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Ethanol extract of the seed of *Zizyphus jujuba var. spinosa* potentiates hippocampal synaptic transmission through mitogen-activated protein kinase, adenylyl cyclase, and protein kinase A pathways

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ABSTRACT

Ethnopharmacological relevance: As the seed of *Zizyphus jujuba var. spinosa* (Bunge) Hu ex H.F. Chow (Rhamnaceae) has been used to sleep disturbances in traditional Chinese and Korean medicine, many previous studies have focused on its sedative effect.
Aim of the study: Recently, we reported the neuroprotective effect of the effect of *Z. jujuba var. spinosa*. However, its effects on synaptic function have not yet been studied. In this project, we examined the action of ethanol extract of the seed of *Z. jujuba var. spinosa* (DHP1401) on synaptic transmission in the hippocampus.
Materials and methods: To investigate the effects of DHP1401, field recordings were conducted using hippocampal slices (400 μm). Object recognition test was introduced to examine whether DHP1401 affect normal recognition memory.
Results: DHP1401 (50 μg/ml) induced a significant increase in synaptic activity in Shaffer collateral pathway in a concentration-dependent manner. This increase of synaptic responses was blocked by NBQX, a broad spectrum α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonist, but not IEM-1460, a Ca^{2+}-permeable AMPAR blocker. Moreover, U0126, a mitogen-activated protein kinase inhibitor, SQ22536, an adenylyl cyclase inhibitor, and PKI, a protein kinase A inhibitor, blocked DHP1401-induced increase in synaptic transmission. Finally, DHP1401 facilitated object recognition memory.
Conclusions: These results suggest that DHP1401 increase synaptic transmission through increase of synaptic AMPAR transmission via MAPK, AC and PAK.

1. Introduction

The seeds of *Zizyphus jujuba var. spinosa* (Bunge) Hu ex H.F. Chow (Rhamnaceae) (English name: spine date seed) have been used as a hypnotic agent (Zhu, 1998) in traditional Chinese and Korean medicine. Recent studies revealed that the seed of the Ziziphus species produced sleep state without inducing convulsion or muscle relaxation (Peng et al., 2000). Moreover, as active constituents, jujuboside A and

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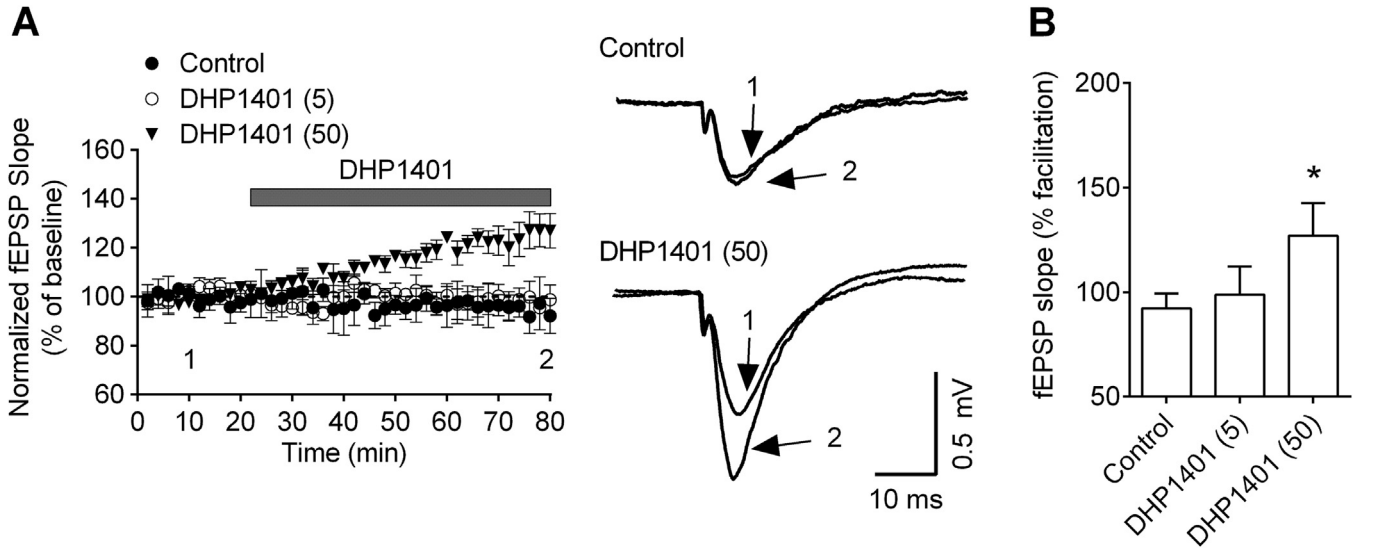

Fig. 1. The effect of DHP1401 on synaptic transmission. A. Normalized fEPSP slope in the hippocampal Shaffer-collateral pathway. DHP1401 (5 or 50 μg/ml) was perfused to recording chamber after 20 min period of stable baseline. B. Bar chart was represented as normalized fEPSP at 80 min time points in A. Data were expressed as mean ± SEM. n=5. $^{*}P < 0.05$ vs. control group.

jujubogenin produced a hypnotic effect through modulations in neurotransmitter systems, such as serotonin and GABA systems (Chen et al., 2008; Wang et al., 2012, 2015; You et al., 2010). However, its precise action mechanism on the brain is still not clear.

Glutamate receptors including *N*-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) are known to play a crucial role in learning and memory (Lussier et al., 2015; Sanderson et al., 2008; Tsien et al., 1996). NMDAR activation causes AMPAR translocation into postsynaptic membrane and this causes long-term potentiation, a cellular model of learning and memory (Bliss and Collingridge, 2013; Lu et al., 2001; Sanderson et al., 2008). The GABAergic system can modulate the actions of these glutamate receptors by limiting postsynaptic depolarization (McBain and Fisahn, 2001; Pouille and Scanziani, 2001; Staley and Mody, 1992). Therefore, many drugs showing sedative effects, including diazepam, a typical hypnotic agent, have been reported to impair memory processing (Kim et al., 2012, 2009). In line with this, the seed of *Z. jujuba var. spinosa* may be speculated to produce amnesic effects. However, we recently found memory-enhancing effects of the seed of *Z. jujuba var. spinosa* and its constituents, including spinosin and swerticin (Jung et al., 2014; Ko et al., 2015; Lee et al., 2016a, 2013, 2016b). To better understand this discrepancy, in the present study we examined the effect of the seed of *Z. jujuba var. spinosa* on synaptic transmission in the hippocampus. Interestingly, we found that ethanol extract of the seed of *Z. jujuba var. spinosa* facilitated basal excitatory transmission and this might have been mediated by mitogen-activated protein kinase, (MAPK) adenylyl cyclase (AC), and protein kinase A (PKA) pathways.

2. Materials and methods

2.1. Animals

We purchased male CD-1 mice (26–28 g, 6 weeks old) from the SAMTAKO biokorea (Osansi, Korea). Mice were housed in the Dong-A University Animal Care Unit for 1 week before the start. Four mice were contained in a cage. Mice were allowed freely access to water and food (temperature: 23 ± 1 °C; humidity: 60 ± 10%; light/dark cycle: from 07:30 to 19:30). Institutional Animal Care and Use Committee of Dong-A University (Korea) approved the protocols of animal experiments.

2.2. Materials

A standardized ethanol extract of the seed of *Z. jujuba var. spinosa* (DHP1401) was donated by DAEHWA Pharmaceutical Co., LTD. (Pangyo, Korea). The seed of *Z. jujuba var. spinosa* were blended first. Extraction was conducted two times with 50% ethanol under reflux (80–85 ℃) for 3 h. Evaporation was conducted under reduced pressure and the extract was dried using spray dryer (DHP1401) (Lee et al., 2013). DHP1401 was standardized with spinosin (MW, 608.55, > 0.5%). A voucher specimen was deposited in the Herbarium of the Traditional Herb Research Center, Korea Food and Drug Administration (No. 11E-1001). NBQX, IEM-1460, D-AP5, KN-62, U0126 and SQ22536 were purchased from Abcam Biochemicals (Cambridge, UK). PKI (5–24) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.3. Acute hippocampal slice preparation

Artificial cerebrospinal fluid (ACSF) was comprised with NaCl, 124 mM; KCl, 3 mM; $NaHCO_3$, 26 mM; NaH_2PO_4, 1.25 mM; $CaCl_2$, 2 mM; $MgSO_4$, 1 mM; D-glucose, 10 mM. We rapidly removed and isolated mouse hippocampus brain and placed in ice-cold ACSF (bubbled with 95% O_2/5% CO_2). Hippocampal slices (450 μm thick) were made using McIlwain tissue chopper (Brinkman, Westbury, NY). Hippocampal slices were recovered in ACSF (25–27 °C) for 2 h before recording.

2.4. Electrophysiology

Field excitatory postsynaptic potentials (fEPSP) were recorded in the hippocampal stratum pyramidale. Stimulating tungsten electrode was located in the Schaffer-collateral commissural pathway. The slope of the evoked field potential responses was averaged from 4 consecutive recordings evoked at 30 s intervals.

2.5. Object place recognition test

Habituation to the open field (25 cm×25 cm×25 cm) was conducted with an internal cue on one wall for 10 min. Immediate after habituation, mice were administered DHP1401 (50, 100, 200 mg/kg, p.o.). Mice were re-located in the open field with two objects (plastic cylinder and a glass box) at 1 h later. The exploration time were monitored for