Effects of synaptic plasticity regulated by 17β -estradiol on learning and memory in rats with Alzheimer's disease

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Abstract: Objective To investigate whether estrogen modulates learning and memory and long-term potentiation (LTP) in the hippocampus of rats with Alzheimer's disease (AD). **Methods** The rats were divided into ovariectomy (OVX) and estrogen replacement therapy (ERT) groups. Rats in the ERT group received OVX, followed by ERT, while rats in the OVX group received only OVX. The rat model of AD was established by injection of 1 μ L (10 μ g/ μ L) amyloid-beta peptide 1-40 ($A\beta_{1-40}$) into the hippocampus. The learning and memory ability and LTP were determined by Morris water maze and electrophysiological method, respectively. **Results** The escape latency in Morris water maze significantly decreased in ERT group compared with that in OVX group ($P < 0.05$). Besides, rats in ERT group exhibited a significant enhancement of the magnitude of LTP at 30 min after high-frequency stimulation (HFS), compared with that in OVX group ($P < 0.01$). **Conclusion** ERT can attenuate the cognitive deficits in the rat model of AD, and estrogen can regulate LTP and synaptic remodeling in AD rats.

Keywords: estradiol; long-term potentiation; learning and memory; rat; Alzheimer's disease

1 Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. The incidence of AD increases in postmenopausal women, which might be a consequence of the plummeting estrogen level^[1]. Both *in vitro* and *in vivo* studies have revealed a significantly higher incidence of AD in postmenopausal women and the neuroprotective effects of estrogen, leading to an increase of interest in their role in cognitive functioning or dementia^[1]. In fact, accumulating experimental data support a positive role of estrogen, notably on neuronal functioning and neurotransmitter systems,

possibly through the interactions with neurotrophic factors^[1,2]. Estrogen can inhibit the accumulation of neurotoxic glutamate or amyloid-beta peptide ($A\beta$), and possesses an antioxidant property. Estrogen also inhibits neuronal apoptosis and modulates apolipoprotein E gene expression^[2]. Based on these activities, estrogen can protect against cognitive dysfunction of AD. According to the experimental studies and observations, there has been a growing belief since the past half-century that replacement of the lost estrogen at menopause would prevent many of the aging manifestations, including osteoporosis, cardiovascular disease, and declines in sexual and cognitive functions. This attractive view initially leads to the prevalence of estrogen replacement therapy (ERT) after menopause. Epidemiological studies have indicated that ERT is associated with reduced risks of AD and cognitive decline in postmenopausal women^[1,2]. Unfortunately, results of the recent Women's Health Initiative (WHI)

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Article ID: 1673-7067(2010)02-0133-07

Received date: 2009-09-13; Accepted date: 2009-12-15

study diminish this enthusiasm, leading to new clinical recommendation. The epidemiological and clinical studies on the effectiveness of ERT in reducing the risk of AD or alleviating AD symptoms are hampered by many complex factors^[3]. Moreover, ERT have potential risks for breast cancer, pulmonary embolism, stroke and possibly coronary heart disease. In fact, these harmful effects are dependent on the age and health status of women as well as on the formulation of used hormones. However, the animal model has become a valuable tool to control over confounding factors, thus being more sensitive to reveal the possible effects of ERT on cognitive functions. Here the effects of 17 β -estradiol treatment on the behavior in Morris water maze test and long-term potentiation (LTP) in rats with A β -induced AD are investigated, to test the hypothesis that estrogen enhances learning and memory functions.

2 Materials and methods

2.1 Animals A total number of 30 sexually naive Sprague-Dawley female rats (weighting 200-250 g, supplied by Animal Center of China Medical University) were employed in the present study. The rats were randomly divided into ovariectomy (OVX) group and ERT group ($n=15$ in each group).

2.2 OVX OVX was performed^[4] in both groups, and rats in ERT group were then subcutaneously implanted with estrogen pellets, which contained 0.72 mg 17 β -estradiol and could continuously supply estrogen for 60 d^[5]. In the OVX group, the rats were subcutaneously implanted with placebo pellets as a control. The 17 β -estradiol and placebo pellets were both purchased from Innovative Research of America.

2.3 Establishment of AD model As described previously^[6,7], 2 weeks after OVX, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate and placed in a stereotaxic instrument (model Kopf 938, USA). A hole was drilled on the skull approximately 3.3 mm after the bregma, 1.5 mm lateral to the bregma, and 4.5 mm in depth. Then 1 μ L (10 μ g/ μ L) A β was injected into the brain using micro sample-adding apparatus at a velocity of 5 μ L/h. The apparatus was kept still for half an hour to ensure the adequate local infiltration.

2.4 Morris water maze The behavioral test was conducted 4 weeks after the injection of A β in the 2 groups^[8]. The training

schedule consisted of 7 consecutive days of testing. A plastic circular pool (120 cm in diameter and 120 cm in height), and a plastic square platform (14 $\times$ 14 cm², 1 cm below the water line) were used. The temperature of the water was maintained at (20.0 $\pm$ 0.5) $^{\circ}$ C throughout the experiment. The platform was located in one quadrant, at the middle point between the pool wall and the center. The platform location was kept constant during the period of training. The starting locations were labeled North, South, East and West, and located randomly on the pool rim. The rat was placed at one of the starting points with its nose pointing toward the wall. If the rat failed to find the platform within the maximum time, it was manually guided to the platform by the experimenter, and stayed there for 10 s. During the platform training, the latency to amount the escape platform (the duration from the entry into the water to the crawling onto the platform) was recorded and indicated as the escape latency. Both hidden and visible platform escape latencies were measured.

2.5 LTP recording LTP recording was performed 5 weeks after A β injection, and then the rats were sacrificed. As described previously^[6], field-excitatory postsynaptic potentials (fEPSPs) were recorded for 40 min from the apical dendrite layer of CA1 region by using glass microelectrodes filled with 2 mol/L NaCl. fEPSPs were evoked by applying single-test pulses (0.05 Hz, 100 μ s) at voltage intensities sufficient to evoke 50% of the maximal fEPSP. LTP was induced by a brief period of high-frequency stimulation (HFS, 5 trains of 20 pulses at 100 Hz) after 40-min pre-HFS induction at the pre-HFS stimulation rate and intensity. Electrophysiological recordings were collected, digitized, and stored in a computer using AXOBASIC software.

2.6 Weighting uterine tissues and analyzing hormonal content The rats were sacrificed by decapitation after LTP recording. To confirm the success of OVX and 17 β -estradiol treatment, the trunk blood was collected for hormonal content analysis through radioimmunoassay (RIA), and the uterine tissues were weighted at the time of killing.

2.7 Statistical analysis Data were analyzed by using SPSS1 1.5 software package (SPSS, Inc, Chicago, IL, USA), and expressed as mean $\pm$ SD.

3 Results

3.1 Uterine weight The effects of OVX and ERT were confirmed by measuring the fresh weight of uterine right after decapitation. The uterine in ERT group showed turgor vitalis, and its surface was smooth and glossy, while the uterine in OVX group showed atrophy and its size was smaller (Fig. 1). As shown in Table 1, uterine weight in ERT group was significantly increased, compared to that in OVX group ($P < 0.001$), which confirmed the success of OVX and ERT.

3.2 Serum estrogen level As shown in Table 1, the serum estrogen level was significantly higher in ERT group than in OVX group ($P < 0.001$), which again confirmed the success of model establishment.

3.3 Morris water maze The escape latencies in both visible and hidden platform tasks were shorter in ERT group than in OVX group ($P < 0.05$), indicating that the ability of learning

Fig. 1 Representative images of fresh uterine in ERT group (A) and OVX group (B). The uterine in ERT group was smooth and glossy, showing turgor vitalis, while the uterine in OVX group was small and showed atrophy.

and memory in AD rats could be improved by estrogen implantation. However, there was no significant difference in the time spent in the target quadrant in the probe trial between ERT group and OVX group ($P > 0.05$).

3.4 LTP Extracellular fEPSPs were recorded in CA1 region for 40 min before application of HFS to the Schaffer collateral

Fig. 2 Changes in LTP in CA1 region in rat hippocampus. A: fEPSPs recorded at the last 10-min baseline period in ERT group; B: fEPSPs recorded at 30 min after HFS in ERT group; C: fEPSPs recorded at the last 10-min baseline period in OVX group; D: fEPSPs recorded at 30 min after HFS in OVX group.

Table 1. Fresh weight of uterine, serum estrogen level, and escape latency in Morris water maze in ERT and OVX groups (mean $\pm$ SD)

Variable	ERT group	OVX group	<i>t</i> value (<i>P</i> value)
Fresh weight of uterine (g)	0.35 $\pm$ 0.03	0.12 $\pm$ 0.02	20.17(1×10^{-18})
Serum estrogen level (pg/mL)	52.61 $\pm$ 7.34	31.89 $\pm$ 4.15	7.77(1×10^{-8})
Escape latency in the hidden platform trial (s)	44.65 $\pm$ 11.7	56.98 $\pm$ 15.42	2.47(0.020)
Escape latency in the visible platform trial (s)	7.59 $\pm$ 2.10	10.06 $\pm$ 2.71	2.28(0.032)
Time spent in the target quadrant in the probe trial (s)	48.27 $\pm$ 4.37	50.02 $\pm$ 3.11	1.264(0.218)

Fig. 3 The fEPSPs amplitude in the 2 groups. a: EPSP amplitude (%) recorded at the last 10-min baseline period in the 2 groups (baseline); b: EPSP amplitude (%) recorded at 30 min after HFS in ERT group; c: EPSP amplitude (%) recorded at 30 min after HFS in OVX group. * $P < 0.05$ vs baseline.

Fig. 4 The change rate of PS amplitude with application time after HFS in ERT and OVX groups. The slope of fEPSP alteration between pre- and post-HFS was extremely lower in OVX group than that in ERT group ($P < 0.05$).

pathway. Posttetanic potentiation (PTP) was recorded immediately after HFS and it gradually declined to a stable potentiation. A significant difference was observed in the magnitude of LTP between the 2 groups (Fig. 2), which indicated that the amplitude of fEPSPs was dramatically stronger in ERT group than in OVX group after HFS (Fig. 3). Besides, the change rate of population spikes (PS) magnitude with application time was higher in ERT rats than in OVX rats after HFS (Fig. 4).

4 Discussion

The present study investigated the effect of estrogen on brain cognitive functions in AD rats. Behavioral testing

revealed that the escape latency was significantly shortened in ERT group, indicating that estrogen could attenuate A β -induced impairment in learning and memory, especially the hippocampal functions of AD rats. Here the water maze result is consistent with the previous study^[9].

Besides, we observed that the magnitude of LTP in CA1 region of hippocampus was significantly increased in ERT group compared to that in OVX group. These results suggest that the estrogen treatment could improve the physiological processes involved in the formation of episodic memories, and regulate LTP and synaptic remodeling in AD rats.

The present results clearly show that estrogen is a major regulator of memory in the brain of AD rats. Firstly, OVX reduced the circulating estrogen level. Secondly, this effect was completely reversed by estrogen pellet implantation. Finally, estrogen shortened the escape latency in Morris water maze test and enhanced LTP. Our results give a hint that estrogen produces various effects on synaptic plasticity in brain, particularly on synaptic connectivity in CA1 region of hippocampus in AD model. Estrogen could also improve cognitive functions in AD rats.

However, studies of the changes in learning and memory processes by estrogen in female are not fully consistent, sometimes even opposite. Many studies have indicated that estrogen affects hippocampus-dependent learning and memory^[10,11]. It exerts beneficial effects on working memory

in humans^[12] and on spatial memory^[13] or delayed matching-to-place^[14] in rats. In the Cache County, women with hormone use at any age exhibit slower rates of cognitive decline, particularly the women aged 85 years or older^[15]. Meta analyses further suggest that hormone use induces a decrease in the rate of cognitive decline by 1/3^[16,17]. Moreover, long-term use of hormone induces a greater reduction in AD risk than short-term use^[1]. Recent studies have indicated an association between ERT and a decrease in the risk of cognitive impairment or AD^[2,18,19]. Our results, consistent with this finding, show that estrogen could shorten the escape latency in Morris water maze test and induce an increase in magnitude of LTP in hippocampus^[20]. The electrophysiological explanations for these effects are further investigated here. However, some scholars do not support the idea that estrogen treatment decreases the risk or alleviates the symptoms of AD. They propose that long-term treatment with estrogen cannot inhibit or decrease hippocampal A β accumulation in the hippocampus of AD female mice^[21]. The largest trial concerning this proposition involved 180 healthy postmenopausal women aged 45-55 years with cognitive symptoms. They were randomized into placebo and conjugated equine estrogens and medroxyprogesterone acetate groups. Results show that there was no significant difference in memory or other cognitive functions between the 2 groups^[22]. Besides, results of observational research are not fully consistent in the putative effects of hormone therapy on cognition in postmenopausal women^[23].

The interpretation and clinical relevance of these reported associations are challenged by seemingly discrepant findings from the Women's Health Initiative Memory Study (WHIMS) trials^[24,25]. Several factors may explain the discrepancies among the studies. Based on the present and the previous studies, it can be found that long-term estrogen treatment may improve the ability of learning and memory and increase A β production at early stage of amyloid pathology. However, it has no effect on A β aggregation, rapid accumulation, or deposition at late stages^[21]. In fact, one key limitation of the previous studies is probably the focus on late-onset hormonal changes, at a time when neurodegeneration has already begun, without taking into account the lifetime

exposure to hormonal variability^[1,26]. Thus, ERT is likely to be ineffective in already diagnosed AD patients, failing to show any cognitive improvement in female AD patients after prolonged ERT^[27], which seems to be inconsistent with recent placebo-controlled clinical trials. On the other hand, differences in methodology regarding the type, dosage, timing, and duration of ERT, as well as the population group, the cognitive measures assessed, and the employed analysis method, could greatly lead to these discrepancies across the findings^[26]. Although estrogen replacement may not slow down the amyloid-induced neuronal dysfunction, it still has beneficial effects on cognitive performance in AD patients, at least preventing mild cognitive impairment caused by sudden drop down of estrogen levels after menopause.

In conclusion, our results show that estrogen is possibly a part of regulation in synaptic remodeling. However, the potential effects may involve different mechanisms. The results concerning the potential value of ERT in reducing age-related cognitive impairment and delaying the onset of dementia should be considered carefully, especially when comparing the findings from clinical trials with those from fundamental research or epidemiologic studies.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (No. 30700938, 30872656, 30700861, 30800451) and the Natural Science Foundation of Liaoning Province, China (No. 20082078).

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雌二醇对阿尔茨海默病大鼠学习记忆能力及长时程增强的影响

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摘要: **目的** 探讨雌二醇对阿尔茨海默病(Alzheimer's disease, AD)大鼠的学习记忆能力及长时程增强(long-term potentiation, LTP)的影响。**方法** 大鼠随机分成卵巢切除 (ovariectomy, OVX)组和雌激素替代治疗(estrogen replacement treatment, ERT)组。两组大鼠均接受卵巢切除手术, ERT组大鼠随后接受雌激素替代治疗。手术2周后, 两组大鼠海马内立体定位单侧注射 $1\ \mu\text{L}\ A\beta_{1-40}$ ($10\ \mu\text{g}/\mu\text{L}$), 建立AD模型。运用水迷宫实验检测大鼠的学习和记忆能力, 并进行LTP记录和比较。**结果** OVX组AD大鼠的水迷宫逃避潜伏期相较于ERT组明显延长($P < 0.05$)。此外, 高频刺激(high frequency stimulation, HFS) 30 min后与HFS刺激前相比, ERT组LTP增加的幅值明显高于OVX组($P < 0.05$)。**结论** 雌激素替代治疗能改善AD大鼠的认知功能, 调整LTP及突触可塑性。

关键词: 雌二醇; 长时程增强; 学习和记忆; 大鼠; 阿尔茨海默病