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Localization and sex-difference of steroid receptor coactivator-1 immunoreactivities in the brain of adult female and male mice

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ABSTRACT

Females and males are different in brain and behaviors. These differences are mediated by steroids and their nuclear receptors which require coactivators to regulate the transcription of target genes. Studies have shown that these coactivators are critical for modulating steroid hormone action in the brain. Steroid receptor coactivator-1 has been implied in the regulation of reproduction, stress, motor learning, and limited studies have reported the sex-specific difference of SRC-1 mRNA or protein expression in specific brain regions, but the expression and differences of SRC-1 immunoreactivities in adult female and male brain remain unclear. In this study we reported that in both sexes, high levels of SRC-1 immunoreactivities were detected in olfactory bulb, cerebral cortex, hippocampus, Purkinje cells, some limited diencephalon and brainstem nuclei. The immunopositive materials were predominantly detected in cell nucleus, but in some regions they were also detected in the processes or fiber-like structures. In most of the brain regions studied, males possessed significantly higher levels of SRC-1 immunoreactivities than that of females. Higher levels of SRC-1 were detected in some nuclei related to learning and memory, motor regulation and reproduction indicated its potential roles in neurodegeneration and sex-dependent behavior and structure; the region- and sex-specific localization of SRC-1 immunoreactivities in agreement with that of some steroid receptors, indicating this coactivator play important roles in these hormone-reactive regions and cell groups related to reproduction, learning and memory, integration of motor and sense.
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1. Introduction

Females and males are different in brain and behavior and many other ways. These sex differences occur early during development due to a combination of genetic and steroid hormonal factors and continue throughout the lifespan. It is well-known that steroids such as testosterone, estrogen and progesterone play pivotal roles in brain function and structure emparting synaptic plasticity, learning and memory and sexually dimorphic differentiation [1–5]. These actions are believed to be predominantly mediated by the nuclear steroid receptors, which require nuclear receptor coactivators for efficient transcriptional activity. Several studies have demonstrated that these steroid receptor coactivators (SRCs) are critical for modulating steroid hormone action in the brain and behavior [6–10]. Among all the SRCs, SRC-1 in particular is the focus of intense research, because its high expression in the brain and only SRC-1 null mice showed neural abnormality [10]. Additional functional studies show that SRC-1 is required for normal neuronal development; reduced SRC-1 protein interferes with the defeminizing actions of estrogen in the neonatal rat brain [11]. The studies of knock-out mice found that adult SRC-$1^{-/-}$ mice exhibit motor dysfunction and delayed development of Purkinje cells [6] and altered hypothalamic-pituitary-adrenal axis function [12].

Several studies have reported the distribution of SRC-1 mRNA/protein in the brain of different animal species. In both male and female quails, similarly high levels of SRC-1 expression were detected in the telencephalon, diencephalon, optic lobes and brainstem; and lower levels of SRC-1 expression were observed in the cerebellum [13]. In embryonic mouse, significant amount of SRC-1 transcript was found in the olfactory epithelium, neocortex and anterior pituitary [14]. In the adult mouse brain, SRC-1 mRNA was highly expressed in the olfactory bulb, hippocampus, piriform cortex, amygdala, hypothalamus, cerebellum, and brainstem [6]. In the rats, SRC-l mRNA was detected in many brain areas, including hippocampus, amygdala, hypothalamus, basal ganglia, and isocortex [15]. Further study showed that in adult hippocampus of rats, SRC-1-immunoreactivities were observed in CA1-CA4 pyramidal cell layers of the hippocampus and the granular cell layer of the dentate

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gyrus [16]. These findings suggest important roles that SRC-1 plays in the brain.

Limited studies have reported the sex-differences of SRC-1 in specific brain regions. For example, SRC-1 expressed at higher levels in the hippocampus of male rats when compared to females [17]; mature female quails had a higher concentration of SRC-1 in the preoptic area/hypothalamus compared with males [7]. However, whether the expression of brain SRC-1 is sex-dependant is currently unclear. To comprehensively understand the roles that SRC-1 plays in the brain of both sexes and to further elucidate the mechanisms that underlay the sex-specific brain structure, function and behavior, in this study we investigated the immunoreactivities in adult male and female mice brain using nickel-intensified immunohistochemistry.

2. Materials and methods

2.1. Animals and tissue preparation

Adult male and female SPF grade C57BL/6 mice (12 weeks old; $n = 5$) 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 females used in the experiments were at diestrus phase as determined by examining vaginal smear.

Tissue preparation was carried out according to our previous reports [18] with slight modifications. In brief, after deep anesthesia with 100 mg/kg sodium pentobarbital, animals were perfused transcardially with saline, followed by 200 ml 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains was carefully dissected, removed, post-fixed overnight in the same fixative, and then transferred to the fixative containing 30% sucrose. The brain was serially cut frozen into 20 μm-thick 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.

2.2. Immunohistochemistry (IHC)

Nickel-intensified SRC-1 IHC was carried out according to our previous description [18] with slight modifications. 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 2% normal goat serum for 30 min at room temperature. The sections were then incubated over night 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 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 in HRP-labeled streptavidin reagent (1:200; ZB2404, Zhongshan Biotech; Beijing) for 1 h at room temperature and then visualized using a DAB-nickel chromogen solution for 5 min at room temperature. Finally, the sections were dehydrated, cleared in xylene and mounted with DPX [18]. Negative controls were carried out using the same procedure, but PBS or normal serum was used instead of the primary antiserum.

2.3. 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). SRC-1 expression pattern was determined from images of the brain regions guided by The Mouse Brain in Stereotaxic Coordinates (2nd edition) [19]. The average optical density from 2–5 sections from each brain region or sub-region was used to represent the regional expression level for each animal. The relative levels of expression presented were the average level from all 5 brains of each group, representative SRC-1 immunostaining in specific brain regions were measured by Image Pro Plus software 5.0, values in each group were averaged and reported as mean ± S.E.M. 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 this study we found that SRC-1 immunopositive materials were predominantly detected in the cell nucleus of mice brain, but in some limited regions it seemed they were also detected in neuritis of fiber-like structures, as shown in Fig. 4G and H. Male brain showed statistically significant higher levels of SRC-1 immunoreactivities when compared with that of the females, in very limited regions female brain showed higher levels of SRC-1 immunoreactivities than males but without statistically significance as shown in and Figs. 6–8.

3.1. Telencephalon

In the females, concentrated positive cell nuclei were detected in most part of the olfactory bulb (Fig. 1A) including interpeduncular nucleus, gigantocellular reticular nucleus, anterior olfactory nucleus and external plexiform layer of the olfactory bulb; dense positive cells were also detected in most part of the cortex (Fig. 1C) as well as in the hippocampal formation (CA1 and dentate gyrus; Fig. 2C). Moderate or weak immunostaining were detected in the ventral taenia tecta (Fig. 1E) and basal forebrain including medial septal (Fig. 1G), the dorsal part of lateral septal nucleus (Fig. 1G) and the nucleus of the vertical limb of the diagonal band (Fig. 2A), very weak or negative staining were detected in the striatum. SRC-1 immunopositive signals were different in subregions of the amygdaloidal complex with strong levels detected in the anterior cortical amygdaloidal nucleus but weak or negative in other subareas.

Similar expression pattern of SRC-1 immunoreactivities was detected in the male telencephalon but in general, higher levels were found in corresponding areas. The highest expression was also detected in the olfactory bulb, cortex and hippocampal formation, as shown in Figs. 1B, D and 2D. In contrary to the females, male taenia tecta (Fig. 1F) and the dorsal part of lateral septal nucleus (Fig. 1H) also showed high levels of SRC-1. Expression of SRC-1 in the bed nucleus of the stria terminalis, medial septal area and the vertical limb of the diagonal band, anterior cortical amygdaloidal nucleus, and subiculum were significantly higher than that of females. No significant sex-differences of SRC-1 immunoreactivities were detected in the olfactory tubercle.

3.2. Diencephalon

In the female thalamus, scattered SRC-1 immunopositive cells were detected in most part of this area, clear and strong SRC-1 immunopositive cells were predominantly detected in the medial habenular nucleus and paraventricular nucleus (Fig. 2E). In the hypothalamus, relative high expression of SRC-1 was detected in the medial optical area (Fig. 2G), ventral medial nucleus (Fig. 3A) and arcuate nucleus (Fig. 3C), paraventricular nucleus (Fig. 3E), medial mammillary nucleus (Fig. 3G), followed by the dorsal medial nucleus, periventricular nucleus and some other part of the mammillary body.

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