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Reproductive neuroendocrinology of mammalian gonadotropin-inhibitory hormone

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Abstract

Background: Gonadotropin-inhibitory hormone (GnIH) was discovered in the Japanese quail brain in 2000 as a hypothalamic neuropeptide that suppresses luteinizing hormone release from cultured quail anterior pituitary.

Methods: The authors investigated the existence of mammalian orthologous peptides to GnIH and their physiological functions in the following 19 years of research.

Main findings: Mammals have orthologous peptide to GnIH, often described RFamide-related peptide, expressed in the hypothalamus and gonads. Mammalian GnIH may also suppress gonadotropin synthesis and release by suppressing gonadotropin-releasing hormone (GnRH) synthesis and release in addition to directly suppressing gonadotropin synthesis and release from the pituitary. Mammalian GnIH may also suppress kisspeptin, a stimulator of GnRH, release. Mammalian GnIH is also expressed in the testis and ovary and suppresses gametogenesis and sex steroid production acting in an autocrine/paracrine manner. Thus, mammalian GnIH may act at all levels of the hypothalamic-pituitary-gonadal axis to suppress reproduction. GnIH may be involved in the regulation of puberty, estrous or menstrual cycle, seasonal reproduction, and stress responses.

Conclusion: Studies suggest that mammalian GnIH is an important neuroendocrine suppressor of reproduction in mammals.

KEYWORDS

gonadotropin-inhibitory hormone, gonadotropin-releasing hormone, kisspeptin, reproduction, RFamide-related peptide

1 | INTRODUCTION

Gonadotropin-inhibitory hormone (GnIH) is a peptide that was isolated from the Japanese quail (*Coturnix japonica*) brain in search for an RFamide peptide in vertebrates by using an antibody against RF-NH_2.[1] The structure of quail GnIH was identified to be SIKPSAYLPLRFamide.[1] FMRFamide peptide is the first RFamide peptide isolated from the ganglia of the venus clam, which had a cardioexcitatory function.[2] Since then, many RFamide peptides were identified in invertebrate species and studies suggested that they act as neurotransmitters, neuromodulators, and hormones.[3,4] It had been suggested that some RFamide peptides may regulate pituitary hormone release because RFamide-immunoreactive (ir) neurons projected close to the pituitary gland in vertebrates.[5,6]

In quail, gonadotropin-inhibitory hormone-immunoreactive (GnIH-ir) neuronal cell bodies were located in the paraventricular nucleus (PVN) of the hypothalamus and sent their axons to the median eminence (ME), suggesting regulation of anterior pituitary

hormone release.[1] It was shown that administration of GnIH to the cultured anterior pituitary suppresses luteinizing hormone (LH) release. Therefore, this peptide was named GnIH.[1] In vivo administration of GnIH to quail further suppressed gonadotropin synthesis and release, and gonadal activity.[7] Following studies have shown that vertebrate animals have orthologous peptides to GnIH that have a characteristic LPXRFamide (X = L or Q) motif at their C-termini. GnIH orthologous peptides of mammals are also described as RFamide-related peptide (RFRP). It was found that mammalian GnIH is also expressed in the gonads and may regulate gonadal activity by paracrine/autocrine manner.[3]

Here, we summarize the structure of GnIH peptide, morphology of GnIH neurons in the brain, function of GnIH in the brain, regulation of pituitary hormone secretion, and expression of GnIH in gonads and its function in mammals in this review. We also describe that expression of GnIH or activities of GnIH neurons are not only endogenously regulated by developmental stages or estrous and menstrual cycles, but also photoperiod and stress in order to regulate reproductive functions according to the environment.

2 | STRUCTURE OF MAMMALIAN GNIH PEPTIDES

A cDNA that encodes GnIH peptide (LPXRFamide peptide) precursor was found by a gene database search in mammals.[8] LPXRF (X = L or Q) sequence is followed by glycine as an amidation signal and arginine or lysine as endoproteolytic basic amino acids in the precursor (Figure 1). LPXRFamide peptide precursor cDNA encodes one LPLRFamide peptide that is described RFRP1 and one LPQRFamide peptide that is described RFRP3 (Figure 1). Human, macaque, sheep, and cattle LPXRFamide precursor cDNA also encodes LPXRFamide

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Rat             MEIISSKRFILLTLATSSFLTSNTLCSDELMMPHFHSKEGYGKYYQLRGIPKGVKERSVT
Mouse           MEIISLKRFILLTVATSSFLTSNTFCTDEFMMPHFHSKEGDGKYSQLRGIPKGEKERSVS
Hamster         MEIISSKRFILLTLATSSLLTSNIFCTEELMMPHFHSKEKEDKYSQPTGISKGEKERSVS
Monkey          MEIISSKLFILLTLATSSLLTSNISCADELMMSSLHSKENYDKYSEPRGYP--KREKRLN
Human           MEIISSKLFILLTLATSSLLTSNIFCADELVMSNLHSKENYDKYSEPRGYP--KGERSLN
Sheep           MEIISLKRFILLMLATSSLLTSNIFCTDESRIPSLYSKKNYDKYSEPRGDLGWEKERSLT
Cattle          MEIISLKRFILLMLATSSLLTSNIFCTDESRMPNLYSKKNYDKYSEPRGDLGWEKERSLT
                ***** * **** :****:****  *::*  :. ::**:  .** :  *      *: :.

                                          RFRP1              (RFRP2)
Rat             FQELKDWGAKKDIKMSPAPANKVPHSAANLPLRFGRNIEDRRSPRARA--------NMEA
Mouse           FQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTIDEKRSPAARV--------NMEA
Hamster         FQEVKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTLEEDRSTRART--------NMEA
Monkey          FEELKDWGPKNVIKMSTPAVNKMPHSVTNLPLRFGRTTEEERSAGATANLPLRSGRNMEV
Human           FEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRFGRNVQEERSAGATANLPLRSGRNMEV
Sheep           FEEVKDWGPK--IKMNTPAVNKMPPSAANLPLRFGRNMEEERSTRVMAHLPLRLGKNRED
Cattle          FEEVKDWAPK--IKMNKPVVNKMPPSAANLPLRFGRNMEEERSTRAMAHLPLRLGKNRED
                *:*:***..*  ***. . .**:* * :********. :: **. . .        * *

                        RFRP3
Rat             GTMSHFPSLPQRFGR-TTARRITKTLAGLPQKSLHSLASSELLYAMTRQHQEIQSPGQEQ
Mouse           GTRSHFPSLPQRFGR-TTAR-SPKTPADLPQKPLHSLGSSELLYVMICQHQEIQSPGGKR
Hamster         RTLSRVPSLPQRFGR-TTARSIPKTLSHLLQRFLHSMATSEVLNAMTCQHGEIQSPGGKQ
Monkey          SLVRQVLNLPQRFGRTTTAKSVCRTLSDLCQGSLHSPCANDLFYSMTCQHQEIQNPDQKR
Human           SLVRRVPNLPQRFGRTTTAKSVCRMLSDLCQGSMHSPCANDLFYSMTCQHQEIQNPDQKQ
Sheep           SLSRRVPNLPQRFGRTIAAKSITKTLSNLLQQSMHSPSTNGLLYSMTCRPQEIQNPGQKN
Cattle          SLSRWVPNLPQRFGRTTTAKSITKTLSNLLQQSMHSPSTNGLLYSMACQPQEIQNPGQKN
                     . .*******  :*:   :  : * *  :**  :. ::  *  :  ***.*. :.


Rat             PRKRVFTETDDAERKQEKIGNLQPVLQGAMKL
Mouse           TRRGAFVETDDAERKPEK---------------
Hamster         PRRQAFMETDDEEGKHEK---------------
Monkey          SRRLVFQKMDDAELKQEK---------------
Human           SRRLLFKKIDDAELKQEK---------------
Sheep           LRRLGFQKIDDAELKQEK---------------
Cattle          LRRRGFQKIDDAELKQEK---------------
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FIGURE 1 A multiple sequence alignment of gonadotropin-inhibitory hormone (GnIH) precursors. Human (*Homo sapiens*), rhesus macaque monkey (*Macaca mulatta*), sheep (*Ovis aries*), cattle (*Bos taurus*), Siberian hamster (*Phodopus sungorus*), rat (*Rattus norvegicus*), and mouse (*Mus musculus*) GnIH (RFRP) precursor polypeptides were aligned by CLUSTAL W Multiple Sequence Alignment. Multiple alignment parameters were as follows: Gap open penalty 10, Gap extension penalty 0.05, Hydrophilic residues GPSNDQERK, and Weight matrix GONNET. Identified or predicted endogenous peptide sequences are underlined. LPXRF (X = L or Q) sequences are underlined. Biochemically identified endogenous peptide sequences are shown in bold. Glycine (G) as an amidation signal and arginine (R) as an endoproteolytic basic amino acid are italicized. Asterisk (*) indicates positions which have a single, fully conserved residue. Colon (:) indicates conservation between groups of strongly similar properties—scoring >0.5 in the Gonnet PAM 250 matrix. Period (.) indicates conservation between groups of weakly similar properties—scoring <0.5 in the Gonnet PAM 250 matrix. Accession numbers are *H sapiens* pro-FMRFamide-related neuropeptide VF precursor [NP_071433.3], *M mulatta* Neuropeptide VF [EHH17432.1], *O aries* pro-FMRFamide-related neuropeptide VF precursor [NP_001120740.1], *B taurus* pro-FMRFamide-related neuropeptide VF precursor [NP_776593.1], *P sungorus* GnIH precursor [AEF16016.1], *R norvegicus* pro-FMRFamide-related neuropeptide VF precursor [NP_076442.1], and *M musculus* pro-FMRFamide-related neuropeptide VF precursor [NP_068692.1]. RFRP, RFamide-related peptide

peptide-like sequences that are LPLRSamide peptide in human and macaque, and LPLRLamide peptide in sheep and cattle (Figure 1). These peptides are described RFRP2 but none of their mature peptide structures are identified at present. Rodents such as rat, mouse, and hamster LPXRFamide peptide precursor cDNAs do not encode RFRP2 as in human, macaque, sheep, and cattle (Figure 1). The mature peptide structures of human RFRP1 and RFRP3,[9] macaque RFRP3,[10] and cattle RFRP1[11] and RFRP3,[12] mouse RFRP1 and RFRP3,[13] rat RFRP3,[14] Siberian hamster RFRP1 and RFRP3[15] are identified by biochemical methods.

3 | GNIH RECEPTOR AND CELL SIGNALING

Hinuma et al[8] identified a specific receptor for GnIH (RFRP) in mammals, which had been an orphan G-protein coupled-receptor (GPCR) GPR147. Bonini et al (2000) reported two GPCRs for neuropeptide FF (NPFF), a neuropeptide that has a C-terminal PQRFamide motif and involved in pain modulation. They were named NPFF1 that is identical to GPR147 and NPFF2 that is identical to GPR74.[16] Genes encoding the precursors of LPXRFamide peptides and PQRFamide peptides are thought to be paralogues from an evolutionary standpoint.[17,18] GPR147 and GPR74 are also thought to be paralogues.[19] Investigations of binding affinities and cell signaling pathways triggered by RFRPs and NPFFs have shown that RFRPs have higher affinities for GPR147, and NPFFs have potent agonistic activity for GPR74, suggesting that GPR147 is the primary receptor for GnIH (RFRP).[12,16,20]

GPR147 is likely to be coupled to $G_{\alpha i}$ protein because GnIH (RFRP) suppresses production of cAMP in Chinese hamster ovarian cells transfected with GPR147 cDNA.[8] Son et al[21] investigated the precise cell signaling pathway triggered by GnIH in a mouse gonadotrope cell line LβT2. It was first confirmed that GPR147 mRNA is expressed in LβT2 cells. Predicted mouse RFRP1 and RFRP3 suppressed cAMP production induced by gonadotropin-releasing hormone (GnRH), suggesting that GnIH (RFRP) inhibits adenylate cyclase (AC) activity. Mouse GnIH (RFRP) further suppresses extracellular signal-regulated kinase (ERK) phosphorylation and gonadotropin subunit gene transcriptions stimulated by GnRH. The results suggest that mouse GnIH (RFRP) suppresses GnRH-stimulated gonadotropin subunit gene transcriptions by inhibiting the AC/cAMP/protein kinase A (PKA)/ERK phosphorylation pathway.[21] This inhibitory pathway of mouse GnIH (RFRP) action was also shown in a GnRH neuronal cell line GT1-7 stimulated by vasoactive intestinal polypeptide (VIP).[22]

4 | MORPHOLOGY OF GNIH NEURONS

In mouse and hamster brains, GnIH precursor mRNA is expressed in neuronal cell bodies located in the dorsomedial hypothalamic area (DMH).[13,15,23,24] In rat brains, GnIH precursor mRNA is expressed in the periventricular nucleus (PerVN) and between the dorsomedial nucleus (DMN) and the ventromedial nucleus (VMN) of the hypothalamus.[8,25] In macaque brains, GnIH precursor mRNA is principally expressed in the intermediate periventricular nucleus (IPe) of the hypothalamus.[10] In sheep brains, GnIH mRNA is expressed in the DMN and PVN.[26]

Gonadotropin-inhibitory hormone neuronal fibers are widely distributed in the diencephalic, mesencephalic, and limbic brain regions in all mammals investigated.[10,15,27,28] Distribution of GnIH neuronal fibers and interaction with neurons in the brain were investigated in detail in the macaque brain.[10] GnIH neuronal fibers were abundant in the stria terminalis in the telencephalon, habenular nucleus, paraventricular nucleus of the thalamus, preoptic area (POA), PVN, IPe, arcuate nucleus (Arc), median eminence, and dorsal hypothalamic area in the diencephalon, medial region of the superior colliculus, central gray substance, and dorsal raphe nucleus in the midbrain. GnIH neuronal fibers are in close proximity to GnRH1, dopamine, pro-opiomelanocortin (POMC), and GnRH2 neurons in the POA, IPe, Arc, and central gray substance of the midbrain, respectively.[10] In sheep, GnIH neurons project to neuropeptide Y (NPY) and POMC neurons in the Arc, orexin, and melanin-concentrating hormone neurons in the lateral hypothalamic area, orexin cells in the DMN, corticotrophin-releasing hormone and oxytocin cells in the PVN, and GnRH neurons in the POA.[29] Kisspeptin is a hypothalamic neuropeptide that stimulates GnRH release.[30] Kisspeptin neurons form two populations in the anteroventral periventricular nucleus (AVPV) and Arc. Approximately 35% of Arc kisspeptin cells are contacted by GnIH neuronal fibers and 25% express GPR147 or GPR74 in mice.[31] Therefore, GnIH may also suppress gonadotropin release by suppressing kisspeptin neurons in the Arc.[32]

5 | REGULATION OF GONADOTROPIN SYNTHESIS AND RELEASE

Gonadotropin-inhibitory hormone neuronal fiber terminals are in probable contact with GnRH neurons in rodents,[15,23] monkeys,[10] and humans.[9] It was further demonstrated that GnIH receptor (GPR147) is expressed in GnRH neurons in hamsters.[15] Central administration of GnIH suppresses the release of gonadotropin in Syrian hamsters,[23] Siberian hamsters,[15] and rats.[27,33] Central administration of RFRP3 and RF9, an antagonist of RFRP, to female mice decreases and increases GnRH mRNA expression, respectively.[34] GnIH also suppresses the stimulatory effect of kisspeptin on GnRH release in mouse hypothalamic culture.[22] Administration of GnIH (RFRP3) to cultured mouse brain slices decreases the firing rate in a subpopulation of GnRH neurons.[35] GnIH (RFRP3) also suppresses the firing of kisspeptin-activated vGluT2 (vesicular glutamate transporter 2)-GnRH neurons as well as of kisspeptin-insensitive GnRH neurons.[36] Suppression of GnRH mRNA expression by GnIH (RFRP3) administration was also shown in a novel GnRH neuronal cell model, mHypoA-GnRH/GFP.[37] Accordingly, GnIH may inhibit the secretion of gonadotropins by decreasing the

activity of GnRH neurons in addition to directly regulating pituitary gonadotropin secretion.

Gonadotropin-inhibitory hormone may not directly regulate the pituitary in rodents, because there are only few or no GnIH neuronal fibers in the median eminence of hamsters [15,23] and rats.[38] Injection of a retrograde tracer Fluoro-Gold intraperitoneally to rats labeled the majority of GnRH neurons but no GnIH neuron.[38] On the other hand, abundant GnIH neuronal fibers are observed in the median eminence of sheep,[26] macaque,[10] and humans.[9] It was demonstrated that GPR147 mRNA is expressed in the gonadotropes in the human pituitary.[9] GnIH (RFRP3) suppresses gonadotropin synthesis and/or release from cultured pituitaries in sheep,[39] cattle,[40] and rats.[33] Peripheral administration of GnIH (RFRP3) also suppresses gonadotropin release in sheep,[26] rats,[41] cattle,[40] and humans.[42] Pulsatile secretion of GnIH (RFRP3) was observed in the hypophyseal portal blood of ewes, and a significant reduction in LH response to GnRH was observed by GnIH (RFRP3) administration in hypothalamo-pituitary-disconnected ewes.[43] Taken together, it is likely that GnIH can directly act on the pituitary to suppress gonadotropin synthesis and/or release from the pituitary at least in relatively large mammalian species.

6 | REGULATION OF PUBERTY

Changes in the expression of GnIH in the brain and the testicular activity were analyzed after birth in male mice.[44] The testicular activity increases progressively until 13 weeks of age and declines in the old mice. GnIH neurons appear in 1-week-old mice and their number and size increase significantly at 3 weeks of age, remain unaltered until 7 weeks of age, followed by a progressive decline until 13 weeks and increase again in the old age.[44] Both GnIH expression and GnIH/c-Fos co-expression decrease markedly in the early prepubertal stage in developing female mice.[45] These results suggest that the decrease in GnIH during postnatal development may facilitate puberty in male and female mice.

In rats, GnIH gene expression increases with developmental age, peaking around the time of puberty in females.[46] Iwasa et al[47] investigated the changes in GnIH and GPR147 mRNA levels in the rat hypothalamus during development in detail. In male rats, mRNA expressions of GnIH and GPR147 both increase from postnatal (P) 12 and P16, and peak at P35 and P42, respectively, and fall on P49. In females, GnIH mRNA expression continues to increase throughout development. On the other hand, GPR147 mRNA expression in female rats increases from P16, peaks at P28, and decreases from P35.[47] Central administration of GnIH (RFRP3) from P28 to P36 twice a day significantly decreases serum LH and estradiol concentration, delay uterine maturation and vaginal opening in female rats.[48] These results suggest that decreases in GnIH and/or GPR147 may also facilitate puberty in male and female rats.

Kiyohara et al[49] investigated whether thyroid dysfunction affects pubertal onset through GnIH in female mice. Hypothyroidism shows delayed pubertal onset with increased GnIH expression. However, GnIH knockout prevents delayed pubertal onset in hypothyroidism, indicating that increased GnIH expression induced by hypothyroidism may delay puberty. Administration of thyroid hormone suppresses GnIH expression in hypothalamic explants, and GnIH neurons express thyroid hormone receptors to convey thyroid status. These findings indicate a novel function of GnIH to mediate interactions of the hypothalamic-pituitary-thyroid (HPT) and gonadal axes that contribute to pubertal regulation.[49]

7 | REGULATION OF ESTROUS AND MENSTRUAL CYCLE

The estrous cycle that has metestrus, diestrus, proestrus, and estrus phases normally cycles in 4-5 days in rats and mice. Estradiol secretion from the ovary gradually increases from the metestrus phase to the proestrus phase and rapidly decreases at the estrus phase. It is known that relatively low estradiol concentration during metestrus and diestrus phases suppresses GnRH/LH pulse. However, high estradiol concentration in the afternoon of the proestrus phase increases the frequency and amplitude of GnRH/LH pulse resulting in GnRH/LH surge that induces ovulation.[50-52]

Estradiol induces kisspeptin precursor mRNA expression in the AVPV possibly via ERα.[53-56] Therefore, kisspeptin precursor mRNA expression in the AVPV peaks during the evening of proestrus in female rats.[57] These results suggest that AVPV kisspeptin neurons are involved in GnRH/LH surge. On the other hand, kisspeptin neurons in the Arc are thought to maintain GnRH pulse, because administration of kisspeptin antagonist in the Arc profoundly attenuates LH pulse frequency.[58]

Gonadotropin-inhibitory hormone neuronal system may also be involved in estrogen feedback to GnRH neurons because GnIH neurons in rodents express ERα.[23] Estradiol administration for 4 days suppresses GnIH precursor mRNA expression in ovariectomized mice.[59] The cellular activity of GnIH neurons and GnIH precursor mRNA expression is low at the time of LH surge in female hamsters or proestrus in female rats and mice, suggesting that reduction in GnIH neuronal activity may contribute to GnRH/LH surge.[60-62]

Intravenous infusion of GnIH (RFRP3) blocks estrogen-induced LH surge in ewes.[63] GnIH gene expression is also reduced in the preovulatory period in ewes.[63] These results suggest that reduction in GnIH may also be involved in the induction of GnRH/LH surge in ewes. On the other hand, GnIH precursor mRNA expression was higher in the late follicular phase just before GnRH/LH surge in female rhesus macaque, suggesting that GnIH fine tunes GnRH/LH surge in primates.[64] The opposite changes of GnIH during GnRH/LH surge between ewe and primates suggest a higher functional role of GnIH in primates. It may be possible that GnIH acts more as a translator of environmental and social information in the regulation of menstrual cycle, such as translating environmental or psychological stress to inhibit ovulation, rather than a member of the rigid periodic machinery that propels the reproductive cycle in primates.

8 | REGULATION OF SEASONAL REPRODUCTION

It has been suggested that photoperiodic mammals rely on the annual cycle of changes in nocturnal secretion of melatonin from the pineal gland to regulate seasonal reproduction. Therefore, how photoperiod and melatonin regulate GnIH synthesis and/release was investigated in photoperiodic mammals such as hamster and sheep. The level of GnIH precursor mRNA and the number of GnIH-ir cell bodies are reduced in sexually quiescent male hamsters acclimated to short-day (SD) photoperiod compared with sexually active animals maintained under LD photoperiod independent from gonadal steroids.[15,24,65-67] The photoperiodic regulation of GnIH gene expression is abolished in pinealectomized (Px) male hamsters, and continuous administration of LD hamsters with melatonin reduces GnIH gene expression to SD levels, indicating that photoperiodic regulation of GnIH is dependent on melatonin.[15,65] GnIH gene expression level can be increased in SD hamsters if they were exposed with dim light at night that can reduce melatonin.[68] On the other hand, GnIH gene expression is not modulated by photoperiod in the laboratory rat, a nonphotoperiodic breeder.[65] Central administration of hamster GnIH peptides (RFRP1 and RFRP3) suppresses LH release 5 and 30 minutes after administration in LD. On the other hand, both peptides stimulate LH release 30 minutes after administration in SD. These results suggest that GnIH peptides (RFRP1 and RFRP3) fine tune LH levels via its receptor expressed in gonadotropin-releasing hormone-immunoreactive (GnRH-ir) neurons in an opposite fashion across seasons in male hamsters.[15]

Gonadotropin-inhibitory hormone-immunoreactive neurons in the dorso-ventromedial hypothalamus are approximately threefold higher in sexually active male jerboa a semi-desert rodent, captured in the spring compared to sexually inactive autumn animals, like hamsters.[69] On the other hand, there is a significant increase in GnIH cell body number during the nonbreeding season (summer) compared to the breeding season (winter) in the adult female brushtail possum brain.[70]

Thyroid hormones are also implicated in the regulation of seasonal reproduction. Triiodothyronine (T_3) is decreased in hamsters housed in SD, and injections of exogenous T_3 stimulate testicular growth in SD Siberian hamsters. Administration of T_3 to SD hamsters also increases GnIH-ir cells in the dorsomedial hypothalamus compared with SD controls.[71] It is thought that thyroid-stimulating hormone (TSH) in the pars tuberalis of the adenohypophysis regulates local thyroid hormone availability in the mediobasal hypothalamus. In SD-adapted male Djungarian and Syrian hamsters, central administration of TSH for 4-6 weeks restores the summer phenotype of testicular activity, kisspeptin, and GnIH expression.[72]

In Soay sheep, a SD breeder, GnIH shows a generally moderate increase in the hypothalamus in LD. However, GnIH expression in the ependymal cells surrounding the base of the third ventricle is highly photoperiodic, with levels being undetectable in animals held on SD but consistently high under LD.[73] In ovariectomized (OVX) ewes treated with estradiol, lesser expression of GnIH and lesser GnIH-GnRH contacts were concurrent with the breeding season.[74] The number of GnIH-ir neurons in female goat hypothalamus is also lower in the follicular phase than in the luteal and anestrous stages.[75] Mean GnIH (RFRP3) pulse amplitude and frequency are also higher during the nonbreeding (anestrous) season compared with the luteal and follicular phases of the estrous cycle in the breeding season in ewes.[43]

9 | MEDIATION OF STRESS RESPONSES

Because stress has inhibitory effects on reproduction,[76] it was hypothesized that stress may suppress the hypothalamic-pituitary-gonadal (HPG) axis by activating the GnIH system. Kirby et al (2009) showed that both acute and chronic immobilization stress up-regulates GnIH expression in the DMH of adult male rats. Adrenal glucocorticoids (GC) may increase GnIH expression because adrenalectomy blocks stress-induced increase in GnIH expression and 53% of GnIH cells express receptors for GC.[77] Administration of a high dose of lipopolysaccharide, an endotoxin, increases GnIH and GPR147 mRNA levels in both OVX and gonadal intact female rats, while kisspeptin and GnRH mRNA levels are decreased.[78] Food restriction also up-regulates GnIH mRNA expression and suppresses ovarian development and follicular growth in prepubertal ewes.[79] Metabolic challenges, such as short-term fasting and high-fat diet, are less effective in decreasing LH secretion in GPR147-deficient male mice, suggesting that the GnIH-GPR147 inhibitory pathway mediates gonadotropin suppression by metabolic stress.[80] Stressful stimuli also activate GnIH-ir neurons or increase GnIH expression in rats and mice.[81-83] It was further shown that GnIH administration activates hypothalamic-pituitary-adrenal (HPA) axis in rats,[81] mice,[84] and rhesus monkeys.[85] Administration of GnIH (RFRPs) induces anxiety-related behavior in rats [81] and mice,[84] suggesting that GnIH also mediates behavioral stress responses, although its mechanism of action should be investigated in future studies.[3,86]

Son et al[87] investigated the mechanism of activation of GnIH precursor mRNA transcription by corticosterone (CORT) using a GnIH-expressing neuronal cell line, rHypoE-23, derived from rat hypothalamus. GR mRNA is expressed in rHypoE-23 cells and CORT treatment for 24 hours increases GnIH mRNA expression. They further characterized the promoter activity of the rat GnIH precursor gene stimulated by CORT. CORT-responsive region at 2000-1501 bp upstream of GnIH precursor coding region includes two GC response elements (GREs) at 1665 and 1530 bp, and mutation of 1530 GRE abolishes responsiveness to CORT. Finally, CORT-stimulated GR recruitment was demonstrated at the GnIH promoter region containing the 1530 GRE.[87]

10 | GONADAL GNIH ACTIONS

Zhao et al[88] investigated the locations of GnIH, GPR147, and GPR74 in the testis of Syrian hamster. GnIH was found in spermatocytes and

round to early elongated spermatids. GPR147 was found in myoid cells and pachytene spermatocytes, maturation division spermatocytes, and round and late elongated spermatids, whereas GPR74 was found only in late elongated spermatids. These results suggest a possible autocrine and/or paracrine role for GnIH in spermatogenesis.[89] In mice, GnIH (RFRP3) treatment causes dose-dependent decrease in germ cell proliferation and survival markers and increase in apoptotic markers. GnIH (RFRP3) administration also suppresses testosterone synthesis in the testis both in vivo and in vitro.[90] It was also shown that GnIH (RFRP3) suppresses synthesis and release of testosterone in the male pig.[91] GnIH and GPR147 are also expressed in the epididymis of male rat. Intratesticular administration of RFRP3 decreases spermatozoa and increases degenerated and vacuolated epididymal epithelial cells.[92]

Gonadotropin-inhibitory hormone and GPR147 are primarily expressed in the granulosa cell layer of large preovulatory follicles as well as in the corpus luteum in primary cultures of granulosa-lutein cells of premenopausal women.[93] Immunoreactivities of GnIH and GPR147 are mainly localized in the granulosa and theca cells of antral follicles during proestrus and estrus and in luteal cells during metestrus and diestrus in female pig.[94] Singh et al[95] investigated the changes in GnIH (RFRP3) expression in the ovary of mice during the estrous cycle. The immunoreactivity of GnIH (RFRP3) was mainly localized in granulosa cells of the healthy and antral follicles during proestrus and estrus, and in luteal cells during diestrus 1 and 2 phases. A significant increase in GnIH (RFRP3) immunoreactivity during late diestrus 2 coincided with the decline in corpus luteum activity and initiation of follicular growth and selection.[95] Singh et al[96] further showed that GnIH (RFRP3) inhibits follicular development and steroidogenesis in the ovary of mice in vivo and in vitro. Oishi et al[93] investigated the role of GnIH and GPR147 in the human ovary. Treatment of human granulosa-lutein cells with GnIH (RFRP3) reduces FSH-, LH-, and forskolin-stimulated progesterone production and steroidogenic acute regulatory protein (StAR) expression. GnIH (RFRP3) also suppresses gonadotropin- and forskolin-induced intracellular cAMP accumulation. These results suggest that GnIH (RFRP3) acts via GPR147 to suppress gonadotropin signaling in human granulosa cells.[93] Wang et al[97] further showed that GnIH (RFRP3) induces cell cycle arrest at G2/M in porcine ovarian granulosa cells.

11 | CONCLUSION

Gonadotropin-inhibitory hormone is a peptide that was first isolated from the quail brain, which has a characteristic LPXRFamide (X = L or Q) motif at their C-termini. Mammals also possess GnIHs having C-terminal LPXRFamide (X = L or Q), which are also named RFRP1 that has a C-terminal LPLRFamide sequence and RFRP3 that has a C-terminal LPQRFamide sequence (Figure 1). The mature structures of human GnIHs (RFRP1 and RFRP3), macaque GnIH (RFRP3), and cattle GnIHs (RFRP1 and RFRP3), mouse GnIHs (RFRP1 and RFRP3), rat GnIH (RFRP3), and Siberian hamster GnIHs (RFRP1 and RFRP3) are currently identified by biochemical methods.

The GPCR, GPR147 is the primary receptor for GnIH. GPR147 is likely to be coupled to $G_{\alpha i}$ protein in target cells. GnIH suppresses GnRH-stimulated gonadotropin subunit gene transcriptions by inhibiting the AC/cAMP/PKA/ERK phosphorylation pathway. GnIH also suppresses the same pathway in GnRH neuronal cell line.[98] Morphological evidence supports that GnIH suppresses the secretion of gonadotropins by decreasing the activity of GnRH neurons in addition to directly regulating pituitary gonadotropin secretion. GnIH may also suppress gonadotropin release by suppressing kisspeptin neurons in the Arc.

Measurements of GnIH and GPR147 contents and gonadal activities during developmental stages suggest that decreases in GnIH and/or GPR147 may facilitate puberty in mammals. GnIH may also mediate interactions of the HPT and HPG axes in pubertal regulation. Reduction in GnIH neuronal activity may contribute to GnRH/LH surge in rodents. GnIH may also be involved in the regulation of seasonal reproduction regulated by melatonin and thyroid hormones.

Various stressors increase GnIH neuronal activity or GnIH precursor gene expression suggesting that stress suppresses the HPG axis by activating the GnIH system. CORT-stimulated GR recruitment in the GnIH promoter region is likely to be the mechanism of activation of GnIH precursor mRNA transcription under stress. GnIH may also mediate behavioral stress responses, although its mechanism of action is less understood at present.

Gonadotropin-inhibitory hormone and GPR147 are expressed in the gonads and likely to suppress gametogenesis and sex steroid synthesis in an autocrine/paracrine manner. Thus, mammalian GnIH may act at all levels of the HPG axis to suppress reproduction. Nineteen years of studies after the discovery of GnIH in quail suggest that GnIH is also an important neuroendocrine suppressor of reproduction in mammals.

CONFLICT OF INTEREST

Takayoshi Ubuka and Kazuyoshi Tsutsui declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with human and animal subjects performed by any of the authors.

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