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Research Report

Rapid increase of spines by dihydrotestosterone and testosterone in hippocampal neurons: Dependence on synaptic androgen receptor and kinase networks

Yusuke Hatanaka[a,1], Yasushi Hojo[a,b,1], Hideo Mukai[a,b], Gen Murakami[a,b], Yoshimasa Komatsuzaki[a], Jonghyuk Kim[a], Muneki Ikeda[a], Ayako Hiragushi[a], Tetsuya Kimoto[a], Suguru Kawato[a,b,*]

[a]Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan

[b]Bioinformatics Project of Japan Science and Technology Agency, The University of Tokyo, Tokyo, Japan

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ABSTRACT

Rapid modulation of hippocampal synaptic plasticity by locally synthesized androgen is important in addition to circulating androgen. Here, we investigated the rapid changes of dendritic spines in response to the elevation of dihydrotestosterone (DHT) and testosterone (T), by using hippocampal slices from adult male rats, in order to clarify whether these signaling processes include synaptic/extranuclear androgen receptor (AR) and activation of kinases. We found that the application of 10 nM DHT and 10 nM T increased the total density of spines by approximately 1.3-fold within 2 h, by imaging Lucifer Yellow-injected CA1 pyramidal neurons. Interestingly, DHT and T increased different head-sized spines. While DHT increased middle- and large-head spines, T increased small-head spines. Androgen-induced spinogenesis was suppressed by individually blocking Erk MAPK, PKA, PKC, p38 MAPK, LIMK or calcineurin. On the other hand, blocking CaMKII did not inhibit spinogenesis. Blocking PI3K altered the spine head diameter distribution, but did not change the total spine density. Blocking mRNA and protein synthesis did not suppress the enhancing effects induced by DHT or T. The enhanced spinogenesis by androgens was blocked by AR antagonist, which AR was localized postsynaptically. Taken together, these results imply that enhanced spinogenesis by DHT and T is mediated by synaptic/extranuclear AR which rapidly drives the kinase networks.

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*Corresponding author at: Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan. Fax: +81 3 5454 6517.
E-mail address: kawato@bio.c.u-tokyo.ac.jp (S. Kawato).

[1]Contributed equally to the present work.

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1. Introduction

Endogenous synthesis of dihydrotestosterone (DHT) and testosterone (T) has been demonstrated in male rat hippocampal neurons (Hojo et al., 2004). Mass-spectrometric analysis has revealed that the level of adult male hippocampal androgen is higher than that of plasma androgen (Hojo et al., 2009). These results suggest that hippocampal T (~17 nM) may be the sum of hippocampus-synthesized T (~3 nM) and circulating T (~14 nM), which penetrates into the hippocampus. The level of hippocampal DHT is approximately 7 nM which is much higher than that of circulating DHT (~0.6 nM). Therefore, it is important to investigate the modulating effects on synaptic plasticity by hippocampal androgen.

For over a decade, slow modulating effects on synaptic plasticity by circulating androgen have been investigated. Gonadectomy decreases spine–synapses in male rat hippocampus, and the replacement of DHT or T through subcutaneous injection rescues the level of spine–synapse density of CA1 neurons in gonadectomized rats, after three days (Leranth et al., 2003). Electrophysiological investigations in rat hippocampal slices have shown that T application rescues excitatory postsynaptic potentiation (EPSP), which is decreased by castration (Smith et al., 2002). For rats castrated during puberty, T decreases hippocampal long-term potentiation (LTP) in vivo (Harley et al., 2000), and an antagonist of androgen receptor (AR), flutamide, suppresses this reduction (Hebbard et al., 2003). Gonadectomized male rats show impaired cognitive performance, and androgen replacement rescues this impairment (Edinger and Frye, 2004; Frye and Seliga, 2001). A direct application of androgen to the rat hippocampus in vivo has anti-anxiety effects mediated via AR (Edinger and Frye, 2005, 2006).

Candidates of receptors for androgen action have been investigated in the hippocampus. The expression of AR in the hippocampal neurons (Brown et al., 1995; Simerly et al., 1990) implies that the hippocampal neurons are the target of DHT and T. In the CA1 area of the hippocampus, AR appears to be primarily located in the pyramidal neurons (Clancy et al., 1992; Kerr et al., 1995). AR is located not only in the cytoplasm and in the nuclei but also within dendritic spines and axon terminals (Tabori et al., 2005). These results suggest that androgen may have rapid effects on synaptic function via synaptic/extranuclear AR, in addition to slow genomic effect.

Molecular mechanisms of rapid effects of androgen in the hippocampus are, however, still largely unknown (Foradori et al., 2008; Hajszan et al., 2008), in contrast to deep understandings of estrogen-induced rapid synaptic effects. Leranth, MacLusky and their coworkers conclude that remodeling of spine–synapses by androgen is not driven via AR (Hajszan et al., 2008). Our earlier study has shown androgen-induced rapid increase of dendritic thorns in CA3 stratum lucidum via MAPK and PKC, but not via PKA (Hatanaka et al., 2009). The rapid effect of androgen on the spinogenesis may play an important role in memory processes via producing new spines for creating new neuronal contacts. Here, we performed experiments to test the hypothesis that androgen rapidly modulates dendritic spines via nongenomic signaling, including activation of synaptic/extranulear AR and several kinases (including MAPK, PKA, PKC, LIMK). We also investigated different effects of DHT and T on spine head diameter distribution.

2. Results

2.1. *Rapid effect of androgen on CA1 spinogenesis*

We investigated the effect of DHT and T on the modulation of the dendritic spine density and morphology in the hippocampal CA1 stratum radiatum. In addition to T, DHT (non-aromatizable androgen) was also used, because T may be partially converted into estrogen, estradiol (E2), by hippocampal endogenous aromatase (Hojo et al., 2004, 2008). Spine analysis was performed for secondary branches of the apical dendrites located 100–200 μm away from the pyramidal cell body, in the middle of the stratum radiatum of CA1 region.

2.2. *Time-dependence and dose dependence*

Following a 0.5–2 h treatment with DHT or T, treated dendrites significantly have more spines than control dendrites (i.e. with no DHT or T) (Fig. 1A). Time dependency of androgen treatment was demonstrated by treating slices for 0.5, 1 and 2 h with 10 nM DHT or 10 nM T (Fig. S1). The increasing effect on the total spine density was approximately proportional to the incubation time, showing 1.06 (0.5 h), 1.14 (1 h) and 1.28 spines/μm (2 h) in DHT-treatments, and 1.05 (0.5 h), 1.10 (1 h) and 1.32 spines/μm (2 h) in T-treatments. Dose dependency was also observed after 2 h incubation (Fig. S1). In DHT-treatment, the increasing effect on the total spine density was strongest at 10 nM DHT (1.28 spines/μm) as compared with 1 nM (1.17 spines/μm) and 100 nM (1.10 spines/μm) DHT. In T-treatments, the increasing effect was strongest at 10 nM T (1.32 spines/μm) compared with 1 nM T (1.28 spines/μm) and 100 nM T (1.29 spines/μm). Since 2 h treatments with 10 nM DHT and 10 nM T were most effective for spinogenesis, these incubation time and concentration were used in the following investigations unless specified (Fig. S1). Applied concentrations of DHT and T are similar to the concentrations of endogenous hippocampal DHT (~7 nM) and T (~17 nM) (Hojo et al., 2009).

Two hours treatments with 10 nM DHT and 10 nM T increased spines from 0.97 (control, i.e. with no DHT or T) to 1.28 (10 nM DHT) and 1.32 spines/μm (10 nM T). These results indicate that the enhancing effect of spinogenesis by DHT and T is nearly identical for affecting the spine density. Blocking androgen receptor (AR) by 1 μM hydroxyflutamide (HF), a specific inhibitor of AR, completely blocked the increasing effect by DHT and T on the spine density (Fig. 1B).

2.2.1. *Spine head diameter analysis*

The morphological changes in spine head diameter, induced by 2 h treatments, were assessed. We classified the spines into three categories depending on their head diameter: 0.2–0.4 μm as small-head spines, 0.4–0.5 μm as middle-head spines, and 0.5–1.0 μm as large-head spines (Fig. 1C). Small-, middle-, and large-head spines may be different in the number of AMPA receptors, and therefore these three types of spines may have different efficiency in signal transduction. The number of