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ORIGINAL PAPER

Investigation of effect peripheral kisspeptin treatment on hypothalamo-pituitary-gonadal axis and hypothalamo-pituitary-adrenal axis in male rats

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Abstract

Kisspeptin is an endogenous peptide hormone that is the most potent stimulator of the hypothalamo-pituitary–gonadal (HPG) axis. The HPG axis can be suppressed by the activation of the hypothalamo-pituitary-adrenal (HPA) axis. The physiological role of kisspeptin in the interaction of the HPG axis and the HPA axis is not fully understood yet. The purpose of the current study was to investigate the possible effects of peripheral injection (intraperitoneally) of kisspeptin on HPG axis and HPA axis activity as well. Adult male Wistar rats were randomly divided into seven groups as sham (control), kisspeptin (10 nmol), p234 (10 nmol), kisspeptin + p234, kisspeptin + antalarmin (10 mg/kg), kisspeptin + astressin2b (100 µg/kg), and kisspeptin + atosiban (0.250 mg/kg) (n = 10 each group). At the end of the experiment, the hypothalamus, pituitary gland, and serum samples of the rats were collected. Serum follicle-stimulating hormone and luteinizing hormone levels of the kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin2b groups were significantly higher than the control group. Serum testosterone levels were significantly higher in the kisspeptin, kisspeptin + antalarmin, kisspeptin + astressin2b, and kisspeptin + atosiban groups that compared to the control group. There was no a significant difference in corticotropic releasing hormone immunoreactivity in the paraventricular nucleus of the hypothalamus, serum adrenocorticotropic hormone and corticosterone concentrations among all groups. Moreover, no significant difference was found in the concentration of pituitary oxytocin. Our results suggest that peripheral kisspeptin injection induces an activation in the HPG axis, but not in the HPA axis in male rats.

Keywords Kisspeptin · HPG axis · HPA axis · Paraventricular nucleus · Corticotropic releasing hormone · Oxytocin

Introduction

Hypothalamo-pituitary-adrenal (HPA) axis has circadian rhythm that is conducted daily by regulation of the hypothalamic suprachiasmatic nucleus, regardless of stress (Kalsbeek et al. 2012). In addition to this regulation, a neuroendocrine response to external or internal stress occurs in the body with the interaction of neuronal and endocrine systems. The most fundamental component of neuroendocrine regulation to response in stress conditions is the HPA axis. The hypothalamus, one of the components of the limbic system, has a primary role in this regulation process. HPA axis activation is initiated by corticotropin-releasing hormone (CRH or CRF (corticotropin-releasing factor)), which is secreted from the paraventricular nucleus of the hypothalamus (PVN) into the hypothalamo-pituitary portal circulation (Stephens and Wand 2012; Van Bodegom et al. 2017). CRH exerts its effects through the CRH 1(CRHR1) and CRHR2 receptors. However, predominant effects of CRH are primarily mediated by CRHR1 (Refojo and Holsboer 2009). Although both CRH receptors are distributed in the limbic system structures, the density and distribution of CRHR1

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receptors in the limbic system is higher. It has been suggested that CRH circulation of the body generally provides the initiation of the stress response by CRHR1 activation, and CRHR2 activation is responsible for the modulation of this response (Caruso et al. 2022). Under the influence of CRH, adrenocorticotropin-releasing hormone (ACTH) is released from the anterior pituitary into the systemic circulation and causes the secretion of glucocorticoids such as cortisol in humans and corticosterone in rodents from the cortex of the adrenal glands (Stephens and Wand 2012; Van Bodegom et al. 2017).

Kisspeptin is produced by enzymatic processing of the 145 amino acid protein encoded by the KiSS1 (Kiss1 for rodents) gene, as well as the peptide called kisspeptin-54 (or metastin) and shorter peptides of 14, 13 or 10 amino acids. Since all of these peptides contain decapeptide (kisspeptin-10 (Kp-10)) with a common C-terminal, they are collectively called kisspeptin/kisspeptins (Kotani et al. 2001). The receptor that mediates the effects of kisspeptin is called KISS1R (Kiss1r in rodents, formerly known as GRP54). In 2003, it was determined by two different research groups that there is a KISS1R mutation in people with delayed puberty and infertility problems (Seminara et al. 2003). Expression of kisspeptin in the body occurs primarily in the hypothalamus, as well as in the organs that covering the CNS and peripheral body tissues (Landgraf and Neumann 2004). In addition to components of the HPG axis, kisspeptin neurons have projections to brain regions of the HPA axis such as PVN (Clarkson et al. 2009). It has been determined that kisspeptin reduces CRH mRNA expression in PVN cell culture (Rao et al. 2011). However, it has been reported that kisspeptin administration does not alter glucocorticoid secretion in monkeys (Ramaswamy et al. 2009) and rats (Rao et al. 2011). Furthermore, there is an opposite relationship between CRH expression and medial preoptic area Kiss1 expression in PVN during puberty in female rats (Kinsey-Jones et al. 2010). These results indicate that reproductive peptides may also play a role in the HPA response and reproductive function may be adversely affected in case of stress. It is not known whether the same effect applies to humans. Knowledge about the effect of kisspeptin on HPA axis activity and the role of CRH receptors is quite limited. There are data showing that kisspeptin leads an increasing of the firing rate of oxytocin neurons and the plasma oxytocin level (Kotani et al. 2001; Scott and Brown 2011). These results suggest that if kisspeptin has a role in HPA axis activation, which can be mediated by an oxytocin-mediated mechanism. Moreover, especially Kp-54 and other members of kisspeptin have been reported to be able to cross the blood–brain barrier. (d'Anglemont de Tassigny and Colledge 2010; Scott and Brown 2011; Comninos et al. 2016). Therefore, it is unknown whether peripheral kisspeptin administration has an effect on HPA and HPG axis. Therefore, our study aimed to investigate the effects of peripheral (intraperitoneally) kisspeptin injection on HPA and HPG activity and the possible role of CRH and oxytocin receptors in these interactions.

Material and method

Animal ethics and research center

A total of seventy adult male Wistar albino rats (250 ± 20 g body weight) were obtained from Karadeniz Technical University Surgical Research Center (Trabzon, Turkey). The rats housed at a regular temperature (22–25 °C) on a standard light/dark cycle (12 h light and 12 h dark). Animals had free access to fresh water and food. The experimental applications were confirmed by Ethical Committee of Karadeniz Technical University (Document No: 2019/35), and the rats were treated in strict compliance with the international laws on the use and care of experimental animals.

Experimental procedure

Animals were randomly divided into seven groups as following; control, kisspeptin, p234, kisspeptin + p234, kisspeptin + antalarmin, kisspeptin + astressin2b, and kisspeptin + atosiban (n = 10 for each group). In the control group, vehicle was injected intraperitoneally in a volume of 0.3 ml. Kisspeptin was injected at a dose of 10 nmol, and its antagonist p234 at a dose of 10 nmol. Antalarmin, a CRH receptor-1 antagonist, was administered at a dose of 10 mg/kg (Esposito et al. 2002). Astressin2b, a CRH receptor-2 antagonist, was injected at a dose of 100 µg/kg (Wang et al. 2013). Atosiban, an oxytocin receptor antagonist, was administered at a dose of 0.250 mg/kg (Kannan et al. 2010). In the combination groups, kisspeptin injection was performed 5 min after the injection of receptor antagonists. Experiments were completed 45 min after the last intraperitoneal injection.

Procedures performed after intraperitoneal injections

At the end of the intraperitoneal injection process, the rats were sacrificed by cervical dislocation under anesthesia (urethane 1.25 g/kg, intraperitoneally). Immediately after sacrification, the abdomen was opened and blood was drawn from the left ventricle, the skull was separated from the body, brain was removed, and pituitary gland was dissected. Serum samples and pituitary tissue were stored at − 20 °C for analysis.

Three animals from each group were used for immunohistochemical analysis. For this purpose, following the

blood sample collection from the left ventricle after cervical dislocation, a mixture of heparin + physiological saline was perfused transcardially. After this procedure, 4% paraformaldehyde solution was delivered to the brain tissue by a transcardial route, and then the brain was removed. Following this procedure, brain tissues were kept in 4% paraformaldehyde at 4 °C for 3–4 h. Then, after the solution was refreshed with fresh 4% paraformaldehyde, fixation of the brain was continued at 4 °C for 16–20 h. After this process was completed, 4% paraformaldehyde solution was replaced with 25% sucrose solution and the brains were incubated at 4 °C for 24–48 h for sucrose saturation. The subsidence of the brain to the bottom of the sucrose solution was taken as a sign of saturation. At the end of this process, the brains removed from the sucrose solution and stored at − 20 °C for use in immunohistochemical analysis.

Immunohistochemical analysis

Brain samples were kept in 4% paraformaldehyde at 4 °C for 3–4 h. After the solution was refreshed with fresh 4% paraformaldehyde, fixation of the brain was continued at 4 °C for 16–20 h. Then, 4% paraformaldehyde solution was replaced with 25% sucrose solution and waited for 24–48 h at 4 °C until the brains were saturated with sucrose. The sign of saturation with sucrose was considered the collapse of the brain to the bottom of the sucrose solution. This process is important in order to preserve the morphology of the brain areas and to obtain high-quality sections. After the process brains tissues were stored at − 20 °C. The brains were placed in a refrigerated microtome for sectioning, so that the cold of the frozen brains was maintained at a constant temperature. By a guidance of stereotaxic atlas, coronal sections of 25 µm thickness were taken from the hypothalamic PVN area and these sections were placed in cryoprotective solution and used for immunohistochemistry analysis. In these procedures, we used the rat brain atlas (Paxinos and Watson 2006) to reach the PVN area, and serial slicing was performed by following the optic chiasm and the 3rd ventricle area (bregma − 1.80, − 1.88 and − 2.12 mm). Serial sections were taken from the sections.

Fluorescent immunohistochemistry

CRH fluorescent immunohistochemistry experiments were performed on 25 µm-thick free-floating sections of brain regions. Sections were washed at room temperature in 0.05 M PBS (pH 7.3, 8 × 15 min), followed by incubation in 3% hydrogen peroxide for 30 min and washed again in 0.05 M PBS (6 × 5 min). Sections were incubated overnight on a shaker in PBS solution containing 5% normal horse serum, 0.1% BSA and 0.05% Triton X-100 at 4 °C to eliminate non-specific binding. After this procedure, the sections were incubated with primary antibodies (Anti-CRF antibody, ab272391, Abcam, UK) prepared in PBS-Donkey serum solution for 48 h at 4 °C on a shaker and then washed (4 × 5 min) in 0.05 M PBS at room temperature. To detect immunofluorescence, the sections were incubated with secondary antibody (AlexaFluor®488, ab150077, Abcam, UK) in PBS-at serum solution for 1 h at room temperature in the dark, and after washing again (4 × 5 min) in 0.05 M PBS at room temperature, the sections were placed on slides. After the sections were dried on the slide in the dark, they were covered with GEL/MOUNT fluid preservative, covered with a coverslip and stored at 4 °C until analysis. For CRH immunofluorescence expression in the hypothalamus, the images taken from the sections with a 20-angle lens were boot in the ImageJ program. Density measurements were analyzed by ImageJ program on digital images. In the first image, a hollow box with a field was created. Then, the created box was added to the ROI manager section by selecting Analysis, Tools, ROI manager. The box is named (Measuring box) and the dimensions of the box are adjusted. Thus, a size and area standardization were made. By selecting mean intensity value from the analysis and set measure section, it is possible to calculate the mean intensity of the area contained in the box. Then, measurements were made in the analysis and measure section. The mean of the obtained densities was taken by moving the box in 5 different areas over the digital images. The operations were repeated using the same size measuring box for each image.

ELISA analysis

Hormone levels in serum and pituitary gland samples were analyzed using commercial ELISA kits. Concentrations of FSH, LH, testosterone (MBS580154, MyBioSource, Inc. San Diego, CA, USA; CSB-E05100r, Cusabio Technology LLC, Houston, TX, USA), ACTH (LS-F10570, LifeSpan BioSciences, Inc. Seattle, WA, USA), and corticosterone (DEV9922, Demeditec Diagnostics GmbH, Kiel, Germany) were measured in the serum samples. In the pituitary gland, oxytocin (MBS700101, MyBioSource, Inc. San Diego, CA, USA) levels were analyzed. Antigen binding was achieved by first adding samples and standards to plates coated with rat FSH, LH, testosterone, ACTH, corticosterone and oxytocin antibodies. Then, a biotinylated detection antibody specific to the relevant rat hormone and Avidin Horseradish Peroxidase (HRP) conjugate were successively added to each microplate well and incubated. After incubation, washing was performed and substrate was added and incubated again. After incubation, optical densities obtained from standards and serum samples were measured at 450 nm

wavelength in an automatic ELISA plate reader (Thermo Scientific, USA). The analyses were carried out taking into account the kit protocols.

Statistical analysis

All data were presented as mean ± standard error. Results were analyzed by the SPSS 26.0 (licensed for Karadeniz Technical University, Turkey). Shapiro–Wilk test was used in all cases to test the normal distribution of the data, the homogeneity of the variances was evaluated using Levene's test. All our data were normally distributed, thus we utilised parametric methods. Therefore, the differences between groups were determined using one-way ANOVA with post hoc the Tukey's honest significance test. Significance was accepted at values of $P < 0.05$.

Results

Serum FSH results are shown in Fig. 1 and Table 1. There was a significant variation among the different experimental groups in the serum FSH levels ($F(6,42) = 3.756$, $p < 0.01$). Post hoc analysis revealed that the serum FSH levels were found to be significantly higher in kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin2b groups compared to the control group ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively). There was no significant change in FSH levels of p234, kisspeptin + p234 and kisspeptin + atosiban groups.

Table 1 Serum FSH, LH, Testosterone levels (The values were expressed as mean ± Standard error of the mean)

HPG axis parameters	Serum FSH (mIU/ml)	Serum LH (mIU/ml)	Serum Testosterone (ng/ml)
Control	17.82 ± 1.20	16.00 ± 0.85	2.89 ± 0.12
Kisspeptin	21.58 ± 0.49	25.41 ± 0.94	4.38 ± 0.14
P234	17.17 ± 0.61	14.29 ± 0.89	2.45 ± 0.11
Kisspeptin + p234	16.01 ± 0.92	15.70 ± 1.10	3.31 ± 0.11
Kisspeptin + antalarmin	20.38 ± 0.62	25.80 ± 1.13	5.18 ± 0.20
Kisspeptin + astressin2b	19.87 ± 1.10	23.21 ± 1.15	4.24 ± 0.25
Kisspeptin + atosiban	17.97 ± 1.00	19.24 ± 1.00	3,67 ± 0,37

Serum LH results are shown in Fig. 2 and Table 1. There was a significant variation among the different experimental groups in the serum LH levels ($F(6,42) = 22.745$, $p < 0.001$). Post hoc analysis determined that the serum LH levels in the kisspeptin ($p < 0.001$), kisspeptin + antalarmin ($p < 0.001$), kisspeptin + astressin2b ($p < 0.001$) groups were significantly higher than in the control group. Although the LH level in the kisspeptin + atosiban group was higher than the control level, this difference was not statistically significant. There was also no significant difference in LH levels of the p234 and kisspeptin + p234 groups compared to the control group.

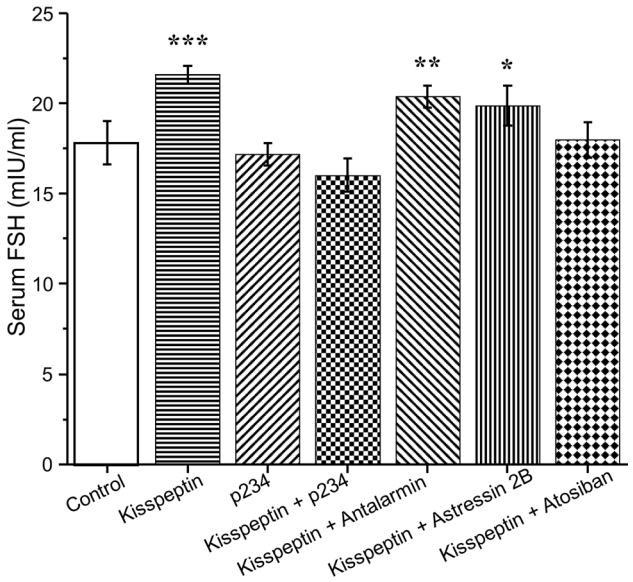


Fig. 1 Serum FSH levels. Peripheral kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin 2B treatments caused a significantly increase in the serum FSH levels compared to control group. (The values were expressed as mean ± Standard error of the mean. **: $p < 0.01$ and ***: $p < 0.001$)

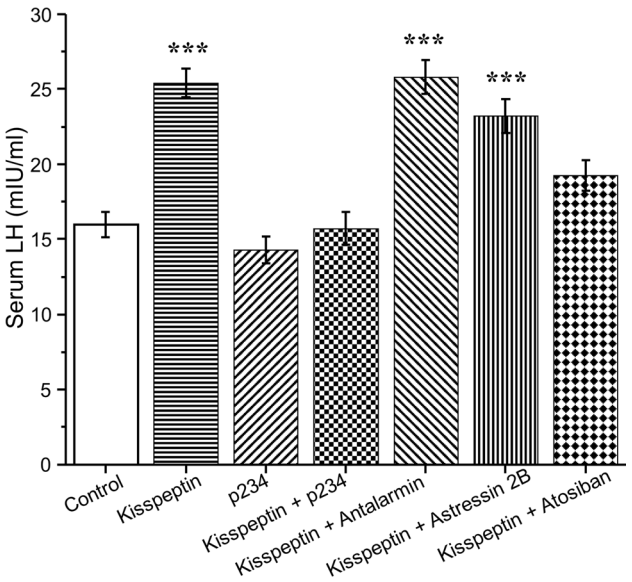


Fig. 2 Serum LH levels. Peripheral kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin 2B treatments caused a significantly increase in the serum LH levels compared to control group. (The values were expressed as mean ± Standard error of the mean. ***: $p < 0.001$)

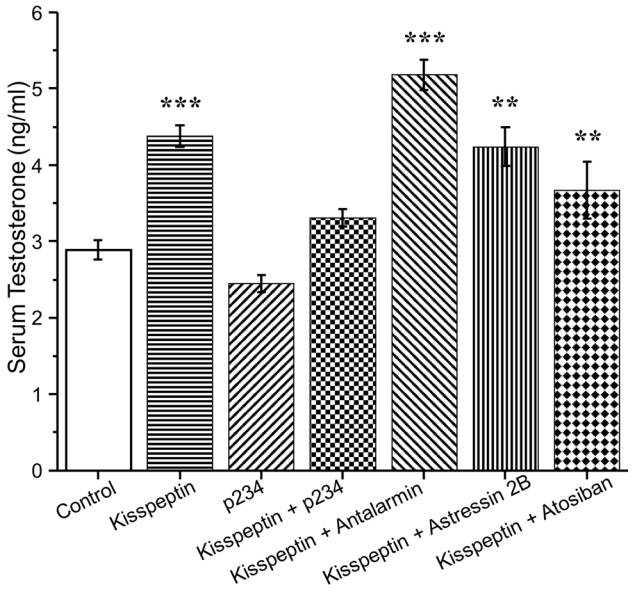


Fig. 3 Serum testosterone levels. Peripheral kisspeptin, kisspeptin + antalarmin, kisspeptin + astressin 2B and kisspeptin + atosiban treatments caused a significantly increase in the serum testosterone levels compared to control group. (The values were expressed as mean ± Standard error of the mean. **: $p < 0.01$, ***: $p < 0.001$)

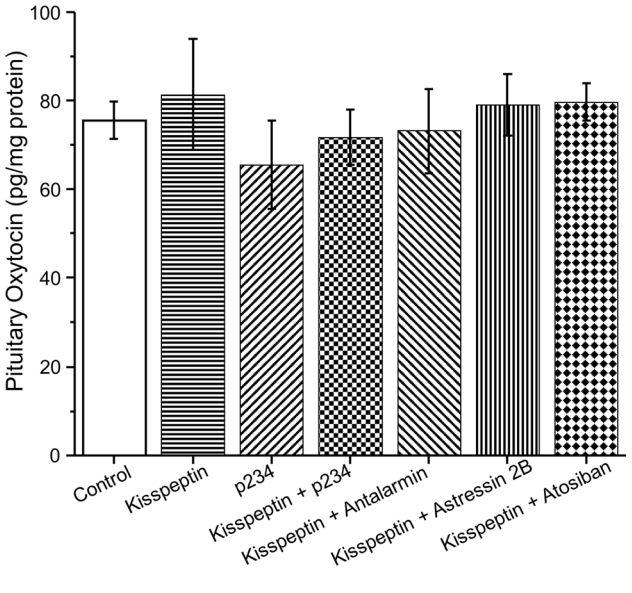


Fig. 4 Pituitary oxytocin levels. There was no significantly difference in the pituitary oxytocin levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean)

Serum testosterone results are shown in Fig. 3 and Table 1. There was a significant variation among the different experimental groups in the serum testosterone levels ($F(6,42) = 21.845$, $p < 0.001$). Post hoc analysis showed that serum testosterone concentrations were significantly higher in the kisspeptin ($p < 0.001$), kisspeptin + antalarmin ($p < 0.001$), kisspeptin + astressin2b ($p < 0.01$), and kisspeptin + atosiban ($p < 0.01$) groups compared to the control group. Testosterone concentration values in kisspeptin + p234, p234 groups were similar to those in the control group.

Pituitary oxytocin results are presented in Fig. 4 and Table 2. There was no significant difference in pituitary oxytocin levels among the all groups compared to the control. Although oxytocin values in the kisspeptin, kisspeptin + astressin2b and kisspeptin + atosiban groups were higher than the control group, and lower in the p234 group, these differences are not significant. Oxytocin levels of kisspeptin + p234 and kisspeptin + antalarmin groups were at the same level as the control group.

CRH immunoreactivity results in PVN are shown in Fig. 5 and Table 2. There was no significantly difference in the kisspeptin, p234, kisspeptin + p234, kisspeptin + antalarmin, kisspeptin + astressin2b and kisspeptin + atosiban groups compared to the control group.

Serum ACTH results are shown in Fig. 6 and Table 2. Although there is no significance, serum ACTH levels of kisspeptin, p234 and kisspeptin + atosiban groups were found higher than the control group. Serum ACTH concentrations in the kisspeptin + p234, kisspeptin + antalarmin and kisspeptin + abstressin2b groups were similar to the control group value.

Serum corticosterone levels are shown in Fig. 7 and Table 2. Serum corticosterone concentrations in the kisspeptin, kisspeptin + p234, kisspeptin + antalarmin, kisspeptin + abstressin2b and kisspeptin + atosiban groups were similar to the control group value. Although there is no statisticallysignificance, serum corticosterone level of the p234 group was found higher than the control group.

Discussion

Kisspeptin is the most potent endogenous stimulator of GnRH neurons. Therefore, the HPG axis stimulating effect of kisspeptin is well known. Regarding the HPG axis stimulating effect of kisspeptin, mutations in the kisspeptin receptor (KISS1R in humans, Kiss1r in rodents, previously known as GPR54) result in sexual immaturity and infertility in both humans and rodents (Seminara et al. 2003). It has been demonstrated that puberty onset is early and gonadotropins are strongly stimulated by experimental injections of kisspeptin (Şahin 2015). These results were generally obtained from experiments in which kisspeptin was administered centrally (intracerebroventricularly (icv)). However, it is known that kisspeptin and its receptor are present not only in the hypothalamus and CNS, but also in the peripheral tissues of the body. In humans, kisspeptin are expressed especially in

Table 2 Pituitary Oxytocin levels, CRH Immunoreactivity in PVN, Serum ACTH and Corticosterone levels (The values were expressed as mean ± Standard error of the mean)

HPA axis parameters	Pituitary Oxytocin (pg/mg protein)	CRH Immuno-reactivity	Serum ACTH (pg/ml)	Serum Corticosterone (ng/ml)
Control	75.58 ± 4.31	3.32 ± 0.05	87.29 ± 3.13	349.65 ± 10.96
Kisspeptin	81.39 ± 12.55	2.57 ± 0.21	95.57 ± 3.61	351.15 ± 12.43
P234	65.51 ± 9.90	3.56 ± 0.17	98.23 ± 1.04	416.81 ± 25.42
Kisspeptin + p234	71.71 ± 6.22	2.72 ± 0.15	89.03 ± 2.76	355.20 ± 9.77
Kisspeptin + antalarmin	73.17 ± 9.50	1.76 ± 0.21	89.91 ± 2.33	345.62 ± 8.36
Kisspeptin + astressin2b	79.09 ± 7.03	3.55 ± 0.2	92.99 ± 3.95	346.47 ± 20.40
Kisspeptin + atosiban	79.66 ± 4.27	5.05 ± 0.25	96.12 ± 2.99	361.84 ± 30.45

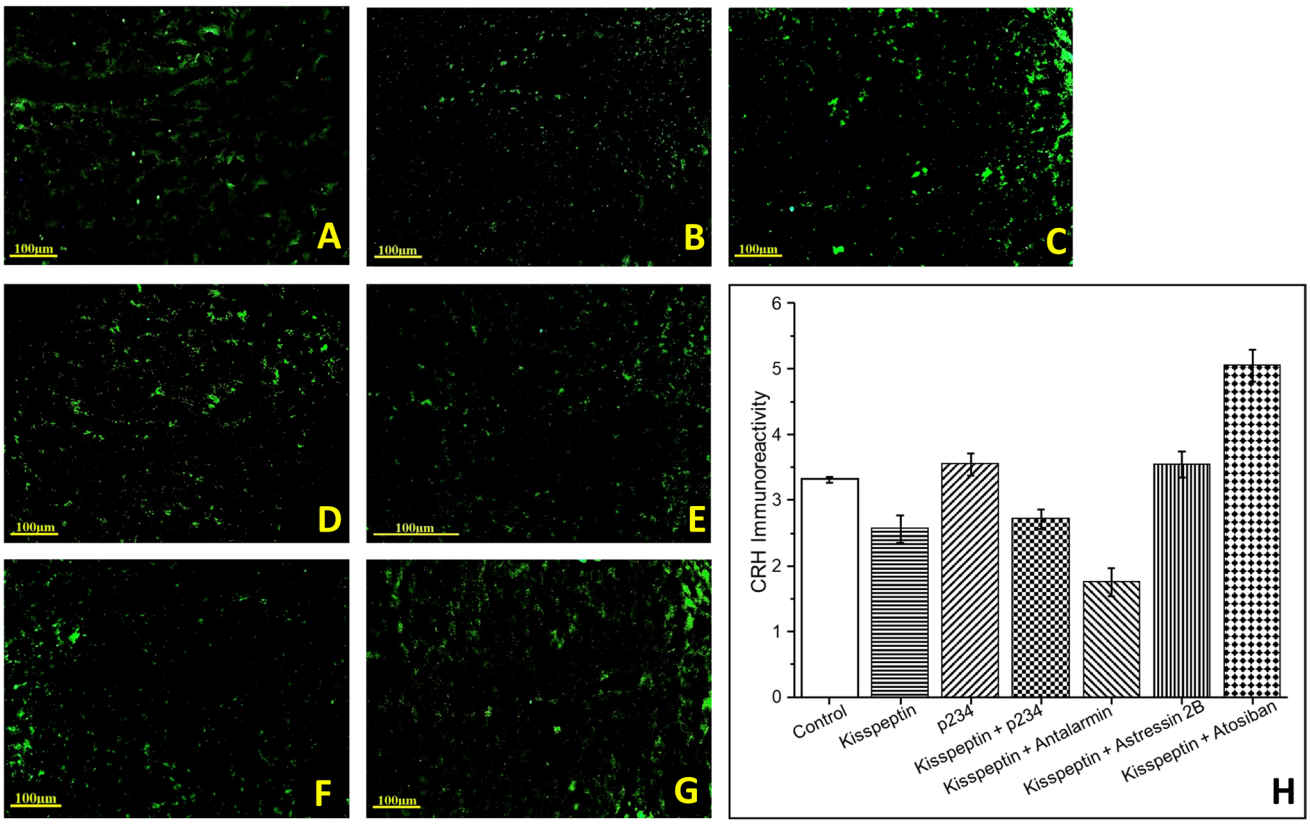


Fig. 5 CRH immunoreactivity in PVN. (A: control, B: Kisspeptin, C: P234, D: Kisspeptin + p234, E: Kisspeptin + antalarmin, F: Kisspeptin + astressin 2B, and G: Kisspeptin + atosiban). There was no significantly difference in the CRH immunoreactivity between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean)

the placenta, gonads, pancreas and liver (Muir et al. 2001; Gaytán et al. 2009). It has been reported that the kisspeptin receptor is also expressed in cardiac and skeletal muscles (Muir et al. 2001; Gaytán et al. 2009). This distribution indicates that kisspeptin in the peripheral area of the body may also have physiological functions. For this reason, in our study, the relation of kisspeptin administered intraperitoneally at a dose of 10 nmol with HPG axis activation and HPA axis activity was evaluated. In current experiments regarding HPG activity, serum FSH level was significantly higher in the kisspeptin group. In addition, the FSH level was statistically higher in the kisspeptin + antalarmin and kisspeptin + astressin2b groups compared to the control group. Regarding serum LH, the level of this hormone was significantly higher in the kisspeptin, kisspeptin + antalarmin, and kisspeptin + astressin2b groups. However, although the LH concentration was higher in the kisspeptin + atosiban group, there was no significant difference. Regarding to testosterone, the level of this hormone was statistically higher in the kisspeptin, kisspeptin + antalarmin, kisspeptin + astressin2b,

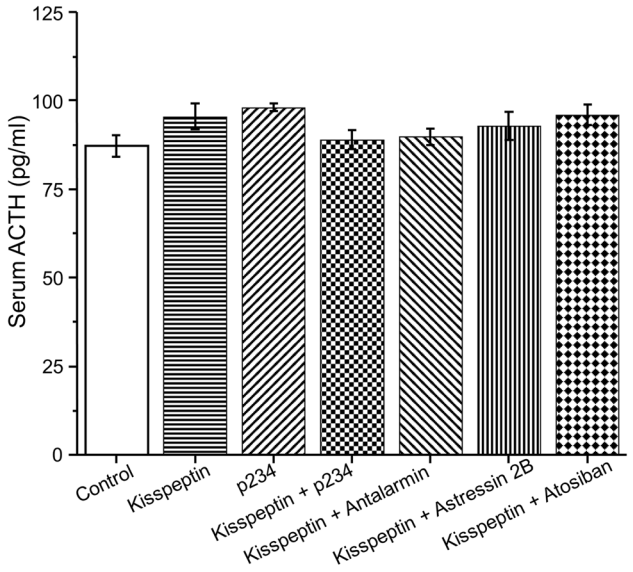


Fig. 6 Serum ACTH levels. There was no significantly difference in the serum ACTH levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean)

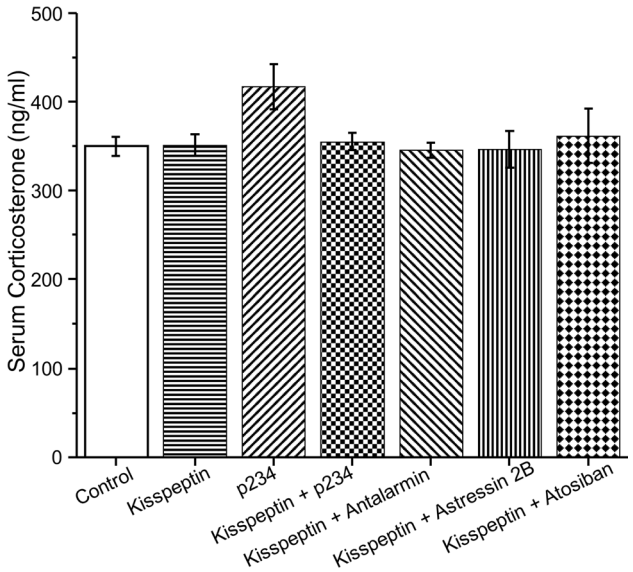


Fig. 7 Serum corticosterone levels. There was no significantly difference in the serum corticosterone levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean)

and kisspeptin + atosiban groups compared to the control. In a study conducted with male rats, it has been shown that both central and peripheral administration of kisspeptin increased plasma LH, FSH, and testosterone levels. In the same study, it has been reported that the effect of kisspeptin can change in a time and dose-dependent manner (Thompson et al. 2004). Although peripheral administration of kisspeptin increased serum concentrations of LH in prepubertal gilts, it did not cause any increase FSH or growth hormone levels. Researchers suggested that similar results occur by icv injection (Lents et al. 2008). It has been shown that exogenous kisspeptin administered intraperitoneally caused strong dose-dependent increases in gonadotropin release in both long-day and short-day hamsters (Greives et al. 2007).

There are many studies showing that kisspeptin can interact with oxytocin neurons. In one of these studies, it was reported that axons of kisspeptin neurons projecting to PVN in the hypothalamus reach CRH as well as oxytocin neurons (Landgraf and Neumann 2004). Moreover, it has been suggested that kisspeptin increases the firing rate of oxytocin neurons and also increases the plasma oxytocin level (Kotani et al. 2001; Scott and Brown 2011). In current study, although oxytocin levels in the kisspeptin, kisspeptin + astressin2b, and kisspeptin + atosiban groups were higher than the control group and lower in the p234 group, these differences were not statistically significant. Moreover, oxytocin levels of kisspeptin + p234 and kisspeptin + antalarmin groups were at the same level as the control group. Therefore, we consider these data as interesting findings in terms of indicating a possible interaction in HPA parameters for oxytocin. However, since we applied a single dose of kisspeptin, we could not reveal whether there was a dose-dependent effect.

Axons of kisspeptin neurons extend to the PVN and reach CRH, AVP and oxytocin neurons (Landgraf and Neumann 2004). These kisspeptin neurons have projections to neurons belonging to limbic system structures that innervate the PVN with GABAergic impulses, such as the bed nucleus of the stria terminalis and central amygdala nucleus (Clarkson et al. 2009). These connections indicate that kisspeptin may have relation with HPA axis activity.

Kisspeptin is widely expressed in the fetal adrenal cortex. It was determined that kisspeptin induced dehydroepiandrosterone sulfate secretion in vitro in human adrenocortical carcinoma cell line and cultured human fetal adrenal cells (Katugampola et al. 2017). Adrenal gland activity is altered in Kiss1 KO mice, resulting in a disorder associated with hypersecretion of corticosterone and aldosterone. Related to this condition, hyperaldosteronism has been found to be permanent in older animals, although corticosterone levels return to normal over time. KISS1 and KISS1R mutations have been identified in some of the human patients with hypercortisolism or hyperaldosteronism caused by adrenal tumors. It has been stated that these patients also have infertility problems (Berthon et al. 2020). These results provide important evidence for a potential role of kisspeptin in adrenal gland development and/or regulation of adrenal gland function. It is known that both physical and psychological stress factors affect reproductive function and suppress LH secretory pulse (Cagampang et al. 1997). The stress response in the body is regulated by the HPA axis. Primary control

of the stress response is mediated by hypothalamic CRH. Indeed, experimentally, central injection of CRH causes rapid suppression of LH secretion. CRH increases the release of ACTH from the anterior pituitary gland. The anterior pituitary gland secretes ACTH, which in turn stimulates the release of corticosteroids from the cortex of the adrenal gland. The glucocorticoid receptor (GR) and the mineralocorticoid receptor, respectively, are the receptors for corticosteroids such as glucocorticoids and mineralocorticoids (De Kloet and Reul 1987). Stress stimulates the adrenal cortex's production of glucocorticoids, which bind to and activate the GR. The precise relationship between the HPA axis and HPG axis has not yet been fully understood, despite the fact that it is well recognized that stress affects the HPG axis and has a negative effect on reproductive function. Moreover, effects of kisspeptin on HPA axis activity and the role of CRH receptors are quite limited and the findings are controversial. In our study, we determined that peripheral injection of kisspeptin did not affect CRH immunoreactivity in PVN. Moreover, there was no change in serum ACTH and corticosterone levels. Serum ACTH concentrations in the kisspeptin + p234, kisspeptin + antalarmin and kisspeptin + abstressin2b groups were similar to the control group value. Although serum ACTH levels of kisspeptin, p234 and kisspeptin + atosiban groups were higher than the control group, there was no statistical significance. Serum corticosterone levels in the kisspeptin, kisspeptin + p234, kisspeptin + antalarmin, kisspeptin + abressin2b and kisspeptin + atosiban groups were same as in the control group. Interestingly, only the serum corticosterone level of the p234 group was higher than the control group, but again there was no statistical significance. In an in vitro study, it was suggested that kisspeptin decreased CRH mRNA expression in PVN cell culture (Rao et al. 2011). However, it has been reported that exogenous kisspeptin treatment does not alter glucocorticoid secretion in both monkeys (Ramaswamy et al. 2009) and rats (Rao et al. 2011). The findings of our study confirm the data obtained from monkeys and rats regarding exogenous kisspeptin administration. Previous study that performed on female rats, suggesting that there is a negative correlation between CRH expression and Kiss1 expression in the hypothalamus during puberty (Kinsey-Jones et al. 2010). For this reason, it is possible that there may be gender and age-related variability in HPA activation. As a result of the presence of only adult male rats in the current study, our findings are far from answering the possibility of gender and age-related variability of the effects of kisspeptin on HPA activity.

It is possible that HPA axis activity that triggered under stress conditions affects kisspeptin signaling in the body. As a matter of fact, it was discovered that both anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) kisspeptin neurons express CRH receptors, which are involved in such stress responses but are not expressed in GnRH neurons (Takumi et al. 2012). Additionally, CRH fibers in both AVPV and ARC project to kisspeptin neurons. These results demonstrate direct interaction between kisspeptin neurons and hormones involved in the control of the stress response, such as CRH and glucocorticoids. The HPG axis, a system that regulates reproduction, and the HPA axis, a stress response system, are believed to communicate with each other through kisspeptin neurons. In addition, direct manipulation of the kisspeptin system by CRH and glucocorticoids can control the suppression of reproductive activity during stress (Ozawa 2022). In our current study, the effects of exogenous kisspeptin application on HPA axis activity were evaluated only under normal conditions. For this reason, we do not know the effects of exogenous kisspeptin treatment in animals exposed to various stress factors. In addition, it is clear that there is a need to investigate how endogenous kisspeptin expression is affected under stress conditions.

Conclusion for future biology

In conclusion, peripheral injection of kisspeptin strongly activated the HPG axis, causes increase of gonadotropins and testosterone levels. However, this strong effect was not valid for HPA axis activation. The activating effects of kisspeptin on HPG axis activation can be modulated by oxytocin receptor antagonism. Therefore, we can suggest that suppression of kisspeptin and oxytocin interaction may play role in HPG axis inhibition due to HPA activation. It is possible that CRH 1 receptor signaling is involved in suppression of the HPG axis due to HPA axis activation. Evaluating our findings with studies investigating endogenous kisspeptin signaling and the effects of different doses of exogenous kisspeptin in stress-treated models may contribute to a better understanding of this issue. In conclusion, it can be suggested that although peripheral injection of kisspeptin at a dose of 10 nmol strongly stimulates the HPG axis, it has no direct effect on HPA axis activation in adult male rats.

Authors' contributions ZS contributed to the study conception and design; ZS, OA and OFK were responsible for the development of animal experiments; GC was responsible for the histological analyses; AA, ES, SE and NS were responsible for the biochemical analyses; ZS and OA were responsible for the statistical analyses and layouts; ZS wrote the manuscript; ZISG and SK were responsible for editing and revising the manuscript.

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Declarations

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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