-dependent relaxation of 3 μ M 5-HT-induced coronary artery contractions in intact samples, while only 20(S)-ginsenoside Rg₃ inhibited coronary artery contraction in endothelium-denuded arteries. 20(S)- but not 20(R)-ginsenoside Rg₃ inhibited L-type Ca²⁺ channel currents in a dose- and voltage-dependent manner. These results indicate that 20(S)- and 20(R)-ginsenoside Rg₃ epimers might exhibit stereospecific relaxation effects on swine coronary artery contractions caused by high K⁺ and 5-HT receptor activation.

Key words *Panax ginseng*; ginsenoside Rg₃ epimer; coronary artery contraction; vasorelaxation

Coronary artery disease (CAD), a leading cause of morbidity and mortality throughout the world, has been associated with a variety of biomedical and psychological factors.^{1,2)} In Western populations, CAD and myocardial ischemia are the most common causes of heart failure, which occurs when the heart fails to pump blood at a rate needed to meet metabolic requirements under normal cardiac filling pressure.³⁾ Patients who survive myocardial infarction resulting from CAD have an approximately 3-fold increased risk of developing left ventricular systolic dysfunction (systolic ejection fraction <45%) thereafter.⁴⁾ However, our understanding of CAD pathophysiology has undergone a remarkable evolution over the past decade, and the likelihood of survival from myocardial infarction has almost doubled in some countries.⁵⁾

Ginseng, a widely recognized herbal medicine, has been used extensively in Korean, Japanese, and Chinese medicine, and has become increasingly popular in the Western world for its alleged tonic effects and possible curative and restorative properties. Accumulating clinical evidence supports the potential benefits of ginseng roots in the cardiovascular system. For example, administration of ginsenosides, the active ingredients extracted from *Panax ginseng*, has been shown to decrease blood pressure in both hypertensive patients and experimental animals.^{6,7)} The anti-hypertensive effects of ginsenosides may be at least partially due to their ability to inhibit vascular tone.⁸⁾ Indeed, ginsenosides have been shown to concentration-dependently relax the prostaglandin F2 α -induced contraction of isolated rabbit pulmonary arteries⁹⁾ and the phenylephrine-induced contraction of isolated rabbit and rat aortas.⁷⁾ Studies examining the effect of various purified ginsenosides on vascular contractions have demonstrated that ginsenoside Rg₃, which is one of the protopanaxadiol ginsenosides, acts as the most potent vasodilator among ginsenosides.¹⁰⁾ Ginsenoside Rg₃ exists as a pair of stereoisomers,

with a change in the position of the hydroxyl group on carbon-20 differentiating between the epimers, 20(R)-ginsenoside and 20(S)-ginsenoside¹¹⁾ (Fig. 1). Although we previously reported that 20(R)- and 20(S)-ginsenoside Rg₃ regulate voltage-dependent ion channel activities in a stereospecific manner,¹²⁾ no previous work has examined the effects of these epimers on vasorelaxation.

Here, we investigated the relaxation effects of 20(S)- and 20(R)-ginsenoside Rg₃ on high K⁺- and 5-HT-induced contraction of swine coronary arteries with intact- or endothelium-denuded artery rings. Our results revealed that 20(S)- and 20(R)-ginsenoside Rg₃ exhibit differential relaxation effects in swine coronary arteries.

MATERIALS AND METHODS

Materials Figure 1 shows the chemical structures of 20(R)- and 20(S)-ginsenoside Rg₃, which were kindly provided by the Korea Ginseng Cooperation (Daejeon, South Korea). L (α_1 C)-type Ca²⁺ channel α subunits and their auxiliary subunits were kindly provided by Dr. K. P. Campbell (University of Iowa, IA, U.S.A.). KCl, serotonin, and other reagents were purchased from Sigma (MO, U.S.A.).

Fig. 1. The Chemical Structure of 20(R)- and 20(S)-Ginsenoside Rg₃

Carbohydrates are abbreviated 'Glc.' Superscripts indicate the carbons in the glucose ring that link the two carbohydrates.

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Preparation of Coronary Artery Strips and Measurement of Isometric Contraction The effects of ginsenoside Rg₃ epimers on swine coronary artery contraction induced by high K⁺ or 5-HT were evaluated in an organ bath chamber. Swine hearts were obtained from an abattoir and transferred to our laboratory immersed in ice-cold Tyrode solution. The left circumflex coronary (LCC) arteries were carefully dissected and placed in modified physiological salt solution (PSS) containing (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, 25.0 NaHCO₃, 0.016 CaEDTA, and 11.1 glucose (control solution). The LCC arteries were cleaned of loose connective tissue and then cut into eight rings 2–3 mm in width. Each specimen was divided into intact and endothelium-denuded samples. The four of the eight was used with endothelium intact and the endothelium of the remaining four was removed. The endothelium was removed by gently rubbing the inner surface of the vessel with a cotton thread moistened with physiological PSS. The removal of the endothelium was confirmed by stabilization of the specimen with 25 mM KCl and further confirm by treatment with acetylcholine (10 μ M), which does not induce relaxation in endothelium-denuded coronary artery.¹³ The prepared LCC artery rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 3 ml of control solution at 37.8 °C (pH 7.4) and bubbled with 95% O₂ and 5% CO₂. One of the stirrups was anchored to the organ chamber and the other was connected to a transducer coupler (FT03; Grass, RI, U.S.A.) connected to a recording system (AD Instrument, Sydney, Australia). The LCC arteries rings were progressively stretched to the optimal tension (2 g) prior to addition of 25 mM KCl or 3 μ M 5-HT.

Measuring the Effect of Ginsenoside Epimers on High K⁺- or 5-HT-Induced Contraction of the Suspended Swine Coronary Artery Sections For experiments involving high K⁺, a 25 mM KCl solution was prepared by replacing the NaCl in the physiological salt solution with an equimolar amount of KCl. The solution was saturated with a mixture of 95% O₂ and 5% CO₂ at 37 °C and pH 7.4. For stabilization of the artery samples, the intact or endothelium-denuded artery rings were treated with the high KCl solution or 3 μ M 5-HT. Once the contraction plateau was achieved, the rings were rinsed five times with warm PSS (37.8 °C) and allowed to rest for 30 min in PSS. This procedure was repeated three to four times to confirm the stability of samples. If the amplitudes of contraction in each test showed a deviation of more than 5%, the specimen was excluded from further testing. We then examined the effects of the two ginsenoside epimers in this system. Intact or endothelium-denuded samples were pre-incubated for 1 min with different concentrations of 20(S)- or 20(R)-ginsenoside Rg₃ (1, 3, 10, 30, 100, or 300 μ M), and the coronary arteries were again contracted by treatment with 25 mM KCl or 3 μ M 5-HT. The inhibitory effects of 20(S)- or 20(R)-ginsenoside Rg₃ were expressed as the percentage of the response with respect to the maximal contraction seen in the presence of 25 mM KCl or 3 μ M 5-HT alone. Experiments were repeated four to five times at each concentration, and concentration curves were generated.

Preparation and Microinjection of *Xenopus* Oocytes *Xenopus laevis* were obtained from Xenopus I (MI, U.S.A.); their care and handling was performed in accordance with the highest standards of institutional guidelines. Oocytes

were surgically isolated from frogs anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester, and separated by treatment with collagenase and agitation for 2 h in a Ca²⁺-free medium containing (in mM) 82.5 NaCl, 2 KCl, 1 MgCl₂, 5 HEPES, 2.5 sodium pyruvate, supplemented with 100 units/ml penicillin and 100 μ g/ml streptomycin. Stage V–VI oocytes were collected and stored in ND96 (in mM; 96 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, and 5 HEPES, pH 7.5) supplemented with 0.5 mM theophylline and 50 μ g/ml gentamycin. This oocyte-containing solution was maintained at 18 °C with continuous gentle shaking and changed daily. Electrophysiological experiments were performed within 5–6 d of oocyte isolation, using bath application of the noted drugs. For experiments involving Ca²⁺ channel recordings, one day after harvest, a 10 μ l VWR microdispenser (VWR Scientific, CA, U.S.A.) fitted with a tapered glass pipette tip (15–20 μ m in diameter) was used to inject 40 nl cRNAs into the animal or vegetal pole of each oocyte.¹⁴

Data Recording A Plexiglas net chamber was custom designed for the two-electrode voltage-clamp recording. The chamber was constructed by milling two concentric wells into the chamber bottom (diameter/height: upper well: 8/3 mm, lower well: 6/5 mm) and gluing plastic meshes (*ca.* 0.4 mm grid diameter) onto the bottom of the upper well. A perfusion inlet (*ca.* 1 mm in diameter) was formed through the wall of the lower well, and a suction tube was placed on the edge of the upper well. When an oocyte was placed on the net separating the upper and lower wells, and the grids of the net served to keep it in place during electrophysiological recording. For experiments, the oocytes were bathed in a recording solution consisting of (in mM) 10 Ba(OH)₂, 90 NaOH, 2 KOH, 5 HEPES (pH 7.0 adjusted with methanesulfonic acid) and 0.3 niflumic acid, and impaled with two microelectrodes filled with 3 M KCl (0.2–0.7 M Ω). The electrophysiological experiments were performed at room temperature with an Oocyte Clamp (OC-725C; Warner Instrument, CT, U.S.A.), and stimulation and data acquisition were controlled with a pClamp 8 (Axon Instruments, Union City, CA, U.S.A.). For most of the electrophysiological experiments on Ca²⁺ channel activity, the oocytes were clamped at a holding potential of –100 mV and depolarized to 10 mV for 500 ms every 10 s. For examination of current–voltage relationships, voltage steps were applied from –80 to +60 mV at 10 mV increments.¹²

In Vitro Transcription of cDNAs for Various Ca²⁺ Channel Subunits Recombinant plasmids containing cardiac L-type calcium channel α_1C subunit cDNA inserts were linearized by digestion with the appropriate restriction enzymes. cRNAs were obtained from the linearized templates with an *in vitro* transcription kit (mMessage mMachine; Ambion, TX, U.S.A.) using SP6, T3, or T7 RNA polymerases. The resulting RNA was dissolved in RNase-free water at 1 μ g/ μ l, divided into aliquots and stored at –70 °C until use.¹²

Data Analysis To obtain concentration–response curves in the presence of 20(S)-ginsenoside Rg₃, the peak current amplitudes were obtained and plotted. The Origin software (Origin; MA, U.S.A.) was then used to fit them to the Hill equation: $y/y_{\max} = [A]^{n_H} / ([A]^{n_H} + [EC_{50}]^{n_H})$, where y is the I_{Ba} channel current at a given concentration of ginsenoside Rg₃, $y_{\max}$ is the peak value of the Ca²⁺ channel current, the IC_{50} is

the concentration of ginsenoside Rg_3 producing a half-maximum effect on the control response to ginsenoside Rg_3 , $[A]$ is the concentration of ginsenoside Rg_3 , and n_H is the interaction coefficient. All values are presented as means $\pm$ S.E.M. The differences between means of control and treatment group data were analyzed using unpaired Student's *t*-tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of 20(*S*)- and 20(*R*)-Ginsenoside Rg_3 on High K^+ -Induced Swine Coronary Artery Contraction We previously demonstrated that ginsenoside Rg_3 epimers differentially regulated voltage-dependent ion channels (e.g. Ca^{2+} , K^+ and Na^+ channels) and ligand-gated ion channels (e.g. 5-HT_{3A} and $\alpha 3\beta 4$ nicotinic acetylcholine receptors).¹²⁾ Here, we examined whether ginsenoside Rg_3 epimers also exhibited differential effects on high K^+ -induced coronary artery contractions. Pretreatment of intact and endothelium-denuded swine coronary artery rings with 20(*S*)-ginsenoside Rg_3 (1 to 300 μM) concentration-dependently inhibited the contraction induced by 25 mM KCl (Figs. 2A, B). The % inhibition by 20(*S*)-ginsenoside Rg_3 on coronary artery contraction in intact artery samples was 2.0 ± 0.9 , 2.2 ± 1.2 , 9.2 ± 1.7 , 21.8 ± 2.2 , 72.6 ± 6.7 and $76.4 \pm 6.0\%$ at ginsenoside doses of 1, 3, 10, 30, 100 and 300 μM , respectively (Fig. 2A), while that in endothelium-denuded samples was 3.1 ± 1.2 , 2.8 ± 2.4 , 5.3 ± 3.6 , 12.8 ± 3.7 , 68.5 ± 7.9 and $72.2 \pm 8.4\%$, respectively (Fig. 2B). In contrast, 20(*R*)-ginsenoside Rg_3 exhibited smaller dose-dependent inhibitions on high K^+ -induced coronary artery contractions in both intact and endothelium-denuded samples. The % inhibition by 20(*R*)-ginsenoside Rg_3 on coronary artery contraction in intact samples was 0.4 ± 0.4 , 1.0 ± 0.8 , 4.2 ± 1.4 , 8.4 ± 2.3 , 26.8 ± 6.6 and $30.8 \pm 7.6\%$ at doses of 1, 3, 10, 30, 100 and 300 μM , respectively, while that in endothelium-denuded samples was 2.1 ± 1.8 , 1.7 ± 2.8 , 2.8 ± 3.8 , 4.9 ± 5.4 , 18.8 ± 8.6 and $22.7 \pm 6.4\%$ at doses of 1, 3, 10, 30, 100 and 300 μM , respectively (Figs. 2A, B). The IC_{50} values of 20(*S*)-ginsenoside Rg_3 were 46.2 ± 7.0 and $42.1 \pm 6.7 \mu M$ in intact and endothelium-denuded samples, respectively. Interestingly, these IC_{50} values were 7–8-fold larger than those obtained from the rat aorta,¹⁰⁾ perhaps due to differences in the utilized organs and species. These results indicate that the inhibitory effect of ginsenoside Rg_3 on high K^+ -induced coronary artery contraction was stereospecific.

Effects of (*S*)- and (*R*)-Ginsenoside Rg_3 on 5-HT-Induced Swine Coronary Artery Contractions As serotonin (5-HT) is known to induce contraction of mammalian coronary arteries *via* activation of 5-HT_{2A} receptors,^{15,16)} we next examined whether 20(*S*)- and 20(*R*)-ginsenoside Rg_3 exerted similar inhibitory effects on 5-HT-induced swine coronary artery contraction in intact and endothelium-denuded samples. Treatment with both epimers significantly and concentration-dependently inhibited 5-HT-induced contraction in intact coronary artery samples, with 20(*S*)-ginsenoside Rg_3 showing a slightly but significantly more potent effect than 20(*R*)-ginsenoside Rg_3 (Fig. 3A). The % inhibition by 20(*S*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction in intact samples was 0.0 ± 0.0 , 1.7 ± 1.2 , 10.5 ± 2.4 , 24.7 ± 4.2 , 54.7 ± 5.0 and $53.2 \pm 5.5\%$ at doses of

Fig. 2. Effect of 20(*S*)- and 20(*R*)-Ginsenoside Rg_3 on 25 mM KCl-Induced Coronary Artery Contraction

(A) Concentration-dependent inhibitory effects of 20(*S*) (■)- and 20(*R*) (●)-ginsenoside Rg_3 on coronary artery ring contractions in intact samples. (B) Concentration-dependent inhibitory effects of 20(*S*) (■)- and 20(*R*) (●)-ginsenoside Rg_3 on coronary artery ring contractions in endothelium-denuded samples. 20(*S*)- but not 20(*R*)-ginsenoside Rg_3 potently inhibited K^+ -induced contractions in both samples. Data are expressed as means $\pm$ S.E.M. ($n=4-5$ /dose). * $p < 0.01$, compared with 20(*R*)-ginsenoside Rg_3 treatment.

1, 3, 10, 30, 100 and 300 μM , respectively ($IC_{50}=31.0 \pm 6.3 \mu M$), while that by 20(*R*)-ginsenoside was 0.0 ± 0.0 , 0.0 ± 0.0 , 4.2 ± 1.6 , 13.1 ± 2.3 , 32.6 ± 7.3 and $35.7 \pm 3.7\%$, respectively ($IC_{50}=38.6 \pm 2.5 \mu M$). Interestingly, we observed a significant epimer-specific difference in the response of endothelium-denuded swine coronary artery samples. In these samples, 20(*S*)-ginsenoside Rg_3 dose-dependently inhibited 5-HT-induced coronary artery contraction, with % inhibition values of 0.0 ± 0.0 , 0.6 ± 2.1 , 6.0 ± 3.9 , 18.7 ± 5.7 , 54.5 ± 6.4 and $58.5 \pm 7.28\%$ at doses of 1, 3, 10, 30, 100 and 300 μM , respectively ($IC_{50}=41.0 \pm 4.3 \mu M$) (Fig. 3B). In contrast, 20(*R*)-ginsenoside Rg_3 showed less effective inhibition of 5-HT-induced coronary artery contraction in endothelium-denuded samples, with % inhibition values of only 0.0 ± 0.0 , 0.0 ± 0.0 , 0.6 ± 0.4 , 7.5 ± 3.8 , 18.7 ± 9.6 and $23.8 \pm 5.9\%$ at doses of 1, 3, 10, 30, 100 and 300 μM , respectively ($IC_{50}=50.2 \pm 4.1 \mu M$) (Fig. 3B). Since 5-HT is known to induce blood vessel contractions in an endothelium-independent manner,^{16,17)} these results further demonstrated that the epimer-specificity of ginsenoside Rg_3 exists through experiments using endothelium-denuded swine coronary arteries.

Effects of 20(*S*)-Ginsenoside Rg_3 on Cardiac L-Type Ca^{2+} Channels Expressed in *Xenopus* Oocytes The in-

ward Ba^{2+} peak current (I_{Ba}) was recorded by the two-electrode voltage clamp technique from oocytes injected with cRNAs encoding the L-type Ca^{2+} ion channel α and auxil-

A.

B.

Fig. 3. Effect of 20(S)- and 20(R)-Ginsenoside Rg_3 on 5-HT-Induced Coronary Artery Contraction

(A) Concentration-dependent inhibitory effects of 20(S) (■)- and 20(R) (●)-ginsenoside Rg_3 on 5-HT-induced coronary artery ring contractions in intact samples; $*p < 0.05$, compared with 20(R)-ginsenoside Rg_3 treatment. (B) Concentration-dependent inhibitory effects of 20(S) (■)- but not 20(R) (●)-ginsenoside Rg_3 on 5-HT-induced coronary artery ring contractions in endothelium-denuded samples. Data are expressed as means $\pm$ S.E.M. ($n = 4-5/\text{dose}$). $*p < 0.01$, compared with 20(R)-ginsenoside Rg_3 treatment.

B.

Fig. 4. Effects of 20(S) and 20(R)-Ginsenoside Rg_3 on L-Type Ca^{2+} Channel Activity

(A) Concentration-response curve for 20(S)-ginsenoside Rg_3 on I_{Ba} in L-type Ca^{2+} channels. Oocytes were injected with cRNAs coding for L (α_{1C} , $\alpha_{2\delta}$ and β_3)-type Ca^{2+} channels, and the I_{Ba} for L-type Ca^{2+} channels was recorded as described in Materials and Methods. (Inset) Representative tracings from six separate oocytes from three different frogs in the absence (Con) and presence of various concentrations of 20(S)-ginsenoside Rg_3 . Concentration-response curves were fitted to the Hill equation. Data are means $\pm$ S.E.M. ($n = 10-13/\text{group}$). (B) Representative current-voltage relationships of L-type Ca^{2+} channels in the absence (Con, ●) and presence of 20(S)-ginsenoside Rg_3 (○) or after wash-out of 20(S)-ginsenoside Rg_3 (▼) from four separate oocytes from three different frogs.

iary subunits. The oocytes were held at -100 mV for L-type Ca^{2+} channels¹⁸⁾ and I_{Ba} was elicited by depolarizations to 10 mV, every 10 s for 500 ms. As shown in Fig. 4A (inset), the depolarizing voltage steps induced large inward I_{Ba} in oocytes expressing L-type Ca^{2+} channels, but application of various doses of 20(S)-ginsenoside Rg_3 reversibly inhibited this I_{Ba} . Interestingly, treatment with 20(R)-ginsenoside Rg_3 had almost no effect on I_{Ba} (Fig. 4A). This is consistent with previous findings in experiments using P/Q-type Ca^{2+} channels.¹⁹⁾

Concentration-Dependent Effect and Current-Voltage Relationship of the Action of 20(S)-Ginsenoside Rg_3 on L-Type Ca^{2+} Channels Treatment with 20(S)-ginsenoside Rg_3 inhibited I_{Ba} in a dose-dependent manner that became saturated at about $300 \mu\text{M}$ (Fig. 4A); the IC_{50} was $39.4 \pm 2.1 \mu\text{M}$ for the L-type Ca^{2+} channels, and the Hill coefficient was 1.3 ± 0.13 . The current-voltage relationships for the L-type Ca^{2+} channels were examined in the presence or absence of $100 \mu\text{M}$ of 20(S)-ginsenoside Rg_3 , with voltage steps from -80 to $+60$ mV at -100 mV holding potentials (Fig. 4B). Our results revealed that 20(S)-ginsenoside Rg_3 voltage-dependently inhibited the peak I_{Ba} of L-type Ca^{2+} channels, beginning at voltages that were depolarized relative to the resting state.

Although ginseng and its active constituents, ginsenosides, have gained worldwide popularity for their myriad beneficial effects, the underlying mechanisms for these effects have not been fully elucidated. In addition, only a few studies have specifically examined the differential effects of ginsenoside stereoisomers, such as 20(R)-ginsenoside Rg_3 and 20(S)-ginsenoside Rg_3 . We previously demonstrated that 20(S)- but not 20(R)-ginsenoside Rg_3 inhibited voltage-dependent Ca^{2+} , K^+ and Na^+ channel activations, whereas both epimers inhibited 5-HT₃ and nicotinic acetylcholine receptor channel activities in *Xenopus* oocytes.¹²⁾ Kim *et al.* (1999 and 2003) reported that ginsenoside Rg_3 inhibited rat aortic ring contractions in endothelium-dependent and -independent manners.^{10,20)} Here, we investigated the effect of ginsenoside Rg_3 epimers against K^+ - and 5-HT-induced swine coronary artery contractions, and the requirement of the endothelium in this response.

Our novel results demonstrated that: (1) 20(S)- but not

20(*R*)-ginsenoside Rg₃ potently and dose-dependently inhibited high K⁺- and 5-HT-induced swine coronary artery contraction; and (2) consistent with previous reports observed in the rat aorta,^{10,20} the inhibitory effect of 20(*S*)-ginsenoside Rg₃ on high K⁺- and 5-HT-induced swine coronary artery contraction was not endothelium-dependent. These results indicate that ginsenoside Rg₃ epimers have stereospecific effects on high K⁺- and 5-HT-induced coronary artery contractions, and that 20(*S*)-ginsenoside Rg₃ is the more active epimer in this system.

The elevation of intracellular free Ca²⁺ concentration, a main factor mediating smooth muscle contractions in vascular and gastrointestinal systems, occurs *via* two major processes. The first, which may be triggered by high K⁺ or treatment with L-type Ca²⁺ channel agonists, is the influx of extracellular Ca²⁺ through activated voltage-dependent L-type Ca²⁺ channels. Thus, our finding that 20(*S*)- but not 20(*R*)-ginsenoside Rg₃ inhibits the voltage-dependent L-type Ca²⁺ channel in *Xenopus* oocytes heterologously expressing L-type Ca²⁺ channels (Fig. 4A), and the previous finding of this effect in neuronal cells²¹ likely provides insight into the mechanism by which ginsenoside Rg₃ inhibits high K⁺ arterial contractions. Furthermore, Bai *et al.* showed that ginsenoside Re inhibited L-type Ca²⁺ channels in guinea-pig cardiomyocytes,²² and we previously demonstrated that 20(*S*)- but not 20(*R*)-ginsenoside Rg₃ is the main regulator of voltage-dependent Ca²⁺ channels in *Xenopus* oocytes.¹² Collectively, the previous reports together with the present findings seem to indicate that 20(*S*)-ginsenoside Rg₃-induced L-type Ca²⁺ channel inhibition might be an underlying mechanism for the observed inhibition of K⁺-induced coronary artery contractions.

The second process for elevating intracellular Ca²⁺, which may be triggered by 5-HT treatment, is the release of Ca²⁺ stored in the endoplasmic reticulum in response to activation of phospholipase C-Gα_{q/11} binding protein-coupled receptors. Activation of 5-HT_{2A} receptors, which are the predominant vascular receptors mediating contractility to 5-HT and its agonists in the mammalian artery, is coupled to phospholipase C-IP₃-induced release of intracellular Ca²⁺ stores from the endoplasmic reticulum.^{15,16} We herein demonstrated that 5-HT-induced swine coronary artery contraction was attenuated by 20(*S*)- and 20(*R*)-ginsenoside Rg₃ in intact samples but in endothelium-denuded samples only 20(*S*)-ginsenoside Rg₃ greatly attenuated 5-HT-induced swine coronary artery contraction (Fig. 3), suggesting that treatment with 20(*S*)-ginsenoside Rg₃ attenuates 5-HT-mediated intracellular Ca²⁺ elevation, resulting in the inhibition of swine coronary artery contraction.

A third alternative may be suggested by the findings that ginsenoside Rg₃ treatment stimulated NO production in the rat aorta,^{20,23} and ginsenoside Re inhibited L-type Ca²⁺ channel activity *via* a cGMP-dependent pathway in guinea-pig cardiomyocytes.²² Thus, it is possible that the ginsenoside Rg₃-induced relaxation of coronary arteries might be mediated *via* cGMP-dependent mechanisms. However, our finding that 20(*S*)- and 20(*R*)-ginsenoside Rg₃ might inhibit swine coronary artery contractions mediated by elevation of intracellular Ca²⁺ levels as well as through L-type Ca²⁺ channels is consistent with our previous report that ginsenosides may regulate intracellular Ca²⁺ levels in different man-

ners between excitable cells (*e.g.* neurons) *versus* non-neuronal cells.¹⁹ For example, ginsenosides were shown to increase intracellular Ca²⁺ levels in non-neuronal cells such as *Xenopus laevis* oocytes, whereas ginsenoside treatment attenuated both L-type Ca²⁺ channel-mediated and stored release-based intracellular Ca²⁺ increases in rat hippocampal neurons.¹⁹ Future studies will be required to assess these possibilities.

Interestingly, the inhibitory potency of 20(*R*)-ginsenoside Rg₃ on 5-HT-induced swine coronary artery contraction in intact samples was 30–40% less than that of 20(*S*)-ginsenoside Rg₃. This difference in potency might be derived from their chemical structures with a change in the position of the hydroxyl group on carbon-20 (Fig. 1). We previously showed that 20(*S*)- but not 20(*R*)-ginsenoside Rg₃ inhibited voltage-dependent Ca²⁺, K⁺ and Na⁺ channel activations, and also showed that 20(*S*)-ginsenoside Rg₃ inhibited 5-HT_{3A}- and nicotinic acetylcholine receptor-mediated ion currents more effectively than 20(*R*)-ginsenoside Rg₃ in *Xenopus* oocytes,¹² suggesting that the position of the hydroxyl group on carbon-20 of ginsenoside Rg₃ might play an important role to exhibit the differential effects in ion channel regulations and in coronary artery relaxation and furthermore indicating that its hydroxyl group might be one of the important functional groups considered in new drug designs or developments using ginsenosides in future.

In summary, we herein show for the first time that 20(*S*)- but not 20(*R*)-ginsenoside Rg₃ potently and dose-dependently inhibited high K⁺- and 5-HT-induced swine coronary artery contractions, suggesting that 20(*S*)-ginsenoside Rg₃ might be a useful agent for vasorelaxation, which is achieved *via* inhibition of the elevation of intracellular Ca²⁺ level mediated by L-type Ca²⁺ channel or 5-HT receptor activation.

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