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Detecting the presence of hippocampus membrane androgen receptors in male SAMP8 mice and their induced synaptic plasticity

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ABSTRACT

Testosterone (T), the principal androgen, and its metabolite, dihydrotestosterone (DHT), are known to mediate their effects through binding to intracellular androgen receptors (iARs). In addition to their well-known genomic effects, androgens rapidly alter neuronal excitability through a non-genomic pathway mediated by membrane androgen receptors (mARs). The existence and specificity of mARs in the hippocampus were investigated in SAMP8 mice. Using T-BSA-FITC, we detected plasma membrane labeling by flow cytometry analysis for the presence of mARs. The specificity of binding was examined with iAR antagonist or anti-iAR antibody. Flow cytometry analysis showed that pretreatment with iAR antagonist, flutamide (F), failed to completely prevent the coupling action of the T-BSA-FITC membrane binding. In addition, we found classical iARs did not localize to the membrane of hippocampal neurons. These data indicate that these mARs might be not identical to classical iARs. Modulation of hippocampal synaptic plasticity by androgen has been attracting much attention. To identify the functional consequences induced by mARs, we analyzed the rapid effects of T on the density of dendritic spines using Golgi staining. The application of 50 μg/5 μl T and 30 μg/5 μl DHT induced a rapid increase in the dendritic spines within 2 h. Almost no difference was observed between T and T-BSA in the effect on thorn density. Next, we explored the protective mechanism and found that T and DHT altered the expression of synaptophysin (SYN) and postsynaptic dense material 95 (PSD95), which play crucial roles in cognitive function and synaptic plasticity.

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1. Introduction

Androgens are important male sex steroid hormones that play a key role in a range of developmental and physiological processes leading to the maintenance of male reproductive organs and spermatogenesis as well as other phenotypes (Heemers et al., 2006; Smith and Walker, 2014). In the classical model of action, androgens diffuse through the plasma membrane, enter the cytoplasm, and bind to intracellular androgen receptors (iARs). iARs are members of the nuclear receptor superfamily, which act as ligand-activated transcription factors modulating the transcription of target genes (Heinlein and Chang, 2002a, b; Lee and Chang, 2003). The binding of androgens to iARs induces receptor dimerization, facilitating the ability of iARs to bind to specific DNA sequences, called hormone response element, and recruit coregulators to promote the expression of target genes. The biological actions of androgens via transcriptional regulation of target genes by the iARs are referred to as "genomic action". In addition to transcriptional or genomic mode of action, accumulating evidence has demonstrated that androgens can exert novel, non-classical effects in various cell types and tissues (Benten et al., 1999a, b; Castoria et al., 2003; Estrada et al., 2003; Kampa et al., 2002; Walker, 2010). This mechanism of androgen action became known as "non-genomic action". Although the molecular identity of membrane androgen-binding sites or receptors (mARs) remains elusive, non-genomic effects of androgens have now been convincingly demonstrated in several tissues, in particular in the reproductive, cardiovascular, immune and musculoskeletal systems.

Senescence-accelerated-prone mouse 8 (SAMP8) has been

Abbreviations: T, testosterone; DHT, dihydrotestosterone; iARs, intracellular androgen receptors; mARs, membrane androgen receptors; F, flutamide; SAMP8, senescence-accelerated-prone mouse 8; AD, Alzheimer's disease; RFI, relative fluorescence intensity; MFI, mean fluorescent intensity; SYN, synaptophysin; PSD, postsynaptic dense material; Ab, antibody.

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proposed as a suitable, naturally derived animal model for investigating the fundamental mechanisms of Alzheimer's disease (AD) (Butterfield and Poon, 2005; Cheng et al., 2014; Nomura and Ohkuma, 1999). In addition, serum testosterone (T) levels decrease quickly in the natural growth process of this model (Flood et al., 1995; Kang et al., 2014). Recently, we reported that androgen plays an important role in sustaining and regulating structural synaptic plasticity in the hippocampus of male SAMP8 mice, whereas androgen deficiency may contribute to the etiology of age-related cognitive impairment, corresponding to the decline in endogenous androgen production (Li et al., 2013). The hippocampus is attractive as a center of learning and memory and is one of the major target areas for the neuromodulatory actions of androgens (Choate et al., 1998; Kerr et al., 1995). There is little doubt that the hippocampus retains some degree of androgen-regulated neuroplasticity, and extensive studies have been performed to investigate their role in modulating hippocampal plasticity and function (Atwi et al., 2014; MacLusky et al., 2006; Ooishi et al., 2012a, b). Classical genomic actions modulate synaptic plasticity via gene transcription and the synthesis of synaptic proteins in neurons. Now, we focus on the problem of whether androgen mediates rapid, non-genomic synaptic plasticity in the hippocampus.

The current study investigated the presence and specificity of mARs of the hippocampus in SAMP8 mice. A fluorescent membrane impermeable testosterone-bovine serum albumin-fluorescein isothiocyanate (T-BSA-FITC) macromolecular was used to label mARs. To further verify whether these mARs are identical to classical iARs, we used flutamide (F, antagonist of the classical AR) and anti-AR antibody (Ab) to determine its binding specificity. Although biochemical, molecular and physiological studies have added to the understanding of the rapid, non-genomic actions of androgens, few studies have addressed how mARs induce synaptic plasticity in the hippocampus. Here we analyzed the rapid effects of T and dihydrotestosterone (DHT) on the density of dendritic spines, and T-BSA was also investigated. To explore the protective mechanisms and neurological basis of T and DHT, we determined whether they altered the expression of synaptophysin (SYN) and postsynaptic dense material 95 (PSD95), which play crucial roles in cognitive function and synaptic plasticity.

2. Materials and methods

2.1. Animals

Experiments were carried out in male SAMP8 mice, obtained from our breeding colonies and maintained as an inbred strain, which was 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 were 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. Surgery

Mice were deeply anesthetized with 6% chloral hydrate (5 mg/kg, i.p. injection) and placed in a motorized stereotaxic apparatus (StereoDrive, NeuroStar, Germany) equipped with a small animal adaptor to keep a flat skull. Cannula systems were implanted stereotaxically into the left lateral ventricle and were attached to the skull surface using dental cement. The stereotaxic coordinates guided by the software coordinates the motor movement of all 3 axes probe placements in relation to a 3-dimensional visual representation of a mouse (Watson, 3rd edition) Brain Atlas were: anterior–posterior (AP), −0.3 mm from bregma; medial–lateral (ML), 1.0 mm from midline; and dorsal–ventral (DV), −2.5 mm from the skull surface.

2.3. Drugs and microinjection procedure

After 7 days, intracerebroventricular (i.c.v.) injections were administered through the guide cannulae using injection needles (27-gauge) connected by polyethylene tubing to 5-μl Hamilton microsyringes. T-BSA-FITC (product code: T5771, Sigma–Aldrich, MO, USA) and BSA-FITC (Sigma–Aldrich, MO, USA) were dissolved in PBS. T (product code: 1616690, International Laboratory, CA, USA), T-BSA (product code: T3392, Sigma–Aldrich, MO, USA), DHT (product code: 2500981, International Laboratory, CA, USA) and F (product code: F0663, Tokyo Chemical Industry Co., Ltd, Japan) were dissolved in dimethyl sulfoxide (DMSO, 20 mg F dissolved in 1 ml DMSO). The drugs were injected at a rate of 1 μl per min. The needles were left in place for an additional 3 min to facilitate the diffusion of the drugs.

2.4. Flow cytometry analysis for the presence and quantification of mARs

Male 7-month-old SAMP8 mice were randomly divided into a control group, BSA-FITC group and T-BSA-FITC group. To ensure consistent dosing, only one batch of stock was used for all of the treatment groups. BSA-FITC was used as the control for the nonspecific FITC signal, whereas T-BSA-FITC measured the specific mAR signal. The mice were implanted with cannulas into the lateral ventricle and injected with PBS (0.5 h), BSA-FITC (0.5 h) and T-BSA-FITC (15 min, 0.5 h, 1 h and 2 h). Groups of mice (n = 5) were deeply anesthetized with 6% chloral hydrate and were rapidly decapitated. Hippocampal tissue samples were immediately dissected from the brains, and the cells were harvested, washed and resuspended in PBS. To determine the levels of FITC, the cells were analyzed with excitation at 488 nm and a 525 nm band-pass filter in the emission path. The mean fluorescent intensity (MFI) was derived using CellQuest Pro software by flow cytometry (BD FACS Calibur, USA). The relative fluorescence intensity (RFI) was calculated as the ratio of the MFI of specific staining to that of control staining.

2.5. Flow cytometry analysis for the binding specificity of mARs using iAR antagonist

Male 7-month-old SAMP8 mice were randomly divided into a control group, BSA-FITC group, T-BSA-FITC group and F + T-BSA-FITC group. Groups of mice (n = 5) were implanted with cannulas into the lateral ventricle and injected with PBS (0.5 h), BSA-FITC (0.5 h), T-BSA-FITC (0.5 h) and F + T-BSA-FITC (pre-administration of F for 0.5 h). The mice were deeply anesthetized with 6% chloral hydrate and rapidly decapitated. Hippocampal tissue samples were immediately dissected from the brains and the cells were harvested, washed and resuspended in PBS. The MFI was derived using CellQuest software by flow cytometry (BD FACS Calibur, USA). The RFI was calculated as the ratio of the MFI of specific staining to that of control staining.

2.6. Flow cytometry analysis for the binding specificity of mARs using anti-AR Ab

Male 7-month-old SAMP8 mice were randomly divided into membrane-intact groups (control group, FITC-IgG group and anti-AR Ab + FITC-IgG group) and permeabilized groups (control

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