

Published in final edited form as:

J Neurosci Res. 2009 June ; 87(8): 1904–1912. doi:10.1002/jnr.22017.

Ginsenosides protect striatal neurons in cellular model of Huntington's disease

Jun Wu¹, Hye Kyoung Jeong², Sarah Elizabeth Bulin¹, Sung Won Kwon², Jeong Hill Park², and Ilya Bezprozvanny^{1,*}

¹Department of Physiology, UT Southwestern Medical Center at Dallas, TX 75390, USA

²College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-741, Korea

Abstract

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is a widely used herbal medicine. Ginsenosides, the active ingredients of ginseng, are the main components responsible for many beneficial actions of ginseng. In the present study, we tested ten different ginsenosides in the previously developed *in vitro* Huntington's disease (HD) assay with primary medium spiny striatal neuronal cultures (MSN) from the YAC128 HD mouse model. We found that nanomolar concentrations of ginsenoside Rb1 and Rc effectively protected YAC128 medium spiny neurons from glutamate-induced apoptosis; and that Rg5 was protective at micromolar concentration. The other seven ginsenosides tested were not effective or exerted toxic effects in MSN cultures. In further experiments we suggested that neuroprotective effects of ginsenosides Rb1, Rc, and Rg5 could correlate with their ability to inhibit glutamate-induced Ca²⁺ responses in cultured MSN. From these results we concluded that ginsenosides Rb1, Rc and Rg5 offer a potential therapeutic choice for the treatment of HD and possibly other neurodegenerative disorders.

Keywords

Huntington's disease; apoptosis; excitotoxicity; Ginseng; Ginsenosides; transgenic mouse

Introduction

Huntington's disease (HD) is an inherited, incurable autosomal dominant disorder caused by a polyglutamine expansion in Huntingtin, a 350-kDa ubiquitously expressed cytoplasmic protein. (The Huntington's Disease Collaborative Research Group, 1993). It is characterized by progressive neurodegeneration resulting in motor abnormalities including chorea and psychiatric disturbance with gradual dementia (Vonsattel and DiFiglia 1998). The pathological hallmark of HD is the loss of medium spiny neurons (MSN) in the striatum which leads to major clinical abnormalities that characterize the disease. The exact cause of neuronal loss is still unknown. Recent evidence indicates that deranged neuronal calcium signaling plays an important role in the pathophysiology of HD (Bezprozvanny and Hayden 2004; Tang et al. 2005). The HD mutation affects Ca²⁺ signaling in medium spiny neurons (MSN) by sensitizing InsP₃R1 to activation by InsP₃ (Tang et al. 2003), stimulating NR1/NR2B NMDAR activity (Chen et al. 1999; Sun et al. 2001; Zeron et al. 2002; Zhang et al. 2008) and destabilizing mitochondrial Ca²⁺ handling (Choo et al. 2004; Panov et al. 2002). Enhanced glutamate-

*Corresponding author: tel: (214) 645-6017 fax: (214) 645-6018 Ilya.Bezprozvanny@UTSouthwestern.edu.

induced Ca^{2+} responses lead to continuous mitochondrial Ca^{2+} overload and eventual apoptosis of HD MSN (Shehadeh et al. 2006; Tang et al. 2005; Zhang et al. 2008).

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), has been used as herbal medicine in Asia to provide benefit in many disease states, including neurodegenerative and aging disorders. Ginsenosides, the main components responsible for the actions of ginseng, have a four-ring, steroid-like structures with sugar moieties attached. More than thirty different ginsenosides have been isolated and identified from ginseng, and they are classified into protopanaxadiol (PPD) and protopanaxatriol (PPT) saponins according to the chemical structures. Ginsenosides Rb1, Rb2, Rc, Rd, and Rg3 belong to PPD saponin, while Rg1, Rg2, and Re belong to PPT saponin. Previous studies demonstrated that ginsenoside Rb1 and Rg3 protected cortical neurons from glutamate-induced cell death by blocking Ca^{2+} influx through glutamate receptors (Kim et al. 1998). It was also demonstrated that saponins extracted from ginseng could inhibit both NMDA-induced and glutamate-induced Ca^{2+} increase in rat hippocampal neurons (Kim et al. 2002). Neuroprotective effects of Rb1, Rb3, and Rd ginsenosides have been demonstrated in the 3-nitropropionic acid (3-NP) model of striatal neurodegeneration in rodents (Lian et al. 2005). The protective effects of ginseng saponins have also been found on 3-NP induced striatal degeneration in rats (Kim et al. 2005). These results suggest that ginsenosides may be useful and potentially therapeutic choices for the treatment of neurodegenerative disorders (Nah et al. 2007).

In the present study, we tested a number of ginsenosides using previously developed *in vitro* HD model (Tang et al. 2005). In these experiments, primary cultures of MSN neurons from YAC128 HD mouse were exposed to glutamate stimulation and the protective effects of ginsenosides on the survival of YAC128 MSN were studied. The effects of ginsenosides on glutamate-stimulated Ca^{2+} responses of primary cultured MSN were also investigated. From these experiments, we concluded that ginsenosides Rb1, Rc and Rg5 may be useful in the treatment of HD and possibly other neurodegenerative disorders.

Materials and Methods

Drugs

Glutamate was from Tocris. Ginsenosides Rb1, Rc, Rd, Re, Rg3, Rg5, Rh1, ReRd mixture, Rk1Rg5 mixture and Rh4Rk3 mixture were isolated as previously described (Kwon et al. 2001; Park et al. 2002). Briefly, raw and steamed ginsengs were refluxed with methanol for 6 h. After removal of methanol by evaporator, the aqueous residue was extracted with butanol. Then butanol fraction was subjected to silica gel chromatography or prep-HPLC (high performance liquid chromatography) for the isolation of ginsenoside. Chemical structure of isolated ginsenoside was confirmed by NMR and MS. For the qualitative and quantitative analysis of ginsenosides, HPLC-ELSD (evaporative light scattering detector) was used. The purity of ginsenosides was decided by calculation of the peak area from HPLC-ELSD data. The purity of the ginsenosides and percentage of various ginsenosides in mixtures used in our experiments were: Rb1 (>85%), Rc (99%), Rd (97%), Re (99%), Rg3 (99%), Rg5 (85%), Rh1 (95%), Re/Rd (Re:Rd 39:50), Rk1/Rg5 (Rk1:Rg5 85:13) and Rh4/Rk3 (Rh4:Rk3 38:50). Ginsenosides were dissolved in DMSO to 10 mM concentrated stock and further diluted to final concentration in Neurobasal-A medium for *in vitro* HD assay or in artificial cerebrospinal fluid (ACSF) for the Ca^{2+} imaging experiments.

In vitro HD assay

YAC128 mice (FVBN/NJ background strain) (Slow et al. 2003) were obtained from Jackson Labs (stock number 004938). The male YAC128 mice were crossed to wild-type (WT) female FVBN/NJ mice and P1-P2 pups were collected and genotyped by PCR. The primary cultures

of striatal medium spiny neurons (MSN) were established from YAC128 and wild type littermates as previously described (Mao and Wang 2001; Tang et al. 2005). Striata were dissected, diced and digested with trypsin. After dissociation, neurons were plated on poly-L-lysine (Sigma) coated 12 mm round coverslips (Assistent) in Neurobasal-A medium supplemented with 2% B27, 1 mM glutamine and penicillin-streptomycin (all from Invitrogen) and kept at 37°C in a 5% CO₂ environment. Ginsenosides were added to the 14-DIV (days in vitro) MSN at the final concentration of 0.01 μM, 0.1 μM and 1.0 μM. After 3 h incubation with ginsenosides, the MSN were exposed for 7h to 250μM glutamate in Neurobasal-A added to the culture medium. Immediately after the treatment with glutamate, neurons were fixed for 30 min in 4% paraformaldehyde plus 4% sucrose in PBS (pH7.4), permeabilized for 5 min in 0.25% Triton-X-100, and stained using the DeadEnd fluorometric TUNEL System (Promega). Nuclei were counterstained with 5 μM propidium iodine (PI) (Molecular Probes). Coverslips were extensively washed with PBS and mounted in Mowiol 4-88 (Polysciences). For quantification, six to eight randomly chosen microscopic fields containing 100-300 MSN each were cell-counted for YAC128 and wild type cultures. The number of TUNEL-positive neuronal nuclei was calculated as a fraction of PI-positive neuronal nuclei in each microscopic field. The fractions of TUNEL-positive nuclei determined for each microscopic field were averaged and the results are presented as mean ± SE (n= number of fields counted).

Calcium imaging

The Ca²⁺ imaging experiments with 13-14 DIV MSN cultures were performed in multiple pulses of glutamate application, using a DeltaRAM illuminator, an IC-300 camera, and IMAGEMASTER PRO software (all from PTI). The WT MSN were loaded with 5μM fura-2 AM (Molecular Probes) for 45 min at 37°C in artificial cerebrospinal fluid (ACSF, containing the following: 140mM NaCl, 5mM KCl, 1mM MgCl₂, 2mM CaCl₂, 10mM Hepes, pH7.3). Coverslips were mounted onto a recording/perfusion chamber (RC-26G, Warner Instruments) and positioned on the movable stage of an Olympus (Melville) IX-70 inverted microscope. Images at 340 and 380 nm excitation wavelengths were acquired every 6 s and shown as 340/380 image ratios as described previously (Tang et al. 2003). Neurons were stimulated with 20 μM glutamate (30s duration) repetitively with 4-5 min ACSF wash in between. Before the third application of glutamate, neurons were incubated with Rb1, Rc, Rg3, Rg5, Re at 10 μM concentration in ACSF for 1 min. Higher concentrations 50 μM or 100 μM and 5 min incubation time were tested for Rc, Rg5 and Re. The Rg3 and Rb1 were also tested in Ca²⁺ imaging experiments at 0.1 μM concentration with 5 min incubation.

Statistical analysis

All experiments were repeated at least three times. All data were evaluated for statistical significance by analysis using SigmaPlot t-test or One-Way ANOVA. Statistical difference was considered to be significant only if P<0.05.

Results

Ginsenosides are protective in *in vitro* HD assay

In the absence of glutamate, approximately 5-15% of neurons were apoptotic in both wild type (WT) and YAC128 MSN cultures. Following exposure to 250 μM glutamate, the fraction of apoptotic WT MSN was increased to 30-45% and the fraction of apoptotic YAC128 MSN was increased to 60-75% (Figs 1A and 1B, Table 1). As we previously described, the difference between glutamate-induced apoptosis of YAC128 and WT MSN is highly significant and constitute a quantitative basis for the *in vitro* HD assay (Tang et al. 2005). In the earlier study, we utilized the *in vitro* HD assay to evaluate neuroprotective effects of five clinically relevant glutamate pathway antagonists – folic acid, gabapentin, lamotrigine, memantine and riluzole (Wu et al. 2006). Here we used similar approach to evaluate neuroprotective effects of ten

isolated ginsenosides. The chemical structures of ginsenosides tested in our experiments are shown on Supplementary Figure 1. Neuroprotective effects of all ten ginsenosides were evaluated at 0.01 μM , 0.1 μM and 1 μM concentrations. In all experiments ginsenosides were added 3h prior to addition of glutamate to WT and YAC128 MSN cultures. We found that ginsenosides Re, Rh1, ReRd, Rk1Rg5 and Rh4Rk3 had no protective effects on glutamate-induced apoptosis of YAC128 MSN at the concentrations tested (Figs 1E and 1F, Table 1). The ginsenosides Re, Rh1 and Rk1Rg5 were toxic to MSN at 1 μM concentration (Table 1). The ginsenosides Rd and Rg3 potentiated glutamate-induced apoptosis of WT and YAC128 MSN (Table 1).

In contrast to other ginsenosides tested, Rb1, Rc and Rg5 had significant protective effects in the *in vitro* HD assay. Rb1 was most effective, with potent protective effects at 0.01 μM and 0.1 μM concentrations (Figs 1C, 1D, 2A, 2B, Table 1). Rc had protective effect at 0.01 μM concentration (Fig 2C, Table 1). Rg5 was not effective at 0.01 μM and 0.1 μM concentrations but was protective at 1.0 μM concentration (Fig 2D, Table 1). The neuroprotective effects of Rb1, Rc and Rg5 were observed when 100 μM or 250 μM of glutamate was used to induce apoptosis of YAC128 MSN (Fig 2).

Ginsenosides inhibit glutamate-induced Ca^{2+} signals in MSN

How do ginsenosides exert their neuroprotective effects? In the previous studies, we demonstrated that inhibitors of Ca^{2+} signaling protect YAC128 MSN from glutamate-induced apoptosis (Tang et al. 2005; Wu et al. 2006; Zhang et al. 2008). It has been previously demonstrated that some ginsenosides inhibit NMDA-induced Ca^{2+} responses in cultured hippocampal neurons (Kim et al. 2002; Lee et al. 2006). Thus, in the next experiments we examined the effects of ginsenosides on glutamate-induced calcium signals in Fura-2 Ca^{2+} imaging experiments with DIV13-14 WT MSN cultures in ACSF. The ratio of Fura-2 signals at 340 and 380nm excitation wavelengths was used to quantitatively determine the concentration of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). The acute application of glutamate (20 μM , a 30s duration) to WT MSN cultures produced a rapid increase of Ca^{2+} (Figs 3A, 4A). Following a 4-5 min washout with ACSF, repetitive applications of glutamate induced consistent Ca^{2+} responses in stimulated MSN (Figs 3A, 4A). The glutamate-induced responses were not significantly affected following a 1 min preincubation with 0.1% DMSO (Figs 3A, 4A). Preincubation with 10 μM Rb1 suppressed glutamate-induced Ca^{2+} responses (Figs 3B, 4B), whereas preincubation with 10 μM Re had no effect (Figs 3C, 4C). The inhibition by Rb1 was reversible as the glutamate-induced Ca^{2+} response could be restored to initial amplitude following a washout of this ginsenoside (Figs 3B, 4B). To quantify the effects of the ginsenosides, we calculated a ratio of peak glutamate-induced Ca^{2+} responses before and after the application of ginsenosides (peak3/peak2 ratio). Using this statistical measurement, we determined that the average peak3/peak2 ratio was equal to 1.08 ± 0.05 ($n=56$) for DMSO control, 0.41 ± 0.03 ($n=50$) for Rb1-exposed cells and 1.25 ± 0.11 ($n=29$) for Re exposed cells (Fig 4D). Preincubation with 10 μM Rc or Rg5 had some modest inhibitory effect on glutamate-induced Ca^{2+} responses, but it did not reach a level of statistical significance when compared to DMSO alone (Fig 4D). However, the trend of inhibition by Rc and Rg5 at 10 μM could be seen. Thus, we evaluated higher concentrations of these ginsenosides. We determined that incubation with 50 μM Rg5 for 1 min reduced the glutamate-induced Ca^{2+} response to $24 \pm 5\%$ ($n=40$) of the previous Ca^{2+} response. The glutamate-induced Ca^{2+} response was also decreased to $70\% \pm 9\%$ ($n=47$) of the previous Ca^{2+} response following 1 min treatment with 100 μM Rc. In contrast, 1 or 5 min incubation with 100 μM Re had no significant effect on glutamate-induced calcium responses. In additional experiments we tested Rg3 and Rb1 in 0.1 μM concentrations with 5 min incubation time. In these experiments we did not observe significant effects of Rg3 or Rb1 on glutamate-induced Ca^{2+} responses evoked in YAC128 MSN (data not shown). Most likely lack of effect in these experiments could be explained by

the low penetrance of the drugs at low concentrations within 5 min time frame allowed. Thus, we concluded that the ability of these ginsenosides to inhibit glutamate-induced MSN Ca^{2+} responses follows the order $\text{Rb1} > \text{Rg5} \sim \text{Rc} \gg \text{Re}$, which is the similar order as their ability to protect YAC128 MSN from glutamate-induced apoptosis (Fig 2, Table 1). At least one exception to this rule appears to be Rg3, which was not protective in the *in vitro* HD assay (Table 1) but effectively blocked glutamate-induced Ca^{2+} responses at the 10 μM concentration (Fig 4D). The inability of Rg3 to exert protective effects in our experiments was most likely related to the toxic effects of this ginsenoside on cultured MSN (Table 1). To rule out potential toxic contamination present in the batch of Rg3 used in our experiments, we repeated apoptosis experiments with an independently isolated preparation of Rg3 but did not observe neuroprotection in these experiments either (data not shown). Furthermore, we separately evaluated 20(R)-Rg3 and 20(S)-Rg3 stereoisomers but did not observe protective effects in *in vitro* HD assay for either stereoisomer (data not shown).

Discussion

Development of small molecules, which can cross the blood-brain-barrier and confer neuroprotection to medium spiny neurons, remains one of the primary objectives in HD research. It has been previously demonstrated that ginsenosides have a variety of beneficial effects in the central nervous system (Nah et al. 2007; Rhim et al. 2002; Wen et al. 1996). Prevention of ischemic neuronal death by intravenous infusion of ginsenoside Rb1 suggests that Rb1 may pass through blood brain barrier (Zhang et al. 2006). It has also been shown that ginsenosides Rb1 and Rg3 can significantly attenuate glutamate-induced neurotoxicity in cultured rat cortical and hippocampal cells (Kim et al. 2004; Kim et al. 1998). *In vivo* studies demonstrated neuroprotective effects of Rb1, Rb3, and Rd ginsenosides in the 3-nitropropionic acid (3-NP) model of striatal neurodegeneration (Lian et al. 2005). The systemic administration of ginseng saponins (GTS) produced significant protections against systemic 3-NP- and intrastriatal malonate-induced lesions in rat striatum (Kim et al. 2005). All these reports indicate that ginsenosides may offer a potential therapeutic option for treatment of HD. Our current results show that ginsenosides Rb1, Rc and Rg5 can efficiently protect YAC128 HD MSN from glutamate-induced apoptosis in the *in vitro* HD assay (Figs 1 and 2, Table 1). In contrast, ginsenosides Re, Rh1, ReRd, Rk1Rg5 and Rh4Rk3 did not show significant protective effects and Re, Rh1 and Rk1Rg5 were toxic at 1 μM concentration (Table 1). Ginsenosides Rd and Rg3 potentiated glutamate-induced apoptosis of MSN (Table 1). While low doses of Rb1 (0.01, 0.1 μM) and Rc (0.01 μM) were protective, the overdoses of the ginsenosides Rb1 (1.0 μM) and Rc (0.1, 1.0 μM) did not show significant protective effects (Table 1). From these results we concluded that there is a fine line between neuroprotective and toxic effects of ginsenosides. Similar dose-dependent phenomenon has been previously observed with Rb1 and Rg3 in experiments with cultured rat cortical neurons, however with somewhat wider therapeutic window (Kim et al. 1998). It appears that at high concentrations ginsenosides are toxic to neurons, which compromises their ability to act as neuroprotective agents at high doses and limits their therapeutic window. The differences observed in our experiments and in previous experiments performed with cortical neuronal cultures are most likely due to the different sensitivity of striatal MSN and cortical neurons to toxicity of ginsenosides. Most striking example of this phenomenon appears to be Rg3, which was neuroprotective in cortical neuronal preparation (Kim et al. 1998) but was toxic in our experiments performed with striatal MSN (Table 1). Previous studies revealed that Rg3 can affect activity of voltage-gated Ca^{2+} channels (Rhim et al. 2002), K^{+} channels (Lee et al. 2008), Na^{+} channels (Jeong et al. 2004), and NMDA receptor (Lee et al. 2006). It is possible that the multiple effects exerted by Rg3 lead to different levels of toxicity in cortical and striatal cultures.

It has been hypothesized that glutamate-induced Ca^{2+} overload contributes to the pathogenesis of HD (Bezprozvanny and Hayden 2004; Tang et al. 2005). Our previous studies demonstrated

that Ca^{2+} signaling inhibitors, including clinically relevant compounds memantine and riluzole, are protective in *in vitro* HD assay (Tang et al. 2005; Wu et al. 2006). Thus, we evaluated the ability of ginsenosides Rb1, Rc and Rg5 to affect glutamate-induced Ca^{2+} responses of MSN. The effects of these ginsenosides were compared to the effects of Re, which was not protective in the *in vitro* HD assay. In our Ca^{2+} imaging experiments we utilized much higher concentrations of ginsenosides (10 – 100 μM) as in these experiments ginsenosides had to be applied acutely. We found that ginsenosides Rb1, Rc and Rg5 could inhibit the glutamate-induced increase of Ca^{2+} in MSN at concentrations ranging from 10 μM to 100 μM (Fig 4). At the same concentrations, Re had no significant effect on glutamate-induced Ca^{2+} signals (Fig 4). These results are in agreement with the previous report of inhibitory effects of Rb1 on NMDA-induced Ca^{2+} responses in cultured rat hippocampal neurons (Kim et al. 2002). Importantly, the ability of these ginsenosides to inhibit glutamate-induced MSN Ca^{2+} responses followed the order $\text{Rb1} > \text{Rg5} \sim \text{Rc} \gg \text{Re}$ (Fig 4D), which is the same order as their ability to protect YAC128 MSN from glutamate-induced apoptosis (Fig 2, Table 1). The exception to this rule appears to be Rg3, which potentially inhibited glutamate-induced Ca^{2+} responses (Fig 4D) but was not protective in apoptosis experiments (Table 1). As discussed above, this discrepancy is likely to be due to the toxic effects exerted by Rg3 on cultured MSN. The effect of ginsenosides Rg3 or Rb1 on glutamate-induced Ca^{2+} responses in MSN could be observed at 10 μM concentrations (Fig 4), however lower concentrations of these ginsenosides (0.1 μM) did not inhibit Ca^{2+} responses in our experiments (data not shown). This could probably be caused by slow membrane partitioning of ginsenosides at low concentrations. Given that the overall pharmacology of ginsenosides is complex, it is likely that other factors in addition to Ca^{2+} stabilizing effects may also contribute to the actions of ginsenosides.

In our experiments we evaluated neuroprotective effects of ginsenosides in striatal YAC128 MSN culture preparation. Although striatal neurons are most affected in HD, cortical neurons also appear to be affected (Cepeda et al. 2007; Macdonald et al. 1997; Sotrel et al. 1991). Moreover, it is becoming increasingly clear that the cortical inputs onto MSN play a significant role in striatal cell loss and dysfunction in HD (Cepeda et al. 2007). Both cell-autonomous toxicity and pathological cell-cell interaction synergistically contribute to neuropathogenesis of the vulnerable cortical and striatal neurons in HD (Gu et al. 2007; Gu et al. 2005). Thus, it will be interesting to evaluate effects of ginsenosides using co-culture of striatal and cortical neurons, which is more close to a physiological situation. This experiment will be performed in the future.

Ginseng has been a popular herbal remedy used in eastern Asia for thousands of years. The use of ginseng, while beneficial, can cause complications when used in combination with other medications. The herb has been documented to induce manic episode in patients taking antidepressants (Vazquez and Aguera-Ortiz 2002). Ginseng also slightly decreases the anticoagulant effects of warfarin, a drug often prescribed to patients with heart disease (Greenblatt and von Moltke 2005). Other adverse effects from long term use and high doses of ginseng have been reported, including nervousness, insomnia, hypertension and diarrhea (Gyllenhaal et al. 2000). Although ginseng has beneficial anti-diabetic effects, decreasing fasting blood glucose levels in type 2 diabetics (Xie et al. 2005), it may interact with treatment for diabetes which is a well-known complication in HD. Therefore, more studies on basic pharmacological mechanisms of actions and toxicity of ginsenosides are required.

In conclusion, our studies demonstrate that at low doses, the ginsenosides Rb1, Rc and Rg5 could attenuate neuronal apoptosis induced by glutamate in the *in vitro* HD model. The same ginsenosides also inhibited glutamate-induced neuronal Ca^{2+} signals in cultured MSN, which suggests that, at least in part, their protective actions were due to inhibition of Ca^{2+} signaling. Although the cellular and molecular mechanisms that underlie the protective actions of

ginsenosides are not fully understood, our results indicate that ginsenosides Rb1, Rc and Rg5 could potentially be useful for treatment of Huntington's disease and possibly other neurodegenerative disorders. Considering the long history of safe usage of Ginseng in the human population, these compounds represent attractive leads for further clinical development. As previously discussed (Nah et al. 2007), the issues related to metabolism of ginsenosides and the blood-brain-barrier permeability of their metabolite will have to be addressed in order to take a full advantage of their therapeutic potential for treatment of neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Xiangmei Kong, Huarui Liu and Yuemei Li for help with maintaining the YAC128 mouse colony and genotyping, Janet Young and Leah Benson for administrative assistance, and Tie-Shan Tang for helpful advice and discussions. The study was supported by the McKnight Neuroscience of Brain Disorders Award, Robert A. Welch Foundation, the CHDI foundation, and the NINDS R01 NS38082 and NS056224 (IB) and Grant R01-2006-000-10593-0 from the Basic Research Program of the Korea Science & Engineering Foundation (SWK and JHP).

References

- Bezprozvanny I, Hayden MR. Deranged neuronal calcium signaling and Huntington disease. *Biochem Biophys Res Commun* 2004;322(4):1310–1317. [PubMed: 15336977]
- Cepeda C, Wu N, Andre VM, Cummings DM, Levine MS. The corticostriatal pathway in Huntington's disease. *Prog Neurobiol* 2007;81(56):253–271. [PubMed: 17169479]
- Chen N, Luo T, Wellington C, Metzler M, McCutcheon K, Hayden MR, Raymond LA. Subtype-specific enhancement of NMDA receptor currents by mutant huntingtin. *J Neurochem* 1999;72(5):1890–1898. [PubMed: 10217265]
- Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. *Hum Mol Genet* 2004;13(14):1407–1420. [PubMed: 15163634]
- Greenblatt DJ, von Moltke LL. Interaction of warfarin with drugs, natural substances, and foods. *J Clin Pharmacol* 2005;45(2):127–132. [PubMed: 15647404]
- Gu X, Andre VM, Cepeda C, Li SH, Li XJ, Levine MS, Yang XW. Pathological cell-cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. *Mol Neurodegener* 2007;2:8. [PubMed: 17470275]
- Gu X, Li C, Wei W, Lo V, Gong S, Li SH, Iwasato T, Itohara S, Li XJ, Mody I, Heintz N, Yang XW. Pathological cell-cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. *Neuron* 2005;46(3):433–444. [PubMed: 15882643]
- Gyllenhaal C, Merritt SL, Peterson SD, Block KI, Gochenour T. Efficacy and safety of herbal stimulants and sedatives in sleep disorders. *Sleep Med Rev* 2000;4(3):229–251. [PubMed: 12531167]
- Jeong SM, Lee JH, Kim JH, Lee BH, Yoon IS, Kim DH, Rhim H, Kim Y, Nah SY. Stereospecificity of ginsenoside Rg3 action on ion channels. *Mol Cells* 2004;18(3):383–389. [PubMed: 15650337]
- Kim JH, Kim S, Yoon IS, Lee JH, Jang BJ, Jeong SM, Lee BH, Han JS, Oh S, Kim HC, Park TK, Rhim H, Nah SY. Protective effects of ginseng saponins on 3-nitropropionic acid-induced striatal degeneration in rats. *Neuropharmacology* 2005;48(5):743–756. [PubMed: 15814108]
- Kim S, Ahn K, Oh TH, Nah SY, Rhim H. Inhibitory effect of ginsenosides on NMDA receptor-mediated signals in rat hippocampal neurons. *Biochem Biophys Res Commun* 2002;296(2):247–254. [PubMed: 12163009]
- Kim S, Kim T, Ahn K, Park WK, Nah SY, Rhim H. Ginsenoside Rg3 antagonizes NMDA receptors through a glycine modulatory site in rat cultured hippocampal neurons. *Biochem Biophys Res Commun* 2004;323(2):416–424. [PubMed: 15369768]

- Kim YC, Kim SR, Markelonis GJ, Oh TH. Ginsenosides Rb1 and Rg3 protect cultured rat cortical cells from glutamate-induced neurodegeneration. *J Neurosci Res* 1998;53(4):426–432. [PubMed: 9710262]
- Kwon SW, Han SB, Park IH, Kim JM, Park MK, Park JH. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J Chromatogr A* 2001;921(2):335–339. [PubMed: 11471818]
- Lee E, Kim S, Chung KC, Choo MK, Kim DH, Nam G, Rhim H. 20(S)-ginsenoside Rh2, a newly identified active ingredient of ginseng, inhibits NMDA receptors in cultured rat hippocampal neurons. *Eur J Pharmacol* 2006;536(12):69–77. [PubMed: 16563373]
- Lee JH, Lee BH, Choi SH, Yoon IS, Pyo MK, Shin TJ, Choi WS, Lim Y, Rhim H, Won KH, Lim YW, Choe H, Kim DH, Kim YI, Nah SY. Ginsenoside Rg3 inhibits human Kv1.4 channel currents by interacting with the Lys531 residue. *Mol Pharmacol* 2008;73(3):619–626. [PubMed: 17959711]
- Lian XY, Zhang Z, Stringer JL. Protective effects of ginseng components in a rodent model of neurodegeneration. *Ann Neurol* 2005;57(5):642–648. [PubMed: 15852378]
- Macdonald V, Halliday GM, Trent RJ, McCusker EA. Significant loss of pyramidal neurons in the angular gyrus of patients with Huntington's disease. *Neuropathol Appl Neurobiol* 1997;23(6):492–495. [PubMed: 9460715]
- Mao L, Wang JQ. Upregulation of preprodynorphin and preproenkephalin mRNA expression by selective activation of group I metabotropic glutamate receptors in characterized primary cultures of rat striatal neurons. *Brain Res Mol Brain Res* 2001;86(12):125–137. [PubMed: 11165379]
- Nah SY, Kim DH, Rhim H. Ginsenosides: are any of them candidates for drugs acting on the central nervous system? *CNS Drug Rev* 2007;13(4):381–404. [PubMed: 18078425]
- Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 2002;5(8):731–736. [PubMed: 12089530]
- Park IH, Kim NY, Han SB, Kim JM, Kwon SW, Kim HJ, Park MK, Park JH. Three new dammarane glycosides from heat processed ginseng. *Arch Pharm Res* 2002;25(4):428–432. [PubMed: 12214849]
- Rhim H, Kim H, Lee DY, Oh TH, Nah SY. Ginseng and ginsenoside Rg3, a newly identified active ingredient of ginseng, modulate Ca²⁺ channel currents in rat sensory neurons. *Eur J Pharmacol* 2002;436(3):151–158. [PubMed: 11858794]
- Shehadeh J, Fernandes HB, Mullins MM Zeron, Graham RK, Leavitt BR, Hayden MR, Raymond LA. Striatal neuronal apoptosis is preferentially enhanced by NMDA receptor activation in YAC transgenic mouse model of Huntington disease. *Neurobiol Dis* 2006;21(2):392–403. [PubMed: 16165367]
- Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Hum Mol Genet* 2003;12(13):1555–1567. [PubMed: 12812983]
- Sotrel A, Paskevich PA, Kiely DK, Bird ED, Williams RS, Myers RH. Morphometric analysis of the prefrontal cortex in Huntington's disease. *Neurology* 1991;41(7):1117–1123. [PubMed: 1829794]
- Sun Y, Savanenin A, Reddy PH, Liu YF. Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D- aspartate receptors via post-synaptic density 95. *J Biol Chem* 2001;276(27):24713–24718. [PubMed: 11319238]
- Tang T-S, Slow EJ, Lupu V, Stavrovskaya IG, Sugimori M, Llinas R, Kristal BS, Hayden MR, Bezprozvanny I. Disturbed Ca²⁺ signaling and apoptosis of medium spiny neurons in Huntington's disease. *Proc Natl Acad Sci U S A* 2005;102:2602–2607. [PubMed: 15695335]
- Tang T-S, Tu H, Chan EY, Maximov A, Wang Z, Wellington CL, Hayden MR, Bezprozvanny I. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. *Neuron* 2003;39:227–239. [PubMed: 12873381]
- The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;72(6):971–983. [PubMed: 8458085]
- Vazquez I, Agüera-Ortiz LF. Herbal products and serious side effects: a case of ginseng-induced manic episode. *Acta Psychiatr Scand* 2002;105(1):76–77. [PubMed: 12086230]discussion 77–78

- Vonsattel JP, DiFiglia M. Huntington disease. *J Neuropathol Exp Neurol* 1998;57(5):369–384. [PubMed: 9596408]
- Wen TC, Yoshimura H, Matsuda S, Lim JH, Sakanaka M. Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia. *Acta Neuropathol (Berl)* 1996;91(1):15–22. [PubMed: 8773141]
- Wu J, Tang T-S, Bezprozvanny I. Evaluation of clinically-relevant glutamate pathway inhibitors in in vitro model of Huntington's disease. *Neurosci Lett* 2006;407:219–223. [PubMed: 16959411]
- Xie JT, McHendale S, Yuan CS. Ginseng and diabetes. *Am J Chin Med* 2005;33(3):397–404. [PubMed: 16047557]
- Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt BR, Brundin P, Hayden MR, Raymond LA. Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* 2002;33(6):849–860. [PubMed: 11906693]
- Zhang B, Hata R, Zhu P, Sato K, Wen TC, Yang L, Fujita H, Mitsuda N, Tanaka J, Samukawa K, Maeda N, Sakanaka M. Prevention of ischemic neuronal death by intravenous infusion of a ginseng saponin, ginsenoside Rb(1), that upregulates Bcl-x(L) expression. *J Cereb Blood Flow Metab* 2006;26(5):708–721. [PubMed: 16163298]
- Zhang H, Li Q, Graham RK, Slow E, Hayden MR, Bezprozvanny I. Full length mutant huntingtin is required for altered Ca²⁺ signaling and apoptosis of striatal neurons in the YAC mouse model of Huntington's disease. *Neurobiol Dis* 2008;31(1):80–88. [PubMed: 18502655]

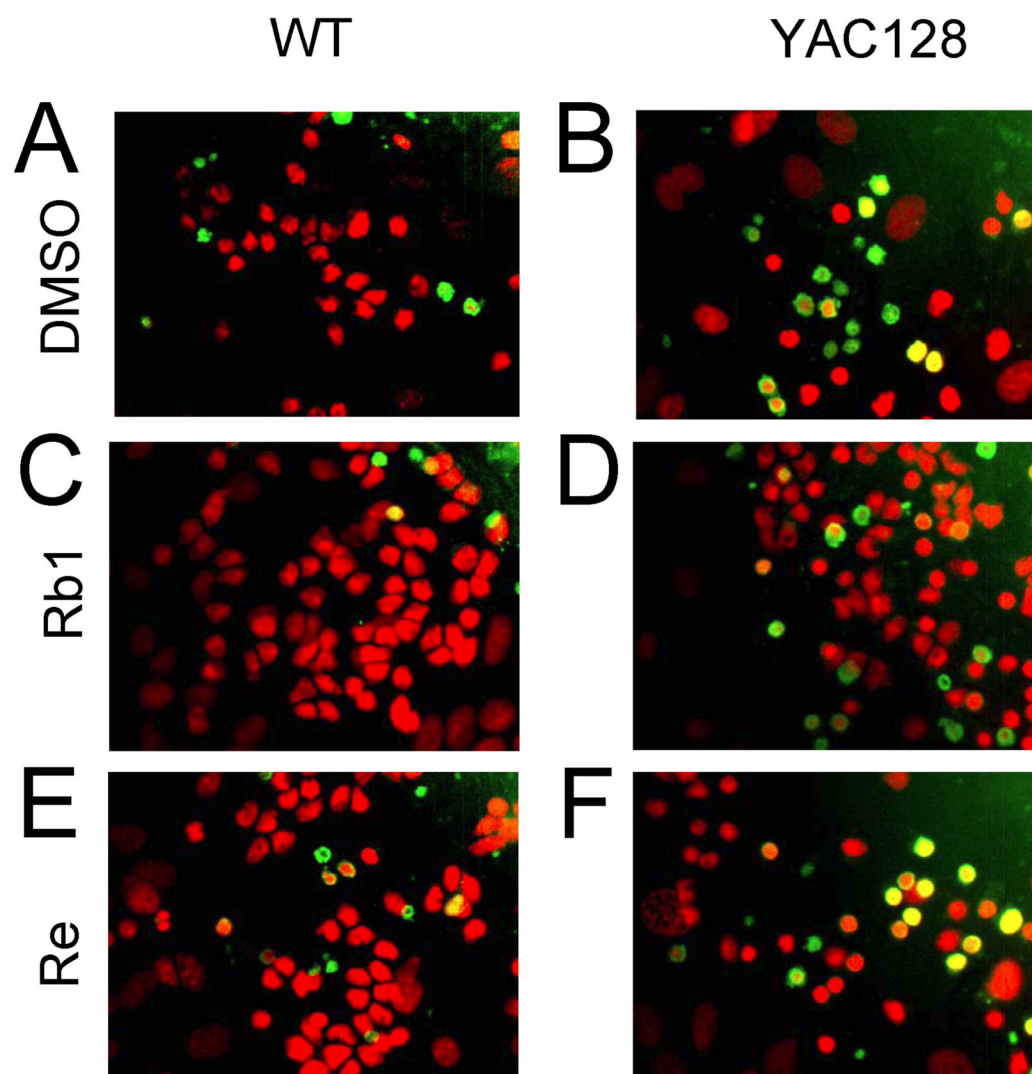


Fig. 1. Effects of ginsenosides in *in vitro* HD assay

Fourteen-DIV medium spiny neurons (MSN) from WT and YAC128 mice were exposed to 250 μ M glutamate for 7 h, fixed, permeabilized, and analyzed by TUNEL staining (green) and PI counterstaining. Representative images showing the TUNEL stained and PI stained medium spiny neurons after glutamate application in (A) WT and (B) YAC128 cultures. The TUNEL-staining images are also shown for WT and YAC128 MSN pre-treated for 3 h with 0.1 μ M Rb1 (C, D) and 0.1 μ M Re (E, F).

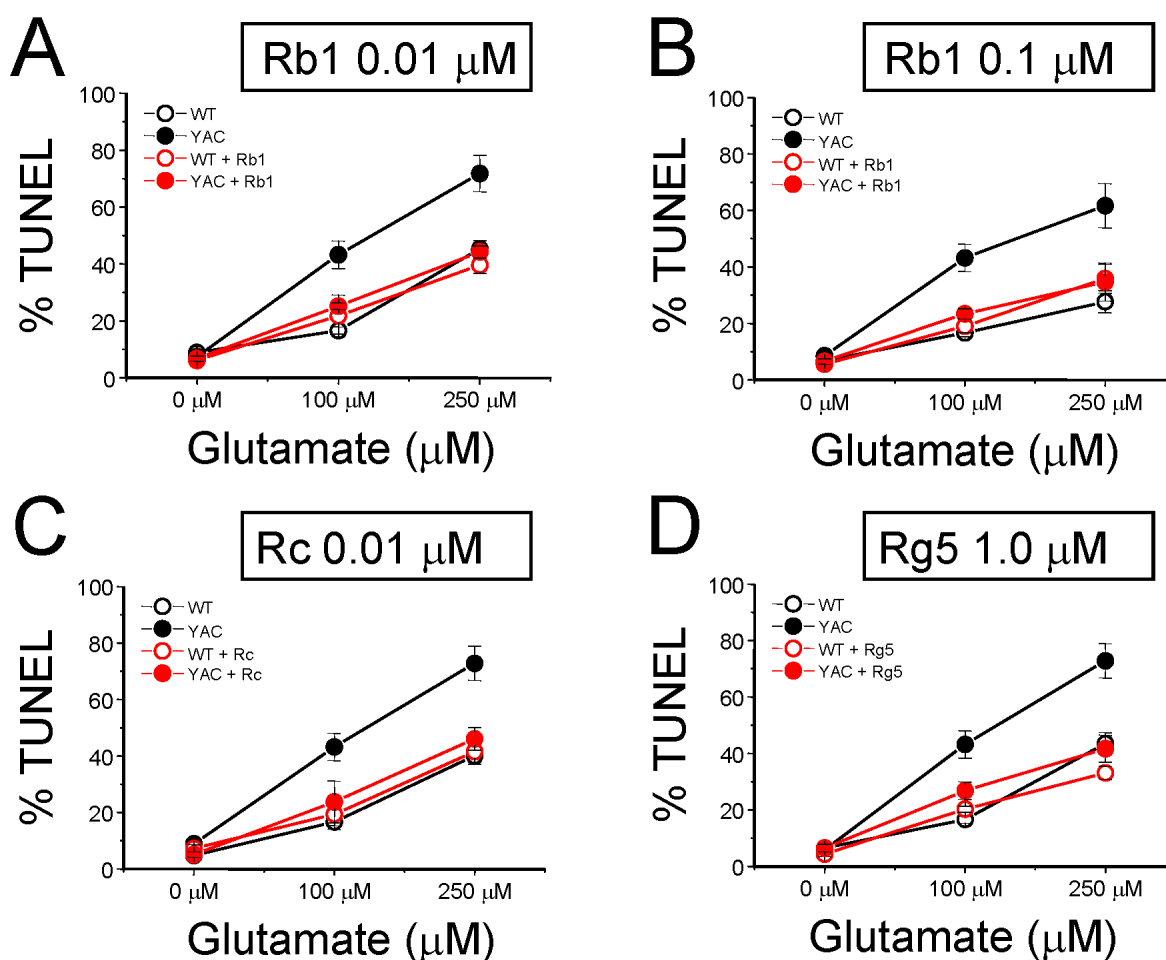


Fig. 2. Protective effects of ginsenosides in *in vitro* HD assay

The fraction of TUNEL-positive MSN is plotted against glutamate concentration for WT (open circle) and YAC128 (YAC, filled circles) mice. The data are shown as mean $\pm$ SE ($n = 6-8$ microscopic fields, 100-300 MSN per field). The results in the absence (black symbols) and in the presence (red symbols) of ginsenosides are shown for 0.01 μ M Rb1 (A); 0.1 μ M Rb1 (B); 0.01 μ M Rc (C); 1.0 μ M Rg5 (D). All ginsenosides were added 3 h prior to the application of glutamate.

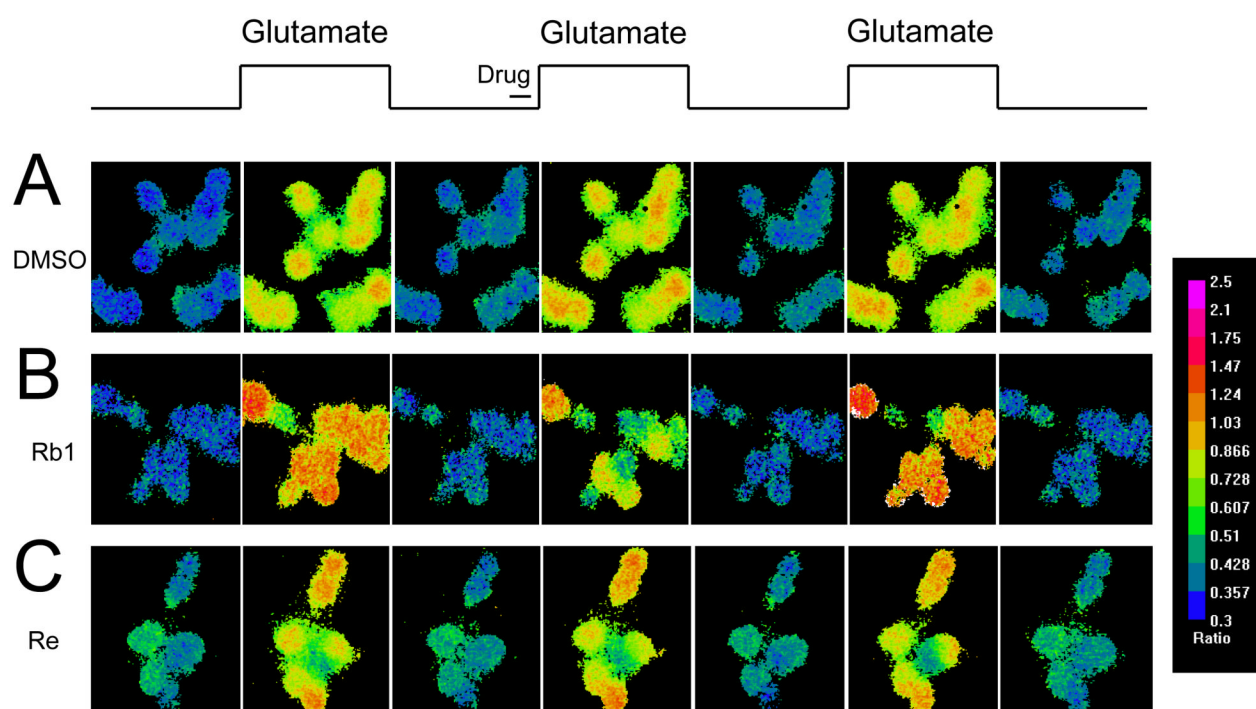


Fig. 3. Effects of ginsenosides on glutamate-induced Ca^{2+} response in cultured MSN
 Representative images showing Fura-2 340/380 ratios in medium spiny neurons (MSN). The pseudocolor calibration scale for 340/380 ratios is shown on the right. The acute application of glutamate (20 μM , a 30s-duration) produced a rapid increase of Ca^{2+} in ACSF. The glutamate-induced increase of Ca^{2+} was reproducible by repeated applications of glutamate with 4-5 min ACSF wash within 30 min. Ratio recordings are shown for the glutamate-induced responses in MSN for the second, third and fourth glutamate application with one minute incubation with DMSO (A), 10 μM Rb1 (B) and 10 μM Re (C) prior to the third glutamate application.

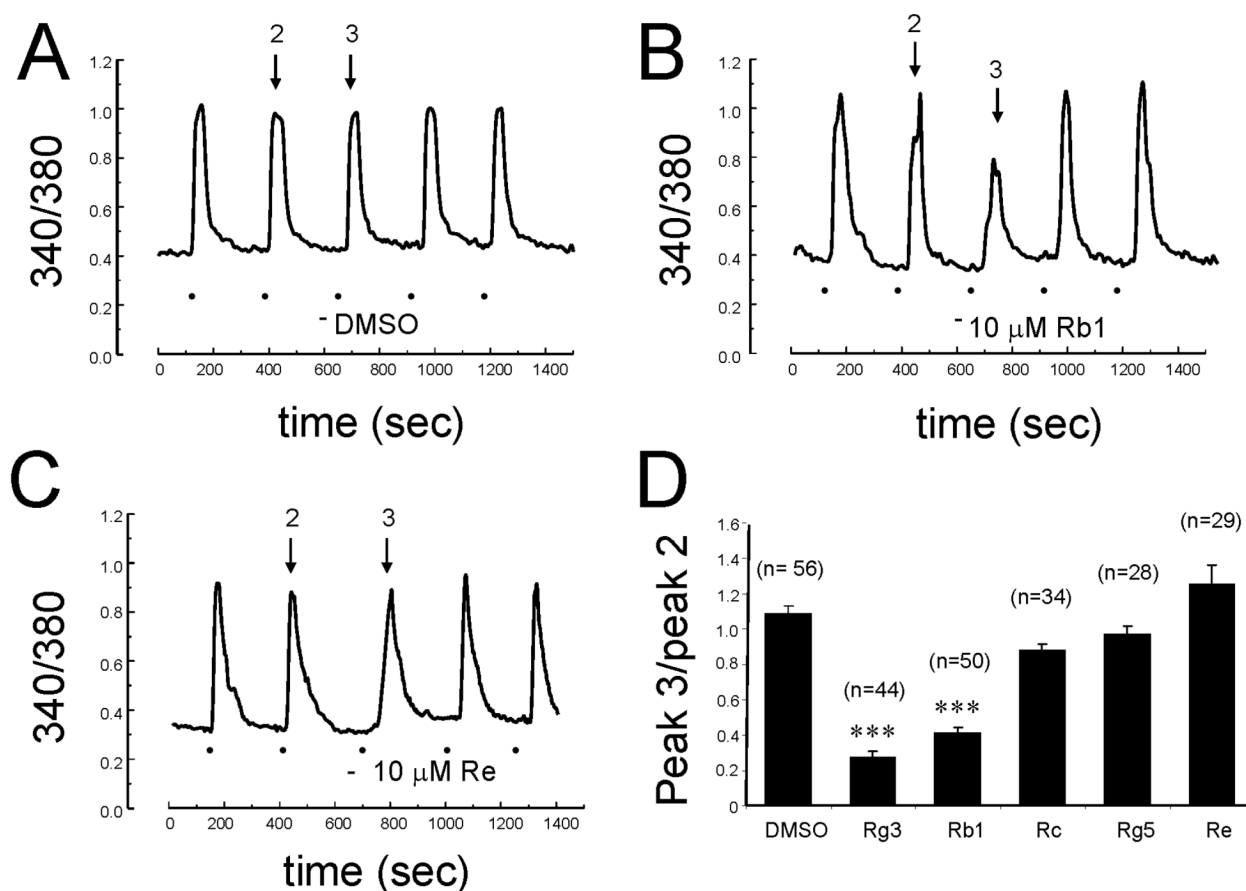


Fig. 4. Ginsenosides inhibit glutamate-induced Ca^{2+} responses in cultured MSN

(A-C) Representative 340/380 Fura-2 ratio traces are shown for MSN repetitively stimulated with 20 μM glutamate pulses. Between 2nd and 3rd glutamate pulse the cells were exposed for 1 min to 0.1% DMSO (A), 10 μM Rb1 (B), or 10 μM Re (C).

(D) The average values of peak3/peak2 ratios are shown for MSN exposed to DMSO or 10 μM of Rb1, Rc, Rg3, Rg5 or Re as mean $\pm$ S.E (n – number of cells analyzed). The peak3/peak2 ratio was significantly ($p < 0.05$) reduced for Rb1 and Rg3 when compared to the DMSO control (***).

Table 1

Effects of ginsenosides on glutamate-induced apoptosis of WT and YAC 128 MSN

Drug treatment	WT (% TUNEL-positive)			YAC128 (% TUNEL-positive)		
	0μM glutamate	250 μM glutamate	0μM glutamate	0μM glutamate	250 μM glutamate	250 μM glutamate
Rb1 0.01μM	6.09 ± 0.59	(8.89 ± 2.02) ^d	39.64 ± 2.87	6.79 ± 0.93	44.25 ± 2.15 [*]	(71.77 ± 6.39)
0.1 μM	5.57 ± 1.03	(6.77 ± 1.31)	35.89 ± 5.11	6.59 ± 0.99	34.67 ± 6.72 [*]	(61.72 ± 7.81)
1.0 μM	9.53 ± 1.86	(6.77 ± 1.31)	24.80 ± 2.90	9.86 ± 1.56	44.11 ± 5.61	(61.72 ± 7.81)
Rc 0.01μM	7.26 ± 1.36	(4.78 ± 1.32)	41.61 ± 4.45	5.13 ± 0.92	46.08 ± 4.01 [*]	(72.83 ± 6.09)
0.1μM	11.05 ± 1.51	(8.84 ± 1.45)	42.74 ± 3.90	8.30 ± 1.46	63.78 ± 5.76	(78.12 ± 3.44)
1.0μM	10.72 ± 1.32	(15.13 ± 2.44)	42.35 ± 3.37	10.87 ± 1.64	52.04 ± 6.44	(63.02 ± 4.45)
Rd 0.01μM	5.17 ± 1.37	(8.84 ± 1.45)	53.84 ± 3.95	5.77 ± 2.27	75.16 ± 4.51	(78.12 ± 3.44)
0.1μM	6.10 ± 1.22	(6.35 ± 1.72)	41.11 ± 4.02	5.91 ± 1.68	86.18 ± 3.18	(53.08 ± 2.56)
1.0μM	—	—	—	—	—	—
Re 0.01μM	5.79 ± 1.42	(8.89 ± 2.02)	48.37 ± 7.04	4.91 ± 1.38	70.58 ± 5.56	(71.77 ± 6.39)
0.1μM	6.02 ± 1.10	(8.99 ± 1.67)	32.58 ± 3.02	5.12 ± 0.88	54.59 ± 5.78	(61.73 ± 7.94)
1.0μM	23.51 ± 2.75	(15.13 ± 2.44)	38.13 ± 2.73	23.82 ± 3.61	59.30 ± 5.82	(63.02 ± 4.45)
Rg3 0.01μM	6.74 ± 1.52	(8.84 ± 1.45)	33.74 ± 4.00	8.74 ± 2.19	66.45 ± 5.71	(78.12 ± 3.44)
0.1μM	7.37 ± 1.41	(8.28 ± 1.55)	52.47 ± 4.01	8.93 ± 1.81	85.29 ± 4.25	(71.74 ± 3.57)
1.0μM	—	—	—	—	—	—
Rg5 0.01μM	8.00 ± 1.95	(6.77 ± 1.31)	28.07 ± 1.37	6.11 ± 0.86	49.13 ± 6.93	(61.72 ± 7.81)
0.1μM	4.18 ± 0.86	(6.77 ± 1.31)	23.24 ± 3.13	5.25 ± 1.01	45.77 ± 7.08	(61.72 ± 7.81)
1.0μM	4.38 ± 0.88	(6.47 ± 1.15)	33.14 ± 2.25	6.50 ± 1.33	41.70 ± 4.82 [*]	(72.83 ± 6.09)
Rh1 0.01μM	6.14 ± 0.36	(8.89 ± 2.02)	46.26 ± 3.48	6.55 ± 1.15	55.57 ± 4.38	(71.77 ± 6.39)
0.1μM	12.19 ± 1.00	(8.99 ± 1.67)	46.49 ± 7.96	7.53 ± 1.26	57.30 ± 2.40	(61.73 ± 7.94)
1.0μM	23.78 ± 4.37	(15.13 ± 2.44)	42.51 ± 2.68	27.05 ± 2.78	60.54 ± 5.23	(63.02 ± 4.45)
Re.Rd	3.70 ± 1.17	(6.47 ± 1.15)	44.10 ± 4.19	6.41 ± 1.40	63.88 ± 6.53	(72.83 ± 6.09)
0.01μM	4.14 ± 0.88	(8.89 ± 2.02)	52.24 ± 6.39	5.27 ± 0.57	61.18 ± 9.16	(71.77 ± 6.39)
1.0 μM	9.47 ± 1.60	(8.89 ± 2.02)	52.41 ± 3.79	8.21 ± 0.55	62.10 ± 8.38	(71.77 ± 6.39)
Rk1.Rg5	4.45 ± 0.82	(6.47 ± 1.15)	27.30 ± 4.47	6.49 ± 1.34	56.92 ± 6.35	(72.83 ± 6.09)
0.01μM	7.08 ± 1.59	(4.78 ± 1.32)	44.39 ± 4.71	4.46 ± 0.77	71.98 ± 5.34	(72.03 ± 6.90)
1.0μM	21.91 ± 2.93	(10.02 ± 1.26)	40.44 ± 3.14	23.48 ± 1.50	69.18 ± 4.09	(65.54 ± 6.06)
Rh4.Rk3	5.43 ± 1.30	(6.47 ± 1.15)	36.69 ± 4.08	4.85 ± 0.73	60.66 ± 9.26	(72.83 ± 6.09)
0.01μM	8.06 ± 1.00	(4.78 ± 1.32)	46.88 ± 3.31	7.18 ± 2.49	57.45 ± 7.15	(72.03 ± 6.90)
1.0μM	11.15 ± 0.99	(10.02 ± 1.26)	27.38 ± 2.73	19.86 ± 1.31	62.83 ± 6.67	(65.54 ± 6.06)

— toxic, total number of cells are much less than control.

n = 6-8 microscopic fields, 100-300 MSN per field

^dThe number in parenthesis is the untreated control cells in the same batch of MSN culture.^{*}P<0.05, compared with control cells and show protective effect.