Chinese Journal of Pharmacology and Toxicology 2002 Aug; **16**(4):246 – 249

Effect of 17β-estradiol on the expression of brain-derived neurotrophic factor and neurotrophin-3 in the hippocampus of ovariectomized mice

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Abstract: AIM To confirm the possible neurotrophomodulatory effect of estrogens. **METHODS** Western blot analysis was employed to determine the levels of brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3). **RESULTS** There was a significant reduction of BDNF expression in the hippocampus of ovariectomized mice compared with that of sham-operated mice ($P < 0.01$). Replacement of 17β-estradiol (2.4 or 4.8 $\mu g \cdot d^{-1}$, sc, for 12 weeks) restored BDNF level in the hippocampus. However, both ovariectomy and estradiol replacement had no effect on NT-3 expression in the hippocampus. **CONCLUSION** The decrease in the expression of BDNF in the hippocampus of ovariectomized mice is closely associated with estrogen deficiency. Estrogen plays an important role in modulating BDNF level in the hippocampus.

Key words: ovariectomy; estradiol; brain-derived neurotrophic factor; neurotrophin 3

CLC number: R977.12
Document code: A
Article ID: 1000-3002(2002)04-0246-04

Neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5, play important roles in the development, differentiation, long-term survival and synaptic activity of neurons[1]. Hippocampal neurons have been known to be rich in BDNF and NT-3[2]. It was found that BDNF was significantly reduced in the hippocampus of patients with Alzheimer's disease[3], suggesting that BDNF is involved in the pathogenesis of the brain or learning and memory deficiency in Alzheimer's disease.

Recent evidence supported a role for estrogen in both normal neural development and neuronal maintenance throughout life. Women spent 25% – 33% of their life in an estrogen-deprived state and retrospective studies have shown an inverse correlation between dose and duration of estrogen replacement therapy and incidence of Alzheimer's disease, indicating that estrogen possessed potential effect in the prevention and treatment of neurodegenerative disease. In recent years, an animal model using ovariectomy (OVX) and OVX with estradiol replacement was developed to mimic postmenopausal changes. OVX in rats resulted in estrogen-reversible impairment of learning and memory behavior[4], which has also been confirmed in mice by our work (not published). In this study, we observed the possible neurotrophomodulatory effect of estrogen by quantifying the effect of 17β-estradiol on the expressions of BDNF and NT-3 in the hippocampus using Western blotting analysis.

1 MATERIALS AND METHODS

1.1 Animals

Swiss female mice, 6 weeks old, 20 – 24 g, were obtained from the Animal Center of Academy of Military Medical Sciences and maintained on a 12 h light/12 h dark cycle with food and water *ad libitum*.

1.2 Ovariectomy

Mice were anesthetized (pentobarbital sodium, 40 $mg \cdot kg^{-1}$, ip) until a toe pinch failed to

Received date: 2002-01-28 **Accepted data**: 2002-04-02
Foundation item: The project supported by National Project of 973 (G1999054401); and Army Elite Foundation
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elicit a reflex reaction. The skin and muscle were incised. The bilateral ovaries were removed and then, the muscle and overlaying skin were sutured using silk. A group of 4 mice had sham OVX (Sham) following the same-procedure as OVX except the ovaries were not removed. One week after OVX, OVX mice were given 17β-estradiol (2.4 or 4.8 $\mu g \cdot d^{-1}$)[5] or placebo (0.9% saline containing less than 3% ethanol) by subcutaneous injection for 12 consecutive weeks.

1.3 Western blot analysis

Hippocampi were dissected, frozen in liquid nitrogen and then cut into very small pieces after weighing. Frozen tissue can be sliced very thinly and thawed in lysis buffer. Hippocampi were further disrupted and homogenized. All the procedures were performed at 4℃. Proteinase inhibitors (100 $mg \cdot L^{-1}$ phenylmethylsulfonyl fluoride, 1 $mg \cdot L^{-1}$ aprotinin) were added and incubated at 4℃ for 30 min, transferred to microcentrifuge tubes and centrifuged at 10 000 × *g* for 15 min at 4℃. The supernatant was removed and it is the total cell lysis. Protein concentration of the samples was determined by Lowry, *et al* assay.

Aliquots of protein sample (50 μg) were solubilized with SDS-polyacrylamide gel electrophoresis sample buffer and electrophoresed through a 15% SDS gel. The resulting protein bands were transferred to nitrocellular membranes using an electroblotting apparatus. Staining procedure as follows: the membranes in Blotto for 1 h at room temperature and overnight at 4℃ and then incubated with rabbit-anti-BDNF or NT-3 polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) of 1 h at room temperature. After rinsing three times for 15 min each with tris-buffered saline(TBS) and 0.05% Tween-20, membranes were incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase at a dilution of 1∶1000 for 1 h at room temperature. Non-specific binding was blocked by incubating membrane in Blotto (1 × TBS, 5% milk, 0.05% Tween-20) for 1 h at room temperature. Subsequently, specific protein bands were detected by chemiluminescence system. Band intensities were quantified by image analysis.

1.4 Statistical analysis

Results were presented as $\bar{x} \pm s$. Statistical analysis was performed with *t* test.

2 RESULTS

2.1 Effect of 17β-estradiol on the expression of brain-derived neurotrophic factor in the hippocampus of ovariectomized mice

Ten weeks after OVX in mice, a significant reduction in BDNF expression in the hippocampus was observed. Treatment with 17β-estradiol (2.4 or 4.8 $\mu g \cdot d^{-1}$, sc, for 12 weeks) restored the expression of BDNF in the hippocampus of OVX mice (Fig 1).

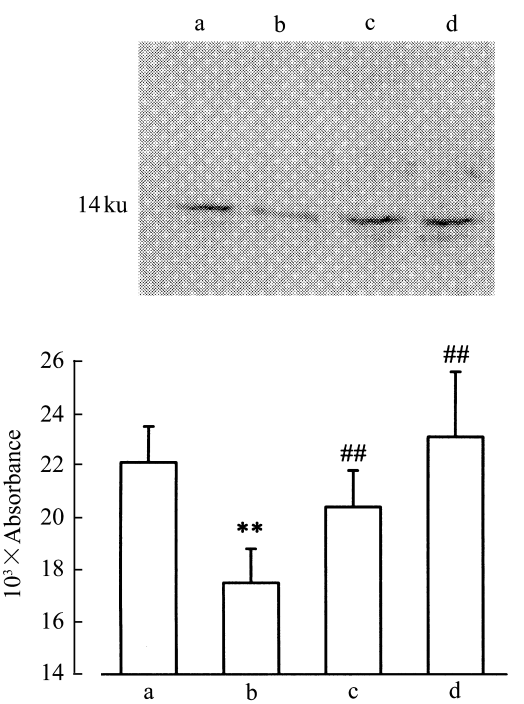

Fig 1. Effect of 17β-estadiol on the expression of brain-derived neurotrophic factor in the hippocampus of ovariectomized mice. 17β-Estradiol or placebo (0.9% saline containing less than 3% ethanol) was given one week after ovariectomy by subcutaneous injection for 12 consecutive weeks. a: Ovary intact mice; b, c, d: ovariectomized mice with placebo or different doses of 17β-estradiol (2.4 or 4.8 $\mu g \cdot d^{-1}$), respectively. $\bar{x} \pm s$, $n = 3$. $^{**}P < 0.01$, compared with (a) group; $^{\#\#}P < 0.01$, compared with (b) group.

2.2 Effect of 17β-estradiol on the expression of neurotrophin-3 in the hippocampus of ovariectomized mice

There was no significant change in hippocampal NT-3 expression 10 weeks after OVX, replacement of 17β-estradiol (2.4 or 4.8 $\mu g \cdot d^{-1}$, sc, for 12 weeks) also showed no effect on the expression of NT-3 in the hippocampus (Fig 2).

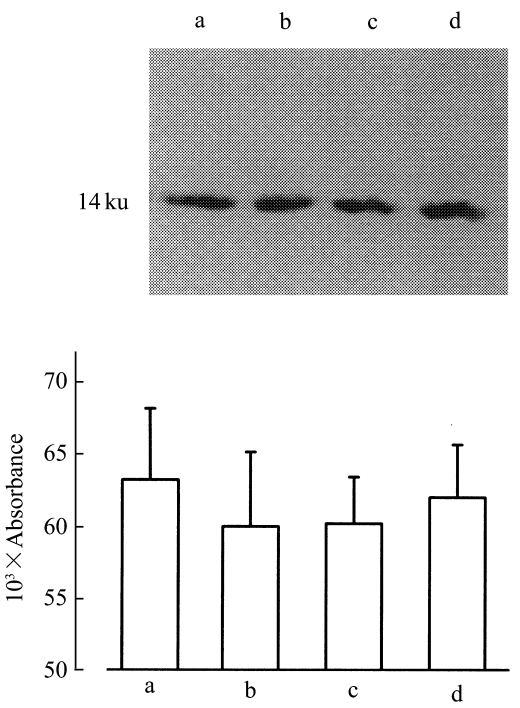

Fig 2. Effect of 17β-estradiol on the expression of neurotrophin-3 in the hippocampus of ovariectomized mice. See Fig 1 for the treatments. $\bar{x} \pm s$, $n = 3$.

3 DISCUSSION

It has been shown that the lack of neurotrophic support may be contributed to neurone-generative changes in Alzheimer's disease. Recent studies suggested that estrogen replacement therapy help to reduce the risk and severity of Alzheimer's disease in postmenopausal women. It is hypothesized that estrogen may enhance the hippocampal and cerebral cortical functions by influencing the expression of specific neurotrophins and neurotrophin receptors[6]. Evidence for the relationship between estrogen and neurotrophins came from the early studies of Sohrabji, *et al*[7, 8] who showed that estrogen increased the expressions of NGF and its receptor trkA mRNA. In addition, estrogen replacement reduced the expression of p75 mRNA in the brain. Gibbs[9] found that the levels of BDNF and trkA mRNA in the hippocampus significantly fluctuates across the estrous cycle by using quantitative *in situ* hybridization technique, the level of BDNF mRNA is the highest in the proestrus. In the acute ovariectomized rats, BDNF mRNA was also up-regulated in the cerebral cortex and olfactory bulb exposed to estrogen[10, 11]. Kaisho, *et al*[12] considered that the impairment of learning and memory in senescence accelerated mouse-prone/8 (SAMP8) was related to the changes in NT-3 level. However, the expressions of BDNF and NT-3 proteins were still not studied in the chronically ovariectomized mice. In this study, the expressions of BDNF and NT-3 proteins were detected by using Western blotting analysis. A significant reduction in BDNF expression in the hippocampus of ovariectomized mice was observed and 17β-estradiol replacement showed significant effect in normalizing BDNF level. However, both OVX and estrogen replacement had no effect on NT-3 level in the hippocampus. These results suggest that the estrogen deficiency mainly affect hippocampal BDNF level. Sohrabji, *et al*[13] reported that estrogen-target neurons widely coexpressed the mRNA for the neurotrophin ligands and their receptors. The gene encoding the neurotrophin-BDNF contains a sequence similar to the canonical estrogen response element found in estrogen-target genes. Gel shift and DNA footprinting assays indicate that estrogen receptor-ligand complexes bind to this sequence in the BDNF gene.

Above data show that estrogen regulated the expression of BDNF protein in the hippocampus of chronically ovariectomized mice, suggesting that estrogen influence neurotrophin-mediated neuronal function by increasing the availability of specific neurotrophins in the brain. Further study is being

conducted in our laboratory.

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17β-雌二醇对卵巢切除小鼠海马内脑源性神经营养因子及神经营养因子 3 表达的影响

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摘要：目的　为确定雌激素可能具有的神经营养调节作用。**方法**　采用蛋白质印迹法检测上述指标的变化。**结果**　与卵巢未切除对照组相比，小鼠卵巢切除 10 周后海马内脑源性神经营养因子（BDNF）表达水平明显下降，给予 17β-雌二醇（2.4 或 4.8 $\mu g \cdot d^{-1}$，sc，连续 12 周）替代具有明显的改善作用（$P < 0.01$）。但卵巢切除及 17β-雌二醇替代对海马内神经营养因子 3 表达水平无明显影响。**结论**　海马内 BDNF 表达水平的改变与雌激素缺乏具有密切关系。雌激素对调节海马内 BDNF 水平具有重要作用。

关键词：卵巢切除术；雌二醇；脑源性神经营养因子；神经营养因子 3

基金项目：国家重点基础研究发展规划项目资助课题（G1999054401）；军队杰出人才基金项目资助课题

（本文编辑　乔　虹）