INTRODUCTION

K⁺ channels play critical roles in a wide variety of physiological processes, including the regulation of neurotransmitter release, neuronal excitability, heart rate, muscle contraction, hormone secretion, epithelial electrolyte transport, cell volume, and cell proliferation [1]. Cardiomyocytes contain two kinds of delayed rectifier K⁺ channels, which are important for cardiac repolarization after cardiac action potential and shorten the action potential duration [2]. Thus, the human ether-a-go-go-related gene (HERG, \( I_{Kr} \)) and KVLQT (KCNQ) (\( I_{Ks} \)) K⁺ channels are mainly responsible for repolarization of the heart cardiac action potential [3]. The genetic or patho-

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logical dysfunctions of HERG or KCNQ K⁺ channels are one of the primary triggers of cardiac diseases such as arrhythmias. Since arrhythmias are a major cause of sudden cardiac death in the world [2], HERG as well as KCNQ K⁺ channels are the principal pharmacological targets for development of therapeutic drugs against cardiovascular diseases including arrhythmias.

Ginseng, the root of Panax ginseng Meyer, has been used as a general tonic to promote longevity and enhance bodily functions against stress, fatigue, diseases, cancer and diabetes mellitus [4]. Among various ginseng components, ginsenosides (also called ginseng saponins) exhibit anti-hypertension and cardio-protective effects [5]. In a previous study, we demonstrated that ginsenoside Rg₃ (Rg₃) enhanced cardiac Iᵦₖ channel currents, which consist of KCNQ1 plus KCNE1 subunits in both concentration- and voltage-dependent manners [6]. In addition, we also demonstrated that Rg₃ enhanced outward currents (Iₜₕ) and transient tail currents (Iₜₙₕ). Rg₃ induced a large persistent deactivating-tail current (Iₜₕ₋ₙₕ) and significantly decelerated deactivating current decay [7].

On the other hand, ginsenosides administered via the oral route can pass into the large intestine without being broken down by either gastric juices or digestive enzymes [8]. But protopanaxadiol (PPD) ginsenosides are metabolized by intestinal microorganisms into compound K (CK) with one glucose at the C-20 position and are further metabolized to form PPD without glucose, whereas protopanaxatriol (PPT) ginsenosides are metabolized to PPT that only maintains the backbone structure of ginsenosides without any carbohydrate component (Fig. 1). The purpose of this study was to investigate how ginsenoside metabolites affect HERG K⁺ channel activity. We report here that CK decelerated HERG K⁺ channel deactivation, whereas PPT accelerated HERG K⁺ channel deactivation. PPD itself had no effect on HERG K⁺ channel activity but antagonized Rg₃-mediated HERG K⁺ channel regulations. These results indicate that ginsenoside metabolites exhibit differential regulation on HERG K⁺ channel activity.

**MATERIALS AND METHODS**

**Materials**

The individual ginsenoside Rg₃ and ginsenoside metabolites such as PPD, PPT, and CK were provided by AMBO Institute (Seoul, Korea) (Fig. 1A). The cDNAs for human HERG K⁺ channels (accession no. U04270) were kindly provided by Dr. Pongs (University of Hamburg, Germany). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Preparation of Xenopus oocytes and microinjection**

Xenopus laevis frogs were purchased from Xenopus I (Ann Arbor, MI, USA). Their care and handling were in accordance with the highest standards of institutional guidelines of Konkuk University. For isolation of oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester followed by removal of ovarian follicles. The oocytes were treated with collagenase and then agitated for 2 h in Ca²⁺-free medium containing 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM HEPES, 2.5 mM sodium pyruvate, 100 units/mL penicillin and 100 µg/mL streptomycin. Stage V-VI oocytes were collected and stored in ND96 medium (in mM: 96 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, and 5 HEPES, pH 7.5) supplemented with 50 µg/mL gentamicin. The oocyte-containing solution was maintained at 18°C with continuous gentle shaking and renewed daily. Electrophysiological experiments were performed within 5-6 days of oocyte isolation, with ginsenoside Rg₃ or ginsenoside metabolites added to the bath. For HERG K⁺ channel experiments, HERG K⁺ channel-encoding cRNAs (40 nL) were injected into the animal or vegetal pole of each oocyte one day after isolation, using a 10 µl microdispenser (VWR Scientific, San Francisco, CA, USA) fitted with a tapered glass pipette tip (15-20 µm in diameter) [9].

**Fig. 1.** Chemical structures of ginsenoside Rg₃ and ginsenoside metabolites used in this study. CK, compound K; PPD, protopanaxadiol; PPT, protopanaxatriol; Glc, glucopyranoside.
**Data recording**
A custom-made Plexiglas net chamber was used for two-electrode voltage-clamp recordings as previously reported [7]. The oocytes were impaled with two microelectrodes filled with 3M KCl (0.2-0.7 MF), and electrophysiological experiments were carried out at room temperature using an Oocyte Clamp (OC-725C; Warner Instruments, Hamsden, CT, USA). Stimulation and data acquisition were controlled with a pClamp 8 (Axon Instruments, Union City, CA, USA). For most electrophysiological experiments, oocytes were initially perfused with ND96 solution (in mM: 96 NaCl, 3 KCl, 2 CaCl², 5 HEPES, pH 7.4 with NaOH) to obtain control current recordings. The oocytes were then clamped at a holding potential of −90 mV, the membrane potential was depolarized to 0 mV for 4 s, followed by repolarization to −60 mV at 20 s intervals, and current values were recorded.

**Data analysis**
To obtain the concentration-response curve of the respective ginsenoside metabolites effects on the K⁺ current from the HERG K⁺ channel, the peak amplitudes at various concentrations of the respective ginsenoside metabolites were plotted, and Origin software (Origin, Northampton, MA, USA) was used to fit the plot to the Hill equation: y/y_{max} = [A]^n/(IC_{50}^n+[A]^n), where y is the peak current at a given concentration of ginsenoside metabolite, y_{max} is the maximal peak current, IC_{50} is the concentration of the respective ginsenoside metabolite producing a half-maximal effect, [A] is the concentration of the respective ginsenoside metabolite, and n is the Hill coefficient. All values were presented as the mean±SEM. The significance of differences between mean control and treatment values was determined using Student’s t-test, where p<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Effects of CK on HERG K⁺ channel currents**
We first examined the effect of intermediate ginsenoside metabolite CK on the HERG K⁺ channel currents using a Xenopus oocyte gene expression system. HERG K⁺ channel currents were recorded using the two-electrode voltage-clamp recording technique at room temperature [10]. Throughout these experiments, the holding potential was maintained at −90 mV and repolarized to −60 mV after depolarizing pulses from 0 mV, unless otherwise indicated. Fig. 2A gives an example of a voltage-clamp recording with the representative current traces of I_{HERG} at end of depolarization, transient peak I_{trans}, and slow I_{deactivating-tail} as indicated by arrows in the absence or presence of various concentrations of CK. CK had almost no effect on I_{HERG} and I_{trans} (Fig. 2A). Interestingly, CK showed a major effect on slow I_{deactivating-tail}. Thus in the presence of CK, I_{deactivating-tail} failed to decay, and instead, a large persistent outward current developed. CK (100 µM) increased slow I_{deactivating-tail} by an average of 97.8±8.13% compared to control (Fig. 2A). CK response was completely reversible upon washing the oocytes with ND96 (data not shown). Next, we examined the concentration-dependent effect of CK on I_{HERG}, I_{trans}, and slow I_{deactivating-tail}. As shown in Fig. 2B, CK had a negligible effect on both I_{HERG} and I_{trans}. CK also increased a persistent I_{deactivating-tail} in a concentration-dependent manner. The EC_{50} value was 16.6±1.3 µM for slow I_{deactivating-tail}.

Next, we investigated the HERG K⁺ current-voltage (I-V) relationship using CK (Fig. 2C, D). The amplitude of I_{HERG} measured at the end of depolarization increased with increasing positive voltage steps, reaching a maximum at −10 mV and then progressively decreased with further voltage steps in the control, indicating that HERG K⁺ channel is activated with rectified manner (Fig. 2C, D). When the amplitude of I_{HERG} was normalized to the maximum amplitude of the I_{HERG} obtained under the control conditions and was plotted against the potential of the step depolarization (Fig. 2C, D), the presence of various concentrations of CK did not show a significant increase of I_{HERG} even at high concentrations of CK, indicating that CK mainly affects slow I_{deactivating-tail}.

We next examined whether CK affects steady-state activation (Fig. 2E). Treatment of 10 and 30 µM CK caused significant leftward shifts in steady-state activation curves compared to control oocytes. The V_{1/2} values were −25.5±0.39 mV in the control and −24.6±0.43, −30.9±0.50 and −32.4±0.82 mV in the presence of 3, 10, and 30 µM CK-treated groups, respectively (p<0.05 at 10 and 30 µM Rg, compared to control, n=5). The slope factors (kg) were not significantly changed, yielding values of 6.7±0.34 in control and 6.3±0.37, 6.1±0.44 and 6.5±0.70 in the presence of 3, 10 and 30 µM CK-treated groups, respectively. We also examined CK (30 µM) and observed that it did not affect steady-state inactivation (data not shown). Therefore, these results indicate that CK affects the activation gating rather than inactivation gating of HERG K⁺ channels.

**Effects of PPT on HERG K⁺ channel currents**
PPT had no effects on I_{HERG} and I_{trans} as observed with CK. However, PPT showed a significant effect on slow
As a result, in the presence of PPT, $I_{\text{deactivating-tail}}$ accelerated to decay in contrast to CK. PPT (100 µM) decreased slow $I_{\text{deactivating-tail}}$ by an average of 88.2±1.3% compared to control (Fig. 3A). PPT response was also completely reversible upon washing the oocytes with ND96 (data not shown). Next, we examined the concentration-dependent effect of PPT on $I_{\text{HERG}}$, $I_{\text{tail}}$, and slow $I_{\text{deactivating-tail}}$. As shown in Fig. 3B, PPT showed a slight inhibition on $I_{\text{tail}}$ but a negligible effect on $I_{\text{HERG}}$, however PPT primarily inhibited $I_{\text{deactivating-tail}}$ in a concentration-dependent manner.
dependent manner. Interestingly, PPT had a considerable effect on $I_{\text{deactivating-tail}}$ compared to $I_{\text{HERG}}$ and $I_{\text{tail}}$. The IC$_{50}$ value was 27.5±2.3 µM for slow $I_{\text{tail}}$ while the IC$_{50}$ value was 10.6±0.5 µM for slow $I_{\text{deactivating-tail}}$.

Next, we examined the HERG K$^+$ current-voltage (I-V) relationship using PPT (Fig. 3C, D). The amplitude of $I_{\text{HERG}}$ measured at the end of depolarization increased with increasing positive voltage steps, reaching a maximum at −10 mV and then progressively decreased with further voltage steps in the control, indicating that HERG K$^+$ channel rectifies (Fig. 3C, D). When the amplitude of $I_{\text{HERG}}$ was normalized to the maximum amplitude of the $I_{\text{HERG}}$ obtained under the control conditions and was plotted against the potential of the step depolarization (Fig. 3D).
concentrations of PPD did not show a significant increase of $I_{\text{HERG}}$ even at high concentrations of PPT, indicating that PPT mainly affects slow $I_{\text{deactivating-tail}}$.

We next set forth to determine whether PPT affects steady-state activation (Fig. 3E). Treatment of 10 and 30 mM PPT did induce slightly but not significant leftward shifts in steady-state activation curves compared to control oocytes. The $V_{1/2}$ values were $-22.6\pm 0.37$ mV in the control and $-28.7\pm 1.35$, $-29.1\pm 1.43$ and $-30.1\pm 0.46$ mV in the presence of 3, 10, and 30 mM PPT-treated groups, respectively ($p<0.08$ at 10 and 30 mM PPT compared to control, $n=5$). The slope factors ($k_g$) were not significantly changed, yielding values of 7.9±0.33 in control and 4.8±0.09, 5.2±1.29 and 6.3±0.39 in the presence of 3, 10, and 30 mM PPT-treated groups, respectively. We also examined PPT (100 mM) and observed that it did not affect steady-state inactivation (data not shown). Therefore, these results indicate that PPT affects the activation gating rather than inactivation gating of HERG $K^+$ channels.

**Effects of PPD on HERG $K^+$ channel currents**

PPD itself had nearly no effects on $I_{\text{HERG}}$, $I_{\text{tail}}$, and slow $I_{\text{deactivating-tail}}$ (Fig. 4A). However, PPD showed an inhibition on $R_g$-mediated enhancement on slow $I_{\text{deactivating-tail}}$. Hence in the presence of PPD, $R_g$-induced persistent $I_{\text{deactivating-tail}}$ was greatly attenuated. PPD effect on $R_g$-induced persistent $I_{\text{deactivating-tail}}$ was completely reversible upon washing the oocytes with ND96 (data not shown).

We subsequently examined the concentration-dependent effect of PPD on $R_g$-induced persistent $I_{\text{deactivating-tail}}$. As shown in Fig. 4B, PPD showed a negligible effect on $I_{\text{HERG}}$ and $I_{\text{tail}}$ but PPD greatly attenuated $R_g$-induced persistent $I_{\text{deactivating-tail}}$ in a concentration-dependent manner. Interestingly, PPD caused a rightward shift of $R_g$-induced persistent $I_{\text{deactivating-tail}}$ from 0.5±0.04 to 2.9±0.57 μM for slow $I_{\text{deactivating-tail}}$ (Fig. 4C).

We then studied the HERG $K^+$ current-voltage (I-V) relationship using PPD on $R_g$-induced enhancement of $I_{\text{HERG}}$ (Fig. 4B). The amplitude of $I_{\text{HERG}}$ measured at the end of depolarization increased with increasing positive voltage steps, reaching a maximum at $-10$ mV and then progressively decreased with further voltage steps in the control, indicating that HERG $K^+$ channel rectifies (Fig. 4B). When the amplitude of $I_{\text{HERG}}$ was normalized to the maximum amplitude of the $I_{\text{HERG}}$ obtained under the control conditions and was plotted against the potential of the step depolarization (Fig. 4B), the presence of various concentrations of PPD did not show a significant increase of $I_{\text{HERG}}$ even at high concentrations of PPT, indicating that PPD mainly affects $R_g$-induced persistent $I_{\text{deactivating-tail}}$.

Our following goal was to examine whether PPD affects steady-state activation (Fig. 3E). In contrast to CK or PPT, co-treatment of 10 and 30 mM PPD with $R_g$ did not affect on $R_g$-caused leftward shifts in steady-state activation curves compared to control oocytes. The $V_{1/2}$ values were $-21.7\pm 0.18$ mV in the control and $-21.2\pm 0.35$, $-24.6\pm 0.27$ and $-24.6\pm 0.28$ mV in the presence of 3, 10, and 30 mM PPD-treated groups, respectively ($p<0.07$ at 10 and 30 mM $R_g$ compared to control, $n=5$). The slope factors ($k_g$) were not significantly changed, yielding values of 7.9±0.16 in control and 8.7±0.31, 7.5±0.24 and 7.5±0.25 in the presence of 3, 10 and 30 mM PPD-treated groups, respectively. We also examined PPD (100 mM) and observed that it did not affect steady-state inactivation (data not shown). These results suggest PPD affects the activation gating rather than inactivation gating of HERG $K^+$ channels.

Ginsenosides consist of aglycone and carbohydrates portions. The aglycone is the backbone of the ginsenoside, with a hydrophobic four-ring steroid-like structure that may be non-polar, whereas the carbohydrates on carbons-3, 6, and 20 of the backbone are polar (Fig. 1A). *In vitro* and *in vivo* studies have shown that ginsenosides administered by an oral route were metabolized and finally become an aglycone such as CK which has one glucose at the C-20, PPD and PPT [8]. Recent report showed that these ginsenoside metabolites might also induce apoptosis of the cancer cells and play a role as an anti-cancer agent [8,11], suggesting that ginsenosides are pro-drugs of these metabolites. However, relatively little is known regarding how ginsenoside metabolites regulate ion channel or receptor activity.

In the present study, we report three major findings. First, we observed that CK decelerated deactivating time constants in both concentration- and voltage-dependent manners, whereas CK had no effects on $I_{\text{HERG}}$ and $I_{\text{tail}}$. Second, we found that PPT accelerated $I_{\text{deactivating-tail}}$ deactivation in both concentration- and voltage-dependent manners without affecting on $I_{\text{HERG}}$ and $I_{\text{tail}}$. Third, PPD itself had no effect on $I_{\text{HERG}}$, $I_{\text{tail}}$ and $I_{\text{deactivating-tail}}$. However, co-treatment of PPD with $R_g$ blocked $R_g$-mediated decelerating effects on $I_{\text{deactivating-tail}}$. Thus, the major findings of the present study are that ginsenoside metabolites had no significant effects on $I_{\text{HERG}}$ and $I_{\text{tail}}$ of HERG $K^+$ channel but they did affect $I_{\text{deactivating-tail}}$ in differing manners, indicating that ginsenoside metabolites show a differential regulation of $I_{\text{deactivating-tail}}$ with respect to the HERG $K^+$ channel.

On the other hand, we demonstrated in previous stud-
gies that ginsenoside metabolites such as CK, PPD and PPT regulate ion channels and receptors. For example, CK but not PPT caused strong inhibition of the voltage-dependent α1G-type Ca$^{2+}$ channel [12]. Similarly, we have also found that CK but not M4 (PPT) inhibited a neuronal Na$^{+}$ (Nav1.2) channel [13]. We demonstrated that M4, but not CK, exhibited an inhibitory effect on 5-HT$_3$A receptor-mediated currents [14]. In addition, CK and PPT exhibited an inhibitory effect on α3β4 nicotinic acetylcholine receptor-mediated currents [15]. All these findings indicate that ginsenoside metabolites as well as ginsenosides have regulatory effects on voltage-dependent ion channel and receptor activities.

Since the HERG K$^+$ channel regulators are clinically important for treatment of cardiac diseases such as arrhythmia, there are numerous reports on the development...
of HERG K⁺ channel regulators from natural compounds [16]. In the previous study, we found that Rg₁ increased \( I_{\text{HERG}} \) and peak \( I_{\text{tail}} \). In addition, Rg₁ profoundly delayed deactivation kinetics by inducing a persistent \( I_{\text{deactivating-tail}} \) and caused a leftward shift of steady-state voltage-dependent activation but not inactivation, leading to the possibility that Rg₁ is an activator to open HERG K⁺ channel at a more negative voltage step than control and that Rg₁ mainly works by slowing closure of the HERG K⁺ channel once it has opened. In the present study, when we examined the effects of ginsenoside metabolites on HERG K⁺ channel currents, we found that CK induced a persistent \( I_{\text{deactivating-tail}} \) and caused a leftward shift of steady-state voltage-dependent activation (Fig. 2). However, the effective concentration of CK was higher than that of Rg₁. In contrast to CK, PPT caused an acceleration of \( I_{\text{deactivating-tail}} \) decay (Fig. 3). Interestingly, co-treatment of PPD with Rg₁ blocked Rg₁-mediated deceleration of deactivation process. It is noteworthy that since Rg₁ became PPD after metabolic process, PPD-mediated antagonism of Rg₁ action on HERG K⁺ channel activity show the possibility that aglycone, PPD, ginsenoside Rg₁, and other PPD ginsenosides might interfere with \textit{in vivo} Rg₁ actions on HERG K⁺ channel activity.

Taken together, we found that CK decelerated the deactivation process by inducing a persistent \( I_{\text{deactivating-tail}} \) with delayed deactivation in HERG K⁺ channel without affecting \( I_{\text{HERG}} \) and \( I_{\text{tail}} \). In contrast, PPT accelerated the deactivation process by inducing an inhibition of \( I_{\text{deactivating-tail}} \) without affecting \( I_{\text{HERG}} \) and \( I_{\text{tail}} \). PPD itself had no observable effects on \( I_{\text{HERG}} \), \( I_{\text{tail}} \) and \( I_{\text{deactivating-tail}} \) but co-treatment of PPD with Rg₁ blocked Rg₁-mediated a persistent \( I_{\text{deactivating-tail}} \) with delayed deactivation in HERG K⁺ channel. These novel findings provide an insight into the molecular action of ginsenoside metabolites on HERG K⁺ channel activity.

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