The effect of intracerebroventricular administration of neuropeptide Y on reproductive axis function in the male Wistar rats: Involvement of hypothalamic KiSS1/GPR54 system

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Abstract

Several studies have shown that neuropeptide Y (NPY) is considered to be one of the key regulators of the hypothalamic-pituitary-gonadal axis in the mammals. Also, kisspeptin is a powerful upstream regulator of gonadotropin-releasing hormone neurons in the hypothalamus. The present study aims to investigate the effects of the intracerebroventricular (ICV) injection of NPY and BIBP3226 (NPY receptor antagonist) on the reproductive axis (either hormonal or behavioral) of the male rats. Furthermore, to see whether NPY signals can be relayed through the pathway of KiSS1/GPR54, the gene expression of these peptides in the arcuate nucleus was measured. The ICV injection of NPY decreased the latencies and increased the frequencies of sexual parameters of the male rats in a significant way. Results obtained from LH and testosterone measurement showed that NPY had a significant increase in comparison with the control group. In this line, BIBP3226 antagonized the stimulative effects of NPY. Furthermore, data from real-time quantitative PCR showed that injection of NPY significantly increased the gene expression of KiSS1 and GPR54, while treatment with BIBP3226 controlled the stimulative effects of NPY on gene expression of KiSS1 and GPR54. Summing up, NPY can exert its impacts on the reproductive axis, this occurs at least partly through affecting KiSS1/GPR54 system.

Introduction

Neuropeptide Y (NPY) is considered to be a powerful 36 amino-acid orexigenic peptide being expressed in distinct areas of the brain especially, in the arcuate nucleus (ARC) of the hypothalamus.1 Neuropeptide Y plays a key role in multiple physiological actions such as regulation of energy balance, maternal behavior, and reproductive activities. Neuropeptide Y signaling is mediated by five G_{i/o}-protein-coupled receptors.2 Recent studies have confirmed that stimulation of Y1-subtype receptor is highly influential on the hypothalamic-pituitary-gonadal (HPG) axis.3,4 Previous reports have concluded that NPY neurons directly influence gonadotropin-releasing hormone (GnRH) cells in the hypothalamus.5 Also, there is some evidence showing the colocalization of Y1 receptor and GnRH neurons.6 Similarly, in the ARC of male rats, the synthesizing cells of NPY mRNA are regulated by testosterone.7 Intracerebroventricular (ICV) injection of NPY increases the release of GnRH and, as a consequence, the level of energy.8-10 Reciprocally, NPY knockout mice do not secrete a normal level of LH.6

Kisspeptin (encoded by KiSS1 gene) is taken to be a neuropeptide being expressed mostly in the hypothalamus. Also, it is necessary for both the maturity and the normal performance of the reproductive axis among the mammals. Kisspeptin signaling acts through the G-protein paired receptor (GPR54) and, accordingly, stimulates the production and release of GnRH.11,12 Some studies have shown that KiSS1/GPR54 system is necessary for reaching normal maturity and sexual activities.13 In this regard, those mutations that end in a loss of KiSS1 receptor gene pave the ground for hypogonadotropic hypogonadism and late maturity in humans and rodents.13-15

According to neuroanatomical considerations, two major populations of kisspeptin neurons can be found in the...
hypothalamus of rodents. These two major populations are involved in maturity and sexual activities. One of them is located in the anteroventral periventricular nucleus (AVPV) in the preoptic area (POA). The neurons of the other population have accumulated in the ARC of medial basal hypothalamus.16-18 The ARC neuronal populations express kisspeptin gene. The kisspeptin secreting neurons play a key role in controlling of the HPG axis. Such neurons are under the influence of metabolic factors. Similar to NPY neurons, they act as a link between energy balance and reproduction.17,19 Also, orexigenic molecules regulate sexual behaviors.20 It was shown earlier that GnRH-immunoreactive fibers in different areas, especially medial POA (mPOA), have a regulating role in sexual behavior.21 According to recent studies, the injection of androgen and gonadotropin facilitates sexual behaviors among mammals.22,23 The fact that GnRH neurons lack androgen receptors but NPY neurons express androgen receptors paved the way for investigating the effects of central injection of NPY on GnRH neurons through the mediation of kisspeptinergic system. Similarly, ARC exerts its influence on the HPG axis and sexual behaviors.

The present study intended to explore ICV injection of NPY and non-peptide antagonists such as BIBP3226 on sexual behavior, serum level of LH, and testosterone. By doing so, the role of NPY as a key neuropeptide in the regulation of the reproductive axis, the gene expression of kisspeptin (KISS1), and its receptor GPR54 among the male rats was examined.

Materials and Methods

Animals. In this experimental study, male Wistar rats (n = 56) weighing 200-250 g (provided by Neuroscience Research Center of Shahid Beheshti University, Tehran, Iran) were housed in cages. These animals were kept under the following controlled conditions in the course of the project: Ambient temperature 22.00 ± 2.00 °C; relative humidity 50.00% and photoperiod of 12:12 darkness and light. Furthermore, there were not any limitations in terms of working with animals. Also, the Ethics Committee of Shahid Beheshti University confirmed the validity of this project [IR.SBU.REC.1397.002; 2018-05-08].

Intracerebroventricular cannulation and injections. The animals were deeply anesthetized by a mixture of ketamine (80.00 mg kg⁻¹; Alfasan, Woerden, The Netherlands) and xylazine (10.00 mg kg⁻¹; Alfasan). It was done to conduct cannulation and intra-cerebral third ventricle injection. To put it briefly, to find the coordinates of the area of injection, the animals were placed in the stereotaxic device after being anesthetized. Then, according to the coordinates given in the Paxinos and Watson atlas (AP = -2.30, ML = 0.00 and DV = 6.50), the area for cannulation was specified.24 After inserting the cannula into the brain, the rats were transferred slowly to their sterile individual cages. They were kept in the animals' room for one week to go through the period of recovery. After a one-week recovery period, the rats were divided into two major groups (behavioral and hormonal/ gene expression). The rats were subdivided into four groups intracerebroventricularly received 3.00 μL vehicle, NPY [YY-36-NH₂] (2.30 nmol), BIBP3226 [RR-1] (7.80 nmol) and BIBP3226 (7.80 nmol) + NPY (2.30 nmol), to study the sexual behavior (n = 8 per groups) and the other rats, received the same treatments for studying gene expression and hormonal assay (n = 6 per groups). For ICV micro-injection, The NPY (GL Biochem Ltd., Shanghai, China) and BIBP3226 (GL Biochem Ltd., Shanghai, China) were dissolved in 0.10% trifluoroacetic in 100% acetonitrile. The prepared solutions were injected (through microinjection method) slowly into the cerebral third ventricle of the rats in 1 min. To do so, Hamilton syringe (5.00 μL) and polyethylene tube were used.

Serum collection and hormonal assay. The blood samples of the animals were collected for testing LH and testosterone at time intervals of 0, 30, and 60 min after injection. Upon being separated by the centrifuge device (Hermle Co., Wehingen, Germany), sera were transferred to a freezer of -20.00 °C. The serum concentrations of LH and testosterone were tested respectively using rLH [125I] RIA (sensitivity and intra-assay coefficients of variation of the kit were 0.09 ng mL⁻¹ and 6.50%, respectively) and testosterone [125I] RIA (sensitivity, intra- and inter-assay coefficients of variation of the kit were 0.04 ng mL⁻¹, 7.30%, and 12.00%, respectively) kit (Isotopes Co., Ltd., Budapest, Hungary) based on the instructions of the manufacturing company.

Sexual behavior test. This experiment was conducted in a chamber with the dimensions of 32.00 × 32.00 × 56.00 cm. The front part of the chamber was made of glass. Each male rat was placed individually inside the chamber 30 min after injection. They were set free for 5 min to attain adaptability with the environment. Then, a sexually active female subject (the receptivity of which was checked before the test) was introduced. Accordingly, the sexual behavior of animals in 30 min was recorded by a digital camera connected to a computer (the operator was unaware of the animal categorization to any particular group). All the tests were conducted during the final phase of photoperiod (15:00 to 19:00) and the below parameters were measured for each animal: The latencies of first mount (male places its forequarters on hindquarters of female from behind; ML), first intromission (mount with vaginal insertion; IL) and first ejaculation (EL); total number of mounts (NM), intromissions (NI) and ejaculations (NE) in 30 min, based on Agmo et al.25 All latencies parameters of sexual behavior were measured in seconds. The chamber was cleaned with diluted ethanol after each test.
Brain dissection and hypothalamus isolation. At the end of the experiment, the rats were euthanized with a mixture of ketamine (150 mg kg⁻¹) and xylazine (10.00 mg kg⁻¹) intraperitonealy. After that, the brain was removed very rapidly, the hypothalamus was isolated and then the ARC nucleus was isolated by previously described method.²⁶ Subsequently, the tissues were immersed in liquid nitrogen immediately. It was stored under a temperature of −80.00 °C until conducting further research.

RNA extraction and gene expression. Total RNA was extracted from the ARC of the hypothalamic tissues of the experimental groups using GeneJET RNA Purification Kit (Thermo Fisher Scientific, Waltham, USA). To omit the possibility of DNA infection, total RNA was treated with DNase I (Thermo Fisher Scientific). The yield of obtained total RNA was determined by spectrophotometer (Hermle Labortechnik Co., Wehingen, Germany) at 260 nm. Then, 1.00 µg extracted RNA was utilized for the synthesis of cDNA with the help of RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). All mentioned stages were implemented according to the manufacturer's instructions.

The RT-qPCR was used to measure the target genes with the specific primers of each gene (Table 1). Relative expression of each sample was defined with SYBR® Ex Taq® (Corbett Research, Mortlake, Australia). For each given transcript, PCR was conducted in a duplicate manner with a final volume of 20.00 µL based on the following thermal cycle: 10 min at 95.00 °C; 40 cycles of 95.00 °C for 30 sec, 60.00 °C for 30 sec and 72.00 °C for 30 sec. Transcript level analysis was corrected by calculating the cycle threshold (Ct) for each reaction based on efficiency. Then, ΔCt was calculated by deducting the average of the calibrator from the Ct of each gene. Ultimately, the relative expression of each target gene was calculated in comparison with the reference gene using formula 2−ΔΔCt.²⁷

Data analysis and statistics. The results were assessed using SPSS Software (version 25.0; IBM Corp., Armonk, USA). The given results were investigated and analyzed as the mean ± average of the standard error from the mean. The normality of the data was tested using the Kolmogorov-Smirnov test. Statistical tests such as one-way and two-way variance analysis and post-hoc Tukey test were used to compare the results. Data differences with p < 0.05 were considered significant. The respective charts were drawn using Microsoft Excel Software (version 16.0; Microsoft Corporation, Redmond, USA).

Results

The average serum concentration of LH increased significantly (p < 0.001) at 30 and 60 min after injection of NPY in comparison with the time before injection (0 times) (Fig. 1A). However, the injection of BIBP3226 reduced the average serum concentration of LH at 30 and 60 min after injection. It should be noted that the given decrease was not significant (Fig. 1B). Lastly, pretreatment with BIBP3226 prevented the stimulative effects of NPY on the secretion of LH (Fig. 1C).

At 30 min after injection of NPY, the concentration of LH was significantly higher than the normal level of given hormone in comparison with the same time in the control group (p < 0.001; Fig. 1A). Injection of BIBP3226 did not undergo a significant change in comparison with the control group at 30 min time interval (Fig. 1B). Pretreatment of the rats with BIBP3226 alongside the combined injection of the BIBP3226 with NPY reduced the stimulative effects of NPY at 30 min after injection. Consequently, the serum level of LH was decreased to the extent that it did not show a significant change in comparison with the control group. The results at 60 min after injection of the drugs followed the same pattern as those at 30 min (Fig. 1C).

The average serum concentration of testosterone increased significantly (p < 0.001) at 30 min after injection of the NPY in comparison with the time before injection. At 60 min after injection of the NPY, the concentration of testosterone decreased in comparison with that of 30 min time interval. However, it showed a higher level of significance in comparison with the 0 times (Fig. 1D). Injection of BIBP3226 decreased the serum level of testosterone at 30 and 60 min after injection in a significant way (p < 0.001 and p < 0.01, respectively) in comparison with that of the 0 times (Fig. 1E). By the same token, the combined injection of the NPY and BIBP3226 produced a similar result. It was suggested that pretreatment with BIBP3226 will control NPYY1 receptors and, by extension, it can prevent the stimulative effects of NPY on the concentration of testosterone (Fig. 1F).

At 30 min after injection of NPY, serum concentration of testosterone was significantly higher than that in the control group at the same time (p < 0.001; Fig. 1D).

On the other side, treatment with BIBP3226 at 30 min time interval reduced the serum level of testosterone in comparison with the same time in the control group.
(p<0.01; Fig. 1E). The suppressive effect was much more in the combined injection of NPY and BIBP3226 in comparison with that in an injection of the BIBP3226 (p<0.001). At 60 min time interval, NPY increased the serum level of testosterone in comparison with the same time in the control group (p<0.001), while BIBP3226 and its combined injection with NPY decreased the concentration of testosterone in a significant way in comparison with the same time in the control group (p<0.01; Fig. 1F).

Table 2 demonstrates the latencies in the sexual behaviors of male rats treated with NPY and BIBP3226. In comparison with the control group, NPY decreased IL and EL in a significant way. However, no significant difference was observed between NPY-received group and control group in terms of ML. Similarly, BIBP3226-received group showed a significant increase in terms of ML, IL, and EL in comparison with the control group. Furthermore, the injection of BIBP3226 brought about a significant increase in the aforementioned indices of sexual behavior in comparison with NPY group.

Combined injection of NPY and BIBP3226 produced no statistically significant change in ML, IL, and EL compared to the control group. Pretreatment with BIBP3226 controlled the decreasing effects of NPY on the latencies of sexual behaviors. Accordingly, there appeared to be a significant increase in ML, IL, and EL in comparison with NPY group. Contrary to these results, the increasing effects of BIBP3226 on ML, IL, and EL were controlled in NPY and BIBP3226 received group. Also, all three indices of sexual behavior underwent a statistically significant decrease in comparison with BIBP3226 group.

According to Table 2, NM decreased significantly in NPY-received group in comparison with the control group. By way of explanation, the increase of NI and NE was not significant in comparison with the control group. Central injection of BIBP3226 increased NM and decreased NI significantly in comparison with control group. However, it did not bring about a significant difference in NE.

The group having the combined injection of NPY and BIBP3226 did not show significant changes in NM, NI, and NE in comparison with the control group. The BIBP3226 increased NM and decreased NI significantly in comparison with NPY-received group. It should be noted that there was not a significant decrease in NE in comparison with this group. The combined injection of NPY and BIBP3226 blocked the increasing effects of BIBP3226 on NM in a significant way. Mutually, it produced a significant statistical increase in the suppressing effect of NPY on NM.

Analyses of the data obtained from RT-qPCR showed that the central injection of NPY can increase the relative gene expression level of KiSS1 (p<0.001; Fig. 2A). Mutually, injection of BIBP3226 decreased the gene expression level of KiSS1 in a significant way (Fig. 2B). Also, a combined

Table 2. Effects of NPY, BIBP3226, and co-administration of NPY + BIBP3226 on sexual behavior parameters in the male Wistar rats. Data are represented as mean ± SEM (n = 8 in each group).

| Groups         | ML (s) | IL (s) | EL (s) | NM | NI | NE |
|----------------|--------|--------|--------|----|----|----|
| Control        | 33.73±1.94 | 67.16±5.77 | 430.9±1.673 | 41.0±0.32 | 17.2±0.97 | 2.2±0.37 |
| NPY            | 18.1±2.65 | 33.6±2.18 | 225.6±1.95 | 2.2±0.38 | 18.6±0.81 | 3.6±0.40 |
| BIBP3226       | 19.67±7.63 | 193.1±8.36 | 628.7±7.30 | 8.8±0.37 | 13.8±0.80 | 2.4±0.24 |
| NPY+ BIBP3226  | 36.9±2.20 | 66.1±5.28 | 431.6±1.54 | 4.0±0.31 | 16.8±0.73 | 2.2±0.36 |

ML: Mount latency; IL: Intromissions latency; EL: Ejaculation latency; NM: Total number of mounts; NI: Total number of intromissions; NE: Total number of ejaculations. abc Different letters show the significant level at p<0.05 (a, vs. control; b, vs. NPY; c, vs. BIBP3226).
injection of BIBP3226 and NPY increased the gene expression of KISS1 significantly (Fig. 2C). It should be noted that the given increase was not as intense as the increasing effect of NPY. It was suggested that pre-treatment with BIBP3226 could block the stimulatory effects of NPY on the transcription level of KISS1 gene to some extent.

The relative gene expression of GPR54 in NPY-treated group showed a significant difference with that of control group (p<0.001; Fig. 2D). The central injection of BIBP3226 reduced the gene expression level of GPR54. However, the given decrease was not significant (Fig. 2E). Pretreatment with BIBP3226 decreased the stimulative effects of NPY on GPR54 gene transcription so that at the time of combined injection of the drugs no significant difference was observed at the level of GPR54 gene expression in comparison with that of the control group (Fig. 2F).

Discussion

According to the obtained results of the present study, the significant increase of LH concentration among male rats in comparison with the control group and the fact that the given effects were antagonized by BIBP3226 altogether show that endocrine activities of the reproductive system are under the influence of NPY. It should be noted that the results are in line with those of previous studies.5,10

Because there are no androgenic receptors in the secreting neurons of GnRH, the feedback effects of gonadal hormones will not be achieved without the presence of intermediary regulators such as NPY.7 Urban et al. have reported that the gene expression of NPY in the ARC will be regulated through testosterone. For this reason, sexual dimorphism will occur in the brain.7 This study confirmed the given fact. In other words, upon 60 min after injecting NPY, the serum level of testosterone decreased significantly in comparison with that at 30 min time. As a consequence, a negative feedback mechanism was at work.

According to the results of this study, the significant decrease in IL and EL shows premature sexual desire among male rats received NPY. As a consequence, the rate of mating success among male sex turns out to increase. A large number of regulating mechanisms are involved in the emergence of sexual behavior. These mechanisms are influenced by external factors like photoperiod, chemical signals, temperature, and nutrition on the one hand and internal factors like hormones, neurotransmitters, and neuropeptides on the other hand. It should be noted that neuropeptides like ACTH, TRH, and oxytocin have a stimulating effect on parameters of sexual behavior, while opioids and ghrelin appear to have an inhibiting effect.20,29

The ML, IL, and EL latencies which are frequently used for evaluating the sexual motivation of male rats30 were specified through synchronizing the increase of sexual desire in the brain and transferring it to the level of sexual behavior.31,32 The results are in contrast to Poggioli et al. about the inhibiting effect of NPY on sexual behaviors of male rats.33 In contrast, the ICV administration of BIBP3226 increased ML, IL, and EL in a significant way. It demonstrated that the capacity of the body to develop the tendency to mate in male rats has decreased. Intraperitoneal and ICV injections of benextramine, a long-lasting NPY receptor antagonist, in male rats helped the improvement of sexual behavior.33 However, it must be highlighted that ICV injection of BIBP3226 increased the temporal indices of sexual behavior in the present study.

Most of the information suggested the direct and indirect stimulatory effects of GnRH and LH on regions of the brain which have a role in regulating male reproduction and sexual behavior.21,24,25 According to this evidences, inhibiting LH synthesis or release may lead to copulatory deficiency. All in all, our data suggest that NPY increases the LH and testosterone synthesis and in turn increases sexual behavior. Although the direct projection of the terminal of NPY neurons on the cell body of GnRH-
secreting neurons in the hypothalamus is considered to be a fact,\textsuperscript{36} it has been demonstrated that there are chemical ligands and receptors involved in the reproductive system in neural networks of hypothalamus.\textsuperscript{37} By the same token, it is posed whether, in these neural networks, NPY neurons indirectly affect GnRH neurons or not.

Recent reports have shown that the gene expression of \textit{KiSS1/GPR54} in the ARC rises before the increase in GnRH level. Gene expression of \textit{KiSS1/GPR54} is regarded as the major generator of reproductive activities among male and female rats. There is a direct relationship between \textit{KiSS1} neurons and GnRH neurons in ARC and AVPV.\textsuperscript{38,39} Immunohistochemical studies have indeed shown that most GnRH neurons in rats express \textit{GPR54} gene both in their own cell body in the ARC and their axonal terminal in median eminence (ME) regions.\textsuperscript{38,40,41} It is a point of contention whether NPY neurons, apart from their direct impact on GnRH neurons, leave any impact via the intermediation of \textit{KiSS1} secreting neurons on reproductive activities including behavioral and hormonal levels or not. For this reason, the gene expression level of \textit{KiSS1} and \textit{GPR54} in the ARC was investigated. The increase in production and release of GnRH will indisputably lead to an increase in the level of LH production in the pituitary gland (hypophysis) and, by extension, an increase in testosterone production in testicles. It should be highlighted that the given fact is in the same line with the result of the present study.\textsuperscript{42}

The data of the current study showed that NPY will increase the gene expression level of \textit{GPR54} in the ARC in a significant way. Accordingly, NPY can have a stimulative effect on the endocrine aspects of reproduction. It occurs through stimulating GnRH neurons to cause gene expression of \textit{GPR54}. As a consequence, the given neurons are influenced much more by kisspeptin. However, it is possible as well that NPY increases the level of \textit{GPR54} mRNA in axonal terminals of GnRH neurons and, correspondingly, it will pave the ground for a further linkage of kisspeptin with \textit{GPR54}. Last but not least, it facilitates the release of GnRH in ME region.

Identifying NPY and its exclusive receptor (NPY Y1) in the hypothalamus on the one hand and specifying its impacts on the secretion of hormones in the reproductive axis on the other made us hypothesize that NPY may have its impacts on facilitating secretion of sexual stimulation hormones as well as gonadal hormones. By the same token, sexual behaviors may change through stimulation of \textit{KiSS1} neurons. According to an \textit{in vitro} study that was conducted in 2009, NPY directly induces gene expression of GnRH from GT1 cells (mHyPOE-38) in the growth culture. It occurs through stimulating Y1 subtype receptors.\textsuperscript{6}

Data from the current study showed that BIBP3226 antagonized the stimulative effects of NPY on the gene expression level of \textit{KiSS1} and \textit{GPR54}. Also, BIBP3226 inhibited the serum level of LH and testosterone. The NPY Y1 receptor in GnRH neurons binds to the receptor antagonist and induces intracellular signaling.\textsuperscript{43,44} As a consequence, the production of GnRH and downstream activities at the levels of hypophysis and gonad will decrease. The results of this study showed that BIBP3226 has paved the ground for the activity of this suppressive pathway. Neuroanatomical data show that there is a close relationship between the synapse of NPY fibers and the \textit{KiSS1} neurons in the hypothalamus.\textsuperscript{45} On the contrary, the transcription level of \textit{KiSS1} gene decreases significantly among NPY knockout rats. It is suggested that NPY neurons in the ARC are considered to be afferent for \textit{KiSS1} neurons. The NPY neurons stimulate the \textit{KiSS1} neurons and thereby they pave the ground for secretion of GnRH/LH and testosterone.\textsuperscript{46,47} The findings of the present study agree with those of previous researches. By way of explanation, ICV injection of NPY increases the gene expression of \textit{KiSS1} and \textit{GPR54} in the ARC in a significant way. In addition, it increases the plasma level of LH and testosterone.\textsuperscript{35,40,49} Previous studies have emphasized that \textit{KiSS1} gene transcription decreases in NPY knockout rats in a significant way. Consequently, these rats are unable to produce LH and, by extension, gonadal steroids at a basic level.\textsuperscript{4,50} The aforementioned point shows that there is a cooperation between \textit{KiSS1} and NPY neurons in the neuroendocrine neural networks in the reproductive system.

Very interestingly, the increase in gene expression of \textit{KiSS1/GPR54} in the ARC, the reversal of the given results by BIBP3226, the increase in the plasma level of LH and testosterone after central injection of NPY and, ultimately, the significant decrease in the concentration of these hormones by BIBP3226 are all in line with the results of the study that dealt with NPY knockout rats.\textsuperscript{6} Data collected in the current study showed that the central injection of NPY increased the gene expression of \textit{KiSS1} and \textit{GPR54} and, accordingly, it activated the reproductive axis much more. Consequently, LH and testosterone levels appeared to increase. In line with the given changes, the level of the external signs of sexual behavior was observed to increase as well. To rationalize this point, it can be argued that NPY has probably antedated the start of sexual activity through increasing LH and testosterone at HPG axis.

Similarly, the given data demonstrated that the effects of NPY on sexual behavior are similar to satiation conditions and positive balance of energy in the animals. The NPY probably regulates the mechanisms of energy balance and, thereby, directs the body of animals toward an increase in sexual behaviors. Likewise, the findings of this study about the general increase of sexual behavior indices (especially the decrease in interval between mating cycles), the given phenomenon is suggested as a good solution to both discharge and balance of energy in the body of the animal. Overall, the findings of this study corroborate the idea that NPY supports, to some extent, the complex mechanism of regulating sexual behavior at the level of the brain.
To sum up, the results showed that the intra-cerebral third ventricle injection of NPY can increase the serum level of sex hormones like LH and testosterone. Furthermore, it increases the sexual behavior among the male rats received NPY. More in-depth studies in this area demonstrated that NPY will increase the relative expression of Kiss1 and GPR54 genes. It seems that NPY leaves its moderating impacts on the reproductive axis. The BIBP3226 will antagonize the facilitating effects of NPY on reproductive activities. This occurs at least partly through affecting Kiss1/GPR54 system.

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Conflict of interest

The authors confirm that there is no conflict of interest.

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