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Original Article

The involvement of kisspeptin in centrally regulatory mechanism of neuropeptide Y on testosterone secretion in male Wistar rats

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Abstract

Introduction: Numerous studies have demonstrated that kisspeptin, a peptide from the KISS1 gene, plays an important role in regulating the secretion of gonadotropin releasing hormone (GnRH). Also, there is some evidence suggesting that kisspeptin can interact with other neuropeptides for the control of the reproductive axis. In the present study, we have investigated the effect of central administration of either kisspeptin or neuropeptide Y (NPY) or both on the mean plasma testosterone concentration in male rats.

Methods: In this experimental study, 66 male Wistar rats were allocated into 11 groups (n=6 per group) receiving saline, kisspeptin (1 nmol), P234 (kisspeptin receptor antagonist, 1 nmol), NPY (2.3 nmol), BIBP3226 (NPY receptor antagonist, 7.8 nmol) or co-administration of them via intracerebroventricular (ICV) injection at 9:00-9:30 A.M. Blood samples were collected at 30 and 60 min following the injections for hormone assay. The serum testosterone concentration was measured using rat testosterone kit and the method of radioimmunoassay.

Results: Kisspeptin or NPY injection significantly increased the mean serum testosterone concentration compared to saline at 30 and 60 min postinjection (P<0.001). The co-injection of kisspeptin+NPY considerably raised the mean serum testosterone concentration compared to NPY in both 30 and 60 min after the administration (P<0.001). This study indicates that P234 or BIBP3226 significantly attenuated (P<0.001) the testosterone increase after the kisspeptin injection compared to kisspeptin while a stimulatory increase effect was observed in the kisspeptin groups compared to either NPY or kisspeptin.

Conclusion: Based upon the results, NPY may modulate the testosterone secretion indirectly via the kisspeptin signaling system.

Keywords:
Neuropeptide Y;
Kisspeptin;
HPG axis;
Testosterone;
Rat

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Introduction

There is a close relationship between gonadal steroids and hypothalamic-pituitary-gonadal (HPG) axis (Navarro et al., 2005). A quintessential question in reproductive neuroendocrinology is the precise identity of the specific sets of neurons and afferent pathways responsible for the gonadal hormone feedback control of the gonadotropin-releasing

hormone (GnRH) secretion. The temporal patterning of the GnRH secretion into the hypophyseal portal blood stream, and its subsequent control of the luteinizing hormone (LH) secretion from the pituitary gland, is under the control of positive and negative feedback actions of gonadal steroid hormones progesterone, estradiol and testosterone (Goodman and Inskeep, 2006; Zeleznik and Pohl, 2006).

Most of the neurohormones and neuromodulator molecules are involved in the control of reproduction. These molecules play a pivotal role in regulating the reproductive axis activity.

Kisspeptin-54 is the product of the KISS1 gene and its further proteolytic cleavage gives rise to the shorter products namely kisspeptin-14, kisspeptin-13 and kisspeptin-10. All kisspeptins share a common COOH-terminal decapeptide and have similar affinity to the G protein-coupled receptor-54 (GPR54) (Navarro et al., 2004; Han et al., 2005) -also known as the KISS1 receptor (KISS1R) (Kotani et al., 2001). Kisspeptin is a neuroendocrine switch for the onset of puberty (Han et al., 2005). Some studies have revealed that GPR54 is essential for normal pubertal development and sexual function. Malfunction in kisspeptin signaling leads to reduction of GnRH signaling and results in hypogonadotrophic hypogonadism (HH) (Bianco and Kaiser, 2009; Silveira and Latronico, 2013; D'Anglemont de Tassigny et al., 2007; de Roux et al., 2003). Reproductive disorders which lead to the reduction of GnRH signaling culminate in HH (Silveira and Latronico, 2013; Matsui et al., 2004). In this regard, loss-of-function mutations of the gene encoding the GPR54 cause HH and delayed puberty in both humans and rodents (Bianco and Kaiser, 2009; D'Anglemont de Tassigny et al., 2007).

Kisspeptin expression has been identified in multiple tissues including pancreas, adipose tissue, gonads and placenta (Kotani et al., 2001; Lee et al., 1996; Roseweir et al., 2009); however its main functional role is mediated by its expression within the central nervous system. Kisspeptin is produced by the neurons in the infundibular (arcuate) nucleus of the hypothalamus. These neurons have been shown to have direct afferent connections to the mediobasal hypothalamus GnRH neurons in rats. It has also been reported that approximately 70% of the GnRH neurons express the GPR54 (Roseweir et al., 2009) and kisspeptin strongly affects the direct regulation of the GnRH/LH release. The kisspeptin activity has been shown to induce up-regulation of KISS1R within these GnRH neurons (Kim et al., 2010). Kisspeptin therefore regulates the activity of GnRH neurons, making it the top level of a final common pathway controlling reproduction. Recently, it has been established that some major factors are involved in the control of the sexual function that include fasting, ghrelin and morphine; they exert their inhibitory effects onto the HPG axis via down-regulation of the kisspeptin/GPR54 signaling system (Simonneaux et al., 2009; Smith et al., 2007).

Kisspeptin greatly stimulates the LH secretion by acting on the preoptic area (POA), where most of the GnRH neurons projecting to the median eminence (ME) are located; this is due to the injection of kisspeptin into the third ventricle or POA increased plasma LH (Kinoshita et al., 2005) and subsequently testosterone concentration in rats (Mahmoudi et al., 2014b).

Neuropeptide Y (NPY) is a 36 amino acid orexigenic peptide which is highly expressed in the hypothalamic nuclei, especially in the arcuate (ARC) nucleus (Sahu et al., 1987; Dhillon et al., 2009). It has been exhibited that the NPY neurons in the ARC nucleus project to the GnRH neurons. Thus, the hypothalamic NPY neurons may participate in the regulation of LH secretion in the rat, thereby indicating that one of the mechanisms of its action may be to increase the pituitary LH in response to GnRH (Crowley et al., 1987). The central injection of NPY increase the LH secretion through raising the GnRH release (Sahu et al., 1987; Karla et al., 1995). However, injection of the NPY receptor (Y1 subtype) antagonist such as BIBP3226 completely block the stimulatory effects of the NPY on the GnRH/LH release (Leupen et al., 1997). Kisspeptin is a hypothalamic neuropeptide which is mainly synthetized in the ARC nucleus in male rats (Navarro et al., 2004; Han et al., 2005). Some researches have reported the stimulatory effects of NPY or kisspeptin on the GnRH/LH secretion. Based on the previous studies, hypothalamic NPY has an overlapped distribution with the GnRH pathways in the hypothalamus and the POA (Rodriguez-Sierra et al., 1987). NPY stimulates both the basal episodic and the pre-ovulatory surge release of LH in rats, rabbits and primates (Sahu et al., 1987). The results demonstrate that the facilitatory effects of NPY on GnRH/LH

secretion which can be manifested under the endocrine conditions required to produce preovulatory LH surges, i.e. after the estrogen and progesterone treatment (Bauer-Dantoin et al., 1992). We previously displayed that NPY noticeably boosted the mean serum LH concentration compared to the saline and the co-administration of kisspeptin and NPY could noticeably raise the mean level of the serum LH in comparison with the saline or NPY (Azizi et al., 2015).

Our hypothesis is that the regulatory effects of kisspeptin are also applied to the GnRH neurons in the arcuate nucleus indirectly by means of the NPY neurons. It is possible that the feedback effects of the gonadal hormones be applied for regulating the reproductive axis in the arcuate nucleus through the NPY neurons.

The main research question addressed in this study is whether kisspeptin regulates the GnRH neuron activity directly or performs its role through modulation of NPY neurons? The purpose of this study was to determine the effects of kisspeptin on the mean serum testosterone in male rats treated with NPY or its antagonist (BIBP3226). Furthermore, whether increase in the testosterone concentration, in the creation of negative feedback of NPY neurons, plays a role as a connecter between the kisspeptin and GnRH neurons in the hypothalamus or not.

Materials and methods

Animal groups

In the present experimental study, male Wistar rats (n=66) weighing 200-250g (provided by the Neuroscience Research Center of Shahid Beheshti University, Tehran, Iran) were housed individually in cages and kept at certain temperature (22±2°C) with limited light hours (12h light/dark cycle, light at 7:00 am). The animals had continual free access to food and water. All procedures for the maintenance and use of the experimental animals were based upon the approval of the Ethical Committee of the Shahid Beheshti University.

Intracerebroventricular (ICV) cannulation and injections

Animal surgery procedures and handling were carried out as previously described (Lewis et al., 2004). The animals were anesthetized by intraperitoneal injection of a mixture of ketamine (80 mg/kg B.W) and xylazine (10 mg/kg B.W). For the central injections, a 22-gauge stainless cannula was implanted in the third cerebral ventricle according to the instructions of Paxinos and Watson Atlas (AP= -2.3, ML=0.0, DV=6.5) (Paxinos and Watson, 2005). The cannula was secured to the skull with three stainless steel screws and dental cement. The animals were kept in individual cages. After one-week recovery period, 66 rats in 11 groups (n=6 in each group) received saline, kisspeptin (1 nmol), P234 (1 nmol), kisspeptin (1 nmol) + P234 (1 nmol), NPY [YY-36-NH_2] (2.3 nmol), BIBP3226 [RR-1] (7.8 nmol), NPY (2.3 nmol) + BIBP3226 (7.8 nmol), kisspeptin (1 nmol) + NPY (2.3 nmol), kisspeptin (1 nmol) + BIBP3226 (7.8 nmol), P234 (1 nmol) + NPY (2.3 nmol) and P234 (1 nmol) + BIBP3226 (7.8 nmol), respectively. For the ICV injection, kisspeptin-10 (Phoenix Pharmaceutical Inc, USA) and P234 (Phoenix Pharmaceutical Inc, USA) were dissolved in the saline. The NPY (GL Biochem Ltd, Shanghi, China) and BIBP3226 (GL Biochem Ltd, Shanghi, China) were dissolved in 0.1% trifluoroacetic in 100% acetonitrile. All solutions in the volume of 3µl were injected using a 27-gauge stainless steel injector that protruded 0.5mm beyond the cannula and was connected to a Hamilton microsyringe by a polyethylene tube via the third cerebral ventricle at 9:00-9:30 A.M.

Hormonal assay and statistical analysis

One milliliter of the blood samples were collected at 30 and 60 min following the injections via the tail vein (Patterson et al., 2006; Thompson et al., 2009). The blood samples were immediately centrifuged for 15 min at 3000 rpm and the serum was stored at –20°C till further assessment. Mean serum testosterone concentration was measured using the rat testosterone kit (Institute of Isotopes Co., Ltd, Budapest, Hungary) and the method of radioimmunoassay. The sensitivity of the kit was 0.05 ng/ml or 0.18 nmol/L. The results were presented as the mean ± SEM. The data analysis was performed using one way-ANOVA test followed by post hoc Tukey's test and SPSS software (version 23). $P<0.05$ was considered to be statistically significant.

Results

The statistical analysis shows that kisspeptin

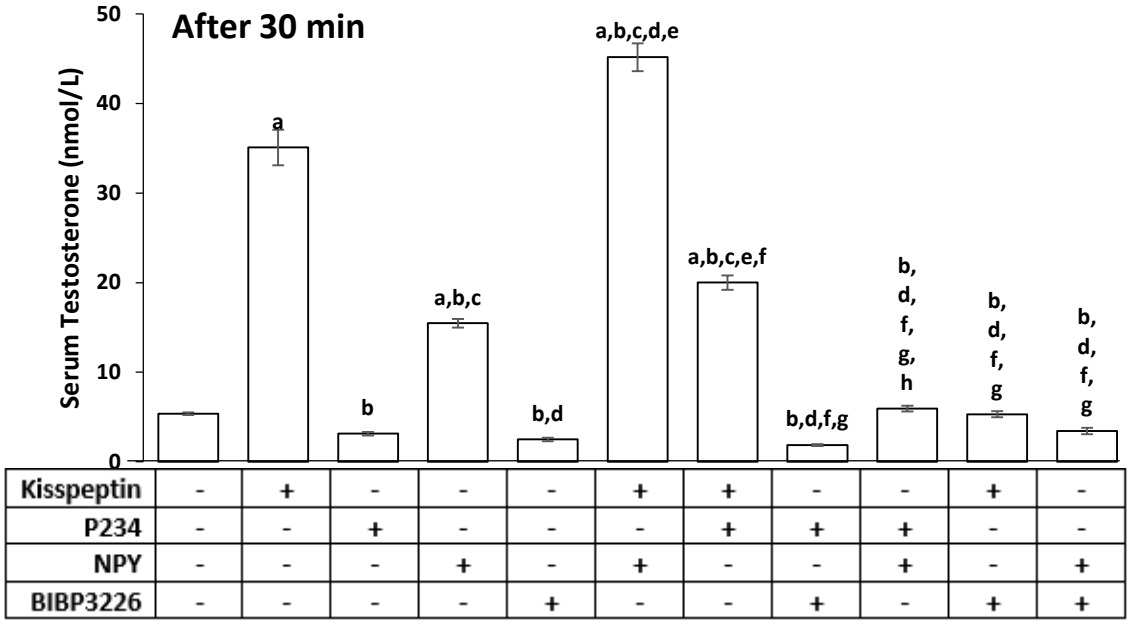

Fig.1. The effect of ICV saline, kisspeptin (1 nmol), P234 (1 nmol), NPY (2.3 nmol) and BIBP3226 (7.8 nmol) or co-administration of them on serum concentration of testosterone in male Wistar rat at 30 min postinjection of solutions. Significance differences are indicated by small caption. a) compared to saline group, b) compared to kisspeptin group, c) compared to P234 group, d) compared to NPY group, e) compared to BIBP3226 group, f) compared to kisspeptin+NPY group, g) compared to kisspeptin+P234 group and h) compared to P234+BIBP3226 group. Data presented as mean ± SEM; *P*<0.05; n=6 per group.

significantly increases the mean serum testosterone concentration when compared to the saline at 30 and 60 min after the injection (*P*<0.001, Fig. 1 and 2), while P234 decreases the serum level of the testosterone concentration. Although this decrement was not statistically significant after 30 min (Fig. 1), it was significant at 60 min (*P*<0.05, Fig. 2). The co-administration of kisspeptin and P234 raised the testosterone compared to the saline in both 30 and 60 min postinjection (*P*<0.01, Fig. 1). Moreover, the co-administration of kisspeptin and P234 made a considerable decrease in the mean serum testosterone concentration in comparison with kisspeptin after 30 and 60 min injection (*P*<0.001, Fig. 1 and 2).

NPY noticeably boosted the mean serum testosterone concentration compared to the saline in both times (*P*<0.001, Fig. 1 and 2). There was an insignificant decrease in the mean serum testosterone concentration following the BIBP3226 injection compared to the saline (Fig. 1 and 2). In addition, our results revealed that in the BIBP3226 pre-treatment groups, NPY considerably raise the testosterone level (*P*<0.001, Fig. 1 and 2), whereas the mean serum testosterone concentration dropped following the simultaneous injection of NPY and BIBP3226 compared to the NPY group (*P*<0.001, Fig. 1 and 2). The co-administration of kisspeptin+NPY could noticeably augment the mean level of the serum testosterone in comparison with saline or NPY (*P*<0.001, Fig. 1 and 2). Furthermore, the mean serum testosterone concentration increased following the co-administration of kisspeptin and NPY compared to kisspeptin, (*P*<0.001, Fig. 1 and 2). Besides, simultaneous administration of kisspeptin and BIBP3226 did not considerably boost the mean serum testosterone level in comparison with saline or BIBP3226 (Fig. 1 and 2). Also, testosterone concentration decreased significantly in the kisspeptin+BIBP3226 group compared to the kisspeptin group (*P*<0.001, Fig. 1 and 2). The co-administration of P234 and NPY slightly enhanced the mean level of the serum testosterone compared to the saline or P234. Additionally, there was a drop in the testosterone concentration after P234+NPY injection in comparison with NPY (P<0.001, Fig. 1 and 2). The co-administration of P234 and BIBP3226 decreased insignificantly the mean serum

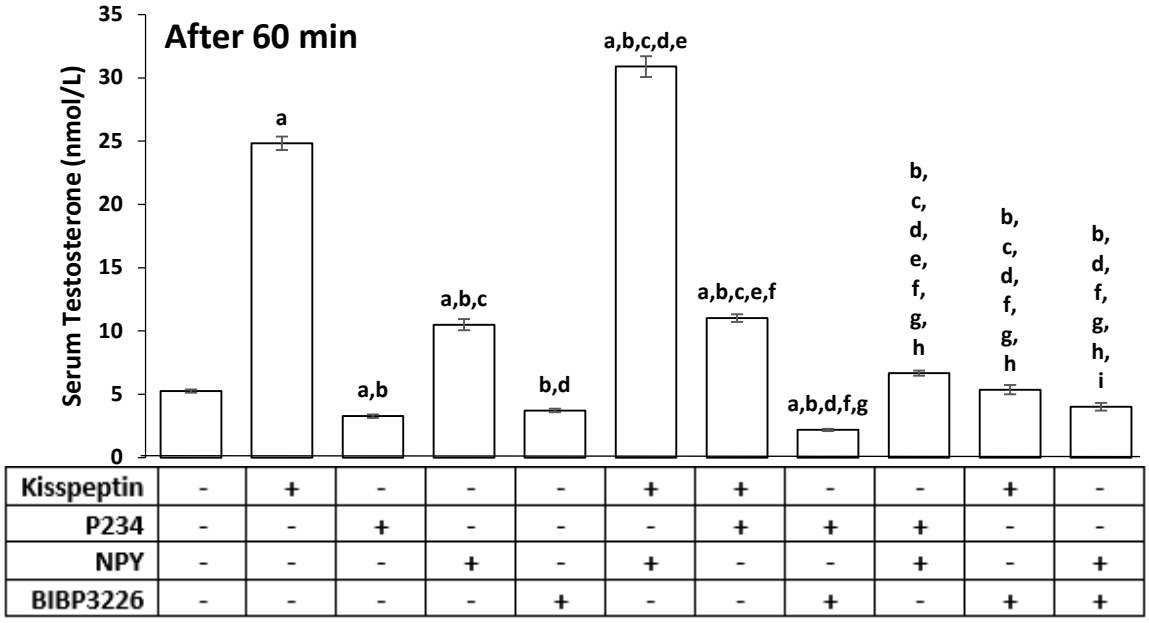

Fig.2. The effect of ICV saline, kisspeptin-10 (1 nmol), P234 (1 nmol), NPY (2.3 nmol) and BIBP3226 (7.8 nmol) or co-administration of them on serum concentration of testosterone in male Wistar rat at 60 min postinjection of solutions. Significance differences are indicated by small caption. a) compared to saline group, b) compared to kisspeptin group, c) compared to P234 group, d) compared to NPY group, e) compared to BIBP3226 group, f) compared to kisspeptin+NPY group, g) compared to kisspeptin+P234 group, h) compared to P234+BIBP3226 group and i) compared to P234+NPY group. Data presented as mean ± SEM; $P<0.05$; n=6 per group.

testosterone concentration compared to the saline, P234 or BIBP3226 (Fig. 1 and 2). P234+BIBP3226 injection led to a considerable decrease in the mean serum testosterone concentration compared to NPY after 30 and 60 min following the injection ($P<0.001$, Fig. 1 and 2).

The comparisons between corresponding 30 and 60 min groups are shown in Fig. 3. In general, after 60 min, the mean serum testosterone concentration was dropped significantly in kisspeptin, kisspeptin+NPY and kisspeptin+P234 groups ($P<0.001$), but slight increase in BIBP3226, P234+BIBP3226 and P234+NPY groups ($P<0.05$) was observed. In other groups no significant change between 30 and 60 min groups was found.

Discussion

We have demonstrated that ICV administration of kisspeptin-10, a fragment of the endogenous agonist for the GPR54 receptor, stimulates the HPG axis and leads to a considerable increase in the mean serum testosterone concentration compared to the saline group in the male adult intact rats. In another article we have reported that kisspeptin-10 stimulates the LH secretion, suggesting that kisspeptin stimulates the reproduction system (Azizi et al., 2015). These results are in accordance with the ones from the previous studies which demonstrated that the peripheral administration of kisspeptin increases gonadotropin release in the mouse (Matsui et al., 2004; Gottsch et al., 2004). The generation of KISS1R knockout mice confirmed the importance of this GPR54 and its ligand, kisspeptin, as key players in the reproductive endocrinology (Seminara et al., 2003; Funes et al., 2003). These evidences certainly suggest that kisspeptin operates the upstream of GnRH/LH.

Thompson et al. (2004) have stated that kisspeptin-10 dose-dependently stimulates the release of GnRH from *in vitro* hypothalamic explants (Thompson et al., 2004) and the ICV actions of kisspeptin can be occluded by the GnRH antagonists, giving credence to the fact that kisspeptin stimulates the HPG axis via GnRH (Matsui et al., 2004; Gottsch et al., 2004).

In 2009, a group of investigators produced a potent KISS1R antagonist (peptide234) that obstructed the effect of kisspeptin in mice, ewes and monkeys. In the present study, we chose the effective dose of P234 (1nM) in our experiment based upon the

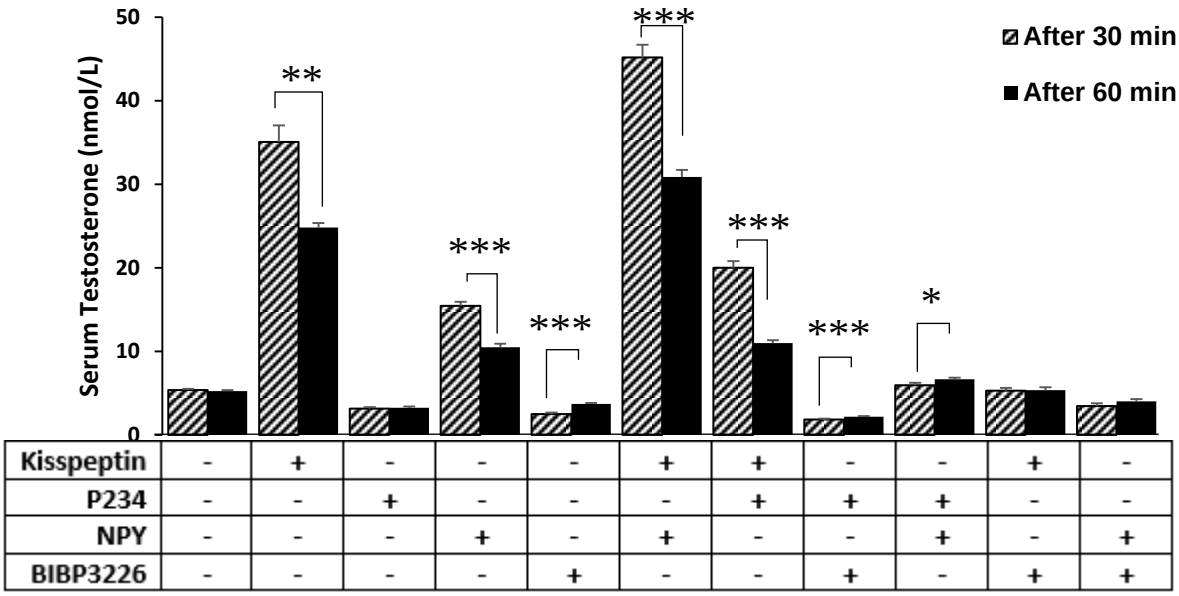

Fig.3. The effect of ICV saline, kisspeptin-10 (1 nmol), P234 (1 nmol), NPY (2.3 nmol) and BIBP3226 (7.8 nmol) or co-administration of them on serum concentration of testosterone in male Wistar rat at 30 and 60 min postinjection of solutions. **P*<0.05, ***P*<0.01 and ****P*<0.001 significance differences between each corresponding groups. Data presented as mean ± SEM; n=6 per group.

previous studies which zeroed in on its inhibitory effects on the HPG axis (Roseweir et al., 2009; Mahmoudi et al., 2014a). The data demonstrated that administration of P234 led to a considerable decrease in the mean serum testosterone concentration (41% in 30 min group and 38% in 60 min group) compared to the saline group. P234, significantly blocks the stimulatory effects of kisspeptin on the androgen release. Our results are in accordance with Roseweir's et al. (2009) data that confirmed that P234, significantly blocked the stimulatory effects of kisspeptin on GnRH/LH release. Also, these results are similar to the previous studies which displayed that the ICV administration of kisspeptin-10 potently elevated the LH secretion *in vivo* over a range of doses from 10 pmol to 1 nmol; the central or peripheral injection of KISS1R antagonist, nonetheless, inhibited it (Roseweir et al., 2009). This antagonist was employed to provide direct evidence for the actions of kisspeptin, concluding that kisspeptin is essential for the generation of the hypothalamic GnRH pulses and that kisspeptin is a target site for the mediation of the negative feedback effects of gonadal sex steroids on the HPG axis. Kisspeptin had no impact on the LH or FSH release from the pituitary fragments. It suggests that the peripheral kisspeptin stimulates the HPG axis via the hypothalamus (Calley and Dhillo, 2014). As it is known, however, the LH stimulates the leydig cells in testis to produce and secretion of the testosterone. The kisspeptin action in the HPG axis is steroid-dependent so that the expression of KISS1 mRNA during the estrous phase is less than other phases of the cycle (Kinoshita et al., 2005; Seminara et al., 2003).

Kisspeptin, notwithstanding, has been proven to be potent gonadotrophin secretagogues through the activation of the HPG axis at the hypothalamic levels and the GPR54 receptor has been localized to the hypothalamic GnRH neurons in the vertebrates, suggesting a direct influence of kisspeptin on these neurons (Parhar et al., 2004). It is probable that the ICV kisspeptin-10 influences the intermediate neurons which regulate the GnRH neurons. Probably, kisspeptin could indirectly stimulate the HPG axis (Navarro et al., 2005; Han et al., 2005).

With the gonadotrophin stimulatory effects of kisspeptin being abolished by preadministration of a GnRH antagonist (Abbara et al., 2013). The GnRH pulsatility is the product of the integration of multiple signals and the number of neuropeptides linked to the regulation of the GnRH are growing apace. (Steiner, 2013). Those that seem to be of paramount importance include the members of the tachykinin

peptide family neurokinin B, substance P, dynorphin A and also neuropeptide Y. The regulatory network is complex, with transsynaptic inputs and the glial cells playing a role. The neurotransmitters glutamates and noradrenaline mainly excite the GnRH neurons, whilst the GABA and endogenous opioids tend to hamper them (Pinilla et al., 2012). The NPY concentration in the arcuate nucleus has been shown to be modulated by gonadal steroids in the male rats (Urban et al., 1993). Also, some studies have reported the co-localization of the NPY receptors and the GnRH neurons (Dhillon et al., 2009).

In the present study, we have investigated the effects of the central injection of kisspeptin on the mean serum testosterone concentration following NPY and BIBP3226-NPY antagonist-injection (Rudolf et al., 1994). Our results signify that the injection of NPY significantly increased the mean serum testosterone concentration compared to the saline, whereas BIBP3226 impeded the stimulatory effects of NPY on the testosterone secretion. Neuroanatomical evidence between NPY and GnRH neurons establishe a possible mechanism in which NPY can directly affect the reproductive axis. Moreover, previous studies have revealed that the Y1 subtype receptor mediates the NPY effects on the HPG axis (Leupen et al., 1997; Jain et al., 1999) and NPY knockout mice are not capable to secret a normal level of LH and gonadal steroids (Dhillon et al., 2009). Be that as it may, the previous studies have produced contradictory results regarding the stimulatory and the inhibitory effects of NPY on KISS1 gene expression. So far no study has mentioned the effects of interaction of NPY/BIBP3226 and kisspeptin/GPR54 signaling systems on hormones controlling HPG axis in male rats.

The results of the present research harmonized with the previous studies which reported the stimulatory effects of NPY and the inhibitory effects of BIBP3226 on endocrine function in the HPG axis (Dhillon et al., 2009). Based on a research conducted in 1995, NPY mRNA levels were enhanced before GnRH release and Y1 subtype receptors were responsible for the increase of the GnRH mRNA levels. The GnRH release injections of antisense oligonucleotides directed against NPY or Y1 receptor antagonists including BIBP3236 into the ARC of different species would block the stimulatory effects of GnRH/LH release (Karla et al., 1995). However, the precise mechanism of the stimulatory effects of NPY on the reproductive axis is controversial; probably, the endogenous NPY or exogenous NPY (YY-36-NH_2) and its antagonists (e.g., BIBP3226) appear to influence the release of the GnRH/LH and subsequently the gonadal sex steroid hormones.

The data in various species demonstrate that NPY can stimulate the release of LH pulse. It has been exhibited to have various effects in animals and acts in a complex fashion with a significant (Hrabovszky et al., 2012) but as yet incompletely understood interaction with kisspeptin. Probably there were detectable direct stimulatory synapses or contacts in kisspeptin expressing neurons arising from NPY expressing neurons in the arcuate nucleus.

The results showed that BIBP3226 significantly attenuated the serum testosterone but the co-administration of BIBP3226 and kisspeptin produced a modest and non-significant decrease in the testosterone concentration compared to the BIBP3226 alone. A stimulatory additive effect, however, was observed in the kisspeptin+NPY group compared to only NPY. Crowley et al. tested whether NPY acts directly on the pituitary gland, either alone or in combination with GnRH, to modify testosterone secretion (Crowley et al., 1987). It is found that the NPY nerve terminals terminate on the GnRH perikarya and dendrites. This possibly exhibits the direct control of NPY neurons on the GnRH-producing neurons. Previous studies revealed that the hypothalamic neuropeptides function in the down-regulation or up-regulation of KISS1 gene expression influence mRNA levels (Crowley et al., 1987; Karla et al., 1995). This indicates the importance of the indirect effects of NPY and its antagonists on the hypothalamic GnRH-producing neurons.

The findings unveiled a stimulatory additive effect in kisspeptin+NPY group compared to the NPY or kisspeptin groups. In agreement with the research carried out in 2005 by Navarro et al., our results provide solid evidence for the potent stimulatory effects of kisspeptin (Navarro et al., 2005) and NPY on testosterone release, acting at central levels (like the hypothalamus+adenohypophysis) and eventually at the testis, and further document an important role of the kisspeptin /GPR54 signal transduction system as a relevant downstream element in the neuroendocrine network governing testosterone secretion. Our results unravel that the kisspeptin and

NPY pathways may interact at hormonal levels to stimulate the HPG axis.

Further studies are warranted to examine the effects of NPY/BIBP3226 and kisspeptin/P234 interaction on testosterone level in orchidectomized male rats or the LH/estradiol levels in ovariectomized (OVX) or OVX-estradiol treated female rats.

It is hypothesized that the gonadal hormones may exert activational effects upon the NPY neurons in the ARC because all serum testosterone levels decrease after 60 min ICV injection of kisspeptin, NPY and the co-administration of them compared to the 30 min injection. The chronic administration of the kisspeptin synthetic analogues (TAK-448 and TAK-683) induces testosterone suppression in male rats (Matsui et al., 2014). The subcutaneous infusion of TAK-683 for 2 weeks in healthy men has been demonstrated to induce rapid and effective reduction in the serum testosterone; it was shown to be reversible upon discontinuation of the drug in a dose-dependent fashion (Scott et al., 2013).

Feedbacks of the gonadal steroid hormones at the level of the hypothalamus and pituitary buttress this hypothesis. The steroid hormone receptors are abundant in the hypothalamus and in many neural systems that impinge on the GnRH neurons including noradrenergic, serotoninergic, β-endorphin-containing and the NPY neurons. The highest density of the androgen receptors was found in the hypothalamic nuclei known to participate in the control of the reproduction and the sexual behaviors, including the ARC, the medial preoptic nucleus and the amygdala (Simerly et al., 1990). The GnRH neurons have generally been reported to lack the steroid hormone receptors. It is likely that the effects of the steroid hormones on the firing rate of GnRH neurons are mediated by the steroid hormone actions in other neural systems that provide afferent input to the GnRH neurons. We postulate that the androgen-mediated negative feedback in the GnRH secretion in rats appears to be regulated by the NPY-containing neurons in the hypothalamus. In this article, we have posited that if a Y1-receptor antagonist, such as BIBP3226, is administrated with kisspeptin, the action of kisspeptin on GnRH secretion can be occluded. The kisspeptin expression is regulated by the testosterone. Probably, kiss1 neurons directly regulate the synthesis and the secretion of NPY in the hypothalamus and the NPY neurons project onto the GnRH neurons in the ARC. Unlike kiss1 neurons, the NPY neurons may play an important role in mediating the effects of kisspeptin on the reproductive system.

Conclusion

In the present study, for the first time, interaction between NPY and kisspeptin on the reproductive axis in male rats has been investigated.

In a nutshell, the main outcomes of the study are as follows:

1) Central injection of kisspeptin or NPY significantly increased the mean serum testosterone concentration compared to saline.
2) Kisspeptin considerably amplified the testosterone response to the NPY injection compared to the NPY group. Hence, NPY and kisspeptin systems may interact with each other to control the testosterone secretion.

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Conflict of interest

The authors declare that they have no competing interests.

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