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RESEARCH ARTICLE

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Intracerebroventricular injection of kisspeptin in male rats activates hypothalamo-pituitary-gonadal axis, but not hypothalamo-pituitary-adrenal axis

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ABSTRACT

Kisspeptin is an important hormone involved in the stimulation of the hypothalamo-pituitary gonadal (HPG) axis. The HPG axis can be suppressed in certain conditions such as stress, which gives rise to the activation of the hypothalamo-pituitary-adrenal (HPA) axis. However, the physiological role of kisspeptin in the interaction of HPG and HPA axis is not fully understood yet. This study was conducted to investigate the possible effects of central kisspeptin injection on HPG axis as well as HPA axis activity. Adult male Wistar rats were randomly divided into seven groups as followed: sham (control), kisspeptin (50 pmol), P234 (1 nmol), kisspeptin + p234, kisspeptin + antalarmin (0.1 μg), kisspeptin + astressin 2B (1 μg), and kisspeptin + atosiban (300 ng/rat) ($n = 10$ each group). At the end of the experiments, the hypothalamus, pituitary, and serum samples of the rats were collected. There was no significant difference in corticotropic-releasing hormone immunoreactivity in the paraventricular nucleus of the hypothalamus, serum adrenocorticotropic hormone, and corticosterone levels among all groups. Moreover, no significant difference was detected in pituitary oxytocin level. Serum follicle-stimulating hormone and luteinizing hormone levels of the kisspeptin, kisspeptin + antalarmin, and kisspeptin + astressin 2B groups were significantly higher than the control group. Serum testosterone levels were significantly higher in the kisspeptin kisspeptin + antalarmin, kisspeptin + astressin 2B, and kisspeptin + atosiban groups compared to the control group. Our findings suggest that central kisspeptin injection causes activation in the HPG axis, but not the HPA axis in male rats.

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Kisspeptin; HPG axis; HPA axis; paraventricular nucleus; corticotropic releasing hormone; oxytocin

Introduction

In case of acute or chronic stress conditions, the main neuroendocrine response is the activation of the hypothalamo-pituitary-adrenal (HPA) axis. This activation is initiated by corticotropin-releasing hormone (CRH or CRF (corticotropin-releasing factor)) that is secreted from the paraventricular nucleus (PVN) of the hypothalamus to the median eminence and hypothalamo-pituitary portal circulation. The CRH secretion from PVN is accompanied by arginine vasopressin (aVP or Antidiuretic Hormone (ADH)) hormone. As a result of CRH secretion (partially with the involvement of aVP), adrenocorticotropin-releasing hormone (ACTH) than releases from the anterior pituitary into the systemic circulation, leading to the secretion of glucocorticoids such as cortisol in humans and corticosterone in rodents from the cortex of the adrenal glands [1,2]. Regardless of stress, the HPA axis also has a circadian rhythm that is governed daily by regulation of the suprachiasmatic nucleus (SCN) in the hypothalamus [3]. One of several inhibitory physiological inputs that affect CRH release from PVN is oxytocin secretion [4–6]. Oxytocin receptor, which is widely distributed in the brain has abundant bioactivity in limbic system structures such as the nucleus accumbens, amygdala, thalamus and hypothalamus [7,8].

Kisspeptin, encoded by the KiSS1 gene (Kiss1 for rodents) [9], is primarily involved in the activation of gonadotropin-releasing hormone (GnRH) and the increase of gonadotropins, as well as in the regulation of many physiological functions. Kisspeptin also contributes to regulation of stress physiology. Primary expression of kisspeptin is in the hypothalamus but it also has bioactivity in the other brain areas and peripheral organs [10]. In addition to components of the HPG axis, kisspeptin neurons have projections to brain regions of the HPA axis such as PVN [10]. Axons of kisspeptin neurons extend to the PVN and reaches the CRH, aVP and oxytocin neurons [11–13]. Although it was stated that kisspeptin administration did not change glucocorticoid secretion in monkeys [14] and rats [15], it decreases CRH mRNA expression in PVN cell culture [15]. It has been reported that there is an inverse relationship between CRH expression and medial preoptic area Kiss1 expression in PVN during puberty in female rats [16]. However, contribution of kisspeptin activity on the HPA axis and participation of CRH receptors are still remain unclear. There are several studies show that

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kisspeptin increases the firing rate of oxytocin neurons and the plasma oxytocin level [9, 17]. These results indicate that if kisspeptin has a role in HPA axis activation, this may occur by oxytocin-mediated mechanism. There is no data on whether kisspeptin has such an effect. Therefore, our study aimed to investigate the effects of intracerebroventricular (icv) kisspeptin injection on HPA and HPG activity. Furthermore, we also investigated the possible role of CRH and oxytocin receptors in this interaction.

Material and method

Animal ethics and research center

A total of 70 adult Wistar albino male rats (250 ± 20 g body weight) were obtained from Karadeniz Technical University Surgical Research Center (Trabzon, Turkey). The animals were housed in classic polycarbonate cages and in a 12:12-h light–dark cycle. The temperature of the room was maintained at 22 °C ± 1 °C. The rats were fed with standard chow diet *ad libitum*. 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 followed: sham (control), kisspeptin, P234, kisspeptin + p234, kisspeptin + antalarmin, kisspeptin + astressin 2B, and kisspeptin + atosiban ($n = 10$ each group). In the sham group, 4 μl artificial cerebrospinal fluid was injected to ventricles. Kisspeptin was injected at dose of 50 pmol, and its antagonist p234 at dose of 1 nmol. Antalarmin, CRH receptor-1 antagonist, was administered at dose of 0.1 μg [18]. Astressin 2B, CRH receptor-2 antagonist, was injected at dose of 1 μg [18]. Atosiban, oxytocin receptor antagonist, was administered at dose of 300 ng/rat (Benoussaidh et al. 2005). In the combination groups, kisspeptin injection was performed 5 min after the injection of receptor antagonists. Experiments were completed 30 min after the last icv injection.

Icv injection procedure

Animals were anesthetized with urethane (1.25 g/kg, intraperitoneal) after 12 h fasting and then placed in on stereotaxic device. Depth of anesthesia was controlled step by step during experimental process. Scalp was perforated by a scalpel and the bone tissue was removed from soft tissues. By determining the Bregma point in the skull, stereotaxic coordinates were determined by means of a guide cannula placed in a holder of the stereotaxic device by a clip. By using a rat brain atlas [19], lateral ventricular coordinates (lateral: 1.70 mm, anteroposterior: − 0.72 mm and ventral: 4 mm) were calculated and marked. Then, skull was drilled by a dental tour device, and injections were applied by a Hamilton injector.

After icv injection process

Following the last icv injection, the rats were sacrificed by cervical dislocation. Immediately after sacrifice, abdomen was opened and blood was drawn from the left ventricle. Following this procedure, skull was separated from the body and brain was completely removed. The brain was then placed dorsally on a chilled plate supported with dry ice. The pituitary gland was dissected on this plate. Tissue and serum samples were kept at −20 °C for further analysis.

Three animals from each group were used for immunohistochemical analysis. In these animals, following the collection of blood from the left ventricle after cervical dislocation, a mixture of heparin + physiological saline was perfused transcardially. After this procedure, 4% paraformaldehyde solution transcardially delivered to the brain tissue, and then the brain was removed.

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 for an additional 16–20 h at 4 °C. Then, 4% paraformaldehyde solution was replaced with 25% sucrose solution and waited for 24–48 h at 4 °C until to the saturation of the brains by sucrose. The saturation sign with sucrose was considered as 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 higher quality sections. Brains removed from sucrose solution were stored at −20 °C. The brain samples were placed in a cooled microtome for sectioning, thus ensuring that the frozen brains were kept at a constant coldness. By using a stereotaxic atlas, coronal sections of 25 μm thickness were taken from the hypothalamic PVN area. The sections were placed in cryoprotective solution and used for immunohistochemistry analysis. In these procedures, serial sections were taken from the sections including the beginning and the end of the 3rd ventricle (bregma −1.80, −1.88 and −2.12 mm), after serial slicing based on the optic chiasm and the 3rd ventricle area to reach the PVN area by using the rat brain atlas [19]. Serial sections were taken from the indicated brain areas.

Fluorescent immunohistochemistry

CRH fluorescent immunohistochemistry experiments were performed on 25 μm-thick free-floating sections obtained from 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 nonspecific bindings. 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 liquid GEL/MOUNT preservative 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 imaged by using ImageJ program. Density measurements were made with 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 being 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 average intensity of the area contained in the box. Then, measurements were made in the analysis and measure section. The average of the obtained densities being taken by moving the box in 5 different areas over the digital picture. The operations were repeated using the same size measuring box for each image.

ELISA analysis

Hormone levels in pituitary gland and serum samples were analyzed using commercial ELISA kits. In the pituitary gland, oxytocin (MBS750178, MyBioSource, Inc. San Diego, CA, USA) levels were analyzed. Levels of ACTH (MBS2502683, MyBioSource, Inc. San Diego, CA, USA), corticosterone (DEV9922, Demeditec Diagnostics GmbH, Kiel, Germany), FSH, LH and testosterone (MBS580154, MyBioSource, Inc. San Diego, CA, USA; CSB-E05100r, Cusabio Technology LLC, Houston, TX, USA) were measured in the serum samples. Antigen binding was achieved by first adding serum samples and standards to plates coated with rat FSH, LH, and testosterone 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 by the guidance of the kit protocols.

Statistical analysis

All results were presented as mean ± standard error. Data were analyzed with the SPSS 26.0 (licensed for Karadeniz Technical University, Turkey). 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

CRH immunoreactivity results in PVN are presented in Figure 1. There was no significantly difference in the kisspeptin (1.68 ± 0.13), p234 (1.43 ± 0.11), kisspeptin + p234 (1.37 ± 0.09), kisspeptin + antalarmin (1.29 ± 0.06), kisspeptin + astressin 2B (1.47 ± 0.06) and kisspeptin + atosiban (1.34 ± 0.09) groups compared to the control group (1.39 ± 0.58).

Serum ACTH results are presented in Figure 2. Although there is no significant difference, serum ACTH levels of kisspeptin (86.73 ± 3.71 pg/ml), p234 (90.23 ± 5.84 pg/ml), kisspeptin + p234 (90.43 ± 6.38 pg/ml) and kisspeptin + atosiban (86.94 ± 5.56 pg/ml) were higher compared to control group (79.6 ± 1.63 pg/ml). Serum ACTH concentrations in the kisspeptin + antalarmin (81.53 ± 4.99 pg/ml) and kisspeptin + astressin 2B (78.14 ± 2.2 pg/ml) groups were similar to the control group value.

Serum corticosterone levels are presented in Figure 3. There was no statistically significant difference in serum corticosterone levels of the kisspeptin (396.65 ± 23.70 ng/ml), p234 (425.33 ± 21.35 ng/ml), kisspeptin + p234 (457.53 ± 36.17 ng/ml), and kisspeptin + astressin 2B (403,71 ± 19.79 ng/ml), kisspeptin + antalarmin (365.55 ± 13.13 ng/ml) and kisspeptin + atosiban (379.92 ± 12.12 ng/ml) groups compared to the control group (364.17 ± 12.15 ng/ml).

Pituitary oxytocin results are presented in Figure 4. No statistically significant difference was determined in the serum pituitary oxytocin levels of kisspeptin (52.19 ± 2.71 pg/mg protein), kisspeptin + p234 (54.21 ± 2.26 pg/mg protein), kisspeptin + astressin 2B (53.61 ± 1.66 pg/mg protein), kisspeptin + atosiban (53.60 ± 2.51 pg/mg protein), p234 (49.92 ± 2.65 pg/mg protein) and kisspeptin + antalarmin (48.43 ± 3.33 pg/mg protein) groups compared to the control group (48.58 ± 1.78 pg/mg protein).

Serum FSH results are presented in Figure 5. It was determined that serum FSH levels of the kisspeptin (23,04 ± 0,40 mIU/ml, $p < 0.05$), kisspeptin + antalarmin (23,91 ± 1,53 mIU/ml, $p < 0.01$) and kisspeptin + astressin 2B (22,66 ± 0,93 mIU/ml, $p < 0.05$) groups were significantly higher than the control group (17,98 ± 0,88 mIU/ml). Although the FSH value was higher in the kisspeptin + atosiban group (20,56 ± 1,82 mIU/ml) than in the control group, there was no statistically difference. FSH values of the p234 (16.95 ± 0.46 mIU/ml) and kisspeptin + p234 (18.16 ± 0.47 mIU/ml) groups were similar to the control group value.

Serum LH results are presented in Figure 6. Serum LH levels were found to be significantly higher in kisspeptin (39,05 ± 1,33 mIU/ml, $p < 0.001$), kisspeptin + antalarmin (39,11 ± 0,92 mIU/ml, $p < 0.001$) and kisspeptin + astressin 2B (36,37 ± 1,41 mIU/ml, $p < 0.01$) groups compared to the control group (26,32 ± 1,35 mIU/ml). There was no statistically significant difference in serum corticosterone levels of the kisspeptin + atosiban (32,06 ± 1,56 mIU/ml), p234 (29.05 ± 1.05 mIU/ml) and kisspeptin + p234 (31.73 ± 2.57 mIU/ml) groups compared to the control group.

Serum testosterone results are presented in Figure 7. Serum testosterone levels were significantly higher in the kisspeptin (6.65 ± 0.24 ng/ml, $p < 0.001$), kisspeptin + antalarmin (6.90 ± 0.23 ng/ml, $p < 0.001$), kisspeptin + astressin 2B (6.74 ± 0.38 ng/

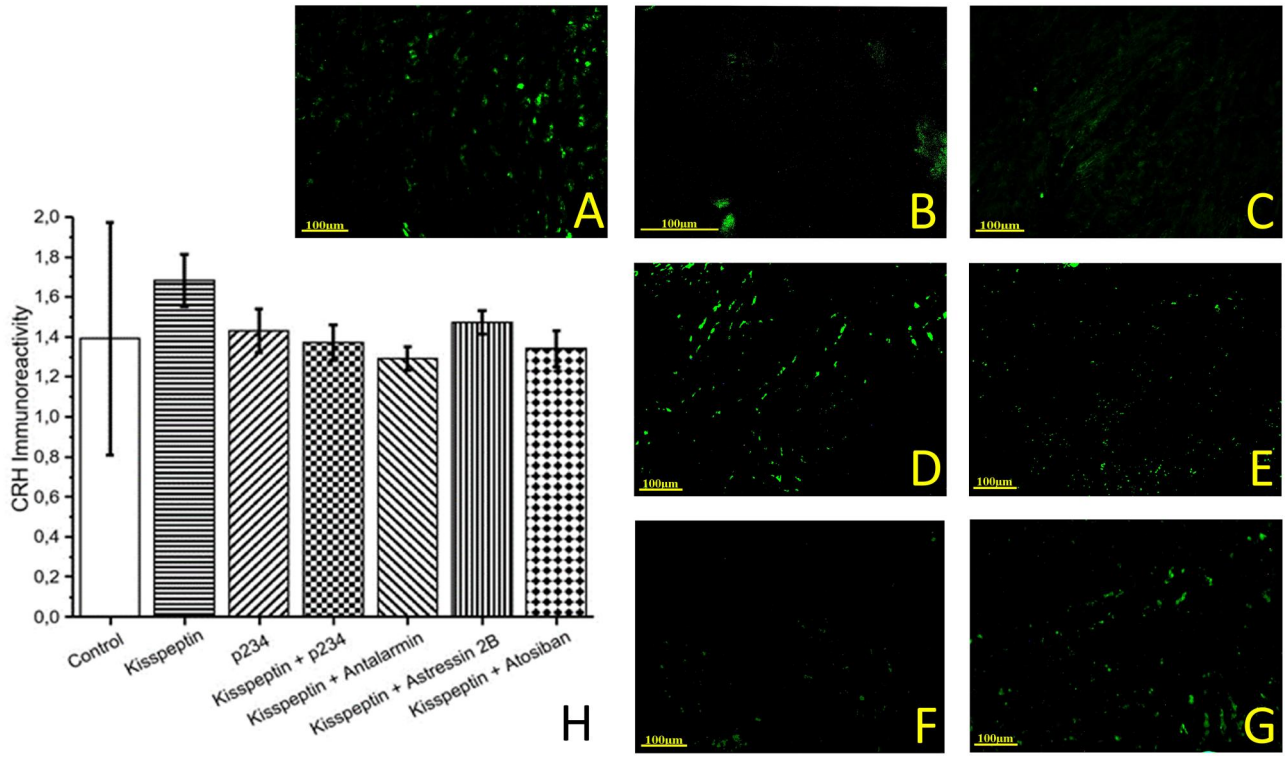


Figure 1. CRH immunoreactivity in PVN. (A: control, B: Kisspeptin, C: P234, D: Kisspeptin + p234, E: Kisspeptin + antalarmin, F: Kisspeptin + astressin 2B, and G: Kisspeptin + atosiban). There was no significantly difference in the CRH immunoreactivity between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean).

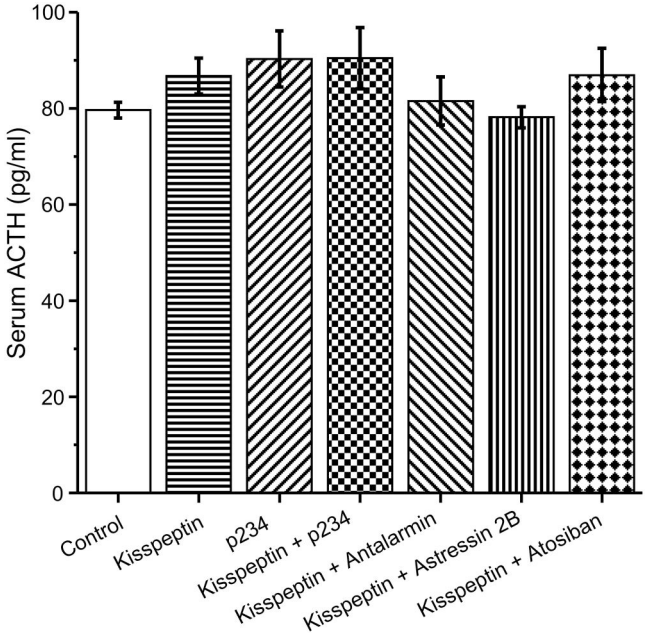


Figure 2. Serum ACTH levels. There was no significantly difference in the serum ACTH levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean).

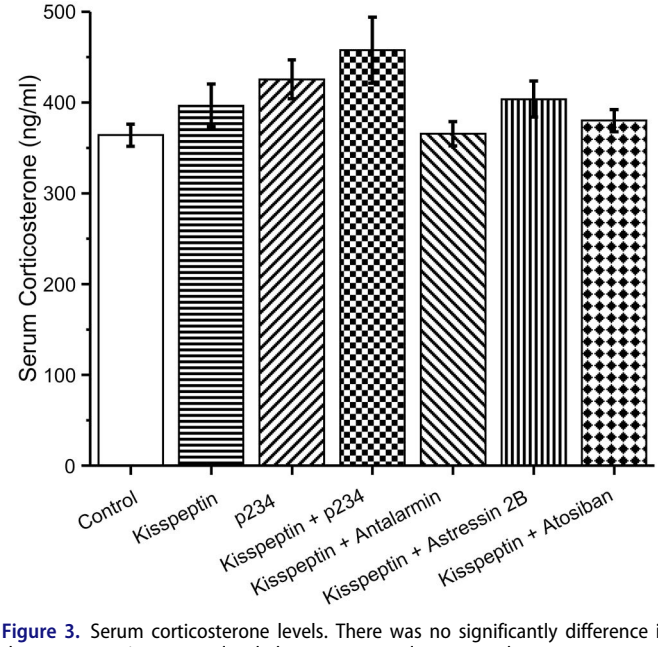


Figure 3. Serum corticosterone levels. There was no significantly difference in the serum corticosterone levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean).

ml, $p < 0.001$) and kisspeptin + atosiban (5.17 ± 0.26 ng/ml, $p < 0.01$) groups compared to the control group (3.21 ± 0.19 ng/ml). Although the testosterone level of P234 (2.37 ± 0.41 ng/ml) was lower than the control group, there was no statistical difference. Testosterone level in the kisspeptin + p234 group (3.65 ± 0.18 ng/ml) was similar to the control group value.

Discussion

There is a close relationship between the HPA and HPG axes, especially in terms of the regulation of reproductive hormones; because increased glucocorticoid levels due to stress typically inhibit reproductive functions [20,21]. Glucocorticoids negatively induce GnRH promoter activity and reduce GnRH receptor transcription [22], thereby

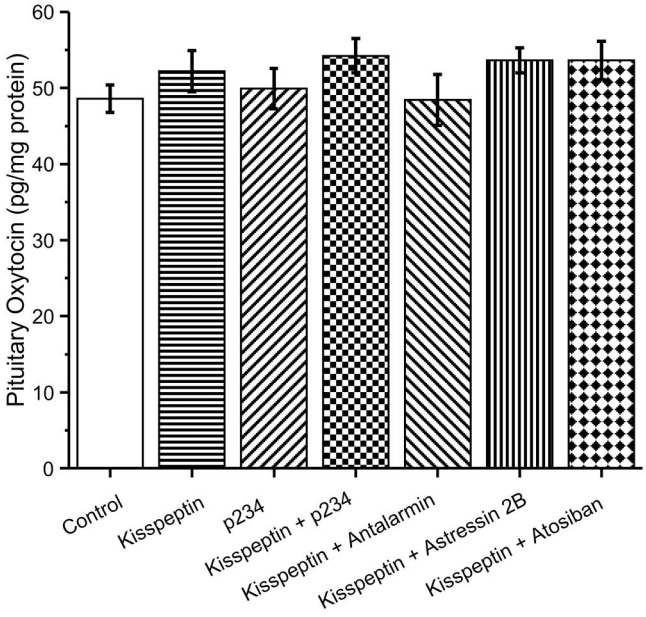


Figure 4. Pituitary oxytocin levels. There was no significantly difference in the pituitary oxytocin levels between control group and treatment groups. (The values were expressed as mean ± Standard error of the mean).

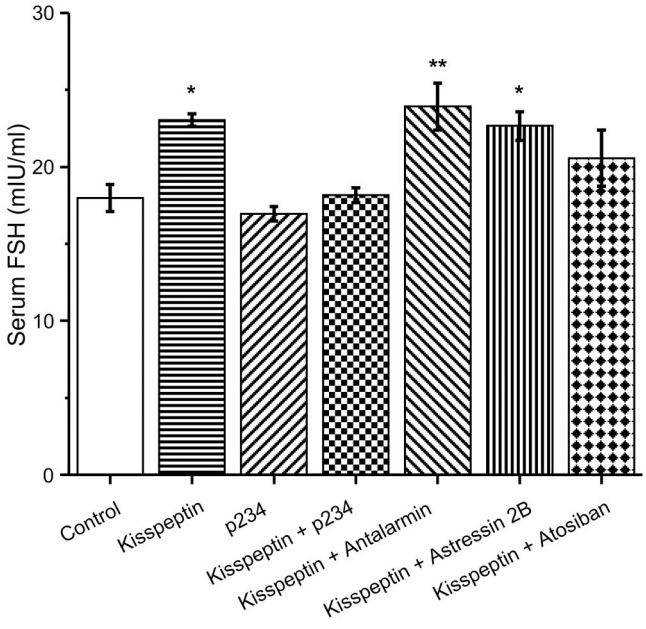


Figure 5. Serum FSH levels. Central kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin 2B treatments caused a significantly increase in the serum FSH levels compared to control group. (The values were expressed as mean ± Standard error of the mean. *: $p < 0.05$ and **: $p < 0.01$).

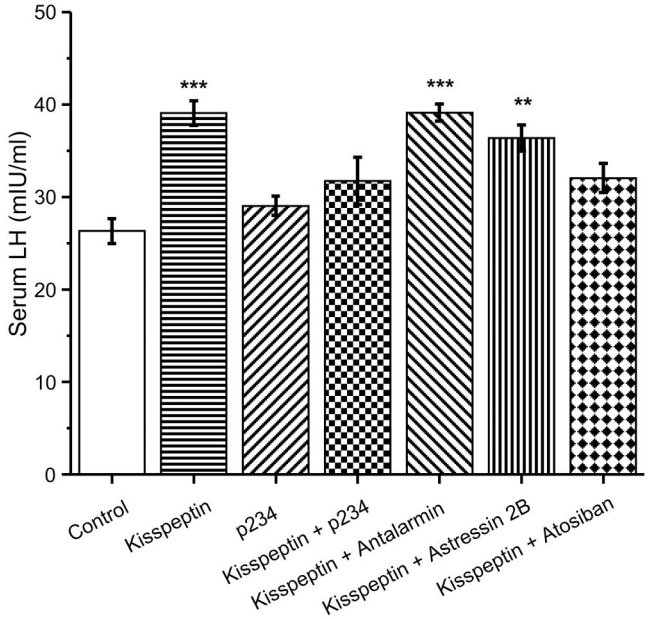


Figure 6. Serum LH levels. Central kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin 2B treatments caused a significantly increase in the serum LH levels compared to control group. (The values were expressed as mean ± Standard error of the mean. **: $p < 0.01$, ***: $p < 0.001$).

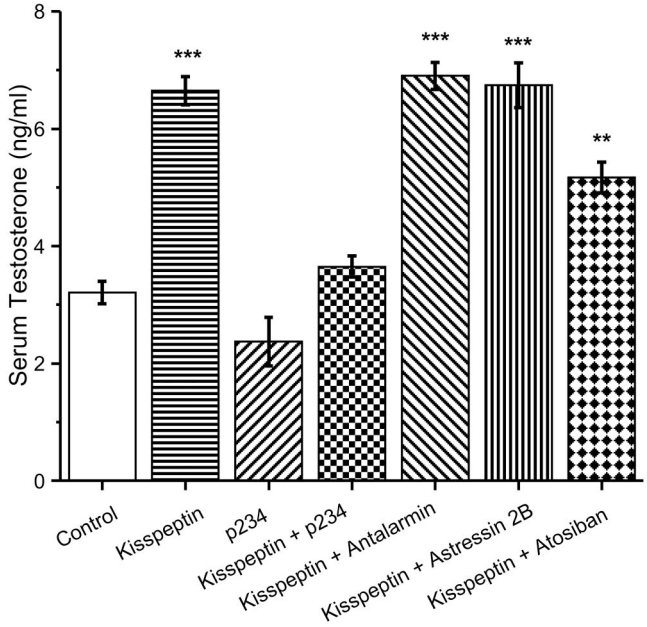


Figure 7. Serum testosterone levels. Central kisspeptin, kisspeptin + antalarmin, kisspeptin + astressin 2B and kisspeptin + atosiban treatments caused a significantly increase in the serum testosterone levels compared to control group. (The values were expressed as mean ± Standard error of the mean. **: $p < 0.01$, ***: $p < 0.001$).

impairing gonadal functions as a result of reduced levels of circulating gonadal steroid hormones. The gonadal hormones can also regulate the physiological parameters of the HPA axis such as, testosterone in men reduces circulating glucocorticoid levels [23], while estradiol has the opposite effect in women [24]. Gonadal steroid hormones also regulate the central expression of CRH, the critical upstream activator of the HPA axis [25,26]. Therefore, a physiologically reciprocal interaction occurs between these two systems, and this pathway establishes an important homeostatic regulation to ensure optimal reproductive success.

Activation of the HPA axis is not only under stress, but also has a circadian rhythm regulated daily by the hypothalamic SCN in a stress-independent manner [3]. This activation begins with the secretion of CRH primarily from the PVN area of the hypothalamus into the hypothalamo-pituitary portal circulation. Considering the particular importance of kisspeptin in the regulation of the HPG axis, as well as the fact that recent data indicate neuronal projections of kisspeptin to the PVN [10], it seems likely that kisspeptin is

involved in the control of the HPA axis. Kisspeptin neurons extend to the PVN and reach to CRH, aVP and oxytocin neurons [11–13]. However, 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 found that central injection of kisspeptin did not change CRH immunoreactivity in PVN. Moreover, there were no changes in serum ACTH and corticosterone levels.

Although it has been suggested that kisspeptin reduces CRH mRNA expression in PVN cell culture [15], it also has been reported that kisspeptin application does not change glucocorticoid secretion in monkeys [14] and rats [15]. The findings of our study confirm the data from monkeys and rats regarding to kisspeptin. However, HPA activation may also show gender-dependent variability. It has been suggested that there is an inverse relationship between CRH expression and medial preoptic area Kiss1 expression in PVN during puberty in female rats [16]. As a result of absence of female rats, our findings are far from answering the possibility of gender-dependent variability.

In our study, even there is no statically difference, we found that the serum ACTH levels of the kisspeptin, p234 and kisspeptin + p234 and kisspeptin + atosiban groups were higher than the control group. Moreover, serum ACTH concentrations in the kisspeptin + antalarmin and kisspeptin + abstresin2B groups were similar to the control group values. Since the tendency to increase ACTH levels in the kisspeptin group was not observed in the kisspeptin + antalarmin and kisspeptin + abstresin 2B groups, we can conclude that kisspeptin interacts with the CRH 1 and CRH 2 receptors, albeit partially. Interestingly, we determined that the trend of increase in serum corticosterone level in the kisspeptin group was not valid in the kisspeptin + antalarmin and kisspeptin + atosiban groups. Considering both the ACTH and corticosterone results, we can say that kisspeptin interacts with the CRH 1 receptor in particular. These results may indicate the possibility of a dose-dependent interaction between kisspeptin and HPA. However, it is unknown whether kisspeptin has a dose-dependent effect on the activation of the HPA axis in male rats. In our study, being able to use single dose was among our limitations.

Kisspeptin neuron axons that project to PVN in the hypothalamus reach oxytocin neurons as well as CRH [11–13]. This indicates that kisspeptin may interact with oxytocin. Regarding the subject, it has been suggested that kisspeptin increases the firing rate of oxytocin neurons and also increases plasma oxytocin level [9, 17]. In our study, even there is no statically difference, we observed that the pituitary oxytocin values of the kisspeptin, kisspeptin + p234, kisspeptin + astressin 2B and kisspeptin + atosiban groups were higher. Oxytocin levels of the P234 and kisspeptin + antalarmin groups were similar to the control group value. An increase trend in pituitary oxytocin levels in the kisspeptin group was not observed in the kisspeptin CRH 1 receptor antagonist group. For this reason, it seems to be an interesting finding as it indicates a possible interaction in HPA parameters for oxytocin.

The well-known physiological function of kisspeptin is potent stimulation of the HPG axis [27]. Mutations in kisspeptin receptor (KISS1R in humans, Kiss1r in rodents, formerly known as GPR54) results in sexual immaturity and infertility indicates the important role of kisspeptin on reproduction [28–30]. The effects of kisspeptin on the onset of puberty and stimulation of gonadotropins have been demonstrated in both male and female rodents [29, 31]. In current experiments, we found that both FSH and LH levels were significantly higher in the kisspeptin, kisspeptin + antalarmin and kisspeptin + astressin 2B groups. Although there is no statically difference, the kisspeptin + atosiban group had higher FSH and LH levels than the control group. Regarding testosterone, we determined that this hormone was significantly higher in the kisspeptin, kisspeptin + antalarmin, kisspeptin + astressin 2B and kisspeptin + atosiban groups. In our study, as expected from kisspeptin, gonadotropins and testosterone levels increased significantly with icv application of this hormone. Likewise, significant increases in gonadotropins and testosterone were observed in the groups in which kisspeptin was administered together with CRH receptor 1 and CRH receptor 2 antagonists. We also determined that kisspeptin-induced gonadotropin and testosterone increase was blocked by kisspeptin and its receptor antagonist p234 co-administration. An interesting finding in our study is that the potent stimulant effect of kisspeptin on gonadotropins was decreased in the oxytocin receptor antagonist group. This result indicates that oxytocinergic interaction contributes positively to the effect of kisspeptin on gonadotropins. However, since we used only a single dose in our study, we do not know whether this effect varies dose-dependently. Again, we think that it is valuable to determine its agonistic effect as well as the oxytocin antagonist in further studies. Oxytocin neurons in supraoptic nucleus increase their firing rate following intravenous administration of kisspeptin-10 [17]. There is a relationship between circulating kisspeptin level and oxytocin because intravenous administration of kisspeptin increases plasma oxytocin levels in non-pregnant female rats [9]. It was reported that when oxytocin is administered intranasally, it increases kisspeptin expression in the hypothalamus. It also reduces gonadotropin-inhibiting hormone (GnIH, mammalian homologue RFamide-related peptide 3 (RFRP3)). Depending on these effects, exogenous oxytocin stimulates the secretion of GnRH [32]. Moreover, it has been reported that endogenous oxytocin stimulates GnRH neurons and stimulates gonadotropin secretion [33]. These results indicate that gonadotropins may be negatively affected due to the effect of kisspeptin and oxytocin signaling in the event of stress-induced HPA axis activation. After oxytocin production its transvers to the posterior pituitary by neurophysin 1-mediated transport [34]. In the PVN area, which is the beginning of the activation pathway of the HPA axis, there are CRH neurons as well as oxytocin neurons. Therefore, oxytocin also has contribution to the activation of the HPA axis. Oxytocin secretion is one of the inhibitory physiological input affecting CRH releasing from PVN [5,6, 35]. Therefore, oxytocin has a suppressive effect on HPA activity [36–38]. Moreover, oxytocin injection decreased

locomotor activity in rats, and it was observed that this hormone was anxiolytic at low doses and sedative at high doses [39]. Oxytocin suppresses HPA and decreases cortisol level [38]. It has also been reported that the inhibitory effects of oxytocin on the HPA axis can be reduced by an oxytocin receptor antagonist, and an increase in corticosterone level in rats due to this antagonistic effect [35]. According to these results, it can be said that the stimulating effect of kisspeptin on gonadotropins may weaken in conditions that affect oxytocin production in the hypothalamus. Considering the possible role of oxytocin in HPA axis activity, this finding is one of the important results of our study.

Conclusion

This study suggests that icv injection of kisspeptin at a dose of 50 pmol has no direct effect on HPA axis activation. However, kisspeptin strongly stimulates the HPG axis. The stimulatory effects of kisspeptin on HPG 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. In summary, although the central injection of kisspeptin does not have direct effect on the HPA axis, it strongly stimulates the HPG axis.

Disclosure statement

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Data availability statement

All data generated or analyzed during this study are included in this published article.

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