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Dose-dependent regulation of steroid receptor coactivator-1 and steroid receptors by testosterone propionate in the hippocampus of adult male mice

Linli Qiu[a,b], Yangang Zhao[a], Qiang Guo[a], Yuanyuan Zhang[a], Li He[a,b], Wei Li[b,**], Jiqiang Zhang[a,*]

[a] Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, China
[b] Department of Filed Nursing, School of Nursing, Third Military Medical University, Chongqing 400038, China

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ABSTRACT

Androgens have been proposed to play important roles in the regulation of hippocampus function either directly, through the androgen receptor (AR), or indirectly, through estrogen receptors (ERs), after aromatization into estradiol. Steroid receptor coactivator-1 (SRC-1) is present in the hippocampus of several species, and its expression is regulated by development and aging, as well as by orchidectomy and aromatase inhibitor letrozole administration, while ovariectomy only transiently downregulated hippocampal SRC-1. However, whether the expression of hippocampal SRC-1 can be directly regulated by testosterone, the principal male sex hormone, remains unclear. In the present study, we investigated the expression of hippocampal SRC-1 after orchidectomy and testosterone treatment using immunohistochemistry and Western blot analysis. We found that while hippocampal SRC-1 was significantly downregulated by orchidectomy (ORX), its expression was rescued by treatment with testosterone in a dose-dependent manner. Furthermore, we noticed that the decreased expression of hippocampal AR, ERs and the synaptic proteins GluR-1 and PSD-95 induced by ORX was also rescued by testosterone treatment in a dose-dependent manner. However, we found that hippocampal membrane estrogen receptor GPR30 and dendritic spine marker spinophilin were not altered by ORX or testosterone treatment. Together, the above results provided the first direct evidence for the androgenic regulation on hippocampal SRC-1, indicating that SRC-1 may be a direct target of androgenic regulation on the hippocampus. Furthermore, because AR and ERs can be differentially regulated by testosterone, and the transcriptional activity requires the involvement of local SRC-1, and considering the complicated regulatory pathway of each individual receptor, the converged hub regulator SRC-1 of these nuclear receptor networks is worthy of further investigation.

1. Introduction

Studies have demonstrated that androgens such as testosterone and dihydrotestosterone play profound roles in the regulation of the hippocampal structure and function in both rodents and human subjects. Androgen deprivation (usually by orchidectomy, ORX) causes significant cognitive deficiency [1] and a significant decrease of hippocampal BDNF and PSD-95 [2]. Testosterone treatment significantly increased the spine synapse density in the hippocampus of rats [3] and regulated hippocampal spinogenesis [4]. A recent study showed that testosterone in the hippocampus of male rats is protective against ORX-induced depressive-like behavior [5]. In humans, age-induced decreased levels of androgens may be one of the causative factors for heightened risk of Alzheimer's disease (AD). This is based on the fact that testosterone replacement has been shown to improve cognitive deficits in rodents [6]. However, the underlying mechanisms of androgen actions remain unclear.

In addition to its direct action pathway of binding to an androgen receptor (AR), testosterone also functions by local neural aromatization into 17beta-estradiol (E2). This process is catalyzed by local aromatase (estrogen synthase), leading to a hippocampal E2 concentration of up to 8 nM for males and 0.5–2 nM for females, which is much higher than circulatory levels, as shown by hippocampal slices from adult male rats [7]. Numerous studies

* Corresponding author.
** Corresponding author.
E-mail addresses: weilee2020@163.com (W. Li), zhangjqtmmu@yahoo.com (J. Zhang).

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have localized aromatase mRNA and/or protein in the hippocampus. It has been detected in the hippocampus of mice [8], rats [9] and humans [10]. Over the last decade, studies have revealed a crucial role of hippocampal aromatization in the regulation of hippocampal structural and functional synaptic plasticity [11,12], as well as in spatial memory performance [13] and protective effects against depression-like behavior [5].

The actions of androgens may be achieved by either binding to AR or estrogen receptors (ERs) after conversion to E2 by aromatase, the alterative pathway for androgen action. Nuclear AR and ERs (ERα and ERβ) have been detected in the hippocampus of several species [14–16]. AR and ERs show unique properties that affect the structure of the hippocampus and its function, including synaptic plasticity, learning and memory, and cognition [17–22]. Additionally, the novel membrane estrogen receptor GPR30 (also called GPER-1) has been shown to mediate rapid, non-classic, estrogenic regulation on the hippocampus [23,24]. However, the changes of these receptors after testosterone treatment have not been fully examined.

Steroid nuclear receptors require coactivators for efficient transcriptional activity. Among these coactivators, steroid receptor coactivator-1 (SRC-1; or NCoA-1) [25] is predominantly localized in the hippocampus [26,27]. Our previous studies have demonstrated that in the female rats, hippocampal SRC-1 was regulated by postnatal development and aging, but not by an ovariectomy [28,29]. We also demonstrated that SRC-1 expression is higher in the brain of male mice compared to females [30] as reported by Kerver et al. [31,32]. However, in the hippocampus, male and female mice displayed a similar postnatal developmental profile of SRC-1 expression [33], but SRC-1 remained depressed only in males after gonadectomy [34]. Recently, we found that letrozole administration and ORX induced a similar downregulation of SRC-1 immunoreactivity in specific brain regions involved in sense of smell, learning and memory, cognition, neuroendocrine, reproduction and motor control [35,36]. Although we have reported that androgen deprivation by ORX or inhibition of hippocampal E2 by letrozole administration downregulated hippocampal SRC-1 [34–36], whether hippocampal SRC-1 can be upregulated by androgens remains unclear. To explore the role of SRC-1 in the mediation of androgenic regulation on the hippocampus, we used immunohistochemistry and Western blots to investigate the expression of hippocampal SRC-1, androgen and estrogen receptors, and synaptic proteins GluR-1 and PSD-95 after the administration of testosterone.

2. Materials and methods

2.1. Animals and testosterone administration

Adult male SPF grade C57BL/6 mice (12 weeks old, 22 ± 2 g) were obtained from the Experimental Animal Center of Daping Hospital, Third Military Medical University. All of the animal-related procedures were conducted in strict compliance with the Approved Institutional Animal Care and Use Protocols of this university. The animals were randomly divided into five groups. Animal surgery was carried out according to our previously reported procedures [34]. In short, mice were anesthetized with 100 mg/kg 4% chloral hydrate, the fur was clipped over the surgical area and scrubbed with Betadine and an ethanol swipe. The skin of the scrotum was then opened, the epididymis was cut and testes were completely removed, followed by suture of the wound. The testosterone treatment groups were injected with a testosterone propionate injection (H31020524, Shanghai General Pharmaceutical Co., Ltd., Shanghai, China) subcutaneously with a dose of 0.5, 1.0, or 2.0 mg/kg body weight. A sham-operation group of animals that received an injection of an equal amount of soybean oil was used as the control group. The injection was carried out every afternoon and lasted 2 weeks.

2.2. Tissue section preparation

Tissue section preparation was carried out according to our previous reports [14,28]. Briefly, two weeks after testosterone administration, the mice in each group ($n = 5$) were deeply anaesthetized with 100 mg/kg 4% chloral hydrate and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffer (PBS, pH 7.4). The brains were carefully dissected, removed, fixed overnight with the same fixative, and transferred to freshly-prepared fixative containing 30% sucrose until they sank to the bottom of the container. Brains were then serially cut into 20 μm-thick coronal sections with a cryostat (CM1900, Leica Microsystems, Germany). Following the principles of unbiased and systematic random sampling, the serially cut hippocampus-containing sections (between Bregma −0.82 and −3.80) were transferred into one six well plate, with every sixth section being placed in the same well.

2.3. Immunohistochemistry

Nickel-intensified immunohistochemistry was carried out as previously described [14]. Briefly, free-floating sections were first washed with PBS (10 mmol/L; pH 7.4), quenched for 15 min in 3% H_2O_2, and washed with PBS at room temperature. The sections were then incubated at 4 °C overnight with the individual primary antibodies diluted in Antibody Diluent (ZLI-9028, Zhongshan Biotech, Beijing, China). After being washed, the sections were incubated with the biotinylated secondary goat-anti-rabbit antibody (1:200; ZB2010, Zhongshan Biotech) 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) for 1 h at room temperature and then visualized using a DAB-nickel chromogen kit (SK-4100; Vector Laboratories Inc., Burlingame, USA) for 5 min at room temperature. Finally, the sections were dehydrated, cleared and mounted.

Images of the stained sections were recorded using a digital camera (DP70, Leica Microsystems, Wetzlar, Germany) equipped with an Olympus microscope (BX60, Tokyo, Japan) as previously reported [31,32]. The average optical density of sections between Bregma −1.46 and −2.46 was measured using Image Pro Plus software 6.0 (Media Cybernetics, Rockville, USA), and the mean value was used to represent the regional expression level for each group.

The primary antibodies used in this study were as follows: rabbit polyclonal anti-SRC-1 (1:200; sc-8995, Santa Cruz, Dallas, USA), rabbit polyclonal anti-AR (1:50; sc-13062, Santa Cruz), rabbit polyclonal anti-ERα (1:50; sc-542, Santa Cruz), rabbit polyclonal anti-ERβ (1:50; sc-8974, Santa Cruz), rabbit mAb-PSD95 (1:200; 3409, Cell Signaling, Danvers, USA) and rabbit mAb-GluR-1(1:100; 04-855, MerkMillipore, Shanghai, China).

2.4. Western blot

Western blot analysis was carried out according to our previous description [29,34]. Proteins of the hippocampus of the mice were extracted using a Protein Extract Kit (P0027, Beyotime Biotech; Beijing, China) and the protein concentration was determined using a BCA Assay Kit (P0010, Beyotime Biotech). Nuclear protein (for SRC-1, AR and ERs) or cytoplasmic protein (for synaptic proteins) samples were diluted in loading buffer and subjected to SDS-PAGE followed by transfer to PVDF membranes. The membranes were blocked with 5% fresh-prepared milk-TBST