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Differential Effects of Quercetin and Quercetin Glycosides on Human α7 Nicotinic Acetylcholine Receptor-Mediated Ion Currents

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Abstract

Quercetin is a flavonoid usually found in fruits and vegetables. Aside from its antioxidative effects, quercetin, like other flavonoids, has a various neuropharmacological actions. Quercetin-3-O-rhamnoside (Rham1), quercetin-3-O-rutinoside (Rutin), and quercetin-3-(2(G)-rhamnosylrutinoside (Rham2) are mono-, di-, and tri-glycosylated forms of quercetin, respectively. In a previous study, we showed that quercetin can enhance α7 nicotinic acetylcholine receptor (α7 nAChR)-mediated ion currents. However, the role of the carbohydrates attached to quercetin in the regulation of α7 nAChR channel activity has not been determined. In the present study, we investigated the effects of quercetin glycosides on the acetylcholine induced peak inward current (I_{ACh}) in *Xenopus* oocytes expressing the α7 nAChR. I_{ACh} was measured with a two-electrode voltage clamp technique. In oocytes injected with α7 nAChR copy RNA, quercetin enhanced I_{ACh}, whereas quercetin glycosides inhibited I_{ACh}. Quercetin glycosides mediated an inhibition of I_{ACh}, which increased when they were pre-applied and the inhibitory effects were concentration dependent. The order of I_{ACh} inhibition by quercetin glycosides was Rutin≥Rham1>Rham2. Quercetin glycosides-mediated I_{ACh} enhancement was not affected by ACh concentration and appeared voltage-independent. Furthermore, quercetin-mediated I_{ACh} inhibition can be attenuated when quercetin is co-applied with Rham1 and Rutin, indicating that quercetin glycosides could interfere with quercetin-mediated α7 nAChR regulation and that the number of carbohydrates in the quercetin glycoside plays a key role in the interruption of quercetin action. These results show that quercetin and quercetin glycosides regulate the α7 nAChR in a differential manner.

Key Words: Flavonoids, Quercetin, Quercetin glycosides, α7 nAChR

INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop family of ligand-gated ion channels. The Cys-loop family also includes serotonin (5-HT_3), gamma-aminobutyric acid ($GABA_A$), and glycine receptors (Jensen *et al*., 2005). nAChRs have been divided into two types: a muscle type and a neuronal type (Dani and Bertrand, 2007). Neuronal nAChRs are widely expressed in the human central and peripheral nervous systems. Eleven different nAChR subunits are currently known, and subunits of nAChR α ($\alpha_{2\text{-}10}$) and β ($\beta_{2\text{-}4}$) have been identified (Nashmi and Lester, 2006). Neuronal nAChRs containing $\alpha_{2\text{-}6}$ subunits are usually expressed as heteromers in combination with $\beta_{2\text{-}4}$ subunits (Boulter *et al*., 1987; Karlin, 2002) and are found throughout the whole nervous system (Gotti and Clementi, 2004). In contrast, the α7 and α9 subunits can form homomeric receptors (Couturier *et al*., 1990; Elgoyhen *et al*., 1994; Karlin, 2002). Homomeric α7 nAChRs are the major binding site for α-bungarotoxin in the central nervous system of mammals and are predominantly expressed in the cortical and the limbic areas including the hippocampus. Homomeric α7 nAChRs are known to play an important role in normal brain function and development (Gotti *et al*., 2000).

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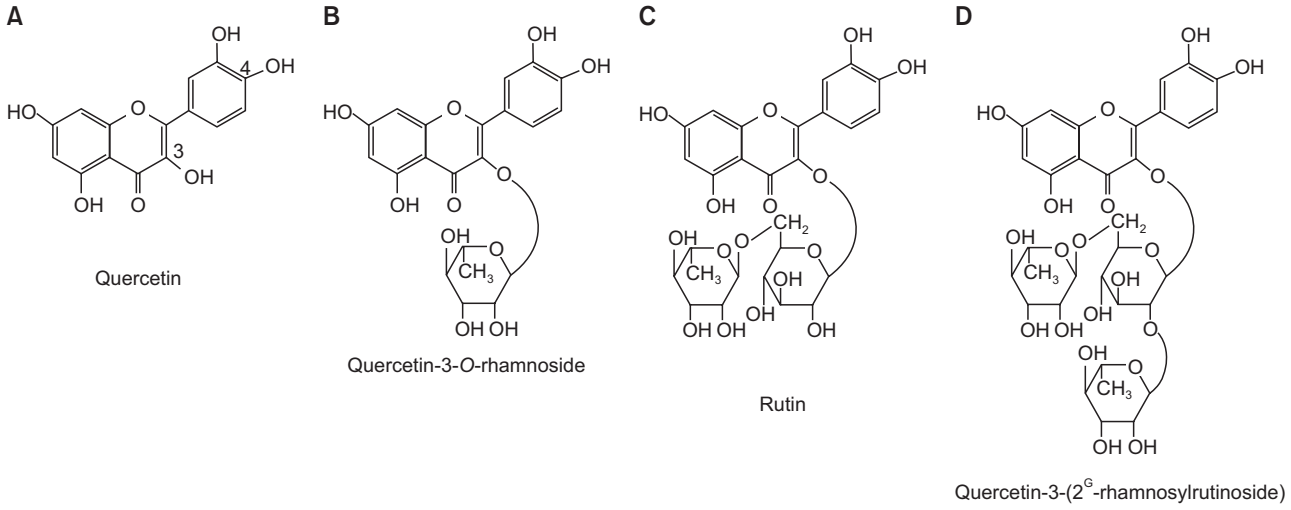

Fig. 1. Chemical structures of quercetin and its glycosides. (A) Quercetin, (B) quercetin-3-*O*-rhamnoside (Rham1), (C) quercetin-3-*O*-rutinoside (Rutin), and (D) quercetin-3-(2^G-rhamnosylrutinoside) (Rham2).

In previous reports, we have shown that the application of the flavonoid quercetin inhibits 5-HT- and glycine-induced peak inward currents ($I_{5\text{-}HT}$ and I_{Gly}) of mouse 5-HT_{3A} and human glycine α receptor channels expressed in *Xenopus laevis* oocytes, respectively. The observed inhibition of $I_{5\text{-}HT}$ by quercetin was competitive and voltage-independent, whereas inhibition of I_{Gly} by quercetin appeared non-competitive and voltage-dependent (Lee *et al*., 2005; Lee *et al*., 2007). In addition, we have found that co- or pre-application of quercetin with acetylcholine (ACh) enhanced I_{ACh} in oocytes expressing human α7 nAChRs. This enhancement appeared independent of ACh concentration and voltage. Furthermore, quercetin enhanced Ca^{2+}-mediated potentiation of I_{ACh}, which was observed to be dependent on extracellular Ca^{2+} concentration (Lee *et al*., 2010).

On the other hand, in addition to quercetin, quercetin glycosides are also compounds of low molecular weight and are mainly found in apples, tomatoes, gingko, other red fruits, and vegetables (Havsteen, 2002). In fruits and vegetables, quercetin naturally exists in glycosylated forms such as Rham 1, Rutin, Rham2, or other glycosidic forms (Azevedo *et al*., 2013). In previous studies using glycine and $5HT_3$ receptors, we have shown that quercetin glycosides can regulate ligand-gated ion channel activity in a differential manner with respect to quercetin. The inhibition of the glycine receptor channel activity by quercetin glycosides was noncompetitive and voltage-sensitive, whereas the inhibition of $5\text{-}HT_3$ receptor channel activity by quercetin glycosides was competitive and voltage-insensitive. Recently, we have also shown that quercetin glycosides inhibit $GABA_C$ receptor channel activity in a non-competitive and membrane voltage-insensitive manner. However, relatively little is known about the effects of quercetin glycosides on α7 nAChR channel activity.

In this study, we investigated the regulation of α7 nAChR channel activity expressed in *Xenopus* oocytes by quercetin glycosides. We first expressed neuronal human α7 nAChR copy RNAs (cRNAs) in *Xenopus* oocytes and examined the effect of quercetin and quercetin glycosides on I_{ACh}. This system was employed because (1) *Xenopus laevis* oocytes have been used widely as a tool to express the membrane proteins encoded by exogenously administered cDNAs or cRNAs including receptors, ion channels, and transporters (Dascal, 1987); and (2) nAChR channels expressed in *Xenopus* oocytes by the injection of nAChR subunit cRNAs have been well studied and characterized (Chavez-Noriega *et al*., 1997).

We found that co- or pre-application of quercetin with ACh enhanced I_{ACh}, whereas co- or pre-application of quercetin glycosides inhibited I_{ACh}. The observed inhibition of I_{ACh} by quercetin glycosides was ACh concentration- and voltage-independent. Interestingly, quercetin-induced enhancement of I_{ACh} was attenuated by co-treatment of quercetin glycosides. Here, we demonstrate that the quercetin glycosides-induced regulation of α7 nAChR channel activity is different from that of quercetin. We further discuss the role of the carbohydrate portion of quercetin glycosides in the differential regulation of α7 nAChR channel activity.

MATERIALS AND METHODS

Materials

Human wild-type α7 nAChR cDNA was kindly provided by Dr. S. Heinemann (Salk Institute, California, USA). Quercetin (Fig. 1) and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of *Xenopus laevis* oocytes and microinjection

X. laevis frogs were purchased from *Xenopus* I (Ann Arbor, MI, USA). Animal care and handling were in accordance with the highest standards of institutional guidelines. To isolate oocytes, frogs were anesthetized with an aerated solution of 3-amino benzoic acid ethyl ester, and the ovarian follicles were removed. The oocytes were separated with collagenase followed by agitation 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 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$,

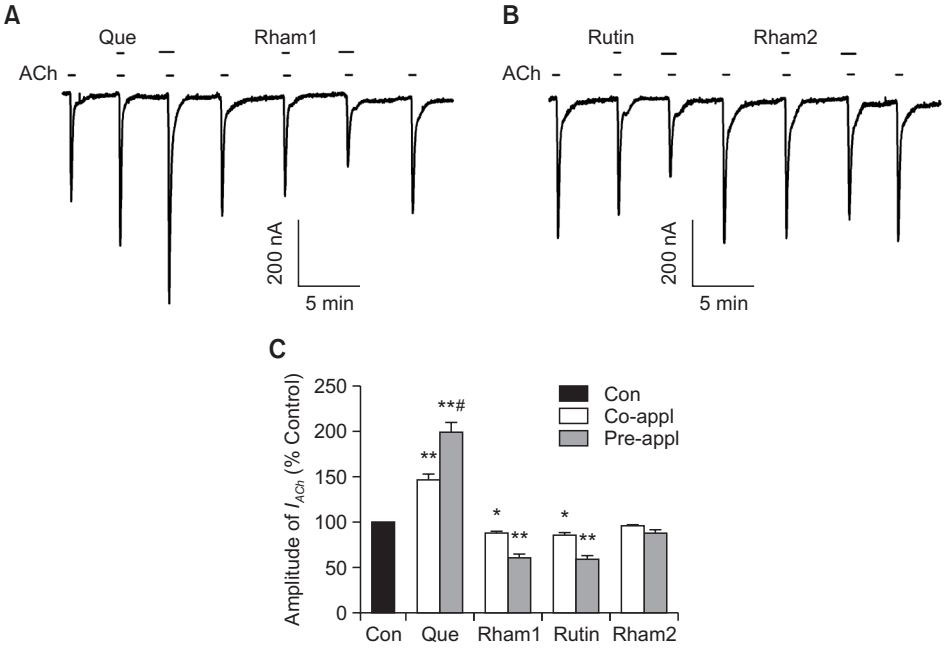

Fig. 2. Effects of quercetin and its glycosides on I_{ACh} in oocytes expressing human α7 nAChRs. (A-B) Acetylcholine (ACh; 200 μM) was applied first, followed by co- or pre-application of quercetin (Que) or quercetin glycosides (Rham1, Rutin, Rham2) and ACh. Co-application of 100 μM quercetin with ACh enhanced I_{ACh} and pre-application of 100 μM quercetin with ACh further enhanced I_{ACh}. Whereas, co-application of 100 μM of quercetin glycosides with ACh inhibited I_{ACh} and pre-application of 100 μM quercetin glycosides with ACh further inhibited I_{ACh}. Traces represent six separate oocytes from three different batches of frogs. (C) Summary of I_{ACh} enhancement by co- or pre-application of quercetin (*p<0.05, **p<0.005 compared to the control; $^{\#}p$<0.005, compared to the co-application of quercetin). Each point represents the means ± S.E.M. (n=9-12/group).

1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) supplemented with 50 μg/ml gentamicin. The solution containing the oocytes was maintained at 18°C with continuous gentle shaking and media was replaced daily. Electrophysiological experiments were performed five to six days after oocyte isolation, during which time the relevant chemicals were added to the media. α7 nAChR-encoding cRNAs (40 nL) were injected into the animal or vegetal pole of appropriate oocytes 1 day after isolation using a 10-μl microdispenser (VWR Scientific, West Chester, PA, USA) fitted with a tapered glass pipette tip (15-20 μm diameter) (Lee *et al*., 2005).

Data recording

A custom-made Plexiglas net chamber was used for two-electrode voltage-clamp recordings, as previously reported (Lee *et al*., 2005). A single oocyte was constantly superfused with ND96 media in the absence or presence of acetylcholine or quercetin during recording. The microelectrodes filled with 3 M KCl giving a resistance of 0.2-0.7 MΩ. Two-electrode voltage-clamp recordings were obtained at room temperature using an Oocyte Clamp (OC-725C, Warner Instrument) and digitized using Digidata 1200A (Molecular Devices, Sunnyvale, CA, USA). Both stimulation and data acquisition were controlled using pClamp 8 software (Molecular Devices). For most electrophysiological experiments, the oocytes were clamped at a holding potential of -80 mV, and 300 ms voltage steps were applied from -100 to +50 mV to assess the relationship between current and voltage. Linear leak and capacitance currents were corrected by means of the leak subtraction procedure. Because α7 nAChRs have a high relative permeability to Ca^{2+} (Séguéla *et al*., 1993; Castro and Albuquerque, 1995), oocytes were incubated in 100 μM 1,2-Bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid tetrakis (acetoxymethyl ester) (BAPTA-AM) for 4 h before recording to avoid α7 nAChR-mediated endogenous Ca^{2+}-activated Cl^- currents.

Data analysis

To obtain the concentration-response curves for quercetin and quercetin glycosides on the inward peak I_{ACh} mediated by α7 AChR, the I_{ACh} peak was plotted at different concentrations of quercetin and its glycosides. Origin software (OriginLab Corp., Northampton, MA, USA) was used to fit the plot to the Hill equation: $I/I_{max}=1/[1+(ED_{50}/[A])^{nH}]$, where I_{max} is maximal current obtained from each ED_{50} value of acetylcholine in wild-type receptors, ED_{50} is the concentration of quercetin or quercetin glycoside required to increase/decrease the response by 50%, [*A*] is the concentration of quercetin or quercetin glycoside, and nH is the Hill coefficient. All values are presented as means ± S.E.M. The differences between the means of control and treatment data were determined using the paired t-test or a one-way ANOVA followed by Tukey test. A value of p<0.05 was considered to be statistically significant.

RESULTS

Effects of quercetin or quercetin glycosides on I_{ACh} in oocytes expressing α7 nAChRs

Treatment of ACh (200 μM) to oocytes injected with human α7 nAChR cRNA induced a large inward current (I_{ACh})

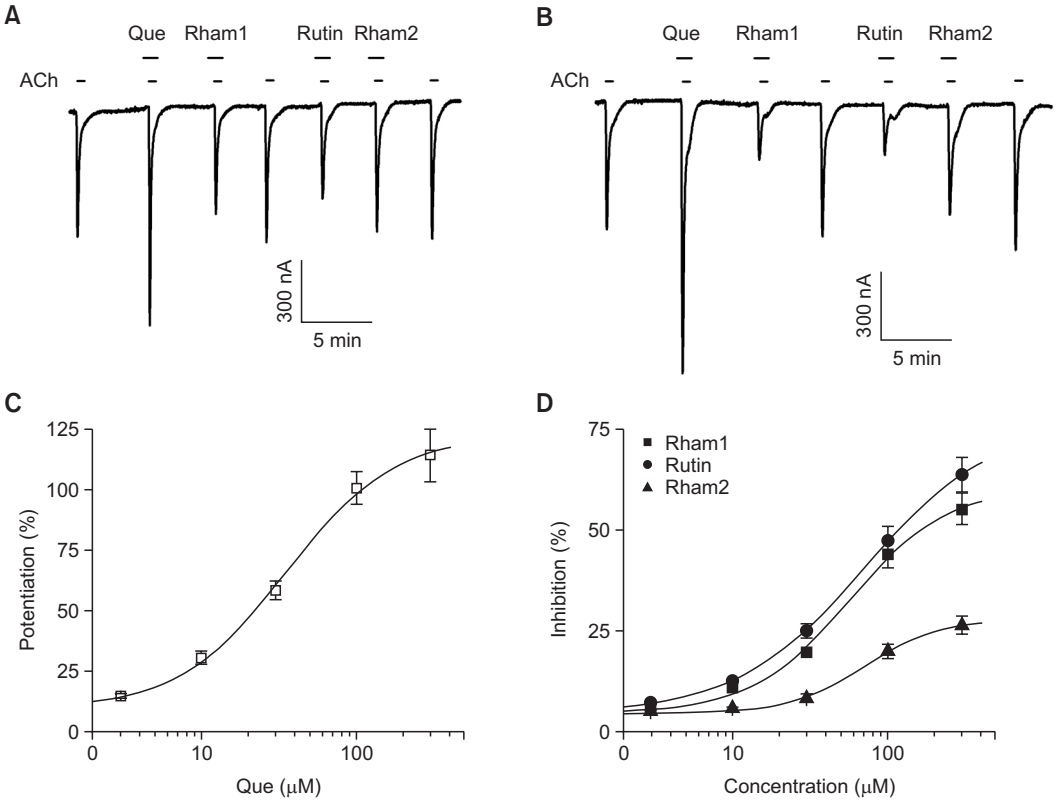

Fig. 3. Concentration-dependent effects of quercetin and its glycosides on I_{ACh}. (A) The representative trace of quercetin- or quercetin glycoside- (30 μM each) mediated effects on I_{ACh}. I_{ACh} in oocytes expressing the α7 nAChR was elicited at a holding potential of -80 mV for 30 s in the presence of 200 μM ACh. Quercetin and its glycosides were pre-applied 30 s before ACh application. (B) The representative trace of quercetin- and quercetin glycoside- (300 μM each) mediated effects on I_{ACh}. I_{ACh} in oocytes expressing the α7 nAChRs was elicited at a holding potential of −80 mV for 30 s in the presence of 200 μM ACh. Quercetin and its glycosides were pre-applied 30 s before ACh application. Traces represent six separate oocytes from three different batches of frogs. (C-D) Concentration-dependent effects of quercetin and quercetin glycosides on I_{ACh}. The solid lines were fit using the Hill equation. Each point represents the mean ± S.E.M. (n=9-12/group).

(Fig. 2A) but the application of ACh did not induce any inward current in H_2O-injected control oocytes (data not shown) (Lee *et al*., 2010). Although quercetin (100 μM) itself had no effect on oocytes expressing α7 nAChRs at a holding potential of -80 mV (data not shown), the co-application of quercetin with ACh enhanced I_{ACh} in oocytes expressing α7 AChR (Fig. 2A, n=9 from three different frogs). The co-application of quercetin with ACh induced an enhancement of I_{ACh} by 46.5 ± 6.5% (Fig. 2B, $^{**}p<0.005$ versus unexposed controls). In addition, pre-application of quercetin (100 μM) alone for 30 s before co-application with ACh (200 μM) induced a much larger enhancement of I_{ACh} in oocytes expressing α7 nAChRs than the enhancement observed after co-application as we previously demonstrated (Fig. 2A, $^{\#}p<0.005$, compared to co-treatment) (Lee *et al*., 2010). Next, we examined the effects of quercetin glycosides on I_{ACh}. Quercetin glycosides (100 μM each) themselves showed no effect on oocytes expressing the α7 nAChRs at a holding potential of -80 mV. Co-application of quercetin glycosides with ACh decreased the amplitude of I_{ACh} reversibly (13.2 ± 2.7%, 15.0 ± 2.9%, and 4.7 ± 1.2% inhibition by Rham1, Rutin, and Rham2, respectively) (Fig. 2C). Pre-application of quercetin glycosides alone for 30 s before co-application with ACh induced a much larger inhibitory effect on I_{ACh} (39.4 ± 3.5%, 42.1 ± 4.7%, and 13.1 ± 4.5% inhibition by Rham1, Rutin, and Rham2, respectively) (Fig. 2B, 2C, n=8-11 from three different frogs). Thus, the I_{ACh} inhibitory potency order appeared where Rutin≈Rham1>Rham2, also indicating that the regulatory pattern of quercetin glycosides on α7 nAChR channel activity is different from that of quercetin (Fig. 2).

Quercetin enhances I_{ACh}, while quercetin glycosides inhibit I_{ACh} in a concentration-dependent manner

In concentration-dependent experiments with quercetin, pre-application with quercetin for 30 s enhanced I_{ACh} in a concentration-dependent manner in oocytes expressing α7 nAChRs (Fig. 3C). Pre-application of quercetin at 3, 10, 30, 100, and 300 μM increased I_{ACh} by 5.9 ± 0.9, 23.1 ± 3.0, 52.9 ± 4.2, 98.7 ± 7.3, and 113.4 ± 11.9% in oocytes expressing α7 AChRs, respectively. Thus, the apparent EC_{50} of I_{ACh} for quercetin pre-application was 35.1 ± 3.8 μM (n=10-11, with samples taken from three different frogs for each point; Fig. 3C). In concentration-dependent experiments with quercetin glycosides, pre-application with quercetin glycosides for 30 s inhibited I_{ACh} in a concentration-dependent manner in oocytes expressing α7 nAChRs (Fig. 3D). For instance, pre-application of Rham1 inhibited I_{ACh} by 1.2 ± 0.3, 6.8 ± 0.8, 16.2 ± 1.1, 41.9 ± 3.5, and 53.9 ± 4.1% at 3, 10, 30, 100, and 300 μM in oocytes expressing α7 AChRs, respectively. Pre-application of Rutin inhibited I_{ACh} by 2.9 ± 0.9, 8.9 ± 0.8, 21.8 ± 1.8, 45.7 ±

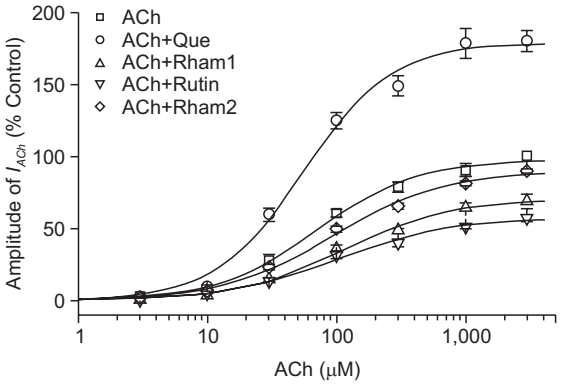

Fig. 4. Concentration-dependent effects of ACh on quercetin- and quercetin glycoside-mediated regulation of I_{ACh}. (A) Concentration-response relationships for oocytes expressing the α7 nAChRs treated with ACh (3-3000 μM) alone or with ACh plus 100 μM quercetin and 100 μM quercetin glycoside. The I_{ACh} of oocytes expressing α7 nAChRs was measured using the indicated concentration of ACh in the absence (□) or presence of quercetin (Que), Rham1, Rutin and Rham2. Oocytes were exposed to ACh alone or to ACh with quercetin and quercetin glycosides for 30 s before application. Oocytes were voltage-clamped at a holding potential of -80 mV.

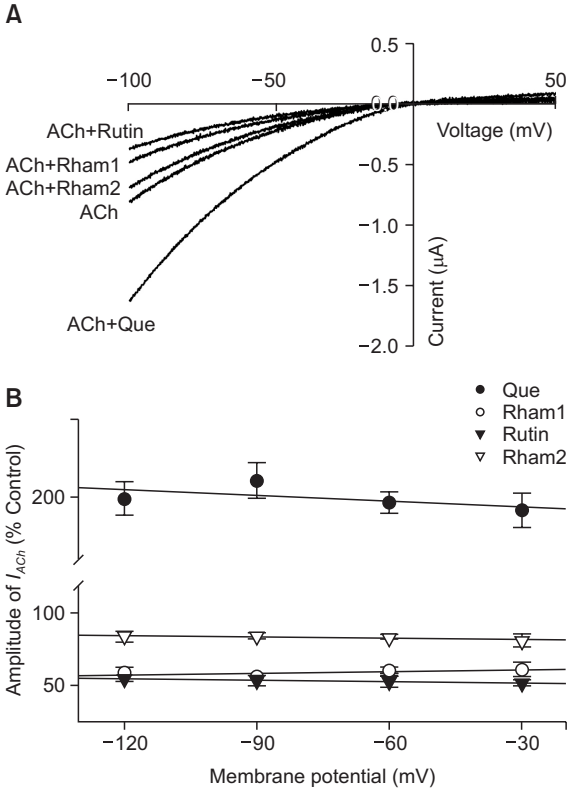

Fig. 5. Current-voltage relationships and voltage-independent inhibition by quercetin and its glycosides. (A) Current-voltage relationships of I_{ACh} regulation by quercetin in the oocytes expressing α7 nAChRs. Representative current-voltage relationships were obtained using voltage ramps of -100 to +50 mV for 300 ms at a holding potential of 80 mV. Voltage steps were applied before and after application of 200 μM ACh in the absence or presence of 100 μM quercetin, Rham1, Rutin, and Rham2. Each point represents the mean ± S.E.M. (n=7-9/group). (B) Voltage-independent regulation of I_{ACh} in oocytes expressing α7 nAChRs by quercetin and quercetin glycosides (100 μM each). The values were obtained in the presence of 200 μM ACh at the indicated membrane holding potentials. Each point represents the mean ± SEM (n=8-12/group).

3.7 and 63.1 ± 4.6% at 3, 10, 30, 100, and 300 μM in oocytes expressing α7 AChRs, respectively. Pre-application of Rham2 inhibited I_{ACh} by 0.7 ± 0.3, 1.3 ± 0.4, 4.1 ± 1.1, 16.3 ± 1.9 and 23.2 ± 2.6% at 3, 10, 30, 100, and 300 μM in oocytes expressing α7 AChRs, respectively. The apparent IC_{50s} of I_{ACh} were 56.4 ± 8.4, 70.4 ± 3.6, 71.3 ± 6.7 μM for Rham1, Rutin, and Rham2 pre-application in oocytes expressing the α7 AChR receptor, respectively (n=10-11, with samples taken from three different frogs for each point; Fig. 3D). These results indicate that quercetin and quercetin glycosides regulate α7 nAChR channel activity in a differential manner.

Concentration-dependent effect of ACh and the relationship between current and voltage in the quercetin- or quercetin glycoside-mediated effects on I_{ACh}

Similar to our previous report (Lee *et al*., 2010), when we analyzed the effect of quercetin on I_{ACh} evoked by different ACh concentrations, we found that pre-application of quercetin with different concentrations of ACh did not significantly shift the concentration-response curve of ACh to the left (EC_{50} values were changed from 70.8 ± 9.6 to 63.5 ± 7.5 μM, *p<0.08, while the Hill coefficient changed from 1.1 to 1.2) in oocytes expressing α7 nAChRs (Fig. 4), suggesting that quercetin did not affect the ACh binding.

Next, to further study the mechanism by which quercetin glycosides inhibit I_{ACh}, we first analyzed the effects of quercetin glycosides on I_{ACh} evoked by different ACh concentrations in oocytes expressing the α7 nAChR (Fig. 4). Pre-application of quercetin glycosides for 30 s with different concentrations of ACh did not significantly shift the dose-response curve of ACh. The apparent EC_{50} values were 70.8 ± 9.6, 78.6 ± 7.1, 76.1 ± 6.5, and 80.6 ± 6.5 μM for ACh alone, ACh + Rham1, ACh + Rutin, and ACh + Rham2, respectively, and the Hill coefficients were 1.1 ± 0.1, 1.0 ± 0.2, 1.0 ± 0.1, and 1.1 ± 0.1, respectively. Thus, Rham1, Rutin, and Rham2 significantly inhibited the I_{ACh} elicited, independent of ACh concentration (n=9-12 from three different frogs) (Fig. 4). These results show that quercetin increases I_{ACh}, whereas quercetin glycosides inhibit I_{ACh} and that these effects probably occur in a non-competitive manner.

In the current-voltage relationship, the membrane potential, which was held at -80 mV, and a voltage ramp was applied from -100 to +50 mV for 300 ms. In the absence of ACh, the inward current (at -100 mV) and the outward current (at +50 mV) were negligible (data not shown). Treatment of ACh to in oocytes expressing the α7 nAChR induced a mainly inward current and outward current at negative- and positive voltages, respectively. Pre-application of quercetin with ACh enhanced both inward and outward currents. The reversal potential was near 0 mV for both ACh alone and for ACh with quercetin. This indicates that Na^+ and Ca^{2+} are main charge carriers (Revah *et al*., 1991; Galzi *et al*., 1992). In addition, the pre-application of quercetin with ACh further increased currents but did not appear to affect α7 nAChR channel properties as quercetin addition did not change the reversal potential of the α7 nAChR (Fig. 5A). Pre-application of quercetin glycosides combined with ACh treatment gave greater inhibition of both inward and outward currents than those achieved when ACh

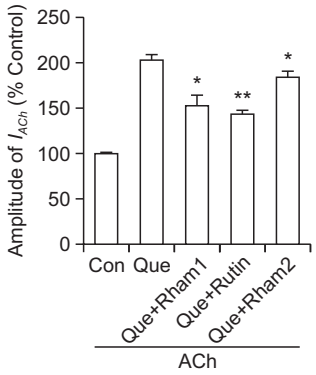

Fig. 6. Effects of quercetin glycosides on quercetin-induced I_{ACh} enhancement. I_{ACh} in oocytes expressing α7 nAChRs was elicited at a holding potential of -80 mV for 30 s in the presence of 200 μM ACh. 100 μM quercetin (Que) alone or with 100 μM quercetin glycoside (Rham1, Rutin, Rham2) were applied for 30 s before ACh addition. Summary histograms are from three different frogs (n=7-12/group). Each point represents the mean ± S.E.M (*p<0.05, **p<0.005 compared to quercetin).

and quercetin glycosides were applied together. The reversal potential was also near -0 mV when ACh was used alone, as well as in ACh+quercetin glycoside treatments (Fig. 5A). In addition, the enhancement of quercetin or the inhibitory effects of quercetin glycosides on I_{ACh} in oocytes expressing α7 nAChRs did not appear to be membrane voltage-sensitive. Quercetin increased I_{ACh} by 98.5 ± 5.4%, and 95.6 ± 6.3%, at -120 and -30 mV, respectively. Rham1, Rutin and Rham2 inhibited I_{ACh} by 41.2 ± 3.8%, 44.9 ± 2.2%, and 16.7 ± 3.7%, respectively, at -120 mV, and by 38.9 ± 4.9%, 48.1 ± 2.1%, and 18.7 ± 4.6%, respectively, at -30 mV (n=10-12, from three different frogs). These results indicate that quercetin enhances I_{ACh}, while quercetin glycosides inhibit I_{ACh} in a voltage-insensitive manner (n=10-12, from three different frogs; Fig. 5B).

Effects of quercetin glycosides on quercetin-induced I_{ACh} enhancement

The above results indicate that quercetin glycosides may be novel regulators of α7 nAChR channel activity and that their actions could be different from those of quercetin. Therefore, we investigated the effect of quercetin on I_{ACh} after co-treatment of quercetin glycosides. As shown in Fig. 6, the enhancing effect of quercetin on I_{ACh} was significantly attenuated in the presence of Rutin, Rham1 and Rham2. The order of potency for the quercetin attenuating effects was Rutin> Rham1>Rham2. The above results show that quercetin-mediated enhancement of I_{ACh} could be affected by the presence of quercetin glycosides.

DISCUSSION

Quercetin is a flavonoid that shows diverse effects in nervous and non-nervous systems (Kandaswami and Middleton, 1994; Harborne and Williams, 2000). For example, quercetin protects the central nervous system against oxidative effects and exerts effects on analgesia, locomotor activity and sleep (Speroni and Minghetti, 1988; Picq *et al*., 1991; Oyama *et al*., 1994), as well as having anticonvulsant, sedative, and anxiolytic effects (Marder *et al*., 1996; Medina *et al*., 1997; Griebel *et al*., 1999; Yao *et al*., 2010). Quercetin glycosides are natural forms of quercetin that are found in colored fruit and vegetables (Fig. 1) (Murota and Terao, 2003; Nemeth and Piskula, 2007). In previous reports, we have shown that quercetin and quercetin glycosides can regulate the activity of several types of ligand-gated ion channels, but the relationship between quercetin and quercetin glycosides and α7 nAChR regulation has not been well characterized.

In the present study, we have investigated the effects of quercetin glycosides on human α7 nAChRs heterologously expressed in *Xenopus* oocytes. We found that: (1) pre-application of quercetin with ACh induced a large enhancement of I_{ACh} in a reversible and concentration-dependent manner; (2) quercetin glycoside pre-application with ACh inhibited I_{ACh}; (3) quercetin-mediated enhancement of I_{ACh} was non-competitive and membrane potential independent, while quercetin glycosides inhibit I_{ACh} in a non-competitive manner and are also membrane potential independent; and (4) quercetin-induced enhancement of I_{ACh} was attenuated in the presence of quercetin glycosides. These results indicate that quercetin and quercetin glycosides show opposite effects on α7 nAChR channel activity and quercetin glycosides are different from quercetin in their regulation of α7 nAChRs. Structural differences between quercetin and quercetin glycosides suggest that the differential regulation of α7 nAChR channel activity might be due to the carbohydrate components.

It will be questioned how structural differences between quercetin and quercetin glycosides induce differential regulations of α7 nAChR channel activity. One possibility is that the carbohydrates attached to quercetin might cause a different behavior in the regulations of α7 nAChRs. For example, quercetin enhances I_{ACh}, whereas quercetin glycosides inhibit I_{ACh} of α7 nAChR (Fig. 3). The other is that the number or different size of carbohydrate attached to quercetin might also induce differential effects on I_{ACh} of α7 nAChRs. Rham1 or Rutin with one or two carbohydrates more potently inhibited I_{ACh} than Rham2, which is tri-glycosylated forms of quercetin (Fig. 3). In addition, quercetin glycosides attenuated the quercetin-induced enhancement of I_{ACh}. Thus, although we found in the present study that carbohydrate component of quercetin could play important roles in the regulations of ligand-gated ion channel such as α7 nAChR, we do not know exactly how carbohydrate(s) attached to quercetin cause an opposite on I_{ACh} of α7 nAChRs or differential effects on I_{ACh} of α7 nAChRs and how quercetin glycosides decreases the quercetin-induced enhancement of I_{ACh}. Further study will be required to elucidate molecular mechanisms how carbohydrate(s) attached to quercetin contribute to quercetin-induced regulations of α7 nAChR.

The activation of the α7 nAChR is known to be linked to many physiological conditions (Khiroug *et al*., 2003; Gotti and Clementi, 2004; Gilbert *et al*., 2009). The α7 nAChR is widely expressed throughout the central nervous systems, including the cortical and limbic areas of the brain. Clearly, the α7 nAChR plays an important role in normal brain function because α7 nAChR dysfunction is associated with neurological disorders such as learning and memory loss, Alzheimer's disease, schizophrenia, and epilepsy (Chini *et al*., 1994; Léna and Changeux, 1997; Weiland *et al*., 2000; Changeux and Edelstein, 2001). However, relatively little is known about the effects of quercetin glycosides on α7 nAChR function. In the

present study, we found that quercetin glycosides inhibited I_{ACh} and furthermore, Rutin and Rham1 could attenuate the quercetin-induced enhancement of I_{ACh}. Interestingly, the inhibitory effects of quercetin glycosides on I_{ACh} as well as the attenuation of quercetin-induced enhancement of I_{ACh}, was observed to be more potent with pre-treatment before ACh addition. Currently we cannot explain the exact physiological or pharmacological role of quercetin glycosides or the reason for their behavior being different from that of quercetin in α7 nAChR regulation. It is known that dietary quercetin glycosides are usually metabolized in two ways. First, they are deglycosylated to the aglycone quercetin; and second, they remain as quercetin glycosides without further metabolism (Havsteen, 2002; Lee *et al*., 2010). Further studies will be required to elucidate the role of quercetin glycosides in the *in vivo* regulation of the α7 nAChR.

In previous studies, we have shown that quercetin inhibits 5-HT_3 receptor-gated ion currents through interactions within the pre-transmembrane domain I. Furthermore, quercetin can inhibit or potentiate glycine receptor-gated ion currents through interactions with the amino acid Ser256 residue in transmembrane domain II (Lee *et al*., 2005; Lee *et al*., 2007). Examination of the effects of quercetin glycosides shows an inhibition of $GABA_C$ receptor channel activity in the order of Rutin>Rham1>Rham2 (Kim *et al*., 2015). In the present study, we found that quercetin glycosides also inhibited α7 nAChR-gated ion currents with the same order of efficacy. Thus, although the Cys-loop family of ligand-gated ion channels such as glycine, $GABA_C$, 5-HT_3, and α7 nAChR all form homomeric receptors, quercetin and quercetin glycosides regulate these homomeric receptors in a different manner.

In conclusion, we found that quercetin increased I_{ACh}, while quercetin glycosides inhibited I_{ACh} in *Xenopus* oocytes expressing α7 nAChRs. The present results indicate that quercetin and quercetin glycosides act differently in the regulation of α7 nAChRs. Finally, these results also suggest that quercetin and quercetin glycosides exhibit their differential regulation of α7 nAChR channel activity through their structural differences.

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