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# 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



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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 ($I_{HERG}$) and transient tail currents ($I_{tail}$). Rg3 induced a large persistent deactivating-tail current ($I_{de\text{-}activating\text{-}tail}$) 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.

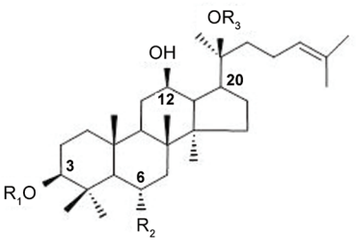
## MATERIALS AND METHODS

### 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




| Ginsenosides | R1 | R2 | R3 |
|---|---|---|---|
| CK | -H | -H | -Glc |
| Rg3 | -$Glc_2$-Glc | -H | -H |
| PPD | -H | | -H |
| PPT | -H | -OH | -H |

**Fig. 1.** Chemical structures of ginsenoside Rg3 and ginsenoside metabolites used in this study. CK, compound K; PPD, protopanaxadiol; PPT, protopanaxatriol; Glc, glucopyranoside.

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: $y/y_{max} = [A]^{nH}/([A]^{nH} + [IC_{50}]^{nH})$, where y is the peak current at a given concentration of CK and PPT, $y_{max}$ 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 nH 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 evoked by this voltage-clamp

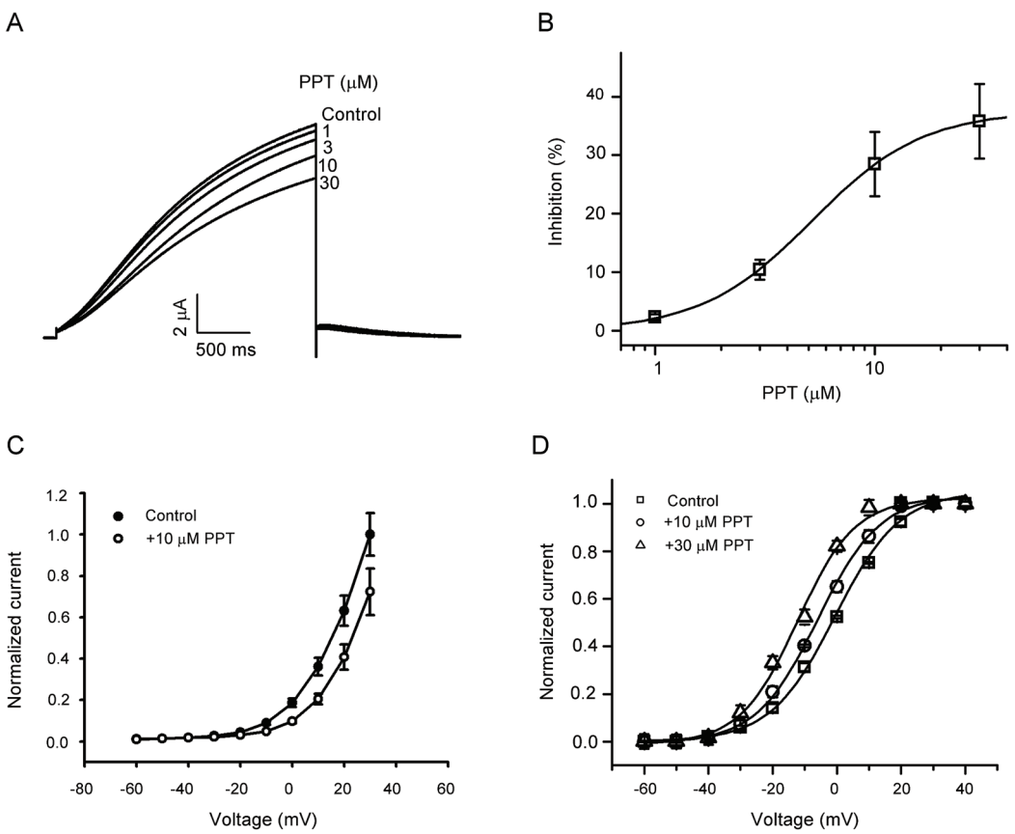



**Fig. 2.** Effects of protopanaxatriol (PPT) on $I_{Ks}$ channel currents. (A) The representative traces on $I_{Ks}$ channel current blocks by different concentrations of PPT. Currents were in response to 2.5-s voltage steps up to +30 mV from a holding potential of –80 mV. (B) Concentration-response curves of PPT on $I_{Ks}$ and KCNQ1 alone channel currents. Solid lines have been fitted to the Hill equation as described in Materials and Methods. Oocytes were clamped at the same as described for (A), and evoked every 10 s. (C) Current-voltage (I-V) relationships of $I_{Ks}$ channel in the absence (●) or presence (○) of 10 μM PPT. Voltage pulses of 3-second duration were applied in 10-mV increments and at 10-second intervals from a holding potential of –80 mV. The peaks of the evoked currents, normalized to the peak current evoked by the voltage step to +30 mV in the absence of PPT, were used in the I-V plot. (D) An example of $I_{Ks}$ channel currents recorded before (control) and after modification by 10 and 30 μM PPT. Currents recorded during 3-second depolarizing pulses to membrane potentials of –60 to +50 mV, applied from a holding potential of –80 mV. Tail currents were measured at –70 mV. Voltage-dependent activation curves were determined from the normalized amplitudes of tail currents. Data were fitted to a Boltzmann function. Data represent the mean±SEM (*n*= 6–7).

protocol were slowly activating delayed rectifier $K^+$ channel ($I_{Ks}$) currents with no apparent inactivation (Fig. 2A) [3]. PPT inhibited $I_{Ks}$ in a concentration-dependent manner over a range of concentrations (i.e., 1 to 30 μM) (Fig. 2A). The $IC_{50}$ and Hill coefficient for the PPT block of $I_{Ks}$ 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 $I_{Ks}$ 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 $I_{Ks}$ 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 $I_{Ks}$ channels. The activation of $I_{Ks}$ channels was fitted to a Boltzmann function. As shown in Fig. 2D, 10 and 30 μM PPT shifted $V_{g0.5}$ 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 $I_{Ks}$ in a concentration-dependent block, the I-V relationship, and the G-V curve (Fig. 3). Thus, ginsenoside-induced regulations on $I_{Ks}$ 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 $I_{Ks}$ 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 $IC_{50}$

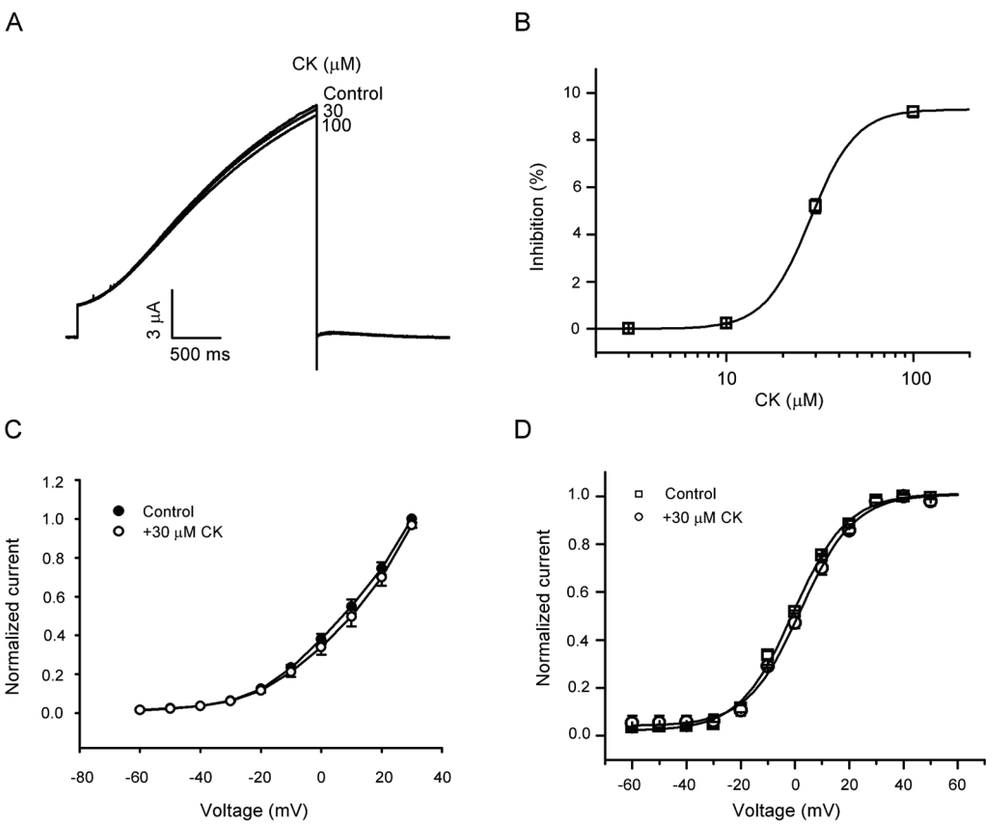



**Fig. 3.** Effects of compound K (CK) on $I_{Ks}$ channel currents. (A) Representative current traces on $I_{Ks}$ channel inhibitions by different concentrations of CK. (B) Concentration-response curves of CK on $I_{Ks}$ channel currents. (C) I-V relationships for KCNQ1 plus KCNE1 channel currents measurement at the end of the 3-second test pulse before and after application of 30 μM CK. (D) The steady-state activation curve for $I_{Ks}$ channel currents by 30 μM CK. Protocols were the same as described for Fig. 2. Data are represented by the mean±SEM ($n$=7).

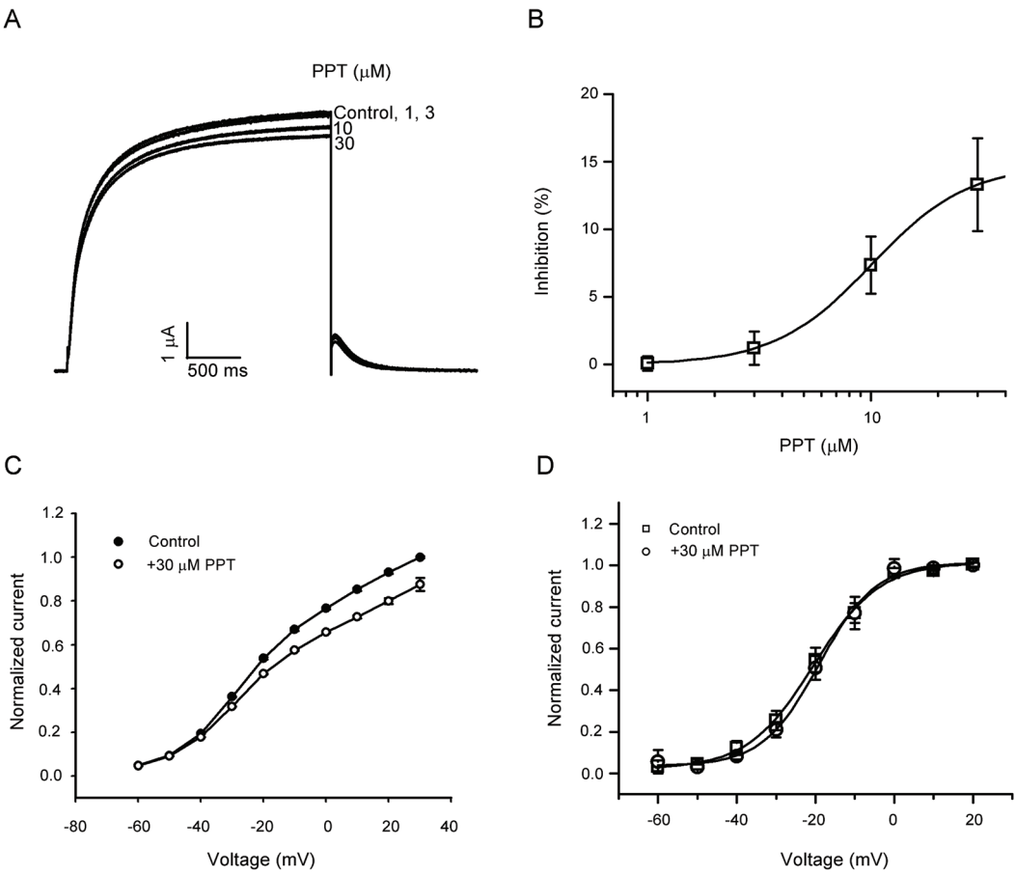



**Fig. 4.** Effects of protopanaxatriol (PPT) on KCNQ1 alone channel. (A) The representative traces on KCNQ1 alone channel current inhibition by different concentrations of PPT. Protocols were the same as described for Fig. 2. (B) Concentration-response curves of PPT on KCNQ1 alone channel currents. (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).

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

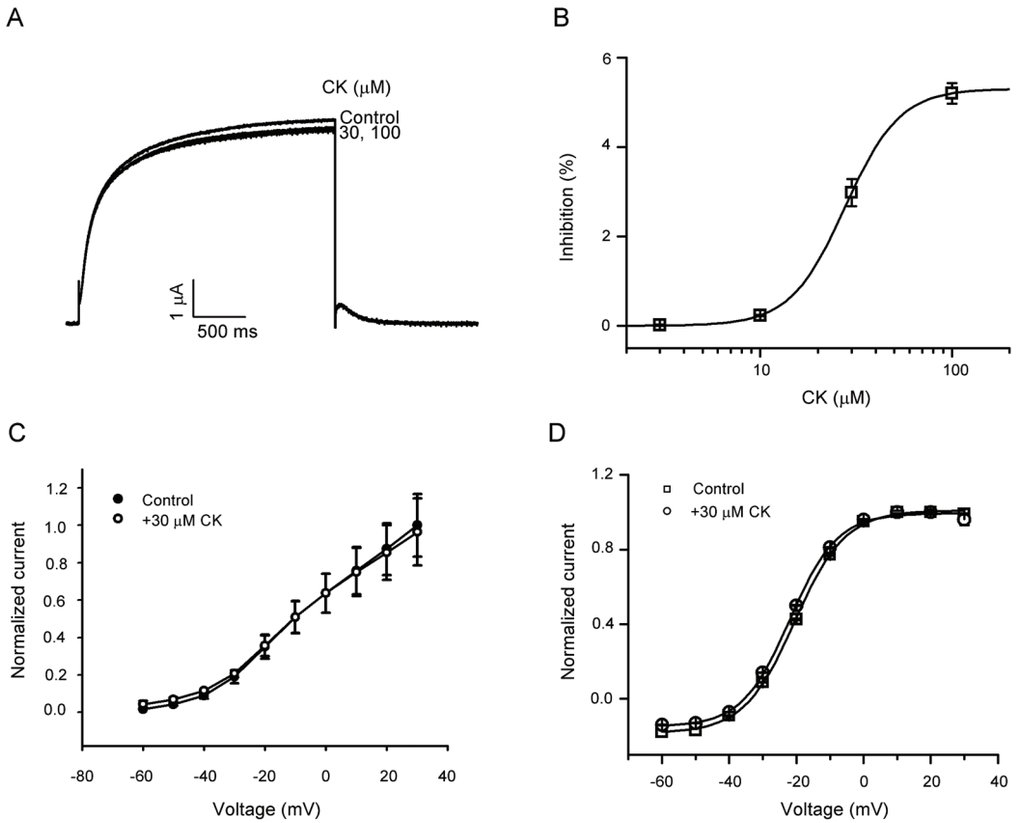



**Fig. 5.** Effects of compound K (CK) on KCNQ1 alone channel currents. (A) Representative current traces on KCNQ1 alone channel inhibition by different concentrations of CK. (B) Concentration-response curves of CK on KCNQ1 alone channel currents. (C) I-V relationships for KCNQ1 alone channel currents measurement at the end of the 3-second test pulse before and after application of 30 μM CK. (D) The steady-state activation curve for KCNQ1 alone channel currents by 30 μM CK. Protocols were the same as described for Fig. 2. Data are represented by the mean±SEM (*n*=6).

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 $I_{deactivating\text{-}tail}$ of the HERG $K^+$ channel [22]. CK induced a persistent $I_{deactivating\text{-}tail}$ and caused a leftward shift of steady-state, voltage-dependent activation. In contrast to CK, PPT caused an acceleration of $I_{deactivating\text{-}tail}$ 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 $K^+$ channel currents. 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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## REFERENCES

1. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 1996;12:17-23.
2. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. Nature 1996;384:78-80.
3. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature 1996;384:80-83.
4. Pusch M, Magrassi R, Wollnik B, Conti F. Activation and inactivation of homomeric KvLQT1 potassium channels. Biophys J 1998;75:785-792.
5. Seebohm G, Scherer CR, Busch AE, Lerche C. Identification of specific pore residues mediating KCNQ1 inactivation. A novel mechanism for long QT syndrome. J Biol Chem 2001;276:13600-13605.
6. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J, Faure S, Gary F, Coumel P, Petit C et al. A novel mutation in the potassium channel gene *KVLQT1* causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat Genet 1997;15:186-189.
7. Mall M, Bleich M, Schurlein M, Kuhr J, Seydewitz HH, Brandis M, Greger R, Kunzelmann K. Cholinergic ion secretion in human colon requires coactivation by cAMP. Am J Physiol 1998;275(6 Pt 1):G1274- G1281.
8. Grahammer F, Herling AW, Lang HJ, Schmitt-Graff A, Wittekindt OH, Nitschke R, Bleich M, Barhanin J, Warth R. The cardiac $K^+$ channel KCNQ1 is essential for gastric acid secretion. Gastroenterology 2001;120:1363-1371.
9. Snyders DJ. Structure and function of cardiac potassium channels. Cardiovasc Res 1999;42:377-390.
10. Cheng JH, Kodama I. Two components of delayed rectifier $K^+$ current in heart: molecular basis, functional diversity, and contribution to repolarization. Acta Pharmacol Sin 2004;25:137-145.
11. Sanguinetti MC. Dysfunction of delayed rectifier potassium channels in an inherited cardiac arrhythmia. Ann N Y Acad Sci 1999;868:406-413.
12. Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. Annu Rev Physiol 2002;64:431-475.
13. Tristani-Firouzi M, Sanguinetti MC. Structural determinants and biophysical properties of HERG and KCNQ1 channel gating. J Mol Cell Cardiol 2003;35:27-35.
14. Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. Pharmacol Ther 2001;90:1-19.
15. Nah SY. Ginseng: recent advances and trends. Korea J Ginseng Sci 1997;21:1-12.
16. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685-1693.
17. Chen X, Gillis CN, Moalli R. Vascular effects of ginsenosides *in vitro*. Br J Pharmacol 1984;82:485-491.
18. Chen X. Cardiovascular protection by ginsenosides and their nitric oxide releasing action. Clin Exp Pharmacol Physiol 1996;23:728-732.
19. Bai CX, Sunami A, Namiki T, Sawanobori T, Furukawa T. Electrophysiological effects of ginseng and ginsenoside Re in guinea pig ventricular myocytes. Eur J Pharmacol 2003;476:35-44.
20. Furukawa T, Bai CX, Kaihara A, Ozaki E, Kawano T, Nakaya Y, Awais M, Sato M, Umezawa Y, Kurokawa J. Ginsenoside Re, a main phytosterol of *Panax ginseng*, activates cardiac potassium channels via a nongenomic pathway of sex hormones. Mol Pharmacol 2006;70:1916-1924.
21. Choi SH, Shin TJ, Lee BH, Chu DH, Choe H, Pyo MK, Hwang SH, Kim BR, Lee SM, Lee JH et al. Ginsenoside Rg3 activates human KCNQ1 $K^+$ channel currents through interacting with the K318 and V319 residues: a role of KCNE1 subunit. Eur J Pharmacol 2010;637:138-147.
22. Choi SH, Shin TJ, Hwang SH, Lee BH, Kang J, Kim HC, Oh JW, Bae CS, Lee SH, Nah SY. Differential effects of ginsenoside metabolites on HERG $K^+$ channel currents. J Ginseng Res 2011;35:191-199.
23. Hasegawa H, Suzuki R, Nagaoka T, Tezuka Y, Kadota S, Saiki I. Prevention of growth and metastasis of murine melanoma through enhanced natural-killer cytotoxicity by fatty acid-conjugate of protopanaxatriol. Biol Pharm Bull 2002;25:861-866.
24. Akao T, Kanaoka M, Kobashi K. Appearance of compound K, a major metabolite of ginsenoside Rb1 by in-

testinal bacteria, in rat plasma after oral administration: measurement of compound K by enzyme immunoassay. Biol Pharm Bull 1998;21:245-249.
25. Bae EA, Park SY, Kim DH. Constitutive beta-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. Biol Pharm Bull 2000;23:1481-1485.
26. Tawab MA, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M. Degradation of ginsenosides in humans after oral administration. Drug Metab Dispos 2003;31:1065-1071.
27. Lee JH, Jeong SM, Kim JH, Lee BH, Yoon IS, Lee JH, Choi SH, Kim DH, Rhim H, Kim SS et al. Characteristics of ginsenoside Rg3-mediated brain $Na^+$ current inhibition. Mol Pharmacol 2005;68:1114-1126.
28. Wakabayashi C, Hasegawa H, Murata J, Saiki I. *In vivo* antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. Oncol Res 1997;9:411-417.
29. Wang CZ, Du GJ, Zhang Z, Wen XD, Calway T, Zhen Z, Musch MW, Bissonnette M, Chang EB, Yuan CS. Ginsenoside compound K, not Rb1, possesses potential chemopreventive activities in human colorectal cancer. Int J Oncol 2012;40:1970-1976.
30. Splawski I, Tristani-Firouzi M, Lehmann MH, Sanguinetti MC, Keating MT. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nat Genet 1997;17:338-340.
31. Seebohm G, Pusch M, Chen J, Sanguinetti MC. Pharmacological activation of normal and arrhythmia-associated mutant KCNQ1 potassium channels. Circ Res 2003;93:941-947.
32. Salata JJ, Jurkiewicz NK, Wang J, Evans BE, Orme HT, Sanguinetti MC. A novel benzodiazepine that activates cardiac slow delayed rectifier $K^+$ currents. Mol Pharmacol 1998;54:220-230.
33. Lee JH, Jeong SM, Kim JH, Lee BH, Yoon IS, Lee JH, Choi SH, Lee SM, Park YS, Lee JH et al. Effects of ginsenosides and their metabolites on voltage-dependent Ca(2+) channel subtypes. Mol Cells 2006;21:52-62.
34. Kim JH, Hong YH, Lee JH, Kim DH, Nam G, Jeong SM, Lee BH, Lee SM, Nah SY. A role for the carbohydrate portion of ginsenoside Rg3 in $Na^+$ channel inhibition. Mol Cells 2005;19:137-142.
35. Shin TJ, Hwang SH, Choi SH, Lee BH, Kang J, Kim HJ, Zukin RS, Rhim H, Nah SY. Effects of protopanaxatriol-ginsenoside metabolites on rat *N*-methyl-d-aspartic acid receptor-mediated ion currents. Korean J Physiol Pharmacol 2012;16:113-118.