学 位 論 文 の 要 旨

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学 位 論 文 名　Changes in Pituitary Gonadotropin Subunits and Hypothalamic Kiss-1 Gene Expression by Administration of Sex Steroids in Ovary-Intact Female Rats
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論 文 内 容 の 要 旨

INTRODUCTION

The pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play pivotal roles in female reproductive function under the control of hypothalamic gonadotropin-releasing hormone (GnRH). Kisspeptin neurons, which are located in two different areas of the hypothalamus, monitor the serum levels of sex steroids and modulate the secretion of GnRH. In rodents, kisspeptin neurons located in the anteroventral periventricular nucleus (AVPV) are involved in the estradiol (E2)-induced surge secretion of GnRH (positive feedback), whereas those in ARC region are involved in the negative feedback mechanism. Progesterone (P4) and androgens also participate in the control of the HPG axis by acting on the hypothalamus. In this study, we examined the direct action of the sex steroids E2, P4, and one of the androgens, dihydrotestosterone (DHT) using LβT2, a mouse gonadotroph cell model. Furthermore, we administered these sex steroids to ovary-intact rats and investigated the changes in gonadotropin subunit expression and examined the alteration in *Kiss1* gene (which encodes kisspeptin) and *Gnrh* gene expression in hypothalamic regions.

MATERIALS AND METHODS

Cell culture

LβT2 cells were incubated with or without (control) the test reagents (E2, P4, and DHT) at the indicated concentrations for 48 h in high-glucose DMEM containing 10% heat-inactivated FBS and 1% penicillin–streptomycin.

Luciferase assay

Reporter constructs, which were generated by fusing −846/0 of the human common glycoprotein alpha (*Cga*) gene, −797/+5 of the rat *Lhb* gene, or −2000/+698 of the rat *Fshb* gene to firefly luciferase (Luc) cDNA in pXP2. The cells were transiently transfected via electroporation with 2.0 μg/well gonadotropin subunit-Luc and 0.1 μg pRL-TK. After incubation with the

indicated concentration of E2, P4, and DHT for 6 h, Firefly Luc activity was measured and normalized to that of *Renilla* Luc to correct for transfection efficiency.

RNA preparation, reverse transcription, and quantitative RT-PCR

Total RNA was extracted using TRIzol-LS and 1.0 μg total RNA was reverse transcribed using oligo-dT primers and prepared cDNA using a First-Strand cDNA Synthesis Kit. *Cga, Lhb,* and *Fshb* subunits as well as *Kiss1* and *Gnrh* mRNA levels were ascertained using quantitative real-time (RT)-PCR. *Gapdh* mRNA was used to normalize the amount of cDNA added per sample.

***In vivo* experiments using ovary-intact rats**

Seven-week-old female wistar rats vaginal smears were assessed daily to evaluate their estrous cycle. After observation for 1 week, a pellet containing 0.25 mg E2 or 50 mg P4 was implanted subcutaneously. For DHT administration, the rats received a daily subcutaneous injection of 25 mg/kg DHT in 140 μL sesame oil. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

Western blot analysis

Proteins from the anterior pituitary or hypothalamus were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were incubated with an anti-rabbit polyclonal GCA/hCGα antibody, anti-rabbit polyclonal LHβ antibody, anti-rabbit monoclonal FSHβ antibody, or anti-mouse monoclonal kisspeptin antibody. An anti-β-actin antibody was used as an internal control.

Hormone measurement

Blood was collected at sacrifice. Serum LH was measured using a Rat LH ELISA Kit. Values are reported as mIU/mL of whole blood.

Statistical analysis

Data were analyzed statistically using Student's t-test, one-way ANOVA, and Bonferroni's post hoc test. Statistical significance was assessed at a threshold of $P < 0.05$.

RESULTS AND DISCUSSION

In the *in vitro* experiments using LβT2 gonadotroph cells, E2 increased the promoter activity of the *Cga* and *Lhb* subunits as well as their mRNA expression; however, E2 did not increase *Fshb* subunit expression. P4 also increased *Cga* and *Lhb* gene expression but had no effect on the *Fshb* gene. DHT inhibited *Cga* and *Lhb* promoter activity and mRNA expression but had no effect on *Fshb*. These observations indicate that the sex steroids E2 and P4 can stimulate, but DHT inhibited *Cga* and *Lhb* gene expression by themselves at the pituitary levels.

Estrous cycle was disrupted by exogenous E2 administration in ovary-intact female rats. In addition, P4 or DHT administration completely eliminated the estrous cycle. Both E2 and P4 administration inhibited the expression of the CGA, LHβ, and FSHβ subunits in the anterior pituitary gland. Serum LH levels were also reduced by E2 or P4 administration. In contrast, DHT

did not modulate gonadotropin subunit expression or serum LH levels *in vivo*. It is reasonable to think that the disruption or elimination of the estrous cycle in E2- or P4-treated rats depends on the decrease of gonadotropin subunit expression and concomitant decrease of LH serum levels *in vivo*; however, the estrous cycle was abolished by DHT administration, even though DHT did not reduce gonadotropin subunit expression or LH levels *in vivo*.

We examined the changes in *Kiss1* gene expression in tissues from the anterior or posterior parts of the hypothalamus, which include the AVPV and ARC regions, respectively. *Kiss1* gene expression in the anterior part of the hypothalamus was significantly repressed by E2 and P4 treatment. However, *Kiss1* gene in the posterior part of the hypothalamus was suppressed by E2 but not by P4. The observation that P4 inhibited *Kiss1* gene expression in the AVPV but not in the ARC indicates that P4 treatment disrupts the normal positive feedback mechanism and prevents GnRH/LH surge-induced ovulation. It is plausible that in addition to the decrease in gonadotropin subunit expression, the rat estrous cycle was eliminated partly because the LH surge and subsequent ovulation were not induced by P4. DHT administration disrupted the estrous cycle without decreasing gonadotropin subunit expression in ovary-intact rats. In addition, *Kiss1* gene expression in hypothalamic tissues from the anterior and posterior parts was not decreased by DHT treatment. These observations suggest that the basal release of kisspeptin and kisspeptin-regulated GnRH secretion are not altered by DHT, which was supported by our observation that gonadotropin subunit expression as well as serum levels of LH were not altered by DHT. DHT-induced morphological changes in vaginal smears might lead to the mischaracterization of the estrous cycle.

We also examined *Gnrh* gene expression in the anterior hypothalamic regions. *Gnrh* gene expression in the hypothalamus appeared to be repressed by treatment with E2 and P4, although the difference was significant only with P4. DHT administration slightly increased *Kiss1* gene expression but had no effect on GnRH gene expression in the anterior hypothalamus. We suspect that both E2 and P4 decrease *Gnrh* gene expression in the hypothalamus because hypothalamic *Kiss1* was decreased by E2 in both the anterior and posterior parts of the hypothalamus and by P4 in the anterior hypothalamus. Because kisspeptin from the Kiss1 neuron governs GnRH synthesis and release, *Gnrh* gene expression should be repressed with a concomitant decrease in *Kiss1* gene expression by E2 and P4 within the hypothalamus.

CONCLUSION

Our findings indicate that female and male sex steroids affect the pituitary and hypothalamus, but they have divergent effects at different levels of the HPG axis. It is plausible that sex steroids, especially E2 and P4, strongly affect the hypothalamus and modulate gonadotropin secretion and regulate reproductive functions.