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Research Article

Ginsenoside Rk1 is a novel inhibitor of NMDA receptors in cultured rat hippocampal neurons

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ABSTRACT

Background: Ginsenoside Rk1, a saponin component isolated from heat-processed *Panax ginseng* Meyer, has been implicated in the regulation of antitumor and anti-inflammatory activities. Although our previous studies have demonstrated that ginsenoside Rg3 significantly attenuated the activation of NMDA receptors (NMDARs) in hippocampal neurons, the effects of ginsenosides Rg5 and Rk1, which are derived from heat-mediated dehydration of ginsenoside Rg3, on neuronal NMDARs have not yet been elucidated.

Methods: We examined the regulation of NMDARs by ginsenosides Rg5 and Rk1 in cultured rat hippocampal neurons using fura-2–based calcium imaging and whole-cell patch-clamp recordings.

Results: The results from our investigation showed that ginsenosides Rg3 and Rg5 inhibited NMDARs with similar potencies. However, ginsenoside Rk1 inhibited NMDARs most effectively among the five compounds (Rg3, Rg5, Rk1, Rg5/Rk1 mixture, and protopanaxadiol) tested in cultured hippocampal neurons. Its inhibition is independent of the NMDA- and glycine-binding sites, and its action seems to involve in an interaction with the polyamine-binding site of the NMDAR channel complex.

Conclusion: Taken together, our results suggest that ginsenoside Rk1 might be a novel component contributable to the development of ginseng-based therapeutic treatments for neurodegenerative diseases.

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1. Introduction

Ginsenosides, a major component of ginseng, are well-known traditional herbal medicines. More than 30 types of ginsenosides that are triterpene derivatives containing sugar moieties have been isolated from *Panax ginseng* Meyer [1]. Ginsenosides produce various pharmacological and therapeutic effects on the central nervous system (CNS) [2–4]. For example, ginsenoside Rb1 protects hippocampal neurons against ischemia, and ginsenoside Rd counteracts ischemic stroke [5]. Ginsenoside Rg1 promotes the regenerative repair of peripheral nerve injury [6]. Moreover, ginsenosides

Rg3 and Rb1 prevent cortical neurons from glutamate-induced neurotoxicity [7].

Dysregulation of glutamate neurotransmitter systems is implicated in disorders of the nervous system such as spinal cord trauma, seizure, ischemia, and Alzheimer's disease [8]. Glutamate induces neuronal death in the extracellular space, and this toxicity has been linked to an enormous calcium influx via both non-NMDARs and NMDARs in neurological disorders [9]. Furthermore, NMDAR antagonists are known to reduce neuronal death in some *in vivo* models of hypoglycemic or ischemic brain injury [10]. Eventually, based on those observations, a number of pharmaceutical companies and

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laboratories have developed NMDAR antagonists and have continued to test them in clinical trials [4].

Ginsenoside Rk1, a unique saponin component isolated from heat-processed *Panax ginseng* Meyer, has been implicated in the regulation of antitumor and anti-inflammatory activities [11]. Ginsenoside Rk1 is also known to regulate endothelial barrier function [12]. Previously, our laboratory described how ginsenosides significantly decreased NMDAR activation in cultured hippocampal neurons [9,13]. Furthermore, we demonstrated that ginsenoside Rg3 and its metabolite ginsenoside Rh2, both constituents of *Panax ginseng*, were the maximally effective components of ginseng on NMDAR inhibition [14]. Ginsenosides Rg3 and Rh2 could protect hippocampal neurons against exogenous NMDA treatment through their contact with the glycine- and polyamine-binding sites of NMDARs, respectively [14]. Interestingly, the effects of a ginsenoside Rg5/Rk1 mixture on memory function and neuroprotective actions against excitotoxicity were previously reported [15]. These studies raise the possibility that ginsenoside Rg5 or Rk1 might inhibit NMDARs in the hippocampus, a brain region responsible for learning and memory. Furthermore, it is possible that ginsenoside Rg5 or Rk1 protects against hippocampal dysfunction, which could lead to neurodegenerative diseases. However, the single forms of ginsenoside Rg5 or Rk1 have not been investigated in the hippocampus. We, therefore, examined the regulation of NMDARs by ginsenosides Rg5 and Rk1 and elucidated the detailed mechanism by which ginsenoside Rk1 modulates the NMDAR channel complex in cultured rat hippocampal neurons. Overall, our results demonstrate that ginsenoside Rk1 produced its inhibitory action via the regulation of NMDAR-mediated signaling; ginsenoside Rk1 appears to be a reasonable NMDAR antagonist through an interaction at the polyamine-binding site.

2. Materials and methods

2.1. Materials

Ginsenosides Rg3, Rg5, Rk1, Rg5/Rk1 mixture (a mixture of Rg5 and Rk1, 1:1, w/w), and protopanaxadiol (PD) were isolated as previously described [16,17]. Ginsenosides were melted in dimethylsulphoxide as a concentrated stock and additionally diluted to their final concentrations in the external recording solution. All the chemicals for cell culture and preparation were purchased from Life Technologies Inc. (Grand Island, NY, USA) except NMDA and glycine (Tocris, Ellisville, MO, USA). Other reagents and chemicals were obtained from Sigma Chemical (St. Louis, MO, USA).

2.2. Cell culture and preparation

Hippocampal neurons were prepared with the technique modified from Kim et al [9]. Briefly, the hippocampi were collected from 18-day-old fetal Sprague–Dawley rats and then incubated using 0.25% trypsin solution at 37 °C for 25 min. Hippocampal cells were mechanically detached with fire-polished Pasteur pipettes through trituration and then plated on previously dried poly-D-lysine-coated coverslips in a 12-well dish. Thereafter, cells were maintained in Neurobasal medium comprising 5% fetal bovine serum (FBS), 2% B-27 supplement, 2 mM glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin inside a humidified atmosphere of 95% air as well as 5% CO₂ at 37 °C. The next day, the medium was replaced with a similar medium containing no FBS, fed twice per week, and treated with fluorodeoxyuridine (10 µM) after 7 days *in vitro*. All experiments were carried out on hippocampal neurons that were cultured between 10 and 16 days. All techniques and protocols were approved by the Institutional Animal Care and Use Committee from Korea Institute of Science and Technology (KIST).

2.3. Intracellular calcium imaging

Fura-2–based intracellular calcium imaging was modified from Kim et al [9]. Cells were incubated for 30 min at room temperature using 5 µM fura-2/AM and 0.001% Pluronic F-127 in a HEPES buffer consisting of 5 mM KCl, 150 mM NaCl, 10 mM HEPES, 2 mM CaCl₂, 0.001 mM glycine, and 10 mM glucose, and finally, the pH was adjusted to 7.4 using NaOH. Hippocampal neurons were illuminated with a xenon arc lamp. In addition, excitation wavelengths (340 and 380 nm) were selected by a computer-controlled filter wheel. Emitter fluorescence was reflected via a 515-nm longpass filter to a frame transfer cooled CCD camera, and the 340/380 ratios of emitted fluorescence were analyzed. All imaging data were collected and analyzed using MetaFluor Fluorescence Ratio Imaging Software (Molecular Devices, San Jose, CA).

2.4. Electrophysiological recordings

Whole-cell voltage-clamp recordings were performed by the patch-clamp method [18] on hippocampal pyramidal neurons at room temperature. Patch electrodes with resistances of 3–4 MΩ were filled with the internal solution containing 120 mM potassium gluconate, 20 mM NaCl, 2 mM MgCl₂, 10 mM HEPES, and 10 mM Mg-ATP, and the pH was adjusted to 7.4 by KOH. The external solution contained 150 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 10 mM HEPES, 10 mM glucose, and 0.001 mM glycine, with pH adjusted to 7.4 using NaOH. To eliminate the blockade of NMDA channels by Mg²⁺, Mg²⁺ was omitted from the external solution when needed. Current recordings were obtained with an EPC-9 amplifier and Pulse/Pulsefit software (HEKA, Germany).

2.5. Data analysis

All data are expressed in mean ± standard error and statistically compared using an unpaired Student *t* test or one-way analysis of variance followed by Tukey's *post hoc* tests. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Ginsenoside Rk1 most effectively suppresses NMDARs in hippocampal neurons

Ginsenosides Rg5 and Rk1 are derived from ginsenoside Rg3 by dehydration or heating processes as shown in Fig. 1A. In the present study, we examined the effects of ginsenoside Rg3 and its metabolites ginsenoside Rg5 and ginsenoside Rk1 on NMDARs in rat hippocampal neurons. To examine the modulation of NMDARs by ginsenosides, intracellular Ca²⁺ concentrations ([Ca²⁺]_i) were recorded in hippocampal neurons using fura-2–based digital imaging systems. Neurons were acutely (10 sec duration) and cyclically treated with 100-µM NMDA for 4 to 5 min through the fast perfusion system. Here, we found that 1 min of pretreatment with ginsenoside Rg3 (10 µM) inhibited NMDA-mediated Ca²⁺ influx by 22.0% (Fig. 1B). However, pretreatment with ginsenoside Rk1 (10 µM) increased inhibition of NMDA-mediated Ca²⁺ influx (54.9% Fig. 1C) compared to ginsenoside Rg3. When we further examined ginsenoside Rg5, ginsenoside Rg5/Rk1 mixture, and PD, ginsenoside Rg3, ginsenoside Rg5, and PD exhibited less than 25% inhibition (Fig. 1D). However, the mean percentage inhibition by the ginsenoside Rg5/Rk1 mixture was 41.5 ± 3.5% (n = 7), whereas ginsenoside Rk1 was 55.8 ± 1.2% (n = 7, Fig. 1D). Therefore, together these findings indicate that ginsenoside Rk1 produced the greatest inhibitory effect on NMDA-mediated Ca²⁺ influx among the five ginsenosides tested.

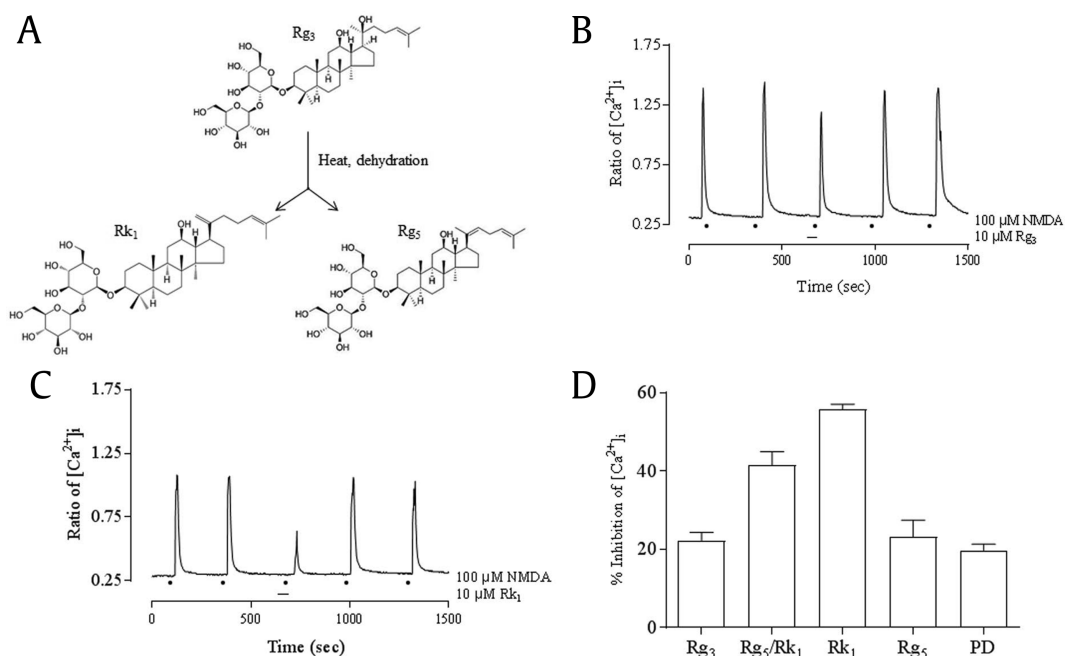


Fig. 1. Effects of ginsenoside Rk1 on NMDA-mediated Ca^{2+} influx in cultured hippocampal neurons. (A) Structures of ginsenoside Rg3 and its metabolites ginsenoside Rg5 and ginsenoside Rk1. (B, C) A sample recording showing the effects of ginsenosides Rg3 and Rk1 on a cultured hippocampal neuron using fura-2–based calcium imaging in a HEPES-buffered solution. After pretreatment with ginsenoside Rg3 (10 μ M) and Rk1 (10 μ M) for 1 min, 100 μ M NMDA was applied for 5 sec with each ginsenoside. (D) Pooled results illustrating the mean percentage inhibition on NMDA-mediated Ca^{2+} influx by ginsenosides Rg3, Rg5, Rk1, Rg5/Rk1 mixture, and protopanaxadiol.

3.2. Ginsenoside Rk1 blocks NMDARs without competing with the NMDA-binding site

We next examined the dose dependency of ginsenoside Rk1 on NMDA-mediated Ca^{2+} influx and found that ginsenoside Rk1 produced dose-dependent inhibition with an IC_{50} value of 13.34 ± 0.02 μ M (Fig. 2A). Based on this IC_{50} value, the additive effect of ginsenoside Rk1 and ginsenoside Rg3 was examined at a submaximal concentration (3 μ M). The mean inhibitions of NMDA-mediated Ca^{2+} influx by ginsenosides Rg3 and Rk1 were $17.9 \pm 0.6\%$ and $28.3 \pm 3.5\%$ ($n = 5$), respectively. On the other hand, the mean inhibition was significantly increased when ginsenosides Rg3 and Rk1 were coapplied ($46.1 \pm 2.8\%$, Fig. 2B). These results indicate that ginsenosides Rg3 and Rk1 might target different NMDAR regulatory sites.

An NMDAR has many control sites which are targets for regulation via exogenous and endogenous substances [19]. For example, there are agonist NMDA-binding sites surrounded by the channel lumen and coagonist glycine-binding sites [20]. We previously showed that ginsenoside Rg3 inhibits NMDARs through a competitive interaction with a glycine-binding site [13]. Therefore, we next examined the detailed mechanism of ginsenoside Rk1-mediated inhibition on NMDA-mediated Ca^{2+} influx. In the presence of varying NMDA concentrations (from 10 μ M to 1 mM), we assessed the contribution of ginsenoside Rk1 to the NMDA-binding sites. Fig. 2C shows the effect of ginsenoside Rk1 on variable concentrations of NMDA. Increasing concentrations of NMDA did not change the ginsenoside Rk1-mediated inhibition of NMDA-mediated Ca^{2+} influx as shown in Fig. 2C1. The relationship between the percentage of inhibition by ginsenoside Rk1 and the concentration of NMDA is illustrated in Fig. 2C2. Therefore, these observations indicate that ginsenoside Rk1 blocks NMDARs without competing with the NMDA-binding site in hippocampal neurons.

3.3. Ginsenoside Rk1-mediated NMDAR inhibition does not occur via the glycine-binding site

Ginsenoside Rg3 was previously reported as a competitive inhibitor of NMDARs with the glycine-binding site [13,14]. Therefore, we tested whether ginsenoside Rk1-mediated inhibition on NMDARs occurs via the glycine-binding site of NMDARs. We compared ginsenoside Rk1-mediated NMDAR inhibition in the absence and presence of glycine (100 μ M) in hippocampal neurons. Although glycine exists as a coagonist with NMDA to produce NMDA-mediated Ca^{2+} influx, Ca^{2+} influx was sufficiently detectable even in the absence of glycine [21]. As shown in Fig. 2D1, ginsenoside Rk1 produced 52.6% inhibition when the experiment was performed in the absence of glycine. However, a similar degree of inhibition was also observed when ginsenoside Rk1 was applied in the presence of 100 μ M glycine (47.5%, Fig. 2D2). The mean inhibitions by ginsenoside Rk1 were $47.6 \pm 2.0\%$ ($n = 10$) and $43.2 \pm 1.4\%$ ($n = 8$) in the absence and presence of glycine, respectively (Fig. 2D3). There was no significant difference between the mean percentages of ginsenoside Rk1 under these experimental conditions. However, in our previous studies with ginsenoside Rg3 [13,14], we showed that ginsenoside Rg3-mediated inhibition was observed in the pattern of glycine concentration dependence when we tested ginsenoside Rg3 and 20(S)-ginsenoside Rg3 in hippocampal neurons. Thus, these results suggest that ginsenoside Rk1 inhibits NMDARs without interacting with the glycine-binding site in hippocampal neurons.

3.4. Ginsenoside Rk1 affects the polyamine-binding site of NMDARs

The central regulatory sites of NMDARs are structurally complex with separate agonist NMDA-, coagonist glycine-, and polyamine-binding sites [22]. Based on the negative involvement of NMDA- and glycine-binding sites, we next examined the involvement of polyamine-binding sites on NMDARs in ginsenoside Rk1-mediated

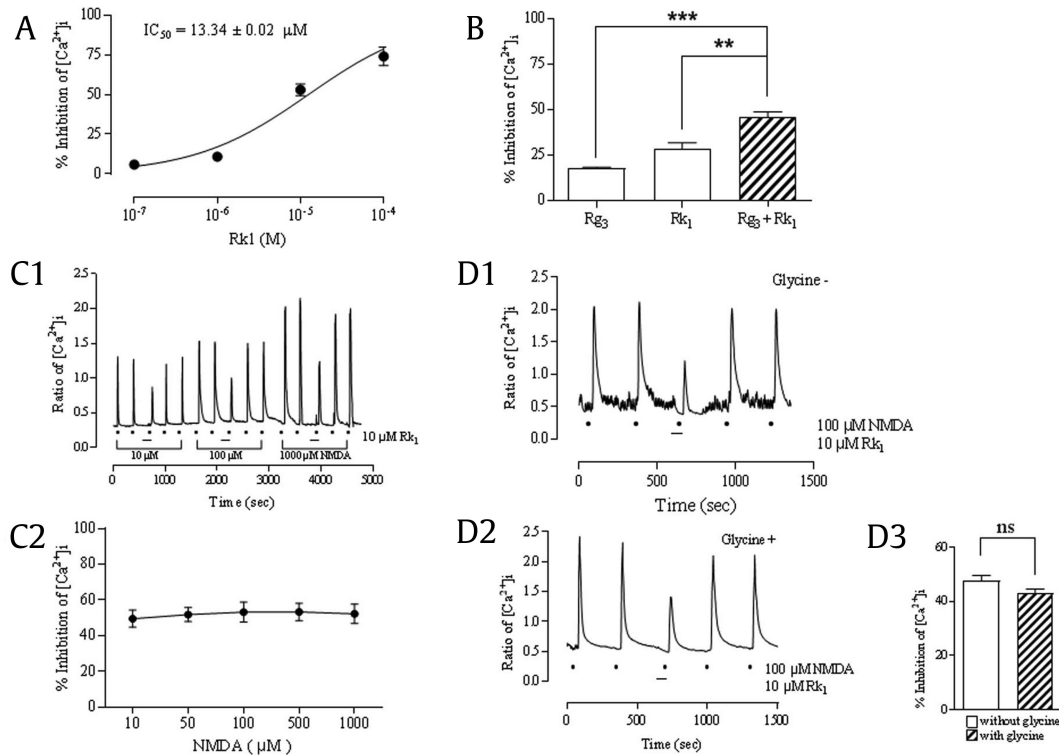


Fig. 2. Effects of ginsenoside Rk1 on the NMDA- and glycine-binding sites of NMDARs. (A) Dose–response relationship of inhibition of NMDA-mediated Ca^{2+} influx by ginsenoside Rk1. (B) The additive inhibitory effect of ginsenosides Rk1 and Rg3 on NMDA-mediated Ca^{2+} influx when used at a submaximal concentration (3 μM). Data were evaluated by unpaired Student *t* test ($***P < 0.001$, $**P < 0.01$). (C1) The effect of ginsenoside Rk1 on variable concentrations of NMDA (10, 100, and 1000 μM). Each concentration of NMDA was acutely (10 sec duration) and cyclically applied from 4 to 5 min through the fast perfusion system. (C2) Mean inhibition by ginsenoside Rk1 in different concentrations of NMDA. (D1, D2) The effects of ginsenoside Rk1 on the glycine-binding site of NMDARs in the absence (Glycine-) and presence (Glycine+) of 100 μM glycine. (D3) Pooled results showing the effects of ginsenoside Rk1 in the absence and presence of glycine. Data were evaluated by unpaired Student *t* test. NMDARs, NMDA receptors; ns, nonsignificant.

NMDAR inhibition. Here, we observed that ginsenoside Rk1 produced 51.3% inhibition on NMDA-mediated Ca^{2+} influx (Fig. 3A). To confirm the effects of ginsenoside Rk1 on polyamine-binding sites, we used the endogenous polyamine, spermine (200 μM), and we found that ginsenoside Rk1-mediated inhibition was 54.4% with spermine treatment (Fig. 3B). Involvement of polyamine sites on ginsenoside Rk1-mediated inhibition was further examined using another endogenous polyamine, spermidine (1 μM). Compared to control solution and spermine-treated cells, ginsenoside Rk1-mediated inhibition was diminished by the addition of spermidine (20.6%, Fig. 3C). From the pooled results, we found that the mean percentage inhibition by ginsenoside Rk1 ($48.7 \pm 3.1\%$) was significantly decreased by spermidine treatment ($19.5 \pm 1.6\%$, $P < 0.001$), but not by spermine treatment ($40.9 \pm 2.4\%$, $P = 0.082$, Fig. 3D).

To confirm the involvement of spermidine in ginsenoside Rk1-mediated inhibition on NMDA-induced Ca^{2+} influx, we directly recorded NMDA-mediated inward currents using perforated whole-cell patch recordings from hippocampal neurons. To decrease the variability in the spermidine responses, we carried out experiments with and without spermidine-treated solutions (5 min) by perforated whole-cell patch recordings from hippocampal neurons. At a holding membrane potential of -60 mV, we found that ginsenoside Rk1 inhibited inward currents induced by NMDA application (100 μM , 5 sec). Fig. 4A shows representative current traces evoked through NMDA alone and when coapplied with ginsenoside Rk1 during 1 min of pretreatment. The application of ginsenoside Rk1 inhibited both NMDA-mediated peak and sustained currents. However, when we next performed the

experiment in the presence of spermidine, ginsenoside Rk1-mediated inhibition of NMDA-induced currents was diminished (Fig. 4B). From a series of similar experiments, we found that the mean percentage inhibition by ginsenoside Rk1 on NMDA-induced peak current was significantly reduced in the presence of spermidine. The inhibitions by ginsenoside Rk1 were $50.3 \pm 2.0\%$ ($n = 4$) without spermidine and $21.0 \pm 1.7\%$ ($n = 4$) with spermidine (Fig. 4C). A similar degree of inhibition of NMDA-gated currents was observed in the presence of spermidine compared to NMDA-mediated Ca^{2+} influx in the presence of ginsenoside Rk1 during calcium imaging experiments.

4. Discussion

Ginsenosides isolated from *Panax ginseng* have numerous pharmacological activities, and an increasing number of studies have reported their importance in regulating neuronal functions in the CNS [23–25]. Accumulating evidence suggests that ginsenosides act on the CNS by restraining the availability of neurotransmitters [23,26] and also exhibit neuroprotective effects against excitotoxicity *in vitro* and *in vivo* [27,28]. To date, ginsenosides Rg1, Rb1, and Rg3 are among the most extensively studied constituents of ginseng in the CNS because of their relatively high abundance in ginseng and robust activities [29,30]. Ginseng is often processed at high temperatures prior to consumption, and the heat processing generates ginsenosides that are normally rarely present in raw or air-dried ginseng. In particular, heat-mediated dehydration of Rg3 produces ginsenosides Rk1 and Rg5, but their contributions to the neuroprotective effects of ginseng in the CNS are currently

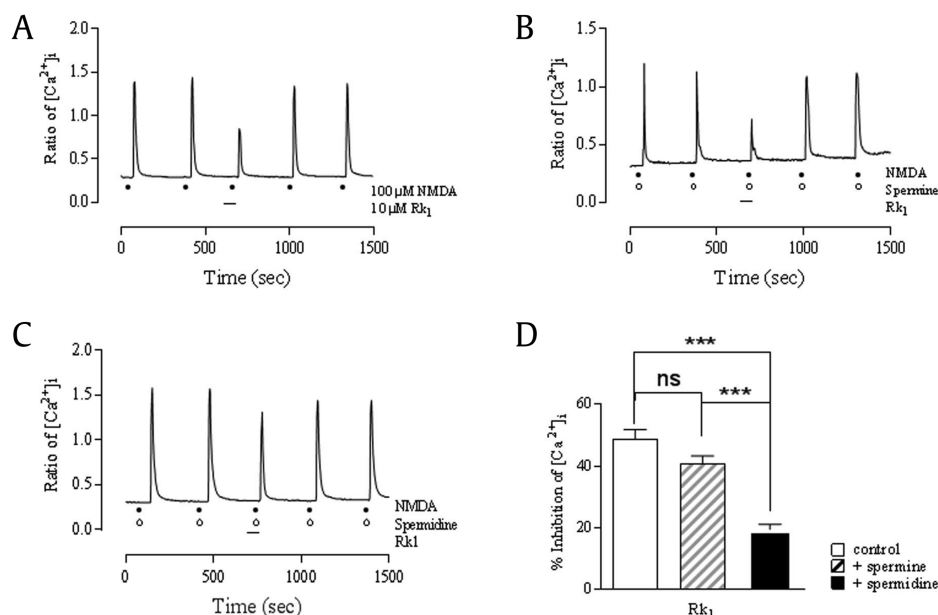


Fig. 3. Effects of ginsenoside Rk1 on the polyamine-binding site of NMDARs. (A) A sample recording showing the effects of ginsenoside Rk1 on a cultured hippocampal neuron using fura-2-based calcium imaging in a HEPES-buffered solution. (B) Sample recordings showing the effects of spermine (200 μM) on ginsenoside Rk1-mediated inhibition. (C) The effect of spermidine (1 μM) on ginsenoside Rk1-mediated inhibition of hippocampal neurons. (D) Pooled results from the polyamine experiments illustrating the mean inhibition of ginsenoside Rk1 on NMDA-mediated Ca^{2+} influx. Data were evaluated by one-way analysis of variance ($^{***}P < 0.001$). NMDARs, NMDA receptors; ns, nonsignificant

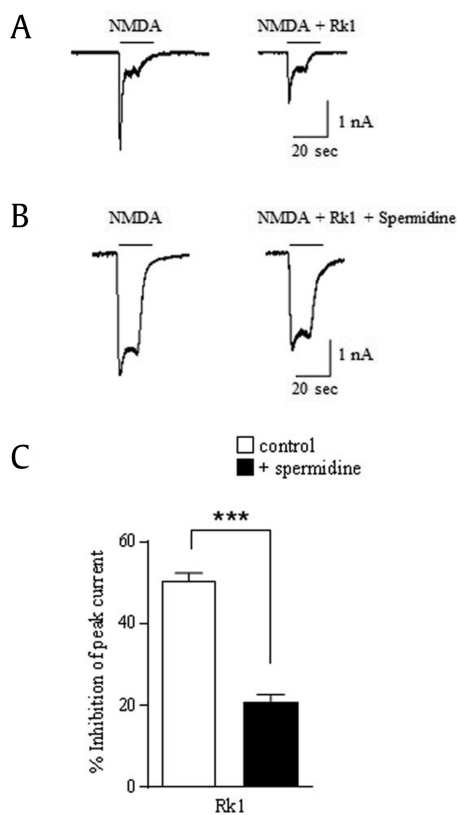


Fig. 4. Effects of ginsenoside Rk1 on NMDA-mediated inward currents. (A) NMDA-mediated inward currents were measured at a holding potential of -60 mV using the whole-cell voltage-clamp technique. (B) Representative currents evoked by NMDA (100 μM) alone and coapplied with spermidine (1 μM). (C) Pooled results show that ginsenoside Rk1-mediated inhibition of NMDA-mediated inward peak currents was significantly diminished in the spermidine-treated recording solution for 5 min. Data were evaluated by unpaired Student *t* test ($^{***}P < 0.001$).

unknown. Therefore, in this study, we examined whether ginsenosides Rk1 and Rg5 inhibit NMDARs in hippocampal neurons and revealed that ginsenoside Rk1 serves as a novel inhibitor of NMDARs with significantly higher potency than ginsenosides Rg3 and Rg5 (Fig. 1).

Cotreatment with ginsenosides Rg3 and Rk1 was more effective in inhibiting NMDARs compared to either ginsenoside Rg3 or Rk1 alone (Fig. 2B), and this additive effect implies that the binding site of ginsenoside Rk1 in NMDARs is different from that of ginsenoside Rg3. A previous study from our laboratory demonstrated that Rg3 antagonizes NMDARs through a glycine-binding site [13], and as expected, the inhibition of NMDARs by ginsenoside Rk1 was not affected by the presence or absence of glycine (Fig. 2D). Moreover, the ginsenoside Rk1-dependent inhibition of NMDARs was unaffected, regardless of changing NMDA concentrations, indicating that ginsenoside Rk1 does not compete for the NMDA-binding site (Fig. 2C). Therefore, we tested whether ginsenoside Rk1 inhibits NMDARs via the polyamine-binding site. Strikingly, treatment with spermidine, an endogenous polyamine, significantly reduced the antagonizing effect of ginsenoside Rk1 on NMDAR activation (Fig. 3). This finding indicates that ginsenoside Rk1 serves as an NMDAR antagonist via a competitive interaction with the polyamine-binding site of NMDARs, which was further confirmed by directly examining the NMDAR-mediated inward current using a patch-clamp technique (Fig. 4). Therefore, this study suggests that ginsenoside Rk1 might protect against NMDA-mediated hippocampal cell death. It should also be noted, however, that another endogenous polyamine, spermine, did not influence the inhibition of NMDARs by ginsenoside Rk1. Strikingly, we previously observed that ginsenoside Rh2-mediated NMDAR inhibition was significantly reduced by spermine treatment [14]. This discrepancy may result from the difference in binding affinities between spermine and spermidine for the polyamine-recognition site. However, the pharmacological validity of spermine should be investigated using lower concentrations of NMDA to rule out the involvement of spermine in ginsenoside Rk1-mediated NMDAR inhibition. Collectively, ginsenoside Rk1 antagonizes NMDARs through the

polyamine-binding site, but ginsenoside Rk1 may also inhibit NMDARs by modulating other regulatory sites that have not been investigated in this study.

The heat-mediated conversion of ginsenoside Rg3 to Rk1 introduces a relatively subtle change in the overall ginsenoside structure. However, the minor structural difference shifted the recognition site from the glycine-binding site to the polyamine-binding site, and ginsenoside Rk1 thus serves as a more potent inhibitor for NMDARs. This substantial change presumably results from the fact that the subtle structural change involves the carbon-20 position, and the local structure around the carbon-20 position plays a significant role in determining the interaction between ginsenosides and NMDARs. We previously demonstrated that ginsenoside Rh2, the main metabolite of ginsenoside Rg3, exhibits stereo-specific effects in which the 20(S)-isomer of ginsenoside Rh2 strongly inhibits NMDARs but the 20(R)-isomer does not [14]. Therefore, the structural rigidity introduced by the double bond at the carbon-20 position in ginsenoside Rk1 most likely contributes to the shift in the competitive interaction from the glycine-binding site to the polyamine-binding site.

In summary, we have demonstrated that the ginsenoside Rk1 produced by heat processing serves as a novel NMDAR antagonist with the highest potency among the ginsenosides we have tested thus far. Given the additive effect of cotreatment with ginsenosides Rg3 and Rk1 in inhibiting NMDARs, targeting both glycine- and polyamine-binding sites simultaneously with ginsenosides can effectively suppress NMDAR activity. Importantly, neuronal death found in ischemic stroke or seizure is attributed to NMDAR hyperactivity, and multiple studies have reported causal links between dysregulated NMDAR activation and neurodegenerative diseases, such as Alzheimer and Huntington diseases. Therefore, the high potency of ginsenoside Rk1 as an NMDAR antagonist along with its synergistic action with ginsenoside Rg3 highlights ginsenoside Rk1 as a promising therapeutic agent to treat pathological conditions associated with neuronal excitotoxicity.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2019.04.002>.

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