学 位 論 文 の 要 旨

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学 位 論 文 名 **Dopamine D_2 Receptor Expression and Regulation of Gonadotropin α-Subunit Gene in Clonal Gonadotroph LβT2 Cells**

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論 文 内 容 の 要 旨

INTRODUCTION

In human, lactotrophs and gonadotrophs have close physical relationship. However, in contrast to the lactotrophs, whose primary regulation is through the negative influence of dopamine, the gonadotrophs are stimulated to produce gonadotropin mainly by hypothalamic GnRH. Another hypothalamic peptide, PACAP also plays a role in stimulating pituitary hormones. Stimulation of c-AMP formation in rat pituitary cells by PACAP may exert its effect on gonadotropin mRNA expression via the cAMP/protein kinase A (PKA) pathway. Giving the close proximity of the gonadotrophs to the lactotrophs, we propose that hypothalamic dopamine may also have an effect on gonadotropin secretion. Indeed in our previous study, the gonadotroph cell line αT3-1, which also posseses dopamine D_2 receptors were responded to GnRH stimulation with gonadotropin α-subunit production, and is negatively regulated by dopamine. It is unclear, however whether dopamine will have a similar effect in a cell capable of synthesizing and secreting the complete repertoire of gonadotropin. This study was performed to characterize the dopamine receptor in LβT2 gonadotrophs, a more developed gonadotroph and to investigate the action of dopamine agonist on gonadotropin promoter activity. Finally, we examined the possible signaling pathway involved and how they regulate gonadotropin synthesis.

MATERIALS AND METHODS

Culture of LβT2 cells

LβT2 cells were cultured in high-glucose DMEM containing 10% heat inactivated fetal bovine serum, 1% penicillin-streptomycin and incubated at 37°C in a humidified atmosphere of 5% CO_2 in 95% air.

Reverse transcription-polymerase chain reaction of dopamine D_2 receptor

Total RNA was prepared from LβT2 cells. Messenger RNA (mRNA) was reversed transcribed into single stranded c-DNA. The reaction mixtures were diluted 20-fold and then subjected to PCR amplification of dopamine D_1 and dopamine D_2 receptor. The PCR products were separated by electrophoresis on a 1.0% agarose gel and stained with ethidium bromide.

Western blotting

The cell extracts were subjected to SDS-PAGE in a 10% acrylamide gel and transferred to nitrocellulose membrane. The membrane was incubated with anti-dopamine D_2 receptor antibody.

Transfection and luciferase assay

LβT2 cells were transiently transfected by electroporation with LHβ-Luc, FSHβ-Luc, or α-GSU-Luc which contains firefly luciferase and PRL-TK which contains Renilla luciferase under the herpes simplex virus thymidine kinase promoter. After 48 h of incubation, cells were treated with chemicals in each experiment, the activities of firefly luciferase and Renilla luciferase were measured by the Dual Luciferase Reporter Assay System with a luminometer.

Measurement of cyclic-AMP accumulation

Cells were plated in 96-well plates at a density of 10^5 cells/well. After 48 h of incubation, cells were treated with chemical as indicated and intracellular c-AMP levels were measured using the c-AMP enzyme immunoassay system.

Statistical evaluation

Values were expressed as means ± SE. Statistical analysis was performed using one-way ANOVA plus Duncan multiple range test. Values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

By RT-PCR and western blotting analysis we identified the dopamine D_2 receptor existence in LβT2 cells. To investigate the dopamine D_2 receptor function, cells were treated with 100 nM GnRH resulted in an increase of all gonadotropin promoter activities, ultimately 10.0 ± 0.3 fold for α-subunit, 7.0 ± 0.4 fold for LHβ and 5.3 ± 0.9 fold for FSHβ. Quinpirol inclusion in ranging concentration from 10 nM – 10μM had no effect on gonadotropin subunit expressions. By PACAP stimulation, only α-subunit promoter was activated to 2.5 ± 0.27 fold but both LHβ and FSHβ were not activated. The stimulatory effect of PACAP on α-subunit promoter was significantly reduced with quinpirol as well as apomorphine. These results suggest that the inhibitory effect of quinpirol in α-subunit promoter activation mediated by the dopamine D_2 receptor takes place only during a stimulus in which c-AMP accumulation is elevated. H89 addition significantly inhibited PACAP-induced α-subunit promoter activation which suggests that c-AMP kinase is involved in the suppression effect. PACAP stimulation increased cAMP accumulation dramatically up to between 10 ± 4-fold and 60 ± 10-fold. GnRH also increased c-AMP accumulation about 1.6 ± 0.25-fold, which is quite modest compared to that of PACAP. The addition of quinpirol strongly inhibited PACAP action to raise c-AMP level, moreover in the presence of quinpirol alone, the intracellular c-AMP level was significantly (75%) reduced. Other dopamine agonists, apomorphine and bromocryptine also showed similar inhibition result, although neither of them alone suppressed basal c-AMP level.

CONCLUSION

In this study, we confirmed the presence of dopamine D_2 receptors in gonadotroph LβT2 cells. Although our results showed ineffective action of quinpirol on GnRH-induced gonadotropin gene expression, the expression of gonadotropin α-subunit which depends on an elevation of intracellular c-AMP is under the inhibitory control of dopamine D_2 receptors. Neither the PKC/MAPK dependent element nor the subtle elevation of c-AMP involved in GnRH signaling participated in the dopamine mediated inhibition of gonadotropin gene expression.