学 位 論 文 の 要 旨

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学 位 論 文 名　Role of Kisspeptin and Kiss1R in the Regulation of Prolactin Gene Expression in Rat Somatolactotroph GH3 Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Hypothalamic kisspeptin, which is encoded by Kiss1 gene, is a known principal activator of gonadotropin-releasing hormone neurons and governs the hypothalamic-pituitary-gonadal axis. Previous reports have shown that kisspeptin is also released into the hypophyseal portal circulation and directly affects the anterior pituitary. Because pituitary gland also expresses the Kiss1 and kisspeptin receptor (Kiss1R), pituitary hormones might also be under the influence of kisspeptin in an autocrine and/or paracrine fashion. Prolactin released from pituitary lactotroph play variety of roles to maintain reproductive functions. Although prolactin is mainly under the inhibitory control of hypothalamic dopamine neurons, hypothalamic factors such as thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide (PACAP) also participate in the regulation of prolactin as prolactin releasing factors. In addition, kisspeptin may play role as pituitary hormone releasing factor. In this study, we examined the direct effect of kisspeptin on pituitary prolactin-producing GH3 cells.

MATERNAL AND METHODS

Cell culture

GH3 cells were plated in 35-mm tissue culture dishes and incubated in high-glucose DMEM containing 10% heat-inactivated FBS and 1% penicillin-streptomycin at 37℃ in a humidified atmosphere of 5% CO2 in air. After 24 h, the culture medium was changed to

high-glucose DMEM containing 1% heat-inactivated FBS and 1% penicillin-streptomycin and incubated without (control) or with test reagents for the indicated times.

Receptor overexpression

Cells were transiently transfected via electroporation with either Kiss1R or PACAP type I receptor (PAC1R) expression vectors. An empty vector (pCI neo) served as the mock control.

Western blotting

The cell extracts were subjected to SDS-PAGE in 10% acrylamide gel and transferred onto polyvinylidene difluoride membranes. The membranes were incubated with anti-Kiss1R antibody. Extracts from rat anterior pituitary tissue and COS7 cells, which are devoid of Kiss1R were used as positive and negative control.

Transfection and luciferase assay

The prolactin promoter reporter constructs used were generated by fusing -609/+12 of the prolactin gene to firefly luciferase cDNA in pGL3 (PRL-Luc). GH3 cells were transiently transfected by electroporation with PRL-Luc and pRL-TK which contains renilla luciferase under the herpes simplex virus thymidine kinase promoter. After stimulation, firefly luciferase and renilla luciferase activities were measured in the supernatants with the Dual-Luciferase Reporter Assay System using a luminometer. To determine the extracellular signal-related kinase (ERK)- and cAMP/protein kinase A (PKA)-mediated signaling activity, pSRE-Luc and pCRE-Luc were applied.

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

Total RNA from untreated or treated GH3 cells was extracted. For the detection of Kiss1R mRNA, after PCR amplification using primers for Kiss1R amplicons were electrophoresed in a 2.0% agarose gel and visualized with ethidium bromide staining. Quantification of Kiss1R and prolactin mRNA was obtained through real-time quantitative PCR utilizing Universal ProbeLibrary Probes and FastStart Master Mix.

Statistical analysis

All experiments were independently repeated at least three times. Data are expressed as the mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test. $p < 0.05$ was considered statistically significant. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULT AND DISCUSSION

We examined whether GH3 cells express Kiss1R. RT-PCR analysis using specific primers for Kiss1R revealed that Kiss1R mRNA could be detected in the extracts from rat anterior pituitary tissues as well as GH3 cells. Western blotting analysis using anti-Kiss1R antibody revealed that Kiss1R protein was also expressed in GH3 cells. GH3 cell express Kiss1R, however, in these cells, kisspeptin failed to stimulate prolactin-promoter activity. We postulated that endogenous Kiss1R was reduced or not functional, probably due to cell immortalization or multiple passages in these immortalized-cell models. Therefore, we used GH3 cells overexpressing Kiss1R as a prolactin-producing cell model in our experiments to determine the effect of kisspeptin. When GH3 cells overexpressed Kiss1R, kisspeptin clearly increased prolactin-promoter activity. In addition, both SRE- and CRE- promoters were dramatically activated by kisspeptin stimulation in these cells. These observations suggested that the overexpressed Kiss1R coupled with Gq and Gs proteins and increased both ERK and cAMP/PKA signaling pathways. In Kiss1R overexpressing GH3 cells, kisspeptin did not potentiate TRH-induced prolactin-promoter activity, but it potentiated the PACAP-induced prolactin-promoter activity. We presume that combined treatment with kisspeptin and TRH did not enhance their prolactin-producing ability because Kiss1R and the TRH receptor share common signaling pathways that are mainly initiated by Gq protein and PLC. Kisspeptin-stimulated prolactin-promoter activity was increased by the amount of Kiss1R overexpression. Endogenous Kiss1R mRNA expression in GH3 cells was significantly increased by treatment with estradiol (E2), but not by TRH. In addition, kisspeptin's ability to stimulate prolactin-promoter activity was restored after E2 treatment in non-transfected GH3 cells. From these observations, we speculated that the increase in Kiss1R number under the influence of E2 might introduce some other influences on prolactin-producing cells.

CONCLUSION

In our study, we used somatolactotroph GH3 cells overexpressing Kiss1R to examine the direct effect of kisspeptin on prolactin-producing cells. We found that Kiss1R is expressed in a pituitary prolactin-producing cell model and obtained evidence that kisspeptin has a direct effect on this cell by stimulating prolactin production. Furthermore, we showed that E2 plays an important role in modulating kisspeptin's effect on these cells. Our current observations suggest an important role of kisspeptin/Kiss1R in the regulation of pituitary lactotroph functions.