

The roles of kisspeptin and gonadotropin inhibitory hormone in stress-induced reproductive disorders

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Abstract. Several kinds of stress suppress the hypothalamic-pituitary-gonadal (HPG) axis and reproductive behavior in humans and animals. These changes can eventually cause diseases and disorders, such as amenorrhea and infertility. In previous studies, it has been shown that stress-related factors, *e.g.*, corticotropin-releasing hormone, cortisol, and pro-inflammatory cytokines, promote the stress-induced suppression of the HPG axis. However, these mechanisms are not sufficient to explain how stress suppresses HPG axis activity, and it has been suggested that some other factors might also be involved. In the early 21st century, novel neuroendocrine peptides, kisspeptin and gonadotropin inhibitory hormone (GnIH)/RFamide-related peptide 3 (RFRP-3), which directly regulate GnRH/gonadotropin synthesis and secretion, were newly discovered. Growing evidence indicates that kisspeptin and GnIH/RFRP-3 play pivotal roles in the stress-induced disruption of the HPG axis and reproductive behavior in addition to their physiological functions. This review summarizes what is currently known about the roles of kisspeptin and GnIH/RFRP-3 in stress-induced reproductive disorders.

Key words: Kisspeptin, Gonadotropin inhibitory hormone, Stress, Gonadotropin

Introduction

Reproductive functions and stress regulation systems are closely linked in most species. Generally, reproductive functions are temporarily suppressed by various kinds of stress, such as infection, psychological burdens, excess exercise, and undernutrition, in humans and animals because reproduction, which requires a large amount of energy, is not essential for survival [1-4]. Such changes are considered to play important roles in the regulation of homeostasis; *i.e.*, in the appropriate allocation of finite energy to prioritized activities. Although reproductive functions are usually restored immediately after stress levels reduce [5, 6], prolonged or excessive stress can sometimes have negative long-term consequences [7].

Reproductive functions are mainly regulated by the hypothalamic-pituitary-gonadal (HPG) axis in humans

and animals. Physical and psychological stressors suppress HPG axis activity, mainly by inhibiting gonadotropin-releasing hormone (GnRH) activity in both males and females, which results in decreased gonadotropin levels [8-13]. Previous studies by our group and others have shown that alterations in the activity levels of some stress-related factors reduce GnRH and gonadotropin secretion and concomitantly promote the stress response [14-16]. However, these mechanisms are not sufficient to explain how stress suppresses HPG axis activity, and it has been suggested that some other factors may also be involved. A breakthrough occurred in the early 21st century, when novel neuroendocrine peptides that directly regulate GnRH/gonadotropin synthesis and secretion were discovered [17-19]. Kisspeptin, which is encoded by the *Kiss1* gene, is a hypothalamic peptide that directly stimulates GnRH synthesis and release [20, 21], whereas gonadotropin inhibitory hormone (GnIH)/RFamide-related peptide 3 (RFRP-3) acts within the hypothalamus and pituitary to suppress the release and synthesis of GnRH and gonadotropins [22-27]. Some studies have shown that these two factors are involved in stress-induced reproductive disorders as well as the regulation of physiological HPG axis activity. In this review,

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we discuss the neuroendocrine mechanisms responsible for stress-induced reproductive disorders, mainly focusing on the relationships between kisspeptin, GnIH/RFRP-3, and HPG axis dysfunction. We also discuss the relationship between GnIH/RFRP-3 and dysfunctional reproductive behavior.

The Roles of Stress-Related Factors in Stress-Induced HPG Axis Dysfunction

As noted above, alterations in the activity levels of some stress-related factors decrease GnRH and gonadotropin secretion and concomitantly promote the stress response (Fig. 1). For example, hypothalamic corticotropin-releasing hormone (CRH), which is the principal driving factor of the HPA axis during stress, is a potent inhibitor of GnRH secretion. In experimental animals, the central injection of CRH suppressed the luteinizing hormone (LH) pulse [28-30], which is an indicator of GnRH pulse activity, and various types of stress-induced LH suppression were reversed by the administration of CRH antagonists [30-34]. Similarly, in women the peripheral

infusion of CRH decreases gonadotropin secretion, and GnRH administration prevents this alteration, indicating that CRH inhibits GnRH secretion in humans [35, 36]. In addition, some studies have reported that cortisol, which is produced by the adrenal gland, suppresses GnRH secretion in humans and monkeys [37-40]; however, some other studies obtained contradictory results [9, 41]. Pro-inflammatory cytokines; *i.e.*, interleukin-1 β and tumor necrosis factor- α , also play pivotal roles in the stress-induced suppression of the HPG axis. The expression levels of these cytokines are upregulated by immune stress, and GnRH release and serum gonadotropin levels are decreased by their central administration [42-47]. Although the primary roles of these stress-related factors are to promote appropriate responses to exogenous and endogenous stressors, they also modulate HPG axis activity in order to save energy. The stress-related factors described above are promptly activated by stress, and they might play roles in the acute phases of stress-induced reproductive disorders. Whereas, it has been assumed that some other factors might also be involved in such disorders, especially in chronic or severe stress

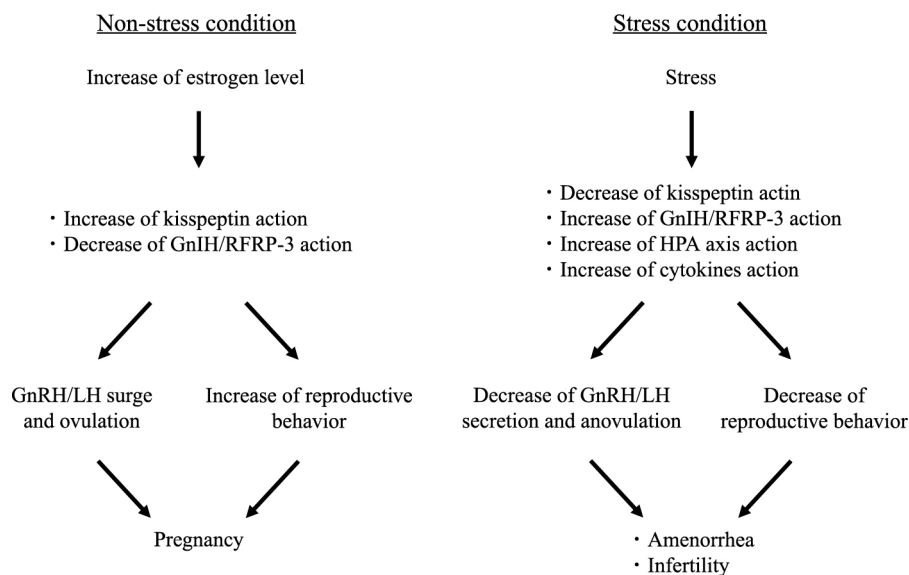


Fig. 1 The roles of kisspeptin, gonadotropin-inhibitory hormone (GnIH)/RFamide-related peptide 3 (RFRP-3), and stress-related factors under physiological and stress conditions. Kisspeptin and GnIH/RFRP-3 participate in the control of physiological GnRH/luteinizing hormone (LH) regulation. The activity levels of kisspeptin and GnIH/RFRP-3 are increased and decreased, respectively, by high estrogen concentrations at the time of the GnRH/LH surge. These alterations promote both ovulation and reproductive behaviors, and consequently, increase the chance of pregnancy. On the contrary, kisspeptin, GnIH, and stress-related factors also play roles in stress-induced reproductive disorders. Kisspeptin and GnIH expression and activity are decreased and increased by stress, respectively, and these changes contribute to suppressing the activity of the hypothalamic-pituitary-gonadal axis. The upregulation of GnIH/RFRP-3 expression decreases reproductive behavior, and consequently, reduces the chances of pregnancy and causes infertility.

conditions. Growing evidence suggests that kisspeptin and GnIH/RFRP-3 might contribute to reproductive disorders in such adverse physiological states. Thus, the physiological and pathological roles of kisspeptin and GnIH/RFRP-3 are discussed in the following sections.

Physiological Roles of Kisspeptin and GnIH/RFRP-3

In 2003, two independent groups reported that a mutation in the G protein-coupled receptor (GPR54); *i.e.*, the kisspeptin receptor, induced hypogonadotropic hypogonadism and pubertal failure in humans and mice [18, 19]. Thus, kisspeptin was assumed to be a key factor for gonadotropin secretion and a modulator of HPG axis activity. Indeed, subsequent studies have shown that kisspeptin stimulates GnRH secretion *via* GPR54 on GnRH neurons [48-50]. The amino acid sequence of kisspeptin, especially the C-terminal 10-amino acid sequence, is well conserved in most mammals [51-57]. Hypothalamic kisspeptin neurons are mainly located in the anterior and posterior nuclei; *i.e.*, the anteroventral periventricular nucleus (AVPV)/preoptic area (POA) and arcuate nucleus (ARC) [55, 56, 58-64]. *Kiss1* mRNA expression in the AVPV/POA is increased in the afternoon during proestrus and is also upregulated by the administration of estrogen, whereas *Kiss1* mRNA expression in the ARC is decreased by the administration of estrogen [61, 63, 65-67]. These results indicate that kisspeptin plays pivotal roles in the regulation of the negative and positive feedback effects of estrogen and that it is indispensable for the maintenance of the ovulation cycle (Fig. 1).

In 2000, Tsutsui *et al.* discovered a novel neuropeptide that suppresses the release of gonadotropins from cultured avian pituitary cells [17]. As this neuropeptide was the first hypothalamic factor that was found to suppress gonadotropin release, it was named GnIH based on its biological activity. GnIH molecules were subsequently identified in other vertebrates, mammals, primates, and humans [68-70]. The mammalian GnIH orthologous gene and peptide are named *Rfp* and RFRP-3, respectively, because the neuropeptide possesses the LPXRFamide (X = L or Q) motif in its C-terminus. Hypothalamic GnIH/RFRP-3 neurons are mainly located in the paraventricular nucleus in birds and the dorsomedial hypothalamic area in mammals [71]. GnIH/RFRP-3 neurons project to the median eminence in birds and female sheep, and suppress the synthesis and secretion of gonadotropins at the

pituitary level *via* the GnIH/RFRP-3 receptor GPR147 under both *in vivo* and *in vitro* conditions in male birds, female rats, and female sheep [17, 26, 69, 72]. In addition, GnIH/RFRP-3 neurons project to GnRH neurons in the hypothalamus and suppress their activity *via* GPR147 in mammals and birds [23, 26, 73]. GnIH/RFRP-3 neurons were activated by the injection of estradiol in ovariectomized female hamsters [23]. However, estradiol has no such effect and GnIH/RFRP-3 neurons are inactivated during the LH surge that occurs during the estrous stage [26, 74]. These results indicate that GnIH/RFRP-3, as well as kisspeptin, plays important roles in the regulation of the negative and positive feedback effects of estrogen, and hence, contributes to the maintenance of the ovulation cycle (Fig. 1).

The Roles of Kisspeptin in Stress-Induced HPG Axis Dysfunction

As noted above, kisspeptin plays pivotal roles in the regulation of HPG axis activity and the maintenance of a regular ovulation cycle in many species. Thus, it had been assumed that the disruption of kisspeptin activity would decrease HPG axis activity and cause reproductive disorders (Fig. 1). In 2008, we reported that immune stress induced by the administration of lipopolysaccharide (LPS) suppressed hypothalamic *Kiss1* mRNA expression and the serum LH level in female rats [75]. In latter study, we also showed that the LPS-induced LH suppression was completely reversed by the co-administration of kisspeptin. As far as we know, our study was the first to describe the effects of stress on the kisspeptin system and the gonadotropin secretion disorders they cause. Thereafter, we and other groups have vigorously evaluated the effects of stress on the kisspeptin system and the neuroendocrine mechanisms that underlie these effects. In agreement with the findings of our aforementioned study, the administration of LPS decreased hypothalamic *Kiss1* mRNA expression and kisspeptin immunoreactivity, and the co-administration of kisspeptin partially restored serum LH levels in female rats [76]. In addition, other kinds of stress, such as psychosocial, unpredictable chronic (six randomly assigned stressors), and hypoglycemic stress, also reduced hypothalamic *Kiss1* mRNA expression and kisspeptin neuron activity [77-79]. Some studies have also evaluated the intermediate factors that transfer stress signals to kisspeptin neurons. As a result, it was found that both the central administration of CRH and the peripheral administration of corticosterone

reduced hypothalamic *Kiss1* mRNA expression and kisspeptin neuron activity in female rats and mice, indicating that activation of the HPA axis is involved in the stress-induced suppression of the kisspeptin system [77, 80]. Interestingly, relatively severe stress protocols were used in these studies; *i.e.*, high-dose LPS (1–5 mg/kg) or repeated LPS administration protocols (three consecutive injections at 24-h intervals), to evaluate the effects of immune stress [75, 76, 81, 82], and chronic (once per day for four weeks) or combined (restraint and isolation) protocols were used to evaluate the effects of unpredictable and psychosocial stress [78, 79]. Similarly, we found that a subseptic dose (500 µg/kg) of LPS did not affect hypothalamic *Kiss1* mRNA expression in female rats, indicating that only chronic or severe stress affects the kisspeptin system [81, 82]. As kisspeptin is indispensable for the regulation of HPG axis activity and maintaining fecundity, it might be rigidly maintained even under stressful conditions. In other words, reproductive disorders caused by disruption of the kisspeptin system might be more serious than those induced by stress-related factors alone.

The Roles of GnIH/RFRP-3 in Stress-Induced HPG Axis Dysfunction

As described above, GnIH/RFRP-3 has suppressive effects on GnRH and gonadotropin activity in many species and plays roles in the regulation of HPG axis activity, especially during the GnRH/LH surge. Recently, it has been shown that some kinds of acute and chronic stress result in increases in the number of GnIH/RFRP-3-immunoreactive cells and *GnIH/Rfrp* mRNA expression in the hypothalamus. It has also been reported that these changes in GnIH/RFRP-3 expression disrupt HPG axis activity and suppress reproductive ability (Fig. 1). Acute (3 h) and chronic (14 days, 3 h/day) psychological (immobilization) stress lead to the upregulation of hypothalamic *GnIH/Rfrp* mRNA expression, and *GnIH/Rfrp* mRNA expression levels are negatively correlated with serum LH levels in male rats [83]. In addition, half of GnIH/RFRP-3 neurons express the glucocorticoid receptor (GR), and adrenalectomy abolishes the increase in *GnIH/Rfrp* mRNA expression seen under chronic psychological stress conditions [83]. Similarly, the administration of corticosterone increases *GnIH/Rfrp* mRNA expression levels in rHypoE23, an Rfrp-expressing cell line [84], and a GR antagonist blocked this effect of corticosterone [85, 86]. In addition, the administration of

corticosterone increased *GnIH/Rfrp* mRNA expression and reduced GnRH mRNA and serum LH levels in fish [87]. These findings suggest that GnIH/RFRP-3 mediates the suppressive effects of glucocorticoids on the HPG axis under stress conditions. Interestingly, a recent study has shown that GnIH/RFRP-3 suppressed sexual maturation in socially non-dominant female rats living in colonies, indicating that GnIH/RFRP-3 is related to social stress-induced reproductive disorders [88]. As has been found for kisspeptin, relatively severe stress protocols are needed to affect the hypothalamic GnIH/RFRP-3 system; *e.g.*, high-dose LPS (2–5 mg/kg) was used to evaluate the effects of immune stress [81, 89], and a repeated immobilization protocol was used to evaluate the effects of psychological stress [83]. As GnIH/RFRP-3 is also essential for the regulation of HPG axis activity, GnIH/RFRP-3 expression might be rigidly maintained even under stressful conditions. We suggest that the neuroendocrine mechanisms responsible for stress-induced reproductive disorders might differ according to the severity of stress, and that GnIH/RFRP-3 and kisspeptin might only contribute to such disorders in severe physiological and/or psychological conditions.

The Roles of GnIH/RFRP-3 in Stress-Induced Reproductive Behavior Disorders

Recently, it has been shown that GnIH/RFRP-3 plays roles in the regulation of reproductive behavior in rodents (Fig. 1). The central administration of GnIH/RFRP-3 decreased sexual behavior in male rats [90], and it reduced sexual motivation in female hamsters [71, 91]. GnIH/RFRP-3 affects neuronal activity in some hypothalamic nuclei (the POA, medial amygdala, and the bed nucleus of the stria terminalis) that are related to female sexual behavior [91]. Thus, it has been speculated that stress-induced upregulation of GnIH/RFRP-3 expression not only disrupts the HPG axis, but also suppresses reproductive behavior and promotes infertility or subfertility. An excellent report about this topic was published in 2017 [92]. In the latter study, it was shown that chronic immobilization stress increased hypothalamic *GnIH/Rfrp* mRNA expression and reduced sexual behavior, the frequency of pregnancy, and litter size in female rats. It was also demonstrated that genetic silencing of *GnIH/Rfrp* with short hairpin RNA during stress led to the normalization of these parameters. These results indicate that GnIH/RFRP-3 is involved in the stress-induced disruption of reproductive behavior, as well as in HPG

axis disorders and that GnIH/RFRP-3 might be a useful clinical target for preventing stress-induced infertility.

Conclusion

Growing evidence indicates that kisspeptin and GnIH/RFRP-3, as well as stress-related factors, play pivotal roles in stress-induced reproductive disorders, such as

disruption of the HPG axis and reproductive behavior. Thus, kisspeptin and GnIH/RFRP-3 might be useful clinical targets for the treatment of stress-induced reproductive disorders, such as menstrual dysfunction and infertility. As the amount of human data available is limited, more evaluations are needed before kisspeptin or GnIH/RFRP-3-based treatments can be developed.

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