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Effects of Ginsenoside Rg_3 Epimers on Swine Coronary Artery Contractions

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Abstract : The previous reports demonstrated that ginseng saponins, active ingredient of *Panax* ginseng, inhibited blood vessel contraction induced by various hormones or high K^+. Recently, we demonstrated that 20(*R*)- and 20(*S*)-ginsenoside Rg_3 regulate ion channel activities with differential manners. The aim of this study was to examine whether ginsenoside Rg_3 isomers also show differential effects on swine coronary artery contractionresponses induced by high K^+, serotonin (5-HT) or acetylcholine. Treatment of 20(*S*)- but not 20(*R*)-ginsenoside Rg_3 caused a concentration-dependent relaxation of coronary artery contracted by 25 mM KCl. 20(*S*)- and 20(*R*)-ginsenoside Rg_3 induced significant relaxations of coronary artery contraction induced by 5-HT (3 μM) in the presence of endothelium with concentration-dependent manner and, also in the absence of endothelium only 20(*S*)-ginsenoside Rg_3 induced a strong inhibition of coronary artery contraction induced by 5-HT in a concentration-dependent manner. 20(*S*)-ginsenoside Rg_3 caused relaxation of coronary artery in the absence and presence of endothelium. In contrast, treatment of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 (100 μM) did not show significant inhibition of coronary artery contraction induced by acetylcholine (0.01 to 30 μM) in the presence of endothelium, whereas both isomers caused significant inhibition of coronary artery contraction induced by acetylcholine (0.01 to 30 μM) in the absence of endothelium in a concentration-dependent manner. These findings indicate that 20(*S*)- or 20(*R*)-ginsenoside Rg_3 exhibits differential relaxation effects of swine coronary artery contractions caused by high K^+, acetylcholine, and 5-HT treatment and that this differential vasorelaxing effects of ginsenoside Rg_3 isomers also might be dependent on endothelium.

Key words : *Panax ginseng*, ginsenoside Rg_3 epimers, coronary artery, vasorelaxation

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of morbidity and mortality throughout the world. A variety of biomedical and psychologic factors are associated with an increased risk for its occurrence[1,2]. During the past decade, our understanding of the pathophysiology of CAD has undergone a remarkable evolution.

Heart failure occurs when abnormalities of cardiac function cause the heart to fail to pump blood at a rate needed to meet metabolic requirements under normal cardiac filling pressure. CAD, resulting in myocardial ischemia, is its most common cause in western populations[3]. Those who survive myocardial infarction resulting from CAD have an approximate threefold increased risk of developing left ventricular systolic dysfunction (systolic ejection fraction $< 45\%$)[4] and the likelihood of survival from myocardial infarction almost doubled in some countries[5].

Ginseng, a widely recognized herbal drug, has been reported to have a wide range of therapeutic and pharmacologic uses. Ginseng's genus name *Panax* is derived from the Greek words *pan* (all) and *akos* (cure), meaning cure-all. Ginseng root has been used extensively in Korean and Chinese medicine and has become increasingly popular in the western world for its alleged tonic effect and possible curative and restorative properties. There are increased clinical evidences concerning the potential benefits of ginseng roots in cardiovascular diseases. Administration of ginsenosides, a mixture of saponins extracted from *Panax ginseng*, decreases blood pressure in both hypertensive patients and experimental animals[6,7]. The antihypertensive effect of ginsenosides may be due, at least in part, to their ability to inhibit vascular tone. Indeed, ginsenosides, with concentration-dependent manner, relax the isolated rabbit pulmonary arteries contracted with prostaglandin $F_{2\alpha}$[8] and the isolated rabbit and rat aorta contracted with phenylephrine[7]. The inhibitory effect of ginsenoside requires the presence

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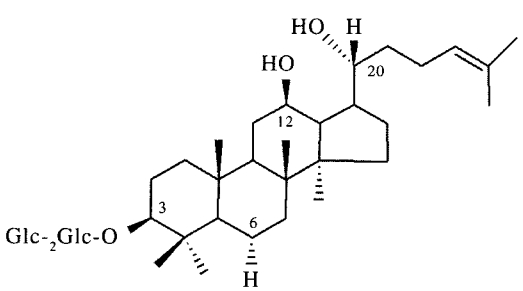
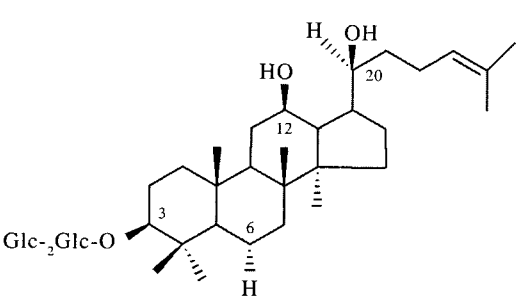
of a functional endothelium and is mediated by an increased formation of endothelium-derived nitric oxide[7]. Studies examining the effect of various purified ginsenosides on vascular tone identified ginsenoside Rg_3, a triterpene glycoside which chemically belongs to the protopanaxadiol ginsenoside group, as the most potent vasodilator[9]. Recently, Kim *et al.*[9] found that, in addition to the endothelium-dependent relaxation, ginsenoside Rg_3 also inhibited the tone of aortic rings without endothelium contracted with 25 mM KCl, whereas only a small relaxation was found in rings contracted with phenylephrine. Ginsenoside Rg_3 exists as stereoisomers; 20(*R*)-ginsenoside and 20(*S*)-ginsenoside are epimers of each other depending on the position of the hydroxyl group on carbon-20[10](Fig. 1). Recently, we have demonstrated that 20(*R*)- and 20(*S*)-ginsenoside Rg_3 regulates ion channel activities with differential manners.

In this study, we investigated the effects of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 on swine coronary arteries with or without endothelium and found that 20(*S*)- or 20(*R*)-ginsenoside Rg_3 exhibits differential relaxation effects of swine coronary artery contracted by high K^+, 5-HT or acetylcholine treatment and that this differential relaxation



20(*S*)-Ginsenoside Rg_3



20(*R*)-Ginsenoside Rg_3

Fig. 1. Chemical structure of 20(S)- or 20(*R*)-ginsenoside Rg_3. There are various ginsenosides. They differ at three side chains attached the common steroid ring. Abbreviations for carbohydrates are as follows: Glc. Superscripts indicate the carbon in the glucose ring that links two carbohydrates.

effects of ginsenoside Rg_3 isomer also might be dependent or independent on the presence of endothelium.

MATERIALS AND METHODS

Materials

Ginsenoside Rg_3 was isolated from an extract of ginsenosides, prepared from *P. ginseng*, by the previously published procedures[11]. KCl, acetylcholine and serotonin were purchased from Sigma (St. Louis, MO, USA)

Organ chamber studies

The inhibitory effects of ginsenoside Rg_3 on swine coronary artery contracted by KCl, 5-HT and acetylcholine were evaluated on organ bath chamber. Swine hearts were obtained at an abattoir and were transferred to our laboratory immersed in ice-cold Tyrode solution. The left circumflex coronary (LCC) arteries were carefully dissected and the LCC arteries were placed in modified physiological salt solution (PSS) containing (in mM) NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; CaEDTA, 0.016; and glucose, 11.1 (control solution). The LCC arteries were cleaned of loose connective tissue and then cut into eight rings 2-3 mm wide. Coronary artery was divided into with or without endothelium. The endothelium was removed mechanically. When the contraction had stabilized by 25 mM KCl, 10 μM acetylcholine was added to test for the presence or absent of the endothelium. And, the LCC arteries rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 3 ml of 37.8, pH 7.4 PSS bubbled with 95% O_2 and 5% CO_2. One of the stirrups was anchored to the organ chamber and the other was connected to a force transducer (Narco bio-system) for the recording of isometric tension. The LCC arteries rings were stretched progressively to the optimal tension (2 g) before the addition of 25 mM KCl, 5-HT (1 to 300 μM) and acetylcholine (0.01 to 30 μM).

Measurement of isometric tension on swine coronary artery by high K^+, 5-HT, or acetylcholine

In 25 mM KCl-induced contraction studies, after the plateau of the contraction elicited by 25 mM KCl was obtained, the aortic rings were rinsed three times in warm PSS (37.8°C). After a 30 min resting period in PSS, the aortic rings were exposed again to 25 mM KCl. After a stable plateau of vasoconstriction had been reached in the presence of 25 mM KCl, the rings were stabilized by PSS. This response was repeated three times. Next, we tested

drug effects; i.e. after following preincubation for 1 min with 20(S)- or 20(*R*)-ginsenoside Rg_3 of 1, 3, 10, 30, 100 and 300 μM, respectively, the rings were again contracted by 25 mM KCl to test inhibition on contraction of coronary artery. A concentration-response curve was obtained from the contraction of coronary artery in the different concentration of ginsenoside Rg_3 epimers with solution containing 25 mM KCl. Inhibitory effects of 20(S)- or 20(*R*)-ginsenoside Rg_3 were expressed as a percentage of the response to a maximal value contracted by 25 mM KCl. In all cases, each experiment was repeated four to five times.

We also evaluated the inhibitory effects of ginsenoside Rg_3 epimers on contractive response induced by 3 μM 5-HT, following dose-dependent manner of 20(S)- or 20(*R*)-gnsenoside Rg_3 on swine coronary artery. Once the plateau of the contraction elicited by 3 μM 5-HT was obtained, the coronary artery rings were rinsed three times for 30 min with PSS (37.8). After a resting period for 30 min, the rings were exposed again to 3 μM 5-HT. After contraction by 3 μM 5-HT, the rings were stabilized by warm PSS. And, the rings were preincubated for 1 min with 20(S)- or 20(*R*)-ginsenoside of 1, 3, 10, 30, 100 and 300 μM, respectively, and the rings were contracted by 3 μM 5-HT to test contraction for the presence or absent of the endothelium, respectively. Thus, after preincubation of 20(S)- or 20(*R*)-ginsenoside Rg_3 in a concentration-dependent manner, contractive responses for 3 μM 5-HT were obtained. The amplitude of contraction induced by 3 μM 5-HT was measured for each concentration. The inhibitory effects of 20(S)- or 20(*R*)-ginsenoside Rg_3 on contraction by 3 μM 5-HT were expressed as a percentage of the response to a maximal value contracted by 3 μM 5-HT administered initially in each aorta. In all cases, each experiment was repeated four to five times.

Also, the inhibitory effects of ginsenoside Rg_3 epimers in the contractive response to acetylcholine was investigated in swine coronary arteries with and without endothelium. After a stable plateau of vasoconstriction had been reached by 25 mM KCl, the rings were stabilized by PSS for 30 min. This response was repeated three times. And, the rings were preincubated for 1 min with 100 μM 20(S)- or 20(*R*)-ginsenoside Rg_3, respectively, and the rings were contracted by acetylcholine with dose-dependent manner (0.01, 0.1, 0.3, 1, 3, 10 and 30 μM) to test inhibitory effects of 20(S)- or 20(*R*)-ginsenoside Rg_3 on contraction of swine coronary artery for the presence or absence of the endothelium, respectively. Acetylcholine of 0.01, 0.1, 0.3, 1, 3, 10 and 30 μM were added to the bath in a cumulative fashion, respectively. The response to each concentration was allowed to reach a plateau before the addition of the next concentration of acetylcholine. The amplitude of contraction induced by acetylcholine was measured for each concentration. The inhibitory effects of 20(S)- or 20(*R*)-ginsenoside Rg_3 on contraction by acetylcholine were expressed as a percentage of the response to a maximal value contracted by 25 mM KCl administered initially in each aorta. In all cases, each experiment was repeated four to five times.

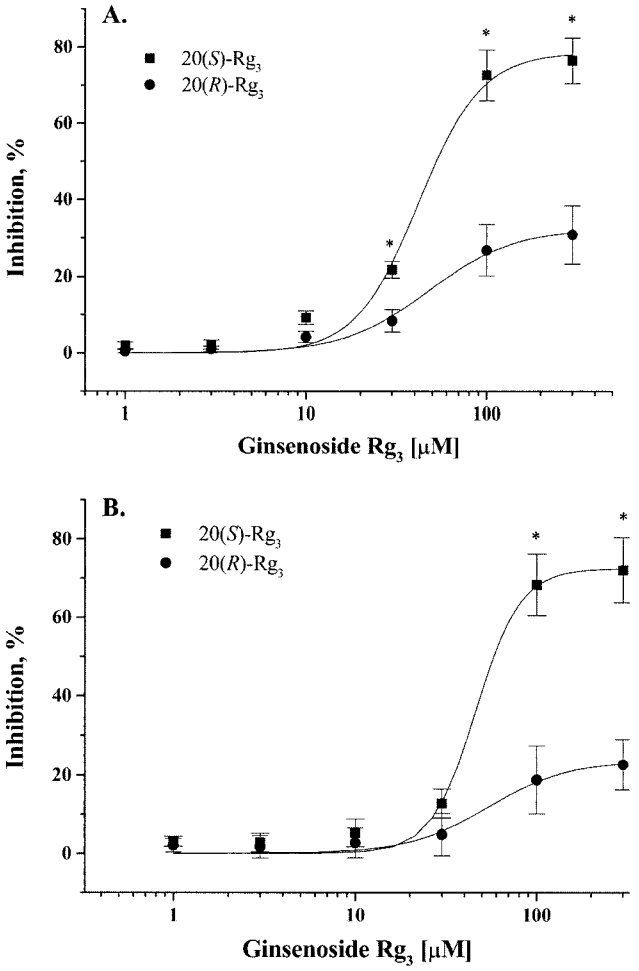


Fig. 2. The inhibitory effect of 20(*S*)-(■) and 20(*R*)-(●) ginsenoside Rg_3 in coronary artery with and without endothelium constricted with 25 mM KCl. The small concentration-dependent inhibition evoked by ginsenoside Rg_3 in endothelium-nuded coronary artery rings was also shown. The constriction induced by 25 mM KCl was inhibited by 20(*S*)- and 20(*R*)-ginsenoside Rg_3 with concentration-dependent manner. Data are expressed as means±S.E.M (n=4-5/dose). Significant from 20(*R*)-ginsenoside Rg_3 (*p<0.01).

RESULTS

Effects of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 on high K^+-induced swine coronary artery contraction

In the previous study, because ginsenoside Rg_3 isomers showed differential effects between voltage-dependent ion channels and ligand-gated ion channels, we first examined the effects of ginsenoside Rg_3 isomers in the inhibition of contraction induced by high K^+. In the presence of 25 mM KCl, the addition of 20(*S*)-ginsenoside Rg_3 (1 to 300 μM) produced a concentration-dependent relaxation of swine coronary artery in the both conditions of absence and presence of endothelium (Fig. 2A and 2B). The inhibitory effect of 20(*S*)-ginsenoside Rg_3 on coronary artery with endothelium was 2.00±0.89, 2.20±1.24, 9.20±1.74, 21.8±2.15, 72.6±6.65 and 76.4±5.97% at 1, 3, 10, 30, 100 and 300 μM, respectively. Also, % the inhibitory effect of 20(S)-ginsenoside Rg_3 on coronary artery without endothelium was 3.10±1.23, 2.81±2.43, 5.34±3.55, 12.83±3.71, 68.45±7.87 and 72.16±8.38% at 1, 3, 10, 30, 100 and 300 μM, respectively. Thus, the inhibitory effect of 20(*S*)-ginsenoside Rg_3 on high K^+-induced contraction was independent in the presence of endothelium. However, 20(*R*)-ginsenoside Rg_3 exhibited a minimal inhibition of high K^+-induced contractions with dose-dependent manner on coronary artery with or without endothelium. The inhibitory effect of R-form on coronary artery with endothelium was 0.40±0.41, 1.00±0.77, 4.20±1.42, 8.40±2.97, 26.83±6.63 and 30.80±7.59% at 1, 3, 10, 30, 100 and 300 μM, respectively. Also, the inhibitory effect of R-form on coronary artery without endothelium was 2.13±1.76, 1.72±2.83, 2.75±3.81, 4.87±5.43, 18.78± 8.58 and 22.65±6.37% at 1, 3, 10, 30, 100 and 300 μM, respectively (Fig. 2B). IC_{50} of 20(*S*)-ginsenoside Rg_3 was 42.08±6.65 and 46.15±7.02 μM, and IC_{50} of 20(*R*)-ginsenoside Rg_3 was 47.59±8.48 and 53.90±13.64 μM on coronary artery in the presence and absence of endothelium, respectively.

Effects of (*S*)- and (*R*)-ginsenoside Rg_3 on 5-HT-induced swine coronary artery contraction.

As a next step, we used another ligand, 5-HT, and did further study to know whether or not 20(*S*)- or 20(*R*)-ginsenoside Rg_3 also exerts their inhibitory effects on 5-HT-induced coronary artery contraction. As shown in Fig. 3A and 3B, treatment of 20(*S*)-ginsenoside Rg_3 showed a slight more potent inhibition in 5-HT-induced coronary artery contraction than that of 20(*R*)-ginsenoside Rg_3 in the presence or absence of endothelium with dose-dependent manner. Inhibition by 20(*S*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction was 0.00±0.00, 1.75±1.18, 10.50±2.46, 24.74±4.26, 54.78±5.02 and 63.35 ±5.54% at 1, 3, 10, 30, 100 and 300 μM, respectively in the presence of endothelium. IC_{50} of 20(*S*)-ginsenoside Rg_3 was 40.75±5.30 μM in the presence of endothelium. Inhibition by 20(*R*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction was 0.00±0.00, 0.00±0.00, 4.20± 1.65, 14.01±2.54, 31.65±7.11 and 45.75 ±3.77% at 1, 3, 10, 30, 100 and 300 μM, respectively in the presence of endothelium. EC_{50} was 72.89±7.22 μM.

Interestingly, in the absence of endothelium, the inhibi-

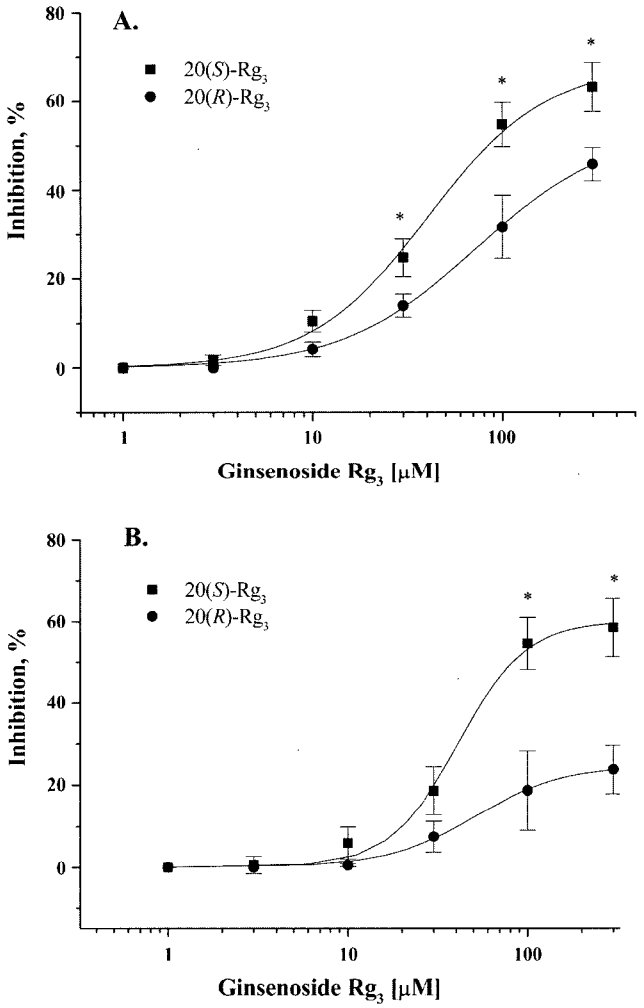


Fig. 3. The inhibitory effect of 20(*S*)-(■) and 20(*R*)-(●) ginsenoside Rg_3 in coronary artery with and without endothelium constricted with 3 μM 5-HT. Also, the constriction induced by 5-HT was inhibited by 20(*S*)- and 20(*R*)-ginsenoside Rg_3 with dose-dependent manner. Data are expressed as means±S.E.M (n=4-5/dose). Significant from 20(*R*)-ginsenoside Rg_3 (*p<0.01).

tory effect of 20(*S*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction still was maintained in a concentration-dependent manner. Thus, % Inhibition by 20(*S*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction was 0.00±0.00, 0.58±2.13, 5.95±3.92, 18.65±5.74, 54.53±6.43 and 58.48±7.18% at 1, 3, 10, 30, 100 and 300 μM, respectively. IC_{50} of 20(*S*)-ginsenoside Rg_3 was 40.95±4.34 μM (Fig. 3B.) Also, in the absence of endothelium, the inhibitory effect of 20(*R*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction was greatly attenuated. Inhibition of 20(*R*)-ginsenoside Rg_3 on 5-HT-induced coronary artery contraction was 0.00± 0.00, 0.00±0.00, 0.58±0.37, 7.51±3.83, 18.68±9.59 and 23.76±5.88% at doses of 1, 3, 10, 30, 100 and 300 μM, respectively (Fig. 4B). IC_{50} of 20(*R*)-ginsenoside Rg_3 was 50.23±4.08 μM.

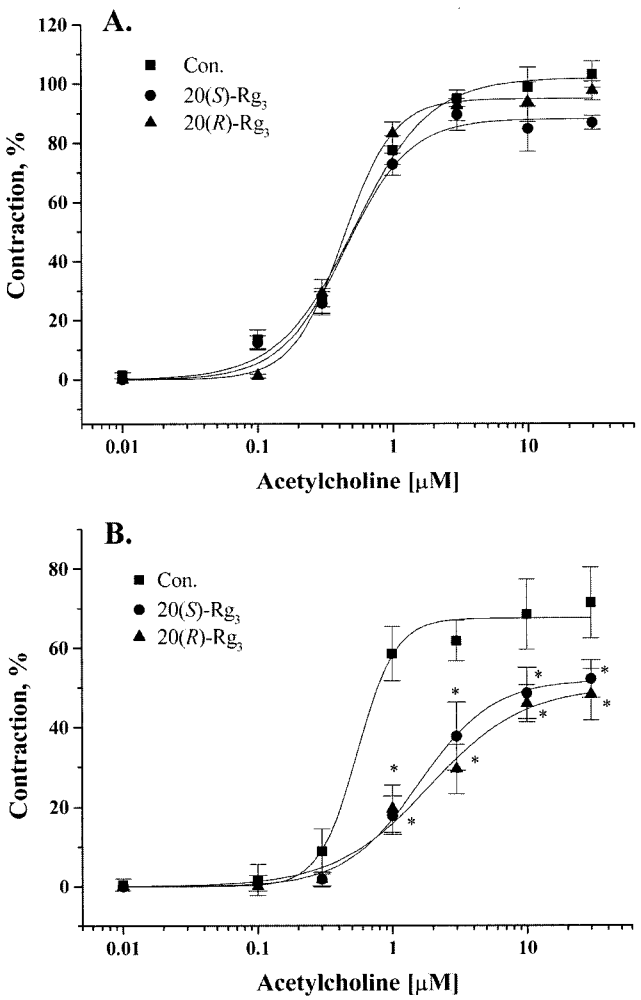


Fig. 4. The inhibitory effect of contraction in coronary artery with or without endothelium preincubated with Con (■), 20(*S*)-(●), or 20(*R*)-(▲) ginsenoside Rg_3 for 1 min, constricted with acetylcholine The inhibitory effect of contraction evoked by acetylcholine was not shown in endothelium-denuded coronary artery rings. The constriction induced by acetylcholine was inhibited by preincubation of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 with dose-dependent manner. Data are expressed as means± S.E.M (n=4-5/dose). Significant from with control group (*p<0.01)

Effects of (S)- and (*R*)-ginsenoside Rg_3 on swine acetylcholine-induced coronary artery contraction.

Since 20(*S*)-ginsenoside Rg_3 inhibited the coronary artery contraction-induced by depolarization with high K^+ and by 5-HT, we next examined whether 20(*S*)- and 20(*R*)-ginsenoside Rg_3 could also inhibit acetylcholine-induced coronary artery contraction (Fig. 4). In contrast to the inhibition by 20(*S*)-ginsenoside Rg_3 on depolarization- or 5-HT-induced coronary artery contraction, both 20(*S*)- and 20(*R*)-ginsenoside Rg_3 had no effect on acetylcholine-induced coronary artery contraction in the presence of endothelium (Fig. 4A). In contrast, both 20(*S*)-ginsenoside Rg_3 inhibited acetylcholine-induced swine coronary artery contraction in the absence of endothelium (Fig. 4B). % Inhibition of 20(*S*)-ginsenoside Rg_3 on acetylcholine-induced coronary artery contraction was 0.00±0.00, 0.87±1.98, 1.85±1.72, 17.91±4.76, 37.68±8.63, 48.50±6.42 and 52.15±4.78% at 0.01, 0.1, 0.3, 1, 3, 10 and 30 μM, respectively in the absence of endothelium (Fig. 4B). EC_{50} of 20(*S*)-ginsenoside Rg_3 was 1.58±0.09 μM.

Also, % inhibition of 20(*R*)-ginsenoside Rg_3 on acetylcholine-induced coronary artery contraction in the absence of endothelium was 0.00±0.00, 0.0±0.00, 1.76±1.85, 19.56±5.87, 29.38±6.19, 45.96±4.68 and 48.17±6.45% at doses of 0.01, 0.1, 0.3, 1, 3, 10 and 30 μM, respectively (Fig. 4B). EC_{50} of 20(*R*)-ginsenoside Rg_3 was 1.87±0.41 μM. Thus, the inhibitory potency on acetylcholine-induced coronary artery contraction between ginsenoside Rg_3 epimers did not also show a significant difference.

DISCUSSION

Although ginseng and its active constituents, ginsenosides, have gained increased popularity worldwide for a myriad of beneficial effects, the underlying mechanisms are still unclear. The information regarding the effects of ginseng on cardiovascular diseases is even more scattered. Herein, we investigated the effect of ginsenosides Rg_3 epimers [20(*S*)- and 20(*R*)-ginsenoside Rg_3] against contraction of coronary artery induced by a various agents in

the absence or presence of endothelium.

In the present study, we demonstrated that (1) 20(*S*)-ginsenoside Rg_3 inhibited high K^+- and 5-HT-induced swine coronary artery contraction in a concentration - dependent manner, (2) the inhibitory effect of 20(*S*)-ginsenoside Rg_3 on high K^+- and 5-HT-induced swine coronary artery contraction is independent of the presence of endothelium, (3) 20(*R*) or 20(*S*)-ginsenoside Rg_3 had no effect on acetylcholine-induced swine coronary artery contraction with endothelium but 20(*R*)- or 20(*S*)-ginsenoside Rg_3 inhibited acetylcholine-induced swine coronary artery contraction with dose-dependent manner without endothelium, (4) finally, (*S*)20-ginsenoside Rg_3 was more effective in the inhibition of high K^+- and 5-HT-induced swine coronary artery contractions than (*R*)20-ginsenoside Rg_3, whereas 20(*R*)-ginsenoside Rg_3 showed almost same inhibitory effects with 20(*S*)-ginsenoside Rg_3 in the inhibition of acetylcholine-induced swine coronary artery contractions without endothelium. These results indicate that ginsenoside Rg_3 epimers showed differential effects on agonist or high K^+-induced swine coronary artery contractions and that endothelium might play a role in ginseng saponins-induced blood vessel relaxation.

Nitric oxide (NO) is a radical produced form L-arginine via NO synthase (NOS), and also serves as an important cellular second messenger[12]. And, NO produced in the vessel was responsible for the vascular relaxation. Therefore, NO production may contribute to the enhancement of vascular relaxation due to ginsenoside Rg_3 epimers treatment The role of NO production by ginsenoside Rg_3 epimers is not yet clear but the role of NO production by ginsenoside Rg_3 in vascular relaxation might be involved in ginsenoside Rg_3 epimers-induced coronary artery contraction. For example, the previous study indicates that, in addition to the endothelium-dependent relaxation[9], ginsenosides were also able to inhibit directly vascular smooth muscle tone in rat aorta [7]. In the present study, although 20(*S*)-ginsenoside Rg_3 still caused the inhibition in high K^+- and 5-HT-induced swine coronary artery contraction without endothelium, both ginsenoside Rg_3 epimers had no effect on acetylcholine-induced coronary artery contraction with endothelium. Thus, these results indicate that endothelium-dependent and independent relaxation pathways in response to ginsenoside Rg_3 might exist in swine coronary artery and further indicate that ginsenoside Rg_3 epimers might utilize the different mechanisms in inhibition of acetylcholine-induced swine coronary artery contraction.

On the other hand, similar observations were obtained in endothelium-intact coronary artery or in endothelium-denuded coronary artery. The inhibitory effect ginsenoside Rg_3 epimers on constriction evoked by 3 μM 5-HT indicated that the effect on coronary artery with endothelium involved NO-dependent mechanism as demonstrated by Sumner[13], Martin *et al.*[14] and Wallis and Martin[15]. It is known that the presence or absence of functional endothelium can influence contractile responses in blood vessels[16, 17, 18, 19]. Thus, these data suggest that a dual endogenous inhibitory mechanism is present in swine coronary artery and that products of the endothelium-derived vascular relaxant factor (EDRF)-NO pathway may interact to regulate 5-HT stimulation *via* 5-HT receptors, as obtained by Yamano *et al.*[20], Hinton *et al.*[21] and Nieto *et al.*[22].

In present results, the endothelium-dependent contraction induced by 5-HT was antagonized by 20(*S*)- and 20(*R*)-ginsenoside Rg_3 on swine coronary artery with endothelium. Moreover, the inhibitory effect of 20(*S*)-ginsenoside Rg_3 on constriction of swine coronary artery induced by 5-HT was not related to the presence of endothelium, though the arterial contraction by 5-HT in the absence of endothelium was slightly antagonized by 20(*R*)-ginsenoside Rg_3.

In summary, we found that 20(*S*)- and 20(*R*)-ginsenoside Rg_3 inhibited high K^+-, 5-HT-, and acetylcholine-induced swine coronary artery contraction with differential manner and that the inhibitory effects of 20(*S*)- and 20(*R*)-ginsenoside Rg_3 on high K^+- or agonists-induced swine coronary artery contraction were dependent or independent on endothelium. Finally, 20(*S*)- rather than 20(*R*)-ginsenoside Rg_3 might be an useful agent for the relaxation of blood vessels that could be contracted by various agents.

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