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Ginsenosides: Are Any of them Candidates for Drugs Acting on the Central Nervous System?

Seung-Yeol Nah,[1] Dong-Hyun Kim,[2] and Hyewhon Rhim[3]

[1] *Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, Korea*
[2] *College of Pharmacy, Kyung Hee University, Seoul, Korea*
[3] *Center for Chemoinformatics Research, Life Sciences Division, Korea Institute of Science and Technology (KIST), Seoul, Korea*

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ABSTRACT

The last two decades have shown a marked expansion in the number of publications regarding the effects of *Panax ginseng*. Ginsenosides, which are unique saponins isolated from *Panax ginseng*, are the pharmacologically active ingredients in ginseng, responsible for its effects on the central nervous system (CNS) and the peripheral nervous system. Recent studies have shown that ginsenosides regulate various types of ion channels, such as voltage-dependent and ligand-gated ion channels, in neuronal and heterologously expressed cells. Ginsenosides inhibit voltage-dependent Ca^{2+}, K^+, and Na^+ channel activities in a stereospecific manner. Ginsenosides also inhibit ligand-gated ion channels such as N-methyl-D-aspartate, some subtypes of nicotinic acetylcholine, and 5-hydroxytryptamine type 3 receptors. Competition and site-directed mutagenesis experiments revealed that ginsenosides interact with ligand-binding sites or channel pore sites and inhibit open states of ion channels. This review will introduce recent findings and advances on ginsenoside-induced regulation of ion channel activities in the CNS, and will further expand the possibilities that ginsenosides may be useful and potentially therapeutic choices in the treatment of neurodegenerative disorders.

Address correspondence and reprint requests to: Dr. Hyewhon Rhim, Center for Chemoinformatics Research, Life Sciences Division, Korea Institute of Science and Technology (KIST), 39-1 Hawholgok-dong Sungbuk-gu, Seoul 136-791, Korea. Tel.:+82-2-958-5923; Fax:+82-2-958-5909; E-mail: hrhim@kist.re.kr

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INTRODUCTION

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), has been used as a representative tonic for 2000 years in Far East countries such as China, Japan, and Korea (Fig. 1). Now, ginseng is one of the most famous and precious herbal medicines consumed around the world (Tyler 1995). Although ginseng exhibits multiple pharmacological actions in both *in vitro* as well as *in vivo* (Attele et al. 1999; Nah 1997), the mechanisms of its various effects are still elusive. However, recently accumulated evidence shows that ginsenosides, the main active ingredients of ginseng, produce their pharmacological actions by modulating membrane proteins such as voltage-dependent or ligand-gated ion channels (Kim et al. 2002; Lee et al. 2004b; Lee et al. 2007a; Nah et al. 1995; Sala et al. 2002). Ginsenosides are derivatives of triterpenoid dammarane, which consists of thirty carbon atoms (Fig. 2). Each ginsenoside has a common four-ring hydrophobic steroid-like structure with attached sugar moieties. About 30 different types of ginsenosides have been isolated and identified from the root of *Panax ginseng* (Baek et al. 1996; Liu and Xiao 1992). They are mainly classified into protopanaxadiol (PD) and protopanaxatriol (PT) ginsenosides according to the position of different carbohydrate moieties at the C-3 and C-6 positions (Nah 1997). Each type of ginsenoside has also at least three side chains at the C-3, C-6, or C-20 position. These side chains are free or coupled to sugar containing monomers, dimers, or trimers. These sugar components might provide specificity for the cellular effects of each ginsenoside (Choi et al. 2001b; Nah 1997; Nah et al. 1995; Rhim 2003; Rhim et al. 2002). However, ginsenosides are hydrophobic compounds, since they are not water-soluble.

As mentioned previously, ginsenosides produce diverse pharmacological effects *in vivo* and *in vitro*. This review article will focus mainly on ginsenoside-induced ion channel regulation, since recent reports show that ginsenosides regulate various types of ion channels. It will cover some recent observations on ginsenoside-induced ion channel regulation and will speculate on possible biological effects of ginsenosides.

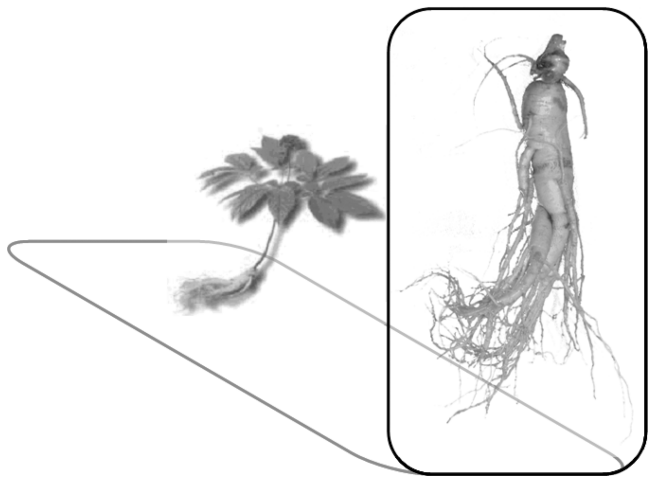
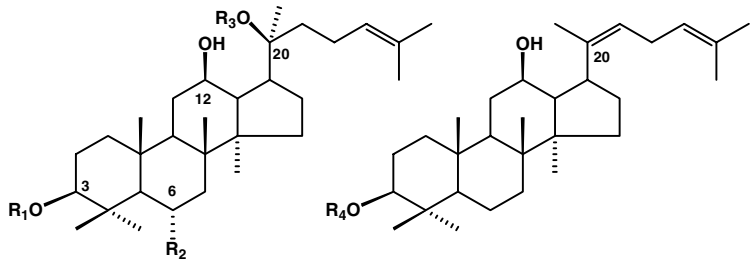


FIG. 1. The root of *Panax ginseng* C.A. Meyer (Araliaceae).



Ginsenosides	R_1	R_2	R_3	R_4	PD or PT
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc	-	PD
Rb_2	-Glc_2-Glc	-H	-Glc_6-Ara(pyr)	-	PD
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)	-	PD
Rd	-Glc_2-Glc	-H	-Glc	-	PD
Re	-H	-Glc_2-Rha	-Glc	-	PT
Rf	-H	-Glc_2-Glc	-H	-	PT
Rg_1	-H	-Glc	-Glc	-	PT
Rg_2	-Hc	-Glc_2-Rha	-H	-	PT
Rg_3	-Glc_2-Glc	-H	-H	-	PD
Rg_5	-	-	-	-Glc_2-Glc	PD
Rh_1	-H	- O-Glc	-Glc_6-Glc	-	PT
Rh_2	-Glc	-H	-Glc_6-Glc	-	PD
Rh_3	-	-	-	-Glc	PD

FIG. 2. Chemical structures of the major types of ginsenosides. Numbers indicate the carbon in the glucose ring that links the two carbohydrates. Abbreviations for carbohydrates are as follows: Glc = glucopyranoside; Ara(fur) = arabinofuranose; Ara(pyr) = arabinopyranoside; and Rha = rhamnopyranoside. PD and PT stand for protopanaxadiol and protopanaxatriol ginsenosides, respectively.

DIVERSITY OF GINSENOSIDES AND METABOLISM

The root of freshly harvested ginseng is called "fresh ginseng," and the dried form of ginseng for long-term storage is called "white ginseng." When it is steamed in a specific way and dried, it is called "red ginseng." The major components of fresh and dried ginsengs are manloyl-ginsenosides Rb_1, Rb_2, Rc, and Rd, and ginsenosides Rb_1, Rb_2, Rc, Re, Rf, Rg_1, and Rg_2 (Tanaka et al. 1972). However, red ginseng also contains ginsenosides Rg_3, Rg_5, Rh_1, and Rh_2 (Fig. 2) (Kitagawa et al. 1983). If ginsengs are orally administered to humans, their constituents cannot be easily absorbed by the intestines due to their hydrophilicity (Akao et al. 1998b; Bae et al. 2002a; Hasegawa et al. 1997). Inevitably, in the intestinal tract they come into contact with and are metabolized by the intestinal microflora. For example, PD ginsenosides Rb_1, Rb_2, and Rc of fresh and white ginsengs are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-PD (compound K) by human intestinal bacteria (Akao et al. 1998b; Bae et al. 2002a; Bae et al. 2000). The PD ginsenosides Rg_3 and Rg_5 of red ginseng are transformed to ginsenosides Rh_2 and Rh_3, respectively (Bae et al. 2002b; Bae et al. 2004a). The PT ginsenosides Re and Rg_1 are transformed to ginsenoside Rh_1 and further to PT (Fig. 3) (Bae et al. 2005; Wang et al. 2000). The metabolites are then easily absorbed from the gastrointestinal tract, since most of the metabolites are nonpolar compared to the parent components. For example, when ginsenoside Rb_1 is orally administered to rats, its metabolite, compound K, but not ginsenoside Rb_1, is absorbed into the circulation (Akao et al. 1998a; Akao et al. 1998b). When standardized extracts of *Panax ginseng* (G-115,

100 mg) are given orally to humans, some metabolites, such as compound K and ginsenosides Rg_1 and Rh_1, are detected in the blood (Tawab et al. 2003). These absorbed metabolites may produce pharmacological actions. Nevertheless, many researchers have not considered the metabolism of ginseng components by intestinal microflora in the evaluation of ginseng's pharmacology.

EFFECTS OF GINSENOSIDES ON VOLTAGE-DEPENDENT CHANNELS

Effects on Voltage-Dependent Ca^{2+} Channels

Ca^{2+} is a second messenger for the regulation of contraction, plasticity, secretion, synaptic transmission, and gene expression (Berridge et al. 1998b; Catterall 2000; Ghosh and Greenberg 1995). Cytosolic Ca^{2+} elevation in excitable cells is mainly achieved through Ca^{2+} influx via presynaptic Ca^{2+} channels, which are activated by membrane depolarization. The elevation of presynaptic Ca^{2+} is closely coupled to neurotransmitter release. Recent reports showed that there are at least five different Ca^{2+} channel subtypes such as L-, N-, P/Q-, R- and T-types, and their precise physiological and pharmacological functions are still under investigation (Miller 2001). Cytosolic Ca^{2+} is very tightly controlled under normal conditions, since cytosolic Ca^{2+} overload leads to the production of oxidative

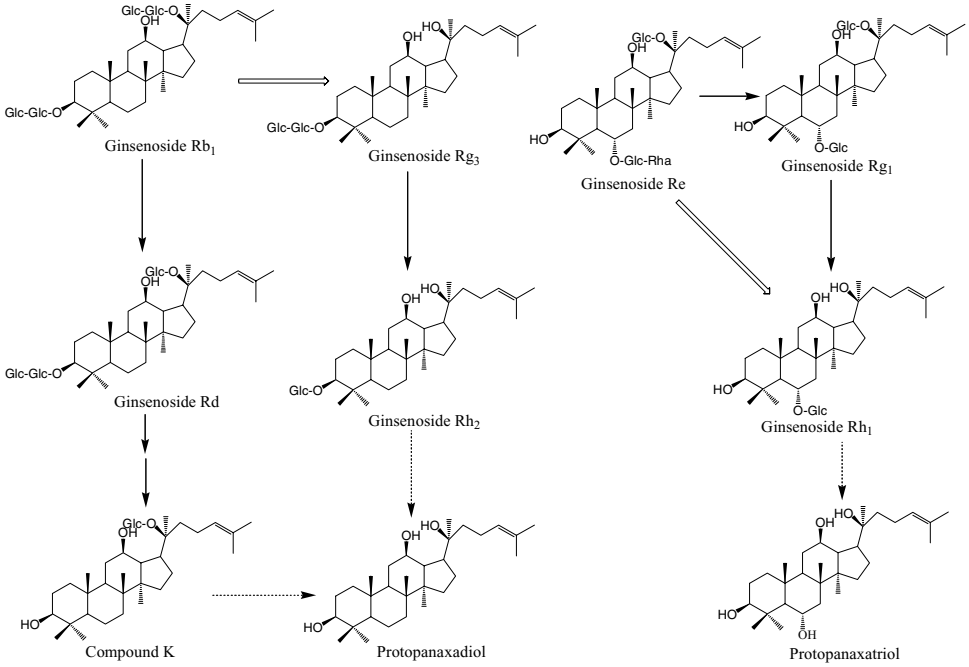


FIG. 3. Structures and metabolic pathways of ginsenoside Rb_1, Re, Rg_1, and Rg_3 by human intestinal microflora (→, main pathway by intestinal microflora; ····>, minor pathway by intestinal microflora; ⇒, chemical transformation by steaming). Adapted from Lee et al. (2006a).

radicals and triggers the activation of various enzymes that are harmful to cells (Berridge 1998a; Berridge et al. 1998b). For example, abnormal conditions such as stroke, ischemia, or excitotoxic insults have been linked to the loss of cytosolic Ca^{2+} homeostasis and are thought to lead to secondary excitotoxicity by activating N-methyl-D-aspartate (NMDA)/non-NMDA receptors (Choi and Rothman 1990; Meldrum and Garthwaite 1990). Since elevation of intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) caused by excessive stimulation of Ca^{2+} channels and/or excitatory NMDA receptors is an early indicator of excitotoxic damage to neuronal cells, agents blocking the elevation of $[Ca^{2+}]_i$ by regulating Ca^{2+} channels and/or NMDA receptors might have neuroprotective effects (Menne et al. 2006; Nikonenko et al. 2005; Rothman and Olney 1995; Sattler and Tymianski 2000).

Recent reports show that ginsenosides inhibit Ca^{2+} channels in neuronal cells and heterologous cell lines. In rat sensory neurons, ginsenosides such as ginsenosides Rb_1, Rc, Re, Rf, and Rg_1 at 100 μM reversibly inhibit N-type and other high-voltage-activated (HVA) Ca^{2+} channels via pertussis toxin (PTX)-sensitive G proteins (Nah and McCleskey 1994; Nah et al. 1995). On the other hand, Kim et al. (1998a) demonstrated that ginsenosides inhibit Ca^{2+} channels in rat chromaffin cells, which are neurosecretory cells involved in the release of catecholamines under various stress situations. When used at 100 μM the order of inhibitory activity of ginsenosides on Ca^{2+} channels of rat chromaffin cells is: ginsenoside Rc > Re > Rf > Rg_1 > Rb_1. In bovine chromaffin cells ginsenosides are selective for N-, P/Q-, and R-, but not L-type Ca^{2+} channels (Choi et al. 2001a). Rhim et al. (2002) showed that in rat sensory neurons ginsenoside Rg_3 at 100 μM more effectively inhibited L-, N-, and P/Q-types of Ca^{2+} channels than other ginsenosides tested. Lee et al. (2006b) have identified the major component(s) of ginsenosides or ginsenoside metabolites regulating cloned Ca^{2+} channel subtypes such as α_{1C} (L)-, α_{1B} (N)-, α_{1A} (P/Q)-, α_{1E} (R)-, and α_{1G} (T)-types using two-microelectrode voltage clamp techniques. They further characterized the effects of ginsenosides and ginsenoside metabolites on Ba^{2+} currents (I_{Ba}) in *Xenopus* oocytes expressing five different Ca^{2+} channel subtypes. This study demonstrated that among various ginsenosides such as Rb_1, Rc, Re, Rf, Rg_1, Rg_3, Rh_2, ginsenoside Rg_3, at 100 μM, effectively inhibited all five Ca^{2+} channel subtypes, whereas ginsenoside Rh_2 inhibited more efficiently α_{1C}- and α_{1E}-type Ca^{2+} channels than other channel types. Compound K, a PD ginsenoside metabolite, strongly inhibited only α_{1G}-type Ca^{2+} channels, whereas M4, a PT ginsenoside metabolite, had almost no effect on any of the subtypes of Ca^{2+} channels examined. Ginsenosides Rg_3, Rh_2, or compound K shifted the steady-state activation curve in the depolarizing direction in α_{1B}- and α_{1A}-types with no shift in the inactivation curve. These results reveal that ginsenosides Rg_3, Rh_2, and compound K are Ca^{2+} channel regulators and are selective in inhibiting certain Ca^{2+} channel subtypes. In addition to Ca^{2+} channel inhibition, ginsenosides also attenuate the stimulated membrane capacitance increase (ΔC_m) in rat chromaffin cells (Kim et al. 1998a). The order of inhibitory activity of ginsenosides, at 100 μM, on ΔC_m was ginsenoside Rf > Rc > Re > Rg_1 > Rb_1. The attenuation of Ca^{2+} channel and membrane capacitance by ginsenosides suggests that they might be closely involved in the regulation of neurotransmitter release from nerve terminals.

Effects on Various K^+ Channels

There are many types of K^+ channels in living cells. The following types of K^+ channels have been identified in neuronal and non-neuronal systems: voltage-dependent,

Ca^{2+}-activated, ATP-sensitive, and G-protein-coupled inwardly rectifying (GIRK) channels (Wickman and Clapham 1995). Most K^+ channels are involved in the regulation of repolarization, duration of depolarization in excitable cells, and the relaxation of smooth muscle by allowing the efflux of K^+ ion from cytosol. It is well known that ginsenosides relax blood vessels and other smooth muscles (Kim et al. 1999), but their mechanism of action has not been clearly demonstrated. Recent reports showed that ginseng total saponins (50–500 μg/mL) and ginsenoside Rg_3 (100 μg/mL) activate Ca^{2+}-activated and ATP-sensitive K^+ channels in rabbit coronary artery smooth muscle cells (Chung and Kim 1999a; Chung and Lee 1999b). Li et al. (2001) demonstrated that ginsenosides (at 50 μg/mL) activated Ca^{2+}-activated K^+ channels in cultured vascular smooth muscle as well as in endothelial cells. In endothelial cells, the potentiation of the activity of Ca^{2+}-activated K^+ channels by ginsenosides may enhance Ca^{2+} influx and increase NO secretion. In the case of vascular smooth muscle cells, this effect may inhibit Ca^{2+} influx and relax vascular smooth muscle cells. These results suggest the possibility that ginsenosides might stimulate membrane components for intracellular Ca^{2+} mobilization. The mobilized Ca^{2+} will activate Ca^{2+}-activated K^+ channels, which in turn would mediate repolarization of smooth muscle cells depolarized by various endogenous or exogenous stimuli.

On the other hand, GIRK channels are known to regulate firing rate, membrane potential, and neurotransmitter responses, resulting in postsynaptic hyperpolarization in the brain. In the brain, GIRK channels are expressed mainly in the olfactory bulb, hippocampus, dentate gyrus, and cortex. In the heart, acetylcholine released from the vagus nerve binds to M_2 receptors in the heart and activates GIRK channels, slowing the heart rate (Dascal 1997). One study showed that ginsenoside Rf activates GIRK channels when GIRK channel genes are co-expressed in *Xenopus* oocytes with rat brain mRNA (Choi et al. 2002a). The effect of ginsenoside Rf on GIRK current was concentration dependent and reversible; the EC_{50} was 34 ± 3 μM, and the maximal effect was obtained at about 100 μM. Other ginsenosides such as ginsenosides Rb_1 and Rg_1 slightly activate this channel. Ginsenoside Rf-induced GIRK current enhancement was blocked by Ba^{2+}, a K^+ channel blocker. Intracellular injection of GDPβS, but not pretreatment with PTX, attenuated ginsenoside Rf-induced GIRK currents (Choi et al. 2002a). These results provide evidence that ginsenoside Rf interacts with unidentified ginsenoside Rf-binding protein(s) in the brain, and the activation of unidentified ginsenoside Rf-binding protein(s) could be coupled to GIRK channels. Thus, the activation of Ca^{2+}-activated K^+ channels through intracellular Ca^{2+} mobilization or the activation of GIRK channels by ginsenosides might provide further evidence that ginsenosides regulate the electrical state of excitable cells. In contrast, Jeong et al. (2004) showed that ginsenoside Rg_3 inhibits $K_v1.4$ voltage-dependent K^+ channels expressed in *Xenopus* oocytes.

Effects on Voltage-Dependent Na^+ Channels

Activation of voltage-dependent Na^+ channels is directly involved in the induction of action potentials in axonal and somatic portions of neurons. They are also involved in actively propagating axonal or dendritic information from one part of a neuron to another. There are two reports on the regulation of Na^+ channel by ginsenosides. Liu et al. (2001) and Jeong et al. (2004) showed that ginsenosides inhibit brain-specific Na^+ channels ($Na_v1.2$) expressed in tsA201 cell lines and *Xenopus laevis* oocytes, respectively. Liu et al. (2001) used

much higher concentrated ginseng extract (3 mg/mL) and ginsenoside Rb_1 (at 150 μM) than those used in other channel regulation studies. Jeong et al. (2004) showed that at 100 μM ginsenoside Rg_3 was much more effective than other ginsenosides tested and suggested that ginsenoside Rg_3 may be a candidate for neuronal Na^+ channel regulation. Further studies on the molecular mechanisms underlying ginsenoside Rg_3-induced Na^+ channel inhibition using site-directed mutagenesis revealed that ginsenoside Rg_3 induces tonic and use-dependent inhibition ($IC_{50} = 32 \pm 6$ μM) of peak Na^+ currents (I_{Na}) (Lee et al. 2005b). Ginsenoside Rg_3 produced a significant depolarizing shift in the activation voltage but did not alter the steady-state inactivation voltage. Mutations in channel entrance, pore region, lidocaine/tetrodotoxin-binding sites, or voltage sensor segments did not affect ginsenoside Rg_3-induced tonic blockade of peak I_{Na}. However, ginsenoside Rg_3 inhibited the peak and plateau I_{Na} in the IFMQ3 mutant, which is inactivation gate deficient, indicating that ginsenoside Rg_3 inhibits both the resting and open states of Na^+ channels. Neutralization of the positive charge at position 859 of the voltage sensor segment domain II abolished the ginsenoside Rg_3-induced activation voltage shift and use-dependent inhibition. These results reveal that ginsenoside Rg_3 is a novel Na^+ channel inhibitor capable of acting on resting and open states of Na^+ channels via interactions with the S4 voltage-sensor segment of domain II.

EFFECTS OF GINSENOSIDES ON LIGAND-GATED ION CHANNELS

Effects on NMDA-Gated Ion Channels

Glutamate, one of the major excitatory neurotransmitter in the central nervous system (CNS), plays an important role in neuronal plasticity and neurotoxicity. Abnormalities in glutamate neurotransmitter systems may be involved in neurological disorders, such as Alzheimer's disease, ischemia, seizures, and head or spinal cord trauma (Chapman 2000; Ikonomidou and Turski 1996; Lee et al. 2002a). The accumulation of glutamate in extracellular space under these neurological disorders can induce neuronal death, and this glutamate toxicity has been clearly attributed to a massive influx of Ca^{2+}, primarily through NMDA receptors (Choi and Rothman 1990; Coyle and Puttfarcken 1993; Sattler and Tymianski 2000). The concept that NMDA receptors are crucial in glutamate neurotoxicity are in agreement with earlier claims that intracellular Ca^{2+} overload is a key component of glutamate-mediated neurotoxicity, as well as with observations indicating that NMDA-antagonist drugs could attenuate neuronal death in animal models of ischemic or hypoglycemic brain injury (Meldrum and Garthwaite 1990; Rothman and Olney 1995; Sattler and Tymianski 2000; Wieloch 1985). Based on these ideas and data, several academic laboratories and pharmaceutical companies developed NMDA receptor antagonists and proceeded to test them in human clinical trials (Lee et al. 1999). In addition to synthetic drugs, alternative medicines, such as herbal products, are being increasingly used for the prevention or treatment of brain injury.

The effectiveness of ginseng in the prevention of neuronal cell death due to ischemia or glutamate toxicity has been reported. In rat cortical cultures, ginsenosides Rb_1 and Rg_3 at 10 μM attenuated glutamate- and NMDA-induced neurotoxicity by inhibiting overproduction of nitric oxide, formation of malondialdehyde, and the influx of Ca^{2+} (Kim

et al. 1998b). Seong et al. (1995) showed that ginseng total saponins attenuated glutamate-induced swelling of rat cultured astrocytes in a concentration-dependent manner. On the other hand, an *in vivo* study using anesthetized rats, showed that by intracerebroventricular (i.c.v.) administration of ginsenoside Rb_1, but not Rg_1, significantly reduced the magnitude of long-term potentiation (LTP) induced by a strong tetanus in the dentate gyrus without any effect on the basal synaptic responses evoked by a low-frequency stimulation (Abe et al. 1994). The inhibitory effects of ginsenoside Rb_1, at 0.5 to 50 nmol, were concentration dependent. Pretreatment with ginsenosides, by intrathecal administration, attenuated NMDA- or substance P- but not glutamate-induced nociceptive behavior (Nah et al. 1999; Yoon et al. 1998). The IC_{50} values of ginsenosides for inhibition of NMDA- or substance P-induced pain were 37 and 43 μg/mouse, respectively. In addition, pretreatment with ginsenosides (50 or 100 mg/kg i.p. for 7 days) attenuated kainic acid-induced death of hippocampal neurons (Lee et al. 2002b). These results indicate that ginsenosides might interact with various excitatory neurotransmitter receptors, and that these interactions might lead to neuroprotection from excitotoxins in the CNS.

Despite the beneficial effects of ginseng on the CNS, little scientific evidence has been obtained at the cellular level. Using fura-2-based digital imaging and whole-cell patch-clamp techniques, a series of studies examining the direct modulation by ginseng of glutamate, and especially NMDA, receptors has been conducted in an attempt to identify the active component(s) of ginseng in rat cultured hippocampal neurons (Kim et al. 2002; Kim et al. 2004; Lee et al. 2006a). Kim et al. (2002) showed that at 100 μg/mL ginseng attenuates glutamate-, or NMDA-induced Ca^{2+} influx, and NMDA-gated currents. Subsequently, they examined the effects of individual ginsenosides on NMDA-induced Ca^{2+} influx. Among the 10 ginsenosides tested at 10 μM (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_2, Rg_3, Rh_1, and Rh_2) (Fig. 2), ginsenoside Rg_3 appeared to be the most effective component of ginseng in cultured hippocampal neurons. However, as previously stated, following oral administration ginsenosides are known to be metabolized in the intestines (Bae et al. 2002b; Hasegawa et al. 1996). In addition, ginsenosides exist as stereoisomers; 20(*R*)-ginsenosides and 20(*S*)-ginsenosides are epimers. Most studies with ginseng examined the activities of mixtures of 20(*R*)- and 20(*S*)-ginsenosides without purification of individual isomers. When the effects of 20(*S*)-ginsenosides Rb_1, Rg_1 and Rg_3, the three most commonly studied ginsenosides in the CNS, and their main metabolites [20(*S*)-ginsenosides Rd, Rh_1, Rh_2, PD, PT, and 20(*S*)-compound K] were examined at 10 μM (Fig. 4), the highest inhibitory effect of 20(*S*)-ginsenoside Rg_3 was confirmed (Lee et al. 2006a). In this study on cultured rat hippocampal cells, NMDA-induced $[Ca^{2+}]_i$ increase was evoked by addition of NMDA (100 μM, for 10 sec) in Mg^{2+}-free and 1 μM glycine-containing solution and measured using fura-2-based intracellular Ca^{2+} imaging techniques. However, at 10 μM 20(*S*)-ginsenoside Rh_2 also selectively inhibited NMDA receptors with similar efficacy as 20(*S*)-ginsenoside Rg_3. The magnitude of inhibition by 20(*S*)-ginsenoside Rg_3 and 20(*R*)-ginsenoside Rg_3 was similar. However, the inhibitory effect of 20(*R*)-ginsenoside Rh_2 was significantly smaller than that of 20(*S*)-ginsenoside Rh_2. These results suggest that ginsenoside Rh_2, unlike Rg_3, has stereospecific effect. When 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2 were tested together at a submaximal concentration (3 μM), they produced additive effects.

The NMDA receptor channel complex has a number of regulatory sites that are targets for modulation by endogenous as well as exogenous compounds. The main regulatory sites include agonist NMDA-, co-agonist glycine-, polyamine-binding sites, and sites within the channel lumen (Lerma et al. 1998). When the effects of ginsenoside Rg_3 (the mixture of

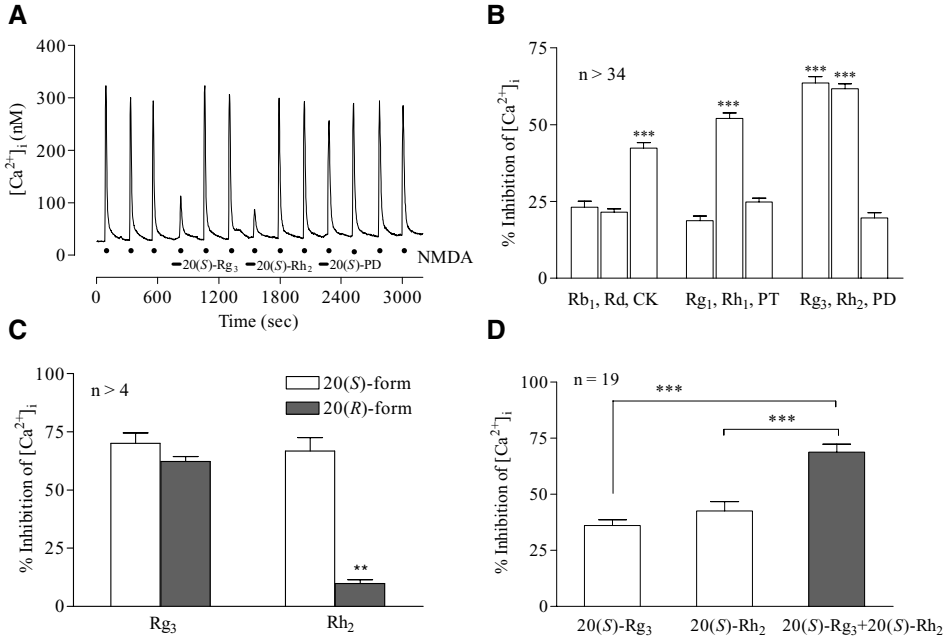


FIG. 4. Identification of active components responsible for ginseng-mediated inhibition on hippocampal NMDA receptors and the additive effect of 20(*S*)-Rg_3 and 20(*S*)-Rh_2 when used at submaximal concentration. In *A*, black dot (•) represents acute application of 100 μM NMDA in cultured rat hippocampal cells. $^{***}P < 0.001$ *versus* 20(*S*)-Rg_1 in *B* and indicated controls in *D*. $^{**}P < 0.01$ *versus* 20(*S*)-Rh_2 in *C*. In *A*-*C*, each ginsenoside was used at 10-μM concentration. In *D*, a submaximal concentration (3 μM) was used for the additive effect. Adapted from Lee et al. (2006a).

20(*S*)- and 20(*R*)-ginsenoside Rg_3, devoid of stereospecificity), or of 20(*S*)-ginsenoside Rh_2, were examined at these regulatory sites in cultured hippocampal neurons (Kim et al. 2004; Lee et al. 2006a), the NMDA-binding site appeared to be the target site modulated by the active components of ginseng. On the other hand, ginsenoside Rg_3 produced its effect in a glycine concentration-dependent manner and shifted the glycine concentration-response curve to the right without changing the maximal response, suggesting that ginsenoside Rg_3 may act as a competitive NMDA receptor antagonist. This pattern of glycine concentration dependence was, however, not observed with 20(*S*)-ginsenoside Rh_2. There was no significant difference between the mean percentage inhibition by 20(*S*)-ginsenoside Rh_2 in the absence and in the presence of a high concentration of glycine (100 μM), suggesting that the mechanism of inhibitory action of 20(*S*)-ginsenoside Rh_2 may be different from that of ginsenoside Rg_3. Further studies showed that 20(*S*)-ginsenoside Rh_2 seems to inhibit the receptors by interaction with polyamine-binding sites as a competitive antagonist. It appears, therefore, that the two main active ingredients of ginseng, 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2, produced their inhibitory effect by modulation of NMDA receptors, but target different regulatory sites of the NMDA receptor channel complex. In hippocampal neurons, 20(*S*)-ginsenoside Rh_2 appears to be a competitive NMDA antagonist at the polyamine-binding site; whereas 20(*S*)-ginsenoside Rg_3 may inhibit NMDA receptors by interacting with the glycine-binding site. If a new and non-metabolizable form of

20(*S*)-ginsenoside Rg_3 could be developed, 20(*S*)-ginsenoside Rh_2, along with the newly modified form of 20(*S*)-ginsenoside Rg_3, would represent potentially useful therapeutic choices for the treatment of neurodegenerative disorders.

Effects on Nicotinic Acetylcholine Ligand-Gated Ion Channels

Nicotinic acetylcholine receptor is one of most extensively investigated receptors among various ligand-gated ion channels. The activation of this receptor channel by acetylcholine allows the influx of cations, mostly Na^+ ions, into the cells. Muscular nicotinic receptor channels consist of $\alpha 1\beta 1\delta\gamma$ (embryonic form) or $\alpha 1\beta 1\delta\varepsilon$ (adult form) subunits (Lindstrom 1995). Neuronal nicotinic receptors consist of α ($\alpha 2 - \alpha 9$) and β ($\beta 2 - \beta 4$) subunits. The α subunit alone can form functional homomeric receptors, and α and β subunits can form functional heteromeric receptors. However, their distribution depends on organ type or the region of the nervous system (Sargent 1993). Interestingly, some reports showed that ginsenosides (1–100 μM) inhibit Na^+ influx into bovine chromaffin cells stimulated by acetylcholine, but not high K^+, and attenuate the release of catecholamines from chromaffin cells, which contain mainly $\alpha 3\beta 4$ nicotinic acetylcholine receptors (Campos-Caro et al. 1997; Tachikawa et al. 1995). Furthermore, at 100 μM, ginsenosides also inhibit acetylcholine-induced inward currents in *Xenopus* oocytes expressed with nicotinic receptors containing $\alpha 1\beta 1\delta\varepsilon$ or $\alpha 3\beta 4$ subunits but not $\alpha 7$ subunit, showing the possibility that ginsenosides differentially regulate nicotinic acetylcholine receptor channels (Choi et al. 2002b). The inhibition of acetylcholine-induced inward currents by ginsenosides in oocytes (expressed with nicotinic acetylcholine receptors containing $\alpha\beta\delta\varepsilon$ or $\alpha 3\beta 4$ subunits) was reversible, voltage independent, and noncompetitive. Ginsenosides themselves had no effect on basal currents in oocytes expressing nicotinic acetylcholine receptors containing $\alpha\beta\delta\varepsilon$ or $\alpha 3\beta 4$ subunits. Interestingly, it appears that PTs, such as ginsenosides Re, Rf, Rg_1, or Rg_2, were more effective than PDs, such as ginsenosides Rb_1, Rb_2, Rc, or Rd, in inhibiting acetylcholine-induced inward currents (Choi et al. 2002b). Sala et al. (2002) also demonstrated that at 100 μM ginsenoside Rg_2 reduces peak current and increases the desensitization of acetylcholine-induced inward currents in oocytes expressing human neuronal nicotinic acetylcholine receptors such as $\alpha 3\beta 4$, $\alpha 3\beta 2$, $\alpha 4\beta 4$, and $\alpha 4\beta 2$ but not $\alpha 7$.

Effects on Serotonin-Gated Ion Channels, 5-Hydroxytryptamine Type 3 (5-HT_3) Receptors

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that is found in both the CNS and the peripheral nervous system. 5-HT mediates its diverse physiological responses through at least 16 different receptors, which are subdivided into seven distinct subfamilies, the 5-HT_{1-7} receptors (Graeff et al. 1996; Martin 1994). Among them, the 5-HT_3 receptor is a ligand-gated ion channel while all other 5-HT receptors are members of the G-protein-coupled receptor (GPCR) superfamily. The activation of this channel renders it permeable to Na^+ and K^+ ions and it is similar in many ways to the nicotinic acetylcholine receptor. 5-HT_3 receptors are sparsely distributed at the primary sensory nerve endings in the periphery, but widely distributed in the mammalian CNS. This receptor is

also clinically significant because antagonists of the 5-HT_3 receptor have important applications as analgesics, antiemetics, anxiolytics, and antipsychotics (Maricq et al. 1991). It has been recently reported that ginsenoside Rg_2 and ginsenoside metabolites (compound K and M4) at 10 μM inhibit 5-HT_3 receptor-gated ion currents in *Xenopus* oocytes expressing 5-HT_3 receptors (Choi et al. 2003; Lee et al. 2004a). The IC_{50} values of ginsenoside Rg_2, compound K and M4 were 22.3 $\pm$ 4.6, 36.9 $\pm$ 9.6 and 7.3 $\pm$ 2.2 μM, respectively. The inhibitory effect by ginsenoside Rg_2 on the 5-HT-induced inward current (I_{5-HT}) was also noncompetitive and voltage independent, which is similar in manner with that of ginsenoside-induced modulation of nicotinic acetylcholine receptors (Choi et al. 2003; Lee et al. 2004a). For the elucidation of the molecular mechanisms underlying ginsenoside Rg_3-induced 5-HT_3 receptor regulation, Lee et al. (2007a) utilized site-directed mutagenesis. They have constructed mutant receptors with alterations at the gating pore region of transmembrane domain 2 (TM2) and found that mutations of V291A, F292A, and I295A in TM2 greatly attenuated or abolished ginsenoside Rg_3-induced inhibition of peak I_{5-HT}. Interestingly, they also showed that mutation in V291A, but not F292A and I295A, induced constitutively active ion currents with a decreased current decay rate. Ginsenoside Rg_3 accelerated the rate of current decay in a dose-dependent manner in the presence of 5-HT. Ginsenoside Rg_3 and TMB-8, an open channel blocker of 5-HT_3 receptor channels, dose-dependently inhibited constitutively active ion currents. Diltiazem, another open channel blocker of 5-HT_3 receptor channels (Gunthorpe and Lummis 1999), did not prevent ginsenoside Rg_3-induced inhibition of constitutively active ion currents in occlusion experiments. These results indicate that ginsenoside Rg_3 inhibits 5-HT_3 receptor channel activity through interaction with residues V291, F292, and I295 in the channel gating region of TM2 and further demonstrate that ginsenoside Rg_3 regulates 5-HT_3 receptor channel activity in the open state at different site(s) from those of TMB-8 and diltiazem.

NEUROPROTECTIVE ACTION

Neuroprotective Action *In Vitro*

Based on the results supporting direct modulation by ginseng and its active components of various ion channels, including voltage-dependent Ca^{2+} channels and NMDA-gated ion channels, ginsenosides may be expected to interact with various channels and excitatory neurotransmitter receptors, and their interactions with these membrane proteins might be coupled to neuroprotection from excitotoxins in the nervous system. Glutamate-mediated neurotoxicity is observed mainly in the ischemic or hypoglycemic brain injuries, and ginseng has been consistently reported to prevent neuronal cell death due to ischemia or glutamate toxicity. In rat cortical cultures, ginsenosides Rb_1 and Rg_3, at 10 μM, attenuated glutamate- and NMDA-induced neurotoxicity by inhibiting the overproduction of nitric oxide, formation of malondialdehyde, and influx of Ca^{2+} (Kim et al. 1998b). Seong et al. (1995) also showed that ginseng total saponins attenuated glutamate-induced swelling of cultured rat astrocytes. Recently, Liao et al. (2002) reported that ginsenosides Rb_1 and Rg_1, at 20 to 40 μM, protect spinal neurons from excitotoxicity induced by glutamate or kainic acid *in vitro*. These results raise the possibility of using ginseng therapeutically to prevent neuronal death linked to neurodegenerative diseases. Except for studies using ginseng total extracts (Lee et al. 2002b; Seong et al. 1995), most *in vitro* studies on the neuroprotective

effects of ginseng were confined to a few ginsenosides, such as ginsenosides Rb_1 and Rg_1 (Kim et al. 1998b; Liao et al. 2002; Lim et al. 1997). These ginsenosides were studied more extensively because they are present in ginseng in relatively high amounts (18.3 and 6.4% of total ginseng saponins, respectively). They can also be obtained in large quantities in a purified form. Ten ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2) were tested at 10 μM *in vitro* on hippocampal neurons for their ability to inhibit NMDA receptors. The inhibitory activity of ginsenosides Rb_1 and Rg_1 was much less pronounced than that of ginsenoside Rg_3, although ginsenosides Rb_1 and Rg_1 could modulate NMDA receptor activity (Kim et al. 2002). At the concentration used, ginsenoside Rg_3 was the most effective among the 10 ginsenosides tested. It inhibited NMDA receptors by interacting with their glycine-binding sites (Kim et al. 2004). Selective blockers of the glycine site on NMDA receptors are considered to be promising therapeutics that may reduce the devastating effects of excitotoxicity (Kemp and Leeson 1993; Lee et al. 1999). Ginsenoside Rg_3 was, therefore, tested for its ability to protect hippocampal neurons in culture from NMDA-induced neurotoxicity by blocking the glycine-binding site. Indeed it was demonstrated that at 1 to 30 μM ginsenoside Rg_3 significantly protects neurons from NMDA insults (Table 1). Recently, 20(S)-ginsenoside Rh_2 was identified as an active ingredient of ginseng that can act at the hippocampal NMDA receptors (Lee et al. 2006a), but its neuroprotective effect has yet to be further examined. Further indication of its neuroprotective activity came from an *in vivo* experiment showing that at 100 mg/kg p.o. 20(*S*)-ginsenoside Rh_2 protects from ischemia-reperfusion-induced brain injury (Park et al. 2004). These results indicate that ginsenoside Rg_3-protects neurons *in vitro* from NMDA-induced neurotoxicity and that *in vivo* ginsenoside Rh_2 protects from ischemia-reperfusion brain injury. The neuroprotective activity of these ginsenosides can be attributed to specific inhibition of NMDA-induced receptor activation.

Ginsenosides may protect cardiovascular system also from homocysteine toxicity. Homocysteine is a sulfur-containing amino acid that is totally absent from any dietary source, but is formed during the metabolism of the essential amino acid, methionine (Finkelstein 1974). Accumulation of high levels of homocysteine (as in hyperhomocysteinemia) appears to be associated with deleterious cardiovascular effects, leading to atherosclerosis and stroke (Dikmen et al. 2006; Tay et al. 2006). In addition to cardiovascular disorders, patients with hereditary homocysteinuria often display cerebral atrophy and suffer from epileptic seizures (Watkins and Rosenblatt 1989). Studies have shown that homocysteine is one of the most potent excitatory agents in mammalian nervous systems (Meweitt et al. 1983; Watkins and Rosenblatt 1989), where it binds to and activates NMDA receptors (Lipton et al. 1997; Pullan et al. 1987), leading to mitochondrial dysfunction, caspase activation, and DNA damage (Ho et al. 2002; Kruman et al. 2000). These effects are believed to be central and due to the excitotoxicity of homocysteine in the CNS (Kim et al. 1987; Lipton et al. 1997; Olney et al. 1987; Pullan et al. 1987). The currently ongoing research attempts to develop new agents that could reduce homocysteine levels in plasma or prevent homocysteine-induced neuronal vascular damage (Dierkes and Westphal 2005; Folbergrova et al. 2005; Lockhart et al. 2000; Weiss et al. 2006). As mentioned above, since ginsenoside Rg_3 attenuates NMDA receptor-mediated currents and NMDA-induced neurotoxicity (Kim et al. 2004), homocysteine could exert its excitotoxicity through NMDA receptor activation. It is, therefore, conceivable that ginsenosides may also protect from homocysteine-induced neurotoxicity. In fact, Kim et al. (2007) examined the effect of ginsenoside Rg_3 on homocysteine-induced hippocampal excitoxicity. *In vitro* studies using rat cultured

TABLE 1. Neuroprotective effects of ginsenoside Rg_3 against NMDA-induced excitotoxicity in primary cultures of rat hippocampal neurons.

Culture	Concentration (μM)	Cell viability (%)[a]
Control		100
NMDA-treated[b]		0
NMDA + D-APV[c]	25.0	72.9 ± 2.6***
NMDA + 7-CK[c]	10.0	92.7 ± 1.7***
NMDA + Rg_3[c]	1.0	65.2 ± 1.5***
	10.0	77.3 ± 1.5***
	30.0	83.1 ± 1.4***

[a]Hippocampal neurons were treated with ginsenoside Rg_3 (Rg_3), D(-)-2-amino-5-phosphonopentanoic acid (D-APV), and 7-chlorokynurenic acid (7-CK) for 1 min before the NMDA insult. The cultures were then exposed to 100 μM NMDA for 15 min and washed with culture medium. After a 24-h incubation, the cultures were assessed for the extent of neuronal death using the MTT assay. Optical densities (OD) of control and NMDA-treated cultures were 1.01 ± 0.06 and 0.51 ± 0.04, respectively. Data were expressed as the percentage of cell viability relative to the control cultures. The values shown are the means ± S.E.M. (n = 19–36). Statistical significance was determined using unpaired Student's t-test.
[b]Significantly different from the control value, $P < 0.001$.
[c]Significantly different from the NMDA-treated cultures, $^{***}P < 0.001$.
Data from Kim et al. (2004).

hippocampal neurons revealed that ginsenoside Rg_3 significantly and dose-dependently inhibits homocysteine-induced hippocampal cell death (IC_{50} = 28.7 ± 7.5 μM). Ginsenoside Rg_3 not only significantly reduces homocysteine-induced DNA damage but *in vitro* it also attenuates concentration-dependently homocysteine-induced caspase-3 activity. In studies designed to examine the underlying *in vitro* neuroprotective effects of ginsenoside Rg_3 from homocysteine-induced hippocampal excitotoxicity, Kim et al. (2007) demonstrated that ginsenoside Rg_3 dose-dependently inhibits homocysteine-induced increase in intracellular Ca^{2+} levels (IC_{50} = 41.5 ± 7.5 μM). In addition, ginsenoside Rg_3 dose-dependently inhibited homocysteine-induced currents in *Xenopus* oocytes expressing NMDA receptors with an IC_{50} of 47.3 ± 14.2 μM. These results collectively suggest that ginsenoside Rg_3 protects from homocysteine-induced neurotoxicity in rat hippocampus and that this effect is likely to be due to inhibition of homocysteine-mediated activation of NMDA receptors.

Neuroprotective Action *In Vivo*

There are few reports on how *in vitro* effects of ginsenosides are related to their *in vivo* neuroprotective actions. It has been reported that ginseng total saponins, at 100 μg/mL, and ginsenoside Rg_3, at 10 μM, inhibit the propagation of status epilepticus (SE; continuous seizure activity for 30 min or longer)-induced neuronal cell death and the development of spontaneous recurrent epileptiform discharges *in vitro* in the hippocampal neuronal culture model of SE (Kim and Rhim 2004). *In vivo*, red ginseng powder (p.o., for 7 days before the induction of ischemia) prevented ischemia-induced decrease in response latency,

as determined by the passive avoidance test, and rescued a significant number of ischemic hippocampal CA1 pyramidal neurons (Wen et al. 1996). By i.p. administration crude ginseng saponin exhibited a similar neuroprotective effect. Ginseng extract (one week, 10 mg/mL in drinking water, 1.6 g/kg/day) has been shown to prevent neuronal death in myocardial ischemia-reperfusion damage induced by hyperbaric oxygen (Maffei Facino et al. 1999). Pretreatment with ginsenosides (50 or 100 mg/kg for 7 days, i.p.) reduced kainic acid-induced death of hippocampal neurons (Lee et al. 2002b). According to Abe et al. (1994) ginsenoside Rb_1, but not Rg_1, by i.c.v. administration at 0.5–50 nmol, significantly inhibited the magnitude of LTP, induced by a strong tetanus in the dentate gyrus of anesthetized rats. It has been also reported that by i.c.v. infusion ginsenoside Rb_1 at 0.1–100 fg/mL (0.09–90 fM) protected hippocampal neurons from ischemia (Lim et al. 1997). Ginsenosides Rg_3 or Rh_2 (100 mg/kg, p.o.) improved ischemia-reperfusion brain injury induced by middle cerebral artery occlusion in rats (Bae et al. 2004b; Park et al. 2004). When the antiinflammatory effect of ginsenosides Rg_3 and Rh_2 were further examined, ginsenoside Rh_2, at 5 or 25 μM, decreased protein and mRNA expression of an inducible NO synthase (iNOS) gene and the expression of COX in lipopolysaccharide (LPS)- and IFN-gamma-induced murine BV-2 microglial cells (Bae et al. 2006). The antiinflammatory effect of ginsenoside Rg_3 against LPS/IFN-gamma-activated BV-2 cells was less pronounced than that of ginsenoside Rh_2. These findings suggest that the *in vivo* antiischemic effect of ginsenoside Rg_3 may be actually produced by ginsenoside Rh_2, the main metabolite of ginsenoside Rg_3 by intestinal microflora, and that the antiischemic effect of ginsenoside Rh_2 may be due to its anti-inflammatory action in brain microglia. Recently, 20(*S*)-ginsenoside Rg_3, at 5 and 10 mg/kg, but not at 2.5 mg/kg, by sublingual injection, has been reported to have a significant neuroprotective effect in rats subjected to focal cerebral ischemic injury (Tian et al. 2005). Ginsenoside Rg_3, 5 and 15 nmol i.c.v. at 1 h prior to homocysteine, reduced homocysteine-induced hippocampal cell death (Kim et al. 2007).

In addition, ginsenosides showed neuroprotective effects against neurotoxins, such as 3-nitropropioninc acid (3-NP) and rotenone (Kim et al. 2005; Leung et al. 2007). 3-NP is a compound found in crops contaminated with fungi (Ming 1995) and causes neurotoxicity in both animals and humans (James et al. 1980; Ludolph et al. 1991). Rotenone, a common pesticide, is a well-known specific and irreversible mitochondria complex I inhibitor and has been suggested to be the causal agent of Parkinson's disease (Heikkila et al. 1985; Sherer et al. 2003). The primary mechanism of 3-NP-caused neurotoxicity involves the irreversible inhibition of mitochondrial succinate dehydrogenase (SDH) and leads to inhibition of ATP synthesis (Alston et al. 1977; Coles et al. 1979). ATP exhaustion via mitochondrial dysfunction couples to the slow secondary excitotoxicity by excitatory neurotransmitters (Pang and Geddes 1997). This secondary excitotoxicity in ATP-deficient neurons is initiated by voltage-dependent Na^+ channel activation, which is coupled to membrane depolarization, Ca^{2+} channel activation, and subsequent NMDA receptor activation by an antagonism of voltage-dependent Mg^{2+} block of NMDA receptors (Novelli et al. 1988; Zeevalk and Nicklas 1991). These serial cascades induced by 3-NP intoxication are accompanied by the impaired mitochondrial Ca^{2+} homeostasis, with an elevation of $[Ca^{2+}]_i$ due to enhanced entry through L-type and other Ca^{2+} channels, and with an impaired buffering capacity of $[Ca^{2+}]_i$ in astrocytes and neurons (Calabresi et al. 2001; Deshpande et al. 1997; Fukuda et al. 1998; Nasr et al. 2003). Moreover, since 3-NP-induced elevation of $[Ca^{2+}]_i$ is known to activate calpain and caspase-9, which are involved in neuronal cell death, 3-NP-induced perturbation of calcium homeostasis in mitochondria and the following activation of these

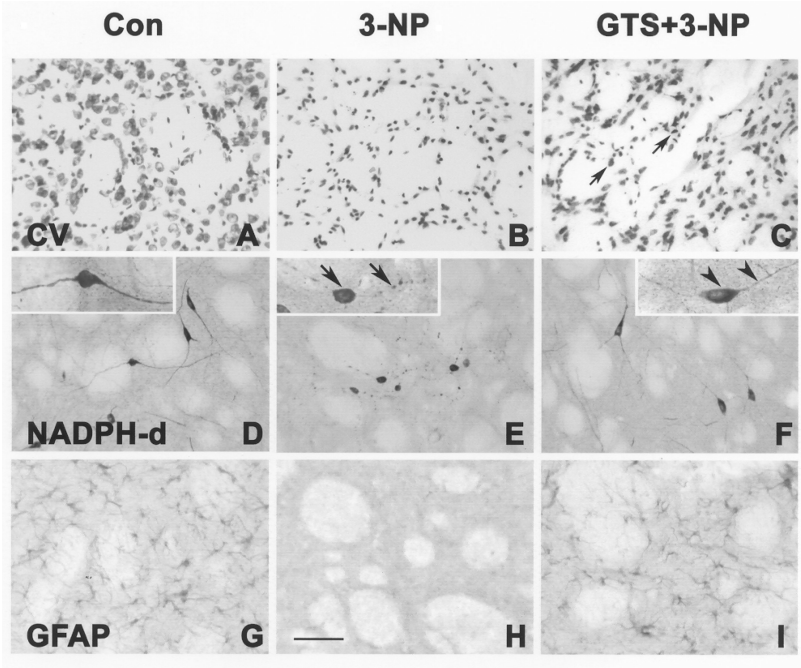


FIG. 5. Neuroprotective effect of ginseng total saponins (GTS) against 3-NP in Cresyl Violet (CV), GFAP, and NADPH-diaphorase staining in coronal rat brain sections at the level of the striatum and anterior commissure from saline (Con), 3-NP-treated, or GTS + 3-NP animals. Adapted from Kim et al. (2005).

enzymes might be the main factors in 3-NP neurotoxicity *in vivo* (Bizat et al. 2003a; Bizat et al. 2003b; Brouillet et al. 1999; Fu et al. 1995). Kim et al. (2005) showed that by i.p. administration ginsenosides, in a dose-dependent manner (at 50 or 100 mg/kg but not at 25 mg/kg), protect from systemic 3-NP- and intrastriatal malonate (a reversible SDH inhibitor)-induced lesions in rat striatum. Ginsenosides also antagonized 3-NP-induced behavioral impairment and extended survival (Fig. 5 and Table 2). To explain the mechanisms underlying the *in vivo* protective effects of ginsenosides in 3-NP-induced striatal degeneration, rat cultured striatal neurons were used. At 100 μg/mL ginsenosides inhibited 3-NP-induced $[Ca^{2+}]_i$ elevation and restored 3-NP-induced reduction of mitochondrial transmembrane potential. It appeared that ginsenosides prevented 3-NP-induced striatal neuronal cell deaths in a concentration-dependent manner. These results suggest that *in vivo* ginsenosides may protect striatal neurons from 3-NP-induced degeneration by inhibiting 3-NP-induced $[Ca^{2+}]_i$ elevation and cytotoxicity.

MECHANISM OF ACTION

Stereospecificity in Ginsenoside-Induced Voltage-Dependent and Ligand-Gated Ion Channel Regulation

Ginsenoside Rg_3 is one of the PD ginsenosides. Its chemical structure is shown in Fig. 2; it has two glucose molecules at the carbon-3 position and no sugars at the carbon-20

TABLE 2. Neuronal counts within the rat striatum lesion area.

Treatment	Total number of neurons (per mm^2, $n = 6$)	Number of NADPH neurons (per mm^2, $n = 6$)
Control	1568.3 ± 45.8	27.4 ± 0.8
3-NP alone	108.7 ± 10.2*	13.8 ± 0.6*
GTS + 3-NP	1270.4 ± 80.5	23.4 ± 0.8

In rats ginseng total saponins (GTS, 100 mg/kg i.p,) produced significant protection from systemic 3-nitropropionic acid (3-NP, 10 mg/kg)-induced lesions in striatum compared with 3-NP only-treated group. The protective effects of GTS were confirmed using NADPH diaphorase histochemistry. Data are expressed as means ± S.E.M. $^*P < 0.001$ compared with GTS + 3-NP by Student's *t*-test. Data from Kim et al. (2005).

position. Unlike ginsenoside Rg_3, ginsenoside Rf has two glucose molecules at the carbon-6 position and no sugars at the carbon-20 position. Ginsenoside Rg_3 has two stereoisomers; the position of the hydroxyl group at the carbon-20 differentiates between the epimers, 20(*R*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rg_3 (Fig. 3). The main reason for the selection of ginsenoside Rg_3 as a model compound is its stereospecificity. It is relatively easy to differentiate the purity between 20(*R*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rg_3 without contamination of the other form. Also ginsenoside Rg_3 is the most potent regulator of various types of ion channels such as voltage-dependent Ca^{2+}, K^+, Na^+ channels, ligand-gated ion channels, such as 5-HT_3, NMDA as well as of nicotinic acetylcholine receptors in muscles and neurons (Choi et al. 2002b; Kim et al. 1999; Kim et al. 2004; Lee et al. 2007a; Lee et al. 2005b; Rhim et al. 2002; Sala et al. 2002).

It has been observed in the experiments examining the stereospecificity of ginsenosides that at 100 μM 20(*S*)-ginsenoside Rg_3, but not 20(*R*)-ginsenoside Rg_3, inhibits voltage-dependent Ca^{2+} (P/Q-type), K^+ ($K_v1.4$), and Na^+ ($Na_v1.2$ and $Na_v1.5$) channel activities (Jeong et al. 2004). The difference between Rg_3 epimers with respect to voltage-dependent ion channel regulation indicates that in voltage-dependent ion channels the hydroxyl group of 20(*S*)-ginsenoside Rg_3 may be geometrically better aligned with the hydroxyl acceptor group than that of the 20(*R*)-ginsenoside Rg_3 (Kang et al. 2005). It has been also found that both ginsenoside Rg_3 stereoisomers inhibit 5-HT_3 and $\alpha3\beta4$ nicotinic acetylcholine receptor channel activities. However, 20(*S*)-ginsenoside Rg_3 is more effective in inhibiting 5-HT_3 and $\alpha3\beta4$ nicotinic acetylcholine receptor-mediated currents than 20(*R*)-ginsenoside Rg_3 (Jeong et al. 2004). These results indicate that ginsenoside Rg_3 stereoisomers have a different stereospecificity with respect to the regulation of voltage-dependent and ligand-gated ion channel activities. In addition, it has been observed that the effects of 20(*R*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rg_3 on mouse 5-HT_3 receptor channel activity are altered after site-directed mutations in the 5-HT_3 receptor facilitation site located at pre-transmembrane domain 1 (pre-TM1). Induction of 5-HT_3 receptor facilitation by point mutations in pre-TM1 amino acid residues R222 to R222A, R222D, R222E, or R222T not only decrease EC_{50} values for I_{5-HT} compared to wild-type, but also abolish 20(*R*)-ginsenoside Rg_3-induced inhibition of I_{5-HT}. These mutations also shifted the IC_{50} values for 20(*S*)-ginsenoside Rg_3 to the right by 2- to 4-fold, compared to the wild-type. These results indicate that 5-HT_3 receptor facilitation differentially affects 20(*S*)- and 20(*R*)-ginsenoside Rg_3-mediated 5-HT_3 receptor channel regulation (Lee et al. 2007b). Moreover, *ex vivo* experiments using swine coronary arteries further demonstrated that treatment with

20(*S*)-ginsenoside Rg_3, but not 20(*R*)-ginsenoside Rg_3, caused a potent concentration dependent, endothelium-independent relaxation of coronary artery contracted by high K^+ (Kim et al. 2006). The IC_{50} values for 20(*S*)-ginsenoside Rg_3 were 46.2 ± 7.0 and 42.1 ± 6.7 μM in intact and endothelium-denuded preparations, respectively. However, both 20(*S*)- and 20(*R*)-ginsenoside Rg_3 induced a significant and concentration-dependent relaxation of intact coronary arteries contracted by 5-HT, while only 20(*S*)-ginsenoside Rg_3 relaxed endothelium-denuded coronary arteries. This finding indicates that, in addition to the differences in their effects on ion channel regulation in single cells, ginsenoside Rg_3 epimers exhibit differential forms of regulation of smooth muscle contraction. These results also suggests that ginsenoside Rg_3 epimers might differ from each other in their *in vivo* actions.

In contrast to the stereospecificity of ginsenoside Rg_3 at voltage dependent, 5-HT_3-gated, and nicotinic acetylcholine-gated ion channels, there appears to be no stereospecificity for 20(*S*)- and 20(*R*)-ginsenoside Rg_3 with respect to the inhibition of hippocampal NMDA receptors (Lee et al. 2006a). Instead, it has been reported that a structural change from (*S*) to (*R*) at the carbon-20 of ginsenoside Rh_2 caused a loss in its NMDA inhibitory activity. Furthermore, the minor structural difference between 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2 changed the target site of the NMDA receptor complex from glycine- to polyamine-binding site. How does the minor structural difference between 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2 change the regulatory sites in the NMDA receptor complex? If it occurred, what is the optimal structure for maximizing the protective effects of ginsenosides? Based on the results from Lee et al. (2006a), carbon-3 and carbon-20 positions of ginsenosides seem to be the important sites for the modulation of NMDA receptors. The structural difference between 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside-Rh_2 is a mono-glycosylated group at the carbon-3 position. Therefore, the three-dimensional structure composed by the mono-glycosylated group at carbon-3 and (*S*)-isomer at carbon-20 seem to be essential for the binding at the polyamine site. These results suggest that two binding sites, glycine- and polyamine-biding sites, modulated by 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2, are located adjacently. The observations using site-directed mutagenesis revealed that both glycine- and polyamine-binding sites are located in the NR1 subunit of NMDA receptors (Hirai et al. 1996; Kuryatov et al. 1994; Williams et al. 1995). On the other hand, it is also possible that the size of three-dimensional ginsenosides determines where they should bind. While 20(*S*)-ginsenoside Rh_2 could fit into the polyamine-binding site, 20(*S*)-ginsenoside Rg_3, which has a more bulky side chain, may not fit in this site, but binds to the glycine-binding site. Although the detailed mechanism remains to be demonstrated, these results clearly show that the two main active ingredients of ginseng, 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2, produce their inhibitory effects by modulating NMDA receptors and targeting different regulatory sites of NMDA receptor channel complex. If a new and non-metabolizable form of 20(*S*)-ginsenoside Rg_3 could be developed, 20(*S*)-ginsenoside Rh_2, along with the modified form of 20(*S*)-ginsenoside Rg_3, could become potentially useful drugs for the treatment of neurodegenerative disorders.

Identification of Ginsenoside Interaction Sites in Ligand-Gated Ion Channel Regulation

Although little is known about the exact mechanisms of ginsenoside-induced regulation of ligand-gated ion channels, recent reports show that ginsenosides might interact with

channel or receptor proteins (Kim et al. 2004; Lee et al. 2007a; Lee et al. 2006a). The evidence that ginsenosides directly interact with channels or receptor proteins comes from competition and site-directed mutagenesis experiments. For example, the inhibitory effects of 20(*S*)-ginsenoside Rg_3 on NMDA receptor-mediated ion currents in cultured hippocampal neurons was greatly attenuated in the presence of glycine, which is a kind of a co-agonist at NMDA receptors. Thus, the degree of attenuation of 20(*S*)-ginsenoside Rg_3-induced NMDA currents appears to be dependent on glycine levels, suggesting that this ginsenoside competes with glycine-binding sites for the regulation of NMDA receptors. More directly, in ginsenoside-induced inhibition of 5-HT_3 receptor-mediated currents, the mutations of V291 to V291A, F292 to F292A and I295 to I295A, which are at the gating pore region of transmembrane domain 2 (TM2), greatly attenuated or abolished ginsenoside Rg_3-induced inhibition of peak I_{5-HT} (Lee et al. 2007a). These results indicate that ginsenoside-induced ligand-gated ion channel regulation is achieved through an interaction with the specific regions or amino acid residues that are involved in ligand-gated ion channel activity. Further site-directed mutagenesis studies will be needed to identify the exact interaction site(s) of ginsenoside Rg_3 in voltage-dependent and ligand-gated ion channels.

SAFETY OF GINSENG

Ginseng (*Panax ginseng* C.A. Meyer) has been a popular herbal remedy used in eastern Asia for thousands of years and now is one of the most famous herbal medicines consumed around the world. In view of the extremely widespread use of ginseng, it seems important to ask whether this herbal medicine involves health risks for the consumer. Based on experimental studies conducted both in animals and humans ginseng is generally considered safe, although some possible side effects have been reported, especially at higher doses (Coon and Ernst 2002; Cuzzolin et al. 2006; Izzo et al. 2005; Kitts and Hu 2000). The most common among them are hypertension, diarrhea, eruptions, mastalgia, extension of menstruation (vaginal bleeding), and sleep disturbances (Buettner et al. 2006; Coon and Ernst 2002). With respect to possible ginseng-drug interactions, it has been reported that ginseng reduced blood levels of alcohol or warfarin and induced mania when used concomitantly with phenelzine (Coon and Ernst 2002; Hu et al. 2005). The analgesic effect of opioids may also be inhibited by ginseng (Abebe 2002). Nevertheless, many studies claimed that, in comparison to other phytomedicines, ginseng has not been shown to produce serious side effects or dangerously interact with other drugs.

In regard to the therapeutic index of ginseng, there are few reported cases of ginseng toxicity (Kitts and Hu 2000). According to the German Commission E, a regulatory body that evaluates the safety and efficacy of medicinal herbs, the recommended daily intake of Asian ginseng, containing 4–5% ginsenosides, is 1 to 2 g/day. Therapeutic doses of individual ginsenosides, especially ginsenosides Rg_3 and Rh_2, have been established for different animal models. In rat models, 20(*S*)-ginsenoside Rh_2 at 50 to 100 mg/kg p.o. protected from ischemia-reperfusion brain injury (Bae et al. 2004b; Park et al. 2004). Ginsenoside Rg_3, at 5 to 15 nmol i.c.v., protected rats from homocysteine-induced toxicity (Kim et al. 2007). In mice with tert-butyl hydroperoxide-induced liver injury, 20(*S*)-ginsenoside Rg_3 and 20(*S*)-ginsenoside Rh_2 were reported to have hepatoprotective effects at 25 to 50 mg/kg p.o. (Lee et al. 2005a).

SUMMARY AND FUTURE DIRECTION

Ginsenosides, which are the pharmacologically active ingredients of *Panax ginseng*, produce reversible and selective inhibitory effects at voltage-dependent and ligand-gated ion channels. In addition, ginsenosides exert *in vitro* and *in vivo* protective actions against acute excessive stimulation by excitatory neurotransmitters and against neurotoxins such as 3-NP and rotenone. Among about 30 different types of ginsenosides, ginsenoside Rg_3 is the most effective component of ginseng at various types of neuronal ion channels. The activity of ginsenoside Rh_2 and ginsenoside Rg_3 at neuronal NMDA receptors is stereospecific. Based on the reported neuroprotective effects ginsenosides may be potentially useful as drugs for the treatment of neurodegenerative disorders. Further studies are, however, needed for their development as drugs. These studies should include systematic blood–brain barrier experiments with ginsenoside Rg_3 or ginsenoside Rh_2. Ginsenoside interaction site(s) in voltage-dependent and ligand-gated ion channel proteins must be more precisely determined to elucidate how they interact or regulate ion channel activities. Also, ginsenoside derivatives with more specific agonistic or antagonistic properties at ion channels may have to be synthesized.

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