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The Role of Gastrodin on Hippocampal Neurons after N-Methyl-D-Aspartate Excitotoxicity and Experimental Temporal Lobe Seizures

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Abstract

Tian ma (*Gastrodia elata*, GE) is an ancient Chinese herbal medicine that has been suggested to be effective as an anticonvulsant and analgesic, and to have sedative effects against vertigo, general paralysis, epilepsy and tetanus. The primary active ingredient isolated from GE is termed gastrodin, which is the glucoside of 4-hydroxybenzyl alcohol (4-HBA). Gastrodin can abolish hypoxia-, glutamate- and N-methyl-D-aspartate (NMDA) receptor-induced toxicity in primary culture of rat cortical neurons, and reduces seizure severity in seizure-sensitive gerbils. We evaluated the effect of gastrodin on NMDA excitotoxicity in hippocampal slice cultures (HSCs) with propidium iodide (PI) fluorescence measurement. We also evaluated the effects of gastrodin for treating active *in vivo* temporal lobe seizures induced by lithium/pilocarpine. Seizure severity, time span to seizure onset, mortality rate and hippocampal histology for survivors were compared. The effect of gastrodin was evaluated for treating *in vitro* seizures induced by Mg^{2+}-free medium in hippocampal slices. Frequencies and amplitudes of epileptiform discharges were compared. The effect of gastrodin on synaptic transmission was evaluated on hippocampal CA1 Schaffer collaterals. Application of 25 μM gastrodin significantly suppressed NMDA excitotoxicity in CA3 but not in CA1 hippocampus and dentate gyrus. Intraventricular gastrodin accelerated seizure onset for 12 min after intraperitoneal pilocarpine injection ($P = 0.051$). Three of five rats (60%) in the gastrodin group, and three of four (75%) in the dimethyl sulfoxide (DMSO) group died within 3 days after status epilepticus (SE). Gastrodin also failed to inhibit epileptiform discharges in hippocampal slices induced by Mg^{2+}-free medium, believed to be NMDA receptor-mediated spontaneous activity. The frequencies of the spontaneous epileptiform discharges were similar under treatments with 25 μM gastrodin, 200 μM gastrodin and DMSO. For the evaluation of gastrodin on synaptic transmission, application of DMSO, 25 μM or 200 μM gastrodin had no significant effect on excitatory postsynaptic potential (EPSP) slopes. Gastrodin at 200 μM decreased paired-pulse facilitation (PPF) from 1.23 ± 0.04 to 1.12 ± 0.04 ($P = 0.002$). In conclusion, gastrodin failed to suppress *in vivo* and *in vitro* seizures in our study. Gastrodin showed no effect on hippocampal Schaffer collateral EPSP. These findings suggest that gastrodin does not interact with ionotropic glutamate receptors to inhibit NMDA receptor-facilitated seizures. However, gastrodin showed protective effects against NMDA toxicity on cultured hippocampal slices. Nevertheless, gastrodin is still a potential neuroprotective agent against NMDA excitotoxicity, with potential benefits for stroke and patients with epilepsy.

Key Words: *Gastrodia elata*, gastrodin, neuroprotection, temporal lobe seizure

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Introduction

Tian ma (*Gastrodia elata*, GE) is an ancient Chinese herbal medicine that has been suggested to be effective as an anticonvulsant and analgesic, and to have sedative effects against vertigo, general paralysis, epilepsy and tetanus (25). Several *in vitro* and *in vivo* animal studies have indicated that GE and its constituents are beneficial in various disorders of the central nervous system (CNS), such as Alzheimer's disease, Parkinson's disease, hypoxia-induced toxicity and cocaine-induced seizures (9, 15, 19, 21, 30, 36). The primary active ingredient isolated from GE, which has been termed gastrodin (PubChem CID 115027), is the glucoside of 4-hydroxybenzyl alcohol (4-HBA). Gastrodin was able to penetrate through the blood brain barrier into the brain, and was rapidly decomposed to p-hydroxybenzyl alcohol (HBA) in the brain, liver and blood (7). Gastrodin can abolish hypoxia-, glutamate- and N-methyl-D-aspartate (NMDA) receptor-induced toxicity in primary cultures of rat cortical neurons (21). Gastrodin also reduced seizure severity in seizure-sensitive gerbils by decreasing the immunodensities of γ-aminobutyric acid (GABA) shunt enzymes, such as GABA transaminase, succinic semialdehyde dehydrogenase (SSADH) and succinic semialdehyde reductase (SSAR) (1).

Temporal lobe epilepsy (TLE) is the most common type of partial-onset epilepsy and results in significant morbidity (33). More than 60% of patients with focal seizures achieve seizure freedom from antiepileptic drugs (AEDs) (20). For those patients with drug-resistant TLE, several molecular mechanisms were reported, such as low brain GABA levels (26), or changes in glutamate levels or neuronal glutamate transporters (5). High extracellular glutamate was also found in human epileptogenic hippocampus (3). *In vitro*, low magnesium medium induced ictal- and interictal-like epileptiform discharges in hippocampal slice preparations, and this can be regarded as an *in vitro* model of TLE (12, 17, 23, 24). These epileptiform discharges were facilitated by NMDA receptors (22). In this study, gastrodin was used to treat *in vivo* and *in vitro* temporal lobe seizures, as well as to rescue NMDA-induced excitotoxicity in hippocampal slice cultures (HSCs). In the *in vivo* study, rats were treated with lithium-pilocarpine to induce generalized tonic-clonic seizures, which were facilitated with muscarinic acetylcholine and NMDA receptor activation (6). With these experiments, the efficacy of gastrodin on NMDAR-facilitated *in vitro* and *in vivo* seizures was evaluated.

Materials and Methods

Animals

The use of animals in this study was approved by the Ethical Committee for Animal Research of the Buddhist Tzu-Chi General Hospital, Taipei branch (IACUC-102-010), in accordance with the National Institutes of Health guidelines. Every effort was made to minimize the number of animals used and suffering of the animals.

Preparation of HSCs for Neurotoxicity Experiments

HSCs for neurotoxicity experiments were prepared from 7- to 9-day-old Sprague Dawley rats (n = 18; BioLASCO Taiwan, Taipei, Taiwan, ROC), using standard methods (32) with modifications. In brief, after being anesthetized with isoflurane, the rats were decapitated and the brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). The hippocampi were transversely sliced into 350-μm sections with a tissue slicer (D.S.K. Super Microslicer™ Zero 1; Dosaka EM, Kyoto, Japan). The entorhinal cortex was removed, and the middle 4 to 6 slices of each hippocampus were placed onto tissue culture membrane inserts (Millicell-CM; Millipore, Billerica, MA, USA) within 6-well culture trays containing 1 ml of slice culture medium per well. The slice culture medium consisted of 50% minimal essential medium, 25% Hank's balanced salt solution, 25% heat-inactivated horse serum, 0.5% glucose, 2 mM GlutaMAX and 2% penicillin-streptomycin. Culture medium was replaced every 2 or 3 days. Seven to eight days later, the HSCs were used in experiments. All media were obtained from Gibco (Life Technologies, Carlsbad, CA, USA).

Propidium iodide (PI) Fluorescence Measurements

Cell viability was determined using PI fluorescence measurements, which is a widely used and reliable marker for neurodegeneration. PI, a polar compound that can only enter into dead and dying neurons, binds to nucleic acid, resulting in a red fluorescence emission at 630 nm upon excitation at 495 nm. The fluorescence intensity is linearly related to the number of dead cells. PI was applied at 2 μM 24 h before fluorescence measurements were taken using an inverted microscope attached to a digital camera. Cell death was expressed as a percent increase of the mean pixel value of matched controls, and recorded using the Photoshop software (Adobe, San Jose, CA, USA). All measurements were taken after subtracting the background fluorescence obtained from a region positioned immediately outside the culture. To assess differential cell death by region, cell regions were identified and circled as regions of interest in the phase contrast image. PI staining was measured densitometrically in each of the three cell regions (CA1,

CA3, dentate gyrus) using the Photoshop software.

Lithium Pilocarpine-Induced Temporal Lobe Seizures

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed in a stereotaxic instrument. The skin was incised to expose the skull, and small holes were drilled into the bone above the target regions. Stainless steel screws were screwed into the frontal cortex region (2 mm from midline, +3 mm from Bregma) and into the hippocampal region (2 mm from midline, -4 mm from Bregma), with a reference screw in the cerebellum. These screws served as electroencephalogram (EEG) electrodes, and were attached to wire-wrapping wire. A 27G stainless steel cannula was placed into the lateral ventricle (anteroposterior [AP] -0.8 mm, mediolateral [ML] -1.8 mm, dorsoventral [DV] 3 mm; coordinates relative to Bregma). Animals were allowed to recover from surgery for 6 days. All rats received lithium chloride (3 mEq/kg i.p.; Sigma-Aldrich, St. Louis, MO, USA). Approximately 20 h later, rats received methylscopolamine bromide (1 mg/kg i.p.; Sigma-Aldrich) and intracerebroventricular (i.c.v.) infusion during isoflurane anesthesia. Intracerebroventricular infusions were performed using a Hamilton syringe coupled to a syringe pump with an injection rate of 1 μl/min. Fifteen-minute baseline EEG recordings were obtained in the hippocampus and frontal cortex prior to pilocarpine. Status epilepticus (SE) was induced by injecting pilocarpine hydrochloride (25 mg/kg s.c.; Sigma-Aldrich), and each animal underwent EEG, electromyography and behavioral recordings for 1 h. Seizure severity was assessed every min using the Racine scale (28). SE was defined as Stage 4 on the Racine scale. Rats were administered diazepam (10 mg/kg) or sodium pentobarbital (30 mg/kg) to terminate SE. Rats that survived SE were allowed to recover for 7 days, after which they were sacrificed for brain histological evaluation. Brain histology was examined with Nissl stain.

For EEG monitoring, a one-channel amplifier (Grass P5 Series AC Amplifier, Grass Instrument Co., Warwick, RI, USA) and an analog–digital converter (PowerLab; ADInstruments Ltd., Quincy, MA, USA) were used. The data were recorded using Chart5 software and analyzed with LabChart8 software (ADInstruments). The sampling rate for the EEG recording was 1000 Hz and the data were amplified 200 times. The low-pass filter was set at 0.3 Hz, and the high-pass filter at 30 Hz. Behavioral state was recorded by direct observation of the animals.

Tissue Preparation for Electrophysiology Experiments

Adult Sprague-Dawley rats (n = 13; 150–250 g) were anesthetized with isoflurane and decapitated. The brains were quickly removed and placed in ice-cold ACSF containing the following: 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$ and 11 mM glucose. The pH was maintained at 7.4 by gassing with a mixture of 95% O_2 and 5% CO_2. The hippocampi were transversely sliced into 450-μm sections with a tissue slicer, and transferred to an interface-type holding chamber at room temperature (25°C). The slices were allowed to recover for at least 90 min, and then transferred to an immersion-type recording chamber and perfused at 2-3 ml/min with ACSF at room temperature.

For epileptiform discharge recordings, bipolar stainless steel stimulating electrodes (Frederick Haer Company, Bowdoinham, ME, USA) were placed in the stratum pyramidale of the CA1 area. Two kinds of spontaneous event can be recorded under Mg^{2+}-free ACSF perfusion: one is a prolonged ictal-like discharge that lasts several seconds and displays both tonic and clonic electrographic components, which are originated from entorhinal cortex; the other type has durations shorter than a few seconds and is thought to be CA3-driven (see ref. 3 for review). Because we cut through the entorhinal cortex during slice preparation, the number of slices with ictal-like discharges was minimal and these were excluded from the study.

For recordings of extracellular field potentials, a glass pipette filled with 3M NaCl was positioned in the stratum radiatum of the CA1 area, and field excitatory postsynaptic potentials (fEPSPs) were amplified by a differential amplifier (DP-301; Warner Instruments, Hamden, CT, USA) and recorded by PowerLab data acquisition hardware (ADInstruments). Data were collected using Scope software (ADInstruments). Bipolar stainless steel stimulating electrodes (Frederick Haer Company) were placed in the stratum radiatum to stimulate Schaffer collateral branches, and stable baseline fEPSP activity was recorded every min for at least 15 min. We averaged all recording sweeps and placed a window of less than 1 millisecond immediately after presynaptic volley to calculate initial slopes of the fEPSPs. Synaptic responses were normalized to the average of the baseline recording for 10 min.

Drugs

The chemicals used for the ACSF were purchased from Merck (Frankfurt, Germany). NMDA, PI, lithium chloride, methylscopolamine bromide and pilocarpine hydrochloride were purchased from Sigma (St. ouis, MO, USA). NMDA was dissolved in distilled water shortly before the experiments were performed. Gastrodin was purchased from Biotic (Taipei, Taiwan, ROC) and dissolved in dimethyl

sulfoxide (DMSO) before experiments.

Data and Statistical Analysis

All data are presented as the mean ± standard error. To analyze PI density after NMDA exposure among different treatment groups, one-way analysis of variance (ANOVA) with *post-hoc* analysis was applied. The amplitudes and frequencies of the spontaneous events induced by Mg^{2+}-free ACSF were determined manually. After a stable recording for 15 min, the spike frequencies and amplitudes recorded in the next 5 min were referred as baseline. Spikes recorded 25–30 min after gastrodin applications were normalized with the baseline and used for statistical comparisons. A paired *t*-test was used to compare spike frequencies and amplitudes before (baseline) and after gastrodin application. One-way ANOVA was used to compare the effects of different gastrodin concentrations. To analyze changes in the EPSP slopes, repeated measures ANOVA was used to compare the changes between baseline and post-treatment. *Post-hoc* analysis was performed to determine the differences between individual treatment groups. The changes in paired-pulse ratio (PPR) before and after gastrodin application were compared by paired *t*-tests. Either least significant difference (LSD) or Games-Howell analysis were used for *post-hoc* analysis. Differences were considered as statistically significant at $P < 0.05$. All statistical analyses were performed with SPSS version 16 (IBM, New York, USA).

Results

Gastrodin Suppressed NMDA Excitotoxicity in Cultured Hippocampal Slices

The effect of gastrodin on excitotoxicity induced by NMDA was explored using PI density quantification in the dentate gyrus, CA3 and CA1. NMDA (40 μM) was added to induce neuronal injury in HSCs. In the experimental group, gastrodin was applied to the hippocampal slices 30 min before the application of NMDA. After 1 h, the culture medium containing NMDA was removed and gastrodin was added again, and the treatment was continued for 24 h. PI density quantification was performed 24 h after NMDA application. These experiments were repeated three times, and for each experiment, five to twenty-three slices were used in the control group and five to eight slices in the treatment groups. PI density quantification and normalization were performed independently in each experiment. The PI density in HSCs in the control medium in each experiment was normalized, which represented the standard condition of the HSCs. Quantified PI densities for each slice were pooled for analysis. Compared with hippocampal slices treated with DMSO only, NMDA induced significant excitotoxicity in the hippocampal dentate gyrus, CA3 and CA1 by increasing PI density to 3.63 ± 0.61 (P = 0.001), 6.99 ± 1.61 (P = 0.005) and 7.40 ± 1.18 (P < 0.001), respectively. Slices treated with 25 μM and 200 μM gastrodin in the presence of 40 μM NMDA had lower PI densities compared with those treated with NMDA only (Fig. 1). PI densities in the dentate gyrus, CA3 and CA1 after treatment with NMDA and 25 μM gastrodin were 2.61 ± 0.32 (P = 0.461), 2.44 ± 0.34 (P = 0.047) and 4.40 ± 0.50 (P = 0.108), respectively. PI densities in the dentate gyrus, CA3 and CA1 after treatment with NMDA and 200 μM gastrodin were 2.98 ± 0.41 (P = 0.810), 3.02 ± 0.45 (P = 0.104) and 5.00 ± 0.46 (P = 0.251), respectively. Application of 25 μM gastrodin significantly suppressed NMDA excitotoxicity in the hippocampal CA3 area but not in the hippocampal CA1 and dentate gyrus.

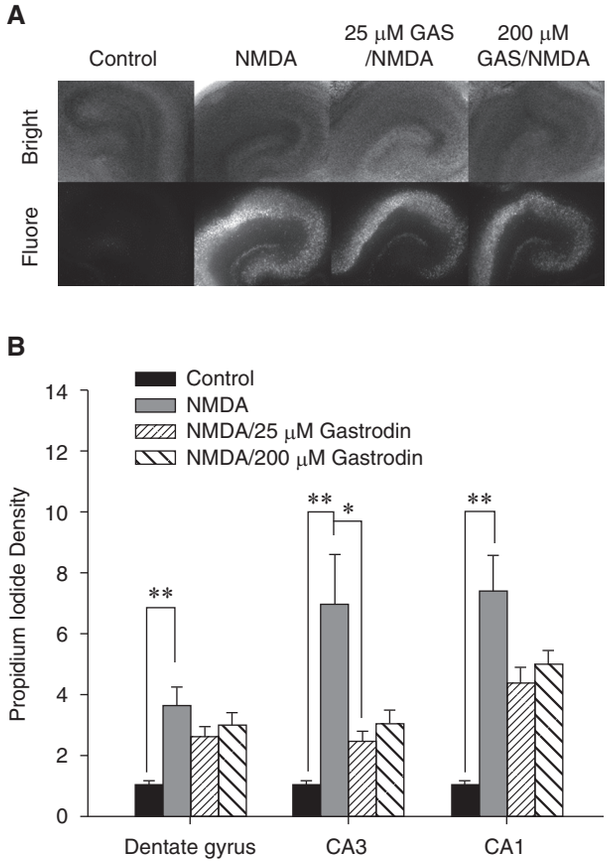

Fig. 1. Gastrodin attenuated NMDA excitotoxicity in cultured hippocampal slices. (A) Representative images of fluorescence intensity after NMDA treatment, with or without gastrodin. Bright: bright-field microscopy, Fluore: fluorescence microscopy, GAS: gastrodin. (B) Quantitative results for fluorescence intensity after NMDA treatment, with or without rosiglitazone rescue. $^{**}P < 0.001$, $^{*}P < 0.05$.

Gastrodin Failed to Suppress SE Induced by Lithium-Pilocarpine in Rats

Because gastrodin was able to be distributed into the brain rapidly (35), gastrodin was directly infused into the intraventricular space to evaluate its effect on SE induced by lithium-pilocarpine. Five of nine rats received 100 mM gastrodin (5 μl; i.c.v.) and the other four received DMSO as a control. Drugs were applied slowly for 15 min under isoflurane anesthesia. Baseline EEG recordings from the frontal and hippocampal cortex were taken for 15 min, after which pilocarpine (25 mg/kg s.c.) was injected. Generalized seizures (Racine 5) could be induced in all nine rats in this experiment. Gastrodin was unable to prevent seizures induced by pilocarpine, and even accelerated seizure attacks. There were no immediate behavior changes after gastrodin infusion. The time spans from pilocarpine injection to Racine 5 seizure for rats in the gastrodin and DMSO groups were 23.2 ± 3.2 and 35.3 ± 3.1 min, respectively (P = 0.051). EEG recordings in both groups showed similar epileptiform discharges (Fig. 2A). Racine 5 seizure continued in all studied animals until pentobarbital or diazepam was injected to stop seizures one hour after the pilocarpine injection. Seizure durations in the gastrodin and DMSO groups were 36.8 ± 7.2 and 24.8 ± 6.3 min, respectively (P = 0.051). Three of the five rats (60%) in the gastrodin group, and three of the four (75%) in the DMSO group, died within 3 days after SE. For rats that survived, neuronal loss in hippocampal CA1 and CA3 was noted in both the gastrodin and DMSO groups comparing with the control animals, and there were no obvious differences between the two experimental groups (Fig. 2B).

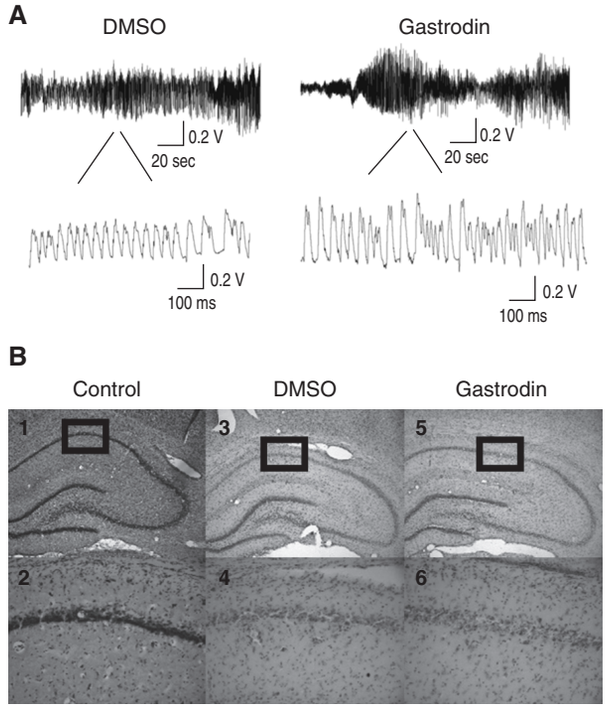

Fig. 2. Gastrodin failed to suppress SE induced by lithium/pilocarpine and did not protect hippocampal injuries. (A) Representative ictal hippocampal EEG recordings induced by lithium-pilocarpine. EEGs from DMSO or gastrodin-treated rats were similar. (B) Nissl staining of hippocampus from a control animal and DMSO/gastrodin-treated animals that survived after SE. B1 & B2: Graph of hippocampal histology from a control rat; B3 & B4: Hippocampal histology from a DMSO-treated rat survived after SE and (B5-6) hippocampal histology from a gastrodin-treated rat survived after SE.

Gastrodin Failed to Reduce Spontaneous Epileptiform Discharges Induced by Mg^{2+}-Free Medium

Acute hippocampal slices were treated with Mg^{2+}-free medium. Stable spontaneous epileptiform discharges could be recorded in CA1 and CA3 pyramidal cells from 15 hippocampal slices of seven animals. Baseline spike frequency was 0.16 ± 0.01 Hz and spike amplitude was 2.25 ± 0.35 V. Six slices were treated with 25 μM gastrodin, six slices with 200 μM gastrodin, and three slices with DMSO as the control. The frequencies of the spontaneous epileptiform discharges under 25 μM or 200 μM gastrodin or DMSO were 119 ± 18%, 117 ± 5% and 109 ± 8%, respectively, compared with baseline. According to the results from ANOVA with *post-hoc* analysis, there were no significant differences in spike frequencies for the 25 μM (P = 0.17) or 200 μM gastrodin group (P = 0.87) compared with the DMSO group. The amplitudes of the epileptiform discharges under 25 μM or 200 μM gastrodin or DMSO were 112 ± 16%, 97 ± 8% and 105 ± 5%, respectively, compared with the baseline (Fig. 3). According to the results from ANOVA with *post-hoc* analysis, there were also no significant differences in spike amplitude for the 25 μM (P = 0.60) or 200 μM gastrodin group (P = 0.73) compared with the DMSO group. Based on these experiments, gastrodin failed to inhibit spontaneous epileptiform discharges induced by Mg^{2+}-free medium in hippocampal pyramidal cells.

Gastrodin Showed No Effect on Synaptic Transmission in the CA1-Schaffer Collateral Pathway

fEPSPs was induced in Schaffer collaterals as shown above by administering electrical stimulation once per minute. Baseline fEPSPs were recorded for 30 min; ligands were then added to the ACSF and fEPSPs were recorded for the next 30 min. The final 5 fEPSP slopes measured during baseline and drug

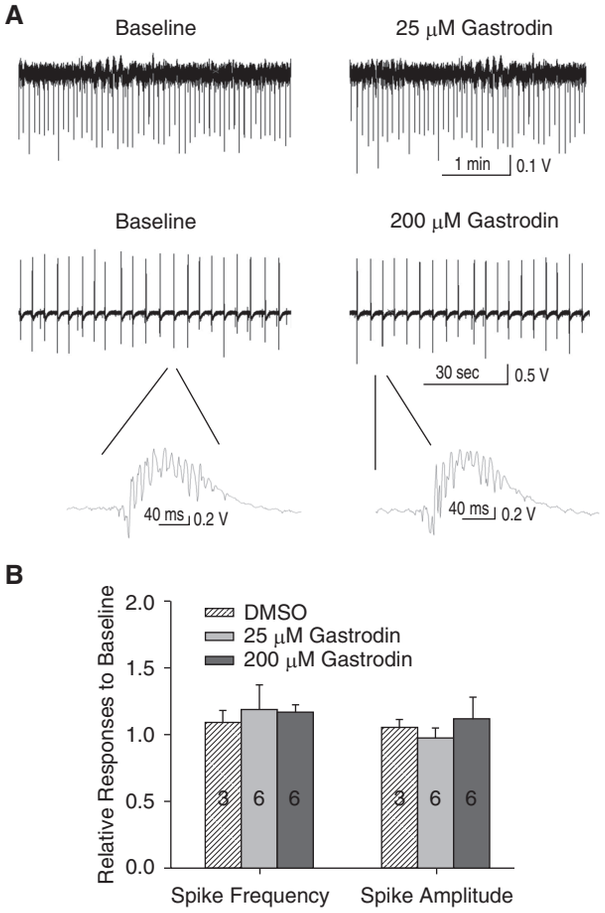

Fig. 3. Epileptiform discharges induced by Mg^{2+}-free ACSF. (A) Field potential recording from the CA1 region in acute hippocampal slices showing regular spontaneous activity induced by Mg^{2+}- free ACSF. (B) Quantification of spike frequency and amplitude before and after application of DMSO, 25 μM or 200 μM gastrodin.

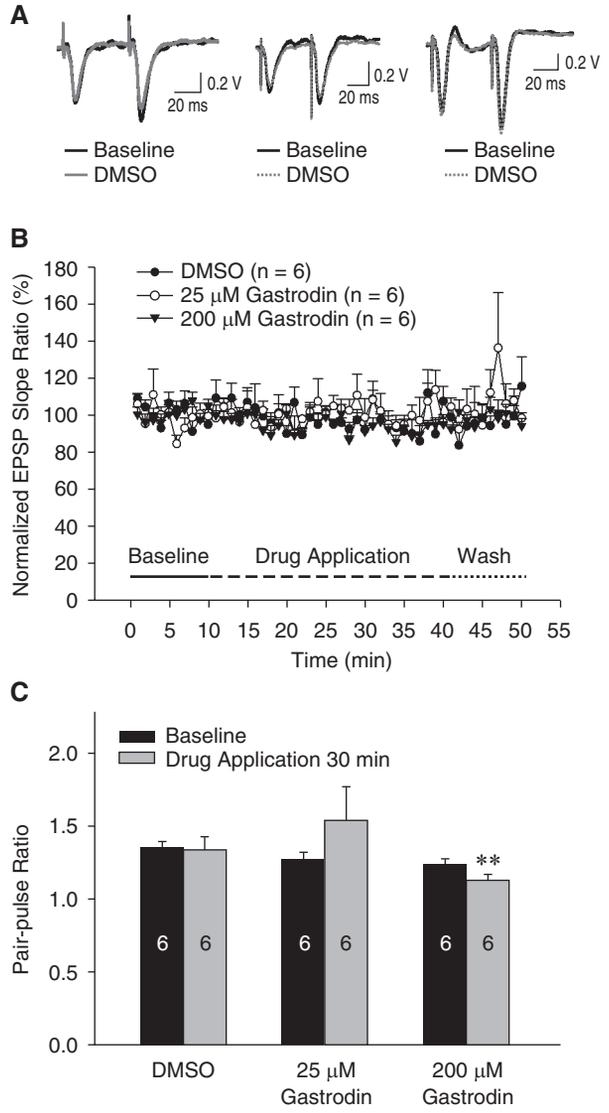

Fig. 4. Effects of gastrodin on CA1-Schaffer collateral fEPSPs (A) Representative traces of fEPSPs before and after application of DMSO, 25 μM or 200 μM gastrodin. (B) The slopes of fEPSPs after treatment with DMSO, 25 μM or 200 μM gastrodin for 30 min. (C) PPR after treatment for 30 min with 200 μM rosiglitazone. ** $P < 0.001$.

application were used for quantification. Application of DMSO, 25 μM or 200 μM gastrodin had no significant effect on EPSP slopes (98.96 ± 5.39%, 119.10 ± 14.53%, and 93.58 ± 1.37% compared with the baseline, respectively, Fig. 4, A and B). Paired-pulse stimulation using a 50-ms interval was also assessed for evaluation of the presynaptic vesicle release probability. Paired-pulse facilitation (PPF) was observed in all recordings. DMSO and 25 μM gastrodin had no effect on PPR. Gastrodin at 200 μM decreased PPF from 1.23 ± 0.04 to 1.12 ± 0.04 ($P = 0.002$), indicating that high-dose gastrodin may affect presynaptic intracellular calcium reservoirs and reduce PPF (Fig. 4C).

Discussion

Gastrodin, the main component of GE, was unable to suppress *in vivo* and *in vitro* TLE in this study. Although gastrodin protected cultured hippocampal slices from NMDA excitotoxicity, it could not abolish epileptiform discharges, which were induced by low magnesium medium in hippocampal slices. Moreover, SE induced by lithium-pilocarpine could not be suppressed by intraventricular gastrodin administration. Gastrodin showed no effects on fEPSP at Schaffer collateral-CA1 synapses, but it suppressed PPF at high concentration, indicating gastrodin may possibly affect presynaptic intracellular calcium reservoirs. In summary, gastrodin was able to protect hippocampal

slices from NMDA *via* mechanisms that did not involve direct suppression of the seizure.

Over-activation of NMDA receptors may trigger rapid Ca^{2+} influx and lead to excitotoxic neuronal death. This excitotoxicity is a common pathological step leading to neuronal loss in many brain disorders, from acute brain injuries such as stroke to chronic neurodegenerative diseases such as Huntington's disease (11). NMDA receptors are also key factors involved in low extracellular magnesium-induced epileptiform activity in hippocampal slices (23, 27). In the present study, gastrodin successfully suppressed NMDA excitotoxicity in cultured hippocampal slices, either *via* suppression of epileptiform discharges or by other neuroprotective mechanisms. However, gastrodin neither suppressed *in vivo* SE induced by pilocarpine, nor low extracellular magnesium-induced epileptiform activity in hippocampal slices. Several mechanisms have been proposed for the neuroprotective effect of gastrodin. Gastrodin protected PC12 cells from apoptosis induced by glutamate through a mechanism involving the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)/p38 mitogen-activated protein kinases (MAPK)/p53-signaling pathway. Glutamate increased intracellular Ca^{2+} concentration and induced oxidative stress, which led to neuronal apoptosis. Gastrodin, which inhibited the phosphorylation of CaMKII, attenuated Ca^{2+}-mediated apoptosis. By phosphorylation of CaMKII, gastrodin also deactivated the ASK-1-p38 MAPK pathway and suppressed oxidative stress (16). In addition, activation of group I metabotropic glutamate receptors attributed to lipopolysaccharide (LPS) or interleukin-1b (IL-1b) induced neuroinflammation and neurodegeneration (8). Gastrodin attenuated levels of neurotoxic proinflammatory mediators and proinflammatory cytokines, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and IL-1b by inhibition of the NF-κB signaling pathway and phosphorylation of MAPKs in LPS-stimulated microglial cells (7). Our data provide evidence for gastrodin as a potential neuroprotective drug against glutamate-related neurodegenerative diseases.

GE is an ancient Chinese herb used for the treatment of epilepsy. The mechanisms of its anticonvulsive effect have been comprehensively reviewed (25). Mice treated with the ether fraction of methanol extracts of GE were resistant to seizures induced by kainic acid and showed reduced neuronal damage in the hippocampus (18). Several main components have been evaluated with regard to their anti-convulsive properties, including vanillin, vanillyl alcohol, 4-hydroxylbenzaldehyde and gastrodin. Intraperitoneal vanillyl alcohol was able to reduce seizure severity induced by intracortical injection of ferric chloride as well as phenytoin (14). Seizure-sensitive Mongolian gerbils pretreated with oral gastrodin had fewer seizures, or lower severity, compared with the control group (1). Gastrodin has also been reported to bind to glutamate receptors (25). In this study, gastrodin, used as a treatment for epileptiform discharges induced by Mg^{2+}-free medium, which is regarded as NMDA receptor-mediated spontaneous activity, showed no efficacy. Low magnesium medium induces epileptiform activity in hippocampal slices, and is commonly used for screening chemicals with anticonvulsive effects (10). Glutamate and NMDA receptors are the key factors for low extracellular magnesium-induced epileptiform activity in hippocampal slices (22, 23). In this study, gastrodin also failed to affect the slopes of Schaffer collateral EPSPs, mediated primarily by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. From these results, we speculate that gastrodin cannot bind to ionotropic glutamate receptors and has no effect on NMDA receptor-mediated seizures. Gastrodin at 200 μM decreased the PPR in two successive stimulations with a 50-ms interval. PPF is a typical finding in hippocampal CA1 pyramidal cells, and calcium plays a prominent role in this form of short-term plasticity (29). Gastrodin decreased PPF either by reducing the intracellular calcium reservoir, or by changing intracellular calcium affinity or saturation. This finding provides a possible mechanism that gastrodin may work *via* calcium channels to attenuate NMDA excitotoxicity in cultured hippocampal slices.

Lithium-pilocarpine can induce SE and trigger further chronic limbic seizures in rodents, and has been regarded as a model for SE and chronic TLE in animals for more than a quarter of a century (34). Injection of pilocarpine in rats induced tonic-clonic generalized seizures that would last for several hours. Lithium pretreatment 24 h before SE induction significantly reduced the pilocarpine dose required to induce seizures. With lithium, there was more consistency in time from pilocarpine injection to SE onset (6). The sequence of behavioral changes observed in animals undergoing SE was very similar for lithium-pilocarpine compared with pilocarpine administered alone. Neuronal damage resulting from SE was also essentially similar (4). In the present study, we examined the efficacy of intraventricular gastrodin on lithium-pilocarpine-induced SE. Gastrodin failed to inhibit seizure behavior or epileptiform activities in hippocampal and frontal EEG tracings. Moreover, gastrodin was unable to shorten SE duration or protect against hippocampal neuronal loss after SE. On the other hand, anticonvulsants in common use, including phenytoin, phenobarbital, valproate and carbamazepine, were also unable to halt SE in lithium-pilocarpine-

treated rats (6). The ability of pilocarpine to induce SE depends on activation of the M1 muscarinic acetylcholine receptors (13). Thus, pilocarpine-induced SE can be blocked by systemic administration of the muscarinic antagonist, atropine (4). Glutamate levels in the hippocampus were elevated after seizure initiation, and the maintenance of SE was considered to have been through activation of the NMDA receptor (31). Gastrodin failed to suppress SE induced by lithium-pilocarpine, which provides further evidence that gastrodin has no effect on NMDA receptors. In a recent study, Zhou and his colleagues reported that gastrodin had a synergistic anticonvulsive effect against penicillin-induced convulsion in rat in combination with phenytoin (37). According to their report, a very high concentration of gastrodin (950.6 mg/kg) alone could produce an anticonvulsive effect. Generalized penicillin epilepsy in cats represented an experimental model of generalized spike-and-wave discharges occurring during clinical absence attack. In this model, $GABA_A$-mediated inhibitory mechanisms are preserved (2). Gastrodin is considered to be involved in GABA metabolism (1). Pentylenetetrazole (PTZ), a non-competitive GABA antagonist, is another CNS stimulant used to study seizure phenomena and to identify compounds that may be effective for the control of seizure susceptibility. Further studies on gastrodin in PTZ-induced seizures are needed for more in-depth evaluation of its anticonvulsant properties.

In conclusion, gastrodin failed to suppress seizures in rats induced by lithium-pilocarpine and epileptiform discharges in hippocampal slices induced by low magnesium medium. It also showed no effect on hippocampal Schaffer collateral fEPSP. These findings suggest gastrodin does not interact with ionotropic glutamate receptors to inhibit NMDAR-facilitated seizures. Nevertheless, gastrodin is still a potential neuroprotective agent against NMDA excitotoxicity, which is potentially beneficial for patients who have survived stroke or who have epilepsy.

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