Experimental Gerontology 48 (2013) 778–785

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

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

# Effects of dihydrotestosterone on synaptic plasticity of hippocampus in male SAMP8 mice

Sha Li [1], Lin Kang [1], Chao Zhang, Guosheng Xie, Nan Li, Yu Zhang, Juan Du, Huixian Cui *

*Department of Human Anatomy, Hebei Medical University, Shijiazhuang 050017, PR China*

ARTICLE INFO

*Article history:*
Received 10 December 2012
Received in revised form 22 April 2013
Accepted 23 April 2013
Available online 4 May 2013

Section Editor: Christian Humpel

*Keywords:*
Dihydrotestosterone
Castration
Hippocampus
Synaptic plasticity
SAMP8

ABSTRACT

The senescence-accelerated-prone mouse 8 (SAMP8) has been proposed as a suitable, naturally derived animal model for investigating the fundamental mechanisms of Alzheimer's disease (AD). In addition, the serum testosterone levels decrease quickly in the natural growth process of this model. This study investigated the effect of androgen deficiency on the synaptic plasticity of hippocampus in male SAMP8 mice after castration and dihydrotestosterone (DHT) administration. We observed the dendritic spines and synapses using Golgi staining and transmission electron microscope. Androgen deficiency after castration significantly reduced the number of apical dendritic thorns, and the abnormal ultrastructure of excitatory synapses was more obvious. Androgen replacement therapy reversed this change. To explore the protective mechanisms and neurological basis of DHT, we researched the changes of expression of GluN1 subunit-containing N-methyl-D-aspartate receptors (NMDARs) and synaptophysin (SYN), which are closely related to synaptic plasticity. Comparisons were made among results observed with immunohistochemistry techniques, Western blots analysis and RT-PCR analysis. The GluN1 and SYN regulation at the protein and mRNA levels probably be related to the DHT-induced morphological synaptic plasticity. This study will be helpful for understanding the function of androgen, and it provides a valuable theoretical basis about the protective and therapeutic targets of androgen in AD.



## 1. Introduction

The senescence-accelerated mouse (SAM) has been previously established as an inbred and accelerated aging mouse model by Takeda et al. (1981). According to aging rate and age-associated pathological phenotypes, SAM is comprised of strains of senescence-accelerated-prone mouse (SAMP) and strains of senescence-accelerated-resistant mouse (SAMR) (Takeda et al., 1991, 1994). Among the SAMP strains, SAMP8 is characterized by an age-related spontaneous deterioration in learning and memory abilities. This deterioration is often preceded or accompanied by cortical and hippocampal changes in gene expression and other various pathological features that are similar to Alzheimer's disease (AD) (Butterfield and Poon, 2005; Del Valle et al., 2010; Nomura and Ohkuma, 1999). Numerous studies have demonstrated that SAMP8 is a good, naturally derived animal model for the investigation of fundamental AD mechanisms.

Flood et al. reported an age-related 71% decrease in serum testosterone levels between 4 and 12-month-old SAMP8 mice, but only a 26% decrease between SAMR1 mice of the same age. It has been previously shown that a testosterone implant can alleviate impaired learning and memory abilities in SAMP8 mice (Flood et al., 1995). Moreover, it is widely accepted that changes in the levels of gonadal hormones over the course of the lifespan contribute not only to variations in cognitive function (Henry and Sherwin, 2012; Janowsky et al., 2000; Matousek and Sherwin, 2010) but also to the incidence of specific types of neurodegenerative disorders. Although relatively less attention has been devoted to the effects of androgens compared to estrogens, androgens clearly have positive effects on cognitive performance and may play a protective role against AD (Cherrier et al., 2005; Rosario and Pike, 2008; Yao et al., 2008). Epidemiological studies show that older men with low serum levels of circulating testosterone appear to be at a higher risk of developing AD compared to men with normal levels of this hormone (Drummond et al., 2009; Moffat et al., 2004; Nagai et al., 2012). Given age-related androgen deficiency of SAMP8 mice in the natural growth process (Flood et al., 1995), which may be a risk factor of AD, it is more reasonable to choose this AD model to study the relationship of androgen and AD. However, it should also be noted that the degree of androgen deficiency in SAMP8 mice is not enough to explain the cognitive deficiencies described elsewhere for this strain. Clearly, a complex interaction among age, environment, and other multiple factors, including androgen deficiency, should be considered and investigated. One of the complexities in understanding the effects of androgen in male SAMP8 mice is that the impaired cognitive function and various pathological features similar to AD is due to an interaction of aging and reduced androgen levels (Flood et al., 1995). The castrated male mice exhibit minute amounts of androgen than intact males. Accordingly, we demonstrated the interventional effect of castration or androgen replacement in male SAMP8 mice on dementia phase.

* Corresponding author. Tel./fax: +86 311 8626 6615.
*E-mail address:* cuihuixianky@yahoo.com (H. Cui).
[1] Contributed equally to this work.

0531-5565/$ – see front matter. 
http://dx.doi.org/10.1016/j.exger.2013.04.014

Morphological and functional studies have demonstrated a strong correlation between a decrease in synaptic density and function in related brain areas and cognitive decline in AD (Gibson, 1983; Hamos et al., 1989; Knafo et al., 2009; Selkoe, 2002; Terry et al., 1991). The hippocampus is involved in learning and memory processes (Simic et al., 1997; van de Pol et al., 2006) and is a target of the modulatory activities of androgen and estrogen. Since the first direct evidence that estradiol modulates the remodeling of spine synapses in the hippocampus in 1992 (Woolley and McEwen, 1992), accumulating experimental studies have demonstrated the effect of estrogen on synaptic plasticity (Adams and Morrison, 2003; Kretz et al., 2004; MacLusky et al., 2005). Nevertheless, structural synaptic plasticity in the male brain and the synaptogenic effect of androgen has received relatively little attention. We focus on the problem whether the effects of androgen on synaptic plasticity of hippocampus in male SAMP8 mice can explain, in part, the protective mechanisms, and also provide a valuable theoretical basis about the therapeutic targets of androgen in AD. To test this hypothesis, we further analyze the effects of androgen-induced spinogenesis in male SAMP8 mice. Because testosterone (T) may be partially converted into estradiol by endogenous aromatase (Hojo et al., 2004; Ishii et al., 2007), we used dihydrotestosterone (DHT), a non-aromatizable androgen, in our studies. Specifically, to explore the protective mechanisms and neurological basis of DHT, we determined whether DHT altered the expression of GluN1 and SYN, which play crucial roles in cognitive function and synaptic plasticity.

## 2. Materials and methods

### 2.1. Animals and study groups

Male 6-month-old SAMP8 mice (n = 84, 28–32 g) were randomly divided into sham-operated control group (P8-sham group), castration group (Cast group), and castration plus dihydrotestosterone group (DHT group). We selected male, 6-month-old SAMR1 mice as the normal contrast of the consanguinity group (R1 group). Bilateral testes were removed from the Cast and DHT groups. Three days after castration, the DHT group was administered standard doses of DHT (product code: 2500981, International Laboratory, CA, USA) for 21 consecutive days by subcutaneous injection (1 mg/kg per day administered between 5:00 PM and 6:00 PM). Other groups were injected aseptically with equal doses of medical maize oil. The mice used in these experiments were obtained from our breeding colonies, which were maintained as an inbred strain and originally provided by Prof. Yew David of The Chinese University of Hong Kong. All of the mice received a standard rodent diet and water ad libitum and were housed under a 12-h light–dark cycle (lights at 6:00 AM) at a room temperature of 21 ± 2 °C. All of the experimental procedures performed in compliance with the Guidelines for the Care and Use of Mammals in Neuroscience Research and approved by the Committee of Ethics on Animal Experiments at Hebei Medical University.

### 2.2. Golgi staining for dendritic spines

Groups of mice (n = 8 per group) were deeply anesthetized with 6% chloral hydrate (5 mg/kg, i.p. injection) and rapidly decapitated. The brains were removed, and tissue samples were immediately dissected from the superior colliculus to the optic chiasma. The tissue blocks were processed for Golgi–Cox staining using a Rapid Golgi Stain Kit (FD Neuro-Technologies, Inc., USA) and stored at room temperature for 2 weeks in the dark before being transferred to a 30% sucrose solution at 4 °C for 2 days. Large specimens were then rapidly frozen and sliced into coronal sections on a cryostat at 100 μm thickness and mounted onto gelatin-coated glass slides. The tissue sections were allowed to dry at room temperature. The stain was developed in the solution mixture for 10 min, and the sections were then rinsed in distilled water. The sections were counterstained with cresyl violet. Finally, the sections were dehydrated in a graded series of ethanol washes, cleared in xylene, and coverslipped using a resinous mounting medium. The sections were viewed using bright field microscopy. Only neurons that were fully impregnated, not obscured by neighboring neurons, and demonstrated no obviously truncated dendrites were analyzed. For each animal, 9 randomly chosen, representative neurons from 3 sections were analyzed and the average values for each animal are presented in the results. Secondary and tertiary apical dendrites were selected for quantitative analysis in which 10 μm segments were magnified 1000× in the digitized images. The number of dendritic spines was independently quantified by two different investigators, and the results were cross-checked to preclude systematic analytical errors. The dendritic spine density values were expressed as the number of thorns/10 μm of dendrite.

### 2.3. Transmission electron microscope analysis for synapses

Groups of mice (n = 5 per group) were anesthetized and perfused with perfusion fluid containing 3% paraformaldehyde and 1% glutaraldehyde in 0.1 M PB, pH 7.4. The brains were removed and postfixed for 2 h in the same fixative. The hippocampus was dissected and cut into sections (100 μm) perpendicular to the longitudinal axis with a vibratome. The sections were fixed using osmium acid, dehydrated in ethanol, embedded in Araldite, and stained with toluidine blue. The cell layer was located in the field of view of the light microscope. Consecutive serial sections at a thickness of 50 nm were prepared from the middle third of the CA1 stratum radiatum using an ultramicrotome and collected on Formvar-coated single slot grids followed by plumbum-uranium staining. Pairs of adjacent parallel electron micrographs ("reference" and "look-up" sections) were evaluated by the Physical Disector, and the synaptic numerical density (Nv), surface density (Sv) and average thickness of postsynaptic dense material (PSD) (Dv) were quantified according to the Disector technique (Braendgaard and Gundersen, 1986; Sterio, 1984). This computer-aided analysis system for synaptic analysis was generated on the basis of Gundersen's Rule (a test frame containing reflective and subsumptive lines) (Gundersen, 1977).

### 2.4. Immunohistochemical staining for GluN1 and SYN

Groups of mice (n = 5 per group) were deeply anesthetized with 6% chloral hydrate (5 mg/kg, i.p. injection) and perfused with 0.9% physiological saline through the left ventricle, followed by a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were carefully removed, and tissue samples were obtained from the superior colliculus to the optic chiasma and postfixed overnight in the same fixative at 4 °C. Next, the tissue block was divided into two halves along the median plane and dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were cut serially on a sliding microtome (Leica-RM2145, Germany) into 5 μm thick coronal sections. Two successive sections were collected every 50 μm and mounted onto polylysine-coated slides for detection of GluN1 and SYN protein expression. After being deparaffinized and hydrated, the sections were subjected to antigen retrieval using a microwave for 30 min, and immersed in 3% hydrogen peroxide in methanol for 30 min to abolish endogenous peroxidase activity. Then the sections were incubated with 5% normal goat serum to block nonspecific binding, followed by an overnight incubation with rabbit anti-GluN1 antibody (1:100, product code: ab28669, Abcam, MA, USA) and rabbit anti-SYN antibody (1:200, product code: ab14692, Abcam, MA, USA) at 4 °C. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG for 2 h. The computer image analysis system Image-Pro Plus 6.0 was used to determine the average values of the optical density (OD) of GluN1 and SYN in the hippocampal CA1 region. Ten sections were analyzed for each mouse and the average OD value was determined for each mouse.