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Chronic administration tetrahydroxystilbene glucoside promotes hippocampal memory and synaptic plasticity and activates ERKs, CaMKII and SIRT1/miR-134 *in vivo*

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ABSTRACT

Ethnopharmacological Relevance: Polygonum multiflorum Thunb is a traditional Chinese medicine with anti-aging effect. 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG) is generally considered as the main active component in Polygonum multiflorum Thunb. However, the effect of TSG on memory in adult is unclear till now.

Aim of study: 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG) is a polyphenols compound from *Polygonum multiflorum* Thunb. The present study aimed to evaluate the effect of chronic administration of TSG on hippocampal memory in normal mice.

Materials and methods: Behavioral test, electrophysiology and golgi staining were used to evaluate the effect of TSG on hippocampus-dependent memory and synaptic plasticity. Western blotting was used to determine the expression of ERK1/2, CaMKII, and SIRT1. Real-time quantitative PCR was explored to measure miR-134.

Results: It was found that TSG enhanced hippocampus-dependent contextual fear memory and novel object recognition, facilitated hippocampal LTP and increased dendrite spine density in the CA1 region of hippocampus. TSG obviously promoted the phosphorylations of ERK1/2, CaMKII, CREB and the expression of BDNF in the hippocampus, with upregulation of silent information regulator 1 (SIRT1) and downregulation of miR-134.

Conclusions: Chronic administration of TSG promotes hippocampal memory in normal mice, suggesting that supplementary of TSG might serve as an enhancement of memory.

1. Introduction

Dietary and environmental factors have a profound impact on shaping the capacity of brain cognition (Benton, 2010; Gomez-Pinilla, 2008; Rendeiro et al., 2012; van Praag, 2009). Therefore, searching for dietary components might be potential strategies for the treatment of cognitive impairments.

Polyphenols are present in high amounts in fruits, vegetables. Recently, much interest has been attracted to the neurocognitive effects of polyphenols (Spencer, 2008; van Praag, 2009). For example, diets of flavanol-rich green tea , blueberries and pomegranates have been shown to enhance synaptic plasticity and memory(Haque et al., 2006; Joseph et al., 1999; Hartman et al., 2006). Besides, specific polyphenol molecules such as fisetin and epicatechin also have the similar effect (Maher et al., 2006; van Praag et al., 2007). The root tuber of *Polygonum multiflorum* Thunb, officially listed in the Chinese Pharmacopoeia, is a traditional Chinese dietary herb that is widely used in the Orient as a tonic and antiaging agent since ancient times (Bounda and Feng, 2015; Lin et al., 2015). The herbal constituents from the roots tuber includes *trans*-Resveratrol, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG), emodin, chrysophanol, aloe-emodin, and physcion. Few clinical studies have revealed the traditional therapeutic claims of these various bioactive constituents. Among them, physcion is for the treatment of inflammatory bowel diseases (Tzeng et al., 2011), and TSG could also be used against Alzheimer's disease and Parkinson's disease, the typical symptom of which is increased impairment of learning and memory (Zhang et al., 2013a, 2013b). As one of the active components extracted from

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P. multiflorum Thunb, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG) has a polyphenolic structure (Ryu et al., 2002; Wang et al., 2009). 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside has neuroprotective, antioxidant and antiapoptotic effects (Wang et al., 2009; Wang et al., 2008). Our previous study also demonstrates that 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside promotes hippocampal long-term potentiation (LTP), a cellular model of learning and memory, in normal mice *in vitro* (Wang et al., 2011a). *In vivo*, administration of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside improves the ultrastructure of hippocampal synapses and enhances cognition in both APP transgenic mice and aged rats (Wang et al., 2007; Zhang et al., 2006). However, the benefits of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside on cognition has only been explored in aged rodents and transgenic mice since the compounds have minimal effects in normal young subjects (Hartman et al., 2006; van Praag, 2009). Therefore, it is tempting to investigate whether 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside enhances cognition in normal rodents *in vivo*.

In present study, we investigated the chronic effect of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside on hippocampus-dependent memory of normal mice and explored the potential mechanisms. We found that 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside promoted hippocampal memory and synaptic plasticity, which correlated with significant activation of ERKs, CaMKII and SIRT1/miR-134 pathways, which have a direct role in regulations of cAMP response element-binding protein (CREB) activity and brain-derived neurotrophic factor (BDNF) expression. Our work reveals that natural compound from dietary herb can promote memory by regulating microRNA function, suggesting that administration of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside might be a strategy for cognitive enhancement therapies.

2. Materials and methods

2.1. Chemicals and drug treatment

2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (purity above 98%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structure of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside is shown in Supplementary Fig. 1. The 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside powder was dissolved into saline freshly every day between 8 and 9 a.m to make the 2, 4 and 8 mg/ml 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside solution. At a level of 100 μl/10 g, the 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside saline–solution was administered via orogastric tube into the stomach. The research was conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. The use of animals for experimental procedures was approved by the Review Committee for the Use of Human or Animal Subjects of Huazhong University of Science and Technology. 8 weeks old C57BL6J mice (20–25 g) were divided into four groups randomly: saline group (NS), low dose (20 mg/kg/day; TSG-L), middle dose (40 mg/kg/day; TSG-M) and high dose of 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (80 mg/kg/day; TSG-H). Saline or TSG was administered to mice daily for 4 weeks.

2.2. Fear conditioning tasks

Fear conditioning was processed according to previous protocols with some modifications (Wang et al., 2011b) with 8–10 mice in each group. On day 1, mice were presented with three pairings of a tone for 30 s as the CS (80 dB) that was coterminated with a foot shocks as the US (1 s). The intertrial interval (ITI) was 60 s and the shock was 0.5 mA. Mice were left in the conditioning chamber for 30 s after termination of the procedure and then returned to their home cage. To assess contextual fear memory, 24 h after conditioning, mice were placed into the conditioning chamber and observed for 3 min. One hour later, the animals were assessed for cued fear conditioning in a novel test chamber with a parameter consists of 8 conditioned stimulus tone (30 s each with a 10-s intertrial interval).

2.3. Novel object recognition

As previously described (Wang et al., 2011b), the objects consisted of two similar wood blocks. During this phase, the frequency and duration of the mouse approached, oriented toward, and sniffed each object was recorded. 24 h later, one object was replaced by a new novel object randomly. Then mouse was again placed in the box, and exposed to the familiar and novel object for a 5 min “test phase”. 12 mice were used in each group.

2.4. Electrophysiological recording

Hippocampal slices were prepared as previously described (Yang et al., 2010). In brief, anaesthetized mice was decapitated and transverse hippocampal slices were made with a Vibrate tissue slicer in ice-cold artificial cerebrospinal fluid. Next, slices were incubated in a holding chamber for at least 1.5 h before using. All solutions were saturated with 95% O_2/5% CO_2. Then, a single slice was transferred to the perfusion-type recording chamber and field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum layer using a glass micropipette filled with 3 M NaCl. The amplitude of the fEPSPs was usually set at 1/3 - 1/2 of the maximal responses. LTP was induced by delivery of three burst of HFS (100 Hz for 1 s; 30 s interval) and measured by the slope of fEPSPs at 60 min after tetanus stimulation in 10–11 slices from 8 mice per group.

2.5. Western blotting

The timing of sacrifice for western blotting is on the day after the 4 weeks 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside administration. The procedures of western blotting were processed according to our previous protocols with some modifications (Wang et al., 2011a). Briefly, hippocampus from 5 mice in each group was homogenized and protein samples (30 μg) were separated by 10% SDS-polyacrylamide gel and then transferred to nitrocellulose membranes. After blocking with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature, transferred membranes were incubated overnight at 4 °C with appropriate primary antibodies. Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies, and immersed in enhanced chemiluminescence (ECL)-detecting substrate (Super Signal West Pico; Pierce Chemical Co., Rockford, IL, USA). Bands intensities were quantified using NIH Image J software and normalized to the quantity of β-actin in each sample lane.

2.6. Real-time PCR

RNAs were extracted from hippocampuses of mice for miR-134 gene expression. Total RNA (0.5 μg) was reverse transcribed using a High Capacity cDNA RT Kit (Applied Biosystems). The SYBR Green reactions were performed in the Agilent Stratagene fluorescent quantitative PCR instrument (Mx3000P, Stratagene, USA) and the data were analyzed using Mxpro-Mx 3000p software. There were 6 mice in each group.