Differential Regulations of Quercetin and Its Glycosides on Ligand-Gated Ion Channels


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Quercetin, one of the flavonoids, is a compound of low molecular weight found in various plants and shows a wide range of diverse neuropharmacological actions. In fruits and vegetables, quercetin exists as monomer-(quercetin-3-O-rhamnoside) (Rham1), dimer- (Rutin), or trimer-glycosides [quercetin-3-(2G-rhamnosylrutinoside)] (Rham2) at carbon-3. In the previous studies, we demonstrated that quercetin inhibits both glycine and 5-hydroxytryptamine type 3, (5-HT3a) receptor channel activities expressed in Xenopus oocytes. However, the effects of quercetin glycosides on glycine and 5-HT3A receptor channel activities are not well known. In the present study, we investigated the effects of quercetin glycosides on the human glycine α1 receptor and mouse 5-HT3A receptor channel activities expressed in Xenopus oocytes using a two-electrode voltage clamp technique. We found that applications of quercetin and its glycosides reversibly inhibited glycine-induced current (Igly) in order of Rham2 > quercetin > Rutin > Rham1. The inhibitions of Igly by quercetin glycosides were non-competitive and voltage-sensitive, whereas the inhibitions of I5HT by quercetin glycosides were competitive and voltage-insensitive manners. These results indicate that quercetin glycosides might regulate the human glycine α1 and mouse 5-HT3A receptors with differential manners.

Key words flavonoid; quercetin glycoside; ligand-gated ion channel; Xenopus oocyte

The glycine and 5-hydroxytryptamine type 3 (5-HT3) receptors are the superfamilies of ligand-gated ion channel receptors with cysteine loop and share a structural similarity with nicotinic acetylcholine and γ-aminobutyric acid A (GABA_A) receptors.1) The glycine receptor is predominantly expressed in spinal cord, brain stem and other regions of the central nervous system.2) The activations of glycine receptor in these regions mediate post-synaptic inhibitions for reflex responses, voluntary motor control, and the processing of sensory signals,3) since the glycine receptor forms a chloride-permeable and -selective transmembrane channel. Thus, the glycine receptor is responsible for fast inhibitory synaptic transmissions.4)

On the other hand, 5-HT3 receptor mediates rapid and transient membrane depolarizing effects of 5-HT in the central and peripheral nervous system like nicotinic acetylcholine receptors.5) 5-HT3A receptor comprises of a N-terminal domain, four transmembrane domains (TM1 to TM4), and an intracellular domain and finally homomeric pentamers of each subunit consist of 5-HT3 receptor as glycine receptor does.6) The physiological and pathological roles of 5-HT3 receptor involve pain transmission, analgesia, vomiting, and mood disorders and drug abuse.7)

Recently, we have reported that quercetin regulates glycine and 5-HT3A receptor channel activities through interaction with amino acid residues existing in pore region of transmembrane domain 2 and N-terminal of pre-transmembrane domain 1, respectively.6,7) Interestingly, in fruits and vegetables quercetin naturally exists as monomer- (quercetin-3-O-rhamnoside), (Rham1) dimer- (Rutin), or trimer-glycosides [quercetin-3-(2G-rhamnosylrutinoside)] (Rham2) at carbon-3 and other glycosidic forms.8,9) When animal or human intakes fruits or vegetables, the dietary quercetin glycosides are metabolized to quercetin or conjugated with glucose or sulfate. A line of evidence showed that quercetin glycosides in addition to quercetin also exert their physiological or pharmacological effects.8,9) However, until now little is known on how quercetin glycosides affect on the regulations of glycine and 5-HT3A receptor channel activities.

In the present study, we examined the effects of quercetin glycosides on the human glycine α1 receptor and mouse 5-HT3A receptor channel activities expressed in Xenopus oocytes using a two-electrode voltage clamp technique. We found that applications of quercetin and its glycosides reversibly inhibited glycine-induced current (Igly) in order of quercetin > Rutin > Rham1 > Rham2. Applications of quercetin and its glycosides inhibited I5HT in order of Rham2 > quercetin > Rutin > Rham1. The inhibitions of Igly by quercetin glycosides were non-competitive and voltage-sensitive, whereas the inhibitions of I5HT by quercetin glycosides were competitive and voltage-insensitive manners. These results indicate that quercetin glycosides might regulate the human glycine α1 and mouse 5-HT3A receptors with differential manners.

MATERIALS AND METHODS

Materials

Figure 1 shows the structure of quercetin and its glycosides used in this study were dissolved in dimethyl
sulfoxide (DMSO) and were diluted with bath medium before use. cDNAs containing the human glycine α1 and mouse 5-HT₃A receptors were kindly provided by Dr. H. Betz (Max-Planck-Institute, Germany) and Dr. D. Julius (UCSF, U.S.A.), respectively. Final DMSO concentration was less than 0.01%. Quercetin and other chemical agents were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Quercetin glycosides were purified from Magnolia obovata.⁶⁰

**Oocyte Preparation** *Xenopus laevis* frogs were purchased from Xenopus 1 (Ann Arbor, MI, U.S.A.). *Xenopus laevis* care and handling were in accordance with the guide for the Care and Use of Laboratory Animals published by NIH, U.S.A. Frogs 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 thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid thiolized with an aerated solution of 3-amino benzoic acid

**RESULTS**

**Effects of Quercetin and Its Glycosides on Human Glycine α1 and Mouse 5-HT₃A Receptor-Mediated Currents** As previous reports, we could observe that application of glycine (100 μM) or 5-HT (1 μM) to bathing medium induced a large inward current (I₆₀₉) in oocytes injected with cRNAs coding glycine or 5-HT₃A receptor (Fig. 2). Strychnine (0.5 μM) and MDL72222 (0.5 μM), of which the respective glycine receptor and 5-HT₃A receptor antagonist blocked I₆₀₉ and I₆₄₉ (data not shown), indicating that the glycine and 5-HT₃A receptor were functionally expressed in this system as previous reports.⁶⁷ In the present study, we examined the effects of quercetin glycosides on I₆₀₉ or I₆₄₉. Quercetin and its glycosides (each 100 μM) themselves had no effect in oocytes expressing the glycine or 5-HT₃A receptor at a holding potential of −80 mV. However, co-application of quercetin (Que) or its glycosides with glycine or 5-HT inhibited I₆₀₉ or I₆₄₉ in a reversible manner (Fig. 2A, n=15 from three different frogs) (Figs. 2A, B). The percent inhibitions were 88.7±2.0, 53.3±5.7, 58.3±5.1, 35.6±8.8% for I₆₀₉ and 74.2±3.0, 52.4±4.9, 58.6±2.1, 83.6±1.6% for I₆₄₉ by quercetin, Rham1, Rutin, and Rham2, respectively.
The inward currents were recorded at a holding potential of −80 mV (A) Glycine (Gly; 100 μM) induced a large inward current in oocytes expressing the glycine α1 receptor. Co-treatment of 100 μM of quercetin, quercetin-3-O-rhamnoside (Rham1), rutin (Rutin) and quercetin-3-(2′′-rhamnosylrutinoside) (Rham2) with glycine inhibited I_{Gly} in the representative trace. Oocytes were exposed to glycine alone, glycine + quercetin, or quercetin+its glycosides treatment for 30 s. (B) 5-HT (1 μM) induced a large inward current in oocytes expressing the 5-HT_3A receptor. Co-treatment of 100 μM of quercetin, Rham1, Rutin and Rham2 with 5-HT inhibited I_{5-HT} in the representative trace. The representative trace shows both quercetin and its glycosides inhibit I_{Gly} and I_{5-HT}. Oocytes were exposed to 5-HT alone or 5-HT+quercetin and+its glycosides treatment for 30 s. (C) Co-treatment of quercetin and its glycosides with glycine or 5-HT exhibits differential inhibitory effects in summary histograms. *p<0.05, **p<0.005 compared quercetin with its glycosides treatment by one-way ANOVA with post-hoc Tukey test. Each point represents the mean±S.E.M. (n=9—12 oocytes/group).

(Fig. 2C). Thus, the inhibitory potency for I_{Gly} was in order of quercetin>Rutin=Rham1>Rham2 and the inhibitory potency for I_{5-HT} was in order of Rham2>quercetin>Rutin=Rham1. Glycosylations of quercetin affect on glycine but not 5-HT_3A receptor channel current inhibitions. In addition, since quercetin more strongly inhibited I_{5-HT} or I_{Gly} than other quercetin glycosides tested, we tested using quercetin whether pre-application of quercetin caused further inhibition on I_{5-HT} or I_{Gly} compared to its co-application with glycine or 5-HT. As shown in Figs. 3A and B, pre-application of quercetin did not induce further inhibition of I_{5-HT} or I_{Gly} compared to co-application of quercetin with glycine or 5-HT. Thus, the percent inhibitions in co-application of quercetin were 52.3±6.4% for I_{Gly} and 49.5±5.0% for I_{5-HT}. The percent inhibitions in pre-application of quercetin were 54.3±3.4% for I_{Gly} and 52.1±3.4% for I_{5-HT} (Fig. 3C), indicating that either co- or pre-application causes the same extent of inhibitions on I_{5-HT} and I_{Gly}.

Concentration-Dependent Effect of Quercetin and Its Glycosides on I_{Gly} and I_{5-HT} Quercetin and its glycosides inhibited both I_{Gly} and I_{5-HT} with concentration-dependent manners (Figs. 4A, B). The IC_{50} for I_{Gly} were 23.8±0.5, 62.5±7.4, 66.4±2.8, 97.4±0.9 μM by quercetin, Rham1, Rutin, and Rham2, respectively. The IC_{50} for I_{5-HT} were 18.8±2.4, 61.4±3.9, 25.5±3.3, 23.6±0.1 μM by quercetin, Rham1, Rutin, and Rham2, respectively (n=8—12 from three different frogs for each point) (Figs. 4A, B, Table 1). The IC_{50} values of quercetin glycosides on glycine receptor were significantly higher than that of quercetin. Especially, it appears that the increase in IC_{50} value was related with number of quercetin glycosylation. Finally, quercetin’s glycosylation caused dose–response curves to rightward shift. These results show a possibility that quercetin rather than quercetin glycosides is the main regulator of glycine receptor channel activity. In 5-HT_3A receptors, the IC_{50} values were not significantly changed between quercetin and its glycosides, except for Rham1 (Fig. 4, Table 1), indicating that glycosylations of quercetin did not much affect on 5-HT_3A receptor channel regulations.

Quercetin Glycosides Inhibit I_{Gly} with Non-competitive, Whereas They Inhibit I_{5-HT} with Competitive Manner To study further the mechanism by which quercetin and its glycosides inhibit I_{Gly} and I_{5-HT}, we first analyzed the effects of quercetin and its glycosides on I_{Gly} evoked by different
The EC₅₀ values were 168.9 ± 25 for glycine alone, 167.4 ± 2.0 for glycine + quercetin, 173.9 ± 0.3 for glycine + Rutin, 193.5 ± 0.1 for glycine + Rham1, 156.5 ± 0.4 for glycine + Rham2, 1.9 ± 0.2 for 5-HT, 1.7 ± 0.1 for 5-HT + Quercetin, 2.6 ± 0.6* for 5-HT + Rutin, and 2.3 ± 0.4 for 5-HT + Rham2. Each point represents the mean ± S.E.M. (n=9—12 oocytes/group).

Table 2. Competitive and Non-competitive Effects of Quercetin and Its Glycosides on Glycine α1 and 5-HT3A Receptors-Mediated Currents Expressed in Xenopus Oocytes

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Drug</th>
<th>EC₅₀</th>
<th>nH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine α1</td>
<td>Glycine</td>
<td>168.9±0.9</td>
<td>2.6±0.1</td>
</tr>
<tr>
<td></td>
<td>Glycine + Quercetin</td>
<td>167.4±2.0</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Glycine + Rutin</td>
<td>173.9±0.3</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td></td>
<td>Glycine + Rham1</td>
<td>193.5±0.1</td>
<td>2.5±0.0</td>
</tr>
<tr>
<td></td>
<td>Glycine + Rham2</td>
<td>156.5±0.4</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>5-HT₃A</td>
<td>5-HT</td>
<td>1.9±0.2</td>
<td>1.9±0.3</td>
</tr>
<tr>
<td></td>
<td>5-HT + Quercetin</td>
<td>4.1±0.1**</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td></td>
<td>5-HT + Rutin</td>
<td>2.6±0.6*</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td></td>
<td>5-HT + Rham1</td>
<td>2.3±0.4</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td></td>
<td>5-HT + Rham2</td>
<td>4.1±0.1**</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E.M. (n=10—12/group). Currents were elicited at a holding potential of −80 mV. IC₅₀, Hill coefficient, and Vₘₐₓ values were determined as described in Materials and Methods. *p<0.05, **p<0.005 compared with glycine alone by one-way ANOVA.

Fig. 5. (A) Dose–Response Relationship for Glycine and Glycine + Quercetin and Its Glycosides in Oocytes Expressing the Human Glycine α1 Receptor

IₙG in oocytes expressing the glycine receptor was measured with the indicated concentration of glycine alone (○) and glycine + 25 μM quercetin (△), +60 μM Rham1 (●), +65 μM Rutin (□) and +100 μM Rham2 (○). Oocytes were voltage-clamped at a holding potential of −80 mV. Oocytes were exposed to glycine alone or glycine + quercetin, + Rham1, + Rutin and + Rham2 treatment for 30 s. (B) Dose–Response Relationship for 5-HT and 5-HT+Quercetin and Its Glycosides in Oocytes Expressing the Mouse 5-HT Receptor

In oocytes expressing the 5-HT₃A receptor was measured with the indicated concentration of 5-HT alone (○) and 5-HT + 20 μM quercetin (△), +60 μM Rham1 (●), +25 μM Rutin (□) and +25 μM Rham2 (○). Oocytes were voltage-clamped at a holding potential of −80 mV. Oocytes were exposed to 5-HT alone or 5-HT+quercetin, + Rham1, + Rutin and + Rham2 treatment for 30 s. Each point represents the mean ± S.E.M. (n=9—12 oocytes/group).
0.005, compared with 5-HT alone) and the Hill coefficients were 1.9, 1.6, 1.5, 1.9 and 1.8, respectively (Table 2). Thus, these results indicate that quercetin and its glycosides inhibit the $I_{Gly}$ in a competitive manner. Altogether, these results indicate that quercetin and its glycosides regulate glycine and 5-HT$_{3A}$ receptor channel activities with differential manners.

**Current–Voltage Relationships in Quercetin and Its Glycosides-Mediated Human Glycine α1 and 5-HT$_{3A}$ Receptor Regulations** In experiments of the current–voltage relationship, the membrane potential was held at $-80 \text{ mV}$ and a voltage ramps for 300 ms were applied from $-100$ to $+40 \text{ mV}$ for glycine receptor. In the absence of glycine, the inward current at $-100 \text{ mV}$ was $<0.1 \mu A$ and the outward current at $+40 \text{ mV}$ was 0.1—0.4 $\mu A$ in the glycine receptor (Fig. 6A). The addition of glycine to the bathing medium resulted in an increase of the inward current at a potential more positive than approximately $-20 \text{ mV}$. In contrast, at a potential more positive than approximately $-20 \text{ mV}$ glycine led to a large increase in outward current. Co-application of quercetin and its glycosides with glycine inhibited both the inward and outward currents more than those induced by glycine treatment alone. The reversal potential was also near $-20 \text{ mV}$ in both glycine alone and glycine + quercetin and its glycosides. The inhibitory effects of quercetin and its glycosides on $I_{Gly}$ in oocytes expressing the glycine receptor were voltage-sensitive (Fig. 6A). Thus, quercetin, Rham1, Rutin, and Rham2 inhibited $I_{Gly}$ by 47.3±2.5%, 45.7±2.4%, 40.6±3.1 and 36.4±2.1%, respectively, at $-100 \text{ mV}$; and by 66.8±3.1%, 58.9±2.1%, 56.7±3.7 and 52.8±2.2%, respectively, at $+40 \text{ mV}$ ($n=10-12$, from three different frogs). These results indicate that quercetin and its glycosides inhibit $I_{Gly}$ in a voltage-sensitive manner.

In experiments of the current–voltage relationship using 5-HT$_{3A}$ receptors, the membrane potential was also held at $-80 \text{ mV}$ and a voltage ramps for 300 ms were applied from $-100$ to $+60 \text{ mV}$ for 5-HT$_{3A}$ receptors, respectively. In the absence of 5-HT, the inward current at $-100 \text{ mV}$ was $<0.1 \mu A$ and the outward current at $+60 \text{ mV}$ was 0.1 $\mu A$ in the 5-HT$_{3A}$ receptor (Fig. 6B). The addition of 5-HT to the bathing medium resulted in an increase of the inward current at negative voltages, and an outward current at positive voltages. The reversal potential was near 0 $\text{ mV}$ with 5-HT alone as well as 5-HT + quercetin and its glycosides. Co-application of quercetin and its glycosides with 5-HT inhibited both the inward and outward currents more than those induced by 5-HT treatment alone. The inhibitory effects of quercetin and its glycosides on $I_{5-HT}$ in oocytes expressing 5-HT$_{3A}$ receptor were voltage-insensitive (Fig. 6B). Thus, quercetin, Rham1, Rutin and Rham2 inhibited $I_{5-HT}$ by 43.1±2.4%, 40.1±2.2%, 33.0±3.0 and 50.7±2.7% at $-100 \text{ mV}$, respectively; and by 45.8±3.0%, 44.1±2.0%, 32.9±3.7 and 54.2±2.1% at $+60 \text{ mV}$, respectively ($n=10-12$, from three different frogs). These results indicate that quercetin inhibits $I_{Gly}$ in a voltage-insensitive manner.

**DISCUSSION**

Quercetin, one of the flavonoids, and its glycosides are substances of low molecular weight mainly found in apple, tomato, gingko and other red fruits (Fig. 1A).$^{12}$ Quercetin shows a wide range of biological activities,$^{13-15}$ with neuromodulatory and sleep,$^{16,17}$ neuronal oxidative modulations,$^{18}$ proconvulsant, anticonvulsant, sedative and anxiolytic effects.$^{19-21}$ The cellular mechanisms underlying quercetin, especially on synaptic transmissions are not well studied. In the previous studies, we could demonstrate that quercetin regulates glycine and 5-HT$_{3A}$ receptor channel activities.$^{6,7}$ However, although most of quercetin in fruits and vegetables exist as quercetin glycosides, the pharmacological and physiological roles of those quercetin glycosides are relatively unknown in nervous systems.

In the present study, we demonstrated that (1) quercetin was more potent for the inhibition of $I_{Gly}$ than other quercetin glycosides, whereas quercetin and its glycosides except Rham1 and rutin were equally potent for the inhibition of $I_{5-HT}$; (2) quercetin glycosides inhibited both $I_{Gly}$ and $I_{5-HT}$ in a concentration-dependent and reversible manner in oocytes expressing the human glycine α1 or mouse 5-HT$_{3A}$ receptors; (3) quercetin glycosides-induced inhibitions of $I_{Gly}$ were non-competitive and voltage-sensitive, whereas quercetin glycosides-induced inhibitions of $I_{5-HT}$ were competitive and voltage-insensitive, indicating that quercetin glycosides regulate glycine and 5-HT$_{3A}$ receptor with differential manners.

It has been known that dietary quercetin and its glycosides are metabolized into three ways. First is to remain in intact quercetin or conjugated with glucoses or sulfates to produce quercetin glucuronides or quercetin sulfates in blood stream; second way is to deglycosylate quercetin to quercetin aglycone; third way is to remain with quercetin glycosides without further metabolisms.$^{8,9}$ Interestingly, quercetin glucuronides or quercetin sulfates maintain their biological activities as antioxidants if they are conjugated with carbon-3 but not carbon-4. These results indicate that the antioxidant effects of quercetin and its metabolites are due to catechol
ring of quercetin backbone structure (Fig. 1). However, it is unknown whether catechol ring of quercetin is also involved in ligand-gated ion channel activity regulations. In future, further investigations using flavonoids, which are conjugated at carbon-4, will be required to evaluate whether intact catechol ring of flavonoids is also necessary for the regulation of ligand-gated ion channel activity.

In the present study, we found the position of glycosylation to quercetin plays important roles in glycine but not 5-HT3A receptor channel activity. As shown in Fig. 4 and Table 1, the IC50 values of quercetin with monomer or dimer of carbohydrate at carbon-3 were about 2.5-fold higher than that of quercetin. Moreover, the IC50 value of quercetin with trimer of carbohydrate was about 3-fold higher than that of quercetin. Thus, glycosylations of quercetin at carbon-3 attenuate quercetin action in quercetin-induced glycine receptor regulations. These results show a possibility that if quercetin glycosides are metabolized into quercetin aglycone, their regulatory effects on glycine receptor channel activity will be recovered as much as quercetin. Interestingly, in case of 5-HT3A receptor, the degree of quercetin glycosylation was more potent for the inhibition of 5-HT3A receptor channel activity except Rham1, since the IC50 values of quercetin with monomer or trimer of carbohydrate were not significantly higher than that of quercetin. In addition, it appeared that the maximum inhibition of Rutin on 5-HT3A receptor channel activity was about 60% compared to quercetin and other quercetin glycosides shown Fig. 4B and Table 1, even though its IC50 was similar to others except Rham1. It seems that Rutin might have a low efficiency in the inhibition of 5-HT3A receptor channel currents compared with quercetin and other quercetin glycosides tested.

From the results of quercetin glycosides’ effects on glycine and 5-HT3A Receptor channel activities, we could observe that the possible interaction site(s) of quercetin glycosides with glycine receptor might be different from that of 5-HT3A receptor, since quercetin glycosides-induced inhibitions of I\textsubscript{Gly} were non-competitive and voltage-sensitive, whereas quercetin glycosides-induced inhibitions of I\textsubscript{SHT} were competitive and voltage-insensitive manners. It has been known that open channel blockers of ligand-gated ion channels are usually non-competitive and voltage-sensitive, due to the charge that they carry in the transmembrane electrical field of channel pore. This notion is again supported by previous report that site-directed mutations of S267 to S267Y at transmembrane domain 2 (TM2), which is forming channel pore region, almost abolished quercetin-induced inhibition of I\textsubscript{Gly}. The present and previous results show a possibility that quercetin and its glycosides might play as open channel blockers of glycine receptor.

On the other hand, we also found that quercetin- and its glycosides-induced inhibitions of I\textsubscript{SHT} were competitive and voltage-insensitive manners. It is also known that the competitive inhibitors of 5-HT3A receptor usually regulate channel activity by inhibiting 5-HT binding to its binding site(s) in N-terminal regions of 5-HT3A receptor. In competition experiments, we could observe that the presence of quercetin- and its glycosides shifted the concentration of 5-HT in oocytes expressed with 5-HT3A Receptor without significant changes of Hill coefficient (Fig. 5B). Thus, the competitive modulation of 5-HT3A receptor channel activity by quercetin and its glycosides shows a possibility that quercetin and its glycosides might have interaction site(s) in N-terminal of 5-HT3A receptor. In previous report, we have shown that site-directed mutations of N-terminal region of pre-transmembrane domain 1 (pre-TM1) such as R246T and R246A but not R246D, R246E and R246K abolished quercetin-inhibited induction of I\textsubscript{SHT}, supporting that quercetin regulates 5-HT3A receptor channel activities through interaction with N-terminal region of 5-HT3A Receptor. However, identification of the exact interaction sites of quercetin and its glycosides involved in the regulation of glycine or 5-HT3A receptor channel activity might require further investigations in future.

Interestingly, it is well known that activations of glycine receptor at postsynaptic sites exhibit fast inhibitory postsynaptic potentials, whereas activations of 5-HT3A at postsynaptic sites exhibit fast excitatory postsynaptic potentials. Therefore, it will be questioned what are the physiological or pharmacological roles of quercetin and its glycosides following the inhibitions of excitatory or inhibitory ligand-gated ion channel activities. Glycine receptors are rich in spinal cord and brain stem, whereas 5-HT3 receptors exist peripheral nervous systems such as intestines and brain stem area related with emesis in central nervous system. Although we could not clearly answer the roles of quercetin and its glycosides that inhibit both ligand-gated ion channel activities in nervous systems, it seems that quercetin and its glycosides might exert their effects with differential manners in different regions of nervous systems.

In summary, we found that quercetin glycosides inhibit I\textsubscript{Gly} and I\textsubscript{SHT} in oocytes expressing the human glycine a1 or mouse 5-HT3A receptors. The glycosylation of quercetin exhibited differential effects on I\textsubscript{Gly} and I\textsubscript{SHT}, in that quercetin was more potent for the inhibition of I\textsubscript{Gly} than other quercetin glycosides, whereas quercetin and its glycosides except Rham1 were equally potent for the inhibition of I\textsubscript{SHT}.

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