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Effects of ginsenosides, active components of ginseng, on nicotinic acetylcholine receptors expressed in *Xenopus* oocytes

Seok Choi [a], Se-Yeon Jung [a], Jun-Ho Lee [a], Francisco Sala [b], Manuel Criado [b], Jose Mulet [a], Luis Miguel Valor [b], Salvador Sala [b], Andrew G. Engel [c], Seung-Yeol Nah [a,*]

[a] *National Research Laboratory for the Study of Ginseng Signal Transduction and Department of Physiology, College of Veterinary Medicine, Chonnam National University, Kwangju 500-757, South Korea*

[b] *Instituto de Neurociencias, Universidad Miguel Hernandez, San Juan de Alicante, Spain*

[c] *Department of Neurology Mayo Clinic, Rochester, USA*

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Abstract

We investigated the effects of ginsenosides, the active ingredient of ginseng, on neuronal or muscle-type nicotinic acetylcholine receptor channel activity expressed in *Xenopus* oocytes after injection of cRNA encoding bovine neuronal α3β4, α7 or human muscle αβδε subunits. Treatment with acetylcholine elicited an inward peak current (I_{ACh}) in oocytes expressing nicotinic acetylcholine receptor subtypes. Cotreatment with ginsenoside Rg_2 and acetylcholine inhibited I_{ACh} in oocytes expressing with α3β4 or αβδε but not in oocytes expressing α7 nicotinic acetylcholine receptors. The inhibition of I_{ACh} by ginsenoside Rg_2 was reversible and dose-dependent. The half-inhibitory concentrations (IC_{50}) of ginsenoside Rg_2 were 60.2 ± 14.1 and 15.7 ± 3.5 μM in oocytes expressing α3β4 and αβδε nicotinic acetylcholine receptors, respectively. The inhibition of I_{ACh} by ginsenoside Rg_2 was voltage-independent and noncompetitive. Other ginsenosides besides ginsenoside Rg_2 also inhibited I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors. The order of potency for the inhibition of I_{ACh} was ginsenoside Rg_2 > Rf > Re > Rg_1 > Rc > Rb_2 > Rb_1 in oocytes expressing α3β4 nicotinic acetylcholine receptors and was ginsenoside Rg_2 > Rf > Rg_1 > Re > Rb_1 > Rc > Rb_2 in oocytes expressing αβδε nicotinic acetylcholine receptors. These results indicate that ginsenosides might regulate nicotinic acetylcholine receptors in a differential manner and this regulation might be one of the pharmacological actions of *Panax* ginseng.

Keywords: Ginseng; Ginsenoside; Nicotinic acetylcholine receptor channel; *Xenopus* oocyte

1. Introduction

Ginseng, the root of *Panax* ginseng C.A. Meyer, is well known in folk medicine as a tonic and restorative, promoting health and longevity. The main molecular components responsible for the actions of ginseng are ginsenosides, which are also known as ginseng saponins. 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 sugar moieties at carbon-3 and -6 (Nah, 1997).

Ginsenosides have been shown to exert various effects on diverse living cells and tissues (Nah, 1997). For example, they increase the intracellular Ca^{2+} concentration in macrophages, NIH3T3 and endothelial cells (Shin et al., 1996; Hong et al., 1998; Li et al., 2000). Also, ginsenosides inhibit high-threshold voltage-gated Ca^{2+} channels in chromaffin cells (Kim et al., 1998; Choi et al., 2001a,b) and sensory neurons (Nah and McCleskey, 1994; Nah et al., 1995) and activate Ca^{2+}-activated K^+ channels in vascular smooth muscle cells (Li et al., 2001).

Recent reports showed that ginsenosides attenuate nicotine-induced dopamine release in the brains of freely moving rats and also inhibit nicotine-induced hyperactivity, reverse tolerance and dopamine receptor supersensitivity (Kim et al., 1999; Kim and Kim, 1999; Shim et al., 2000). In the periphery, in cells expressing nicotinic acetylcholine receptors, such as bovine chromaffin cells, ginsenosides also

* Corresponding author. Tel.: +82-62-530-2832; fax: +82-62-530-2832.
E-mail address: synah@chonnam.ac.kr (S.-Y. Nah).

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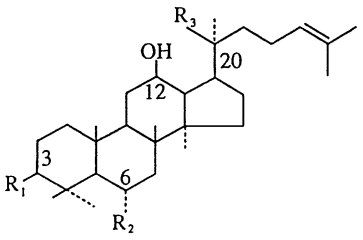
inhibit acetylcholine-stimulated catecholamine release (Tachikawa et al., 1995; Kudo et al., 1998; Tachikawa et al., 1999). Presently, it is unknown how ginsenosides attenuate nicotine-induced behavioral changes and how ginsenosides inhibit acetylcholine-induced catecholamine release at central or peripheral sites. Thus, the precise mechanism underlying the antinicotinic effect of ginsenosides remains to be elucidated.

In this study, we examined whether ginsenosides exerted effects on the nicotinic acetylcholine receptor channel activity of several nicotinic acetylcholine receptor subtypes and whether these effects were mediated through competition with acetylcholine binding sites. For this study, we injected neuronal bovine $\alpha3\beta4$, $\alpha7$ or human muscle $\alpha\beta\delta\varepsilon$ nicotinic acetylcholine receptor subunit cRNAs into *Xenopus* oocytes and examined the effect of ginsenosides on acetylcholine-elicited inward peak currents (I_{ACh}). 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).

2. Materials and methods

2.1. Materials

Fig. 1 shows the chemical structure of representative ginsenosides, such as Rb_1, Rb_2, Rc, Re, Rf, Rg_1 and Rg_2.



Ginsenosides	R_1	R_2	R_3
Ginsenoside-Rb_1	-O-Glc^2-Glc	-H	-O-Glc^6-Glc
Ginsenoside-Rc	-O-Glc^2-Glc	-H	-O-Glc^6-Ara (pyr)
Ginsenoside-Re	-OH	-O-Glc^2-Rha	-O-Glc
Ginsenoside-Rf	-OH	-O-Glc^2-Glc	-OH
Ginsenoside-Rg_1	-OH	-O-Glc	-O-Glc

Fig. 1. Structure of the five representative ginsenosides. They differ at three side chains attached to the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara(pyr), arabinopyranoside; Rha, rhamnopyranoside, Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

They were obtained from Korea Ginseng and Tobacco Research Institute (Taejon, Korea). Ginsenosides used in this study were dissolved in dimethyl sulfoxide (DMSO) and were diluted with bath medium before use. Final DMSO concentration was less than 0.05%. Other chemical agents were obtained from Sigma (St. Louis, MO).

2.2. Collection of Xenopus oocytes

X. laevis care and handling were in accordance with the guide for the Care and Use of Laboratory Animals published by NIH, USA, and with the highest standards of institutional guidelines, ROK (Republic of Korea). 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 U of penicillin/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.

2.3. Recording of acetylcholine-induced inward currents

A single oocyte was placed in a small Plexiglass net chamber (0.5 ml) and was constantly superfused with ND96 medium in the absence or presence of acetylcholine or ginsenosides 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) with Digidata 1200A. For most of the electrophysiological experiments, the oocytes were clamped at a holding potential of −80 mV and 400-ms voltage steps were applied from −100 to +60 mV in 20-mV increments for current and voltage relationship. Linear leak and capacitance currents were corrected by means of the leak subtraction procedure.

2.4. In vitro synthesis of RNA

Recombinant plasmids containing $\alpha3\beta4$, $\alpha7$ or $\alpha\beta\delta\varepsilon$ cDNA insert were linearized by digestion with appropriate restriction enzymes. The cRNAs from linearized templates were obtained by using an in vitro transcription kit (mMessage mMachine; Ambion, Austin, TX) with a SP6 RNA, T3 or T7 polymerase. The RNA was dissolved in RNase-free water at 1 μg/μl, divided into aliquots and stored at −70 °C until used.

2.5. Oocyte injection

Oocytes were injected with H_2O or various nicotinic acetylcholine receptor cRNAs (5–20 ng) by using a Nanoject Automatic Oocyte Injector (Drummond Scientific, Broomall, PA). The injection pipette was pulled from glass capillary tubing used for recording electrodes and the tip was broken to ≈ 20-μm OD.

2.6. Data analysis

All values are presented as means ± S.E.M. The differences between means of control and ginsenoside(s) cotreatment data were analyzed using unpaired Student's *t* test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4, α7 or muscle αβδε nicotinic acetylcholine receptors

The addition of acetylcholine to the bathing solution induced a large inward current in oocytes injected with α3β4, α7 or αβδε nicotinic acetylcholine receptor subtypes, indicating that these nicotinic acetylcholine receptors were functionally expressed in this system (Fig. 2). Ginsenoside Rg_2 itself had no effect in oocytes expressing these nicotinic acetylcholine receptors at a holding potential of −80 mV (data not shown). But cotreatment with ginsenoside Rg_2 and acetylcholine inhibited I_{ACh} in oocytes expressing α3β4 or αβδε but not in oocytes expressing homomeric α7 nicotinic acetylcholine receptors (Fig. 2A, B and C; $n = 6$ from three different frogs). The inhibition of I_{ACh} by ginsenoside Rg_2 in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors was reversible with a slight desensitization (Fig. 2A and B). Other ginsenosides besides ginsenoside Rg_2 did not affect I_{ACh} in oocytes expressing with homomeric α7 nicotinic acetylcholine receptors ($n = 6$ from three different frogs) (data not shown). Thus, these results suggest the possibility that Rg_2 regulates nicotinic acetylcholine receptors in a different manner.

3.2. Dose-dependent effect of ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors

In dose-dependent experiments with ginsenoside Rg_2, cotreatment with ginsenoside Rg_2 and acetylcholine inhibited I_{ACh} in a dose-dependent manner in oocytes expressing both α3β4 and αβδε nicotinic acetylcholine receptors (Fig. 3A and B). The IC_{50} of I_{ACh} were 60.2 ± 14.1 and 15.7 ± 3.5 μM in oocytes expressing α3β4 and αβδε nicotinic acetylcholine receptors, respectively ($n = 9–12$ from three different frogs) (Fig. 3C).

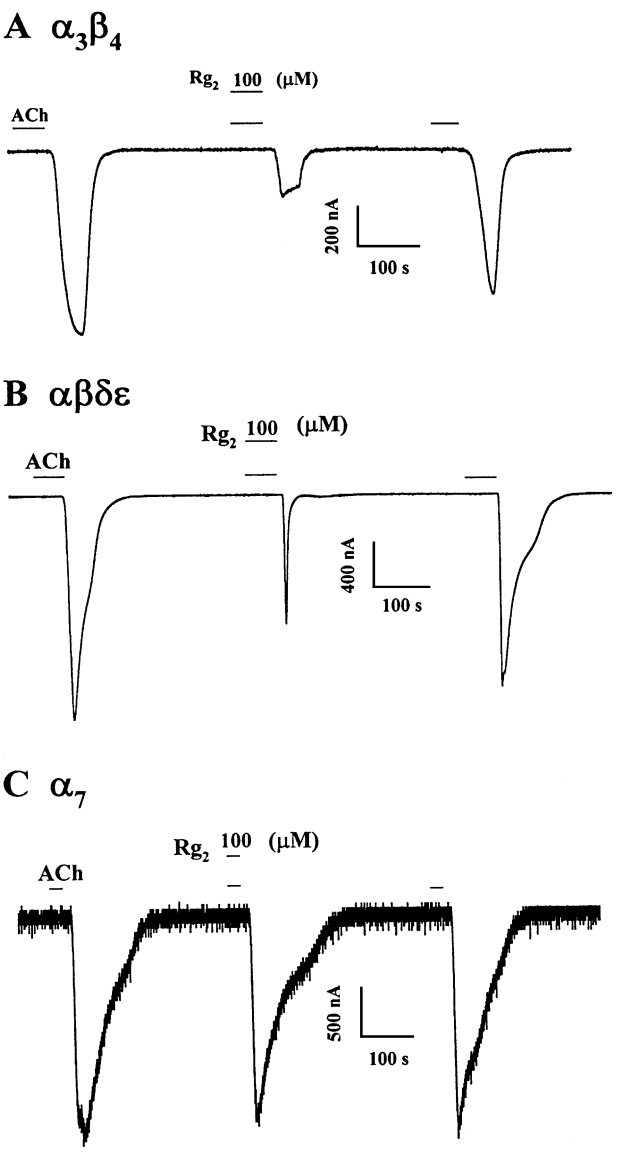


Fig. 2. Effect of ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4, α7 or αβδε nicotinic acetylcholine receptors. (A) Acetylcholine (10 μM) was first applied and then acetylcholine was coapplied with ginsenoside Rg_2. Thus, coapplication of ginsenoside Rg_2 with acetylcholine inhibited I_{ACh}. (B) Acetylcholine (100 μM) first was applied and then acetylcholine and ginsenoside Rg_2 were coapplied. Coapplication of ginsenoside Rg_2 with acetylcholine had no effect on I_{ACh}. (C) Acetylcholine (100 μM) was first applied and then acetylcholine was coapplied with ginsenoside Rg_2. Coapplication of ginsenoside Rg_2 with acetylcholine inhibited I_{ACh}. 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 six separate oocytes from three different frogs.

3.3. Current–voltage relationship and voltage-independent inhibition in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors by ginsenoside Rg_2

As shown in Fig. 4A and B, the current–voltage relationship induced by acetylcholine with voltage steps from −100 to +60 mV showed a rectification at positive potentials in

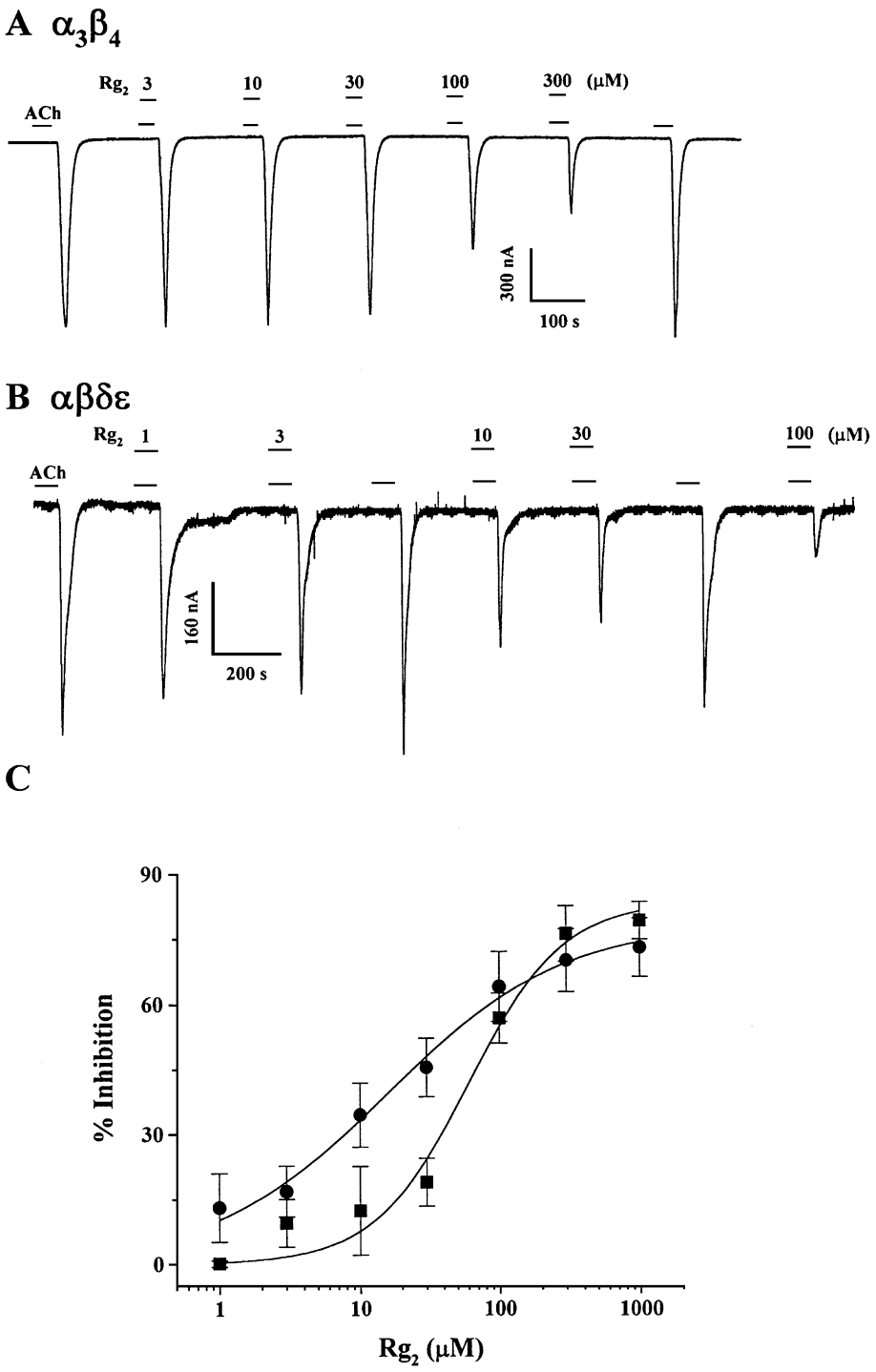


Fig. 3. Ginsenoside Rg_2 concentration–I_{ACh} relationship in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors. (A) I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors was elicited at −80 mV holding potential with indicated time in the presence of 10 μM acetylcholine and then the indicated concentration of ginsenoside Rg_2 was coapplied with acetylcholine. (B) I_{ACh} in oocytes expressing αβδε nicotinic acetylcholine receptors was elicited at −80 mV holding potential with indicated time in the presence of 100 μM acetylcholine and then the indicated concentration of ginsenoside Rg_2 was coapplied with acetylcholine. (C) Percent inhibition by ginsenoside Rg_2 of I_{ACh} was calculated from the average of the peak inward current elicited by acetylcholine alone before ginsenoside Rg_2 and the peak inward current elicited by acetylcholine alone after ginsenoside Rg_2 coapplication with acetylcholine. The continuous line shows the curve fitted according to the equation. y/y_{max}=[ginsenoside Rg_2]/[ginsenoside Rg_2]+$K_{1/2}$), where y_{max} is the maximum inhibition (99.2 ± 28.1% S.D.) for α3β4 (■) and (83.5 ± 8.8% S.D.) for αβδε (●), $K_{1/2}$ is the concentration for half-maximum inhibition (43.7 ± 4.1 μM S.D. for α3β4 and 9.7 ± 4.6 μM S.D. for αβδε) and [ginsenoside Rg_2] is the concentration of ginsenoside Rg_2. Each point represents the mean ± S.E.M. (n=9–12/group).

oocytes expressing α3β4 but not αβδε nicotinic acetylcholine receptors. The patterns of reversal potential of α3β4 and αβδε nicotinic acetylcholine receptors were also different ($V_r = -20 \pm 2$ mV for α3β4 and 7 ± 3 mV for αβδε, means ± S.D., $n = 6$ from three different frogs). Cotreatment with ginsenoside Rg_2 and acetylcholine did not modify the reversal potential of α3β4 nicotinic acetylcholine receptors with a reduction of I_{ACh} ($n = 6$ from three different frogs), whereas cotreatment with ginsenoside Rg_2 and acetylcholine shifted the reversal potential in a negative direction (from 7 ± 3 to -10 ± 2 mV) in oocytes expressing αβδε nicotinic acetylcholine receptors with a reduction of I_{ACh} ($n = 6$ from three different frogs) (Fig. 4A and B). The inhibitory effect of ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors was independent of the membrane holding potential (Fig. 4C). Thus, ginsenoside Rg_2 inhibited I_{ACh} by 43.3 ± 5.5, 38.7 ± 5.1, 42.2 ± 7.0 and 33.0 ± 7.0% at -120, -90, -60 and -30 mV membrane holding potential in oocytes expressing α3β4 nicotinic acetylcholine receptors, respectively ($n = 9$–12 from three different frogs). Ginsenoside Rg_2 also inhibited I_{ACh} by 42.1 ± 7.3, 47.7 ± 3.7, 45.3 ± 6.9 and 40.7 ± 6.5% at -120, -90, -60 and -30 mV membrane holding potential in oocytes expressing αβδε nicotinic acetylcholine receptors, respectively ($n = 9$–12 from three different frogs).

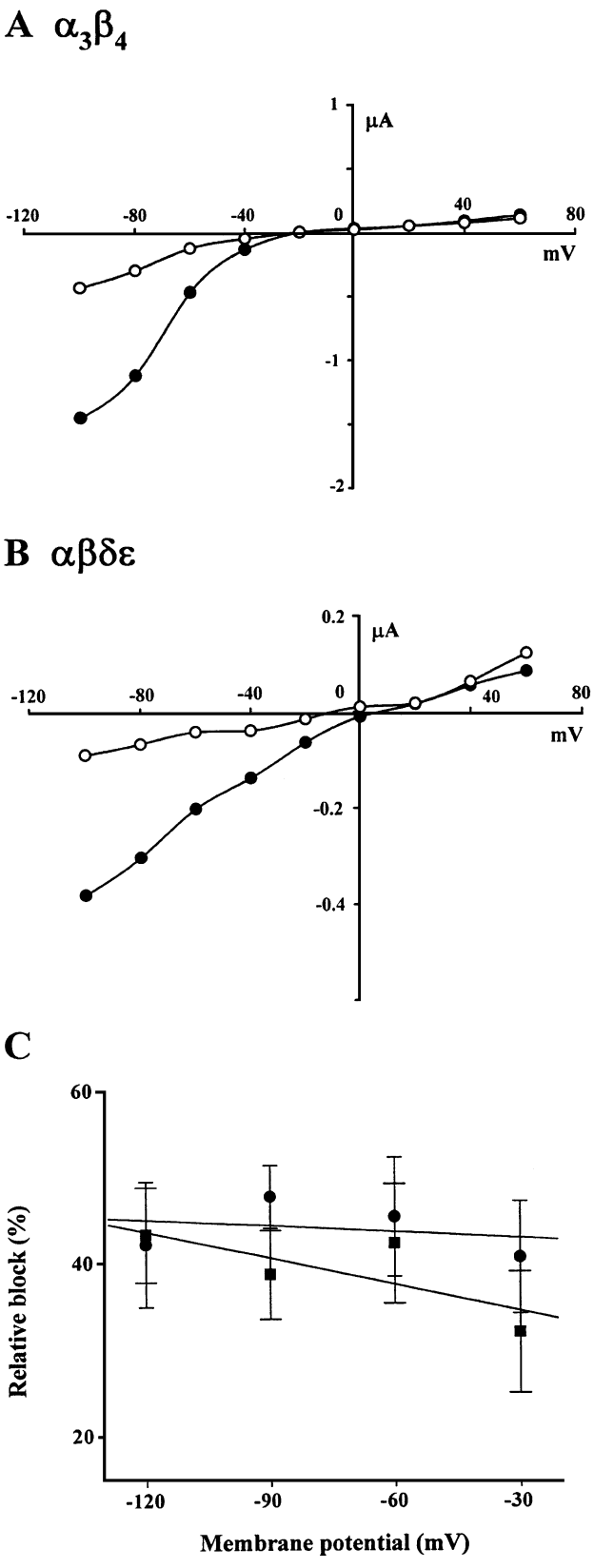


Fig. 4. Current–voltage relationship and voltage-independent inhibition. (A) The representative current–voltage relationship was obtained using voltage steps between −100 and +60 mV with 20-mV increments. Voltage steps were applied before and after application of 10 μM acetylcholine in the absence (●) or presence (○) of 100 μM ginsenoside Rg_2. (B) The representative current–voltage relationship was obtained using voltage steps between −100 and +60 mV with 20-mV increments. Voltage steps were applied before and after application of 100 μM acetylcholine in the absence (●) or the presence (○) of 100 μM ginsenoside Rg_2. (C) Summary of the inhibition induced by ginsenoside Rg_2 at different holding membrane potentials in oocytes expressing α3β4 (●) or αβδε (▲) nicotinic acetylcholine receptors. Each point represents the mean ± S.E.M. ($n = 9$–12/group).

3.4. Noncompetitive inhibition of α3β4 and αβδε nicotinic acetylcholine receptors by ginsenoside Rg_2

To study further the mechanism by which ginsenoside Rg_2 inhibits I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors, we analyzed the effect of 100 μM ginsenoside Rg_2 on I_{ACh} evoked by different acetylcholine concentrations in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors (Fig. 5A and B). Coapplication of ginsenoside Rg_2 with different concentrations of acetylcholine did not shift the dose–response curve of acetylcholine to the right (EC_{50} from 14.4 ± 0.34 to 22.2 ± 0.5 μM and Hill coefficient from 2.3 to 2.4) in oocytes expressing α3β4 nicotinic acetylcholine receptors, although ginsenoside Rg_2 significantly inhibited I_{ACh} at a concentration of 10 and 30 μM but not at 100 μM of acetylcholine. In oocytes expressing αβδε nicotinic acetylcholine receptors, coapplication of ginsenoside Rg_2 with different concentrations of acetylcholine also reduced I_{ACh} with equal potency, independently of the acetylcholine concentration ($n = 9$–12 from three different frogs) (Fig. 5A and B).

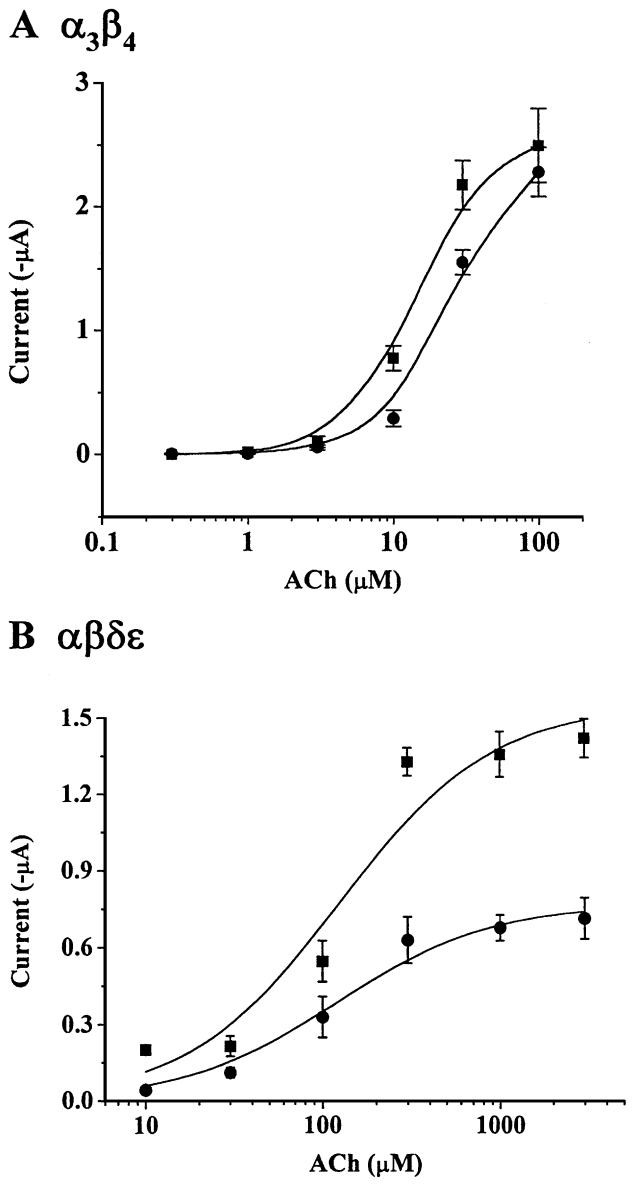


Fig. 5. Dose–response relationship for acetylcholine and acetylcholine plus 100 μM ginsenoside Rg_2 in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors. (A) I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors was measured with the indicated concentration of acetylcholine in the absence α3β4 (■) or the presence (●) of 100 μM ginsenoside Rg_2. (B) I_{ACh} in oocytes expressing αβδε nicotinic acetylcholine receptors was measured with the indicated concentration of acetylcholine in the absence (■) or the presence (●) of 100 μM ginsenoside Rg_2. Oocytes were voltage-clamped at a holding potential of −80 mV. Oocytes were exposed to acetylcholine alone or acetylcholine plus ginsenoside Rg_2 for 1 min. Each point represents the mean ± S.E.M. (n = 9–12/group).

3.5. Comparison of the inhibitory potency of various ginsenosides on I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors

We compared the effect of various ginsenosides on I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors because other ginsenosides besides ginsenoside Rg_2 also have biological activity (Nah, 1997). These ginsenosides also had no effect in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors at a holding potential of −80 mV (data not shown). But cotreatment with these ginsenosides and acetylcholine inhibited I_{ACh} in oocytes expressing α3β4 or αβδε but not in oocytes expressing homomeric α7 nicotinic acetylcholine receptors. As shown in Fig. 6, the inhibitory potency of various ginsenosides on I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors was in the order ginsenoside $Rg_2 \geq Rf > Re > Rg_1 > Rc > Rb_2 > Rb_1$. In contrast, the inhibitory potency of various ginsenosides on I_{ACh} in oocytes expressing αβδε nicotinic acetylcholine receptors was in the order ginsenoside $Rg_2 = Rf > Rg_1 > Re > Rb_1 > Rc > Rb_2$. Interestingly, ginsenosides Re, Rf, Rg_1, and Rg_2 are protopanaxatriol ginsenosides, whereas ginsenosides Rb_1, Rb_2

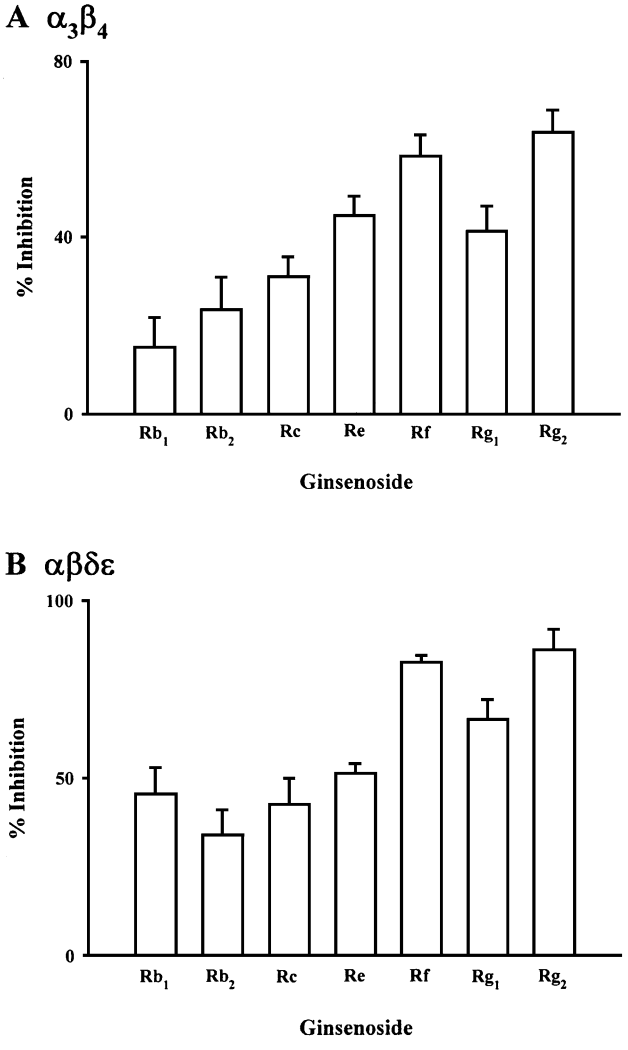


Fig. 6. Blockade by various ginsenosides of peak I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors. The histograms show the percent blockade of peak I_{ACh} by various ginsenosides. Ginsenosides (each 100 μM) were coapplied with 10 μM acetylcholine (A) or 100 μM acetylcholine (B). Each point represents the mean ± S.E.M. (n = 9–12/group).

and Rc are protopanaxadiol ginsenosides. Thus, these results show that protopanaxatriol ginsenosides are more potent than protopanaxadiol ginsenosides in causing inhibition of I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors ($n = 9-12$ from three different frogs) (Fig. 6).

4. Discussion

In the present study, we demonstrated that (1) cotreatment with ginsenoside Rg_2 and acetylcholine inhibited I_{ACh} in oocytes expressing bovine α3β4 or human αβδε nicotine acetylcholine receptors but not in oocytes expressing the bovine α7 nicotinic acetylcholine receptor subtype in reversible and dose-dependent manner; (2) the inhibition of I_{ACh} by ginsenoside Rg_2 occurred in a noncompetitive and voltage-independent manner in oocytes expressing α3β4 or human αβδε nicotinic acetylcholine receptors; (3) protopanaxatriol ginsenosides, such as Re, Rf, Rg_1 or Rg_2 were more potent in causing inhibition of I_{ACh} than were protopanaxadiol ginsenosides, such as Rb_1, Rb_2 and Rc, in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors, demonstrating that the number and the position of sugar moieties attached to aglycone could be associated with the inhibitory potency of ginsenosides on I_{ACh} in oocytes expressing α3β4 or αβδε nicotinic acetylcholine receptors (Fig. 1).

From the present results, however, it is unclear precisely how ginsenosides act to inhibit I_{ACh} in oocytes expressing α3β4 or human αβδε nicotinic acetylcholine receptors. One possible mechanism is that ginsenosides may act as open channel blockers of nicotinic acetylcholine receptors but this may be not the case because the inhibitory effect of ginsenosides on I_{ACh} in oocytes expressing α3β4 or human αβδε nicotinic acetylcholine receptors was not voltage dependent. It is known that open channel blockers, such as local anesthetics or hexamethonium, are strongly voltage dependent, due to the charge that they carry in the transmembrane electrical field (Sine and Taylor, 1982; Heidmann et al., 1983; Arias, 1996).

Another possibility is that ginsenosides may work as a competitive inhibitor by inhibiting acetylcholine binding to its binding site(s) in α3β4 or αβδε nicotinic acetylcholine receptors. In competition experiments, we observed that the presence of ginsenoside Rg_2 did not shift the concentration of acetylcholine in oocytes expressing α3β4 nicotinic acetylcholine receptors without changing the Hill coefficient (Fig. 5). In oocytes expressing αβδε nicotinic acetylcholine receptors, ginsenoside Rg_2 also inhibited I_{ACh} in a noncompetitive manner. Thus, the noncompetitive modulation of α3β4 and αβδε nicotinic acetylcholine receptor channel activity by ginsenoside Rg_2 shows that ginsenoside Rg_2 might have different binding or interaction site(s) as a noncompetitive inhibitor at muscle and neuronal nicotinic acetylcholine receptors, respectively.

It has been reported that the α3β4 nicotinic acetylcholine receptor is one of the endogenous nicotinic acetylcholine receptor subtypes expressed in adrenal chromaffin cells and is involved in catecholamine release (Campos-Caro et al., 1997). Previous reports showed that protopanaxatriol ginsenosides including ginsenoside Re, Rf or Rg_2 inhibited acetylcholine-stimulated catecholamine release in the range of IC_{50} of 4–20 μM (Kudo et al., 1998). Interestingly, in the present study it appeared that the IC_{50} of ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors was about four to ten times higher than that for the inhibition of acetylcholine-stimulated catecholamine release in bovine chromaffin cells (Fig. 3C). The discrepancy in IC_{50} values between the ginsenoside Rg_2-induced inhibition of I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors and the ginsenoside Rg_2-induced inhibition of acetylcholine-stimulated catecholamine release is not clearly understood. One possibility is that ginsenosides might not only inhibit acetylcholine-stimulated catecholamine release through nicotinic acetylcholine receptors, but also suppress the voltage-dependent Ca^{2+} channels involved in catecholamine release in cultured chromaffin cells (Kim et al., 1998; Choi et al., 2001a,b). Thus, the dual actions of ginsenosides might be an explanation for the low IC_{50} value for the inhibition of acetylcholine-stimulated catecholamine release compared to that for ginsenoside Rg_2 on I_{ACh} in oocytes expressing α3β4 nicotinic acetylcholine receptors.

Recent reports show that a wide variety of agents, such as serotonin, strychnine, Ca^{2+} channel blockers, polyamines, steroids such as progesterone and hydrocortisone, ethanol, and metal ion like Zn^{2+}, regulate muscle or neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes (Colunga-Garcia and Miledi, 1995, 1999; Herrero et al., 1999; Haghighi and Cooper, 2000; Valera et al., 1992; Kindler et al., 2000; Cardoso et al., 1999; Palma et al., 1998). Interestingly, the mechanism (i.e., voltage dependence or competition with acetylcholine for binding site) by which these substances regulate nicotinic acetylcholine receptors depend on the receptor subunit composition. The present study showed that ginsenosides also regulate the nicotinic acetylcholine receptor channel activity of heteromeric α3β4 and αβδε but not homomeric α7 nicotinic acetylcholine receptors. These results indicate that ginsenosides may play an important role in modulating the neurotransmitter release or muscular cell excitability induced by nicotinic acetylcholine receptor activation in presynaptic or postsynaptic site(s), since activation of presynaptic nicotinic acetylcholine receptors induces the release of several neurotransmitters in the central nervous system (CNS), including norepinephrine and dopamine (Wannacott, 1997), and activation of postsynaptic nicotinic acetylcholine receptors excites postsynaptic neurons or induces muscle contraction (Brehm et al., 1984).

In a previous study, we showed that ginsenosides inhibit voltage-dependent Ca^{2+} channels in rat sensory neurons

(Nah and McCleskey, 1994; Nah et al., 1995). We also showed that ginsenosides attenuate the pain-related behavior produced by intrathecal injection of substance P (Yoon et al., 1998) or *N*-methyl-D-aspartic acid (NMDA) (Nah et al., 1999) at the spinal cord level. Thus, it can be suggested, on the basis of in vitro or in vivo experimental evidence, that ginsenoside might exert its effect on both pre- and post-synaptic sites in the nervous system through an interaction with channels or receptors.

We also showed that ginsenosides activate the endogenous calcium-activated chloride channels with voltage-dependent manner in *Xenopus* oocytes (Choi et al., 2001a,b). This result might provide the possibility that the chloride current induced by ginsenosides affects the ginsenoside action on I_{ACh} in oocytes expressing the nicotinic acetylcholine receptors, in particular for the highly calcium permeable α7. This may be not the case because ginsenosides used in this study did not elicit an inward current at a holding potential of −80 mV in oocytes expressing the nicotinic acetylcholine receptors (data not shown). We also observed that the ginsenoside-induced activation of calcium-activated chloride channels in *Xenopus* oocytes is mediated via the mobilization of intracellular free calcium but not via the influx of extracellular calcium (Choi et al., 2001a,b). Thus, these results indicate that the chloride current elicited by ginsenosides is not related with the blocking properties of ginsenosides on I_{ACh} in oocytes expressing the nicotinic acetylcholine receptors.

In summary, we found that ginsenosides inhibited I_{ACh} in oocytes expressing bovine neuronal α3β4 or human muscle αβδε nicotinic acetylcholine receptors but not in oocytes expressing bovine α7 nicotinic acetylcholine receptors. These inhibitory effects of ginsenosides on I_{ACh} might provide the single cellular basis for the attenuation of nicotine-induced behavioral changes or for the inhibition of acetylcholine-induced catecholamine release at central or peripheral sites. Finally, these different effects of ginsenosides on nicotinic acetylcholine receptor subtypes may be a mechanism for the pharmacological effect of *Panax* ginseng.

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