# 学 位 論 文 の 要 旨

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| 学　位　論　文　名 | Role of Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and Its PACAP Type I Receptor (PAC1R) in the Regulation of Prolactin Synthesis in Rat Pituitary Somatolactotroph Cells |
| 発　表　雑　誌　名<br>（巻，初頁～終頁，年） | 別紙のとおり |
| 著　者　名 | 別紙のとおり |

## 論 文 内 容 の 要 旨

## INTRODUCTION

Prolactin is secreted by lactotrophs and smatolactotrophs in the pituitary gland. GH3 cells are a clonal strain derived from rat pituitary tumor cells that synthesize and secrete both prolactin and growth hormone. Since these cells have many properties common to normal lactotrophs and somatolactotrophs, they are frequently used as a model to study the regulation of prolactin secreting cells. Although it is well known that dopamine, a prolactin inhibiting factor, largely participates in the control of prolactin release, prolactin secretion is also regulated by several hypothalamic hormones such as thyrotropin-releasing hormone (TRH). TRH, a primary secretagogue for thyrotropin-stimulating hormone from thyrotrophs, also regulates prolactin synthesis and secretion, although it is unclear at present whether TRH is a physiological regulator of prolactin. Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated from extracts of ovine hypothalamus on the basis of its ability to stimulate cAMP formation in rat pituitary cells. Because PACAP immunostained fibers are present in the median eminence and PACAP is detected in the hypophysial portal blood, PACAP was thought to work as an hypophysiotropic factor. Previous studies suggest that PACAP might regulate prolactin; however, the stimulatory effect of PACAP on prolactin synthesis and release in single prolactin-producing cells is inconclusive in experimental models. In this present study, we investigated the action of PACAP and the function of PACAP type I receptor (PAC1R) in single clones of prolactin-producing cells using somatolactotroph, GH3 cells. In addition, we investigated the relation between TRH receptor and PAC1 receptor.

It is well known phenomenon that continuous exposure of pituitary gonadotropin-secreting cells to gonadotropin-releasing hormone (GnRH) leads to desensitization of GnRH receptors and

decreased gonadotropin secretion. Similar to the case with the GnRH receptors, it was reported that prolonged treatment with TRH also decreases the number of its own receptors in GH3 cells. To examine how the functions of TRH receptor and PAC1R changed after prolonged treatment of GH3 cells with TRH and PACAP, we determined the effect of TRH and PACAP on prolactin promoter activity after prolonged treatment with these peptides.

## MATERIALS AND METHODS

### *Cell culture*

GH3 cells, a rat prolactinoma cell line, 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 °C in a humidified atmosphere of 5% $CO_2$ 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 or with test reagents for the indicated times.

### *Western blotting*

The cell extracts were subjected to SDS-PAGE in 10% acrylamide gel and the protein was transferred onto polyvinylidene difloride membranes. The membranes were incubated with anti-PAC1R or phosphorylated-extracellular signal-regulated kinases (ERK) antibody. For total ERK determination, after strip washing, membranes were re-probed with anti-ERK antibody.

### *Transfections and luciferase assays*

The reporter constructs used ware generated by fusing -609/+12 of the prolactin gene to firefly luciferase cDNA in pGL3. GH3 cells were transiently transfected by electroporation with pRL-TK vector containing renila luciferase. When PAC1R expressed to GH3 cells, PAC1R expressing vector (HA-tagged PAC1/pEF-BOS in pCAM17) was used. In some experiments, serum response element (Sre)-luciferase or c-AMP response element (Cre)-luciferase was transfected. Luciferase activities were measured using luminometer and determined by the ratio of firefly to the Renilla luciferase activities.

### *Real-time quantitative RT-PCR*

Total RNA from untreated or treated GH3 cells was extracted using the extraction method Trizol-S. Messenger RNA (mRNA) was reverse transcribed into single stranded cDNA. Quantification of prolactin, growth hormone and PAC1R mRNA was obtained through real-time quantitative PCR using specific primers for prolactin, growth hormone and PAC1R.

### *Statistical analysis*

Values were expressed as means±SEM. Statistical analysis was performed using Student t-test or one-way repeated ANOVA followed by Dunnett test or Newman-Keuls test for multiple comparisons. $P<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

In GH3 cells, the effect of PACAP was dose dependent on prolactin promoter activity; however, this effect was limited. After transfect PAC1R expressing vectors to the GH3 cells, PACAP increased prolactin promoter activity dramatically compared with mock transfected cells,

and the increases were PAC1R dose dependent. These results suggest that the response to PACAP depends on the density of PAC1R on the cell surface. In GH3 cells, TRH increased prolactin promoter activite as expected. PACAP failed to modify the action of TRH in mock transfected GH3 cells. On the other hand, in PAC1R expressing GH3 cells, PACAP stimulated prolactin promoter activity and modified TRH action. Because ERK activation is important for the induction of prolactin promoter, we examined ERK phosphorylation by western blotting. Both TRH and PACAP increased ERK phosphorylation and the combination of TRH and PACAP had a more effect on ERK phosphorylation. TRH increased prolactin mRNA expression and decreased growth hormone (GH) mRNA level in GH3 cells. In contrast to the action of TRH, PACAP increased both GH and prolactin mRNA expression in GH3 cells. PACAP significantly increased its own PAC1R gene expression and protein expression in GH3 cells, but TRH didn't modified PAC1R expression. In PAC1R expressed GH3 cells, the effect of TRH on prolactin promoter and ERK phosphorylation were further potentiated compared to the effect of TRH in mock transfected cells. These results hypothesis that existence of PAC1R itself might augment the ability of TRH to stimulate prolactin synthesis.

Next we examined the function of TRH receptor and PAC1R after prolonged treatment with TRH or PACAP. TRH and PACAP stimulated prolactin promoter activity as described above, and this effect was almost abolished by pretreatment for 48 h with TRH or PACAP in PAC1R transfected GH3 cells. In PAC1R-transfected cells, PACAP and TRH significantly increased both activity of the serum response element (SRE) promoter and cAMP response element (CRE) promoter. After pretreatment for 48 h with TRH or PACAP, the basal activity of the SRE promoter and CRE promoter was increased but the response to both TRH and PACAP was eliminated. These results suggest that prolonged treatment of TRH or PACAP desensitized their own receptors. Additionally, pretreatment with TRH desensitized the functions of the receptor for PACAP; whereas pretreatment with PACAP desensitized TRH receptor functions. These observations demonstrated that sustained stimulation with TRH and PACAP desensitizes their own and each other's receptors.

## CONCLUSION

PACAP had a stimulatory effect on prolactin gene expression in pituitary prolactin producing cells when PAC1R was adequately expressed. As PACAP/PAC1R has a direct effect on pituitary prolactin producing cells, it is likely a regulator of prolactin synthesis and secretion in physiological conditions. Actions of TRH were also potentiated in the presence of PAC1R. Also we have found that prolonged pretreatment with both TRH and PACAP desensitized their own and each other's receptor functions.

別紙

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| 論　文　名 | 1. Stimulatory effect of pituitary adenylate-cyclase activating polypeptide (PACAP) and its PACAP type I receptor (PAC1R) on prolactin synthesis in rat pituitary somatolactotroph GH3 cells.<br>2. Prolonged stimulation with thyrotropin-releasing hormone and pituitary adenylate cyclase-activating polypeptide desensitize their receptor functions in prolactin-producing GH3 cells |
| 発表雑誌名<br>（巻，初頁～終頁，年） | 1. Molecular and Cellular Endocrinology, 339:172-179, 2011<br>2. Molecular and Cellular Endocrinology, 365:139-145, 2013 |
| 著　者　名 | 1. Mijiddorj T, Kanasaki H, Purwana IN, Oride A, Miyazaki K<br>2. Mijiddorj T, Kanasaki H, Purwana I, Unurjargal S, Oride A, Miyazaki K |