Effect of Ginseng Saponins on the Recombinant Serotonin Type 3A Receptor Expressed in Xenopus Oocytes: Implication of Possible Application as an Antiemetic

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ABSTRACT

Objectives: Nausea and vomiting are the most frequently reported side-effects by patients who are given general anesthesia perioperatively and patients with cancer who undergo chemotherapy or radiotherapy. Serotonin (5-hydroxytryptamine, 5HT) type 3A receptor (5HT3A receptor) is known to mediate nausea and vomiting and its antagonists have been used effectively to prevent and/or reduce the incidence and severity of nausea and vomiting. However, the adverse effects on cardiac function, such as QT interval prolongation, limit their routine use by these patients. This study was designed to elucidate the effect of ginseng saponins on the recombinant 5HT3A receptor expressed in the xenopus oocyte.

Design: After in vitro transcription of the recombinant human 5HT3A receptor in the Xenopus laevis oocyte, we examined Panax ginseng saponins (total saponin [TS], panaxadiol saponin [PD] fraction, panaxatriol saponin [PT] fraction, and ginsenoside-Rb1 and -Rg1) for their ability to inhibit current flow through the 5HT3A receptor using the voltage-clamp technique.

Results: All saponin fractions (TS, PD, PT fraction, as well as ginsenoside-Rb1 and -Rg1) inhibited the peak current induced by the agonist 5HT on the 5HT3A receptor in a concentration-dependent, reversible, and voltage-independent manner. The PT fraction inhibited 5HT-induced currents in 5HT3A receptor more than the PD fraction; meanwhile, there was a similar degree of inhibition between ginsenoside-Rg1 and -Rb1, the main substitutes of PT fraction and PD saponin fractions, respectively.

Conclusions: These results indicate that ginseng saponins, especially PT fraction, have substantial inhibitory effects on the recombinant 5HT3A receptor, suggesting that some of the specific types of ginsenoside might have an antagonistic action against 5HT3A receptor related to nausea and vomiting.

INTRODUCTION

The root of Panax ginseng C.A. Meyer has been a well-recognized Oriental herbal medicine for centuries. Among its various constituents, ginseng saponins, specifically called ginsenosides, exert a variety of pharmacologic actions on the central nervous, cardiovascular, endocrine, and immune systems, among others (Attele et al., 1999). The ginseng saponins

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are structurally fractionated into two groups: the panaxadiol (PD) fraction (e.g., ginsenoside-Rb1, -Rb2, -Rc, -Rd) and the panaxatriol (PT) fraction (e.g., ginsenoside-Rg1, -Rg2, -Re, -Rf, depending on their glycons) (Shibata, 2001). Today, more than 30 types of ginsenosides have been identified and isolated, and among them, ginsenoside-Rb1 and ginsenoside-Rg1 are believed to be the most pharmacologically effective compounds of the PD and PT fractions, respectively (Attele et al., 1999).

5-Hydroxytryptamine type 3A receptors belong to the ligand-gated ion channel (LGIC) superfamily and share many structural similarities with nicotinic acetylcholine (nACh), glycine, and γ-aminobutyric acid (GABA A) receptors (Maricq et al., 1991). These receptors are diffusely distributed in both the central and peripheral nervous systems. 5HT3 receptors in the postrema area of the brain and in the visceral afferent nerves are thought to mediate nausea and vomiting associated with anticancer therapy and postoperative periods (Derkach et al., 1989). Currently, the selective 5HT3 receptor antagonists, such as ondansetron and granisetron, are used to prevent/reduce the incidence and severity of nausea and vomiting (Balfour and Goa, 1997; Karim et al., 1996; Watcha and White, 1992). However, controversy exists regarding their efficacy on delayed vomiting, and their high cost and possible detrimental effects on cardiac rhythms, such as prolongation of QT wave interval (Q-wave and T-wave interval), limit their routine use for patients with cancer treated with cardiotoxic chemotherapy (Keefe, 2002; Zarate et al., 2000). Based on the reports that nACh receptor antagonists, such as D-tubocurarine and granisetron, cross-reacted with the 5HT3A receptor (Min et al., 1998) and that some ginsenosides inhibited nACh receptors (Choi et al., 2002; Sala et al., 2002), a study of the inhibitory effect of ginseng saponins on the 5HT3A receptor was considered a valuable addition to the search for alternative and nontoxic antiemetics.

**MATERIALS AND METHODS**

Korean red ginseng saponin fractions (TS, PD, and PT) and ginsenoside-Rb1 and -Rg1 were supplied by Korea Ginseng and Tobacco Research Institute (Daejeon, Korea). Compositional ratio of PD/PT fraction in TS was approximately 1.6. Ginseng saponin fractions were dissolved in water, and ginsenoside-Rb1 and -Rg1 in dimethyl sulfoxide (DMSO) and were serially diluted with bath perfusate before use. The highest concentration of DMSO was less than 0.01% (v/v) and proved to have no effect on the 5HT3A receptor. Unless noted, all chemicals used were obtained from Sigma (St. Louis, MO).

Expression of 5HT3A receptor in Xenopus oocytes and electrophysiologic recordings

Human 5-HT3A receptor cDNA (Yamanouchi Pharmaceutical Co., Tokyo, Japan) was subcloned into a custom oocyte expression vector, pCR-Script SK(+) and linearized by SalI digestion to prepare template cDNA. cRNA was synthesized in vitro using T3 RNA polymerase (Message Machine, Ambion, TX) following the manufacturer’s recommended protocol. The Yonsei University Committee on Animal Care approved the protocol for oocyte harvesting. *Xenopus laevis* oocytes were taken via laparotomy under cold-immersion anesthesia with 0.15% 3-p-aminobenzoic acid for 30 minutes. Oocytes were dissected and placed in and treated with 0.5 mg/mL collagenase IA for 30 minutes to remove the follicular-cell layer. After the oocytes were rinsed several times, approximately 50 ng of cRNA was injected into stage V–VI oocytes by using a microinjector (Nanojector, Drummond Scientific, Broomall, PA). After a 48–96-hour incubation period at 18°C, an oocyte was placed into a plexiglass recording chamber approximately 300 μL in volume and continuously perfused with Ca2+-free, 1.8 mmol Ba2+ frog Ringer’s solution (in 115 mmol of NaCl, 2 mmol of KCl, 1.3 mmol of Na2HPO4, 1.8 mmol of BaCl2; pH 7.4) at 3–7 mL/min. The oocytes were penetrated with two glass electrodes with resistance of 1–3 MΩ when filled with 3 mol KCl solution. Two electrodes voltage clamp recordings at −40 mV−60 mV were obtained with an Oocyte Clamp (OC 725C, Warner Instruments, Hamden, CT). Serotonin with or without ginseng saponins was loaded on a bath by a computer-controlled solenoid valve. The bath solution exchange time constant was approximately 500 ms. The time
of drug application until the peak current and current digitization was controlled by Clampex v 5.7 (Axon Instruments, Burlingame, CA). Experiments were performed at intervals of 5–30 minutes to avoid the cumulative desensitization by previously applied drugs during all experiments. For the current-voltage relationships, fast ramps going from −90 to +40 mV were performed during 500 ms.

Data analysis and statistics

The oocytes showing leakage current of more than 0.02 μA were excluded. Peak currents induced by the drug applications were measured and concentration-response curves were fitted (SigmaPlot v.7.00; SPSS Science, Chicago, IL) to the equation, $I/I_{\text{max}} = C^n/(C^n + EC_{50}^n)$ where $I$ is the normalized peak current to control of 5HT-induced current, and $I_{\text{max}}$ is the maximal normalized peak current; $C$ is the concentration of 5HT, $n$ is the Hill coefficient and $EC_{50}$ is the concentration at which half-maximal peak current was induced. For the currents induced by coapplication of 5HT and ginseng saponins, we normalized them to the control response of 5HT alone. All values were represented as means ± standard error of the mean (SEM). Statistical analysis was performed by Student’s $t$ test with Bonferroni correction for multiple comparisons. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Characteristics of inhibition of ginseng saponin fractions and ginsenoside-Rb1 and -Rg1 on the 5HT$_{3A}$ receptor

5HT induced inward currents in the 5HT$_{3A}$ receptor cRNA injected oocytes but not in the control oocytes (data not shown). Ginseng saponins inhibited the peak current induced by 5HT and accelerated the desensitization of the inward current induced by 5HT. TS shifted the 5HT concentration–response curve to the right. Coapplication of 0.5 mg/mL TS and 100 μmol 5HT suppressed the inward currents down to 77.8% ± 5.36% of the maximal currents induced by 5HT alone (Fig. 1A and 1B). All ginseng saponins inhibited the 5HT-induced currents in concentration-dependant manner (Figs. 2A and 3A). This inhibition was completely restored after washing out (data not shown). Ginseng saponins (0.1 mg/mL TS, 50 μmol ginsenoside-Rb1 or -Rg1) had reversal potentials for 5HT$_{3A}$ receptor unaltered (approximately 0 mV) and inhibited 5HT-induced currents in a voltage-independent manner by the current-voltage relationships (Fig. 2B).

Comparisons of the inhibitory effect of PD and PT fractions and their main ginsenosides

Although the PD and PT fractions inhibited 2.5 μmol (Fig. 2A) or 1.0 μmol (Fig. 3B) 5HT-
induced currents in a concentration-dependent manner, the PT fraction inhibited 5HT-induced currents more strongly than PD fraction. Meanwhile, the ginsenoside-Rg1 of PT fraction to ginsenoside-Rb1 of PD fraction at 10 μmol or 100 μmol was not as prominent as the PD over PT (see right side plots of Fig. 3B).

DISCUSSION

In this study we have demonstrated that the ginseng saponins have an inhibitory effect on the 5HT₃A receptor expressed in Xenopus oocytes. Coapplication of TS-induced reduction of inward current down to 77.8% of the maximum response induced by serotonin at 30 μmol with shifting of the concentration–response curve of 5HT to the right (ED₅₀ 0.87 to 5.74; Hill coefficient, 1.78 to 1.27). This result leads to the hypothesis that ginseng saponins might be noncompetitive inhibitors of the 5HT₃A receptor. To prove this hypothesis further, inhibitory concentration–response with sequentially increased concentrations of TS must be applied. Perfusion of TS at a concentration higher than 1.0 mg/mL, however, slowed down the flow rate of the inline solenoid valve used in this study, thereby preventing us from performing the further experiments at a higher concentration of TS. In a comparison of the inhibitory effect of TS, PD, and PT, we found that the inhibitory action obtained by the PT fraction was much stronger

![FIG. 2. Further experimental evidence of inhibitory effect of ginseng saponin fractions and ginsenoside-Rb1 and -Rg1 on serotonin type 3A receptor expressed in Xenopus oocytes. A: Total saponin (TS) (0.1, 0.5, 1.0 mg/mL), panaxadiol saponin (PD) (0.05, 0.25, 0.5 mg/mL), and panaxatriol saponin (PT) (0.05, 0.25, 0.5 mg/mL) fraction inhibited currents induced by the 2.5 μmol serotonin in a concentration-dependent manner. The PT fraction inhibited more than the PD fraction at all concentrations (0.05 ~ 0.5 mg/mL) (p < 0.05). Bars represent standard error of the mean. Each datum was obtained from 4 to 6 oocytes. B: Current-voltage plots obtained by 500 ms ramp voltage commands from −90 mV to +40 mV during application of serotonin, 1.0 μmol alone (solid line) or coapplication of 1 μmol of serotonin and ginseng saponins (0.1 mg/mL of TS [dash-dot-dot line], 50 μmol of ginsenoside-Rb1 (dotted line), and 50 μmol of ginsenoside-Rg1 [dashed line]). The I–V curves of coapplication of ginseng saponins demonstrate the voltage-independent response and unaltered reversal potential of 5HT₃A receptor approximately at 0 mV.

![FIG. 3. Comparisons of inhibitory effects of total saponin (TS), panaxadiol saponin (PD), and panaxatriol saponin (PT) fractions, and their main ginsenosides, ginsenoside-Rb1 and ginsenoside-Rg1. A: From left to right, current traces recorded in response to coapplication of increased concentrations of TS (0, 0.13, 0.25, and 0.50 mg/mL), PD (0, 0.06, 0.13, and 0.25 mg/mL), PT fraction (0, 0.06, 0.13, and 0.25 mg/mL), ginsenoside-Rb1 (0, 1, 10, and 100 μmol), and ginsenoside-Rg1 (0, 1, 10, and 100 μmol) with 1 μmol of serotonin in oocytes at −50 mV. B: In the left graph, the PT fraction (gray bar) inhibited serotonin-induced currents more than the PD fraction (black bar) at all concentrations (*p < 0.001). The right graph shows no significant inhibitory differences between ginsenoside-Rg1 (black bar) and -Rb1 (gray bar) both were α = 10 and 100 μmol. Bars represent standard error of the mean. Each datum was obtained from 6 oocytes.
than that by the PD fraction. Considering that the overall ratio of PD/PT fraction in TS used in this experiment was 1.6 and that there were no inhibitory summations of PD and PT fraction compared to TS, it is reasonable to assume that the inhibitory effect of TS might have arisen mainly from the PT fraction and there might be a common pathway in the inhibitory mechanism of ginseng saponins on 5HT3A receptor. It is also worth noting that there was no marked difference between ginsenoside-Rb1 and -Rg1 in inhibition of 5HT3A receptor (Fig. 2B).

We have observed that both receptors cross-react with the nACh receptor antagonist, d-tubocurarine (Min et al., 2000; Yan et al., 1998) and the 5HT3A receptor antagonist, ICS-205,930 (Rothlin et al., 1999) probably because of the high degree of structural homology between the nACh and the 5HT3A receptor (Mariq et al., 1991). Ginsenosides are reported to inhibit both of the ionotrophic and metabotropic ion channels (Tachikawa et al., 1999) as well as the voltage-independent Na+ channel (Tachikawa et al., 1995). Regarding the nACh receptors, there are reports that cotreatment with ginsenoside-Rg2 and acetylcholine inhibited ACh-induced currents in oocytes expressing with α3β4 or αβδε but not with α7 nACh receptors. The inhibitory properties of ginsenoside-Rg2 in nACh receptor were reversible, dose-dependent, voltage-independent, and noncompetitive (Choi et al., 2002; Sala et al., 2002), but the lack of an inhibitory effect of ginsenosides on the homomeric nACh receptor contradicts our findings of an inhibitory effect of ginsenosides on homomeric 5HT3A receptor. Although it is not known how ginseng saponins act on the 5HT3A receptor, ginsenosides are likely to act on the polar side of the cell membrane because the 5HT3A receptor is an LGIC situated in the phospholipid layer of the cell membrane. The OH− of the ginsenoside is likely to react largely with the OH− of cholesterol-rich plasma membrane rather than the cholesterol-poor sarcoplasmic Ca2+-adenosine triphosphatase (ATPase) in cytoplasm (Schroeder et al., 1991). Ginseng saponins are less likely to act on homomeric 5HT3A receptor as open-channel blocker because the current–voltage relationship has voltage-independent inhibition and unaltered reversal potential (approximately 0 mV) (Yakel et al., 1993). The steroidal structure of ginsenosides might modify the physical properties of the phospholipid bilayer and modulate the activity of membrane protein structure by changing the fluidity of plasma-membrane dynamics and modulating activity of ion channels, membrane-bound receptors, and enzymes (Brann et al., 1995). This may be comparable to our findings that all ginseng saponins accelerate the desensitization of 5HT-induced currents.

The 5HT3 receptor is well-known to mediate the nausea and vomiting associated with anesthesia and surgery, and anticancer chemotherapy. In a survey of patients with cancer, 80% experienced nausea and 57% experienced vomiting, which resulted in deterioration of the quality of their life (de Boer-Dennert et al., 1997). Currently, 5HT3A receptor-selective antagonists, such as ondansetron and granisetron, have been effectively used as antiemetics but their therapeutic effect for treating delayed vomiting is not clear (Roila and Del Favero, 1997). Some clinicians suggest cautious use of 5HT3A receptor-selective antagonists for patients with cancer because of the possible cardiac toxicity such as arrhythmias (Keefe, 2002).

Ginseng saponins as a natural product have shown fewer and, if any, mild side-effects. The inhibitory effect of ginseng saponin on 5HT3A receptor suggests a new and interesting approach to the management of nausea and vomiting in patients with cancer or those receiving anesthesia during surgery as well as patients with gastrointestinal problems such as irritable bowel syndrome. Although further experiments are needed to determine which types of ginsenoside are specific to the 5HT3A receptor, this study suggests that ginseng saponins may be effective alternatives to the currently available 5HT3A receptor antagonists because ginseng saponins have been used safely in Oriental countries for many centuries and more recently in Western countries.

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