Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Oleuropein Improves Long Term Potentiation at Perforant Path-Dentate Gyrus Synapses *in vivo*

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ARTICLE INFO

Article history

Received: January 30, 2015

Revised: March 26, 2015

Accepted: May 04, 2015

Available online:

July 14, 2015

DOI:

ABSTRACT

Objective To investigate the effect of oleuropein (OE) on long term potentiation (LTP) at hippocampal perforant path-dentate gyrus synapses *in vivo*. **Methods** An outer guide cannula, a monopolar recording electrode, and a bipolar stimulating electrode were implanted in the skull and extracellular recording technique was used to record the population spike in the dentate gyrus of anesthetized rats. **Results** Oleuropein significantly increased the basal synaptic transmission and the amplitude of population spike was increased from (117.6 ± 2.3)% to (134.9 ± 3.7)% after administration with OE. OE also accelerated LTP induction and maintenance, the population spike amplitude after high frequency stimulation was increased from (167.2 ± 12.8)% to (225.5 ± 15.5)% and the maintenance phase of LTP was from (182.1 ± 15.1)% to (210.5 ± 9.0)% respectively after administration with OE. **Conclusion** The present study showed that OE significantly improved different stages of LTP, which could be the molecular mechanism of its efficacy on attenuating AD-like pathology and delaying cognitive decline. OE can be a promising drug for AD and dementia.

Key words

Alzheimer's disease; dentate gyrus; long term potentiation; oleuropein; population spike

1. Introduction

Cerebral plaques and neurofibrillary tangles (NFTs) are considered to be the definitive diagnosis of Alzheimer's disease (AD). Cerebral plaques containing the amyloid-β (Aβ) peptide as the primary component are dense proteinaceous cores. The amyloid cascade hypothesis posits that Aβ plays a critical role in the pathogenesis of AD (Nalivaeva et al, 2014; Morishima-Kawashima et al, 2014). Numbers of therapeutic studies in the laboratory and in the clinic aiming to control Aβ levels in the brain are currently being examined (Marr and Hafez, 2014; Menting et al, 2014). For example, MK-8931, a promising β-secretase inhibitor, reduced Aβ cerebral spinal fluids levels through blocking Aβ production and was well tolerated by patients. Besides, the β-secretase inhibitor, neprilysin-2 (NEP2), which cooperates with NEP to control Aβ levels in the brain may also contribute to the future goal of gene therapy for AD.

In a recent study, it was demonstrated that oleuropein (OE) reduced the Aβ oligomers and leaded to markedly

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Fund: Ministry of Science and Technology (No. 2013ZX09201022); National Natural Science Foundation of China (No. 81073137, 81373418)

elevated levels of sAPPα which suggested a possible protective role for OE against AD (Kostomoiri et al, 2013; Luccarini et al, 2015). Oleuropein, a secoiridoid glucoside characterisic of Oleaceae, is the main phenolic component of Mediterranean extra virgin olive oil. A number of Chinese herbal medicines such as *Syringa pubescens* Turcz., *Syringa oblata* Lindl., also contain OE (Soler-Rivas et al, 2000). Although the exact molecular and cellular mechanisms underlying OE protective activity against AD are still unclarified, mounting evidence supports studies in rodents that diet supplementation with foods rich in red wine and in extra virgin olive oil improves learning and memory associated deficits and has anti-oxidant effect in the brain (Farr et al, 2012; Papandreou et al, 2009) and beneficial to AD-like pathological and cognitive conditions in the Tg2576 AD mouse model (Ladiwala et al, 2011).

The most common early symptom of the Alzheimer's disease is difficulty in remembering recent events often mistakenly thought to be manifestations of stress (Kumar et al, 2015). As the disease advances, gradual progressive trouble with language, mood swings and long-term memory loss are characteristic symptoms for which patients often seek medical advice. Almost from the beginning of modern neuroscience, many researchers believe that an activity-driven change in synapses is a key molecular mechanism underpinning memory. A central tenet of this correlation between the properties of long term potentiation (LTP) and memory was belatedly satisfied by the discovery (Bliss and Lomo, 1973) that the strength of excitatory synapases in the brain was relevant to the intensity of activity. The accumulation of overwhelming correspondences between LTP effect and learning and memory have been rewarded with spectacular breakthroughs and showed that manipulations of the former have predictable effects on the latter (Martin et al, 2000). These discoveries that LTP of excitatory synapses involving in synaptic transmission and triggering the synaptic plasticity, accepted as many commonplace forms of memory have shed new light on the effort to invent mechanism-based memory drugs. Hippocampal LTP is the most researched form of synaptic plasticity, specially, dentate gyrus (DG) of hippocampus fit well with hippocampus dependent memory formation (Kesner, 2007).

Based on the above ideas, it is important to clarify the relationship between OE and LTP. Therefore, in the present study, we used the *in vivo* electrophysiological recording to investigate the effect of OE on different stages of LTP in perforant path-DG synapses in anesthetized rats.

2. Materials and methods

2.1 Animals

Male Sprague-Dawley rats (180 ± 20 g) were provided by the Experimental Animal Center of Chinese Academy of Medical Sciences, Beijing, China. Rats were housed in a temperature and light-control room (23 ± 1 °C, 12 h light cycle) and had free access to food and water. All animals were handled in accordance with the standards established in the Guide for the Care and Use of Laboratory Animals published by Chinese Animal Use and Care committee and executed according to the National Animal Law.

2.2 Drugs and reagents

The following chemicals were used: OE from herbal medicine *Ilex pubescens* Hook. et Arn. (purity > 99%) was isolated and purified by the institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. All other chemicals in this study were purchased locally and were of analytical-reagent grade.

2.3 Surgical procedure

The animals were prepared as previously described (Wang et al, 2001). Briefly, rats were ip anesthetized with urethane carbamate (1.5 g/kg) before being fixed in a stereotaxic frame. Three holes were sequentially drilled at 0.8, 3.5, and 7.8 mm posterior to the bregma and 1.8, 2.5, and 4.5 mm lateral to the midline for an outer guide cannula, a monopolar recording electrode, and a bipolar stimulating electrode, respectively. The cannula was placed into the lateral cerebral ventricle at a depth of 3.5–4.0 mm, the recording electrode was placed in the granular cell layer of dentate gyrus at a depth of 3.0–4.0 mm, and the stimulating electrode was lowered into the perforant path to a depth of 3.0–4.0 mm. The synaptic responses were monitored by a Digidata 1440A Oscilloscope. Once the locations of the cannula and electrodes were verified, they were kept in place for the whole experimental duration.

2.4 Recording of evoked potentials at dentate gyrus and LTP induction

The population spike (PS) amplitude was employed to assess the excitation level of the granular cell population in the DG. An evoked response was generated in the DG granular cell layer by stimulating the PP at low frequency (0.033 Hz) with single constant current pulses (200 μs in duration) triggered by a Master-8 Electrical Stimulator through an isolator. And the stimulus intensity was set to a level which evoked a population spike of 30%–50% of the maximal amplitude. The PS amplitude was measured as the difference in voltage between the peak of the first positive wave and the peak of the first negative deflection. After ensuring a steady state baseline response, which was taken about 30 min between the last two steady state response recordings, LTP was induced using a high-frequency stimuli protocol of 100 Hz (10 bursts of 5 stimuli, 0.2 ms stimulus duration, and 200 μs interburst interval) (Wang et al, 2001). Following delivery of tetanic stimuli, application of the test stimuli was continued at a frequency of 0.033 Hz. The percentage of the ratio of absolute PS amplitude to basal value was used to represent the level of PS amplitude. It was

defined as a successful induction of LTP if the amplitude of PS change exceeded 20%. For each time-point, 10 consecutive evoked responses were averaged at 30 s stimulus interval. Saline or OE was injected over a 10 min period, 10 min before HFS and 40 min after HFS to examine the effect of saline or OE on LTP induction and LTP maintenance, respectively. Besides, after ensuring a steady state baseline response, saline or OE was injected into the lateral cerebral ventricle over a 10 min period and application of the test stimuli was continued up to 30 min at a frequency of 0.033 Hz to examine the effect of saline or OE on baseline activity of the DG cells of the hippocampus.

2.5 Drug delivery

Drugs or vehicle injections were delivered via a cannula in the lateral cerebral ventricle. Injections were performed using a 5 μL volume over a 5 min period via a microsyringe, The dose of OE was calculated based on the theoretical concentration that the drug would have in the brain (assuming a brain volume of approximately 2 mL) (Sun et al, 2012). The drug was dissolved in DMSO and diluted with physiological saline, thus, 5 μL of 37 μmol/L OE was injected and the final concentration of OE for the bain was 92.5 nmol/L. Control rats were injected with 0.06 % DMSO.

2.6 Statistical analysis

All data were shown as $\bar{x} \pm s$. Group differences of PS amplitude in electrophysiological assays were evaluated using Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1 Effects of OE on basal synaptic transmission in hippocampal DG of anesthetized rats

The effect of OE on the basal synaptic transmission induced by stimulation of perforant path in the hippocampus of rats *in vivo* were first examined. We stimulated the PP coming from entorhinal area and recorded the evoked PS in DG regions of anesthetized rats. After establishing a stable baseline for 30 min, OE or vehicle was injected and PS amplitude was monitored for 30 min. After the injection, the PS amplitude of OE group at 30 min was (134.9 ± 3.7)% and that of control group was (117.6 ± 2.3)%. As shown in Figure 1(b)−(d), OE did affect the basal PS amplitude significantly ($n = 6$, $P < 0.05$ *vs* control).

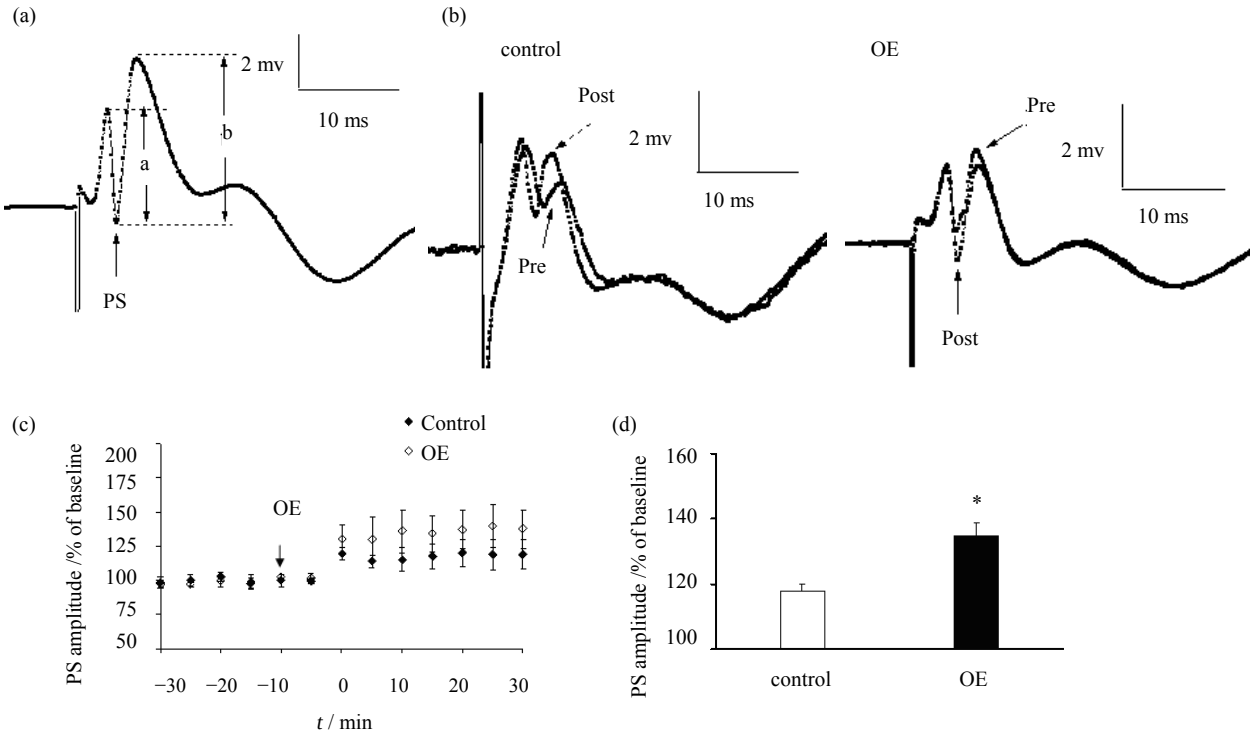

Figure 1 Effects of OE on basic synaptic transmission in hippocampal DG of anesthetized rats ($\bar{x} \pm s$, $n = 6$)

(a) Typical PS in the hippocampal DG of rats in response to electrical stimulation *in vivo*. Amplitude of PS was calculated by taking the average of the mean value of two peak-to-peak amplitudes identified by a and b. (b) Representative traces of PS before (pre) and after (post) the administration. (c) Time-course of changes in PP-DG population spikes of the control group and OE group. The baseline was recorded for 30 min before OE administration. At 0 min, 5 μL 37 μmol/L OE (intracerebroventricular, final concentration was 92.5 nmol/L) was injected in the test subject, whereas vehicle (0.06% DMSO) was injected in control animals. The arrow shows the time point of intracerebroventricular injection. (d) The data in (c) were summarized by calculating the average of population spike amplitude 0−30 min after injection. $^*P < 0.05$ *vs* control.

3.2 Effects of OE on LTP induction in PP-DG pathway of anesthetized rats

To determine whether OE was involved in synaptic plasticity, the effect of OE on the induction of LTP in the DG region of rats *in vivo* was investigated. As shown in Figure 2 (b), HFS was applied 10 min after vehicle administration, which resulted in successful induction of LTP in control-treated rats (169.3 ± 13.3)% of baseline at 55 min after HFS, $n = 6$). In contrast, in the OE-treated animals, OE facilitated the induction of LTP significantly at 55 min after HFS (226.4 ± 27.8 % of baseline, $n = 6$). The PS amplitude of OE-treated rats at 10, 30, 50 min was (220.6 ± 15.5)%, (225.4 ± 15.5)%, (226.4 ± 27.1)% respectively and that of control group was (148.9 ± 10.1)%, (167.2 ± 12.8)%, (169.3 ± 13.4)% respectively. There was a significant main effect in the control and OE groups ($n = 6$, $P < 0.01$).

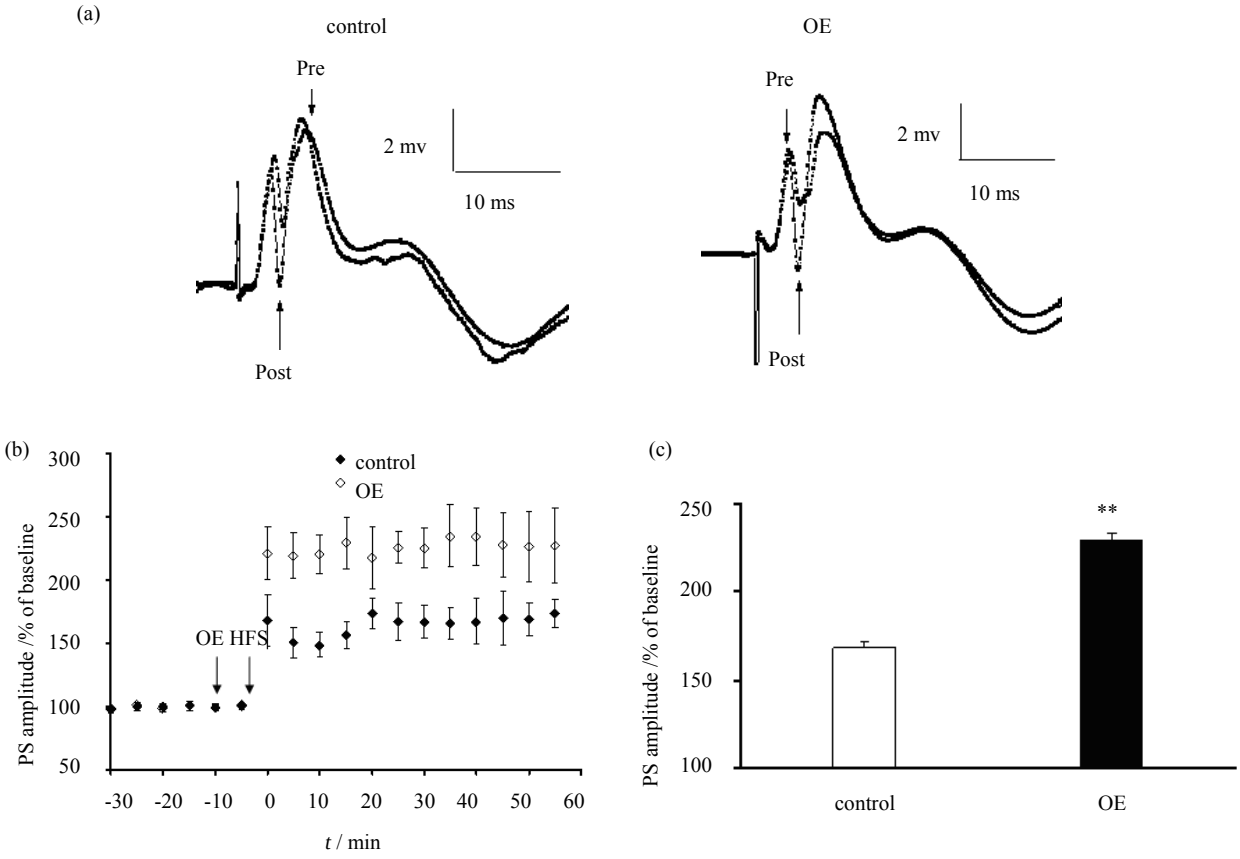

Figure 2 Effects of OE on induction of LTP in PP-DG pathway of anesthetized rats ($\bar{x} \pm s$, $n = 6$)

(a) Representative traces of PS before (pre) and after (post) HFS. (b) Time-course of changes in PP-DG population spikes. Vehicle (n = 6) or OE (n = 6) was injected into the lateral cerebral ventricle 10 min before the application of HFS. The amplitude of population spike was expressed as the percentage of baseline value. (c) The data in (b) were summarized by calculating the average of population spike amplitude 30–60 min after HFS as an index of LTP magnitude. $^{**}P < 0.01$ *vs* control.

3.3 Effects of OE on maintenance phase of LTP in PP-DG synaptic transmission pathway of anesthetized rats

To further understand the effect of OE on synaptic plasticity in the hippocampal dentate gyrus area, OE was injected 40 min after the application of HFS to assess the effect of OE on the maintenance of LTP. Effects of OE on the maintenance of LTP and PS amplitude were shown in Figure 3 (b) –(c). The PS amplitude of OE group at 40, 50, 60 min was (210.5 ± 9.0)%, (205.1 ± 15.5)%, (207.3 ± 11.2)% respectively and that of control group was (189.9 ± 18.0)%, (182.1 ± 15.1)%, (181.5 ± 14.3)% respectively. OE did have significant effect on the maintenance of LTP in anesthetized rats compared with the control group ($n = 6$, $P < 0.05$).

4. Discussion

OE has been shown to possess a broad range of biochemical and beneficial pharmacological properties *in vivo*, such as anti-oxidant, anti-inflammatory, antibacterial, anticancer, anti-amyloidogenic, and hypoglycemic effects (Sarbishegi and Mehraein, 2014; Janahmadi et al, 2015; Casamenti et al, 2015). With pathophysiology of AD, it is characterized by intracellular neurofibrillary tangles and extracellular amyloidal protein deposits contributing to senile plaques. Numerous studies show that OE interferes with tau, Aβ aggregation, and OE administration results in a significant reduction of soluble and aggregated Aβ in AD-relevant brain areas with a significant attenuation of Aβ-mediated cognitive

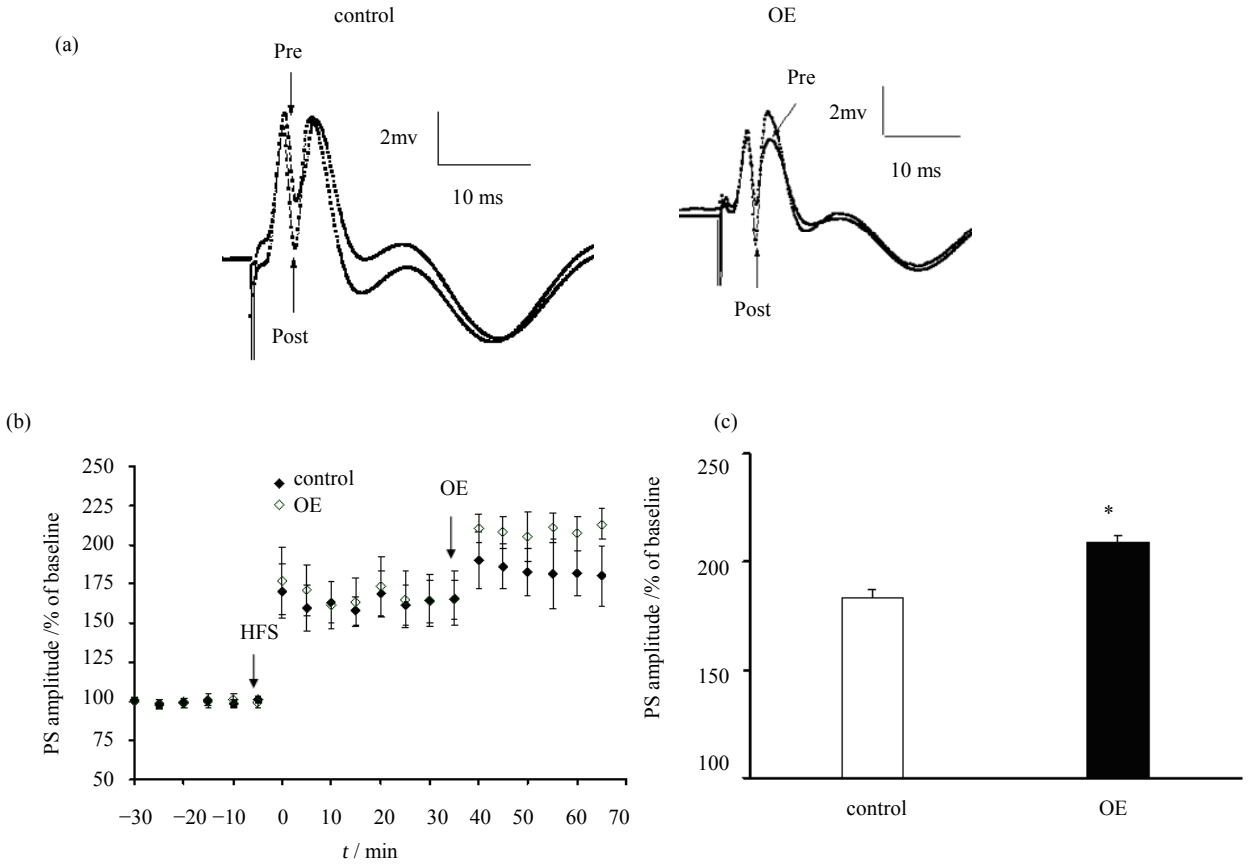

Figure 3 Effect of OE on maintenance of LTP in dentate gyrus of hippocampus after tetanic stimulation ($\bar{x} \pm s$, $n = 6$)

(a) Representative traces of PS before (pre) and after (post) the administration. (b) Time-course of changes in PP-DG population spikes. Vehicle (n = 6) or OE (n = 6) was injected into the lateral cerebral ventricle 40 min after the application of HFS. The amplitude of population spike was expressed as the percentage of baseline value. (c) The data in (b) were summarized by calculating the average of population spike amplitude 40-65 min after the injection. $^{*}P < 0.05$ *vs* control.

deterioration (Luccarini et al, 2015; Kostomoiri et al, 2013). Not only OE, the metabolites of OE but also showed anti-aggregation and neuroprotective activities, confirming its beneficial properties against neurodegeneration (Luccarini et al, 2014; Casamenti et al, 2015), and the related metabolizing data about both ig and iv administration of OE *in vivo* were reported in our previous work (Zhou et al, 2011; Lin et al, 2013).

In the present study, we investigated the effect of OE on synaptic transmission *in vivo*. The main goal of this experiment was to confirm if OE could affect LTP qualitatively, as a molecular predominant experimental model of learning and memory, dose-response relationship did not needed during LTP induction in the present study. Therefore, we designed intracerebroventricle administration of single dose of OE, and we observed the first time that OE significantly increased hippocampal basal synaptic transmission, facilitated LTP induction, and improved the LTP maintenance phase in dentate gyrus of hippocampus. This result is in accordance with the studies which suggested a possible protective role for OE against AD from different aspects. According to Rowan's hypothesis, misprocessing of amyloid precursor protein (APP) results in the accumulation of soluble Aβ, impairs hippocampal LTP and may lead to the cognitive decline seen early in AD (Walsh et al, 2002), therefore, the effect of OE on LTP was considered as the consequence of OE action on Aβ accumulation. And for our results, it seems like that OE improved different stages of LTP was because of its anti-aggregation efficacy. However, we do not think there is direct causality among Aβ accumulation, LTP and cognition declining of AD.

AD may also impair LTP through mechanisms distinct from Aβ, for example, ApoE4 could impair hippocampal L-LTP by reducing p-CaMKIIα and p-CREB (Qia et al, 2014), a pathologic marker of AD, p-PKMζ, which also accumulates in neurofibrillary tangles, is of critical importance in the maintenance of late LTP (Chen et al, 2015). Therefore, the detained molecular mechanism of OE acted on basal synaptic transmission, and LTP induction and maintenance need further investigation.

Aksoy-Aksel et al (2015) reported that group II mGlu receptor activation affected basal synaptic tonus, but not LTP at pp-DG synapses *in vivo*, Kennard et al (2014) reported that redistribution of ionotropic glutamate receptors was associated with LTP induction. Also, it was reported that OE could block L-type calcium channel (Leila et al, 2012). The rise in Ca^{2+}, amplified by voltage-dependent calcium channels

or other factors, is very important to the induction of LTP. So the effect of OE against AD may be correlative with the expression of calcium channel. Above all, except Aβ, OE may act on basal synaptic plasticity and LTP by a numerous pathway or protein factors.

On the other hand, manipulations of LTP have predictable effects on the memory (Stuchlik et al, 2014) and LTP has received much attention among those who study Alzheimer's disease, some have suggested that the cognitive decline seen in individuals with AD may result from impaired LTP. Therefore, in the present study, OE offered a protective or therapeutic alternative against AD and can be a promising candidate drug for AD therapy.

5. Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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