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Androgen rapidly increases dendritic thorns of CA3 neurons in male rat hippocampus

Yusuke Hatanaka [a,c], Hideo Mukai [a,b,c], Kenji Mitsuhashi [a,c], Yasushi Hojo [a,b,c], Gen Murakami [a,c], Yoshimasa Komatsuzaki [a], Rei Sato [a,c], Suguru Kawato [a,b,c,*]

[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] Core Research for Evolutional Science and Technology Project of Japan Science and Technology Agency, The University of Tokyo, Japan
[c] Bioinformatics Project of Japan Science and Technology Agency, The University of Tokyo, Japan

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ABSTRACT

Modulation of hippocampal synaptic plasticity by androgen has been attracting much attention. Thorns of thorny excrescences of CA3 hippocampal neurons are post-synaptic regions whose presynaptic partners are mossy fiber terminals. Here we demonstrated rapid effects of dihydrotestosterone (DHT) and testosterone (T) on the density of thorns, by imaging Lucifer Yellow-injected neurons in adult male rat hippocampal slices. The application of 10 nM DHT or T induced rapid increase in the density of thorns within 2 h. The androgen-mediated increase was suppressed by blocking several kinases, such as Erk MAPK, p38 MAPK, PKC, and CaMKII. On the other hand, PKA, PI3K were not involved in the signaling of thorn-genesis. The increase in the thorn density by androgen was also blocked by the inhibitor of classical androgen receptor. Almost no difference was observed between DHT and T in the effect on the thorn density. We observed that the androgen-induced thorn-genesis is opposite to estrogen-induced thorn-degeneration.

Introduction

The hippocampus is essentially involved in learning and memory processes, and is known to be a target for the modulatory actions of androgen and estrogen from not only the gonads but also the hippocampus [1–6]. Extensive studies have been performed to investigate their role in modulating hippocampal plasticity and function, slowly and genomically (over 1–5 days). The density of dendritic spines of pyramidal neurons in the CA1 region of the hippocampus is modulated *in vivo* by the depletion and replacement of androgens [3]. Compared to the CA1 region (responsible for spatial memory), the effect of androgen on CA3 pyramidal neurons remains almost unknown. The CA3 was considered as a region where control associative memory [7,8]. In the stratum lucidum of the CA3, pyramidal neurons have huge and complex post-synaptic structures, named thorny excrescences. One thorny excrescence consists of multiple heads named thorns with one neck along a dendritic branch [9,10]. One mossy fiber terminal of dentate granule cells contacts multiple thorns of thorny excrescences of CA3 neuron. Thorny excrescences may play essential roles on hippocampal function. Chronic restraint stress has induced retraction of thorny excrescences, which has subsequently been reversed after water maze training. On the other hand, water maze training alone has increased the volume of thorny excrescence as well as the number of thorns per thorny excrescence [11].

As a preceding study, we observed that estradiol (estrogen) induced rapid decrease of thorns in CA3 stratum lucidum within 2 h, and this rapid estradiol modulation of thorny excrescences was mediated by Erk MAPK [4–6,12]. We here demonstrate that androgens rapidly induce the increase of the thorns by driving several kinases. Because testosterone (T) may be partially converted to estradiol by hippocampal endogenous aromatase [4,6,13–18], possible difference of dihydrotestosterone (DHT), non-aromatizable androgen, and T is also investigated.

Materials and methods

Animals. Twelve-week-old adult male Wistar rats were purchased from Saitama Experimental Animal Supply. All experiments using animals in this study were conducted according to the institutional guidelines.

Chemicals. Cyclosporin A, dihydrotestosterone, Lucifer Yellow CH, LY-294,002, SB203580, SP600125, testosterone, and U0126

* Corresponding author. Address: 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@phys.c.u-tokyo.ac.jp (S. Kawato).

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were purchased from Sigma (USA). Chelerythrine, KN-93, and Rottlerin were purchased from Calbiochem (Germany). H-89 was purchased from Biomol (USA). Hydroxyflutamide was purchased from Wako Pure Chemicals (Japan).

Current injection of Lucifer Yellow. Adult male rats were anesthetized with ethyl ether and decapitated. The brains were removed and placed in artificial cerebrospinal fluid (ACSF) at 4 °C. The hippocampus was dissected and 400 μm transverse slices to the long axis from the middle third of the hippocampus were cut with a vibratome (Dosaka, Japan). ACSF consisted of (mM): 124 NaCl, 5.0 KCl, 1.25 NaH_2PO_4, 2.0 $MgSO_4$, 2.0 $CaCl_2$, 22 $NaHCO_3$, and 10 glucose, and was equilibrated with 95% O_2/5% CO_2. Hippocampal slices were transferred into an incubating chamber containing ACSF held at 25 °C for 2 h for recovery. Slices were then incubated with 0.1–10 nM DHT or T together with several protein kinase inhibitors. Slices were then fixed with 4% paraformaldehyde in PBS at 4 °C overnight. Neurons within slices were visualized by an injection of Lucifer Yellow (Molecular Probes, USA) under Nikon E600FN microscope (Japan) equipped with a C2400-79H infrared camera (Hamamatsu Photonics, Japan) and with a 40× water immersion lens (Nikon, Japan). Dye injection was performed with glass electrode filled with 5% Lucifer Yellow for 15 min, using Axopatch 200B (Axon Instruments, USA). Approximately five neurons within a depth of 100–200 μm from the surface of a slice were injected with Lucifer Yellow [19].

Confocal laser scan microscopy and analysis. The imaging was performed from sequential z-series scans with confocal laser scan microscope (LSM5; Carl Zeiss, Germany) at high zoom (×3.0) with a 63× water immersion lens, NA 1.2. For Lucifer Yellow, the excitation and emission wavelengths were 488 nm and 515 nm, respectively. Three-dimensional image was reconstructed from approximately 40 sequential *z*-series sections of every 0.45 μm with a 63× water immersion lens, NA 1.2. The applied zoom factor (×3.0) yielded 23 pixels per 1 μm. The *z*-axis resolution was approximately 0.71 μm. The confocal lateral resolution was approximately 0.26 μm. Our resolution limits were regarded to be sufficient to allow the determination of the density of thorns. Confocal images were then deconvoluted using AutoDeblur software (AutoQuant, USA).

In each slice, 2–3 neurons with more than 100 thorns were analyzed, and at least 90 thorns were counted on each frame. In total, $N = 4–13$ neurons and $N = 300–1000$ total were analyzed for each drug treatment. The density of thorns was analyzed by tracing neurons with Neurolucida software (MicroBrightField, USA). The single apical dendrite which had thorns was analyzed separately. These dendrites were present within the stratum lucidum, within 100 μm from the soma. The density of thorns was calculated from the number of thorns along both primary and secondary dendrites having a total length of 20–100 μm. While counting the thorns in reconstructed images, the position and verification of thorns were aided by three-dimensional reconstructions and by observation of the images in consecutive single planes.

Statistical analysis. The significance of DHT, T, or drug effect was examined via statistical analysis using Tukey–Kramer post-hoc multiple comparisons test when one-way ANOVA tests yielded $P < 0.05$.

Result

We investigated the effect of DHT or T on the modulation of the thorn density in the hippocampus CA3 stratum lucidum. Lucifer Yellow-injected neurons in hippocampal slices from 12 week-old male rats were imaged using confocal laser scan microscopy (Fig. 1). Thorny excrescences were located on apical dendrites within 100 μm from the soma, on which mossy fiber terminals attached.

Androgens increased the density of thorns in CA3 stratum lucidum

Following a 0.5–4 h treatment with DHT or T, treated dendrites had significantly more thorns than control dendrites (i.e. with no DHT or T). Time dependency was examined by treating slices for

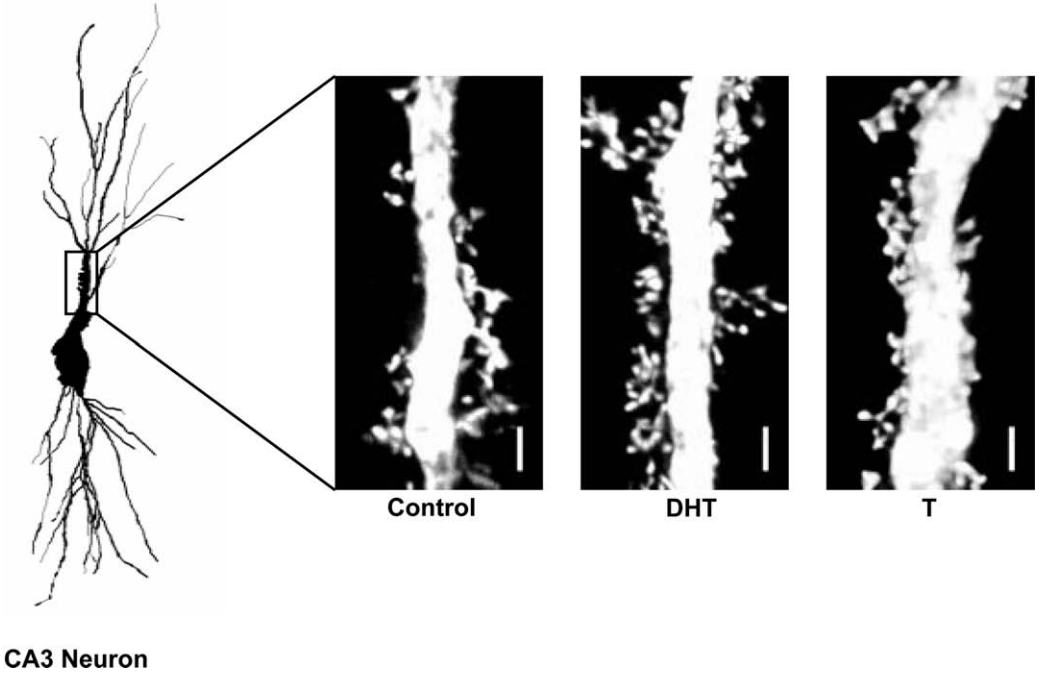

Fig. 1. Changes in the density of thorns by androgens in hippocampal slices. Maximal intensity projections onto *XY* plane from *z*-series confocal micrographs, showing thorns along the primary dendrites of hippocampal CA3 pyramidal neurons. Left image shows a traced whole image of Lucifer Yellow-injected CA3 neuron. Dendritic thorns without drug-treatments (Control), dendritic thorns after 10 nM DHT- (DHT) or T-treatment (T) for 2 h, bar 2 μm.

0.5, 1, 2, and 4 h with 10 nM DHT or 10 nM T. The enhancing effect on the total thorn density was approximately proportional to the incubation time, showing 2.6 (0.5 h), 3.0 (1 h), 3.2 (2 h), and 3.0 thorns/μm (4 h) in DHT-treatments, and 2.5 (0.5 h), 2.6 (1 h), 3.2 (2 h), and 3.1 thorns/μm (4 h) in T-treatments (Fig. 2A and B). Dose dependency was also examined after a 2 h incubation. In DHT-treatment group, the enhancing effect was most significant at 10 nM DHT (3.2 ± 0.2 thorns/μm) compared with 1 nM (2.5 thorns/μm) and 100 nM (3.1 thorns/μm) DHT. In T-treatment group, the enhancing effect was most significant at 10 nM T (3.2 ± 0.2 thorns/μm) compared with 1 nM (2.5 thorns/μm) and 100 nM (2.9 thorns/μm) T. Because a 2 h treatment with 10 nM DHT or 10 nM T was most effective for thorn-genesis, these incubation time and concentration were used in the following investigations unless specified. (Fig. 2C and D).

A 2 h treatment with DHT or T increased thorns from 2.2 thorns/μm (control, i.e. with no DHT or T) to 3.2 (10 nM DHT) or 3.2 thorns/μm (10 nM T). These results indicate that the enhancing effect of thorn-genesis by DHT and T is nearly identical. Blocking of androgen receptor (AR) by 1 μM hydroxyflutamide, a specific inhibitor of AR, completely abrogated the enhancing effect of DHT or T on the thorn density (2.2 or 2.2 thorns/μm, respectively). Washing DHT or T by treating slices with ACSF for another 2 h after DHT- or T-treatment abolished the effect of DHT or T (2.3 or 2.6 thorns/μm, respectively). (Fig. 3A and B).

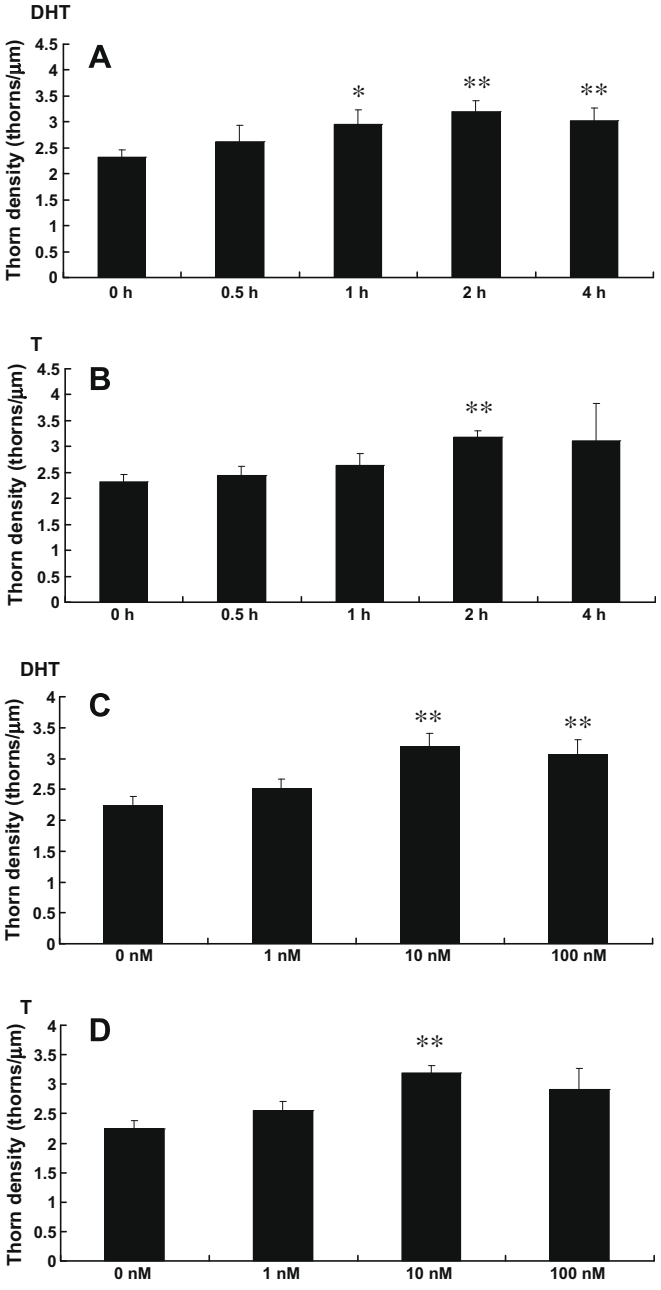

Fig. 2. Effect of androgens on the thorn density of CA3 neurons. (A and B) Time dependency of DHT and T. (A) No treatment with DHT (0 h), 0.5 h treatment (0.5 h), 1 h treatment (1 h), 2 h treatment (2 h), and 4 h treatment in ACSF with 10 nM DHT (4 h). (B) No treatment with T (0 h), 0.5 h treatment (0.5 h), 1 h treatment (1 h), 2 h treatment (2 h), and 4 h treatment in ACSF with 10 nM T (4 h). (C and D) Dose dependency of DHT and T. (C) A 2 h treatment in ACSF without DHT (0 nM), with 1 nM DHT (1 nM), with 10 nM DHT (10 nM), and with 100 nM DHT (100 nM). (D) A 2 h treatment in ACSF without T (0 nM), with 1 nM T (1 nM), with 10 nM T (10 nM), and with 100 nM T (100 nM). Vertical axis is the average number of thorns per 1 μm dendrite. Results are reported as mean ± SEM. The significance yielded $^{*}P < 0.05$, $^{**}P < 0.01$ to 0 h or 0 nM.

The effect of androgens was blocked by several kinase inhibitors

Next we investigated kinase signaling pathways involved in the androgen-induced thorn-genesis by using specific inhibitors for kinases. Blocking of Erk MAPK, by application of 25 μM U0126, abolished the DHT or T effect on the increase of the thorn density, resulting in 2.4 or 2.3 thorns/μm, respectively (Fig. 4A and B). Application of 10 μM SB203580, a p38 MAPK inhibitor, also prevented the effect by DHT or T resulting in 2.4 or 2.5 thorns/μm, respectively. Further, 1 μM KN-93, an inhibitor of CaMKII, reversed the effect of DHT or T (2.4 or 2.3 thorns/μm, respectively) (Fig. 4A and B). 1 μM cyclosporin A, an inhibitor of calcineurin (protein phosphatase 2B), abolished the effect of DHT or T (2.3 or 2.3 thorns/μm, respectively). On the other hand, 10 μM H-89, an inhibitor of protein kinase A (PKA), did not inhibit the enhancing effect but even increased the thorn density to 4.1 (DHT) or 4.1 (T) thorns/μm, respectively. A PI3 kinase inhibitor, 10 μM LY294002, also did not alter the increase of thorn density induced

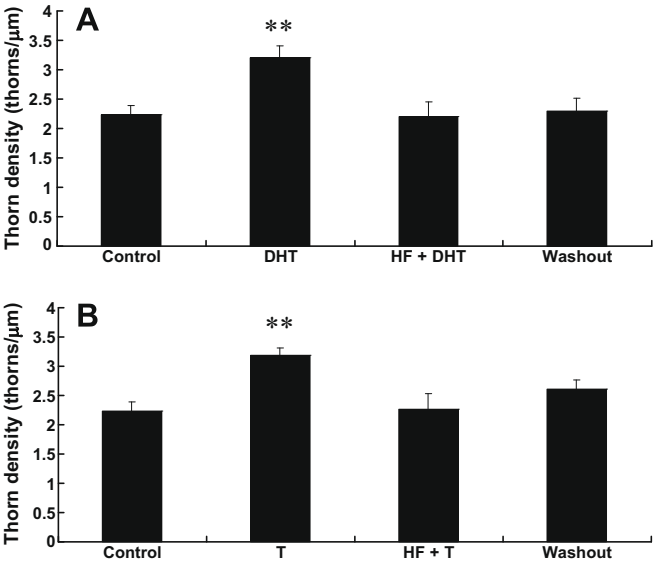

Fig. 3. Effect of receptor blocker on the thorn density in the CA3 neurons. (A) A 2 h treatment in ACSF without drugs (Control), with 10 nM DHT (DHT), with 10 nM DHT and 1 μM hydroxyflutamide (HF + DHT), and with 10 nM DHT followed by 2 h washout with ACSF (Washout). (B) A 2 h treatment in ACSF without drugs (Control), with 10 nM T (DHT), with 10 nM T and 1 μM hydroxyflutamide (HF + T), and with 10 nM T followed by 2 h washout with ACSF (Washout). Vertical axis is the average number of thorns per 1 μm. Results are reported as mean ± SEM. The significance yielded $^{*}P < 0.05$, $^{**}P < 0.01$.

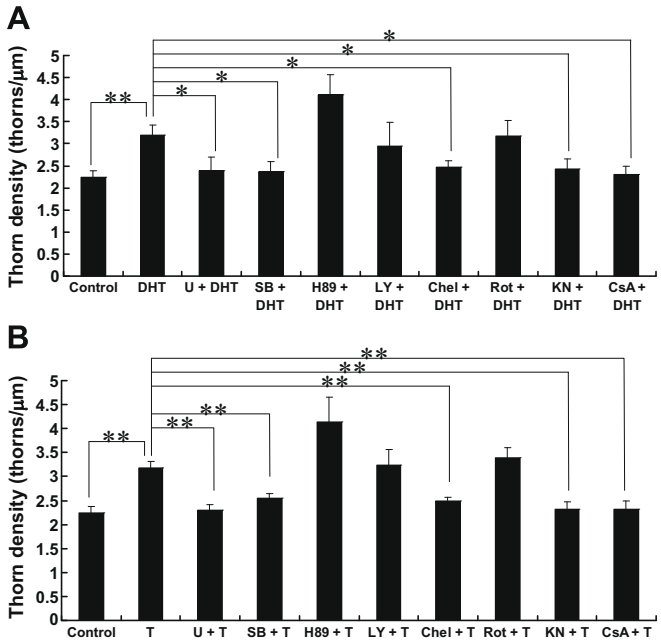

Fig. 4. Effect of kinase inhibitors on the changes in the density of thorns by DHT or T. (A) Effect of kinase inhibitors in the presence of DHT on the total thorn density in CA3 neurons. A 2 h treatment in ACSF without drugs (Control), with 10 nM DHT (DHT), with 10 nM DHT and 25 μM U0126 (Erk MAPK inhibitor) (U + DHT), with 10 nM DHT and 10 μM SB203580 (p38 MAPK inhibitor) (SB + DHT), with 10 nM DHT and 10 μM H-89 (PKA inhibitor) (H-89 + DHT), with 10 nM DHT and 10 μM LY294002 (PI3K inhibitor) (LY + DHT), with 10 nM DHT and 10 μM Chelerythrine (PKC inhibitor) (Chel + DHT), with 10 nM DHT and 5 μM Rottlerin (PKCδ inhibitor) (Rot + DHT), with 10 nM DHT and 1 μM KN-93 (CaMKII inhibitor) (KN + DHT), and with 10 nM DHT and 1 μM cyclosporin A (calcineurin inhibitor) (CsA + DHT). (B) Effect of kinase inhibitors in the presence of T on the total thorn density in CA3 neurons. A 2 h treatment in ACSF without drugs (Control), with 10 nM T (T), with 10 nM T and 25 μM (U + T), with 10 nM T and 10 μM SB203580 (SB + T), with 10 nM T and 10 μM H-89 (H-89 + T), with 10 nM T and 10 μM LY294002 (LY + T), with 10 nM T and 10 μM Chelerythrine (Chel + T), and with 10 nM T and 5 μM Rottlerin (Rot + T), with 10 nM T and 1 μM KN-93 (KN + T), and with 10 nM T and 1 μM cyclosporin A (CsA + T). Vertical axis is the average number of thorns per 1 μm. Results are reported as mean ± SEM. The significance yielded $^{*}P < 0.05$, $^{**}P < 0.01$.

by DHT or T (2.9 or 3.2 thorns/μm, respectively). When all subfamilies of protein kinase C (PKC) were blocked by 10 μM chelerythrine, a non-selective PKC subfamily inhibitor, the enhancing effect of DHT or T on the thorn density was considerably abrogated (2.5 or 2.5 thorns/μm, respectively). However, selective inhibition of PKCδ (by 5 μM rottlerin) did not suppress both DHT- and T-effect (3.2 and 3.4 thorns/μm, respectively).

Discussion

The current study demonstrated that the activation of AR by both DHT and T induced a rapid increase of thorns of thorny excrescences in CA3 pyramidal neurons of the adult male rat hippocampus. In addition, almost no difference was observed between DHT and T concerning thorn-genesis depending on kinase signaling. These results suggest that the conversion of T to estradiol is not significant within 2 h incubation with hippocampal slices. Androgen-induced changes have not been investigated in the stratum lucidum of the CA3, until the current study. An extremely concentrated distribution of thorny excrescences, as compared with sparse distribution of spines located in other regions, such as CA1, may have prevented detailed analysis of thorny excrescences by previous studies using Golgi staining methods [1,20]. We were able to analyze the number of thorns by the high-resolution image analysis of Lucifer Yellow-injected neurons, using deconvolution, and digital three-dimensional analysis. The rapid modulation of thorns observed in the current study is a novel phenomenon and is essential for consideration of the synaptic plasticity affected by androgen. There is much evidence that mossy fiber terminals originating from granule cells in the dentate gyrus provide excitatory inputs to CA3 neurons via thorny excrescences in the stratum lucidum [10,21–24]. Our data imply that DHT or T may significantly enhance the excitatory input to CA3 from dentate gyrus by increasing the density of thorns. Because the effect of T had been blocked by HF as well as that of DHT, we confirmed that T-effect was directly mediated by AR, not by its aromatase metabolite, estradiol. The expression of AR in the CA3 region has been demonstrated by immunoelectron microscopy as well as immunohistochemistry and *in situ* hybridization [25–27]. The intracellular distribution of AR has been found not only in the nuclei and cytoplasm but also in axon terminals and dendritic thorns [25], supporting the possibility that androgen-induced acute thorn-genesis may be mediated by extranuclear AR located in synapses.

It is important to compare the effect of androgen and estrogen on CA3 thorns. Our preceding study indicated that 1 nM estradiol application for 2 h significantly decreased the thorn density in hippocampal CA3 from 2.2 to 1.5 thorns/μm (by approximately 30%) [12]. On the other hand, current study revealed that androgens oppositely increased the thorns by approximately 50%. It is interesting that both androgen and estrogen activated Erk MAPK, but its outcomes on the thorn density are completely opposite. In this report, we demonstrated that androgen-induced thorn-genesis mediated by p38 MAPK, CaMKII, calcineurin, and PKC subfamilies, and that PKA, PI3K, and PKCδ were not included in the signaling. Such enzymes other than Erk MAPK may contribute to induce different effects of androgen and estrogen.

Over a decade, only a few reports have appeared regarding investigations of the effect of androgens on CA3 neurons. Neonatal castration has decreased the dendritic length, number of branches, and volume of neurons in adult hippocampal CA3, and androgens replacement has restored these deficits [28]. On the other hand, in the CA1 region, a number of investigations have been performed concerning the change in the spine density induced by androgens. Gonadectomy decreased the synapses in male rat hippocampus CA1, and the replacement of androgens by subcutaneous injection has restored the level of the total spine density after three days [3,29,30]. In addition to endocrine-derived hormones, we have recently demonstrated the endogenous *de novo* synthesis of androgens in male adult rat hippocampal neurons [13–15]. By using liquid chromatography tandem mass spectrometry, we have determined the concentrations of DHT and T in freshly isolated rat hippocampal slices to be 7 nM and 17 nM, respectively [4]. However, because the slices were incubated for 2 h in ACSF before exogenous androgen application, the concentration of androgens in the hippocampal slices decreased down to below 0.5 nM during the incubation. Accordingly, the increase of thorns after supplementation of 10 nM DHT or T in the current study may reflect restorative effect of DHT or T. These results imply that hippocampal neurons are exposed to local DHT and T, which might regulate synaptic plasticity rapidly.

References

[1] E. Gould, C.S. Woolley, M. Frankfurt, B.S. McEwen, Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood, J. Neurosci. 10 (1990) 1286–1291.
[2] C.S. Woolley, B.S. McEwen, Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat, J. Neurosci. 12 (1992) 2549–2554.
[3] C. Leranth, O. Petnehazy, N.J. MacLusky, Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats, J. Neurosci. 23 (2003) 1588–1592.

[4] Y. Hojo, G. Murakami, H. Mukai, S. Higo, Y. Hatanaka, M. Ogiue-Ikeda, H. Ishii, T. Kimoto, S. Kawato, Estrogen synthesis in the brain–role in synaptic plasticity and memory, Mol. Cell. Endocrinol. 290 (2008) 31–43.
[5] M. Ogiue-Ikeda, N. Tanabe, H. Mukai, Y. Hojo, G. Murakami, T. Tsurugizawa, N. Takata, T. Kimoto, S. Kawato, Rapid modulation of synaptic plasticity by estrogens as well as endocrine disrupters in hippocampal neurons, Brain Res. Rev. 57 (2008) 363–375.
[6] H. Ishii, T. Tsurugizawa, M. Ogiue-Ikeda, M. Asashima, H. Mukai, G. Murakami, Y. Hojo, T. Kimoto, S. Kawato, Local production of sex hormones and their modulation of hippocampal synaptic plasticity, Neuroscientist 13 (2007) 323–334.
[7] D.A. Henze, N.N. Urban, G. Barrionuevo, The multifarious hippocampal mossy fiber pathway: a review, Neuroscience 98 (2000) 407–427.
[8] M.R. Bennett, W.G. Gibson, J. Robinson, Dynamics of the CA3 pyramidal neuron autoassociative memory network in the hippocampus, Philos. Trans. R. Soc. Lond. B Biol. Sci. 343 (1994) 167–187.
[9] D.G. Amaral, A Golgi study of cell types in the hilar region of the hippocampus in the rat, J. Comp. Neurol. 182 (1978) 851–914.
[10] M.E. Chicurel, K.M. Harris, Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus, J. Comp. Neurol. 325 (1992) 169–182.
[11] M.G. Stewart, H.A. Davies, C. Sandi, I.V. Kraev, V.V. Rogachevsky, C.J. Peddie, J.J. Rodriguez, M.I. Cordero, H.S. Donohue, P.L. Gabbott, V.I. Popov, Stress suppresses and learning induces plasticity in CA3 of rat hippocampus: a three-dimensional ultrastructural study of thorny excrescences and their postsynaptic densities, Neuroscience 131 (2005) 43–54.
[12] T. Tsurugizawa, H. Mukai, N. Tanabe, G. Murakami, Y. Hojo, S. Kominami, K. Mitsuhashi, Y. Komatsuzaki, J.H. Morrison, W.G. Janssen, T. Kimoto, S. Kawato, Estrogen induces rapid decrease in dendritic thorns of CA3 pyramidal neurons in adult male rat hippocampus, Biochem. Biophys. Res. Commun. 337 (2005) 1345–1352.
[13] Y. Hojo, T.A. Hattori, T. Enami, A. Furukawa, K. Suzuki, H.T. Ishii, H. Mukai, J.H. Morrison, W.G. Janssen, S. Kominami, N. Harada, T. Kimoto, S. Kawato, Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons, Proc. Natl. Acad. Sci. USA 101 (2004) 865–870.
[14] T. Kimoto, T. Tsurugizawa, Y. Ohta, J. Makino, H. Tamura, Y. Hojo, N. Takata, S. Kawato, Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: *N*-methyl-D-aspartate and calcium-dependent synthesis, Endocrinology 142 (2001) 3578–3589.
[15] K. Shibuya, N. Takata, Y. Hojo, A. Furukawa, N. Yasumatsu, T. Kimoto, T. Enami, K. Suzuki, N. Tanabe, H. Ishii, H. Mukai, T. Takahashi, T.A. Hattori, S. Kawato, Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction, Biochim. Biophys. Acta 1619 (2003) 301–316.
[16] H. Mukai, N. Takata, H.T. Ishii, N. Tanabe, Y. Hojo, A. Furukawa, T. Kimoto, S. Kawato, Hippocampal synthesis of estrogens and androgens which are paracrine modulators of synaptic plasticity: synaptocrinology, Neuroscience 138 (2006) 757–764.
[17] H. Mukai, T. Tsurugizawa, M. Ogiue-Ikeda, G. Murakami, Y. Hojo, H. Ishii, T. Kimoto, S. Kawato, Local neurosteroid production in the hippocampus: influence on synaptic plasticity of memory, Neuroendocrinology 84 (2006) 255–263.
[18] G. Murakami, N. Tanabe, H.T. Ishii, M. Ogiue-Ikeda, T. Tsurugizawa, H. Mukai, Y. Hojo, N. Takata, A. Furukawa, T. Kimoto, S. Kawato, Role of cytochrome p450 in synaptocrinology: endogenous estrogen synthesis in the brain hippocampus, Drug Metab. Rev. 38 (2006) 353–369.
[19] H. Duan, S.L. Wearne, J.H. Morrison, P.R. Hof, Quantitative analysis of the dendritic morphology of corticocortical projection neurons in the macaque monkey association cortex, Neuroscience 114 (2002) 349–359.
[20] C.S. Woolley, E. Gould, M. Frankfurt, B.S. McEwen, Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons, J. Neurosci. 10 (1990) 4035–4039.
[21] D.G. Amaral, N. Ishizuka, B. Claiborne, Neurons, numbers and the hippocampal network, Prog. Brain Res. 83 (1990) 1–11.
[22] B.J. Claiborne, D.G. Amaral, W.M. Cowan, A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus, J. Comp. Neurol. 246 (1986) 435–458.
[23] D.G. Amaral, J.A. Dent, Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions, J. Comp. Neurol. 195 (1981) 51–86.
[24] T.H. Brown, D. Johnston, Voltage-clamp analysis of mossy fiber synaptic input to hippocampal neurons, J. Neurophysiol. 50 (1983) 487–507.
[25] N.E. Tabori, L.S. Stewart, V. Znamensky, R.D. Romeo, S.E. Alves, B.S. McEwen, T.A. Milner, Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation, Neuroscience 130 (2005) 151–163.
[26] L. Xiao, C.L. Jordan, Sex differences, laterality, and hormonal regulation of androgen receptor immunoreactivity in rat hippocampus, Horm. Behav. 42 (2002) 327–336.
[27] J.E. Kerr, R.J. Allore, S.G. Beck, R.J. Handa, Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus, Endocrinology 136 (1995) 3213–3221.
[28] C. Isgor, D.R. Sengelaub, Effects of neonatal gonadal steroids on adult CA3 pyramidal neuron dendritic morphology and spatial memory in rats, J. Neurobiol. 55 (2003) 179–190.
[29] E.G. Kovacs, N.J. MacLusky, C. Leranth, Effects of testosterone on hippocampal CA1 spine synaptic density in the male rat are inhibited by fimbria/fornix transection, Neuroscience 122 (2003) 807–810.
[30] N.J. MacLusky, T. Hajszan, J.A. Johansen, C.L. Jordan, C. Leranth, Androgen effects on hippocampal CA1 spine synapse numbers are retained in Tfm male rats with defective androgen receptors, Endocrinology 147 (2006) 2392–2398.