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Involvement of growth hormone secretagogue receptor (GHS-R1a) in regulation of RFRP-3 and GPR147 mRNA expression in hypothalamus of male rats

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Introduction

Growth hormone secretagogues (GHSs), including some natural and synthetic peptidyl or non-peptide molecules, exhibit somatotrope-secretion activity at the pituitary and hypothalamus levels. Ghrelin is one of these secretagogues molecules. It is well established that ghrelinerghic system has an inhibitory effect on reproductive activity. RFamide-related peptide-3 (RFRP-3) is one of the most important neuropeptides that regulates the mammalian reproduction. Studies have indicated the presence of the ghrelin receptor, called the growth hormone secretagogue receptor (GHS-R1a), in the RFRP-3 containing areas in the brain. Thus, it may be possible that ghrelinergic system involves in the control of reproduction through the

RFRP-3. The aim of this study was to investigate the possible role of the GHS-R1a receptors in the regulation of RFRP-3 and its receptor GPR147 mRNA expression in the hypothalamus of male rats.

Methods & Materials

Forty male Wistar rats were divided in 8 groups (n=5) and each group received saline, 2, 4 or 8 nmol ghrelin (the agonist of GHS-R1a), 5, 10 or 20 nmol D-Lys3-GHRP-6 (DLS) (the antagonist of GHS-R1a), or concomitantly of ghrelin (4 nmol) and DLS (10 nmol) via the stereotaxically implanted cannula to 3th cerebroventricle (AP = - 2.3, ML = 0.0, DV = 6.5). The hypothalamus of rats was dissected 2h after treatment for evaluation of RFRP-3 and GPR147 mRNA levels and stored at -80 ˚C untile evaluation. Total RNA from hypothalami was extracted using a commercial RNA extraction kit, based on the kit's instructions (Denazist Asia, Iran). Then, the complementary DNA (cDNA) synthesis and amplification were carried out using the RT-PCR Kit (Vivantis Technologies, Malaysia). The RT-PCR was run by a thermocycler (Bio-Rad, USA) using a reaction mixture containing the primers RFRP-3, GPR147, GAPDH. In this process, the reaction mixture contains 5 ng of template cDNA, 0.5 µl of each forward and reverse primer (10 pM), 1 unit (U) of Taq DNA polymerase, 0.75 µl $MgCl_2$ (50 mM), 2.5 µl of PCR buffer (10 X), 0.5 µl of a mixture of 10 mM dNTPs and diethyl pyrocarbonate (DEPC) treated water, and the final volume was 20 µl. PCR-amplified products mixed with the safe-red solution were analyzed on 1.5 percent agarose gel using electrophoresis (Bio-Rad Co., USA). Then, the bands were visualized under UV light and quantified by ImageJ software (version 1.41, USA).

All data were analyzed by SPSS software (version: 21.00) by one-way analysis of variance (ANOVA) and Tukey's post-hoc test. A difference at $P < 0.05$ was considered statistically significant.

Results & Discussion

The results showed that treatment with 2 nmol ghrelin increased the relative RFRP-3 mRNA expression compared to the saline group, but this effect was not significant ($P > 0.05$). Relative RFRP-3 mRNA expression significantly ($P < 0.01$) increased following the 4 nmol ghrelin injection, while the 8 nmol ghrelin injection exacerbated the increasing effects of ghrelin and significantly ($P < 0.001$) enhanced the RFRP-3 mRNA level compared to the saline group. Also, 5 or 10 nmol of DLS had no significant effects ($P > 0.05$) on the RFRP-3 mRNA expression compared to the saline group. Relative RFRP-3 mRNA expression significantly ($P < 0.01$) decreased following the injection of 20 nmol DLS compared to the saline-received animals. Co-administration of 4 nmol ghrelin and 10 nmol DLS had no significant effect ($P > 0.05$) on RFRP-3 mRNA level as compared to the saline group. Furthuremore, the results showed that 2, 4 or 8 nmol ghrelin increased the GPR147 mRNA expression compared to the saline group, but these effects were not significant ($P > 0.05$). Also, 5, 10 or 20 nmol DLS had decreasing but not significant effects ($P > 0.05$) on the GPR147 mRNA expression compared to the saline group. The results showed that injection of 4 nmol ghrelin increased the GPR147 mRNA levels in hypothalamus, while the injection of 10 nmol DLS decreased this parameter; but these effects were not significant ($P > 0.05$). Co-administration of 4 nmol ghrelin and 10 nmol DLS did not exert changes on GPR147 mRNA level in comparison to the saline group ($P > 0.05$). In conclusion, results indicated that 4 nmol ($P < 0.05$) or 8 nmol ($P < 0.01$) ghrelin injection significantly increased the RFRP-3 mRNA expression compare to saline group. While, the injection of 20 nmol DLS significantly ($P < 0.05$) decreased the RFRP-3 mRNA level compare to saline group. Pretreatment of ghrelin-received animals with DLS prevented the increasing effects of ghrelin on RFRP-3 gene expression. Ghrelin or DLS had no significant effects on hypothalamic GPR147 mRNA levels. The presence of ghrelin receptors, GHS-R1a, in the dorsomedial nucleus of the hypothalamus (an area containing the RFRP-3 expressing neurons) suggests that GHS-R1a may be involved in regulating the mammalian reproductive function through the synthesis and release of RFRP-3 in the hypothalamus. Therefore, in the present study, we investigated whether

the ghrelinergic system could affect the RFRP-3 expression and its functional receptors GPR147. In this regard, the present study showed for the first time that the GHS-R1a agonist, ghrelin, dose-dependently increased the RFRP-3 mRNA expression in the hypothalamus of male rats. Furthermore, the GHS-R1a antagonist, DLS, dose-dependently decreased the hypothalamic RFRP-3 mRNA expression. Also, it was demonstrated that the pretreatment of the animals receiving the lowest effective concentration of ghrelin with the highest ineffective concentration of DLS prevented the additive effects of ghrelin on RFRP-3 gene expression. These results suggest that Growth hormone secretagogues's receptor, GHS-R1a, may beinvolved in the regulation of RFRP-3 synthesis in the hypothalamus.

Conclusion

Considering the ghrelin receptor distribution in the hypothalamic areas containing RFRP-3 neurons, and the parallel effects of ghrelin and RFRP-3 on many physiological processes, such as food intake and the reproductive axis, the present results suggest that the ghrelinergic system may act at upstream of the RFRP-3/GPR147 system and affects the reproductive axis through the up-regulation of this system; also, GHS-R1a receptors are involved in regulating the expression of hypothalamic RFRP-3 and GPR147 genes, at least in male rats.

Keywords: *D-Lys3-GHRP-6, Ghrelin, Hypothalamus, R*reproduction, *RFamide-related peptide-3*

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