

Original Article:**The effects of interaction of Dopaminergic and Kisspeptin neural pathways on Ghrelin secretion in rats****Azizeh Sadeghzadeh¹, Abolfazl Bayrami^{1,*}, Fariba Mahmoudi¹, Hodayoun Khazali², Asadollah Asadi¹**¹Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran²Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran*Corresponding author: email address: a_bayrami@uma.ac.ir (A. Bayrami)**ABSTRACT**

Dopamine, kisspeptin and ghrelin are important factors for regulating the reproduction and food intake. Finding the effective central or peripheral factors on ghrelin secretion attracted the attention of most researchers. In the present experimental study, the effects of dopamine, kisspeptin and the GPR54 receptor signaling pathway role in the mediating the dopamine effects was determined on ghrelin secretion. Forty Wistar male rats weighing 220-250g in 8 groups received saline, 1nmol kisspeptin, 5, 15 or 45 microgram dopamine hydrochloride, simultaneous injections of 1nmol peptide 234 and kisspeptin, 15microgram dopamine and kisspeptin or peptide234 and 15microgram dopamine via third cerebral ventricle respectively. Blood samples were collected via tail vein. Mean serum ghrelin concentrations were determined by rat ghrelin kit and ELISA method. Kisspeptin significantly decreased mean serum concentration compared to saline group, while 15 or 45 microgram dopamine significantly increased mean serum ghrelin level compared to saline group. Kisspeptin significantly blocked the stimulatory effects of dopamine on ghrelin secretion compared to dopamine group. Dopaminergic and kisspeptin/GPR54 signaling pathways may interact to control the ghrelin secretion at hypothalamic level. Stimulatory effects of dopamine on ghrelin secretions could exert partly via decreasing the activity of hypothalamic kisspeptin neurons.

Keywords: peptide234; dopamine; kisspeptin; ghrelin**INTRODUCTION**

Hypothalamus is a key regulator of a number of physiological processes including the regulation of feeding behavior, reproduction, and different hormones or neurotransmitters secretion. Dopamine is a member of catecholamine neurotransmitters which is synthesized from tyrosine amino acid by the tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase enzymes. Dopaminergic neurons in different cell groups are distributed in the central nervous system, especially in the midbrain and hypothalamus, projected from these structures to several parts of the brain. Hypothalamus contains A₁₁ and A₁₂ dopaminergic cell groups which are

located in the periventricular, posterior, arcuate and paraventricular nuclei [1]. Also dopamine is synthesized in peripheral organs including gastrointestinal, reproductive tracts and so on [2-3]. It has been found that in addition to locomotion, emotion and cognition, dopamine regulates several neuroendocrine functions via binding to D₁-like (receptors D₁ and D₅) or D₂-like (receptors D₂, D₃ and D₄) receptors [94-5]. Kisspeptin is 54 (in human) or 52 (in rodents) amino acids neuropeptide which is synthesized by the neurons of hypothalamic nuclei involved in the control of food intake and reproduction, especially in arcuate nucleus (Arc), and anteparaventricular nucleus (PVN) [6]. Kisspeptin

stimulates reproductive activity via controlling gonadotropin releasing hormone (GnRH) secretion [7]. However, it suppresses food intake response by activating the hypothalamic anorexigenic pro-opiomelanocortin (POMC) where *Kiss1r* knock-out animals developed obesity [8-10]. Peptide 234(P234) is a 10-amino acid synthetic peptide which acts as a kisspeptin receptor antagonist named GPR54. It has absolutely been established that P234 is able to block the kisspeptin influences on reproduction or other neuroendocrine axis (7).

Ghrelin, 28 amino acid peptide is mainly synthesized through the stomach and hypothalamus nuclei [11]. It is synthesized during fasting and acts as endogen ligand for growth hormone secretagogues receptor (GHSR-1a) [11]. In addition to increasing food intakes, stimulating growth hormone secretion and inhibition reproduction process, it plays an important role in regulating and pathogenesis of obesity or other different metabolic or endocrinal conditions including diabetes, polycystic ovary syndrome and so on [11-13]. Recently, controlling ghrelin secretion or finding the effective factors involved in regulating ghrelin secretion has been the center of attention in most researches. Several studies try to use its receptor antagonist including D-Lys³-GHRP-6 to prevent or treat obesity and other diseases which partly correlate with ghrelin signaling pathway. In the present study, the effects of intracerebral ventricular injections of kisspeptin10, dopamine hydrochloride and the GPR54 receptor signaling pathway role in the mediating the dopamine effects was determined on ghrelin secretion.

MATERIALS AND METHODS

Animals and experimental procedure

The present experimental study was performed from July to December in Shahid Beheshti University of Iran in 2016. Fifty male Wistar rats weighing 230- 250g (provided by the Center of Neuroscience Research of Shahid Beheshti University of Medical Sciences, Iran) were housed in the cages under controlled temperature (22 ± 2 C°) and light (12h light/ dark cycle, light on 0700h). Animals had free access to food and water all the time. All procedures for the

maintenance and the use of experimental animals were approved by Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences. Following anesthetization via a mixture of ketamine and xylezine (ketamine 80 mg/kg BW+ xylezine 10 mg/ kg BW), a 22- gauge stainless cannula was implanted into third cerebral ventricle coordinates (AP = - 2.3, ML= 0.0, DV=6.5) [14]. After surgery, animals were kept in individual cages for one week. Then forty rats in 8 groups (n=5 in each group) received saline (3μl), kisspeptin (1nmol/3μl), dopamine hydrochloride (5, 15 or 45 μg/3μl), kisspeptin (1nmol/1.5μl)/peptide234 (1nmol/1.5μl), kisspeptin (1nmol/1.5μl)/dopamine hydrochloride (15μg/1.5μl) or peptide 234 (1nmol/1.5μl)/dopamine hydrochloride (15μg/ 1.5μl). Dopamine hydrochloride (Sigma Aldrich, U.S.A.), peptide234 (Phoenix Pharmaceutical Co, U.S.A.) and kisspeptin (AnaSpec Co, U.S.A.) were dissolved in distilled water and were then injected by a 27- gauge stainless steel injector through using Hamilton micro syringe via third cerebral ventricle at 8:00-9:00. In co-administrated group peptide, 234 were injected 10min before dopamine or kisspeptin injections. The used drugs doses were selected based on previous studies [5, 7, 14].

Hormone assays

Blood samples were collected at 60min following injections via tail vein. Blood samples centrifuged to 15min at 3000 rpm and the serum stored at -20°C until assayed for ghrelin concentration. Mean serum ghrelin concentration was measured via using rat ghrelin kit (East Biopharm Co., China) and the method of enzyme-linked immunosorbent assay (ELISA). The sensitivity of the kit was 25.59ng/L.

STATISTICAL ANALYSIS

The results are presented as mean \pm SEM. The data were analyzed through SPSS software (version 16), using one- way ANOVA followed by post hoc Tukey test. In all cases, significance was defined by $p < 0.05$.

RESULTS

Mean serum ghrelin levels significantly decreased following kisspeptin infusion by 0.33 times compared to saline ($P < 0.05$, Fig1). During simultaneous injection of kisspeptin and P234, the mean ghrelin concentration did not significantly decline in comparison to saline group. On the other hand, the simultaneous

injection of the two significantly blocked the inhibitory effects of kisspeptin on ghrelin secretion ($P < 0.05$, Fig1). Serum ghrelin levels was significantly increased by 0.46 times following simultaneous injection of kisspeptin and P234, compared to only kisspeptin group ($P < 0.05$, Fig1).

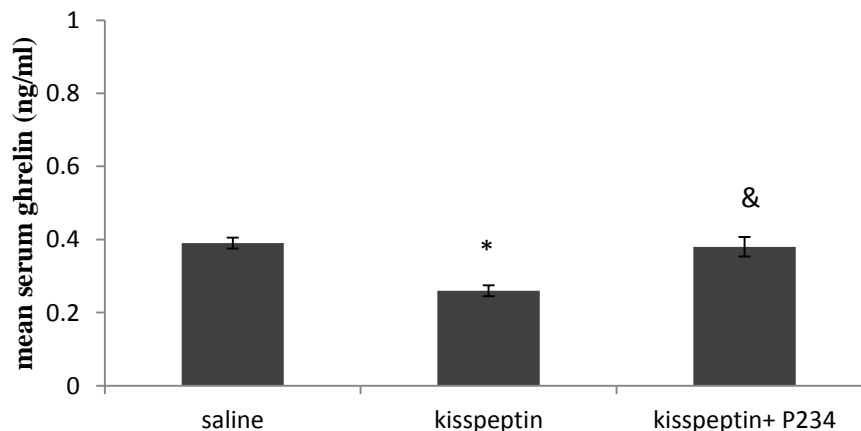


Figure 1. Effects of third cerebral ventricular injections of 1nmol kisspeptin or simultaneous injections of 1nmol kisspeptin and 1nmol peptide 234 (P234) on mean serum ghrelin concentration in male wistar rats. *: compared to saline; &: compared to kisspeptin (data presented mean \pm SEM, $P < 0.05$, $n = 5$ in each group).

Third cerebral ventricular injections 5, 15 or 45 μ g of dopamine hydrochloride increased the mean serum ghrelin concentration by 0.20, 0.38 or 0.69 times compared to saline respectively. This decrease was statistically significant following 15 or 45 μ g infusion of dopamine compared to saline group ($P < 0.05$, Fig2). Mean

ghrelin concentration increased 0.15 times following injections of 15 μ g dopamine compared to 5 μ g group while this increase was not statistically significant (Fig2). Infusion of 45 μ g dopamine significantly increased the mean ghrelin level by 0.23 or 0.40 times compared to 5 or 15 μ g respectively ($P < 0.05$, Fig2).

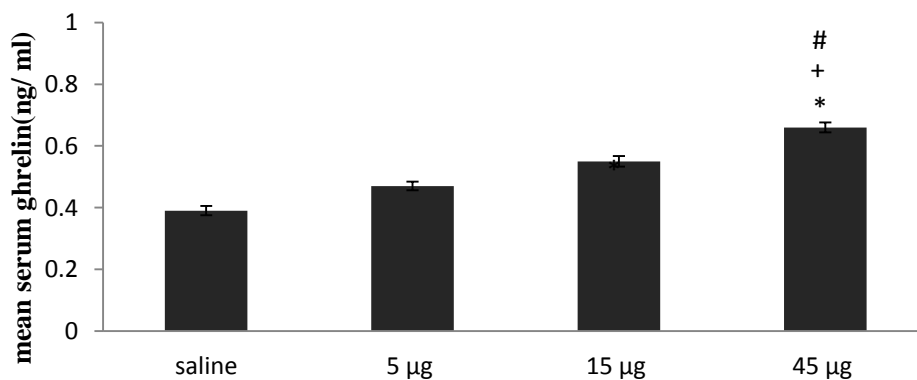


Figure 2. The effects of third cerebral ventricular injections of different doses of dopamine hydrochloride on mean serum ghrelin concentration in male wistar rats. *: compared to saline; +: compared to 5 μ g dopamine; #: compared to 15 μ g dopamine (data presented mean \pm SEM, $P < 0.05$, $n = 5$ in each group).

Kisspeptin significantly decreased the serum ghrelin levels by 0.45 times compared to 15µg dopamine. Simultaneous injection of 1nmol kisspeptin and 15µg dopamine increased ghrelin level by 0.05 or 0.57 times compared to saline or 1nmol kisspeptin group respectively. This increase was statistically significant only in comparison to kisspeptin group ($P < 0.05$, Fig3). Also ghrelin levels showed a significant decrease (0.24 times) following simultaneous injection of 1nmol kisspeptin and 15µg dopamine compared

to only 15µg dopamine group ($P < 0.05$, Fig3). Simultaneous injection of 1nmol peptide234 and 15µg dopamine significantly increased the mean ghrelin levels by 0.28, 0.91 or 0.22 times compared to saline, 1nmol kisspeptin or simultaneous injections of dopamine and kisspeptin groups respectively ($P < 0.05$, Fig3). While a significant decrease was not observed in simultaneous injection of 1nmol peptide234 and 15µg dopamine compared to only dopamine group (Fig3).

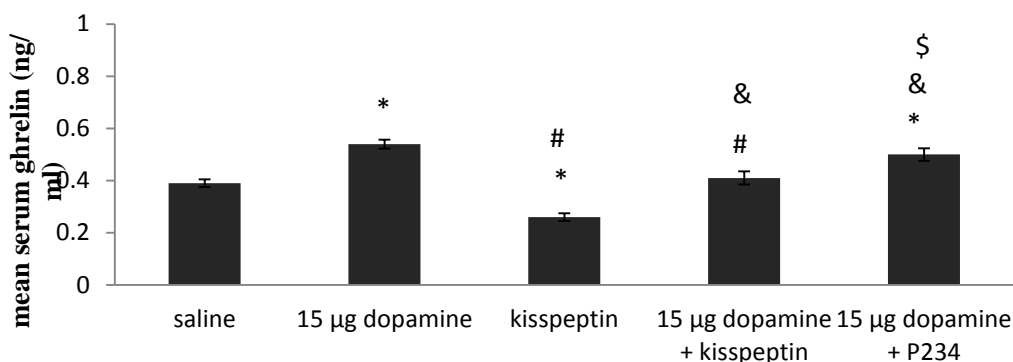


Figure 3. The effects of third cerebral ventricular injections of dopamine hydrochloride, kisspeptin10, or simultaneous injections of kisspeptin and dopamine or simultaneous injections of P234 and dopamine on mean serum ghrelin concentration in male wistar rats. *: compared to saline; &: compared to 1nmol kisspeptin; #: compared to 15µg dopamine; \$: compared to simultaneous injections of kisspeptin and dopamine (data presented mean \pm SEM, $P < 0.05$, $n = 5$ in each group).

DISCUSSION

The findings showed that the injections of dopamine significantly increased mean serum ghrelin concentrations compared to control group, while kisspeptin significantly decreased mean serum ghrelin concentrations compared to control group. Ghrelin secretion followed a dose response related manner by infusions of different doses of dopamine. For the first time, in vivo study of dopamine effects was determined on ghrelin secretion in male rats. However the results are consistent with the only in vitro study which reported the stimulatory effects of dopamine on ghrelin secretion from mouse ghrelinoma3-1 cells (MGN3-1 cells) isolated from a gastric ghrelinoma [15]. There are neurons in the brain which co-express ghrelin receptor, GHSR, and dopamine receptors and infusions of ghrelin increases the dopamine D₁R-induced cAMP accumulation [16-17]. In addition, GHSR is expressed in dopaminergic neurons in

hypothalamus and other parts of central nervous system including ventral tegmental area which is related to the reward-associated behaviors such as food intake [5]. Central injections of different dopamine receptor antagonists (D₁-like or D₂-like receptor antagonists) attenuate hypothalamic ghrelin pathway and they significantly blocked the stimulatory effects of ghrelin on food intakes [5]. Likewise, dopamine D₁ and D₂ receptor subtypes are expressed on MGN3-1 cells and dopamine D₁ receptor antagonists significantly decrease the stimulatory influence of dopamine on ghrelin secretion [15]. These studies indicate a direct interaction between dopamine and ghrelin systems in controlling the energy balance and food intake. However, indirect intra-neuronal pathways might be involved in the mediating dopamine action on ghrelin secretion. Insulin is a peptide which, in addition to pancreas gland is synthesized in central nervous system including hypothalamic periventricular nucleus, which

exerts inhibitory effects on ghrelin secretions [18-19]. It has been revealed that dopamine inhibits insulin secretion and dopamine receptor knock-out mice show an increase in plasma insulin levels [20]. Consequently, decreasing insulin secretion by dopamine injection might be one of central mechanisms through which dopamine could exert stimulatory effects on ghrelin secretion. Based on several previous studies, an interaction has been established between ghrelin and kisspeptin signaling pathways, in a way that central injections of ghrelin regulates hypothalamic kisspeptin gene (*Kiss1*) expression in rats [21]. Ghrelin exerts stimulatory effects on food intakes while kisspeptin infusion inhibits it [9, 11]. The present results showed that the secretion of ghrelin was inhibited in kisspeptin treated rats. The results are according to the study which reports the inhibitory effects of intraperitoneal injection of kisspeptin on ghrelin secretion [22]. The results exhibited that kisspeptin receptor antagonist named peptide234 significantly blocked the inhibitory influences of kisspeptin on ghrelin secretion. It is evident that understanding the involved mechanisms in controlling the ghrelin secretion by kisspeptin needs further studies. However, one could suggest that an increase in plasma growth hormone (GH) levels following kisspeptin injections might be involved in decreasing ghrelin neurons activity. Growth hormone is a peptide which is synthesized via somatotroph cells of pituitary and in addition to growth effects, it plays an important role in regulating the metabolism of carbohydrates, proteins and lipids. Its synthesis and secretion is mainly controlled by hypothalamic growth hormone (GHRH) and somatostatin. Different peripheral or central neuropeptides, neurotransmitters or hormones including ghrelin, kisspeptin, and dopamine influence its secretion. Several studies established that both ghrelin and kisspeptin infusions significantly increase GH secretion [23-24]. And increasing plasma GH levels, in turn exerts inhibitory effects on ghrelin secretion via a negative feedback mechanism [25]. Accordingly, an increase in GH secretion following kisspeptin injection might be partly involved in the mediating its inhibitory effects on ghrelin secretions in rats. Recent studies have established

a close interaction between kisspeptin and dopaminergic pathways in regulating several neuroendocrine functions such as prolactin or gonadotropin releasing hormone, luteinizing hormone secretions and the like in different animal species. It has been revealed that dopaminergic receptors are widely expressed in kisspeptin/neurokinin/dynorphin (KNDy neurons) neurons [26]. And hypothalamic kisspeptin neurons have the ability of highly receiving the projections of dopaminergic ones [27-28]. For the first time, the present study investigated whether dopaminergic pathways might exert stimulatory effects on ghrelin secretion by declining kisspeptin signaling pathway activity. The results demonstrated that mean serum ghrelin concentrations significantly decreased following the injection of dopamine plus kisspeptin compared to dopamine groups while injection of GPR54 receptor antagonist significantly increased ghrelin secretion compared to dopamine plus kisspeptin groups. No previous report has focused on the interaction of these signaling pathways in controlling ghrelin secretion. And further studies are needed to determine the exact mechanism involved. However, the data of present research demonstrated the interference of hypothalamic kisspeptin and dopaminergic pathway for regulating ghrelin secretion.

CONCLUSION

The results revealed that intra-third cerebral injections of dopamine hydrochloride significantly increased ghrelin secretion in a dose-related response while cerebral infusion of kisspeptin significantly suppressed its secretion. Moreover, the results of blocking the kisspeptin/GPR54 signaling pathway suggested that dopamine might stimulate ghrelin secretion partly via declining hypothalamic kisspeptin activity.

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CONTRIBUTION OF THE AUTHORS

Experimental designing, providing material for experiment and analyzing the data were done by Dr. Bayrami and Dr. Mahmoudi. Collecting data were done by Mrs. Sadeghzadeh. Dr. Khazali contributed as scientific counselor and provider of apparatus. Dr. Asadi assisted as the scientific counselor.

“The authors declare no conflict of interest”

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