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Original Article

The antidepressant-like effects of kisspeptin-10 are reversed by kisspeptin antagonist peptide 234 in male rats

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Abstract

Kisspeptins are reported to be the most potent activators of the hypothalamus-pituitary-gonadal (HPG) axis known to date. Kisspeptin potently elicits gonadotropin-releasing hormone (GnRH) release and luteinizing hormone (LH) secretion, even in the pre-pubertal period. Beyond the hypothalamus, kisspeptin is also expressed in limbic and paralimbic brain regions, which are areas of the neurobiological network primarily implicated in emotional behaviors alongside sexual functions. Therefore, an increasing body of studies has implicated kisspeptin as having many influences on emotional behaviors. The study was set out to explore if the kisspeptin/GPR54 signaling system is required for the anti-depressant-like effect of kisspeptin-10 (KP-10), besides the regulation of the HPG axis. To test this concept, peptide 234 (P234), a kisspeptin antagonist, was given to the male rats, and its modulatory effect on the anti-depressant-like effects of kisspeptin was investigated by using a forced swimming test (FST). The study has also sought to know whether kisspeptin can exert its effects through adrenergic and serotonergic receptors. To investigate this, the agents yohimbine (Yoh), an alpha-2 adrenergic receptor antagonist, and cyproheptadine (Cry), a non-selective 5-HT_2 serotonergic receptor antagonist, were administered in the experiments. Our results indicate that, in rats, the anti-depressant-like effects of KP-10 in a modified rat FST are mediated by GPR54 receptors since the kisspeptin antagonist peptide 234 reversed kisspeptin-induced anti-depressant-like effects. Our data also demonstrate that the anti-depressant-like effects of kisspeptin, at least in part, are mediated by an interaction of the alpha-2 adrenergic and 5-HT_2 serotonergic receptors.

Keywords: Kisspeptin, Hypothalamus-pituitary-gonadal axis, Peptide 234, Yohimbine, Cyproheptadine, Forced swimming test, Antidepressant.

1. Introduction

Kisspeptins were formerly called metastin since they were originally identified as products of the metastasis suppressor gene *KiSS1* [1]. Kisspeptins encoded by the *KiSS1* gene produce a peptide of 145 amino acids that can be cleaved into four peptides, which are termed kisspeptin-10, -13, -14, and -54 according to the number of amino acids in length. The decapeptide kisspeptin-10 (KP-10), which is shared by all the members of the kisspeptin family, is required for biological activity [2-4]. Kisspeptins are the natural ligands for *KiSS1R*, a G protein-coupled receptor 54 (GPR54) [2, 5, 6]. Although previous studies implicated kisspeptins as antimetastatic factors, recent studies have focused on their exciting role in reproduction. Gonadotropin-releasing hormone (GnRH) neurons have been shown to express the GPR54 receptor [7], through which kisspeptins activate GnRH secretion [8]. In our recent study, peptide 234 (P234) (an antagonist of GPR54 receptors) has been found to inhibit kisspeptin-induced changes in reproductive functions [9]. Kisspeptins are reported to be the most potent activators of the hypothalamus-pituitary-gonadal (HPG) axis known to date [10, 11]. *KiSS1*/*KiSS1R*-inactivating mutations in humans or *KiSS1R*-targeted deletions in transgenic mice resulted in a phenotype of delayed puberty and idiopathic hypogonadotropic hypogonadism [3, 12, 13]. Conversely, activating mutations in *KiSS1R* cause central precocious puberty [14]. These pivotal observations established the essential role that kisspeptin plays in the timing of puberty and the regulation of the HPG axis. Kisspeptins potently elicit GnRH release and luteinizing hormone (LH) secretion even during the pre-pubertal period [11, 15]. In our previous study, KP-10 was shown to cause a triphasic change characterized by an initial small increase followed by a significant decrease

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and increase in intracellular free calcium concentrations ($[Ca^{2+}]_i$) in the immortalized GnRH-secreting GT1-7 hypothalamic neurons. Thus, the involvement of intracellular calcium flux downstream from GPR54 in the GnRH-secreting hypothalamic neurons has been shown, which has also provided novel data that the changes in intracellular calcium in response to KP-10 are, at least in part, through the PKC signaling pathway [16].

Beyond the hypothalamus, kisspeptin is also expressed in limbic and paralimbic brain regions, which are areas of the neurobiological network implicated in sexual and emotional behaviors. Kisspeptin and its cognate receptor GPR54 are expressed in certain emotional structures of the limbic system in both rodents [17-19] and humans [2, 5, 20]. Therefore, many studies conducted in animals and humans have implicated kisspeptin in the integration of reproductive hormones with influences on reproductive behaviors [21, 22]. A recent study of ours has shown that KP-10 causes $[Ca^{2+}]_i$ transients in hippocampal neurons, which suggests that kisspeptin may have a role in hippocampal neuron functions [23]. It has been suggested that there is a temporal relationship between mood and reproduction, and a negative mood impairs reproductive success [24]. There is little information about the involvement of kisspeptin in mood and emotion. There is only one study investigating the modulatory effect of kisspeptin on depressive effects, which was carried out in male mice [25]. The dose-dependent anti-depressant-like effects of kisspeptin were observed when administered by intracerebroventricular (i.c.v.) injection during a modified forced swimming test (FST), as signified by significantly decreased immobility but increased climbing and increased swimming times [25].

There is no data on whether kisspeptin/GPR54 signaling is required for the anti-depressant-like effects of kisspeptin. The primary objective of this study is to determine if these effects necessitate kisspeptin/GPR54 signaling. To this end, P234, a kisspeptin antagonist, was pre-administered to evaluate its modulatory impact, assessed using the FST. Additionally, the study aims to investigate the roles of specific neurotransmitter systems by including pre-treatments with yohimbine (Yoh), an alpha-2 adrenergic receptor antagonist, and cyproheptadine (Cry), a non-selective 5-HT_2 serotonergic receptor antagonist. In the current experiments, KP-10, the C-terminal 10 amino acid sequence of kisspeptins with full intrinsic activity for binding and activation, was used instead of kisspeptin-13.

2. Materials and Methods

2.1. Animals

A total of 42 male Wistar Albino rats, aged 2-3 months and weighing 250-300 g, were obtained from the Firat University Experimental Research Unit (Elazig, Turkey). All animal experiments were approved by the Firat University Ethical Committee (FUHADEK 2013/18), and the rats were treated in accordance with national and international laws and policies on the care and use of laboratory animals. The animals were housed in three or four per cage in polypropylene cages kept in a controlled environment with a temperature of 21 ± 1 °C and a 12 h light/dark cycle (lights on at 7:00 a.m.). Pelleted food and tap water were given *ad libitum*. The rats were randomly divided into six groups: Control rats (n = 7) did not receive any treatment; Sham control rats (n = 7) received i.c.v. 10 µl of saline; Kisspeptin rats (n = 7) received i.c.v. 1 nmol/10 µl of KP-10; Kisspeptin+P234 rats (n = 7) received i.c.v. 1 nmol/10 µl of KP-10 and 1 nmol/10 µl of P234 (kisspeptin antagonist); Kisspeptin+Yoh: rats (n = 7) received i.c.v. 1 nmol/10 µl of KP-10 and i.p. 5 mg/kg of Yoh (alpha-2 adrenergic receptor antagonist); Kisspeptin+Cry: rats (n = 7) received i.c.v. 1 nmol/10 µl of KP-10 and i.p. 3 mg/kg of Cry (5-$HT_{2A/2C}$ receptor antagonist). The antagonists were administered 1 h before the test session, followed 30 min later by KP-10.

2.2. Drug treatment

KP-10, P234, Yoh, and Cry were obtained from Sigma (St. Louis, MO, USA). They were freshly prepared in saline immediately before the injections on the day of the experiment. For i.c.v. surgery, rats were anesthetized with a xylazine (12 mg/kg) and ketamine (80 mg/kg) cocktail and placed in a stereotactic frame. A stainless steel cannula was positioned in the lateral ventricle according to stereotaxical coordinates (0.6 mm posterior to the bregma, 1.6 mm lateral to the midline, and 4.0 mm below the outer surface of the skull). The cannula was held in position by dental cement fixed to one stainless steel screw placed into the skull. A silastic tube attached to a short length of cannula was implanted into the nape of the neck. Rats were allowed to recover 7 days before injections. Post-operatively, the rats received analgesia for two days with an oral dose of ibuprofen (1 mg/rat).

2.3. Forced swimming test

The forced swimming test (FST) is one of the most commonly used assays for the study of depressive-like behavior in rats. A previous protocol reported for the FST [26] was modified in our study. In brief, all animals were placed into plexiglass cylinders (60 cm height, 25 cm diameter) that were filled with water at 25 ± 1 °C (depth 39 cm) for two consecutive days. On the first day of the experiment, control and untreated rats were placed individually for 15 min into the plexiglass cylinders for habituation. After a pretest session of 15 min, the rat was removed, dried with a towel, and returned to their home cage. On the second day (24 h after their first exposures), acute saline, kisspeptin, P234, Yoh, and Cry were applied, and 30 min later, FST was performed in all rats separately at 10:00 a.m. The cylinders were washed, rinsed, and refilled with fresh water for every test. Each test was managed for 5 min and behaviors were recorded by a videocamera placed above the cylinder for the following analysis. The observer was blind to the experimental conditions being scored. Immobility (floating), swimming, and climbing were measured [27]. When rodents are forced to swim in an inescapable situation, they generally exhibit an immobile posture. When the animals become immobile, that is, floating in an upright position and making only small movements to keep their heads above water, the total duration of immobility was scored. Immobility means a state of "behavioral despair". Climbing behavior consisted of struggling movements to get out of the cylinder with its forepaws above the surface of the water. Swimming behavior was defined as active swimming motions (usually horizontal) throughout the swim cylinder.

2.4. Statistical analysis

Data are presented as the mean ± standard error of the

mean (S.E.M.). Results were analyzed using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test. For all analyses, differences were considered significant at $P < 0.05$.

3. Results

The values regarding the effects of kisspeptin, P234, Yoh, and Cry on the FST are shown in Figure 1.

In male rats receiving kisspeptin, the duration of immobility was significantly reduced (22.1 ± 1.8 vs. 35.4 ± 2.2 s for the control group, $P < 0.05$). P234 blocked the anti-depressant-like effect of kisspeptin and significantly increased the duration of immobility (51.5 ± 3.3 vs. 22.1 ± 1.8 s for the kisspeptin group, $P < 0.01$). Yoh, an alpha-2 adrenergic receptor antagonist, and Cry, a 5-HT_2 receptor antagonist, significantly increased the duration of immobility and reversed the antagonistic effects of kisspeptin (64.6 ± 2.2 and 45.4 ± 4.4 vs. 22.1 ± 1.8 s for the kisspeptin group, respectively, $P < 0.01$). Additionally, in Kiss.+P234. and Kiss.+Yoh., the duration of immobility significantly increased compared to the control group (51.5 ± 3.3 and 64.6 ± 2.2 vs. 35.4 ± 2.2 s for the control group, respectively, $P < 0.01$). Also, the same parameter significantly increased in the Kiss.+Cry., Kiss.+P234., and Kiss.+Yoh. groups compared to the Sham group (45.4 ± 4.4, 51.5 ± 3.3, and 64.6 ± 2.2 vs. 31.2 ± 1.4 s for the Sham group, $P < 0.01$). On the other hand, Yoh treatment significantly raised the duration of immobility compared to Kiss.+Cry. and Kiss.+P234 groups (64.6 ± 2.2 vs. 45.4±4.4 and 51.5 ± 3.3 s, $P < 0.01$, respectively).

Considering the climbing time, although kisspeptin showed an overall increase in climbing time, no significant difference was observed with the control group (97 ± 11.9 vs. 73.8 ± 7.9 s for the control group). Pretreatment with P234 reversed the kisspeptin-induced change in the climbing time (55.1 ± 8.5 vs. 97 ± 11.9 s for the kisspeptin group, $P < 0.05$). Yoh significantly reduced the enhancing effect of kisspeptin, while Cry partially reversed the kisspeptin-induced reduction in climbing time (22.1 ± 4.0 and 70.8 ± 11.2 vs. 97 ± 11.9 s for the kisspeptin group, respectively, $P < 0.01$). Also, Yoh treatment significantly decreased climbing time compared to the control, Sham, and Kiss.+Cry. groups (22.1 ± 4 vs. 73.8 ± 7.9, 80.4 ± 7.6, and 70.8 ± 11.2 s, respectively, $P < 0.01$). There were no differences between the groups in swimming time (190.7 ± 8.2, 188.2 ± 7.2, 180.8±10.9, 183.7±9.4, 193.2±7.7, and 213.1±3.7 s for control, sham, Kiss., Kiss.+Cry., Kiss.+P234, Kiss.+Yoh. groups, respectively, Figure 1).

4. Discussion

Our results showed that KP-10 has an anti-depressant-like effect in the modified FST. In male rats receiving kisspeptin, the duration of immobility was significantly reduced. Thus, it can be said that kisspeptin causes an anti-depressant-like effect in these animals. The anti-depressant-like effect of kisspeptin was reversed by the kisspeptin antagonist P234, which shows the KiSS1/GPR54 signaling system is required for the anti-depressant-like effect of kisspeptin. Although the anti-depressant-like effect of kisspeptin was shown in male mice, this is the first time that it has been shown that kisspeptin exerts its effects through the kisspeptin receptor, GPR54. Activation of GPR54 by kisspeptins in the hypothalamus results in the activation of GnRH neurons and stimulates GnRH secretion [4, 28, 29].

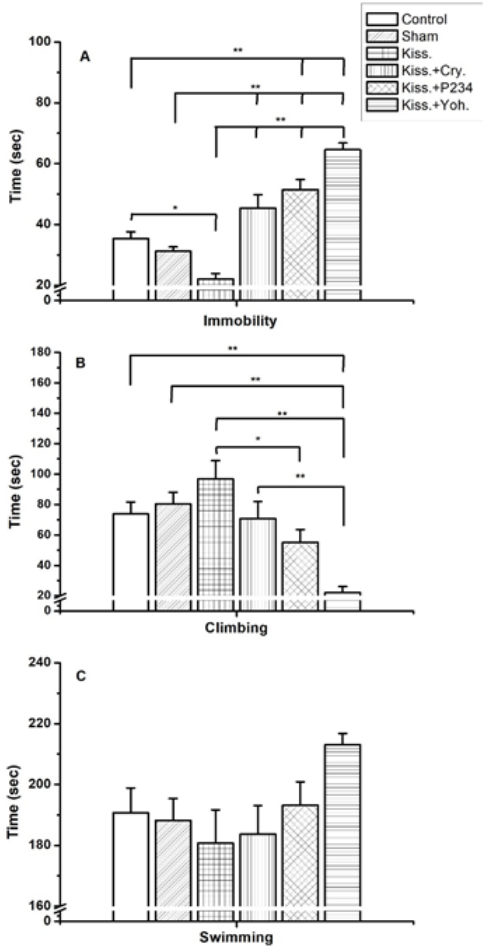

Fig. 1. The anti-depressant-like effects of KP-10 and the effects of a kisspeptin antagonist, P234, a non-selective alpha-2-adrenergic receptor antagonist, Yoh, and a non-selective 5-HT_2 serotonergic receptor antagonist, Cry, on KP-10-induced antidepressant-like action on **A)** immobility, **B)** climbing, and **C)** swimming in a modified rat FST. **P*<0.05 and ***P*<0.01 vs. control.

Kisspeptins and their receptors play a key role in the negative and positive feedback effects of gonadal steroids on the hypothalamus [30]. In contrast to kisspeptin neurons, GnRH neurons lack receptors for sex steroids [31-33]. Sex steroids stimulate or inhibit the *KiSS1* mRNA concentration in the hypothalamus to mediate positive and negative feedback, respectively [34]. I.c.v. administration of kisspeptin results in a robust increase in LH secretion and less prominent follicle-stimulating hormone (FSH) release that ultimately leads to the activation of the HPG axis [35, 36]. Taking this into account, together with the possible role of GnRH in mood disorder pathologies [37, 38], kisspeptin seems to exert its effect through GnRH release. Additionally, kisspeptin has been reported to increase the firing rate of oxytocin neurons [39], which could also explain the anti-depressant-like effect of kisspeptin since oxytocin is well known to have an anxiolytic action in the brain [40].

The KiSS1/GPR54 system is essential for signaling increased gonadotropin secretion during puberty and establishing mammalian reproductive function and regulation of the HPG axis [28, 30]. *KiSS1* expression is negligible in prepubertal ovaries and abruptly increases at the time of a preovulatory surge of gonadotropins [41]. As a consequence, kisspeptin administration in immature rodents and primates was able to induce precocious activation of the gonadotropic axis and precocious pubertal

development [42-44]. Additionally, kisspeptin levels are higher in girls with central precocious puberty [45, 46]. In gonadal juvenile male monkeys, however, the continued kisspeptin administration decreases the LH levels, which suggests that kisspeptin secretion is pulsatile and the continuous stimulation may induce receptor desensitization [47, 48]. Puberty is a progressive period during which behavioral changes also occur along with reproductive maturation. Thus, kisspeptin, which reaches its highest value near puberty, may be related to psychological and social maturation together with reproductive maturation.

The anti-depressant-like effects of kisspeptin in rodents raise the possibility that similar benefits may occur in humans. In a functional neuroimaging study in men, peripheral kisspeptin administration was shown to enhance frontal brain activity in response to negative pictures [49]. Additionally, kisspeptin administration has been found to reduce negative mood [49]. Together, these findings have important implications for considering the potential therapeutic role of kisspeptin-based therapies in mood disorders.

The study has also sought to determine whether kisspeptin can exert its effects through adrenergic and serotonergic receptors, as in other species. To clarify the mechanisms of the antidepressant-like actions of KP-10, various receptor blockers were applied before KP-10 administration. The doses of receptor blockers were selected to effectively block the action of a neuropeptide, as described in a previous study [50]. Pre-treatment with an alpha-2 adrenergic receptor antagonist Yoh or a non-selective 5-HT_2 serotonergic receptor antagonist Cry blocked these effects. Yoh and Cry prevented KP-10 from decreasing the duration of immobility, suggesting that kisspeptin signaling mediates anti-depressant-like effects in rodents via alpha-adrenergic and 5-HT_2 serotonergic systems. Our results confirm the results of the study carried out in male mice [25]. But, differently from that study, in the current experiment, KP-10 did not significantly affect climbing and swimming times. Climbing time was slightly increased by the KP-10 treatment, while swimming time remained unchanged. Swimming and climbing behaviors are reported to be increased by serotonergic and noradrenergic antidepressants in rats, respectively [51]. Thus, the present findings suggest that KP-10 exerts an anti-immobility effect differently from that of selective serotonin reuptake inhibitors in the FST. This discrepancy may result from species differences and the use of different kisspeptin forms. The kisspeptin receptor GPR54 distribution in mice is limited to GnRH neurons in the periventricular region of the hypothalamus and the dentate gyrus of the hippocampus [17, 19]. In contrast, rat brain expression studies showed that the kisspeptin receptor is heavily expressed in other hypothalamic nuclei and the pituitary as well [52]. Therefore, the different expression patterns of GPR54 in these species may be responsible for the difference in kisspeptin actions in the studies. Another explanation for the discrepancy between our results and those of the previous one [25] might be the use of KP-10 in our experiments instead of kisspeptin-13 (KP-13). It has been reported that receptor binding of kisspeptins [53] depends on the length of the peptide. KP-10, therefore, proved to be a less potent activator of the kisspeptin receptor than KP-13 [53].

In conclusion, central treatment with KP-10 produces an anti-depressant-like effect, which is reversed by the kisspeptin antagonist, P234. To our knowledge, this study provides the first evidence that kisspeptin exerts its anti-depressant-like effect through its receptor, GPR54. Additionally, our work indicates that KP-10 induces an anti-depressant-like effect by modulating serotonergic and noradrenergic activity. Further studies are necessary to explore the therapeutic potential of kisspeptin for treating depression.

The limitations of this study encompass the lack of dose-response comparisons and investigations into various forms of kisspeptin. Additionally, the precise mechanism underlying the anti-depressant-like effects of KP-10 remains incompletely elucidated, highlighting the necessity for further research to assess its clinical applicability.

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Declaration of interest
The authors declare that they have no conflict of interest.

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