Discovery of gonadotropin-inhibitory hormone (GnIH), progress in GnIH research on reproductive physiology and behavior and perspective of GnIH research on neuroendocrine regulation of reproduction

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**Abstract**

Based on extensive studies on gonadotropin-releasing hormone (GnRH) it was assumed that GnRH is the only hypothalamic neurohormone regulating gonadotropin release in vertebrates. In 2000, however, Tsutsumi’s group discovered gonadotropin-inhibitory hormone (GnIH), a novel hypothalamic neuropeptide that inhibits gonadotropin release, in quail. Subsequent studies by Tsutsumi’s group demonstrated that GnIH is conserved among vertebrates, acting as a new key neurohormone regulating reproduction. GnIH inhibits gonadotropin synthesis and release through actions on gonadotropes and GnRH neurons via GnIH receptor, GPR147. Thus, GnIH is not the sole hypothalamic neurohormone controlling vertebrate reproduction. The following studies by Tsutsumi’s group have further demonstrated that GnIH has several important functions in addition to the control of reproduction. Accordingly, GnIH has drastically changed our understanding about reproductive neuroendocrinology. This review summarizes the discovery of GnIH, progress in GnIH research on reproductive physiology and behavior and perspective of GnIH research on neuroendocrine regulation of reproduction.

1. Introduction

In the 1920’s, a novel concept of ‘neurosecretion’ was proposed by Sharrer suggesting that neurons in the hypothalamus terminating in the neurohypophysis produce and secrete neurohormones. Subsequently, Bargmann established this seminal concept in 1949 by selectively staining the neurosecretory pathway from the perikarya in the nucleus supraopticus and paraventricularis to their processes in the neurohypophysis. Since then, important hypothalamic neuropeptides, such as oxytocin (Livermore and Du Vigneaud, 1949) and vasopressin (Turner et al., 1951), were identified as neurohormones secreted from the neurohypophysis in mammals. Harris (1948) also proposed a new important concept indicating that hypothalamic neurons terminating at the median eminence (ME) may also secrete neurohormones from the ME into the hypophysial portal system to regulate anterior pituitary hormones such as gonadotropins, i.e. luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thyroid stimulating hormone (TSH), growth hormone (GH) and adrenocorticotropic hormone (ACTH) secretion. Subsequently, Sharrer’s and Guillemin’s groups demonstrated this seminal concept by the discovery of important neurohormones, including thyrotropin-releasing hormone (TRH) (Burgus et al., 1969; Bolier et al., 1969), gonadotropin-releasing hormone (GnRH) (Matsuo et al., 1971; Burgus et al., 1972) and growth hormone-inhibiting hormone (somatostatin) (Brazeau et al., 1973), in the mammalian brains.

As mentioned above, GnRH, a hypothalamic neuropeptide that stimulates the release of gonadotropins, i.e. LH and FSH from gonadotropes in the anterior pituitary was discovered as a neurohormone in mammals by Schally’s (Matsuo et al., 1971) and Guillemin’s (Burgus et al., 1972) groups at the beginning of the 1970s. GnRH was also isolated in other vertebrates (King and Millar, 1982; Miyamoto et al., 1982, 1984; Sherwood et al., 1986). Based on extensive GnRH studies over the next three decades after the discovery of GnRH in mammals, the commonly held belief was that GnRH is the sole hypothalamic neurohormone regulating gonadotropin release in vertebrates.

In 2000, however, Tsutsumi’s group discovered gonadotropin-inhibitory hormone (GnIH), a hypothalamic neuropeptide that inhibits gonadotropin release, in quail, an avian species (Tsutsumi et al., 2000). The discovery of GnIH has opened a new research era of reproductive neuroendocrinology challenging the notion that GnRH is the sole hypothalamic neurohormone regulating gonadotropin release in vertebrates. Subsequent studies of Tsutsumi’s group demonstrated that GnIH is conserved among vertebrates from fish to humans and acts as a new key neurohormone inhibiting reproduction (for reviews, see Kriegsfeld...
et al., 2015; Tsutsui, 2009, 2016; Tsutsui and Ubuka, 2012; Tsutsui and Ukena, 2006; Tsutsui et al., 2006, 2007, 2010a, b, 2012, 2013c, 2015, 2018; Ukena and Tsutsui, 2005). Recent studies by Tsutsui’s group have further demonstrated that GnIH has important functions in addition to the control of reproduction (Tobari et al., 2014; Ubuka et al., 2014). It now appears that GnIH acts on the pituitary and the brain to control not only reproduction but also reproductive behavior through changes in the biosynthesis of neurosteroids in the brain (Ubuka et al., 2014). Thus, the following 19 years of GnIH research has advanced our understanding of the neuroendocrine control of reproductive physiology and behavior (for reviews, see Kriegsfeld et al., 2015, Tsutsui, 2009, 2016; Tsutsui and Ubuka, 2012, 2016; Tsutsui et al., 2006, 2007, 2010a, 2010b, 2012, 2013c, 2015, 2018; Ubuka et al., 2013).

Herein we describe the discovery of GnIH, a new key neurohormone of reproductive physiology and behavior, and progress and perspective of GnIH research on reproductive neuroendocrinology.

2. Discovery of gonadotropin-inhibitory hormone (GnIH)

2.1. Background

GnIH is a novel hypothalamic neuropeptide having a C-terminal Arg-Phe-NH2 motif (RFamide peptide) (Tsutsui et al., 2000). In invertebrates, the identification of an RFamide peptide Phe-Met-Arg-Phe-NH2 (FMRFamide) as a cardioexcitatory peptide from the ganglia of the venus clam dates back to the late 1970s (Price and Greenberg, 1977). After this initial discovery, various RFamide peptides had been isolated in other invertebrates. On the other hand, immunohistochemical studies on vertebrates (Raffa, 1988; Rastogi et al., 2001) suggested the presence of some unknown RFamide peptide in the hypothalamus that may act as a new neurohormone and regulate the secretion of anterior pituitary hormones because FMRFamide-immunoreactive (IR) neurons terminate in the vicinity of the anterior pituitary gland. Based on this background, Tsutsui’s group searched for this novel RFamide peptide in the quail brain.

In 2000, Tsutsui’s group isolated a novel neuropeptide having a having a C-terminal RFamide motif, Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH2 (SIKPAYLPLRFamide), from the quail hypothalamus by using high-performance liquid chromatography (HPLC) and a competitive enzyme-linked immunosorbent assay with an antibody against RFamide (Tsutsui et al., 2000). Importantly, this isolated novel hypothalamic RFamide peptide inhibited gonadotropin release from the quail anterior pituitary by in vitro analysis (Tsutsui et al., 2000). This is the first demonstration of a hypothalamic neuropeptide that inhibits gonadotropin release in any vertebrate (Tsutsui et al., 2000). Given its action on gonadotropin release and its localization in the hypothalamo-hypophysial system, this novel hypothalamic RFamide peptide was named GnIH (Tsutsui et al., 2000) (Fig. 1). Cell bodies and terminals for GnIH neurons exist in the paraventricular nucleus (PVN) and ME, respectively in birds (Tsutsui et al., 2000). The C-terminal structure of quail GnIH is identical to the chicken LPLRFamide peptide that was first reported to be the RFamide peptide isolated in vertebrates (Dockray et al., 1983), although the chicken LPLRFamide peptide can be a fragment of the chicken GnIH peptide SIKPAYLPLRFamide that was identified in a recent study (McConnell et al., 2014).

Subsequently, a cDNA encoding the precursor for GnIH was identified in quail (Satake et al., 2001) and other avian species (for reviews, see Tsutsui, 2009; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015). The GnIH precursor encodes one GnIH and two GnIH-related peptides (GnIH-RP-1 and GnIH-RP-2) that possess a common C-terminal LPRxRFamide (X = L or Q) motif in all avian species studied. GnIH was further isolated as a mature peptide in other avian species, such as European starlings (Ubuka et al., 2008), zebra finches (Tobari et al., 2010), chicken (McConnell et al., 2014), and GnIH-RP-2 was also isolated in quail (Satake et al., 2001).

GnIH is a key neurohormone of avian reproduction because GnIH inhibits gonadotropin release in most avian species studied (for reviews, see Tsutsui, 2009; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015) (Fig. 1). To investigate the biological action of GnIH by in vivo analysis, chronic GnIH administration to mature male quail was further conducted (Ubuka et al., 2006). Chronic GnIH administration decreases the expressions of common α, LHβ, and FSHβ subunit mRNAs and the circulating LH concentration. In addition, chronic GnIH administration induces testicular apoptosis and regression of seminiferous tubules in mature male birds (Ubuka et al., 2006). Chronic GnIH administration inhibits testicular growth in immature male birds (Ubuka et al., 2006). These findings indicate that GnIH suppresses, as a new key neurohormone, gonadal development and maintenance by decreasing gonadotropin synthesis and release in birds (Fig. 1).
2.2. Structure and biological action of GnIH

In addition to birds, Tsutsui’s group further identified GnIHs in mammals and primates (Kriegsfeld et al., 2006; Ubuka et al., 2009a,b; 2012a; Ukena et al., 2002). The identified mammalian and primate GnIHs also possess a common C-terminal LPXRFAmide (X = L or Q) motif, like avian GnIH and GnIH-RPs (for reviews, see Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015). On the basis of GnIH structures, GnIHs identified in birds, mammals and primates were therefore designated as LPXRFAmide peptides. Mammalian and primate GnIHs are also called RFamide-related peptide 1 and 3 (RFRP-1 and -3).

To clarify the biological action of GnIH in mammals, several physiological studies were conducted as follows. In vivo administration of avian GnIH centrally or peripherally to Syrian hamsters inhibits LH release (Kriegsfeld et al., 2006). Central administration of hamster GnIHs (RFRP-1 and -3) also inhibits LH release in Siberian hamsters (Ubuka et al., 2012a). Central administration of rat GnIH (RFRP-3) inhibits LH release (Janvier, 2006) and GnRH-elicited gonadotropin release in rats (Murakami et al., 2008; Rizwan et al., 2009). LH pulse amplitude is reduced, and GnRH-elicited gonadotropin synthesis and release are inhibited by mammalian GnIH (RFRP-3) in sheep (Clarke et al., 2008; Sari et al., 2009) and cows (Kodakawa et al., 2009). Because the structure of human GnIH (RFRP-3) is the same as ovine GnIH (RFRP-3) (Ubuka et al., 2009b), the biological action of human/ovine GnIH (RFRP-3) was examined in the ovine pituitary. Human/ovine GnIH (RFRP-3) inhibits GnRH-stimulated secretions of both LH and FSH (Clarke et al., 2008). Based on these physiological findings, it now appears that mammalian and primate GnIHs inhibit gonadotropin synthesis and release and GnIH-elicited gonadotropin secretion (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015) (Fig. 1).

To extend GnIH findings in vertebrates, Tsutsui’s group further identified GnIHs in the brain of reptiles, amphibians and fish. All of the identified GnIHs in these vertebrates also possess a common C-terminal LPXRFAmide (X = L or Q) motif, like avian, mammalian and primate GnIHs (Chartrel et al., 2002; Chowdhury et al., 2011; Koda et al., 2002; Sawada et al., 2002a, 2002b; Shahjan et al., 2011; Ukena et al., 2003a, 2016). We now know that GnIHs exist in the brain of vertebrates from fish to humans (for reviews, see Tsutsui, 2009, 2016; Tsutsui and Ubuka, 2012, 2016; Tsutsui and Ukena, 2006; Tsutsui et al., 2006, 2007, 2010a, 2010b, 2012, 2013c, 2015; Ukena and Tsutsui, 2005). In fish, Sawada et al. (2002b) reported that goldfish GnIH precursor cDNA encodes three GnIHs, gLPXRFA-1, -2 and -3. Subsequently, several physiological studies on fish showed that these goldfish GnIHs (gLPXRFA-1, -2 and -3) have both inhibitory and stimulatory effects on gonadotropin synthesis and release depending on reproductive conditions (Amano et al., 2006; Moussavi et al., 2012, 2013; Qi et al., 2013). Zebrafish GnIH, zLPXRFA-3, has an inhibitory effect on gonadotropin release (Zhang et al., 2010).

In contrast to extensive studies on representative species of gnathostomes, the presence of GnIH had not been identified in agnathans, the most ancient lineage of vertebrates (Janvier, 2006). Tsutsui’s group therefore searched for agnathan GnIH. Because synten analysis showed the existence of the GnIH gene in sea lamprey, Osugi et al. (2012) cloned lamprey GnIH precursor cDNA that encodes three GnIHs in sea lamprey. Osugi et al. (2012) further isolated these mature GnIHs from the brain of sea lamprey by immunoaffinity purification and mass spectrometry (Osugi et al., 2012). The isolated lamprey GnIHs possess a common C-terminal PQRFamide motif (Osugi et al., 2012), unlike GnIHs isolated in gnathostomes. GnIH neurons are located in the hypothalamus and GnIH fibers project to GnRH3 neurons in the brain of sea lamprey and few lamprey GnIH fibers are located in the neurohypophysis compared to abundant lamprey GnRH3 fibers (Osugi et al., 2012). Based on these morphological findings of GnIH neurons and fibers, Osugi et al. (2012) analyzed the biological actions of lamprey GnIHs on the expressions of lamprey GnRHs and the gonadotropin β subunit and showed that one of the lamprey GnIHs increases the expressions of lamprey GnRH3 and gonadotropin β subunit (Osugi et al., 2012). These findings in lampreys indicate that GnIH is present in the hypothalamic area of lamprey, the oldest lineage of vertebrates and acts on GnRH3 neurons to stimulate gonadotropin β expression in the pituitary (Osugi et al., 2012). Accordingly, it is considered that GnIH emerged in agnathans as a stimulatory neuropeptide for the regulation of gonadotropin secretion and changed into an inhibitory neuropeptide during vertebrate evolution.

2.3. Molecular evolution of GnIH

Most identified GnIHs are LPXRFAmide (X = L or Q) peptides as a member of the RFamide peptide family (for reviews, see Tsutsui, 2009, 2016; Tsutsui and Ubuka, 2012, 2016; Tsutsui and Ukena, 2006; Tsutsui et al., 2006, 2007, 2010a, 2010b, 2012, 2013c, 2015; Ukena and Tsutsui, 2005). Four more groups in the RFamide peptide family, i.e., the neuropeptide FF (NPFF; PQRFamide peptide) group, the kisspeptin group, the pyroglutamylated RFamide peptide (QRFP)/26RFamide group, and the prolactin-releasing peptide (PrRP) group have also been documented in vertebrates (for reviews, see Tsutsui, 2009, 2015 Ukena and Tsutsui, 2006; Ukena and Tsutsui, 2005).

NPFF peptides have a C-terminal PQR motif. Because the C-terminal structure of GnIH peptides (LPXRFAmide (X = L or Q) peptides) is similar to that of NPFF peptides (PQRFAmide peptides) in vertebrates, further clarification of the NPFF peptide gene in agnathans was warranted. Tsutsui’s group therefore identified the cDNAs of NPFF peptides in the brain of lamprey and hagfish (Osugi et al., 2006, 2011). Phylogenetic analysis showed that agnathan possess both GnIH and NPFF genes. Subsequently, agnathan NPFF peptides were identified in sea lamprey and hagfish. The identified agnathan NPFF peptides and GnIH peptides had the same C-terminal PQRFamide motif (Osugi et al., 2006, 2011, 2012).

Because agnathan GnIHs share both NPFF genes and their mature peptides have the same C-terminal PQRFamide motif (Osugi et al., 2006, 2011, 2012), it is possible that the GnIH and NPFF genes are derived from a common ancestral gene in protchordates. To demonstrate this possibility, Tsutsui’s group further identified an amphioxus PQRFamide peptide precursor cDNA that encodes three PQRFamide peptides (Osugi et al., 2014). These mature amphioxus PQRFamide peptides were also identified by immunoaffinity purification and mass spectrometry (Osugi et al., 2014). Osugi et al. (2014) showed that the amphioxus PQRFamide peptide precursor occurs before the divergence between the GnIH and NPFF groups in vertebrates by phylogenetic analysis. Osugi et al. (2014) further showed the conserved synteny region existing around the loci of the amphioxus PQRFamide peptide gene, GnIH gene and NPFF gene in vertebrates by synteny analysis. Importantly, the amphioxus PQRFamide peptide gene is located near the HOX cluster and the GnIH and NPFF genes in vertebrates are located near the HOXA and HOXC clusters, respectively (Osugi et al., 2014). These findings indicate that the GnIH and NPFF genes have duplicated through the whole-genome duplication event. Accordingly, it is considered that the amphioxus PQRFamide peptide gene is close to the ancestor of the GnIH and NPFF genes (Osugi et al., 2014, 2015). Thus, the GnIH and NPFF genes may have diverged from a common ancestral gene in the protchordate through the whole-genome duplication event during vertebrate evolution.

3. Progress in GnIH research on reproductive physiology and behavior

3.1. Molecular mechanisms of GnIH actions on target cells

3.1.1. Discovery of GnIH receptor

To investigate the mode of GnIH action on gonadotropin secretion, Tsutsui’s group identified the receptor for GnIH in quail. The identified
GnIH receptor, GPR147, is a member of the G-protein coupled receptor superfamily (Yin et al., 2005), which is also called neuropeptide FF receptor 1 (NPFFF1). Yin et al. (2005) showed that membrane fraction of COS-7 cells transfected with GnIH receptor cDNA binds to GnIH and GnIH-RPs. Because the identified GnIH receptor is expressed in gonadotropes in the anterior pituitary, GnIH can act directly on gonadotropes to reduce gonadotropin release in birds (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009, 2015; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015; Ubuka et al., 2013) (Fig. 1). GnIH neurons further project to GnRH1 neurons that also express GnIH receptor in birds (Bentley et al., 2003; Ubuka et al., 2008) and mammals (Ubuka et al., 2009a,b, 2012a) (Fig. 1). Therefore, GnIH acts not only on gonadotropes but also on GnRH1 neurons to inhibit gonadotropin synthesis and release in birds (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015; Ubuka et al., 2013) (Fig. 1).

Hinuma et al. (2000) also identified a receptor for mammalian GnIH, RFRP, which is identical to GPR147 and named it OT7T022. Bonini et al. (2000) found two GPCRs for NPF and designated them as NPF1 (identical to GPR147) and NPF2 (identical to GPR74). It is known that NPF has a C-terminal PQRFamide motif and is involved in pain modulation. As described above, it is considered that the GnIH (LPXRamide peptide) and NPF (PQRFamide peptide) genes have diverged from a common ancestral gene through gene duplication (Ouagi et al., 2012, 2014, 2015). It has been demonstrated that GPR147 and GPR74 are paralogous (Fredriksson et al., 2003). Biochemical analyses showed that GnIH has a higher affinity for GPR147, whereas NPF has potent agonistic activity for GPR74 (Bonini et al., 2005; Liu et al., 2001; Yoshida et al., 2003). These biochemical findings indicate that GPR147 (NPF1, OT7T022) is the primary receptor for GnIH.

3.1.2. GnIH signaling pathways in target cells

The molecular mechanisms of GnIH actions in the target cells, gonadotropes in the anterior pituitary and GnRH1 neurons in the hypothalamus, were characterized (Son et al., 2012, 2016). Son et al. (2012) found the expression of GnIH receptor mRNA in the gonadotropes cell line, LβT2 by RT-PCR analysis. Son et al. (2012) further showed the inhibitory effects of GnIH on GnIH-induced signaling pathways by using LβT2 cells as follows: GnIH effectively reduces GnRH-induced cAMP production and extracellular signal-regulated kinase (ERK) phosphorylation; GnIH reduces GnRH-induced LHβ expression and LH release. Thus, GnIH reduces GnRH-stimulated gonadotropin secretion by interfering with GnRH actions via a adenylate cyclase (AC)/cAMP/protein kinase A (PKA)-dependent ERK pathway in the gonadotrope cell line, LβT2 (Son et al., 2012). The same inhibitory mechanism of GnIH action on the AC/cAMP/PKA pathway was also found in the GnRH neuronal cell line GT1-7 that expresses GnIH receptor (Son et al., 2016).

Following the discovery of GnIH inhibiting hypothalamo-pituitary-gonadal (HPG) axis, kispeptin was discovered in mammals. In contrast to GnIH, kispeptin has a stimulatory action on GnRH neurons and up-regulates the HPG axis in mammals (de Roux et al., 2003; Kauffman et al., 2007; Pinilla et al., 2012; Seminara et al., 2003). GnIH neurons may regulate the actions of both GnRH neurons and kispeptin neurons because GnIH neurons project not only to GnRH neurons in the preoptic area (POA) but also to kispeptin neurons in the hypothalamus (for reviews, see Kriegsfeld et al., 2015; Poling et al., 2013; Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015) (Fig. 1). Furthermore, GnIH neurons also project to GnRH2 neurons and many other neurons in the brain, indicating multiple actions of GnIH (for reviews, see Kriegsfeld et al., 2015; Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015) (Fig. 1).

3.2. Multiple actions of GnIH

3.2.1. Local action of gonadal GnIH on reproduction

As mentioned above, GnIH is a key hypothalamic neurohormone regulating reproduction across vertebrates, reducing gonadotropin synthesis and release by decreasing the activities of pituitary gonadotropes and hypothalamic GnRH1 neurons (Fig. 1). In addition to these central actions of GnIH, there are several reports indicating that gonads express GnIH that is directly involved in the local regulation of gonadal activity (for reviews, see Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015; Ubuka et al., 2013, 2014; Kriegsfeld et al., 2015) (Fig. 1). In the gonads of birds and mammals, both GnIH and GnRH receptor are expressed in steroidogenic cells and germ cells (Anjum et al., 2014; Bentley et al., 2008; Oishi et al., 2012; Singh et al., 2008, 2011a; 2011b; Zhao et al., 2010). Furthermore, physiological studies have further demonstrated that GnIH acts on the gonads in an autocrine and/or paracrine manner to suppress sex steroid production and germ cell differentiation and maturation (Anjum et al., 2014; Bentley et al., 2008; Oishi et al., 2012; Singh et al., 2008, 2011a; 2011b; Zhao et al., 2010) (Fig. 1).

3.2.2. Central action of hypothalamic GnIH on reproductive behavior

Central GnIH also acts on the brain to regulate not only reproduction but also sexual and aggressive behaviors (Bentley et al., 2006; Ubuka et al., 2012b, 2014) (Fig. 1). In birds, Bentley et al. (2006) found in estrogen-primed female white-crowned sparrows exposed to the song of males that a centrally administered GnIH inhibits copulation solicitation. It is known that in estrogen-primed female white-crowned sparrows exposed to the song of males GnRH2 enhances copulation solicitation (Maney et al., 1997). Because GnIH neurons terminate in close proximity of GnRH2 neurons and GnRH2 neurons express GnIH receptor in songbirds (Ubuka et al., 2008), it is possible that GnIH inhibits copulation solicitation by inhibiting the activity of GnRH2 neurons in songbirds (Bentley et al., 2006) (Fig. 1). Ubuka et al. (2012b) therefore investigated this possibility by applying RNA interference (RNAi) of the GnIH gene in male and female white-crowned sparrows in collaboration with Wingfield’s and Bentley’s groups. GnIH RNAi reduces resting time, spontaneous production of complex vocalizations, and stimulates agonistic vocalizations (Ubuka et al., 2012b). These avian findings indicate that GnIH gene silencing induces arousal. Ubuka et al. (2012b) further showed that the activities of male and female birds are negatively correlated with GnIH mRNA expression in the PVN. The density of GnIH neuronal fibers in the ventral tegmental area is decreased by GnIH RNAi in female birds, and the number of GnRH1 and GnRH2 neurons receiving close appositions of GnIH neuronal fiber terminals is negatively correlated with the activity of male birds (Ubuka et al., 2012b) (Fig. 1). Ubuka et al. (2014) have further found GnIH inhibits aggressive behavior in male quail. Thus, it now appears that GnIH inhibits not only reproduction but also sexual and aggressive behaviors in birds (for reviews, see Kriegsfeld et al., 2015; Tsutsui et al., 2013c; Ubuka et al., 2013; Tsutsui and Ubuka, 2016) (Fig. 1).

In mammals, Johnson et al. (2007) also reported that ICV administration of GnIH inhibits male sexual behavior in rats. ICV administration of GnIH reduces sexual motivation and vaginal scent marking, but not lordosis behavior in female hamsters (PiekarSKI et al., 2013). GnIH administration alters fos expression in key neural loci including the medial POA, medial amygdala and bed nucleus of the stria terminals implicated in female sexual behavior (PiekarSKI et al., 2013). These mammalian findings indicate that GnIH is an important key neurohormone of female proceptive sexual behavior and motivation (Fig. 1). Accordingly, GnIH acts to regulate not only the HPG axis but also socially motivated behavior in mammals, as in birds.

3.2.3. Central action of hypothalamic GnIH on neurosteroid biosynthesis

The interaction of neuropeptides and neurosteroids may be important for the regulation of brain functions (for a review, see Do-Rego
et al., 2009). Ubuka et al. (2014) found that GnIH stimulates the activity of cytochrome P450 aromatase (P450arom) and increases neuroestrogen synthesis in the quail brain (Ubuka et al., 2014) (Fig. 1). Importantly, the action of GnIH on the stimulation of neuroestrogen synthesis changes the expression of aggressive behavior in quail (Ubuka et al., 2014) (Fig. 1), providing a new concept that GnIH modifies the neurosteroid milieu in the brain to regulate aggressive behavior.

It is known that male quail actively fight with intense aggressiveness, unlike female quails (Mills et al., 1997; Selinger and Bermant, 1967). Aggressive behavior depends on testicular androgen in male quail (Mills et al., 1997; Selinger and Bermant, 1967; Tsutsui and Ishii, 1981). It is also known that aggression in male birds is activated by aromatizable androgens, such as testosterone (T) and androstenedione (AD), but not by non-aromatizable androgens, such as dihydrotestosterone (DHT), and that administration of P450arom inhibitors block T-induced aggression in birds (Schlinger and Callard, 1990; Tsutsui and Ishii, 1981). Based on these findings, the action of testicular androgen on aggressive behavior requires its aromatization into estrogen (neuroestrogen) in the brain (Balthazart et al., 2009, 2011; Yahr, 1979).

Importantly, GnIH neurons project to the ME and other brain areas, such as the POA (Tsutsui et al., 2000; Ubuka et al., 2003; Ukena et al., 2003b) and the periaqueductual central gray (PAG;Ubuka et al., 2008) that express GnIH receptor in birds (Schlinger and Callard, 1990; Tsutsui and Ishii, 1981). Because GnIH decreases aggressive behavior in male birds as described above (Ubuka et al., 2012b, 2014), Tsutsui’s group hypothesized that GnIH may decrease aggressive behavior by regulating P450arom activity and neuroestrogen synthesis in the brain (Fig. 1). Ubuka et al. (2014) found that abundant GnIH-ir neuronal fibers are in the vicinity of P450arom-ir cells in the POA and GnIH receptor is expressed in P450arom-ir cells in the POA. Ubuka et al. (2014) further showed that GnIH stimulates P450arom activity and increases neuroestrogen synthesis in the POA via GnIH receptor (Ubuka et al., 2014) (Fig. 1). This is the first evidence that GnIH, a hypothalamic neuropeptide, decreases aggressive behavior by stimulating neuroestrogen synthesis in the brain. Ubuka et al. (2014) further found that central administration of estradiol-17β (E2) at higher doses decreases aggressive behavior unlike E2 at lower doses (Ubuka et al., 2014). Thus, the action of neuroestrogen is essential for the expression of aggressive behavior, but higher concentrations of neuroestrogen in the brain decrease aggressive behavior. It is therefore considered that GnIH decreases aggressive behavior by activating P450arom and increasing neuroestrogen synthesis in the brain beyond its optimum concentration for the expression of aggressive behavior of male birds (Ubuka et al., 2014) (Fig. 1).

Findings of Balthazart’s group indicate that P450arom activity in the hypothalamus of male quail is rapidly down-regulated by phosphorylation (Balthazart et al., 2001a, 2001b; 2003, 2006; Charlier et al., 2011). It is possible that GnIH activates P450arom by dephosphorylation of phosphorylated P450arom. Importantly, Ubuka et al. (2014) showed that ICV administration of GnIH reduces phosphorylated P450arom in the POA in a short term and that the action of GnIH on neuroestrogen synthesis in the POA is abolished by administration of RF9, a potent antagonist of GnIH receptor (Pineda et al., 2010; Simonin et al., 2006) or fadrozole, an inhibitor of P450arom (Steele et al., 1987; Wade et al., 1994). These findings indicate that GnIH stimulates neuroestrogen synthesis in the POA by activating P450arom through dephosphorylation after binding to GnIH receptor in P450arom cells (Fig. 1).

3.3. Regulatory mechanisms of GnIH biosynthesis in the brain

3.3.1. Melatonin action on GnIH biosynthesis in the brain

Reproductive activity depends on the annual cycle of changes in the nocturnal secretion of melatonin in photoperiodic mammals (Bronson, 1989). Melatonin also contributes to the regulation of seasonal processes including gonadotropin secretion and gonadal activity in birds (Ohta et al., 1989; Guyomarc'h et al., 2001; Rozenboim et al., 2002; Greives et al., 2012), despite the accepted dogma that birds do not use seasonal changes in melatonin secretion (Wilson, 1991; Juss et al., 1993). Based on these background, Tsutsui’s group investigated the action of melatonin on the regulation of GnIH expression in quail, a highly photoperiodic avian species. Ubuka et al. (2005) first found that melatonin removal by pinealectomy, combined with orbital enucleation (Px plus Ex), decreases the expressions of GnIH mRNA and GnIH peptide in the brain of quail (Ubuka et al., 2005). By contrast, melatonin administration increases the expressions of GnIH mRNA and GnIH peptide in the quail brain (Ubuka et al., 2005). Importantly, Mel1α, a melatonin receptor subtype, is expressed in GnIH neurons in quail (Ubuka et al., 2005). Thus, melatonin acts directly on GnIH neurons via Mel1α to induce GnIH expression in this avian species (Fig. 1).

Chowdhury et al. (2010) further found that melatonin increases not only GnIH expression but also GnIH release in quail (Fig. 1). Interestingly, GnIH release is controlled photoperiodically in quail with diurnal changes that are negatively correlated with plasma LH concentration (Chowdhury et al., 2010). Thus, it appears that melatonin derived from the pineal gland and eyes acts directly on GnIH neurons via Mel1α to induce GnIH expression and release in birds (Chowdhury et al., 2010; Ubuka et al., 2005; Tsutsui et al., 2013b,c) (Fig. 1). There are also reports showing that GnIH expressed in the gonads also responds directly to melatonin in a seasonal manner in songbirds (McGuire and Bentley, 2010; McGuire et al., 2011, 2013).

In contrast to birds, melatonin suppresses GnIH expression in Syrian and Siberian hamsters, highly photoperiodic mammals (Revel et al., 2008; Mason et al., 2010; Ubuka et al., 2012a) (Fig. 2). GnIH expression is reduced in sexually quiescent hamsters exposed to short day (SD) photoperiods, compared to sexually active hamsters under long day (LD) photoperiods. These photoperiodic changes in GnIH expression are abolished in Px hamsters and melatonin injections to LD hamsters decrease GnIH expression to SD level (Revel et al., 2008; Ubuka et al., 2012a). Similar seasonal patterns of GnIH expression have also been found in European (Simonneaux et al., 2013) and Turkish (Piekarski et al., 2014) hamsters as well as the semi-desert rodent, Jerboa (Janati et al., 2013). In addition, there are also reports showing that GnIH expression is regulated by melatonin in sheep (Dardente et al., 2008; Smith et al., 2008) and rats (Gingerich et al., 2009). Accordingly, GnIH expression is photoperiodically modulated via a melatonin-dependent process in mammals and birds although there is some species difference (for reviews, see Tsutsui, 2009, 2016; Tsutsui et al., 2010a, 2010b, 2012, 2013c, 2015; Kriegsfeld et al., 2015) (Fig. 1).

In Syrian, Siberian, European, Turkish hamsters and the semi-desert rodent Jerboa, GnIH is more highly expressed in the breeding season than the non-breeding season possibly regulated by melatonin. It was experimentally demonstrated in Siberian hamster that GnIH inhibits LH release in the breeding season (LD) but stimulates LH release in the non-breeding season (SD) (Ubuka et al., 2012a). It is therefore possible that GnIH fine-tunes reproductive activities according to environmental conditions across breeding and non-breeding seasons in these seasonally breeding rodents. It was also shown that the electrophysiological effect of GnIH on a small population of GnRH neurons was stimulatory in mice (Do-Rego et al., 2009). These results indicate the existence of molecular mechanisms that underlies the stimulatory effect of GnIH on reproduction. One possibility is that GnIH stimulates the electrophysiological activity of GnRH neurons by increasing neuroestrogen concentration in the hypothalamus (Ubuka et al., 2014; Ubuka and Parhar, 2018). Other possibilities are that GnIH receptors may form
Birds and sheep, which have broader breeding seasons in seasonally breeding rodents. Rodents may be more according to environmental conditions across breeding and non-breeders. We speculate that GnIH expression is opposite in seasonally breeding rodents that are LD breeders and sheep are SD breeders. However, the direction of GnIH expression in sheep and seasonally breeding rodents. The direction of GnIH expression induced by hypothyroidism may delay pubertal onset.

In summary, melatonin or SD stimulates GnIH expression and/or release in birds. On the other hand, melatonin or SD inhibits GnIH expression in sheep and seasonally breeding rodents. The direction of GnIH expression is logical in birds and sheep because birds are LD breeders and sheep are SD breeders. However, the direction of GnIH expression is opposite in seasonally breeding rodents that are LD breeders. We speculate that GnIH fine-tunes reproductive activities according to environmental conditions across breeding and non-breeding seasons in seasonally breeding rodents. Rodents may be more vulnerable to the change in environmental conditions compared to birds and sheep which have broader field of activities.

3.3.2. Glucocorticoid action on GnIH biosynthesis in the brain

Stress reduces reproduction across vertebrates (Chand and Lovejoy, 2011). Kirby et al. (2009) showed that immobilization stress increases GnIH expression in the dorsomedial hypothalamic area (DMH) associated with inhibition of the HPG axis in rats (Fig. 1). Kirby et al. (2009) also found that adrenalectomy abolishes stress-induced increase in GnIH expression (Kirby et al., 2009). Adrenal glucocorticoids may act directly on GnIH neurons to increase GnIH expression because GnIH neurons express glucocorticoid receptor (GR) (Kirby et al., 2009) (Fig. 1). These findings indicate that GnIH is an important integrator of stress-induced suppression of reproductive function in mammals (Kirby et al., 2009).

Son et al. (2014) found that in quail GR is expressed in GnIH neurons in the PVN and the treatment with corticosterone (CORT; the major glucocorticoid in birds and rodents) increases GnIH expression, indicating that glucocorticoids can directly regulate GnIH expression (Fig. 2). Furthermore, Son et al. (2014) clarified the mechanism of activation of GnIH expression by CORT using a GnIH-expressing neuronal cell line, RHypoE-23, derived from rat hypothalamus. Importantly, GR is expressed in RHypoE-23 cells and CORT treatment also increases GnIH expression in these cells (Son et al., 2014). It thus appears that stress reduces gonadotropin secretion through the increase in GnIH expression in mammals and birds.

These stimulatory effects of glucocorticoids on GnIH expression may be functionally significant especially during metabolic stress because food restriction up-regulated GnIH mRNA expression and significantly inhibited ovarian development and follicular growth in sheep (León et al., 2014). Short-term fasting and high-fat diet were less effective in decreasing LH secretion in GnIH receptor knockout mice (León et al., 2014).

3.3.3. Norepinephrine action on GnIH biosynthesis in the brain

Not only photoperiod and stress but also social environment may influence GnIH expression (Fig. 1). Calisi et al. (2011) reported that European starlings with nest boxes have fewer numbers of GnIH cells than those without nest boxes. In contrast, GnRH content does not vary with nest box ownership (Calisi et al., 2011). These findings suggest that GnIH is involved in reproductive function in response to social environment.

Reproductive physiology and behavior are variable, both within and between individuals. There are several reports showing that the presence of a female bird rapidly decreases plasma T concentrations in male quail (Delville et al., 1984; Cornil et al., 2009). Based on these findings, Tsutsui's group investigated the neurochemical mechanism that alters reproductive physiology by social interaction (Fig. 1). Tobari et al. (2014) first showed that the release of norepinephrine (NE) increases rapidly in the PVN of male quail when viewing a conspecific female (Fig. 1). GnIH expression also increases in the PVN of male quail, with associated decreases in plasma LH concentrations, when males view a female (Tobari et al., 2014) (Fig. 1). Subsequently, Tobari et al. (2014) found that NE application to male quail stimulates GnIH release via a2A-adrenergic receptor. Accordingly, it is considered that female presence increases NE release in the PVN and stimulates GnIH release, resulting in the decrease in circulating LH and T levels in males (Tobari et al., 2014) (Fig. 1).

4. Perspective of GnIH research on neuroendocrine regulation of reproduction

4.1. Role of hypothalamic GnIH in hypothyroidism-induced delayed puberty

Thyroid disorder is known to be associated with abnormal pubertal development. However, the mechanism of thyroid hormone (TH) action on pubertal onset still remains unclear, although interactions between the hypothalamo-pituitary-thyroid (HPT) and HPG axes have been suggested (Anasti et al., 1995; Niedziela and Korman, 2001; Dittricha et al., 2011) (Fig. 2). Some paper showed that the elevated TRH level of in hypothyroidism induces hyperprolactinemia and alters GnRH pulsatile secretion, which lead to delayed LH response, resulting in delayed puberty (Dittricha et al., 2011). Other papers reported that the increased TSH level activates gonadinal function by stimulating FSH receptor in gonads, because the structure of TSH and FSH receptors is similar (Anasti et al., 1995; Niedziela and Korman, 2001). Tsutsui's group challenged to demonstrate the hypothesis that TH-mediated HPG regulation may be initiated by the change in GnIH expression, which acts at the most upstream level of the HPG axis by inhibiting the activity of GnRH neurons to reduce gonadotropin secretion from gonadotropes (for reviews, see Tsutsui, 2009, 2016; Tsutsui et al., 2010a,
4.2. Mechanism of hypothalamic GnIH action on hypothyroidism-induced pubertal delay

To clarify the regulatory mechanism of GnIH action on hypothyroidism-induced pubertal delay, molecular studies were further conducted as follows. Firstly, it was found that GnIH neurons in the hypothalamus express both TH receptors (TRα and TRβ) (Kiyohara et al., 2017). Secondly, several putative TH-response elements (TREs) were found within 3 kb upstream region from the mouse GnIH open reading frame (Kiyohara et al., 2017). However, unexpectedly both TRs cannot directly bind to these TREs present in the GnIH promoter region in chromatin immunoprecipitation (ChIP) assays (Kiyohara et al., 2017), suggesting that TH (T3) may act via non-genomic action by membrane TRs. Importantly, H3 acetylation (H3Ac) status, which is correlated with gene activation, is increased in hypothryoid female mice compared to control mice (Kiyohara et al., 2017). Accordingly, thyroid status may regulate chromatin modification in the GnIH promoter region resulting in the change in GnIH gene expression. This is a novel function of GnIH as a mediator between the HPT and HPG axes (for a review, see Tsutsui et al., 2018) (Fig. 2).

4.3. Perspective of GnIH research on drug development

As described above significant decrease in GnIH neuronal function can cause central precocious puberty (Kiyohara et al., 2017; Tsutsui et al., 2018). We are further investigating if cessation or decrease in GnIH expression induces central precocity by precocious development of the gonads using GnIH-KO mice that were made by Tsutsui’s group. In addition, analyses of how failure in GnIH expression and function causes precocious puberty are in progress by identifying the target factors controlled by GnIH signal through knockdown of GnIH receptor gene in cells.

On the other hand, previous researches (Ubuka et al., 2014; Kiyohara et al., 2017; Tsutsui et al., 2018) suggest that significant acceleration in the function of GnIH neurons causes central reproductive dysfunction. We are currently investigating how excessive GnIH expression in the brain induces reproductive dysfunction using mice that overexpress GnIH by genetic manipulation.

A previous study has shown that GnIH receptor knockout mice do not have precocious or delayed puberty (León et al., 2014). However, metabolic challenges such as short-term fasting and high-fat diet were less effective in decreasing LH secretion in GnIH receptor knockout mice (León et al., 2014). These results suggest that GnIH receptor antagonists may be useful to treat fasting- and obesity-associated hypogonadotropism.

4.4. Development of a novel diagnostic method for reproductive dysfunction

Mutation in GnIH and GnIH receptor genes may be related to reproductive dysfunction in humans. We have collected DNA samples from 500 patients of central reproductive dysfunction due to failure in gonadotropin secretion protecting donor rights and in compliance with related laws. We have therefore planned to analyze mutations in human GnIH and GNIH RECEPTOR (GPR147, NPFFR1) genes in these DNA samples by CGH array and next generation sequencing. We are investigating the functions of mutants by reporter assay of cAMP responsive elements, intracellular Ca2+ assay, and binding assay based on functional analytic methods for mutants that our collaborator has developed (Fukami et al., 2006; Narumi et al., 2016). Analysis of mutations in GNIH and GNIH RECEPTOR (GPR147, NPFFR1) genes can become a novel diagnostic method for reproductive dysfunction if it will be revealed that in GNIH and GNIH RECEPTOR (GPR147, NPFFR1) genes are related to central reproductive dysfunction.

4.5. Development of novel diagnostic strategies for precocious puberty and reproductive dysfunction

It is possible that the decrease in GnIH function causes central precocious puberty and the increase in GnIH function induces central reproductive dysfunction. Therefore, researches are in progress to develop novel diagnostic strategies for precocious puberty and reproductive dysfunction.

First, GnIH agonist has a potential to be used as a medicine to treat central precocious puberty. We are developing GnIH agonists that are effective as a medicine for central precocious puberty. We have already synthesized human GnIH agonists considering their activity and structural stability and analyzing its inhibitory effect on gonadotropin secretion in mice.

On the other hand, GnIH antagonist has a potential to be used as a medicine for central reproductive dysfunction. We are developing GnIH antagonists like RP9, a potent antagonist of GnIH receptor (Pineda et al., 2010; Simonin et al., 2006) that are effective as a medicine for central reproductive dysfunction. We have synthesized a human GnIH antagonist considering its activity and structural stability and analyzing its stimulatory effect on gonadotropin secretion in mice.

5. Conclusions and future directions

GnIH is a newly discovered hypothalamic neuropeptide that inhibits gonadotropin synthesis and release in birds. GnIH studies in the past 19 years have demonstrated that GnIH is highly conserved among vertebrates from agnathans to humans, acting as a key neuropeptide inhibiting reproduction across vertebrates. GnIH inhibits gonadotropin synthesis and release through actions on gonadotropes and GnRH neurons via the GnIH receptor GPR147. Thus, GnIH has marked advanced the progress of reproductive neuroendocrinology.

Following the discovery of GnIH, kisspeptin was also discovered in mammals. GnIH and kisspeptin are new members of the RFamide peptide family. GnIH down-regulates and kisspeptin up-regulates the HPG axis, respectively. Thus, we now know that GnRH is not the single hypothalamic neuropeptide regulating reproduction. Importantly, GnIH neurons project not only to GnRH neurons but also to kisspeptin neurons that express GnIH receptor. Future studies are needed to reveal previously unknown interactions among GnIH, GnRH and kisspeptin.

Furthermore, it now appears that GnIH acts on the pituitary and the brain to regulate not only reproduction but also reproductive behavior in vertebrates. GnIH activates P450 arom to increase neuroestrogen synthesis in the brain. GnIH may also change the formation of other...
neurosteroids by activating or inactivating steroidogenic enzymes. Steroidogenic enzymes are also expressed in the pineal gland, an endocrine organ located close to the brain (Hatori et al., 2011; Haraguchi et al., 2012; Tsutsui et al., 2013a,b). As GnIH receptor is expressed in the pineal gland (Sato, M., Narihira, M., Ubbuka, T., Haraguchi, S., Tsutsui, K., unpublished observation), GnIH may regulate neurosteroidogenesis in the pineal gland as in the brain. Future studies are needed to develop the emerging concept that GnIH may modify the neurosteroid milieu in the brain and the pineal gland to regulate brain functions.

More recent studies have further indicated that GnIH is involved in puberty disorder induced by thyroid dysfunction. It is a novel function for GnIH in abnormal puberty mediating the interaction of the HPT-HPG axes. Cessation or decrease in GnIH expression may cause central reproductive dysfunction. Therefore, research is in progress to develop a novel diagnostic method for precocious puberty and reproductive dysfunction by analyzing GnIH and GnIH receptor genes. Furthermore, GnIH agonist has a potential to be used as a medicine for central precocious puberty. On the other hand, signification in the function of GnIH neurons may cause central reproductive dysfunction. Therefore, research is in progress to develop novel diagnostic strategies for precocious puberty and reproductively.

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Declaration of competing interest
The authors have nothing to disclose.

The neuroendocrine integration of environmental factors, such as photoperiod, stress and social interaction, and internal factors, such as GnIH, melatonin, glucocorticoid and norepinephrine (NE), is important for the control of reproduction and reproductive behaviors. Melatonin increases GnIH expression in quail and rats, whereas melatonin decreases GnIH expression in hamsters and sheep. Stress increases GnIH expression mediated by the actions of glucocorticoids in birds and mammals. Thus, GnIH is an internal mediator of stress-induced reproductive dysfunction. The social environment also changes GnIH expression and release mediated by the action of NE.

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References
Abel, P., Ritters, L.V., Balthazart, J., 2001. Prosopotic amastopin cellus and the mesencephalic central gray in the male Japanese quail (Coturnix japonica). Horm. Behav. 40, 389–393.
Amano, M., Moriyama, S., Iigo, M., Kitamura, S., Amiya, N., Yamamori, K., Ukena, K., Tsutsui, K., 2006. Novel fish hypothalamic neuropeptides stimulate the release of gonadotrophins and growth hormone from the pituitary of the oviparous sockeye salmon, Oncorhynchus gorbuscha. J. Endocrinol. 188, 417–423.
Anast, J.N., Flack, M.R., Froehlich, J., Nelson, L.M., Niula, B.C., 1995. A potential novel mechanism for precocious puberty in juvenile hypothalamiophyidism. J. Clin. Endocrinol. 80, 276–277.
Anjani, S., Krishna, A., Tsutsui, K., 2014. Inhibitory roles of the mammalian GnIH ortholog RRF3 in testicular activities in adult mice. J. Endocrinol. 223, 79–91.
Balthazart, J., Sirlenmont, C., 1990. Androgen and estrogen action in the preoptic area and activation of copulatory behavior in quail. Physiol. Behav. 48, 599–609.
Balthazart, J., Bailleul, M., Ball, G.F., 2001a. Rapid and reversible inhibition of brain aromatase activity. J. Neuroendocrinol. 13, 63–73.
Balthazart, J., Bailleul, M., Ball, G.F., 2001b. Phosphorylation processes mediate rapid changes of brain aromatase activity. J. Steroid Biochem. Mol. Biol. 79, 261–277.
Balthazart, J., Bailleul, M., Charlier, T.D., Ball, G.F., 2003. Calcium-dependent phosphorylation processes control brain aromatase activity in quail. Eur. J. Neurosci. 17, 1591–1606.
Balthazart, J., Bailleul, M., Ball, G.F., 2006. Rapid control of brain aromatase activity by g lutamateric inputs. Endocrinology 147, 359–366.
Balthazart, J., Cornell, C.A., Charlier, T.D., Taziaux, M., Ball, G.F., 2009. Estradiol, a key endocrine signal in the sexual differentiation and activation of reproductive behavior in quail. J. Exp. Zool. A Ecol. Genet. Physiol. 311, 323–345.
Balthazart, J., Charlier, T.D., Cornell, C.A., Dickens, M.J., Harada, N., Konkle, A.T., Voigt, C., Ball, G.F., 2011. Sex differences in brain aromatase activity: genomic and non-genomic controls. Front. Endocrinol. 2, 34.
Bentley, G.E., Perfetto, N., Ukena, K., Tsutsui, K., Wingfield, J.C., 2003. Gonadotropin-inhibitory peptide in song sparrows (Melospiza melodia) in different reproductive conditions, and in house sparrows (Passer domesticus) relative to chicken-gonado-
tropin-releasing hormone. J. Neuroendocrinol. 15, 794–802.
Bentley, G.E., Jensen, J.P., Kaar, G.J., Wacker, D.W., Tsutsui, K., Wingfield, J.C., 2006. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Horm. Behav. 49, 550–555.
Bentley, G.E., Ubeuki, T., McGuire, N.L., Chowdhury, V.S., Morita, Y., Yano, T., Hasunuma, I., Bailleul, M., Wingfield, J.C., Tsutsui, K., 2008. Gonadotropin-inhibitory hormone and its receptor in the avian reproductive system. Gen. Comp. Endocrinol. 156, 34–43.
Bolder, J., Eizmann, F., Bowers, C.Y., Schally, A.V., 1969. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyro-glu-thalamyl-histidyl-proline amide. Biochem. Biophys. Res. Commun. 37, 705–710.
Bonin, A.J., Jones, K.A., Adham, N., Foray, C., Arinumshyn, R., Durkin, M.M., Smith, K.E., Tamm, J.A., Roteju, L.W., Lakhani, P.P., Raddatz, R., Yao, W.J., Ogazoalex, K.L., Boyle, N., Kouranova, E.V., Quan, Y., Vaysse, P.J., Weizel, J.M., Branchek, T.A., Geral, C., Borowsky, B., 2000. Identification and characterization of two G protein-coupled receptors for neuropeptide FF. J. Biol. Chem. 275, 39324–39331.
Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., Guillemin, R., 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179, 77–79.
Bronson, F.H., 1989. Mammalian Reproductive Biology. University of Chicago Press, Chicago.
Burgus, R., Dunn, T.F., Desiderio, D., Guillemin, R., 1969. Molecular structure of the hypothalamic hypophysiotrophic TRF factor of ovine origin: mass spectrometry demonstration of the PCA-His-Pro-NH2 sequence. C. R. Acad. Sci. 269, 1870–1873.
Burgus, R., Butcher, M., Amoss, M., Ling, N., Monahan, M., Rivier, J., Fellows, R., Blackbird, B., Vale, W., Guillemin, R., 1972. Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF) (LH-hypophalmus-LRF-gas chromatography-mass spectrometry-decapeptide-Edman degradation). Proc. Natl. Acad. Sci. U.S.A. 69, 278–282.
Calisi, R.M., Díaz-Muñoz, S.L., Wingfield, J.C., 2007. Social and breeding status are associated with the expression of GnIH. Gene Brain Behav. 10, 557–564.
Chand, D., Lovejoy, D.A., 2011. Stress and reproduction: controversies and challenges. Gen. Comp. Endocrinol. 171, 253–262.
Charlier, T.D., Harada, N., Balthazart, J., Cornell, C.A., 2011. Human and quail aromatase activity is rapidly and reversibly inhibited by phosphorylating conditions.

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peptide-3 suppresses gonadotropin-induced progestrone production in human granulose cells. Endocrinology 153, 3435–3445.

Osugi, T., Ukena, K., Sower, S.A., Kwasuch, H., Tsutsui, K., 2006. Evolutionary origin and divergence of PFRFamide peptides and LPXRFamide peptides in the RFamide peptide family. Insights from novel lamprey RFamide peptides. FEBs J. 273, 1731–1743.

Osugi, T., Uchiha, K., Nozaki, M., Tsutsui, K., 2011. Characterization of novel RFamide peptides in the central nervous system of the brown hagfish: isolation, localization, and functional analysis. Endocrinology 152, 4522–4524.

Osugi, T., Dauks, D., Garda, K., Ukena, T., Kostig, T., Nozaki, M., Sower, S.A., Tsutsui, K., 2012. Evolutionary origin of the structure and function of gonadotropin-inhibitory hormone: insights from lampreys. Endocrinology 153, 2362–2374.

Osugi, T., Okamura, T., Son, Y.L., Oishi, M., Ubuka, T., Henmi, Y., Tsutsui, K., 2014. Evolutionary origin of GnIH and NPF in chordates: insights from novel amphioxus RFamide peptides. PLoS One 9, e160962.

Osugi, T., Ukena, K., Tsutsui, K., 2015. An evolutionary scenario for GnIH in chordates. J. Neuroendocrinol. 27, 556–560.

Panizca, G.C., Gigliotti-Panizca, C., Balthazart, J., 1996. The sexually dimorphic preoptic nucleus of quail: a key area mediating steroid action on male sexual behavior. Front. Neuroendocrinol. 17, 51–125.

Piekarski, D.J., Zhao, S., Jennings, K.J., Iwasa, T., Legan, S.J., Mikkelsen, J.D., Tsutsui, K., Pankazza, G.C., Viglietti-Panizca, C., Balthazart, J., 1996. The sexually dimorphic medial preoptic area of the chicken brain: a novel medium for seasonal breeding in the sheep. Endocrinology 149, 5770–5782.

Son, Y.L., Ukena, T., Millar, R.P., Kanasaki, H., Tsutsui, K., 2012. Gonadotropin-inhibitory hormone inhibits GnIH-induced gonadotropin subunit gene transcriptions by inhibiting AC/AMP-PKA-dependent ERK pathway in L27 cells. Endocrinology 153, 2323–2343.

Son, Y.L., Ukena, T., Narihira, M., Fukuda, Y., Hasunuma, I., Yamamoto, K., Belsham, D.D., Tsutsui, K., 2014. Molecular basis for the activation of gonadotropin-inhibitory hormone gene expression by corticosterone. Endocrinology 155, 1815–1826.

Son, Y.L., Ukena, T., Soga, T., Yamamoto, K., Bentley, G.E., Tsutsui, K., 2016. Inhibitory action of gonadotropin-inhibitory hormone on the signaling pathways induced by kisspeptin and vasotocin in the pituitary gland. PLoS One 11, e0157712.

Steele, R.E., Mellor, L.B., Sawyer, W.K., Vaswani, J.M., Browne, L.J., 1987. In vitro and in vivo studies demonstrating potent and selective estrogen inhibition with the non-steroidal antiestrogenic GHS 169/49A. Steroids 50, 147–161.

Tobari, Y., Okuma, G., Nishikawa, K., Uchida, K., Qin, X., Lu, D., Yi, S., Xie, R., Liu, X., Zhang, Y., Lin, H., 2013. Evidences of gonadotropin-inhibitory hormone from amphibian brain. Eur. J. Biochem. 269, 6000–6010.

Tobari, Y., Iijima, N., Tobiume, K., Inoue, K., Tsutsui, K., Ozawa, H., 2010. Identification of gonadotropin-inhibitory hormone in the sea urchin (Strongylocentrotus purpuratus): peptide sequence, CDNA cloning and brain distribution. Peptides 31, 616–626.

Tobari, Y., Son, Y.L., Ukena, T., Hasegawa, Y., Tsutsui, K., 2014. A new pathway mediating social effects on the endocrine system: female presence acting via nor-epinephrine release stimulates gonadotropin-inhibitory hormone in the parasagittal nucleus and suppresses luteinizing hormone in quail. Neurosci. 34, 9433–9441.

Tsutsui, K., 2009. Review: a new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH); biosynthesis, mode of action and functional significance. Prog. Neurobiol. 88, 76–88.

Tsutsui, K., 2016. Review: how to contribute to the progress of neuroendocrinology: new insights from discovering novel neuropeptides and neurosteroids regulating pituitary and brain functions. Gen. Comp. Endocrinol. 227, 3–15.

Tsutsui, K., Shihi, S., 1981. On the reproductive behavior of adult male Japanese quail. Gen. Comp. Endocrinol. 44, 480–486.

Tsutsui, K., Ukena, K., 2006. Review: hypothalamic LPXRFamide-amide peptides in vertebrates: identification, localization and hypophysiotropic activity. Peptides 27, 1121–1129.

Tsutsui, K., Ubuka, T., 2012. Gonadotropin-inhibitory hormone. In: Kastin, A.J., Vaudry, H. (Eds.), Handbook of Biologically Active Peptides. Academic Press, London, pp. 802–811.

Tsutsui, K., Ubuka, T., 2016. Review: GnIH control of feeding and reproductive behaviors. Mol. Cellular Endocrinol. 7 art. 1359–1359.

Tsutsui, K., Saigoh, E., Ukena, K., Teranishi, H., Fujisawa, Y., Kikuchi, M., Shihi, S., Sharp, P.J., 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 275, 661–667.

Tsutsui, K., Ukena, T., Yin, H., Osugi, T., Ukena, K., Bentley, G.E., Ciccone, N., Inoue, K., Chowdhury, V.S., Sharp, J.P., Wingfield, J.C., 2006. Review: mode of action and functional significance of avian gonadotropin-inhibitory hormone (GnIH). J. Exp. Zool. 305A, 801–806.

Tsutsui, K., Bentley, G.E., Ubuka, T., Saigoh, E., Yin, H., Osugi, T., Inoue, K., Chowdhury, V.S., Ukena, K., Ciccone, N., Sharp, J.P., Wingfield, J.C., 2007. Review: the genetic and comparative biology of gonadotropin-inhibitory hormone (GnIH). Gen. Comp. Endocrinol. 153, 365–370.

Tsutsui, K., Bentley, G.E., Bedecarrats, G.T., Osugi, T., Ukena, T., Tsutsui, K., Tiefel, J.F., 2010a. Review: gonadotropin-inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. Front. Neuroendocrinol. 31, 284–295.

Tsutsui, K., Bentley, O.E., Kriegsfeld, L.J., Osugi, T., Seong, J.Y., Vaudry, H., 2010b. Novel discovery and evolutionary history of gonadotropin-inhibitory hormone and kisspeptin: new key neuropeptides controlling reproduction. J. Neuroendocrinol. 22, 716–727.

Tsutsui, K., Ubuka, T., Bentley, G.E., Kriegsfeld, L.J., 2012. Review: gonadotropin-inhibitory hormone (GnIH): discovery, progress and prospect. Gen. Comp. Endocrinol. 177, 305–314.

Tsutsui, K., Haraguchi, S., Fukuda, Y., Vaudry, H., 2013a. Review: brain and pineal 7α-hydroxyprogrenene stimulating locomotor activity: identification, mode of action and regulation of biosynthesis. Front. Neuroendocrinol. 34, 179–189.

Tsutsui, K., Haraguchi, S., Hatori, M., Hirota, T., Fukuda, Y., 2013b. Review: biosynthesis and biological actions of pineal neurosteroids in domestic birds. Neuroendocrinology 98, 97–105.
