Steroids 88 (2014) 7–14

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Regional specific regulation of steroid receptor coactivator-1 immunoreactivity by orchidectomy in the brain of adult male mice

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ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 13 May 2014
Accepted 4 June 2014
Available online 16 June 2014

Keywords:
Androgen
Orchidectomy
Sex hormone
Nuclear receptor

ABSTRACT

Androgens including testosterone and dihydrotestosterone play important roles on brain structure and function, either directly through androgen receptor or indirectly through estrogen receptors, which need coactivators for their transcription activation. Steroid receptor coactivator-1 (SRC-1) has been shown to be multifunctional potentials in the brain, but how it is regulated by androgens in the brain remains unclear. In this study, we explored the effect of orchidectomy (ORX) on the expression of SRC-1 in the adult male mice using nickel-intensified immunohistochemistry. The results showed that ORX induced dramatic decrease of SRC-1 immunoreactivity in the olfactory tubercle, piriform cortex, ventral pallidum, most parts of the septal area, hippocampus, substantia nigra (compact part), pontine nuclei and nucleus of the trapezoid body ($p < 0.01$). Significant decrease of SRC-1 was noticed in the dorsal and lateral septal nucleus, medial preoptical area, dorsomedial and ventromedial hypothalamic nucleus and superior paraolivary nucleus ($p < 0.05$). Whereas in other regions examined, levels of SRC-1 immunoreactivity were not obviously changed by ORX ($p > 0.05$). The above results demonstrated ORX downregulation of SRC-1 in specific regions that have been involved in sense of smell, learning and memory, cognition, neuroendocrine, reproduction and motor control, indicating that SRC-1 play pivotal role in the mediating circulating androgenic regulation on these important brain functions. It also indicates that SRC-1 may serve as a novel target for the central disorders caused by the age-related decrease of circulating androgens.

1. Introduction

It is well known that male sex steroids androgens, including testosterone and dihydrotestosterone, are necessary for the development and maintenance of normal brain structure and function such as neural circuits and plasticity, behavioral and sexual modulation, cognition, neuroendocrine, feeding and sleep-wakefulness [1–4]. In the humans, age-related decrease of androgens is associated with increased risk of Alzheimer's disease (AD), and testosterone replacement has been shown to improve cognitive deficits in rodents [5]. In the brain, androgens can be aromatized into 17-beta-estradiol, therefore function through androgen receptor (AR) directly or estrogen receptors (ERα and ERβ) indirectly [6]. Studies have revealed different levels of AR or ERs expression in specific brain regions that are mostly related to neurogenesis, learning and memory, cognition, motor control, reproduction, neuroendocrine, pain and social decision-making [7–9]. Furthermore, ERβ knockout induces changes of hippocampal synaptic plasticity and impairs long-term potentiation [10].

It is well established that steroid receptors need coactivators for their efficient transcriptional activity. Among which steroid receptor coactivator-1 (SRC-1; or NCoA-1) has been shown to dramatically enhance the transcriptional activity of nuclear receptors including AR and ERs in a ligand-dependent manner [11–13]. Accumulated studies have shown that brain SRC-1 may play a role in the modulation of neural plasticity, development of olfactory epithelium and cerebellar Purkinje cells [14,15], the defeminizing actions of estrogen [16], HPA axis function and thyroid hormone function [17,18]. It might also function to regulate reproduction and acute stress [19–21], motor learning [15], the anti-obesity effects of estrogen-ERα signals [22], optical and auditory regulation [23,24]. In our previous studies, we have demonstrated age-related significant decrease of SRC-1 in specific brain regions related to learning and memory, motor and sense [25]. Furthermore, in the hippocampus, males and females shared similar postnatal developmental profiles for SRC-1 [26] and levels of

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http://dx.doi.org/10.1016/j.steroids.2014.06.006
0039-128X/

hippocampal SRC-1 were regulated by postnatal development but not ovariectomy [27]. In the mice brain, a significant male-predominant expression profile of SRC-1 when compared to the females was also reported [28].

Limited references have reported the testosterone regulation of brain SRC-1 expression in specific regions with discrepant results. For example, testosterone did not regulate expression of SRC-1 in the adult Siberian hamster [23] or zebra finch brain [29]; however, in the male preoptic area/hypothalamus of Japanese quail it was up-regulated by testosterone [30]. In a recent study, we found that expression of hippocampal SRC-1 was significantly regulated by gonadectomy (GDX) in a sex-dependent manner: while orchidectomy (ORX) caused persistent decreases of SRC-1, ovariectomy (OVX) only induced a transient decrease of SRC-1 at two weeks after surgery [31]. These results indicated that androgens are the main regulator of hippocampal SRC-1; but how about the androgenic regulation on SRC-1 in other brain regions is not clear. In order to explore the multiple roles that SRC-1 plays in steroids regulation on the central nervous system, in this study we investigated the effects of androgens deprivation on the expression of SRC-1 immunoreactivities in the brain of adult male mice.

2. Materials and methods

2.1. Animals and surgery

Adult male SPF grade C57BL/6 mice (12 weeks old, 22 ± 2 g, $n = 10$) 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 two groups. ORX was carried out according to previous surgical procedures [31]. In brief, mice were anesthetized with 100 mg/kg sodium pentobarbital, the hair was clipped over the surgical area and scrubbed with Betadine and ethanol swipe, the skin of the scrotum was opened, the epididymis was cut and the testes were removed completely, then the wound was sutured. A sham-operated group animal was used as control.

2.2. Tissue preparation

Four weeks after surgery, the mice were deeply anaesthetize with 100 mg/kg sodium pentobarbital, perfused transcardially with saline and followed by 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were 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. Tissue preparation was carried out according to our previous reports [7,25,28]. In brief, brains were serially cut frozen into 25 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 [7,25,28]. 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 washes, the sections were incubated with the biotinylated secondary goat-antirabbit antibody (1:200; ZB2010, Zhongshan Biotech; Beijing, China) 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, China) 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. Blank control was carried out using the same procedure, but PBS was used instead of the primary antiserum.

2.4. Data analysis and statistics

Data analysis and statistics were conducted according to our previous studies [25,28]. All the images were recorded by using a digital camera (DP70, Leica Microsystems, Germany) equipped with an Olympus microscope (BX60, Japan) as previous reports [26,31]. SRC-1 expression pattern was determined from images of the brain regions guided by The Mouse Brain in Stereotaxic Coordinates (2nd edition) [32]. 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. The representative SRC-1 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

In the sham animals, expression of SRC-1 immunopositive materials was in well agreement with our previous observations [28]; and it was distinctly regulated by ORX in a region-specific manner as indicated in Figs. 1–3 and summarized in Figs. 4–6.

3.1. Telencephalon

3.1.1. Sham animals

Concentrated SRC-1 immunopositive cell nuclei were detected in most part of the olfactory bulb, including anterior olfactory nucleus, mitral cell layer of the accessory olfactory bulb, granular cell layer of the accessory olfactory bulb and external plexiform layer (Fig. 1A). In the cerebral cortex, sense positive cells were detected in most part of the cortex including cingulate cortex (Cg1) and motor cortex (M1 and M2) as shown in Fig. 1C, piriform cortex, ventral pallidum and olfactory tubercle (Fig. 1E). In the septal area, moderate immunostaining nuclei were detected in the dorsal part of lateral septal nucleus; relative weak levels of SRC-1 were detected in the medial septal nucleus (Fig. 1G) and the horizontal limb of the diagonal band, while strong expression of SRC-1 was detected in the vertical limb of the diagonal band (Fig. 2A). In the hippocampal formation, the CAs and dentate gyrus (Fig. 2C) and the ventral taenia tecta showed the highest expression, lower levels of SRC-1 were detected in the bed nucleus of stria terminalis and subiculum. Different levels of SRC-1 immunopositivities were also detected in other parts of the forebrain such as accumbens nucleus (shell; Fig. 2A) and amygdaloid.

3.1.2. ORX animals

In the olfactory bulb and cerebral cortex, dense SRC-1 immunopositive cells were detected as that of the sham animals as shown in Fig. 1B, D and F. Significant decrease of SRC-1 immunoreactivity was only detected in the piriform cortex and olfactory tubercle ($p < 0.01$; Fig. 4). In the septal area, ORX induced significant