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Short communication

Gintonin, a novel ginseng-derived lysophosphatidic acid receptor ligand, stimulates neurotransmitter release

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HIGHLIGHTS

- Application of gintonin to PC12 cells induced intracellular calcium transients.
- Gintonin treatment in PC12 cells increased the release of dopamine.
- Gintonin-induced $[Ca^{2+}]_i$ transients are coupled to dopamine release.
- Intraperitoneal administration of gintonin to mice increased serum dopamine level.
- Gintonin may regulate neurotransmitter release via LPA receptor activation.

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ABSTRACT

Gintonin is a novel ginseng-derived G protein-coupled lysophosphatidic acid (LPA) receptor ligand. Gintonin elicits an intracellular calcium concentration $[Ca^{2+}]_i$ transient via activation of LPA receptors and regulates calcium-dependent ion channels and receptors. $[Ca^{2+}]_i$ elevation by neurotransmitters or depolarization is usually coupled to neurotransmitter release in neuronal cells. Little is known about whether gintonin-mediated $[Ca^{2+}]_i$ transients are also coupled to neurotransmitter release. The PC12 cell line is derived from a pheochromocytoma of the rat adrenal medulla and is widely used as a model for catecholamine release. In the present study, we examined the effects of gintonin on dopamine release in PC12 cells. Application of gintonin to PC12 cells induced $[Ca^{2+}]_i$ transients in concentration-dependent and reversible manners. However, ginsenoside Rg_3, another active ingredient of ginseng, induced a lagged and irreversible $[Ca^{2+}]_i$ increase. The induction of gintonin-mediated $[Ca^{2+}]_i$ transients was attenuated or blocked by the LPA1/3 receptor antagonist Ki16425, a phospholipase C inhibitor, an inositol 1,4,5-triphosphate receptor antagonist, and an intracellular Ca^{2+} chelator. Repeated treatment with gintonin induced homologous desensitization of $[Ca^{2+}]_i$ transients. Gintonin treatment in PC12 cells increased the release of dopamine in a concentration-dependent manner. Intraperitoneal administration of gintonin to mice also increased serum dopamine concentrations. The present study shows that gintonin-mediated $[Ca^{2+}]_i$ transients are coupled to dopamine release via LPA receptor activation. Finally, gintonin-mediated $[Ca^{2+}]_i$ transients and dopamine release via LPA receptor activation might explain one mechanism of gintonin-mediated inter-neuronal modulation in the nervous system.

1. Introduction

Ginseng is a traditional herbal medicine that possesses a variety of physiological and pharmacological effects as a tonic [12]. Recent studies have shown that ginseng contains a novel G protein-coupled lysophosphatidic acid (LPA) receptor ligand, known as gintonin [10,13,15]. Gintonin induces a $[Ca^{2+}]_i$ transient through LPA receptor activation but not S1P and other fatty

Abbreviations: GT, gintonin; LPA receptor, lysophosphatidic acid receptor; GPCR, G protein-coupled receptor; PC12, pheochromocytoma cell 12; $[Ca^{2+}]_i$, intracellular calcium concentration.

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acid receptors with high affinity in cells expressing LPA receptors endogenously or heterologously. Gintonin-mediated LPA receptor activation is also accountable for diverse downstream events, including stimulation of phospholipase C, protein kinase C (PKC), mitogen-activated protein kinases, and phosphoinositide 4-kinase (PI4 kinase) through multiple G proteins, such as $G\alpha_{i/o}$, $G\alpha_{12/13}$, and $G\alpha_{q/11}$ [2,10,13,16]. In addition, gintonin has been reported to regulate various ion channels (e.g., Ca^{2+}-activated Cl^-, Ca^{2+}-activated K^+, and voltage-gated Kv1.2) and receptors (e.g., NMDA and $P2X_1$) through LPA receptor activation [9,10]. In addition, gintonin can induce long-term potentiation (LTP) in hippocampal slices [16]. Although, previous reports raise the possibility that gintonin-mediated $[Ca^{2+}]_i$ transients and ensuing NMDA receptor activation leading to LTP induction might be closely related to the regulation of neurotransmitter release in the nervous system, it has not been demonstrated that the activation of G protein-coupled LPA receptors by gintonin is coupled to the regulation of neurotransmitter release.

PC12 cells are derived from the pheochromocytoma of the rat adrenal chromaffin. These cells are widely used to study the modulation of neurotransmitter release by a variety of neurotransmitters or drugs because they possess vesicles containing catecholaminergic neurotransmitters, primarily dopamine [7,11], and the release of these catecholamines is associated with elevation of cytosolic calcium, similar to adrenal chromaffin cells [8]. For example, stimulation of PC12 cells by a cholinergic agonist or depolarization by high extracellular K^+ induces a $[Ca^{2+}]_i$ transient and results in the release of dopamine [8]. Currently, PC12 pheochromocytoma cells are utilized to obtain information about the role of bioactive compounds that could affect in vitro neurotransmitter release [6,20]. In the present study, we examined the effects of gintonin on a $[Ca^{2+}]_i$ transients and the release of dopamine in PC12 cells. We report here that gintonin induces a $[Ca^{2+}]_i$ transient via membrane signaling transduction pathways of LPA receptors, and that gintonin-mediated $[Ca^{2+}]_i$ transients are coupled to the stimulation of dopamine release. Additionally, we discuss the physiological and pharmacological role of gintonin-mediated dopamine release in the nervous system.

2. Materials and methods

2.1. Materials

Gintonin, devoid of ginseng saponins, was prepared from Panax ginseng according to previously described methods [15]. Gintonin was dissolved in deionized water and then diluted with medium before use. RPMI1640 medium, DMEM, fetal bovine serum (FBS), horse serum (HS), penicillin, and streptomycin were purchased from Invitrogen (Camarillo, CA, USA). Lysophosphatidic acid (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate, 857130P) was purchased from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA). All other reagents used, including dopamine and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell culture

PC12 cells, a rat pheochromocytoma cell line, were obtained from Korean Cell Line Bank (KCLB, Seoul, Korea) and were grown in RPMI 1640 supplemented with 10% heat-inactivated HS, 5% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3. Measurement of intracellular calcium concentration

The intracellular free calcium concentration was measured by dual excitation spectrofluorometric analysis of cell suspensions loaded with Fura-2 AM as previously described [10]. Briefly, PC12 cells (either untreated or treated with NGF) were harvested with trypsin/EDTA solution and resuspended in HEPES-buffered saline solution (HBS: 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose, 25 mM HEPES, and pH 7.4). The cells were incubated with Fura-2 AM (final concentration 2.5 μM) in HBS at 37 °C for 30 min. Extracellular Fura-2 AM was removed by centrifugation. Each aliquot of 3×10^6 cells was loaded into a cuvette and free calcium mobilization was measured using a RF-5301PC spectrofluorophotometer and Supercap software (Ex: 340 nm and 380 nm; Em: 520 nm) (Shimadzu, Tokyo, Japan).

2.4. Measurement of dopamine release

PC12 cells were treated with Lock's buffer [145 mM NaCl, 5.6 mM KCl, 12 mM $MgCl_2$, 22 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES (pH adjusted to 7.4 with NaOH)] in the presence or absence of gintonin at 37 °C. The samples were centrifuged at 12,000 rpm for 5 min and the levels of released dopamine in the supernatant were determined by an HPLC system, as previously described [19]. Six-week-old male balb/c mice (Koatech Technology Corporation, Seoul, Korea) were housed under specific pathogen-free conditions. Mice were intraperitoneally administered saline solution (Sal) or gintonin (100 mg/kg). Mice were anesthetized with Zoletil 50 and Rompun, sacrificed, and blood samples were collected. Dopamine levels in serum were determined by HPLC [19]. Animal experiments were conducted in strict accordance with the recommendations in the guide for the care and use of Laboratory Animals of the National Institutes of Health.

2.5. Data analysis

To obtain concentration-response curves of the effects of gintonin on $[Ca^{2+}]_i$ transients, the peak increase of $[Ca^{2+}]_i$ transient amplitudes at different concentrations of gintonin were plotted, and Origin software (OriginLab, Northampton, MA, USA) was used to fit the data to the Hill equation: $y/y_{max} = [A]^{nH}/([A]^{nH} + [EC_{50}]^{nH})$, where y is the peak at a given concentration of gintonin, y_{max} is the maximal peak in the absence of gintonin, EC_{50} is the concentration of gintonin producing a half-maximal effect, $[A]$ is the concentration of gintonin, and nH is the Hill coefficient. All values are presented as the mean ± S.E.M. The significance of differences between control and treatment values was determined using Student's *t*-test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of gintonin on $[Ca^{2+}]_i$ transients in PC12 pheochromocytoma cells

In the present study, we first examined the effects of gintonin on $[Ca^{2+}]_i$ transients in PC12 cells. As shown in Fig. 1A and B, gintonin treatment induced a transient rise of $[Ca^{2+}]_i$ in PC12 cells in a reversible and concentration-dependent manner. The EC_{50} was 0.06 ± 0.01 μg/mL. Gintonin-induced $[Ca^{2+}]_i$ transients initiated without a detectable lag and reached peak values within a few seconds, and $[Ca^{2+}]_i$ gradually decreased but did not return to its basal level until the end of time point (100 s). We observed that treatment of PC12 cells with LPA $C_{18:1}$ also induced a $[Ca^{2+}]_i$ transient, similar to gintonin (Fig. 3A and B). When we examined the effect of acetylcholine on $[Ca^{2+}]_i$ transients, we found that acetylcholine application also induced a $[Ca^{2+}]_i$ transient, similar to gintonin