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Steroid receptor coactivator-1 mediates letrozole induced downregulation of postsynaptic protein PSD-95 in the hippocampus of adult female rats

Mengying Liu[a,b], Xuhong Huangfu[a,b], Yangang Zhao[a], Dongmei Zhang[a,c,**], Jiqiang Zhang[a,*]

[a] Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, China
[b] Cadet Brigade, Third Military Medical University, Chongqing 400038, China
[c] Department of Dermatology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

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ABSTRACT

Hippocampus local estrogen which is converted from androgen that catalyzed by aromatase has been shown to play important roles in the regulation of learning and memory as well as cognition through action on synaptic plasticity, but the underlying mechanisms are poorly understood. Steroid receptor coactivator-1 (SRC-1) is one of the coactivators of steroid nuclear receptors; it is widely distributed in brain areas that related to learning and memory, reproductive regulation, sensory and motor information integration. Previous studies have revealed high levels of SRC-1 immunoreactivities in the hippocampus; it is closely related to the levels of synaptic proteins such as PSD-95 under normal development or gonadectomy, but its exact roles in the regulation of these proteins remains unclear. In this study, we used aromatase inhibitor letrozole in vivo and SRC-1 RNA interference in vitro to investigate whether SRC-1 mediated endogenous estrogen regulation of hippocampal PSD-95. The results revealed that letrozole injection synchronously decreased hippocampal SRC-1 and PSD-95 in a dose-dependant manner. Furthermore, when SRC-1 specific shRNA pool was applied to block the expression of SRC-1 in the primary hippocampal neuron culture, both immunocytochemistry and Western blot revealed that levels of PSD-95 were also decreased significantly. Taking together, these results provided the first evidence that SRC-1 mediated endogenous estrogen regulation of hippocampal synaptic plasticity by targeting the expression of synaptic protein PSD-95. Additionally, since letrozole is frequently used to treat estrogen-sensitive breast cancer, the above results also indicate its potential side effects in clinical administration.

1. Introduction

Studies have demonstrated that hippocampus is profoundly regulated by estrogens especially 17β-estradiol (E2) from sex gonads (circulating) and/or local synthesis from androgen testosterone that is catalyzed by aromatase [1,2]. In the past decades, it is well established that circulating E2 regulated hippocampal synapse spine density, synapse density and synapse transmission such as long-term potentiation (LTP) [3–5] during the estrous cycle or estrogen replacement after ovariectomy [6,7]. Furthermore, recent studies have also shown that local estrogen derived from androgen in the hippocampus play pivotal roles in the regulation of hippocampal synaptic plasticity. For example, in hippocampal slice cultures, aromatase specific inhibitor letrozole treatment resulted a significant decrease in the density of spine synapses and in the number of presynaptic buttons; it also caused a downregulation of spinophilin (dendritic spines marker) and synaptophysin (a protein of presynaptic vesicles) as well as growth-associated protein 43 [8,9]. Our recent in vivo studies revealed that letrozole injection caused significant decrease of hippocampal androgen receptor, estrogen receptor α (ERα) and ERβ that have been profoundly implicated in the regulation of synaptic plasticity, learning and memory [10].

PSD-95 is one of the prominent protein components of the postsynaptic density fraction of synapse [11,12]. Studies have shown that overexpression of PSD-95 induces the maturation of

* Corresponding author at: Department of Neurobiology, Third Military Medical University, Chongqing 400038, China. Tel.: +86 23 68752223.
** Corresponding author at: Department of Dermatology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China. Tel.: +86 23 68773025.
E-mail addresses:
574235375@qq.com (D. Zhang), zhangjqtmmu@yahoo.com (J. Zhang)

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presynaptic terminal as well as glutamatergic synapses, enhances postsynaptic clustering and activity of excitatory glutamate receptors. Additionally, overexpression of PSD-95 also increases the number and size of dendritic spines and lacking PSD-95 induced neuronal death, indicating its important roles in synaptogenesis, synapse maturation and stabilization as well as plasticity [12–15]. Previous studies have reported that PSD-95 can be regulated by exogenous estrogens pathway. For example, hippocampal PSD-95 synthesis can be induced by estradiol administration [16,17]. It is inhibited by knockout of ERβ [18] but activated by estrogen nuclear receptor agonists [19,20]. Our work also found that expression of hippocampal PSD-95 could be regulated by ovary estrogens in a time-dependent manner [21]. However, whether endogenous local estrogen can regulate PSD-95 and relative mechanisms remains unclear.

Steroid receptor coactivator-1 (SRC-1) is required for the transcriptional activity of the steroid receptors like estrogen receptors (ERα and ERβ), androgen receptor and thyroid receptor [22–25]. Accumutaed studies have revealed that SRC-1 is widely distributed in the brain. For example, in the mice brain high levels of SRC-1 immunoreactivities were detected in olfactory bulb, cerebral cortex, hippocampus, some limited diencephalon and brainstem nuclei in a male-predominant manner [26]. Striking age-related expression profile of SRC-1 was noticed in the learning and memory and motor regulation areas [27]. We also found that SRC-1 is regulated by development in both sexes and by orchidectomy [28,29] but not ovariectomy in the hippocampus of rats [29]. Furthermore, orchidectomy and letrozole treatment induced decrease of brain SRC-1 in a similar manner [10,30].

Developmentally, hippocampal SRC-1 and PSD-95 share similar highly correlated profile, indicating SRC-1 may be deeply involved in the regulation of hippocampal synaptogenesis [29]. Additionally, in the female and male hippocampus, gonadectomy induced alterations of SRC-1 and PSD-95 were positively correlated [31]. Previously we have shown that letrozole induced significant decrease of SRC-1 in the hippocampus of mice, indicating SRC-1 is an important downstream target of endogenous estrogen [10]. However, whether SRC-1 mediated endogenous estrogens regulation of PSD-95 has not been elucidated. In order to further elucidate the mechanisms under local estrogen regulation on hippocampal synaptic plasticity, in this study we investigated the role of SRC-1 in the regulation of hippocampal synaptic protein PSD-95 using aromatase inhibitor letrozole in vivo injection and SRC-1 RNA interference in vitro.

2. Materials and methods

2.1. Animals and letrozole administration

Adult female SPF grade Wistar rats (3 months old, 240 ± 20 g, $n = 5$ in each group) were obtained from the Experimental Animal Center of Third Military Medical University. All the animal-related procedures were conducted in strict compliance with Approved Institutional Animal Care and Use Protocols. The animals were randomly divided into 4 groups. The control animals were injected intraperitoneally with dimethyl sulfoxide (DMSO; D8418, Sigma-Aldrich Shanghai Trading Co Ltd, Shanghai, China), the letrozole animals were intraperitoneally injected letrozole (L6545, Sigma-Aldrich) with a dose of 0.5, 1.0 and 2.0 mg/kg, respectively, dissolved in DMSO. The injection was conducted between 17:00 and 18:00 at every evening and lasted for 1 week.

2.2. Tissue preparation

Tissue preparation was carried out according to our previous report [32]. In brief, after deep anesthesia with 100 mg/kg sodium pentobarbital, animals were perfused with 4% paraformaldehyde in phosphate buffer (PBS, pH 7.2). The brains were carefully dissected, post-fixed overnight in the same fixative then transferred to the fixative containing 30% sucrose until they sank to the bottom of the container. Then they were serially cut frozen into 30 μm-thickness coronal sections with a cryostat (CM1850, Leica Microsystems, Germany). By following the principles of unbiased, systematic random sampling, the serially cut sections were transferred into one of six wells, with every sixth section being placed in the same well.

2.3. Primary hippocampal neuron culture

The embryonic day 18 pregnant rat fetus were used to isolate hippocampal neurons. In brief, the hippocampus was removed and dissociated into a single cell suspension. These cells were plated on collagen and poly-D-lysine (Sigma-Aldrich) coated plates at a density of 10×10^4 cells/cm^2. Cells were maintained in Neurobasal media supplemented with B27 and 0.5 μM L-glutamine (Gibco/Life Technologies, Grand Island, NY). On day 2 post seeding, 1.5 μM AraC (SigmaC1768; Sigma-Aldrich) was added to inhibit the proliferation of glial cells. The culture medium was changed in half every 2–3 days.

2.4. Immunofluorescence

Immunofluorescence was used to identify the cell purity and localization of SRC-1 and PSD-95 in the cultured cells. Briefly, after 7 d culture, coverslips were washed with PBS, incubated with primary antibody SRC-1 (1:100; sc-8995, Santa Cruz Biotech; Dallas, USA), NSE (ZA-0202, Zhongshan Biotech, Beijing, China), GFAP (ZA-0117, Zhongshan Biotech) or PSD-95 (MAB1596; MerkMillipore, Darmstadt, Germany) at 4 °C overnight. After PBS wash the coverslips were incubated with AlexaFluor 488® (A0423, Molecular Probes, Eugene, USA) goat anti-rabbit IgG (for SRC-1) or AlexaFluor 568® (A-11011; Molecular Probes) goat anti-mouse IgG (for PSD-95, NSE and GFAP; 1:600) for 1 h, and counterstained with DAPI (H1200, Molecular Probes) then examined under Olympus microscope (BX60, Olympus, Japan).

2.5. Immunohistochemistry/immunocytochemistry

Nickel-intensified immunostaining was carried out according to our previous description [32] with slight modifications. For tissue sections, free-floating sections were first washed with PBS; for cultured neurons, cells on coverslips were fixed with 4% paraformaldehyde in phosphate buffer (PBS, pH 7.2) for 20 min then washed with PBS. Tissue sections and cultured cells were then quenched for 15 min in 3% H_2O_2 in PBS, blocked in 5% normal goat serum for 30 min at room temperature, incubated over night at 4 °C with the primary rabbit polyclonal antiserum SRC-1 (1:100; sc-8995, Santa Cruz Biotech; Dallas, USA), ERα (1:100; SC-542, Santa Cruz), ERβ (1:100; SC-8974, Santa Cruz), mouse monoclonal PSD-95 (1:100; MAB1596; MerkMillipore, Darmstadt, Germany) or aromatase (1:100; SM2222PS, Acris, San Diego, USA), diluted with Antibody Diluent (S3022, Dako Inc., Glostrup, Denmark), respectively. After several washes with PBS, the sections or cells were incubated with biotinylated secondary goat-anti-rabbit antibody (1:200; ZB2010, Zhongshan Biotech) or goat-anti-mouse antibody (1:200; ZB2020, Zhongshan Biotech) for 1 h at room temperature, respectively. The sections or cells were washed in PBS again, incubated in HRP-labeled streptavidin reagent (1:200; ZB2404, Zhongshan Biotech) for 1 h at room temperature and then visualized using a DAB-nickel chromogen solution for 5 min at room temperature. Finally, the sections or cells were dehydrated, cleared in xylene and mounted with DPX. Blank control was carried out