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Research Paper

ERα and/or ERβ activation ameliorates cognitive impairment, neurogenesis and apoptosis in type 2 diabetes mellitus mice

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ABSTRACT

Estrogen receptors (ERs) are thought to be associated with the onset and progression of neurodegenerative injuries and diseases, but the relationship and mechanisms underlying between ERs and cognition in type 2 diabetes remain elusive. In the current study, we investigated the effects of ERα and ERβ on the cognition, neurogenesis and apoptosis in high-fat diet and streptozocin-induced diabetic mice. We found that ERα and/or ERβ activation using their agonists (0.5 mg/kg E2, PPT or DPN) ameliorate memory impairment in the Morris water maze and Y-maze tests, increase hippocampal neurogenesis and prevent hippocampal apoptotic responses. Importantly, treatment with the pharmacologic ERs agonists caused significant increases in the membrane ERα and ERβ expression and subsequent PI3K/Akt, CREB and BDNF activation in the hippocampus of type 2 diabetes mellitus mice. Our data indicate that ERα and ERβ are involved in the cognitive impairment in type 2 diabetes, and that activated ERs, such as application of ERs agonists, could be a novel and promising strategy for the treatment of diabetic cognitive impairment.

1. Introduction

Type 2 diabetes mellitus (T2DM) is associated with cognitive decrements and an increased risk to develop dementia (Pasquier et al., 2006; Spauwen et al., 2013); these will become a major worldwide clinical problem in the future. In several recent clinical and animal experimental studies, older women or female mice with T2DM and obesity have a higher frequency of cognitive decline compared with the men or male mice of the same age (Gregg et al., 2000; Sakata et al., 2010; Yaffe et al., 2004). Especially, epidemiological studies have shown increased risk of cognitive impairment with the age-related loss of sex steroid hormones, while cognitive impairment is more prevalent in postmenopausal women than in age-matched men (Vina and Lloret, 2010). The sharp decline of estrogens after menopause has been presumed to account for the increased female susceptibility to cognitive impairment in T2DM.

Estrogens are the primary female sex hormones and involved in female sexual development and maintenance of normal reproductive functions. They also play very important roles in the immune system as well as in the central nervous system (CNS) in human body (Warner and Gustafsson, 2015). A lot of evidence has documented profound effects of estrogens on learning, memory and neurodevelopmental processes (Brann et al., 2007; Craig et al., 2008; Craig and Murphy, 2007a, 2007b). Animal studies have shown that endogenous estrogen levels changed by reproductive experience in females are associated with enhanced hippocampus-dependent memory (Li et al., 2013). Furthermore, women who underwent surgical menopause or had menopause before 47 years old without hormone treatments had an increased risk for global cognitive impairment and dementia in later life, suggesting that earlier menopause is associated with a higher risk for cognitive impairment (Hogervorst, 2013). Moreover, it has reported that 17β-estradiol the most potent estrogen increased neurogenesis in various brain regions such as dentate gyrus of hippocampus, and decrease both brain inflammation and the activation of apoptosis, these effects in the brain contribute to region-specific learning and memory (Gatson et al., 2009; McClure et al., 2013). Usually, the beneficial effects of estrogens on memory are likely mediated through classical estrogen receptors (ERs), designated as ERα and ERβ (Gronemeyer et al., 2004). In more recent years, the focuses on ERs have intensified, because its novel pathophysiological role has emerged in the CNS disorders including spinal cord injury, multiple sclerosis, Parkinson's disease, and Alzheimer's disease (Chakrabarti et al., 2014). It has been shown that ER agonists possess neuroprotective effects in enhancing memory and cognition and ameliorating neurodegenerative diseases, it not only provide neuroprotection by inhibition of microglia activation, but also modulation of cell survival mechanisms, synaptic reorganization,

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regenerative responses to axonal injury, and neurogenesis process. These effects of ER agonists might be a useful therapeutic option for delaying the onset or progression of neurodegenerative injuries and diseases. However, very few reports on the association of ERs with cognition in type 2 diabetes are found yet. In this study, we investigated the relationship between ERs and cognition in type 2 diabetes, and then explored the possible mechanisms of ERs in memory impairment, neurogenesis and apoptosis.

2. Materials and methods

2.1. Animals and reagents

Female ICR mice (Yangzhou University Medical Center, China), weighing 18–22 g (6–8 weeks old) were used for the experiments. Mice were housed in a constant room with maintained temperature (22 ± 2 °C), humidity (55 ± 5%), and lighting (12-h light/dark cycle) and allowed access to water and food freely. All experiments were approved by the Institutional Review Committee for the use of Animal Subjects of China Pharmaceutical University and experimental procedures are subjected to the guidelines of the institutional animal care and use committee of China.

17β-Estradiol (E2, Cat.No.E2758) and streptozocin (STZ, Cat.No.S0130) were purchased from Sigma Aldrich (St. Louis, MO, USA). ERα selective agonist 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT, Cat.No.1426) and ERβ selective agonist 2,3-Bis (4-hydroxyphenyl)-propionitrile (DPN, Cat.No.1494) were purchased from Tocris Bioscience (Ellisville, MO, USA). High-fat diet (HFD) was purchased from Medical Center of Yangzhou University (Yangzhou, China), consisted of 13% lard, 2% sesame, 20% sugar, 3% cholesterol, 0.1% sodium cholate, 5% peanut. Other reagents have been described in the methods.

2.2. Animal model and treatment

The mice were randomly divided into seven groups: (1) sham operated control mice (Veh + Veh), (2) STZ and high-fat diet induced type 2 diabetes mellitus mice (DM + Veh), (3) ovariectomized mice (OVX + Veh), (4) DM mice with OVX operation (DM&OVX + Veh), (5) DM mice with OVX operation and E2 treatment (DM&OVX + E2), (6) DM mice with OVX operation and PPT treatment (DM&OVX + PPT), (7) DM mice with OVX operation and DPN treatment (DM&OVX + DPN). The mice were anesthetized and then carried out bilateral ovariectomy surgery except the group (1) and (2) mice with sham operation. One week later, the mice except the group (1) and (3) were fed with HFD for 4 weeks and then injected once with low dose of STZ (in the tail vein at 100 mg/kg body weight) to induce partial insulin deficiency, followed by continued HFD feeding for an additional 4 weeks, and then the mice presence of hyperglycemia (> 11.0 mmol/L) were used for the next experiments (Jiang et al., 2012).

Mice in group (5) (6) and (7) were injected subcutaneously with E2 (0.5 mg/kg), PPT (0.5 mg/kg) and DPN (0.5 mg/kg) respectively, and other groups were injected vehicle (bean oil) every other day for 4 weeks. The dose used was as previously reports (Bliedtner et al., 2010; Mancuso et al., 2011; Sakata et al., 2010). After treatment, one part of mice was submitted to the behavior tests, a further part was tested for hippocampal neurogenesis and neural differentiation, a third part was used to evaluate the fasting blood glucose and insulin serum levels, and the fourth part was used for assays of ERα, ERβ, caspase-3, Bcl-2, Bax, PI3K, p-PI3K, Akt, p-Akt, CREB, p-CREB and BDNF.

2.3. Morris water maze (MWM) task

Spatial learning and memory was assessed by the MWM test (Tang et al., 2013). The test consisted of 5 d training (visible and invisible platform training sessions) and a probe trial on day 6. Mice were individually trained in a circular pool (diameter 120 cm, height 50 cm) containing 30 cm-high water maintained at 25 °C. A platform (9 cm diameter) was placed in the centre of one quadrant of the pool and its position was fixed throughout the training sessions. Each mouse was individually trained in both visible-platform (days 1–2) and hidden-platform (days 3–5) versions. Visible-platform training was performed for baseline differences in vision and motivation; the platform was placed 1 cm below the surface of the water and was indicated by a small flag (5 cm in height). The hidden-platform version (the flag was removed) was used to evaluate spatial learning and determine the retention of memory to find the platform. On each day, the mice were subjected to four trials with a 2 h interval between trials. Each trial lasted for 90 s unless the mice reached the platform first. The time (escape latency) that elapsed until the mouse reaches the platform was noted. If an animal failed to find the platform within 90 s, the test was ended and the animal was gently navigated to the platform by hand for 30 s. In the probe trial (day 6), the platform was removed and the mice had 90 s to search for the previous platform. The time spent in the target quadrant (i.e. the quadrant where the platform was previously located) and the number of platform location crossings was recorded. Data of the escape latency, the time spent in the target quadrant, the number of platform location crossings and swim speed were collected by the video tracking equipment and processed by a computer equipped with an analysis-management system (Viewer 2 Tracking Software; Ji Liang Instruments, China).

2.4. Y-maze test

This was performed as descried previously (Tang et al., 2013). The Y-maze was constructed of black plastic walls (height 10 cm), consisting of three compartments (10 × 10 cm) connected with passages (4 × 5 cm), with the floor of 3.175 mm stainless steel rods (8 mm apart). The test was conducted for two consecutive days. On day 1 (learning trial), each mouse was placed in one of the compartments and allowed to move freely for 5 min (habituation) before moving to the next session with electric power on. During the training, electric shocks (2 Hz, 125 ms, 10 V) were available through the stainless steel grid floor in two of the compartments and the light was on in the shock-free compartment. Each mouse was trained 10 times. The training was stopped once the mouse entered the shock-free compartment and stayed for 30 s, which was recorded as a correct choice. If the mouse did not enter this compartment, it was gently navigated to the compartment and allowed to stay for 30 s. On day 2 (testing trial), each mouse was also tested 10 times following the same procedures as on day 1. The numbers of correct choices during the 10 trials and the latency to enter the shock-free compartment on day 2 were recorded manually.

2.5. Open-field test

The test was used to evaluate the general locomotor activity of the mice associated with each treatment and performed as descried previously (Chen et al., 2016). The open-field chamber was made of Plexiglas, with a black-painted floor (50 × 50 cm) and transparent walls (height 40 cm). The experiments were carried out in a sound-attenuated room under low-intensity light (7 lx). At the beginning, the mice were gently placed at the center of the open field and allowed to explore for 5 min. The total distance traveled (m) was recorded in the 5 min period by the ANY Maze® video tracking. After each trial the chamber was cleaned with ethanol solution (10% v/v) and dried with paper towels in order to avoid odor impregnation.

2.6. BrdU administration and immunofluorescence

For analyzing hippocampal neurogenesis and neural differentiation, mice were injected intraperitoneally with 50 mg/kg of bromodeoxyuridine (BrdU, Cat.No.B5002, Sigma-Aldrich) thrice, at 2 h interval. BrdU