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# Differential Effect of Bovine Serum Albumin on Ginsenoside Metabolite-Induced Inhibition of α3β4 Nicotinic Acetylcholine Receptor Expressed in *Xenopus* Oocytes

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Ginsenosides, major active ingredients of *Panax* ginseng, that exhibit various pharmacological and physiological actions are transformed into compound K (CK) or M4 by intestinal microorganisms. CK is a metabolite derived from protopanaxadiol (PD) ginsenosides, whereas M4 is a metabolite derived from protopanaxatriol (PT) ginsenosides. Recent reports shows that ginsenosides might play a role as pro-drugs for these metabolites. In present study, we investigated the effect of bovine serum albumin (BSA), which is one of major binding proteins on various neurotransmitters, hormones, and other pharmacological agents, on ginsenoside $Rg_2$-, CK-, or M4-induced regulation of α3β4 nicotinic acetylcholine (ACh) receptor channel activity expressed in *Xenopus* oocytes. In the absence of BSA, treatment of ACh elicited inward peak current ($I_{ACh}$) in oocytes expressing α3β4 nicotinic ACh receptor. Co-treatment of ginsenoside $Rg_2$, CK, or M4 with ACh inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptor with reversible and dose-dependent manner. In the presence of 1% BSA, treatment of ACh still elicited $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptor and co-treatment of ginsenoside $Rg_2$ or M4 but not CK with ACh inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptor with reversible and dose-dependent manner. These results show that BSA interferes the action of CK rather than M4 on the inhibitory effect of $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptor and further suggest that BSA exhibits a differential interaction on ginsenoside metabolites.

**Key words**: Ginsenosides, Ginsenoside metabolites, CK, M4, Bovine serum albumin, Nicotinic acetylcholine receptor-gated ion channels, *Xenopus* oocytes

## INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer, is well known in folk medicine as a tonic. Ginsenosides or ginseng saponins have been regarded as the principal components responsible for the various physiological and pharmacological actions of ginseng. Ginsenosides have a four-ring, steroid-like structure with sugar moieties attached, and about 30 different forms have been isolated and identified from the root of *Panax* ginseng. They are classified into protopanaxadiol and protopanaxatriol ginsenosides according to the position of carbohydrate components at carbon-3 and -6 (Attele *et al.*, 1999).

Recent reports showed that ginsenosides administered via oral route might pass into large intestine without decomposition by either gastric juice or digestive enzymes (Hasegawa *et al.*, 1996). By intestinal microorganisms, protopanaxadiol (PD) ginsenosides are metabolized into compound K (CK) with a glucose at C-20 position, whereas protopanaxatriol (PT) ginsenosides are metabolized into M4 leaving only backbone structures of ginsenosides without carbohydrate components (Fig. 1). These metabolites are absorbed into the blood in humans and rats (Kanaoka *et al.*, 1994; Karikura *et al.*, 1991). *In vitro* and *in vivo* studies, a line of evidences provides a possibility

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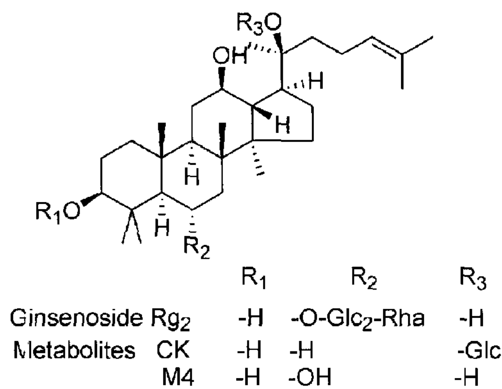


**Fig. 1.** *Structure of ginsenoside* $Rg_2$, *and ginsenoside metabolite CK, or M4. The chemical structure of CK is 20-S-Protopanaxadiol-20-O-β-D-glucopyranoside and M4 is 20-S-Protopanaxatriol. They differ at three side chains attached to the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; rhamnopyranoside, Rha, rhamnopyranoside, Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.*

that ginsenosides might play a role as pro-drugs for these metabolites, since ginsenoside metabolites also inhibit metastasis of caner cells and induce apoptosis of tumor cells (Wakabayashi *et al.*, 1997; Wakabayashi *et al.*, 1998 Hasegawa *et al.*, 2002).

On the other hand, it is well known that most of hydrophobic compounds like cholesterol or fatty acids bind serum proteins in biological fluid (Bojesen and Bojesen, 1994 Bojesen and Bojesen, 1996; Choi *et al.*, 2002a). Since ginsenoside metabolites, especially M4, are structurally similar to cholesterol and are also hydrophobic in chemical nature (Fig. 1), they might bind to plasma protein(s) in biological fluid as do ginsenoside $Rb_1$ and ginsenoside Rc (Kim *et al.*, 1983). However, it is not yet known whether or not serum protein might exert an effect on the pharmacological or physiological actions of ginsenosides or ginsenoside metabolites.

In this study, we examined the effect of bovine serum albumin (BSA), which is one of major plasma binding proteins in biological fluids, on ginsenoside $Rg_2$-, CK-, or M4-induced regulation of α3β4 nicotinic ACh receptor channel activity expressed in *Xenopus* oocytes. For this study, we injected neuronal bovine α3β4 nicotinic ACh receptor cRNAs into *Xenopus* oocytes and examined the effect of ginsenoside $Rg_2$, CK, or M4 on ACh-elicited inward peak currents ($I_{ACh}$) in the absence or presence of BSA. The reason we used this system was that: (1) *Xenopus laevis* oocytes have widely been used as a tool to express membrane proteins encoded by exogenously administered cDNAs or mRNAs including receptors, ion channels, and transporter (Dascal, 1987) and (2) nicotinic acetylcholine receptor channels expressed in *Xenopus* oocytes by injection of homomeric or heteromeric nicotinic acetylcholine receptor cDNAs or cRNAs subunits are well studied and characterized (Kullberg *et al.*, 1990; Sargent, 1993). We found that the presence of BSA inhibits the action of CK rather than M4 on the inhibitory effect on $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptor.

## MATERIALS AND METHODS

### Materials

Ginsenoside $Rg_2$ were kindly provided from Korea Ginseng and Tobacco Research Institute (Taejon, Korea). Fig. 1 shows the structures of ginsenoside $Rg_2$, CK, and M4. Ginsenoside $Rg_2$, CK, or M4 used in this study were dissolved in dimethyl sulfoxide (DMSO) and were diluted with bath medium before use. CK and M4 were prepared according to the procedure of Hasegawa *et al.* (1996). Final DMSO concentration was less than 0.01%. Fatty acid free BSA (Fraction V) and other chemical agents were obtained from Sigma (St. Louis, MO, USA).

### Oocyte preparation

*Xenopus laevis* care and handling were in accordance with the guide for the *Care and Use of Laboratory Animals* published by NIH, USA. Frogs were underwent surgery only twice, separated by at least 3 weeks. To isolate oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester. Oocytes were separated by treatment with collagenase, by gentle shaking for 2 h in $CaCl_2$-free medium containing 82.5 NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM sodium pyruvate, 100 units of penicillin per mL, and 100 μg streptomycin/mL. Only stage 5 or 6 oocytes were collected and maintained at 18°C with continuous gentle shaking in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) supplemented with 0.5 mM theophylline and 50 μg gentamycin/mL. All solutions were changed every day. All experiments were performed within 2-4 days following isolation of the oocytes (Choi *et al.*, 2002b).

### Oocyte recording

A single oocyte was placed in a small Plexiglas net chamber (0.5 mL) and was constantly superfused with ND96 medium in the absence or presence of ginsenoside $Rg_2$, CK or M4 during recording. The microelectrodes were filled with 3 M KCl and had a resistance of 0.2-0.7 MΩ. Two-electrode voltage-clamp recordings were performed at room temperature with Oocyte Clamp (OC-725C, Warner Instrument, Hamden, CT, USA) with Digidata 1200A. For most of the electrophysiological experiments, the oocytes were clamped at a holding potential of 80 mV and 300-ms voltage steps were applied from -100 to +40 mV in 20-mV increments for current and voltage relationship (Choi *et al.*, 2002b).

### cRNA preparation of α3β4 nicotinic acetylcholine receptor and microinjection

The cDNAs encoding bovine α3β4 nicotinic acetylcholine receptor were linearized with appropriate restriction enzyme. The cRNAs were transcribed from linearized templates with *in vitro* transcription kit (mMessage mMachine; Ambion, Austin, TX, USA) using a T7 polymerase. The cRNA was dissolved in RNase-free water at a final concentration of approximately 1 μg/μL and stored at -70°C until used. Oocytes were injected with $H_2O$ or bovine α3β4 nicotinic acetylcholine receptor cRNAs (5-10 ng) by using a Nanoject Automatic Oocyte Injector (Drummond Scientific, Broomall, PA, USA). The injection pipette was pulled from glass capillary tubing used for recording electrodes and the tip was broken to ~20-mm-OD (Choi *et al.*, 2002b).

### Data analysis

All values are presented as means ± S.E.M. The differences between means of control, ginsenoside $Rg_2$, CK and M4 treatment data were analyzed using unpaired Students *t*-test. A value of $P<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

### Effect of ginsenoside $Rg_2$ on $I_{ACh}$ in *Xenopus* oocytes expressing α3β4 nicotinic acetylcholine receptors in the absence or presence of BSA

In the absence of BSA, the addition of acetylchoine to the bathing solution induced a large inward current in oocytes injected with α3β4 nicotinic ACh receptor, indicating that this nicotinic ACh receptors were functionally expressed in this system (Fig. 2A). Ginsenoside $Rg_2$ itself had no effect in oocytes expressing α3β4 nicotinic acetylcholine receptors at a holding potential of -80 mV (data not shown). But co-treatment with ginsenoside $Rg_2$ and acetylcholine inhibited $I_{ACh}$ in oocytes expressing α3β4 with dose-dependent manner by 5.9 ± 5.1, 51.2 ± 6.2, and 56.7 ± 4.5% at 10, 30, and 100 μM, respectively (Fig. 2A and 2C *left panel*, n = 9-12 from three different frogs; $^{*}P<0.05$, $^{**}P<0.01$). The inhibition of $I_{ACh}$ by ginsenoside $Rg_2$ in oocytes expressing α3β4 nicotinic acetylcholine receptors was reversible (Fig. 2A). Thus, these results are well consistent with the previous reports that ginsenosides regulate nicotinic ACh receptors (Choi *et al.*, 2002b; Sala *et al.*, 2002). In the presence of 1% BSA, the addition of ACh to the bathing solution still induced a large $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptors, indicating that BSA did not interfere ACh action for α3β4 nicotinic ACh receptor activation. However, the presence of 1% BSA greatly attenuated the inhibitory effect of ginsenoside $Rg_2$ on $I_{ACh}$. Thus, only 100 μM ginsenoside $Rg_2$ significantly inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors (Fig. 2B and 2C, *right panel*).

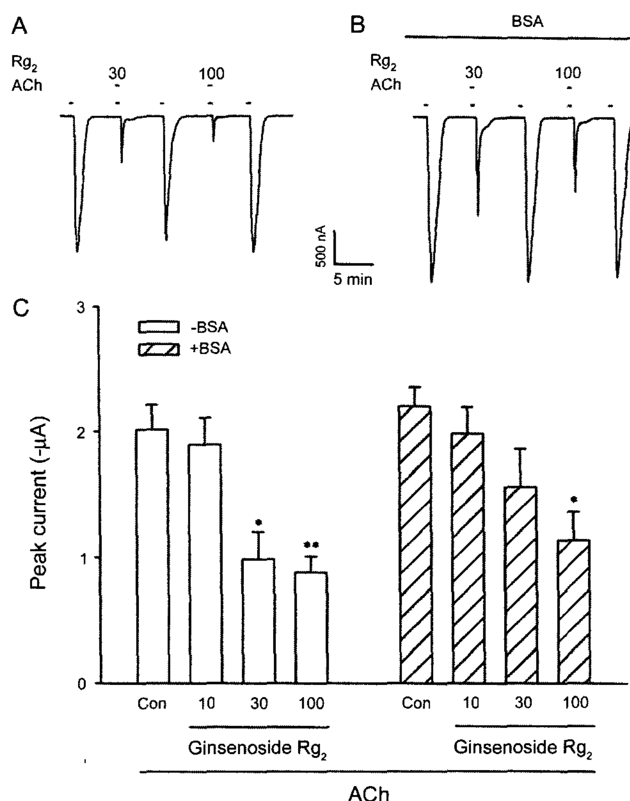


**Fig. 2.** Effect of ginsenoside $Rg_2$ on $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptor in the absence or presence of bovine serum albumin (BSA). (A) Acetylcholine (100 μM, ACh) was first applied and then same concentration of acetylcholine was co-applied with ginsenoside $Rg_2$ of indicated concentration (μM). Thus, co-application of ginsenoside $Rg_2$ with acetylcholine inhibited $I_{ACh}$. (B) In the presence of 1% BSA, acetylcholine (100 μM) first was applied and then ginsenoside $Rg_2$ and acetylcholine were co-applied. In the presence of BSA, the inhibitory effect of ginsenoside $Rg_2$ on $I_{ACh}$ was greatly diminished. (C) Summary of ginsenoside $Rg_2$-induced inhibition of $I_{ACh}$ in the absence or presence of 1% BSA. Each point represents the mean ± S.E.M. (n = 9-12/group). The resting membrane potential of oocytes was about -30 to -35 mV and oocytes were voltage-clamped at a holding potential of -80 mV prior to drug application. Tracings are representative of nine to ten separate oocytes from three different frogs. $^{*}P<0.05$, $^{**}P<0.01$ compared with acetylcholine treatment alone in the absence or presence of 1% BSA.

### Effect of CK on $I_{ACh}$ in *Xenopus* oocytes expressing α3β4 nicotinic acetylcholine receptors in the absence or presence of BSA

CK itself had no effect in oocytes expressing α3β4 nicotinic ACh receptors at a holding potential of -80 mV as did ginsenoside $Rg_2$ (data not shown). But co-treatment with CK and ACh inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptors with dose-dependent manner by 16.1 ± 6.5, 58.6 ± 6.2, and 61.7 ± 6.3% at 10, 30, and 100 μM, respectively (Fig. 3A and 3C *left panel*, n = 18

from three different frogs; *$P$< 0.05, *$P$< 0.01). The inhibition of $I_{ACh}$ by CK in oocytes expressing α3β4 nicotinic acetylcholine receptors was also reversible (Fig. 3A). Thus, these results indicate that ginsenoside metabolite CK derived from protopanaxadiol ginsenosides still maintains the regulatory effect on nicotinic ACh receptor channel activity. However, we could not observe the significant inhibitory effect of CK on $I_{ACh}$ in the presence of 1% BSA in the range of concentrations tested (Fig. 3B and 3C, *right panel*).

### Effect of M4 on $I_{ACh}$ in *Xenopus* oocytes expressing α3β4 nicotinic acetylcholine receptors in the absence or presence of BSA

M4 itself also had no effect in oocytes expressing α3β4 nicotinic ACh receptors at a -80 mV holding potential as did ginsenoside $Rg_2$ and CK (data not shown). But co-treatment with M4 and ACh inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptors with dose-dependent manner by 35.8 ± 7.1, 56.6 ± 4.2, and 72.4 ± 4.7% at 10, 30, and 100 μM, respectively (Fig. 4B and 4C *left panel*, n = 15 from three different frogs; *$P$< 0.05, **$P$< 0.01). Thus, the inhibition of $I_{ACh}$ by M4 in oocytes expressing α3β4 nicotinic acetylcholine receptors was also dose-dependent and reversible (Fig. 4A and 4C). These results indicate that protopanaxatriol ginsenoside metabolite, M4, still maintains the regulatory effect on nicotinic ACh receptor channel activity. Interestingly, in contrast to CK, we could still observe the inhibitory effect of M4 on $I_{ACh}$ in

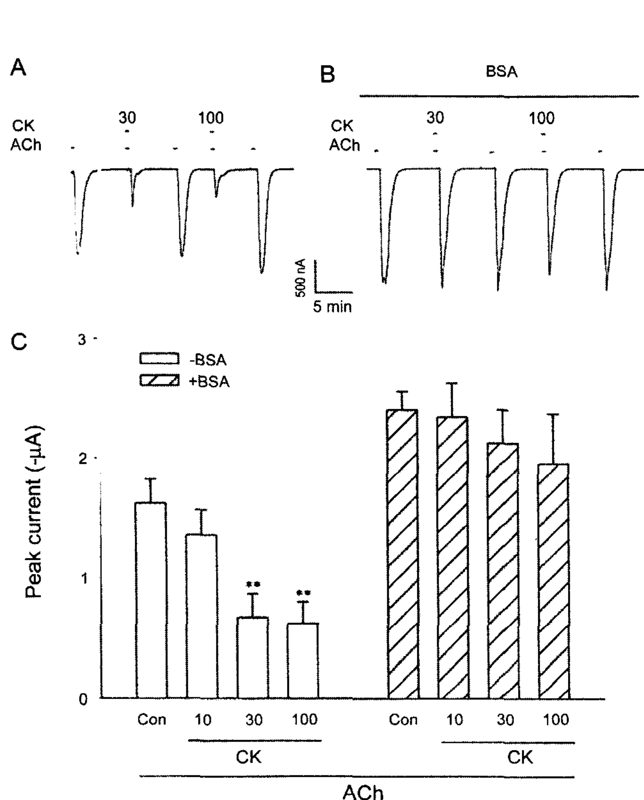


**Fig. 3.** Effect of CK on $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptor in the absence or presence of bovine serum albumin (BSA). (A) Acetylcholine (100 μM, ACh) was first applied and then same concentration of acetylcholine was co-applied with CK of indicated concentration (μM). Thus, co-application of CK with acetylcholine inhibited $I_{ACh}$. (B) In the presence of 1% BSA, acetylcholine (100 μM) first was applied and then CK and acetylcholine were co-applied. In the presence of BSA, CK had no effect on $I_{ACh}$. (C) Summary of CK-induced inhibition of $I_{ACh}$ in the absence or presence of 1% BSA. Each point represents the mean ± S.E.M. (n = 18/group). The resting membrane potential of oocytes was about -30 to -35 mV and oocytes were voltage-clamped at a holding potential of -80 mV prior to drug application. Tracings are representative of nine separate oocytes from three different frogs. *P< 0.05, *$P$< 0.01 compared with acetylcholine treatment alone in the absence of 1% BSA.

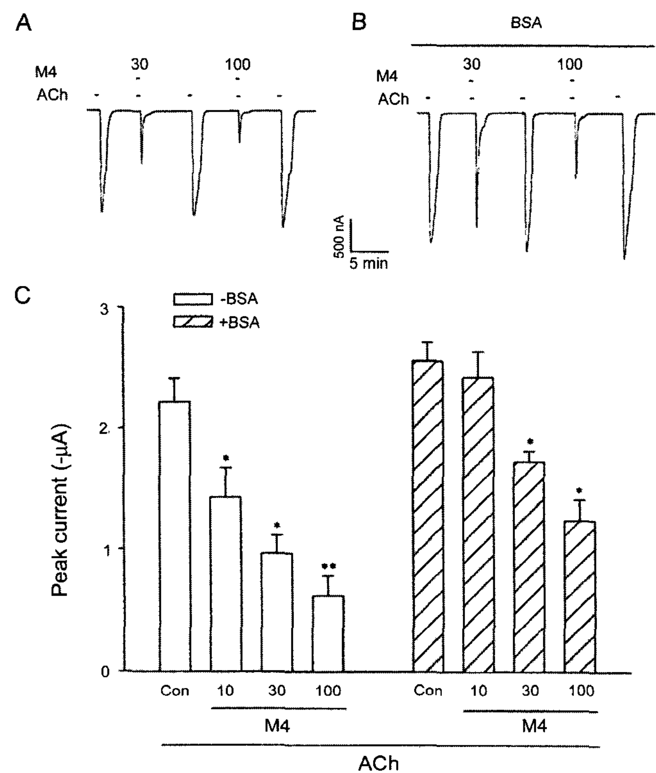


**Fig. 4.** Effect of M4 on $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptor in the absence or presence of bovine serum albumin (BSA). (A) Acetylcholine (100 μM, ACh) was first applied and then same concentration of acetylcholine was co-applied with M4 of indicated concentration (mM). Thus, co-application of M4 with acetylcholine inhibited $I_{ACh}$. (B) In the presence of 1% BSA, acetylcholine (100 μM) first was applied and then M4 and acetylcholine were co-applied. In the presence of BSA, the inhibitory effect of M4 on $I_{ACh}$ was slightly diminished. (C) Summary of different concentration of M4-induced inhibition of $I_{ACh}$ in the absence or presence of 1% BSA. Each point represents the mean ±S .E.M. (n = **15**/group). The resting membrane potential of oocytes was about -35 mV and oocytes were voltage-clamped at a holding potential of -80 mV prior to drug application. Tracings are representative of nine separate oocytes from three different frogs. *$P$< 0.05 or **$P$< 0.01 compared with acetylcholine treatment alone in the absence or presence of 1% BSA.

the presence of 1% BSA in the range of 30 and 100 μM M4 (Fig. 4B and 4C, *right panel*).

In the present study, we demonstrated that (1) CK and M4 derived from both respective protopanaxadiol and protopanaxatriol ginsenosides as well as ginsenoside $Rg_2$ inhibited $I_{ACh}$ in a reversible and dose-dependent manner in oocytes expressing α3β4 nicotinic ACh receptors; (2) the potency of order for the inhibition of $I_{ACh}$ was M4 > CK > ginsenoside $Rg_2$ at 100 μM in the absence of BSA; (3) in the presence of 1% BSA, CK induced-inhibition of $I_{ACh}$ was abolished, whereas ginsenoside $Rg_2$ (100 μM) and M4 (30 and 100 μM) induced-inhibition of $I_{ACh}$ was still maintained.

From the present results, however, it is unclear precisely why BSA abolished the effect of only CK but not M4 on the inhibitory effect of $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors. One possibility is that BSA may bind and coat cell membrane to interfere the binding of CK. However, it may be not the case, since ACh or ginsenoside $Rg_2$ and M4 except CK still induce $I_{ACh}$ or exhibit the inhibitory effect on $I_{ACh}$ in oocytes expressing α3β4 nicotinic ACh receptors (Figs. 2 and 4).

The other possibility may be derived from the chemical structural difference among ginsenoside $Rg_2$, CK, and M4 and BSA might differentially interact with them. The difference of chemical structure between CK and M4 is that CK has a glucose at the C-20 position and that M4 has a hydroxyl group attached to C-6 position (Fig. 1). The chemical difference between ginsenoside $Rg_2$ and M4 is that ginsenoside $Rg_2$ has rhamnose and glucose at the C-6 position (Fig. 1). The carbohydrate components attached to C-6 position of ginsenoside $Rg_2$ might not display an important role in the interaction between ginsenoside $Rg_2$ and BSA, since at 100 μM ginsenoside $Rg_2$ still inhibited $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors. In contrast, it seems, rather, that a glucose attached at C-20 position of CK might play an important role in binding or interacting with BSA rather than the hydroxyl group attached to C-6 position of M4, since BSA abolished CK effect on $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors. However, it might require further investigations on binding site(s) or affinity of CK to BSA for confirmation.

On other hand, it is known that plasma proteins play important roles. As a carrier role, they bind and transport various hydrophobic endogenous and exogenous biologically active compounds to target organs to allow various cellular events. The other is that they might bind with biologically active agents and attenuate their effects by reducing free active forms in plasma. In plasma, the concentration of albumin is approximately 640 μM and 630 μM in rat and human, respectively (Habgood *et al.*, 1992; Peters, 1996). If molecular weight of BSA is assumed to be 67000 Da, the concentration of BSA used in present study is about one-fourth of plasma concentration of albumin. In fact, we have chosen 1% BSA to investigate the effect of BSA on ginsenoside $Rg_2$-, CK-, or M4-induced regulation on $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors, since at higher concentration of BSA than 1% we could observe some fluctuations of $I_{ACh}$ in oocytes expressing α3β4 nicotinic acetylcholine receptors batch by batch of oocytes and it was hard to estimate the exact effect of ginsenoside metabolites in the presence of BSA (data not shown). These results also suggest, furthermore, that a higher concentration of ginsenoside $Rg_2$ or M4 in plasma might be required than those used in present study for their biological activity.

The previous *in vitro* reports have shown that ginsenosides inhibit voltage-dependent $Ca^{2+}$ channels in rat sensory neurons and rat chromaffin cells (Nah and McCleskey, 1994; Nah *et al.*, 1995; Kim *et al.*, 1998). Several individual ginsenosides also inhibit or enhance ligand-gated ion channel activity (Abe *et al.*, 1994; Seong *et al.*, 1995; Tachikawa *et al.*, 1995; Kim *et al.*, 1998; Kudo *et al.*, 1998; Kim *et al.*, 2002; Choi *et al.*, 2002b; Sala *et al.*, 2002; Choi *et al.*, 2003a; Noh *et al.*, 2003; Choi *et el.*, 2003b). Since most of those data obtained from above experiments using ginseng total saponins or individual ginsenosides were performed in the absence of plasma proteins, it is worth while to consider the influence of plasma protein(s) in biological fluid in estimating various pharmacological and physiological effect of ginseng or ginsenosides.

In summary, we found that in the absence of BSA ginsenoside $Rg_2$, CK and M4 showed the inhibition of $I_{ACh}$ in oocytes expressing bovine neuronal α3β4 nicotinic acetylcholine receptors but in the presence of 1% BSA ginsenoside $Rg_2$ and M4 but not CK exhibited an inhibition of $I_{ACh}$ in oocytes expressing bovine neuronal α3β4 nicotinic acetylcholine receptors. This result suggests that BSA exhibits a differential interaction on ginsenoside metabolites.

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## REFERENCES

Abe, K., Cho, S. I., Kitagawa, I., Nishiyama, N., and Saito, H., Differential effects of ginsenoside $Rb_1$ and malonylginsenoside $Rb_2$ on long-term potentiation in the dentate gyrus of rat. *Brain Res.*, 649, 7-11 (1994).

Attele, A. S., Wu, J. A., and Yuan, C. S., Ginseng pharmacology: Multiple constituents and multiple actions. *Biochem. Pharmacol.*, 58, 1685-1693 (1999).

Bojesen, I. N. and Bojesen, E., Binding of arachidonate and oleate to bovine serum albumin. *J. Lipid Res.*, 35, 770-778 (1994).

Bojesen, I. N. and Bojesen, E., Albumin binding of long-chain fatty acids: Thermodynamics and kinetics. *J. Phys. Chem.*, 100 17981-17985 (1996).

Choi, J. K, Ho, J., Curry, S., Qin, D. H., Bittman, R., and Hamilton, J. A., Interactions of very long-chain saturated fatty acids with serum albumin. *J. Lipid Res.*, 43, 1000-1010 (2002a).

Choi, S., Jung, S.Y., Kim, C.H., Kim, H.S., Rhim, H., Kim, S. C., and Nah, S. Y., Effect of ginsenosides on voltage-dependent $Ca^{2+}$ channel subtypes in bovine chromaffin cells. *J. Ethnopharmacol.,* 74, 75-81 (2001).

Choi, S. Jung, S. Y., Lee, J. H., Sala, F., Criado, M., Mulet, J., Valor, L. M., Sala, S., Engel, A. G., and Nah, S. Y., Effects of ginsenosides, active components of ginseng, on nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *Eur. J. Pharmacol.*, 442, 37-42 (2002b).

Choi, S., Lee, J. H., Oh, S., Rhim, H., Lee, S. M., and Nah, S. Y., Effects of ginsenoside $Rg_2$ on the 5-$HT_{3A}$ receptor-mediated ion current in *Xenopus* oocytes. *Mol. Cells*, 15, 108-113 (2003a).

Choi, S. E., Choi, S., Lee, J. H., Whiting, P. J., Lee, S. M., and Nah, S. Y., Effects of ginsenosides on $GABA_A$ receptor channels expressed in *Xenopus* oocytes. *Arch. Pharm. Res.*, 26, 28-33 (2003b).

Dascal, N., The use of *Xenopus* oocytes for the study of ion channels. *CRC Critical Rev. Biochem.,* 22, 317-387 (1987).

Habgood, M. D., Sedgwick, J. E. C., Dziegielewska, K. M., and Saunders, N. R., A developmentally regulated blood-cerebrospinal fluid transfer mechanism for albumin in immature rats. *J. Physiol (Lond.).*, 456, 181-192 (1992).

Hasegawa, H., J., Sung J. H., Matsumiya, S., and Uchiyama, M., Main ginseng saponin metabolites formed by intestinal bacteria. *Planta Med.*, 62, 453-457 (1996).

Hasegawa, H., Suzuki, R., Nagaoka, T., Tezuka, Y., and Kadota, S. and Saiki, I., Prevention of growth and metastasis of murine melanoma through enhanced natural-killer cytotoxicity by fatty acid-conjugate of protopanaxatriol. *Biol. Pharm. Bull.*, 25, 861-866 (2002).

Kanaoka, M., Akao, T., and Kobashi, K., Metabolism of ginseng saponins, ginsenosides by human intestinal flora. *J. Trad. Med.*, 11, 241-245 (1994).

Karikura, M., Miyase, T., Tanizawa, H., Taniyawa, T., and Takino, Y. Studies on absorption, distribution, excretion and metabolism of ginsenog saponins. VII. Comparison of the decomposition modes of ginsenoside $Rb_1$ and $Rb_2$ in the digestive tract of rats. *Chem. Pharm. Bull.*, 39, 400-404 (1991).

Kim, C. K., Ahn, H. Y., Han, B. H., and Hong, S. K., Drug-biomacromolecule interaction V: Binding of ginsenosides to human and bovine serum albumins by fluorescence probe technique. *Arch. Pharm. Res.*, 6, 63-68 (1983).

Kim, H. S., Lee, J. H., Goo, Y. S., and Nah, S. Y., Effects of ginsenosides on Ca channels and membrane capacitance in rat adrenal chromaffin cells. *Brain Res. Bull.*, 46, 245-251 (1998).

Kim, S., Ahn, K., Oh, T. H., Nah, S. Y., and Rhim, H., Inhibitory effect of ginsenosides on NMDA receptor-mediated signals in rat hippocampal neurons, *Biochem. Biophysical. Res. Comm.*, 296, 247-254 (2002).

Kudo. K., Tachikawa, E., Kashimoto, T., and Takahashi, E., Properties of ginseng saponin inhibition of catecholamine secretion in bovine chromaffin cells. *Eur. J. Pharmacol.,* 341, 139-144 (1998).

Kullberg, R., Owens, J. L., Camacho, P., Mandel, G., and Brehm, P., Multiple conductance classes of mouse nicotinic acetylcholine receptors expressed in *Xenopus* oocytes, *Proc. Natl. Acad. Sci., USA,* 87, 2067-2071 (1990).

Nah, S. Y. and McCleskey, E. W., Ginseng root extract inhibits calcium channels in rat sensory neurons through a similar path, but different receptor, as μ-type opioids. *J. Ethnopharmacol.*, 42, 45-51 (1994).

Nah, S. Y., Park, H. J., and McCleskey, E. W., A trace component of ginseng that inhibits $Ca^{2+}$ channels through a pertussis toxin-sensitive G protein. *Proc. Natl. Acad. Sci., USA,* 92, 8739-8743 (1995).

Noh, J. H., Choi, S., Lee, J. H., Betz, H., Kim, J. I., Park, C. S. Lee, S. M., and Nah, S. Y., Effects of ginsenosides on glycine receptor a1 channels expressed in *Xenopus* oocytes. *Mol. Cells,* 15, 34-39 (2003).

Peters, T. Jr., All about Albumin Biochemistry, *Genetics and Medical applications.* San Diego: Academic Press, 256-260 (1996).

Sala, F., Mulet, J., Choi, S., Jung, S. Y., Nah, S. Y., Rhim, H., Valor, L. M., Criado, M., and Sala, S., Effects of ginsenoside $Rg_2$ on human neuronal nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.*, 301, 1052-1059 (2002).

Sargent, P. B., The diversity of neuronal nicotinic acetylcholine receptors. *Annu. Rev. Neurosci.,* 16, 403-443 (1993).

Seong, Y. H., Shin, C. H., Kim, H. S., and Baba A., Inhibitory effect of ginseng total saponins on glutamate-induced swelling of cultured astrocytes. *Biol. Pharm. Bull.*, 18, 1776-1778 (1995).

Tachikawa, E., Kudo, K., Kashimoto, T., and Takashshi, E., Ginseng saponins reduce acetylcholine-evoked $Na^+$ influx and catecholamine secretion in bovine adrenal chromaffin cells. *J. Pharm. Exp. Ther.,* 273, 629-636 (1995).

Wakabayashi, C., Hasegawa, H., Murata, J., and Saiki, I., *In vivo* antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. *Oncology Res.*, 9, 411-417 (1997).

Wakabayashi, C., Murakami, K., Hasegawa, H., Murata, J., and Saiki, I., An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biochem. Biophys. Res. Commun.*, 249, 725-730 (1998).