学 位 論 文 の 要 旨

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学 位 論 文 名　Role of Activin, Follistatin and Inhibin in the Regulation of Kiss-1 Gene Expression in Hypothalamic Cell Models

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論 文 内 容 の 要 旨

INTRODUCTION

The hypothalamic-pituitary-gonadal (HPG) axis regulates the modulation and maintenance of reproductive functions. In mammals, early studies on the regulation of the HPG axis have emphasized the pivotal role of gonadotropin-releasing hormone (GnRH) neurons located in the preoptic area (POA) of the hypothalamus. After the discovery of the loss of function of the gene encoding the receptor for the hypothalamic peptide kisspeptin (Kiss1R), it is generally agreed that kisspeptin, which is produced in hypothalamic Kiss-1 (the gene that encodes kisspeptin) neurons, is positioned at the highest level in the HPG axis and controls the release of GnRH from neurons. In rodents, Kiss-1 neurons are mainly located in two different areas of the hypothalamus, the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC). Kiss-1 neurons in these two areas are implicated in the estradiol (E2)-induced positive and negative feedback mechanisms based on the observations that Kiss-1 expression in the AVPV nucleus is upregulated by E2, whereas those within the ARC nucleus is repressed by E2. The HPG axis also consists of activin, inhibin and follistatin produced by the gonads. Activin has been identified as a gonadal peptide that stimulates follicle-stimulating hormone (FSH) secretion from the pituitary. In contrast, inhibin and follistatin have an antagonistic effect on activin and reduce FSH synthesis and secretion. Inhibin competitively binds the activin receptor, thus suppressing activin activity. Furthermore, these ovarian peptides also modulate pituitary gonadotropin synthesis and secretion, suggesting that these peptides also participate in the control of the HPG axis. In this study, we used two hypothalamic Kiss-1-expressing cell models and examined the effects of activin, inhibin and follistatin on the expression of kisspeptin.

MATERIALS AND METHODS

Cell culture and stimulation

mHypoA-55 cells are a model for mouse Kiss-1-expressing neurons in the ARC region of the hypothalamus. Kiss1-expressing cells that originated from the AVPV region of mouse hypothalamus were named mHypoA-50. Both cells were plated in 35-mm tissue culture dishes and stimulated with the test reagents in high-glucose DMEM containing 1% heat-inactivated FBS and 1% penicillin-streptomycin for the indicated concentrations and time periods.

RNA preparation, reverse transcription, and real-time quantitative RT-PCR

Total RNA from untreated or treated mHypoA50 or mHypoA55 cells was extracted using the extraction method Trizol-S. To obtain cDNA, 1.0 μg of total RNA was reverse transcribed using an oligo-dT primer, and was prepared using a First Strand cDNA Synthesis Kit in reverse transcription (RT) buffer. Messenger RNA (mRNA) was reverse transcribed into single stranded cDNA. Quantification of Kiss-1, inhibin α, inhibin βA, inhibin βB subunit and follistatin mRNA was obtained through real-time quantitative PCR using specific primer for mouse.

Western blotting

The cell extracts were subjected to SDS-PAGE in 10% acrylamide gel and the protein was transferred onto polyvinylidene difluoride membranes. Membranes were incubated with anti-kisspeptin antibody, anti-inhibin α antibody, anti-inhibin βA antibody, anti-βB antibody, or anti-follistatin. Fetal rat brain tissue or rat ovary was used as positive control. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

Statistical analysis

Data are expressed as means ± SEM. Statistical analysis was performed using Student t-test or one-way repeated (ANOVA) with the Bonferroni post hoc test, as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

First, we examined the effect of activin, follistatin and inhibin on Kiss1 mRNA expression in the ARC cell model mHypoA-55. Activin stimulation significantly increased Kiss-1 mRNA expression. Follistatin and inhibin A both of which are known to act as antagonists for activin

reduced basal expression of Kiss-1 in mHypoA-55 cells. Kisspeptin expression was increased by activin and reduced by follistatin and inhibin A at protein level. Follistatin and inhibin A also had an inhibitory effect on activin-induced Kiss-1 gene expression. These observations suggest that gonadal peptides, activin, follistatin and inhibin A also participate in the regulation of kisspeptin in the hypothalamus.

Next, we tested the effect of follistatin and inhibin A on kisspeptin-10 (KP10) or GnRH-induced Kiss-1 gene expression. Both KP10 and GnRH significantly increased the expression of Kiss-1 mRNA in mHypoA-55 ARC cells. Significant increases in Kiss-1 mRNA induced by KP10 or GnRH were almost completely abolished in the presence of follistatin and inhibin A. This observation explains that follistatin and inhibin A also prevents the increase in Kiss-1 gene expression induced by stimulants other than activin.

We also examined the effect of activin, follistatin and inhibin A on mHypoA-50, a Kiss-1-expressing AVPV cell models. In contrast with the phenomenon observed in the mHypoA-55 ARC cell model, activin did not increase Kiss-1 gene expression in mHypoA-50 cells. Both follistatin and inhibin A did not modulate Kiss-1 gene expression in mHypoA-50 cells, implying that these peptides do not have a pivotal role in E2-induced positive feedback mechanisms.

In both hypothalamic ARC cell model mHypoA-55 and AVPV cell model mHypoA-50, inhibin α, inhibin βA, inhibin βB subunits and follistatin genes and proteins were detected by RT-PCR analysis and western blotting analysis. Inhibin subunits and follistatin genes expressed in mHypoA-55 ARC cells were influenced by the sex steroid E2. Stimulation of E2 at 10 nM failed to increase the expression of these three inhibin subunits and follistatin. However, a higher concentration of E2 did induce changes in the expression of some of these peptides in these cells. Inhibin α subunit gene expression was significantly increased by stimulation of 100 nM E2 compared with nontreated cells. However, inhibin βA and βB subunits were not significantly increased when cells were treated with 100 nM E2. Follistatin gene expression in mHypoA-55 cells was significantly upregulated by E2 compared with non-treated cells. These results suggesting that activin's effects or the inhibitory effect of inhibin A and follistatin on Kiss-1 expression is influenced by E2 within the ARC region of the hypothalamus.

CONCLUSION

Our observations using the hypothalamic Kiss-1-expressing cell model from the ARC region, mHypoA-55, we found that activin could increase the expression of Kiss-1, whereas both follistatin and inhibin A could decrease Kiss-1 expression. The activin/inhibin/follistatin system might work not only in pituitary FSH regulation, but also at the level of the hypothalamus, and maintain the HPG axis.