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RF9: May it be a new therapeutic option for hypogonadotropic hypogonadism?

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ABSTRACT

Hypogonadotropic hypogonadism (secondary hypogonadism), congenital or acquired, is a form of hypogonadism that is due to problems with either the hypothalamus or pituitary gland affecting gonadotropin levels. Pulsatile secretion of gonadotropin-releasing hormone (GnRH) by hypothalamus is a primer step to initiate the release of pituitary gonadotropins. Kisspeptin and gonadotropin-inhibitory hormone (GnIH) are accepted as two major players in the activation and inhibition of GnRH regarding the neuroendocrine functioning of the hypothalamic pituitary gonadal axis. Kisspeptin is known as the most potent activator of GnRH. Regarding the inhibition of GnRH, RF-amide-related peptide-3 (RFRP-3) is accepted as the mammalian orthologue of GnIH in avian species. RF9 (1-adamantane carbonyl-Arg-Phe-NH2) is an antagonist of RFRP-3/GnIH receptor (neuropeptide FF receptor 1 (NPFFR1; also termed as GPR147). In recent years, several studies have indicated that RF9 activates GnRH neurons and gonadotropins in a kisspeptin receptor (Kiss1r, formerly known as GPR54) dependent manner. These results suggest that RF9 may have a bimodal function as both an RFRP-3 antagonist and a kisspeptin agonist or it may be a kiss1r agonist rather than an RFRP-3/GnIH receptor antagonist. These interactions are possible because Kisspeptin and GnIH are members of the RF-amide family, and both possibilities are not far from explaining the potent gonadotropin stimulating effects of RF9. Therefore, we hypothesize that RF9 may be a new therapeutic option for the hypogonadotropic hypogonadism due to its potent GnRH stimulating effects. A constant or repeated administration of RF9 provides a sustained increase in plasma gonadotrophin levels. However, applications in the same way with GnRH analogues and kisspeptin may result in desensitization of the gonadotropic axis. The reasons reported above contribute to our hypothesis that RF9 may be a good option in the GnRH stimulating as a kisspeptin agonist. We suggest that further studies are needed to elucidate the potential effects of RF9 in the treatment of the hypogonadotropic hypogonadism.

Introduction

Hypogonadism may arise from a primary gonadal failure (hypergonadotropic hypogonadism) or may occur secondary to hypothalamic-pituitary dysfunction (hypogonadotropic hypogonadism). It can be congenital or acquired. Congenital hypogonadotropic hypogonadism is divided into congenital normosmic idiopathic (or isolated) hypogonadotropic hypogonadism and anosmic hypogonadotropic hypogonadism (Kallmann syndrome). Acquired hypogonadotropic hypogonadism may arise from traumatic brain injury, drugs, infectious or infiltrative pituitary lesions, brain radiation, abusive alcohol or illicit drug intake, and systemic [1] and metabolic [2] diseases.

In the hypogonadotropic hypogonadism, secretion of gonadotropin-releasing hormone (GnRH) is absent or inadequate. Inadequate biosynthesis and isolated lack of production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) may also result in the hypogonadotropic hypogonadism [3]. Pulsatile secretion of GnRH by hypothalamus is a primer step of the reproductive cascade, which initiates the release of pituitary gonadotropins, gonadal secretion of sex steroids, and gametogenesis [4].

During the last two decades, two major players have been involved in the activation and inhibition of GnRH for the neuroendocrine functioning of the hypothalamic pituitary gonadal axis. Regarding the activation of GnRH, kisspeptin (formerly known as metastin) is encoded by the metastasis suppressor gene KiSS-1 in humans (kiss-1 in animals). This peptide is the endogenous ligand for the kisspeptin receptor (KISS1R (kiss1r in animals), formerly called as GPR-54), a G protein-coupled receptor, to regulate puberty onset and fertility [5,6]. Another player is RF-amide-related peptide-3 (RFRP-3), which is accepted as the mammalian orthologue of gonadotropin-inhibitory hormone (GnIH) in avian species [7]. Many researchers have suggested that either central or peripheral administration of RFRP-3/GnIH causes significant inhibition of gonadotropin secretion in hamsters [8], rats [9,10], sheep [7], and human [11]. RF9 (1-adamantane carbonyl-Arg-Phe-NH2) is an antagonist of RFRP-3/GnIH receptor (neuropeptide FF receptor 1 (NPFFR1; also termed GPR147) [12]. This antagonist has a potent role on the gonadotropin secretion in mammals [13]. Moreover, RF9 activates GnRH neurons and gonadotropins in a Kiss1r dependent manner [14–16]. In 2015, we defined that RF9 elicited significant elevations in circulating LH levels and coadministration of RF9 with kisspeptin

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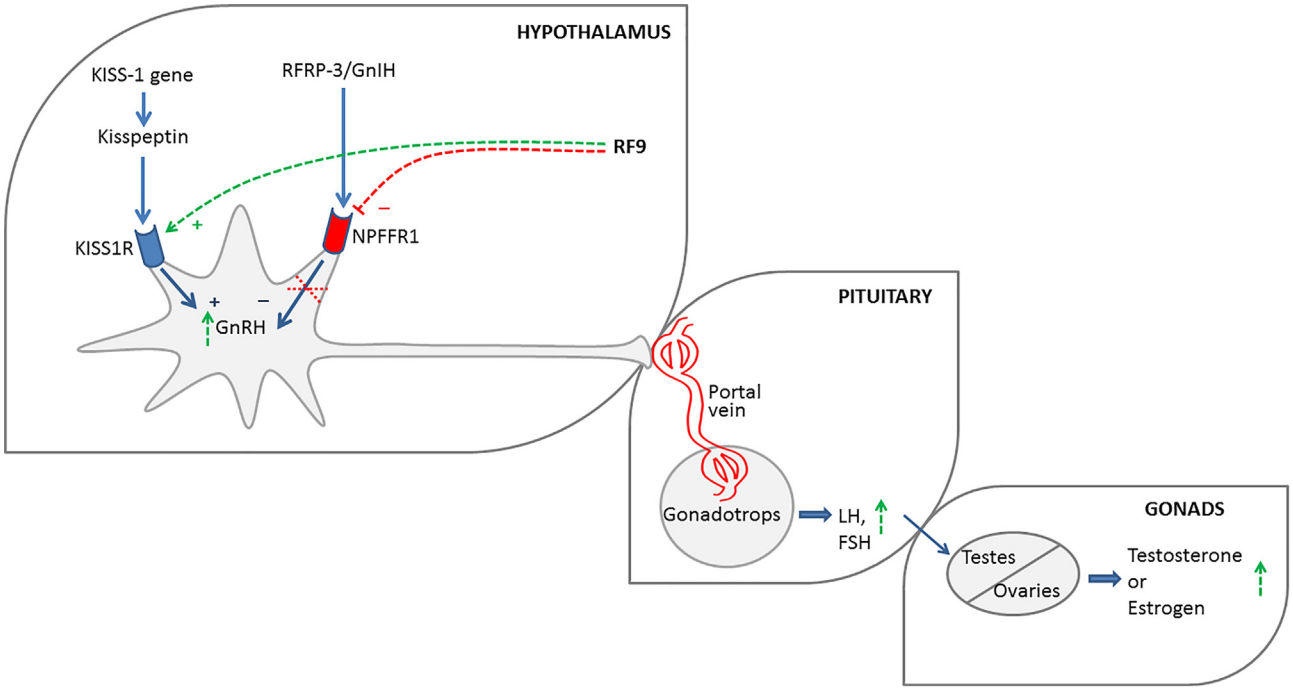

Fig. 1. Physiological control of the HPG axis by kisspeptin and RFRP-3 and possible stimulating effects of the RF9 on GnRH, gonadotropins and gonadal activity. Blue solid arrows indicate the physiological regulation of the HPG axis by kisspeptin and RFRP-3. Kisspeptin has a stimulating effect on GnRH, resulting in the secretion of both gonadotropins and testosterone or estrogen. However, RFRP-3 has an inhibitory effect on GnRH. Dashed arrows signify possible stimulating effects of the RF9 on GnRH neurons, gonadotropins and testosterone or estrogen. Green dashed arrows define a stimulatory effect of the RF9 on GnRH neurons by kisspeptin receptor like a kisspeptin agonist and red dashed arrows represent an inhibitory effect of the RF9 on GnRH neurons by RFRP-3/GnIH receptors as an RFRP-3 antagonist. RF9 can increase gonadotropin secretion and gonadal activity by stimulating GnRH neurons directly via KISS1R (Green dashed arrows). It is also possible that since RF9 is an RFRP-3 antagonist, it can indirectly increase gonadotropin secretion and gonadal activity by eliminating the inhibitory effect of RFRP-3/GnIH on GnRH neurons (Red dashed arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

receptor antagonist p234 decreased LH levels significantly [15]. These results indicate that RF9 exerts a gonadotropin stimulating function not only as the RFRP-3 antagonist, but also as a kiss1r agonist.

The hypothesis

In light of the fact that RF9 stimulates GnRH and gonadotropins, we hypothesize that RF9 may be a new therapeutic option for hypogonadotropic hypogonadism (Fig. 1).

Evaluation of the hypothesis

Since the discovery of mammalian GnRH at the beginning of the seventies, many new neuropeptides and neurotransmitters have been described in the neuroendocrine regulation of reproductive axis, but none of them has had such a dramatic role as kisspeptin [17,18]. GnRH neurons have been shown to express kiss1r [19], through which kisspeptin activate GnRH secretion [20]. Actually, kisspeptin or kisspeptins are the common names of the structurally related peptides (kisspeptin-54, 14, 13 and 10) according to the number of amino acids in length, and the decapeptide kisspeptin-10, which is shared by all the members of kisspeptin family, is required for the physiological activity [21]. In 2003, it was reported that loss-of-function mutations in kiss1/kiss1r signaling are associated with hypogonadotropic hypogonadism both in humans [5,6] and rodents [22]. In the experimental studies, kisspeptin [23] or kiss1r knockout mice [20] were found to be infertile. Moreover, there is a case report that indicates an activating mutation of the KISS1R in a patient with central precocious puberty [24]. The same result is true for central treatment by kisspeptin. There are several studies that intracerebroventricular injection of this peptide to immature female rats induces premature activation of the gonadotropic axis [15,25]. In vitro studies also provide evidence that kisspeptin directly stimulates GnRH neurons [26,27]. Therefore, kisspeptin might be accepted as the most potent activator of HPG axis known to date [28].

Unlike kisspeptin, RFRP-3 inhibits gonadotropin secretion in mammals [7,9]. It is interesting that these two hormones, which are opposite regarding their effects but have common points in terms of sequence properties, belong to the same family, known as the RF-amide neuropeptide family [29]. This family is characterized by a common carboxy-terminal motif that consists of an arginine (R) and an amidated phenylalanine (F). RF-amide family, which are also present in other organisms including humans, are divided into five subgroups: neuropeptide FF (NPFF and NPAF), GnIH (RFRP-1 (NPSF) and RFRP-3 (NPVF), 26RFa, prolactin releasing peptide (PrRP) and kisspeptin/kisspeptins. As related to our hypothesis, RF9 has a bimodal function as both an RFRP-3 antagonist and a kisspeptin agonist, and its effects are based on the biological characteristics of the RF-amide family because these two endogenous hormones are members of the mentioned family. Therefore, although RF9 was developed as an antagonist of NPFFRs (particularly GPR147 and partly GPR74), which are activated by RFRP-3 and many other neuropeptides, studies also demonstrated that administration of RF9 augmented the gonadotropins in a kisspeptin receptor dependent mechanism [14–16]. As expected, RFRP-3 antagonism induced by RF9 may result in an increase in the gonadotropin secretion. This is an indirect effect of the RF9 on GnRH or gonadotropins. The interesting point is the direct effects of the RF9 on GnRH via the kisspeptin-mediated mechanism. In 2006, Simonin et al. reported [12] that RF9 may be structurally related to BIBP3226 (NPY receptor subtype Y1 antagonist). Since BIBP3226 has structural similarity to RFamide-derived peptides and it can bind to NPFFR subtypes, this assay was also carried out for RF9. Thus, they aimed to evaluate the binding activity of RF9 to human NPY receptor subtype Y1, three receptors of three RFamide neuropeptide families (GPR10, GPR54 and

GPR103), and opioid (μ, δ, κ) receptors. It was stated that RF9 did not show any affinity on these mentioned receptors at doses up to 10 μM except for its weak competition to opioid μ and κ receptors. However, the bioactivity of NPFFR ligands for kiss1r has been shown to have an opposite cross reactivity because kisspeptin receptor ligands can exhibit cross-reactivity for NPFFR [30], which indicate that RF9 may have direct effects on kiss1r. In 2015, we tried to explore whether kiss1r antagonism affects the reproductive responses to RF9. In our experiment, p234 inhibited advancement of puberty caused by RF9, which was the same response as to kisspeptin-induced pubertal acceleration [15]. In the same year, Min et al. reported [16] that RF9 acts as a KISS1R agonist both in vivo and in vitro conditions. They performed an in vitro experiment using KISS1R-transfected Chinese Hamster Ovary cells and noticed that RF9 binds specifically to kisspeptin receptor and stimulates an increase in intracellular calcium and inositol phosphate activity. They also reported RF9 stimulated a robust LH increase in Npffr1$^{-/-}$ mice, similar to that in wild-type littermates, whereas the stimulatory effects of RF9 were markedly reduced in Kiss1r$^{-/-}$ and double Kiss1r$^{-/-}$/Npffr1$^{-/-}$ mice. These results are important to show that RF9 is a kisspeptin receptor agonist rather than an RFRP-3 antagonist.

Consequences of the hypothesis and discussion

If our hypothesis is correct, it would have an impact on contributing to the treatment of hypogonadotropic hypogonadism, which is one of the important causes of infertility problems in humans. However, there are important considerations to be emphasized. One of them is that the use of RF9 in treatment would not be applicable in cases associated with the anomaly in embryonic development of GnRH neurons (anosmic hypogonadotropic hypogonadism or Kallmann syndrome). Apart from this exception, RF9 may have a high therapeutic potential in congenital and acquired hypogonadism cases. As a second important point, although RF9 is a kisspeptin-mediated treatment option in the hypogonadism, data on possible other effects on the body are quite limited. However, these obscurities cannot be a justification for ignoring the potential of RF9 as a new therapeutic agent.

In addition to the effects of kisspeptin on puberty, reproductive function and suppression of metastasis, there are also data indicating that it has an essential function on the regulation of energy balance in the body [2,31–33]. An important concern is that metabolic signals reflecting the energy balance of the body are important in the physiological regulation of puberty and reproductive function. Therefore, dysregulation of energy balance (energy insufficiency or obesity) may lead to reproductive disorders [31]. It has been suggested that a decrease in the endogenous kisspeptin secretion can be an important pathway associated with metabolic and endocrine factors in the pathophysiology of hypogonadism in men due to obesity and type 2 diabetes [2]. All these results are important reasons for the therapeutic use of kisspeptin or agonists in such disorders. Continuous or repeated stimulation of the gonadotropic axis with pharmacological boluses of GnRH or its agonists is a well-known phenomenon leading to desensitization [34]. The same phenomenon may also be valid to kisspeptin or its agonists because increased evidence suggests that continuous exposure to kisspeptins may cause desensitization to their potent gonadotropin-releasing effects [35–37]. On the other hand, there are different results particularly in females [38,39]. Peripheral administration of RF9 as a bolus and as a constant intravenous infusion to ewes induces a sustained increase in plasma gonadotrophin concentrations [40].

These reasons reported above contribute to our hypothesis that RF9 has a potent GnRH stimulating effect as a kisspeptin agonist feature. Further studies are needed to expose the potential effects of RF9 because all the discussions in light of evidence indicate that RF9 may be clinical significance for the treatment of the hypogonadotropic hypogonadism.

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Declaration of Competing Interest

As the authors of this manuscript, we disclose that we have no financial or personal relationships with people or organizations that could inappropriately influence (bias) our work. There are no potential conflicts of interest including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. We also declare that there is no role of sponsors, if any, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.05.016.

References

[1] Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic hypogonadism revisited. Clinics (Sao Paulo) 2013;68(Suppl. 1):81–8.
[2] George JT, Millar RP, Anderson RA. Hypothesis: kisspeptin mediates male hypogonadism in obesity and type 2 diabetes. Neuroendocrinology 2010;91:302–7.
[3] Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotropic hypogonadism? Clin Endocrinol (Oxf) 2010;72:731–7.
[4] Seminara SB, Hayes FJ, Crowley WF. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. Endocr Rev 1998;19:521–39.
[5] de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. PNAS 2003;100:10972–6.
[6] Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. N Engl J Med 2003;349:1614–27.
[7] Clarke IJ, Sari IP, Qi Y, et al. Potent action of RFamide-related peptide-3 on pituitary gonadotropes indicative of a hypophysiotropic role in the negative regulation of gonadotropin secretion. Endocrinology 2008;149:5811–21.
[8] Kriegsfeld LJ, Mei DF, Bentley GE, et al. Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. Proc Natl Acad Sci USA 2006;103:2410–5.
[9] Johnson MA, Tsutsui K, Fraley GS. Rat RFamide-related peptide-3 stimulates GH secretion, inhibits LH secretion, and has variable effects on sex behavior in the adult male rat. Horm Behav 2007;51:171–80.
[10] Murakami M, Matsuzaki T, Iwasa T, et al. Hypophysiotropic role of RFamide-related peptide-3 in the inhibition of LH secretion in female rats. J Endocrinol 2008;199:105–12.
[11] George JT, Hendrikse M, Veldhuis JD, Clarke IJ, Anderson RA, Millar RP. Effect of gonadotropin-inhibitory hormone on luteinizing hormone secretion in humans. Clin Endocrinol (Oxf) 2017;86:731–8.
[12] Simonin F, Schmitt M, Laulin JP, et al. RF9, a potent and selective neuropeptide FF receptor antagonist, prevents opioid-induced tolerance associated with hyperalgesia. Proc Natl Acad Sci USA 2006;103:466–71.
[13] Pineda R, Garcia-Galiano D, Sanchez-Garrido MA, et al. Characterization of the potent gonadotropin-releasing activity of RF9, a selective antagonist of RF-amide-related peptides and neuropeptide FF receptors: physiological and pharmacological implications. Endocrinology 2010;151:1902–13.
[14] Liu X, Herbison AE. Rf9 excitation of gnrh neurons is dependent upon Kiss1r in the adult male and female mouse. Endocrinology 2014;155:4915–24.
[15] Sahin Z, Canpolat S, Ozcan M, Ozgocer T, Kelestimur H. Kisspeptin antagonist prevents RF9-induced reproductive changes in female rats. Reproduction 2015;149:465–73.
[16] Min L, Leon S, Li H, et al. RF9 acts as a KISS1R agonist in vivo and in vitro. Endocrinology 2015;156:4639–48.
[17] Seminara SB, Kaiser UB. New gatekeepers of reproduction: GPR54 and its cognate ligand, KiSS-1. Endocrinology 2005;146:1686–8.
[18] Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 2012;92:1235–316.
[19] Parhar IS, Ogawa S, Sakuma Y. Laser-captured single digoxigenin-labeled neurons of gonadotropin-releasing hormone types reveal a novel G protein-coupled receptor (Gpr54) during maturation in cichlid fish. Endocrinology 2004;145:3613–8.
[20] Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci USA 2005;102:1761–6.
[21] Kotani M, Detheux M, Vandenbogaerde A, et al. The metastasis suppressor gene

KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 2001;276:34631–6.
[22] Funes S, Hedrick JA, Vassileva G, et al. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun 2003;312:1357–63.
[23] d'Anglemont de Tassigny X, Fagg LA, Dixon JP, et al. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci USA 2007;104:10714–9.
[24] Teles MG, Bianco SD, Brito VN, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358:709–15.
[25] Navarro VM, Fernández-Fernández R, Castellano JM, et al. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. J Physiol 2004;561:379–86.
[26] Kroll H, Bolsover S, Hsu J, Kim SH, Bouloux PM. Kisspeptin-evoked calcium signals in isolated primary rat gonadotropin-releasing hormone neurones. Neuroendocrinology 2011;93:114–20.
[27] Ozcan M, Alcin E, Ayar A, Yilmaz B, Sandal S, Kelestimur H. Kisspeptin-10 elicits triphasic cytosolic calcium responses in immortalized GT1-7 GnRH neurones. Neurosci Lett 2011;492:55–8.
[28] Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci 2005;25:11349–56.
[29] Findeisen M, Rathmann D, Beck-Sickinger AG. RFamide peptides: structure, function, mechanisms and pharmaceutical potential. Pharmaceuticals 2011;4:1248–80.
[30] Oishi S, Misu R, Tomita K, et al. Activation of neuropeptide FF receptors by kisspeptin receptor ligands. ACS Med Chem Lett 2011;2:53–7.
[31] Castellano JM, Tena-Sempere M. Metabolic regulation of kisspeptin. Adv Exp Med Biol 2013;784:363–83.
[32] De Bond JA, Smith JT. Kisspeptin and energy balance in reproduction. Reproduction 2014;147:53–63.
[33] Tolson KP, Garcia C, Yen S, et al. Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. J Clin Invest 2014;124:3075–9.
[34] Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science 1978;202:631–3.
[35] Seminara SB, Dipietro MJ, Ramaswamy S, Crowley Jr WF, Plant TM. Continuous human metastin 45–54 infusion desensitizes G protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male Rhesus monkey (Macaca mulatta): a finding with therapeutic implications. Endocrinology 2006;147:2122–6.
[36] Thompson EL, Murphy KG, Patterson M, et al. Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats. Am J Physiol Endocrinol Metab 2006;291:E1074–82.
[37] Ramaswamy S, Seminara SB, Pohl CR, DiPietro MJ, Crowley Jr WF, Plant TM. Effect of continuous intravenous administration of human metastin 45–54 on the neuroendocrine activity of the hypothalamic-pituitary-testicular axis in the adult male rhesus monkey (Macaca mulatta). Endocrinology 2007;148:3364–70.
[38] Roa J, Vigo E, García-Galiano D, et al. Desensitization of gonadotropin responses to kisspeptin in the female rat: analyses of LH and FSH secretion at different developmental and metabolic states. Am J Physiol Endocrinol Metab 2008;294:E1088–96.
[39] Clarke IJ, Li Q, Henry BA, Millar RP. Continuous kisspeptin restores luteinizing hormone pulsatility following cessation by a neurokinin B antagonist in female sheep. Endocrinology 2018;159:639–46.
[40] Caraty A, Blomenröhr M, Vogel GM, Lomet D, Briant C, Beltramo M. RF9 powerfully stimulates gonadotrophin secretion in the ewe: evidence for a seasonal threshold of sensitivity. J Neuroendocrinol 2012;24:725–36.