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Kisspeptin Increases Luteinizing Hormone Secretion in Food Deprived D-Lys3-GHRP6-Treated Rats

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Abstract

Background: Kisspeptin stimulates the reproductive axis while food deprivation or ghrelin inhibits it. The aim of the study was to determine the effects of third cerebral ventricle injection of kisspeptin on mean plasma luteinizing hormone (LH) levels in food deprived D-Lys3-GHRP6-treated rats. **Materials and Methods:** In this experimental study, five fed rats received third cerebral ventricular injection of saline at 09:00- 09:30 h. Also, twenty food deprived rats in four groups (n=5 in each group) received third cerebral ventricular injection of saline, kisspeptin (1nmol), D-Lys3-GHRP-6 (20nmol) or D-Lys3-GHRP-6 (20nmol) and kisspeptin (1nmol). Blood samples were collected via the tail vein. Plasma LH concentration was measured by using LH kit and the method of radioimmunoassay (RIA). **Results:** Mean plasma LH level in food-deprived rats decreased significantly compared to fed rats. Kisspeptin significantly increased the mean plasma LH concentration compared to fed or food deprived saline group. Injection of kisspeptin in D-Lys3 -GHRP-6-pre-treated rats significantly increased the mean plasma LH concentration compared to fed saline, food deprived saline, alone kisspeptin or alone D-Lys3 -GHRP-6 groups. **Conclusion:** Decrease of ghrelin pathway activity may partly be involved in the mediating the stimulatory effects of kisspeptin on hypothalamic-pituitary-gonadal axis. [GMJ. 2017;6(1):39-43]

Keyword: Kisspeptin; D-Lys3 -GHRP-6; Food Deprivation; Luteinizing Hormone

Introduction

Hypothalamic arcuate nucleus (ARC) is a major part of the brain in regulating energy balance and reproduction. Several different neural pathways including kisspeptin and ghrelin integrate into the ARC nucleus to control sexual hormones secretions [1]. G-protein coupled receptor 54 (GPR54) is expressed in ARC nucleus and also on gonadotropin-releasing hormone (GnRH) neurons. It is necessary for normal hypothalamic-pituitary-gonadal (HPG) axis function [2-3]. Kisspeptin is

the endogenous ligand of GPR54 and central or peripheral injection of it increases pulsatile GnRH/luteinizing hormone (LH) release and male or female sexual hormones secretions in humans, rodents, ruminants and so on [4-7]. Ghrelin is a 28 amino acid peptide which exerts its physiological functions via growth hormone secretagogues receptor 1a (GHS-R1a) [8-9]. Ghrelin is synthesized mainly in the stomach, hypothalamus especially ARC and paraventricular nucleus (PVN) and other central and peripheral tissue [10]. Ghrelin receptor is expressed in high density in the ventro-

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medial hypothalamus (VMH), ARC, PVN and other brain and peripheral organs [11]. It has been established that VMH and ARC nucleus include GnRH pulse generator which controls LH secretion [12]. Peripheral ghrelin crosses the blood brain barrier. It enters the brain, and it increases food intakes and suppresses the reproductive function [13-14]. The D-Lys3-GHRP-6 is a synthetic peptide which acts as a GHSR-1a receptor antagonist [15-17], and central or peripheral injection of it inhibits food intakes and body weight in food deprived or ghrelin-treated mice [15]. Previous studies have been shown that ghrelin downregulates kisspeptin gene expression [26] and exogenous injections of kisspeptin decrease ghrelin secretion [18-19]. In the present study, the effects of kisspeptin were investigated on mean plasma LH concentration in food deprived D-Lys3-GHRP6-treated rats.

Materials and Methods

Animals

In the current experimental study, male Wistar rats ($n=25$) weighing 230-250g (provided by the Neuroscience Research Center of Shahid Beheshti University, Iran) were housed in the cages under controlled temperature ($22\pm 2^\circ\text{C}$) and 12h light/dark cycle. Animals had free access to food and water all the time except in the start of the experiment which rats were food deprived for 24 hours before the experiment. All procedures for the maintenance and the use of experimental animals were approved by the ethical committee of Neuroscience Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Intra Cerebral Ventricular (ICV) Cannulation and Injections

Animals were anesthetized by intraperitoneal (IP) injection of a mixture of Ketamine and Xylazine (Ketamine 80 mg/kg BW + Xylazine 10 mg/kg BW). A 22-gauge stainless cannula was implanted in the third cerebral ventricle according to coordinates of Paxinos and Watson Atlas (AP = -2.3, ML = 0.0, DV = 6.5). After one-week recovery period, five fed rats in one group received ICV injection of saline in a volume of $3\mu\text{l}$ at 09:00-09:30 h. Also, twenty

food deprived rats in four groups ($n=5$ in each group) received saline, kisspeptin (1nmol), D-Lys3-GHRP-6 (20nmol) or kisspeptin (1nmol) and D-Lys3-GHRP-6 (20nmol) in a volume of $3\mu\text{l}$. Kisspeptin10 (Ana Spec Co, USA) and D-Lys3-GHRP-6 (Ana Spect, U.S.A) were dissolved in saline. The peptides were injected by a 27-gauge stainless steel injector (protruded 1mm beyond the cannula to reach the third ventricle) which connected to Hamilton microsyringe by PE-20 tubing. In co-administrated group D-Lys3-GHRP-6 was injected 15min before kisspeptin10 injection.

Hormones Assays

Blood samples were collected in a volume of 0.5cc at 60min following injections via tail vein. Heparin was used to the samples to prevent clotting. Blood samples immediately centrifuged to 15 min at 3000 rpm and the plasma stored at -20°C until assayed for LH concentration. Plasma LH concentration was measured by using LH kit (Institute of Isotopes Co., Ltd, Budapest, Hungary) and the method of radioimmunoassay (RIA).

Statistical Analyses

The results are presented as mean \pm SEM. The data were analyzed by one-way-ANOVA test. The comparison of the mean plasma LH levels between the different groups was done by Tukey's test. The SPSS software (version 16) was used to analyze the data. A $P\leq 0.05$ was set as significant level.

Results

The results demonstrated that the mean plasma LH concentration in food-deprived rats decreased significantly by 0.45 times compared to fed rats ($P\leq 0.05$, Figure-1). Injection of 1nmol kisspeptin significantly increased the mean plasma LH concentration by 1.07 or 2.8 times compared to fed or food deprived saline group respectively ($P\leq 0.05$, Figure-1). Injection of 20nmol D-Lys3-GHRP-6 increased the mean plasma LH concentration by 0.24 times compared to the fed saline group which this increase was not statistically significant (Figure-1). While mean plasma LH concentration in D-Lys3-GHRP-6 treated group in-

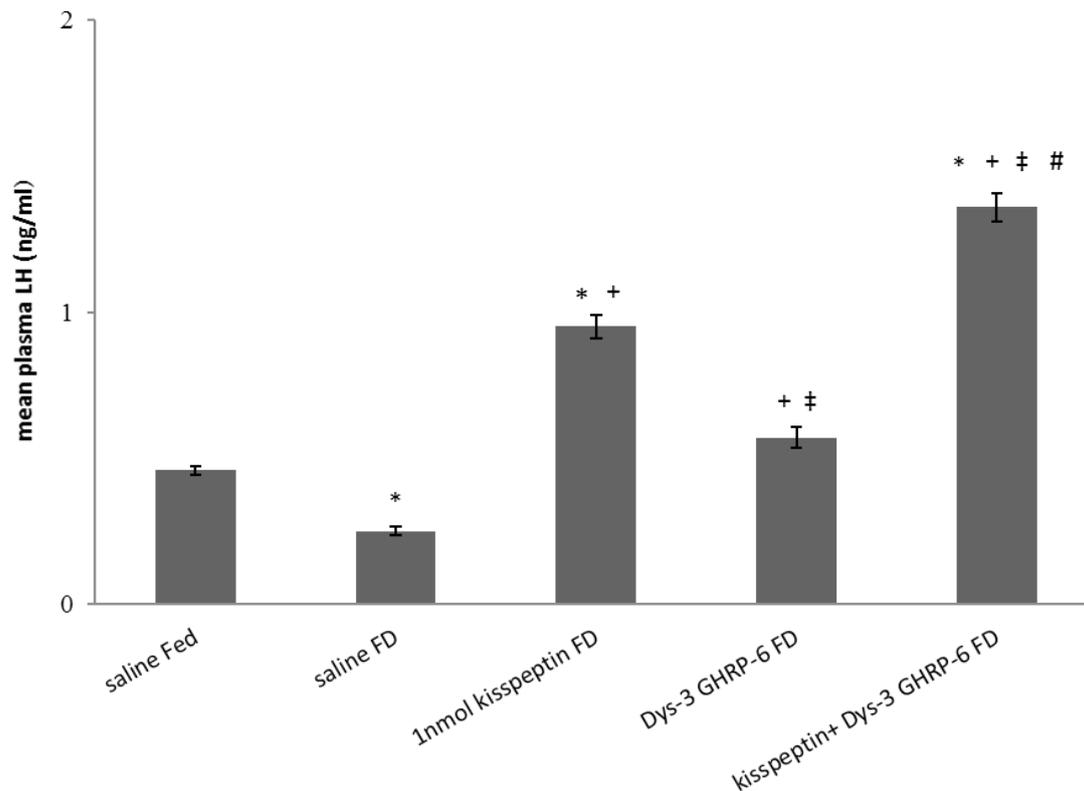


Figure 1. The effects of kisspeptin, D-Lys3 -GHRP-6 or co- administration of D-Lys3 -GHRP-6 and kisspeptin on mean plasma LH concentration. Significant differences are indicated by letters. *: compared to fed saline group; +: compared to food deprived saline group; ‡: compared to kisspeptin group. #: compared to D-Lys3 -GHRP-6 groups (data presented mean \pm SEM, $P < 0.05$, $n = 5$ in each group).

creased significantly by 1.28 times compared to food deprived saline group ($P \leq 0.05$, Figure-1). Also, mean plasma LH concentration in D-Lys3-GHRP-6 treated group decreased significantly by 0.40 times compared to alone kisspeptin group ($P \leq 0.05$, Figure-1). Injection of kisspeptin in D-Lys3-GHRP-6-pre-treated rats significantly increased the mean plasma LH concentration by 1.97, 4.4, 0.43 or 1.38 times compared to fed saline group, food deprived saline group, alone kisspeptin or alone D-Lys3 -GHRP-6 groups respectively ($P \leq 0.05$, Figure-1).

Discussion

In the present study, the mean plasma LH levels increased following kisspeptin injection compared to food deprived saline treated group. The dose of kisspeptin was chosen based on the dose-response previous studies which reported the stimulatory effects of it on the reproductive hormones [4, 6, 20]. This

result is in agreement with previous studies which showed that kisspeptin is one of the most important factors controlling the reproductive axis and it stimulates the LH secretion via a hypothalamic GnRH-dependent mechanism [7, 21]. By expressing the kisspeptin receptor, GPR54, in GnRH neurons, kisspeptin directly stimulates GnRH/LH release [2-3, 22]. However previous studies have shown that up regulation or down regulation of different inter-neural pathways partly have a role in mediating the kisspeptin function on HPG axis. Moreover, hypothalamic kisspeptin/GPR54 signaling system play a major role in the mediating the effects of several factors involving in the control of sexual function including steroid hormones, fasting, ghrelin leptin and photoperiod on HPG axis [23-26]. Recent studies have demonstrated that IP or ICV injections of kisspeptin decrease the mean plasma levels of ghrelin [18-19]. Moreover, 24-hour food deprivation increased mean plasma ghrelin concentrations as same as the

1nmol intraperitoneal injections of ghrelin in male Wistar rats [9]. Based on this information, the effects of kisspeptin in D-Lys3-GHRP-6 pre-treated rats were investigated on LH secretion. The dose of D-Lys3-GHRP-6 was chosen based on dose response previous studies which exert anorexigenic effects in food deprived or ghrelin treated rats [9, 15]. The results demonstrated that blocking the ghrelin receptor by D-Lys3-GHRP-6, increased the stimulatory effects of kisspeptin on LH secretion compared to alone kisspeptin injection. So, down-regulation of ghrelin signaling pathway may partly be involved in the mediating the stimulatory effects of kisspeptin on HPG axis. However further studies are needed to determine the different neural pathway roles involved in the mediating kisspeptin effects on the reproduction, the results of the present study might be useful in improving infertility problems concerned to LH secretion by kisspeptin injection under fasting conditions.

Conclusion

Central injection of kisspeptin stimulates LH secretion in food-deprived rats. Blocking the GHSR-1a receptor in food-deprived rats by D-Lys3-GHRP-6, increased the stimulatory effects of kisspeptin on LH secretion. Down-regulation of ghrelin signaling pathway may partly be involved in the mediating the stimulatory effects of kisspeptin on HPG axis.

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Conflict of Interest

There is no conflict of interest in this article.

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