Differential effects of ginsenoside metabolites on slowly activating delayed rectifier K$^+$ and KCNQ1 K$^+$ channel currents

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Channels formed by the co-assembly of the KCNQ1 subunit and the mink (KCNE1) subunit underline the slowly activating delayed rectifier K$^+$ channels ($I_{Ks}$) in the heart. This K$^+$ channel is one of the main pharmacological targets for the development of drugs against cardiovascular disease. Panax ginseng has been shown to exhibit beneficial cardiovascular effects. In a previous study, we showed that ginsenoside Rg3 activates human KCNQ1 K$^+$ channel currents through interactions with the K318 and V319 residues. However, little is known about the effects of ginsenoside metabolites on KCNQ1 K$^+$ alone or the KCNQ1 + KCNE1 K$^+$ ($I_{Ks}$) channels. In the present study, we examined the effect of protopanaxatriol (PPT) and compound K (CK) on KCNQ1 K$^+$ and $I_{Ks}$ channel activity expressed in Xenopus oocytes. PPT more strongly inhibited the $I_{Ks}$ channel currents than the currents of KCNQ1 K$^+$ alone in concentration- and voltage-dependent manners. The $IC_{50}$ values on $I_{Ks}$ and KCNQ1 alone currents for PPT were 5.18±0.13 and 10.04±0.17 µM, respectively. PPT caused a leftward shift in the activation curve of $I_{Ks}$ channel activity, but minimally affected KCNQ1 alone. CK exhibited slight inhibition on $I_{Ks}$ and KCNQ1 alone K$^+$ channel currents. These results indicate that ginsenoside metabolites show limited effects on $I_{Ks}$ channel activity, depending on the structure of the ginsenoside metabolites.

Keywords: Panax ginseng, Ginsenoside metabolites, Slowly activating delayed rectifier K$^+$ channels ($I_{Ks}$), Human heart

INTRODUCTION

KCNQ1 was first discovered by positional cloning [1]. Coassembly with the β-subunit KCNE1 modified a very slowly activating delayed rectifier K$^+$ current, $I_{Ks}$, with no apparent pattern of inactivation [2-5]. Physiologically, $I_{Ks}$ channels involve repolarization of cardiac action potentials, modulation of H$^+$ secretion into the stomach, secretion of Cl$^-$ into the colon, and secretion of K$^+$ into the stria media of the inner ear [6-8]. In particular, $I_{Ks}$ channels constitute the major outward current involved in ventricular repolarization and HERG ($I_{Kr}$) channels [9-11]. Dysfunction or mutation of delayed rectifier K$^+$ channels ($I_{Ks}$ and $I_{Kr}$) underlines the long QT syndrome (LQT) with increased risk of Torsades de Pointes [11-13]. Since arrhythmia is one of the major causes of sudden cardiac death worldwide [14], KCNQ K$^+$ and hERG channels are primary pharmacological targets for the development of therapeutic drugs against cardiovascular disease, including arrhythmia.

Ginseng, the root of Panax ginseng Meyer, is a representative herbal medicine and exhibits a variety of pharmacological effects, including antistress, antifatigue, anticancer, and antidiabetes mellitus [15]. Ginsenosides (also
called ginseng saponins), as a representative ingredient of ginseng, also exhibit antihypertension and cardioprotective effects [16-18]. For example, administration of ginseng extract shortened action potential duration and ginsenoside Re regulates the $I_{kr}$ and $I_{Ks}$ channel currents of guinea pig myocytes [19,20].

In a previous study, we showed that ginsenoside Rg3 activated human cardiac $I_{Ks}$ channel currents in concentration- and voltage-dependent manners [21]. Moreover, we demonstrated that ginsenoside Rg3 enhanced the outward currents ($I_{vap}$) and transient tail currents ($I_{tail}$). Rg3 induced a large persistent deactivating-tail current ($I_{de}$) and significantly decelerated the deactivating current decay [22]. According to the number and position of sugar moieties, ginsenosides are divided into two main categories (i.e., the 20[S]-protopanaxadiol [PPD] and 20[S]-protopanaxatriol [PPT] families). On the other hand, ginsenosides are metabolized by colonic bacteria [23]. The intestinal bacterial metabolites, including compound K (CK), PPD, and PPT, were easily absorbed and appeared in the plasma of rats or humans after the oral administration of ginsenosides [23-26].

In the present study, we examined the effects of ginsenoside metabolites such as PPT and CK on $I_{Ks}$ and KCNQ1 K$^+$ alone channel activity by using the Xenopus oocyte gene-expression system. We found that CK and PPT exhibited a differential effect on $I_{Ks}$ and KCNQ1 K$^+$ alone channel activity and further discussed the role of ginsenoside metabolites in cardiovascular systems.

MATERIALS AND METHODS

Materials

Ginsenoside metabolites, such as CK and PPT, were provided by the AMBO Institute (Seoul, Korea) (Fig. 1). The cDNAs for human KCNQ channels (Gene Bank ID. NM_000218) were kindly provided by Dr. Pongs (University of Hamburg, Germany). Other agents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of Xenopus oocytes and microinjection

* X. laevis* frogs were purchased from *Xenopus* I (Ann Arbor, MI, USA). Their care and handling procedures were performed in accordance with the institutional guidelines of Konkuk University. For the isolation of oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester, followed by the removal of ovarian follicles. The oocytes were treated with collagenase, and then agitated for 2 h in a Ca$^{2+}$-free medium containing 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl$_2$, 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 a ND96 medium (in mM: 96 NaCl, 2 KCl, 1 MgCl$_2$, 1.8 CaCl$_2$, and 5 HEPES, pH 7.5) supplemented with 50 μg/mL gentamicin. The oocyte-containing solution was maintained at 18°C with gentle continuous shaking and renewed daily. Electrophysiological experiments were performed within 5 to 6 d of oocyte isolation, with ginsenoside metabolites applied to the bath. For K$^+$ channel experiments, Kv channel-encoding cRNAs (40 nL) were injected into the animal or the vegetal pole of each oocyte 1 d after isolation using a 10-µL microdispenser (VWR Scientific, San Francisco, CA, USA) fitted with a tapered glass pipette tip (15 to 20 µm in diameter) [27].

Data recording

A custom-made Plexiglas net chamber was used for two-electrode voltage-clamp recordings as previously reported [27]. The oocytes were impaled with 2 microelectrodes filled with 3M KCl (0.2 to 0.7 MΩ), and electrophysiological experiments were carried out at room temperature by 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 a ND96 solution (in mM: 96 NaCl, 3 KCl, 2 CaCl$_2$, 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 the currents were recorded.

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

RESULTS AND DISCUSSION
Effects of protopanaxatriol and compound K on $I_{ks}$
We first examined the effect of the ginsenoside metabolites PPT and CK on the $I_{ks}$ channel currents by using a Xenopus oocyte gene-expression system. As shown in previous reports, $I_{ks}$ channel currents were recorded using a two-electrode voltage-clamp recording technique at room temperature. In these experiments, cells were held at −80 mV and depolarized to +30 mV for 2.5 s to elicit the currents. The currents evoke by this voltage-clamp
protocol were slowly activating delayed rectifier K⁺ channel (Iₖ) currents with no apparent inactivation (Fig. 2A) [3]. PPT inhibited Iₖ in a concentration-dependent manner over a range of concentrations (i.e., 1 to 30 µM) (Fig. 2A). The IC₅₀ and Hill coefficient for the PPT block of Iₖ were 5.18±0.13 µM and 1.72±0.05, respectively (Fig. 2B). The effect of PPT on the current-voltage relationship of the Iₖ channel was estimated by normalizing current values plotted against the test potential in the absence and presence of 10 µM PPT [21]. Normalized currents were obtained from the peak current amplitudes in response to depolarizing pulses, in the range of −60 to +30 mV in 10-mV increments with a holding potential of −80 mV. The blockage of Iₖ at 10 µM PPT was voltage dependent (Fig. 2C). Next, we examined the effects of PPT on the voltage dependency of steady-state channel activation. As described above, a voltage-clamp protocol consisted of 3-s depolarizing steps from −60 to +40 mV (10-mV increments and 10-s intervals with a holding potential of −80 mV) was used to determine the activation of Iₖ channels. The activation of Iₖ channels was fitted to a Boltzmann function. As shown in Fig. 2D, 10 and 30 µM PPT shifted V₅₀,₅ in the hyperpolarizing direction (control, −0.62±1.12 mV; 10 and 30 µM PPT, −5.93±0.66 mV, −12.25±0.86 mV, respectively; n=5, p<0.05). Interestingly, CK exhibited only a slight inhibition of Iₖ in a concentration-dependent block, the I-V relationship, and the G-V curve (Fig. 3). Thus, ginsenoside-induced regulations on Iₖ might be derived from PPT rather than from protopanaxadiol ginsenoside metabolites (i.e., PPD).

**Effects of protopanaxatriol and compound K on KCNQ1 alone K⁺ channel activity**

We next examined the effect of PPT and CK on KCNQ1 alone channel currents. Similar to the Iₖ channels, PPT also exhibited the concentration-dependent inhibition of KCNQ1 alone K⁺ channel currents and showed by 5% inhibition of the KCNQ1 alone K⁺ channel current (Fig. 4A, B). The fitting curve of PPT for IC₅₀
was 10.0±0.17 μM with a Hill coefficient of h=2.01±0.06 (n=5) (Fig. 4B). The current–voltage relationship was examined, and the current responses evoked by voltage steps (i.e., a series of voltage pulses of 3-s duration given in 10-mV increments and 10-s intervals with a holding potential of −80 mV) were used to construct the I-V curve. KCNQ1 alone K⁺ current by PPT observed voltage-dependent inhibition (Fig. 4C). Unlike the I_{Ks} channels, the effect of PPT on the KCNQ1 alone K⁺ channel induced a minimal shift in the G-V curve (Fig. 4D). Fig. 5 shows the effects of the ginsenoside metabolite CK on KCNQ1 alone K⁺ currents. CK also had no effect on KCNQ1 alone as it did on the I_{Ks} channels. The IC_{50} was 27.65±0.05 μM with a Hill coefficient of h=3.04±0.03 (n=7) (Fig. 5B). CK minimally affected the current–voltage relationship and voltage-dependent activation (Fig. 5C, D). Thus, these results suggest that KCNQ1 alone is more sensitive to PPT than CK with regard to blocking.

In vitro and in vivo studies have shown that an orally administered ginsenoside was metabolized and finally converted to aglycone such as CK, which has a glucose residue at the C-20 of PPD or PPT [23]. Recent reports have shown that such ginsenoside metabolites might exhibit pharmacological effects [23,28,29]. However, relatively little is known about the effects of ginsenoside metabolites on K⁺ ion channel activity, such as I_{Ks} in the heart.

We have previously reported that ginsenoside Rg3 activated human KCNQ1 K⁺ channel currents through interactions with the K318 and V319 residues [21]. In the present study, we examined the effects of ginsenoside metabolites such as CK and PPT on human KCNQ1 K⁺ channel activity. We found that ginsenoside metabolites showed a differential effect of PPT on I_{Ks} and KCNQ1 alone K⁺ channels currents. Thus, we observed that PPT inhibited I_{Ks} and KCNQ1 alone K⁺ channels currents.

Fig. 4. Effects of protopanaxatriol (PPT) on KCNQ1 alone channel. (A) The representative traces on KCNQ1 alone channel current inhibition by different concentrations of PPT. Protocols were the same as described for Fig. 2. (B) Concentration-response curves of PPT on KCNQ1 alone channel currents. (C) Current-voltage (i-V) relationships of KCNQ1 alone channels in the absence (●) or presence (○) of 30 μM PPT. (D) Example of KCNQ1 channel currents recorded before (control) and after modification by 30 μM PPT. Protocols were the same as described for Fig. 2. Data represent the mean±SEM (n=5–7).
in both concentration- and voltage-dependent manners, but the PPT blockade of the $I_{Ks}$ current had an IC$_{50}$ value of 5.18±0.13 µM, which was 2-fold less than that of KCNQ1 alone $K^+$ current. In addition, we found that steady-state activation curves of $I_{Ks}$ channel currents by PPT shifted in the direction of hyperpolarization in a dose-dependent manner, but the KCNQ1 alone $K^+$ channel currents rarely did. This observation suggests that PPT affects the steady-state activation of $I_{Ks}$ channels only. Taken together, these results suggest that the KCNE1 subunit has an important role in PPT-induced regulation of the $I_{Ks}$ channel.

Native cardiac $I_{Ks}$ channels are involved in cardiac diseases such as arrhythmia [30]. $I_{Ks}$ channel regulators are clinically important for the treatment of cardiac diseases [31,32]. In a previous study, we reported that ginsenoside metabolites exhibited differential regulations on $I_{deactivating-tail}$ of the HERG $K^+$ channel [22]. CK induced a persistent $I_{deactivating-tail}$ and caused a leftward shift of steady-state, voltage-dependent activation. In contrast to CK, PPT caused an acceleration of $I_{deactivating-tail}$ decay. In the present study, we found that PPT mainly inhibited the $I_{Ks}$ $K^+$ channel, caused a leftward shift in the activation curve of the $I_{Ks}$ channel, and minimally affected KCNQ1 alone. Therefore, when KCNQ1 co-assembled with KCNE1, the sensitivity to PPT increased 2-fold, thus indicating that ginsenoside metabolites exhibit a differential effect on HERG $K^+$ and $I_{Ks}$ channels.

In previous studies, we also showed that ginsenoside metabolites such as CK, PPD, and PPT regulate ion channels and receptors. PPT, but not CK, induced an inhibition of the voltage-dependent L-type Ca$^{2+}$ channel currents [33]. We have found that CK, but not PPT, inhibited a neuronal Nav1.2 channel [34]. In contrast, M4, but not CK, caused an inhibition of N-methyl-D-aspartic acid receptor-mediated currents [35]. In the present study, CK exhibited a negligible effect on both $I_{Ks}$ and KCNE K$^+$ channels. Thus, ginsenoside metabolite-induced regulations on various ion channels and receptors might be dependent on ion channel or receptor types.
In summary, our results show that PPT, rather than CK, is the main component in the inhibition of $I_{Ks}$ channels. Further, different types of ginsenoside metabolites exhibit differential effects on the regulation of $I_{Ks}$ and KCNQ1 K$^+$ channels.

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