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Tenuigenin ameliorates learning and memory impairments induced by ovariectomy

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HIGHLIGHTS

- Tenuigenin ameliorates cognitive deficiency induced by ovariectomy.
- Tenuigenin decreases the loss of NOS positive neurons in OVX mice.
- Tenuigenin prevents and reverses synaptic morphological ovariectomy-induced changes.
- Tenuigenin exerts a potential therapeutic value for menopause cognitive dysfunction.

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ABSTRACT

Estrogen deficiency is associated with cognitive impairment. Hormone replacement therapy (HRT) has proven to be effective in preventing and reversing the memory and learning deficiencies. However, conventional estrogenic treatment could increase the risks of breast cancer and venous thromboembolism. Tenuigenin (TEN) is putatively believed as the active component extracted from a Chinese herb *Polygala tenuifolia* root. Although TEN has been shown to enhance learning and memory in healthy mice, it remains unknown whether or not TEN could ameliorate learning and memory impairments. In the present study, mice were divided into four groups: sham-operated (sham), ovariectomized (OVX), OVX + estradiol benzoate (EB) and OVX + TEN groups. Step-through passive avoidance and Y-maze tests were used to assess learning and memory abilities, and the number of nitric oxide synthase (NOS) positive neurons and the synaptic measurement of hippocampal CA1 area were examined. The results showed that TEN was given orally to OVX mice, leading to the improvement of learning and memory in step-through passive avoidance and Y-maze tests. TEN could reduce the loss of NOS positive neurons and prevent the synaptic morphological changes induced by ovariectomy. Our results suggest that TEN may exert a potential therapeutic value for menopause cognitive dysfunction.

1. Introduction

Previous studies have confirmed that ovarian steroid hormones can affect learning and memory [1]. Estrogen deficiency, associated with cognitive and emotional impairments [2], may result in the synaptic morphological change in the hippocampus and prefrontal cortex [3]. Hormone replacement therapy (HRT) has proven to be effective in preventing the memory and learning deficiencies [4], and reversing the loss of synaptic and dendritic spine density [5]. Additionally, estrogenic treatment can increase the number of spine density [6] and trigger neuronal nitric oxide synthase (nNOS) expression [7]. However, the increased risks of breast cancer [8] and venous thromboembolism [9] produced by conventional estrogenic treatment remains a major drawback and a strong motive to find new therapeutic strategies.

Tenuigenin (TEN), the chemical structure has been mentioned in references [10,11], is putatively believed as the active component extracted from a Chinese herb *Polygala tenuifolia* root that has been used to improve memory and cognitive function in Traditional Chinese Medicine for centuries [12]. Pharmacological data indicate that TEN could suppress secretion of β-amyloid (A-β) in SH-SY5Y APP 695 cells [13], promote proliferation and differentiation in hippocampal neural stem cell [11], and protect SH-SY5Y cell against the injury induced by 6-OHDA [14]. In term of cognitive function, our study has shown that TEN could enhance learning and memory in healthy mice [15]. However, it remains unknown whether or not TEN could ameliorate learning and memory impairments.

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In order to provide a new window into the pharmacological properties of TEN, the present study is designed to investigate the effect of TEN against ovariectomy-induced learning and memory deficiencies in mice. We hope to expand the understanding of the potential therapeutic value of TEN for menopause cognitive dysfunction.

2. Materials and methods

2.1. Drugs

Tenuigenin (purity > 98%) was purchased from the National Institute of Pharmaceutical and Biological Products (Beijing, China). Other chemical reagents were purchased from Sigma (St. Louis, USA).

2.2. Animals, treatments and groups

Six to eight-week-old female Chinese Kunming (KM) mice (20–25 g) were obtained from the Sun Yat-sen University, China. The animals were housed at 22 ± 3 °C, 55 ± 5% humidity, and 12 h light/dark cycle from 08:00 to 20:00 with free access to water and food. All animals were given three days to adapt to this new environment before any procedure was initiated. Experimental procedures were approved by the Committee on Animal Care and Usage of South China Normal University, and every effort was made to minimize animal suffering.

The ovariectomized (OVX) model was described previously [16]. In brief, mice were anesthetized with sodium pentobarbital (35 mg/kg) via intraperitoneal injection and then both ovaries removed. Two weeks later, we confirmed the success of the model according to the absence of periodic changes in vaginal cells following the performance of vaginal smear examination for 7 days. In order to determine the period of drug-application after OVX surgery, we evaluated the effect of ovariectomy on learning and memory activities in different periods (4, 8 and 12 weeks) after surgery using Y-maze test at first, in preliminary experiments.

In drug-application experiments, mice were randomly divided into sham-operated (sham), OVX, OVX + estradiol benzoate (EB) and OVX + TEN groups. In the sham group, an equivalent of adipose tissue around the ovary was taken away. Oral treatment of TEN (4 mg/kg) was started at 6 weeks after operation and continued twice a day for 4 weeks, and the animals of sham and OVX groups were orally given the same amount of double distilled water. Each mouse of the EB group was injected subcutaneously with 20 μg EB per day for 4 weeks [16].

2.3. Behavioral tests

Behavioral tests were conducted after 4 weeks of drug treatment.

2.3.1. Passive avoidance test

A step-through type passive avoidance test was used to evaluate the effect of TEN on memory of OVX mice. As described previously [17], the apparatus consisted of a chamber with two compartments (illuminated and darkened compartments), which were connected by a guillotine door. The experiment consisted of training and testing sessions. During training, the mouse was placed in the illuminated compartment, facing away from the closed guillotine door, for 1 min before the door was raised. The latency to enter the darkened compartment was recorded. After the mouse entered the darkened compartment, the door was closed and an electric shock (0.5 mA, 3 s) was delivered from the steel-rod floor. At days 1 and 7 after training, the testing session was performed, respectively. The mouse was again placed in the illuminated compartment, with the guillotine door open. The retention latency to enter the darkened compartment was recorded for up to 300 s. If a mouse did not enter the dark chamber over 300 s, it was assigned a latency value of 300 s. No shock was delivered during the testing session.

2.3.2. Y-maze test

As described previously [18], Y-maze learning test (brightness/darkness discrimination test) was assessed using radial-arm maze (90 cm long × 90 cm wide × 76 cm high). Radial-arm maze, which consists of three identical arms, was placed in a darkened room. Animals placed in the intersection of three arms were trained to choose entering the randomly bright arm, which was illuminated by a 15 W lamp suspended in the end of each arm. During the 10-day period, the animal was trained to choose entering the randomly bright arm in the first 10 s, and the choice of the darken arm was count as a 'discrimination error'. Whenever the animal made an error, it received brief electric footshocks (35 V AC, 4.5 mA; duration 1 s, every 5 s) until it entered the bright arm. The training procedure was performed during 10-day period and mice received 20 trials at 25–35 s random intervals every 24 h. Ninety percent correct rate served as learning criterion.

2.4. Counted NADPH-d positive neurons

After performing the passive avoidance test, eight mice from each group were randomly selected for counting NADPH-d positive neurons. In brief, mice were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and transcardially perfused with 0.9% saline, followed by ice-cold fixative containing 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB, pH 7.4), for 15 min. Brains were removed, thoroughly rinsed in PB. The brain tissue was coronally sliced. The thickness of slices was 40 μm. The sections were stained for NADPH-d activity as previously described [19]. The staining intensities of the sections that stained specifically for NADPH-d were quantitatively assessed according to a microdensitometry method based on optical density and using an image analyzer [20]. Considering that hippocampal slices can show a little difference in shape due to the angle and location of the cut, five hippocampal slices in the present study were taken randomly from each mouse brain and the numerous samples (each group n = 40) were used to statistically analyze.

2.5. Observation in synaptic ultrastructural morphology

We observed hippocampal CA1 synaptic ultrastructural morphology using the mice which had finished Y-maze learning task. In brief, five mice were taken randomly from each group. Mice were anesthetized by intraperitoneal injection pentobarbital sodium (50 mg/kg), which was followed by perfusion and fixation with 4% paraformaldehyde + 1% glutaraldehyde via the carotid artery. Then, brains were removed, embedded in EPON812, sectioned into 70–80 nm per micrograph with LKB ultramicrotome (LKB Corporation, Switzerland), and stained with uranyl acetate followed by lead citrate, then observed and photographed using a Hitachi H-7500 microscope. Six sheets of each sample were selected randomly from electron micrographs. Main parameters of Gray type I synapses, including surface density ($\mu m^2/\mu m^3$), synaptic density ($N/\mu m^3$), thickness of postsynaptic density (PSD) (μm), length of the active zones (μm), curvature of the synaptic interface and ratio of perforated synapses, were measured and examined as described previously [21,22].

2.6. Statistical analysis

With application of SPSS 13.0 software (SPSS Inc, Chicago, IL, USA), passive avoidance result was analyzed using rank sum test, and ratio of perforated synapses expressed as percentage was evaluated by chi-square test. Other data, expressed as mean ± SD, was statistically analyzed using two-way ANOVA with group and time as factors. Differences between the groups were considered to be statistically significant when p value < 0.05.