J Ginseng Res 40 (2016) 55–61

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Hippocampus-dependent cognitive enhancement induced by systemic gintonin administration

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in Revised form
3 May 2015
Accepted 4 May 2015
Available online 12 May 2015

Keywords:
fear conditioning
ginseng
long-term potentiation
lysophosphatidic acid receptor
synaptic plasticity

ABSTRACT

Background: A number of neurological and neurodegenerative diseases share impaired cognition as a common symptom. Therefore, the development of clinically applicable therapies to enhance cognition has yielded significant interest. Previously, we have shown that activation of lysophosphatidic acid receptors (LPARs) via gintonin application potentiates synaptic transmission by the blockade of K^+ channels in the mature hippocampus. However, whether gintonin may exert any beneficial impact directly on cognition at the neural circuitry level and the behavioral level has not been investigated.
Methods: In the current study, we took advantage of gintonin, a novel LPAR agonist, to investigate the effect of gintonin-mediated LPAR activation on cognitive performances. Hippocampus-dependent fear memory test, synaptic plasticity in the hippocampal brain slices, and quantitative analysis on synaptic plasticity-related proteins were used.
Results: Daily oral administration of gintonin for 1 wk significantly improved fear memory retention in the contextual fear-conditioning test in mice. We also found that oral administration of gintonin for 1 wk increased the expression of learning and memory-related proteins such as phosphorylated cyclic adenosine monophosphate-response element binding (CREB) protein and brain-derived neurotrophic factor (BDNF). In addition, prolonged gintonin administration enhanced long-term potentiation in the hippocampus.
Conclusion: Our observations suggest that the systemic gintonin administration could successfully improve contextual memory formation at the molecular and synaptic levels as well as the behavioral level. Therefore, oral administration of gintonin may serve as an effective noninvasive, nonsurgical method of enhancing cognitive functions.

1. Introduction

Cognitive enhancement has yielded significant interest as disrupted learning and memory is one of several widely shared deficits found in many neurological and neurodegenerative diseases. So far, a few manipulations have been proposed to enhance cognitive function [1]. Lysophosphatidic acids (LPAs) are extracellular phospholipidic molecules that bind to G-protein coupled LPA receptors (LPARs) [2]. LPARs-associated signaling plays a number of roles in the early development of central nervous system [3]. Recent studies have revealed that activation of LPARs in adult brain exerts a significant role in intellectual processing. For example, a study using intrahippocampal LPA infusion to activate Rho pathway showed enhanced long-term spatial memory in water maze test [4]. These studies suggest that the activation of LPARs could contribute to enhanced cognitive performances.

Recently, we have shown that a newly identified active component of ginseng, gintonin, enhances synaptic transmission in

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p1226-8453 e2093-4947/$ – see front matter
http://dx.doi.org/10.1016/j.jgr.2015.05.001

mature hippocampal synapses via the activation of LPARs [5]. Gintonin, a subset of glycolipoproteins, consists of LPAs and other protein complexes with highly abundant acidic amino acids [6,7]. Recent investigations suggest that gintonin is a potential candidate to mediate the beneficial actions of ginseng [8]. Previous studies showed that the application of gintonin induces a transient Ca^{2+} increase through the binding of LPARs (LPAR1-6) in oocytes preparation [6,9]. We have shown that gintonin-mediated LPAR activation increases the neuronal excitability and slightly depolarizes the resting membrane potential of the hippocampal pyramidal neurons [5]. These observations strongly suggest that gintonin-mediated LPAR activation possibly may modulate synaptic plasticity and/or learning processes.

Here, we aimed to investigate the effect of systemic gintonin administration on cognitive function. We administered gintonin daily by oral gavage to mice for 7 d and evaluated their memory employing the contextual fear-conditioning test. We also examined whether chronic gintonin administration enhanced long-term synaptic plasticity and accompanied by synaptic plasticity-related proteins in the hippocampus.

2. Materials and methods

2.1. Study participants

Male DBA/2 mice (22–25 g) were used in all experiments (Charles River, Gapyeong, Republic of Korea). Mice were 7 wks old at the time of arrival and maintained for at least 1 wk before the start of the experiments. They were housed in a controlled vivarium on a 12-h light/dark cycle with controlled temperature (22 ± 1°C) and humidity (50 ± 10%). They were allowed free access to food and water. Behavior testing was conducted during the light cycle. Among strains of mice available, the DBA/2 mouse strain was chosen due to their poor performance in the contextual fear conditioning [10]. All experiments were approved by the Institutional Animal Care and Use Committee of Konkuk University, Seoul, Korea (KU14154).

2.2. Gintonin preparation

Gintonin was extracted from *Panax ginseng* without ginseng saponin as previously described [7]. We used the similar median effective dose (ED_{50}) concentrations, that exerted LPA receptor activation in *Xenopus* oocyte preparation in a previous study [7]. Crude gintonin was dissolved in vehicle solution for oral administration at the concentration (50 mg/kg) that was previously shown to have behavioral effect *in vivo* [5]. For acute treatment, gintonin was dissolved in dimethyl sulfoxide (DMSO; final concentration of DMSO, 0.1%).

2.3. Administration of gintonin and experimental procedures

To test the effect of gintonin on cognitive function, mice were randomly separated into two groups: vehicle (saline, $n = 8$) and gintonin (50 mg/kg, $n = 7$). After 5 d of handling, vehicle or gintonin was administered daily by oral gavage for 7 d. The experimenters were blind to the administration condition. The mice were sacrificed the day after the final treatment; 30 min after the last testing on the behavioral test (see Fig. 1).

2.4. Slice preparation

Seven-wk-old mice were sacrificed after daily oral administration of either vehicle or gintonin for electrophysiology recordings, 1 d after the final treatment. Researchers were blind to the

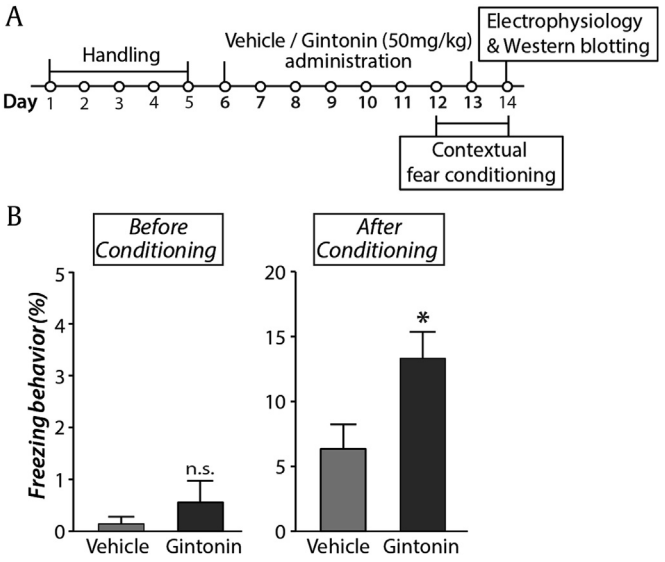

Fig. 1. Gintonin enhances contextual fear memory. (A) Experimental scheme. (B) On the testing day of fear conditioning task, freezing levels in vehicle or gintonin administered mice were quantified. The gintonin-administered mice ($n = 8$) showed an increased fear memory retention when compared with vehicle-administered mice ($n = 7$, $^*p < 0.05$) whereas there is no difference in basal anxiety ($p > 0.4$). Error bars represent standard error of the mean. n.s., not significant.

experimental group of mice. Brain slices (350 μm) were prepared with a microtome (VT 1000S, Leica, Nussloch, Germany) in ice-cold sucrose cutting buffer (212mM sucrose, 3mM KCl, 26mM $NaHCO_3$, 1.25mM $NaHPO_4$, 7mM $MgCl_2$, and 10mM glucose) bubbled with 95% O_2/5% CO_2 mixed gas. Brain slices are placed in a chamber containing artificial cerebrospinal fluid (aCSF: 1mM NaH_2PO_4, 26.2mM $NaHCO_3$, 118mM NaCl, and 2.5mM KCl, and freshly added 11mM glucose, 2mM $CaCl_2$, and 1mM $MgCl_2$) and recovered in a 35°C water bath for 1 h, after which they were maintained at room temperature. Temperature was maintained between 31°C and 33°C during recording. For acute gintonin experiments, brain slices were incubated with 3 μg/mL concentration of gintonin, which we have shown to induce rapid, LPAR-dependent synaptic enhancement.

2.5. Electrophysiology

Field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 dendrites using glass pipettes (0.5–1.5 MΩ) filled with aCSF with two bipolar stimulating electrodes being placed in the stratium radiatum. fEPSPs were alternately evoked through each bipolar electrode with 30-s intervals. Theta burst stimulation (TBS, 20 bursts of 4 pulses at 100 Hz) was delivered at one pathway to induce LTP while the other pathway remained unstimulated during the TBS stimulation.

2.6. Behavioral apparatus

The contextual fear-conditioning task was carried out in square chambers (17.78 cm W × 17.78 cm D × 30.48 cm H, Coulbourn Instruments, Allentown, PA, USA). The chamber was equipped with a grid floor that transmits a foot shock during training. The chamber was surrounded with soundproofed shells and a ventilation fan provided background noise. The foot shock was generated via a Coulbourn programmable tone generator (model #A69-20; Coulbourn Instruments). The stimulus was controlled by Coulbourn Graphic State software (Coulbourn Instruments). The chamber was cleaned with 70% alcohol before and after each trial.