学 位 論 文 の 要 旨

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学 位 論 文 名　Activation of Extracellular Signal-Regulated Kinase (ERK) and Induction of Mitogen-Activated Protein Kinase Phosphatase-1 (MKP-1) by Thyrotropin-Releasing Hormone (TRH) Stimulation, and Their Role for Prolactin Gene Expression in Rat Pituitary GH3 Cells

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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. Thyrotoropin-releasing hormone (TRH), which is secreted from the hypothalamus, is a potential stimulator of prolactin synthesis and secretion from anterior pituitary. TRH stimulates inositol phospholipid metabolism by activating its membrane receptors, which results in the protein kinase C (PKC) activation, and finally increases extracellular signal-regulated kinase (ERK) activation. ERK is widely distributed in eukaryotes as a serine/threonine protein kinase, and there is much evidence supporting the involvement of ERK in TRH-induced prolactin gene expression in prolactin-secreting cells. ERK is activated by reversible phosphorylation of tyrosine and tyreonine residues by upstream MEK and inactivated by dephosphorylation of residues by its physiologic regulators, the mitogen-activated protein kinase phosphatases (MKPs). MKP-1 is the most ubiquitously expressed and well studies MKP to date. In this study, we focused on ERK activation and MKP-1 protein expression by TRH stimulation. We examined how ERK was activated, and how MKP-1 was induced by different patterns of TRH stimulation using rat pituitary somatolactotroph cell line, GH3 cells. In addition, the pharmacological blocker of MKP-1, triptolide and MKP-1 siRNA were used to clarify the

interaction among MKP-1, ERK, and prolactin synthesis.

MATERIALS AND METHODS

Cell culture and Perifusion system

GH3 cells were plated in perifusion chambers mounted on glass slides having been previously coated with Matrigel. The cells were the incubated for 24 h in high-glucose DMEM contain 10% heat-inactivated fetal bovine serum (FBS) and 1 % penicillin-streptomycin at 37℃ in a humidified atmosphere of 5 % CO_2 in air. The chambers were then mounted in perifusion system and continuously perifused with high-glucose DMEM containing 1 % heat-inactivated FBS and 1% penicillin-streptomycin at a constant flow rate of 0.25 ml/min. TRH pulses ware delivered by a set of peristaltic pumps controlled by a time controller. In the static culture experiments, GH3 cells were cultured in the presence of TRH in 1% heat-inactivated FBS and 1% penicillin-streptomycin containing DMEM for indicated times at 37℃ in a humidified atmosphere of 5 % CO_2 in 95 % air.

Western blotting

The cell extracts were subjected to SDS-PAGE in 10% acrylamide gel and protein was transferred onto polyvinylidene difloride membranes. The membranes were incubated with either phospho-ERK antibody, or MKP-1 antibody. For non-phospho-ERK (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. After incubation firefly luciferase and renilla luciferase activities were measured in the supernatants with the dual-luciferase reporter assay system, using luminometer. To study the effect of gene knockdown, MKP-1 siRNA was co-transfected.

Statistical analysis

All experiments were independently repeated at least three times. Values were expressed as means ± SEM. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In static culture, ERK activation by continuous TRH stimulation increased significantly and reached its maximal level at 10 min and persisted for 60 min, with a return to the basal level by 2 h. Stimulation with continuous TRH in perifused cells resulted in a similar level of ERK phosphorylation. In contrast, MKP-1 was expressed 60 min following either static or perifused, in continuous TRH stimulation. Using the perifusion system, cells were stimulated with palsatile

TRH, at a frequency of one pulse every 30 min. ERK phosphorylation occurred 10 min after the start of a TRH pulse and rapidly decreased to the basal level at 20 min. ERK was phosphorylated again with each subsequent pulsatile TRH pulse did not induce MKP-1 expression. Prolactin promoter activity followed continuous, static TRH stimulation was higher than that following perifused, continuous TRH stimulation. TRH at a frequency of one pulse every 30 min increased prolactin promoter activity similar to that of perifused, continuous TRH stimulation. Additionally, changes in pulse frequency resulted in alterations in the level of prolactin promoter. Following static stimulation, a 10 min exposure of TRH was sufficient to obtain full activation of prolactin promoter. Additionally, a 5-10 min exposure of TRH was sufficient to maintain ERK activation. A single 5-min pulse of TRH stimulation resulted in low activation of prolactin promoter.

Next, we investigated the possible role for MKP-1 in TRH-induced prolactin gene expression. MKP-1 protein was induced significantly from 60 min after static TRH stimulation, and remained elevated at 4 h. The effect of TRH on MKP-1 expression was completely prevented in the presence of specific MEK inhibitor, U0126. In the experiments using triptolide, MKP-1 induction by TRH was completely inhibited in a dose-dependent manner. TRH-induced ERK activation was significantly enhanced in this condition. Prolactin promoter activity, activated by TRH, was reduced to the control level in the presence of triptolide in a dose-dependent manner. In addition, GH3 cells which were transfected with MKP-1 specific si-RNA, both the basal and TRH-stimulated activities of the prolactin promoter were significantly reduced compared to the cells transfected with negative control si-RNA. The basal activity of prolactin promoter was significantly reduced compared to the negative control.

CONCLUSION

In the present study, we examined ERK phosphorylation and MKP-1 expression following different patterns of TRH stimulation. The different mode of TRH delivery differently regulate ERK activation. In the transient activation of ERK following 5-min pulse of TRH, dephosphorylation by MKP-1 was not involved. Although ERK is obviously a key kinase for prolactin transcriptional activity, duration of ERK activation were not obligatory for full activation of prolactin promoters. There is insufficient evidence, however, to conclude that the TRH induction of ERK phosphorylation is only mechanism for prolactin gene induction. We also shown that TRH increased the dual phosphatase MKP-1 by ERK dependent manner, resulted in inactivated ERK through dephosphorylation. Thus, inhibition of MKP-1 by its blocker, tritolide, potentiated ERK activation. However, because TRH-induced prolactin promoter activity was completely prevented in the presence triptolide, and it was also inhibited by MKP-1 siRNA expression, MKP-1 induced by TRH functions not only as an ERK-inactivating phosphatase, but also as an important mediator in prolactin gene expression regulation.

別紙

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論　　文　　名

1. Activation of Extracellular Signal-Regulated Kinase (ERK) and Induction of Mitogen-Activated Protein Kinase Phosphatase-1 (MKP-1) by Perifused Thyrotropin-Releasing Hormone (TRH) Stimulation in Rat Pituitary GH3 Cells

2. Possible Involvement of Mitogen-Activated Protein Kinase Phosphatase-1 (MKP-1) in Thyrotropin-Releasing Hormone (TRH)-Induced Prolactin Gene Expression

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1. Molecular and Cellular Endocrinology 296, 78-86, 2008

2. Biochemical and Biophysical Research Communications 382, 663-667, 2009

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