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Aromatase inhibitor letrozole downregulates steroid receptor coactivator-1 in specific brain regions that primarily related to memory, neuroendocrine and integration

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ABSTRACT

As one of the third generation of aromatase inhibitors, letrozole is a favored drug for the treatment of hormone receptor-positive breast cancer with some adverse effects on the nervous system, but the knowledge is limited and the results are controversial, the mechanism underlying its central action is also unclear. Accumulated evidences have demonstrated that estrogens derived from androgens by aromatase play profound roles in the brain through their receptors, which needs coactivator for the transcription regulation, among which steroid receptor coactivator-1 (SRC-1) has been shown to be multifunctional potentials in the brain, but whether it is regulated by letrozole is currently unknown. In this study, we examined letrozole regulation on SRC-1 expression in adult mice brain using immunohistochemistry. The results showed that letrozole induced dramatic decrease of SRC-1 in the medial septal, hippocampus, medial habenular nucleus, arcuate hypothalamic nucleus and superior colliculus ($p < 0.01$). Significant decrease was detected in the dorsal lateral septal nucleus, bed nucleus of stria terminalis, ventral taenia tecta, dorsomedial and ventromedial hypothalamic nuclei, dorsomedial periaqueductal gray, superior paraolivary nucleus and pontine nucleus ($p < 0.05$). In the hippocampus, levels of estradiol content, androgen receptor, estrogen receptor α and β also decreased significantly after letrozole injection. The above results demonstrated letrozole downregulation of SRC-1 in specific regions that are primarily related to learning and memory, cognition and mood, neuroendocrine as well as information integration, indicating that SRC-1 may be one important downstream central target of letrozole. Furthermore, these potential central adverse effects of letrozole should be taken into serious considerations.

1. Introduction

In the past decades, it has been well established that estrogens play pivotal roles in brain function and structure including synaptic plasticity, learning and memory, neuroendocrine and sexually dimorphic differentiation [1–3]. The biosynthesis of estrogens is catalyzed by the enzyme named aromatase (AROM), which is localized in the endoplasmic reticulum and consisted of a cytochrome P450 (P450 Arom) and a NADPH cytochrome P450 reductase [4]. In the early 1970s, Naftolin's studies provided direct evidences for brain aromatization of androgens to estrogens which created the foundation for the brain AROM hypothesis [5]. Later studies have demonstrated extensive distribution of AROM in specific brain regions such as in the cerebral cortex, limbic system especially hippocampus, hypothalamus, amygdale and midbrain [6–9].

Since AROM is the limiting enzyme for estrogen synthesis, many selective AROM inhibitors have been used to explore the function of brain estrogens, among which letrozole (LET; CGS 20267) is the frequently used one. LET is a non-steroidal compound which potently inhibits AROM but does not affect adrenal steroidogenesis [10], it is now a favored drug for the treatment of hormone receptor-positive breast cancer [11]. However, its adverse effects have been noticed by many studies. For example, LET affected neuroendocrine function [12,13], inhibited cell proliferation and increased apoptosis [14]. In the open field tests, LET has been shown to induce mild anxiety and increased latency [11]. Several clues targeting hippocampal synaptic plasticity revealed that LET administration reduced spine synapse and axon outgrowth, decreased expression of estrogen receptor α (ERα) and some synaptic proteins such as GAP-43 [15–17], impaired long-term potentiation (LTP; the electrophysiological indicator of memory) [18]. However, some studies showed that LET could rescue spatial

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learning and memory deficiency induced by ovariectomy [11,19]. Therefore, the exact role of LET on brain function especially hippocampal structure and function needs to be clarified.

The actions of estrogens are believed to be mediated by the estrogen receptors, which require coactivators for efficient transcriptional activity. Steroid receptor coactivator-1 (SRC-1) or nuclear receptor coactivator-1 (NCoA-1) is the first discovered member of the steroid receptor coactivator family, which has been demonstrated to dramatically enhance the transcriptional activity of a variety of nuclear receptors including ERα and ERβ in a ligand-dependent manner [20,21]. Studies have shown that in the brain, SRC-1 plays important roles in the regulation of Purkinje cell development and maturation as well as motor learning [22], female sexual behavior, neural plasticity and reproductive functions [23–26], acute stress [27] and the defeminizing actions of estradiol [28]. Additionally, SRC-1 has been shown to be involved in the regulation of HPA axis and thyroid function [29–31] as well as the anti-obesity effects of estrogen-ERα signals [32]. Our previous studies have shown that in the rat brain, age-related significant decrease of SRC-1 was detected in specific regions related to learning and memory, motor and sense [33]. We also noticed that hippocampal SRC-1 and some synaptic proteins were regulated by postnatal development but not ovariectomy [34]. In the mice brain, a significant male-predominant expression of brain SRC-1 was detected [35] and in the hippocampus of mice, females and males shared similar postnatal developmental profiles for SRC-1 and selected synaptic proteins [36]. Additionally, we found that gonadectomy affected hippocampal SRC-1 and selected synaptic proteins in a sex-dependent manner [37].

The above findings indicate the multifunctional potentials of SRC-1 in the regulation of learning and memory, motor activity and neuroendocrine. Hoverer, how about LET administration on the expression of brain SRC-1 remains unclear. Since male breast cancer shows similar pathology and treatment strategy to that found in the matched female breast cancers [38,39] and brain SRC-1 might be regulated by estrus cycle [40], in this study we investigated the immunoreactivities of SRC-1 in LET-treated adult mice brain using nickel-intensified immunohistochemistry.

2. Materials and methods

2.1. Animals and LET administration

Adult male SPF grade C57BL/6 mice (12 weeks old, 20–22 g) were purchased from the Experimental Animal Center of Third Military Medical University. All the animal-related procedures in this study were conducted in strict compliance with Approved Institutional Animal Care and Use Protocols. The animals were randomly divided into two groups ($n = 5$). The control animals were injected intraperitoneally with dimethyl sulfoxide (DMSO; D8418, Sigma-Aldrich Shanghai Trading Co Ltd, Shanghai, China), the LET animals were intraperitoneally injected LET (L6545, Sigma-Aldrich Shanghai Trading Co Ltd, Shanghai, China) with a dose of 80 μg/kg dissolved in DMSO. The injection was conducted at every morning and lasted for 4 weeks.

2.2. Tissue section preparation

Tissue preparation was carried out according to our previous reports [33,36,37,41]. In brief, after deep anesthesia with 100 mg/kg sodium pentobarbital, animals were perfused transcardially with saline, followed by 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains was carefully dissected, removed, post-fixed overnight with the same fixative, and then transferred to the fixative containing 30% sucrose until they sank to the bottom of the container. The brain was serially cut frozen into 20 μm-thick coronal sections with a cryostat (CM1900, Leica Microsystems, Germany). By following the principles of unbiased and 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. Immunohistochemistry (IHC)

Nickel-intensified SRC-1 IHC was carried out according to our previous description [33,36,37,41] with slight modification. Free-floating sections were first washed with PBS (phosphate buffered saline, 10 mmol/L; pH 7.4), quenched for 15 min in 3% H_2O_2 in PBS, and blocked in 5% normal goat serum for 30 min at room temperature. The sections were then incubated overnight at 4 °C with the primary rabbit polyclonal antiserum (1:200; sc-8995, Santa Cruz, USA) diluted with Antibody Diluent (S3022, Dako Inc., Glostrup, Denmark). After several washes with PBS, the sections were incubated with the biotinylated secondary goat-anti-rabbit antibody (1:200; ZB2010, Zhongshan Biotech; Beijing) for 1 h at room temperature. The sections were washed in PBS again, incubated with the HRP-labeled streptavidin reagent (1:200; ZB2404, Zhongshan Biotech; Beijing) for 1 h at room temperature and then visualized using a DAB-nickel chromogen kit (SK-4100; Vector Laboratories Inc., USA) for 5 min at room temperature. Finally, the sections were dehydrated, cleared in xylene and mounted with DPX. Negative control was carried out using the same procedure, but PBS or normal serum was used instead of the primary antiserum.

Hippocampus sections between Bregma-2.52 and -3.12 were carefully selected and stained with primary antibody against 17β-estradiol (1:200; AB924, Millipore, USA) [42], AR (1:100; sc-13062, Santa Cruz, USA) [43], ERα (1:50; sc-542; Santa Cruz, USA) [44] and ERβ (1:100; sc-6821, Santa Cruz, USA) [45], respectively. The specificity of these antibodies has been verified and they have been successfully applied in the above studies [42–45].

2.4. Data analysis and statistics

All the images were recorded by using a digital camera (DP70, Leica Microsystems, Germany) equipped with an Olympus microscope (BX60, Japan) as previous reports [36,37]. SRC-1 expression pattern was determined from images of the brain regions guided by The Mouse Brain in Stereotaxic Coordinates (2nd edition) [46]. The average optical density from 2 to 5 sections from each brain region or sub-region was used to represent the regional expression level for each animal. For the hippocampus sections stained for estradiol, AR and ERs, only those stained sections between Plates 54 (Bregma-2.52) and 59 (Bregma-3.12) were used for data analysis, ensuring the highest possible correspondence between the two groups [34]. The representative immunostaining in specific brain regions was measured by Image Pro Plus software 6.0, values in each group were averaged and reported as means ± SEM. Independent-sample *T*-test was carried out with software SPSS (version 13.0) and a level of $p < 0.05$ was considered to be statistical significant.

3. Results

SRC-1 immunopositive materials were extensively detected in the mice brain as our previous observations [33,35]. Generally, LET treatment induced significant decrease of SRC-1 in some specific brain regions (about 50% among all the nuclei examined), in some other regions expression of SRC-1 was slightly increased by LET but without statistical significance as indicated in Figs. 4–6. LET treatment also decreased immunoreactivities for 17β-estradiol, AR, ERα and ERβ as shown in Fig. 7.