

# The control of reproductive physiology and behavior by gonadotropin-inhibitory hormone

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**Synopsis** Gonadotropin-releasing hormone (GnRH) controls the reproductive physiology and behavior of vertebrates by stimulating synthesis and release of gonadotropin from the pituitary gland. In 2000, another hypothalamic neuropeptide, gonadotropin-inhibitory hormone (GnIH), was discovered in quail and found to be an inhibiting factor for gonadotropin release. GnIH homologs are present in the brains of vertebrates, including birds, mammals, amphibians, and fish. These peptides, categorized as RF amide-related peptides (RFRPs), possess a characteristic LPXRF-amide (X=L or Q) motif at their C-termini. GnIH/RFRP precursor mRNA encodes a polypeptide that is possibly cleaved into three mature peptides in birds and two in mammals. The names of these peptides are GnIH, GnIH-related peptide-1 (GnIH-RP-1) and GnIH-RP-2 in birds, and RFRP-1 and RFRP-3 in mammals. GnIH/RFRP is synthesized in neurons of the paraventricular nucleus of the hypothalamus in birds and the dorsomedial hypothalamic area in mammals. GnIH neurons project to the median eminence, thus providing a functional neuroanatomical infrastructure to regulate anterior pituitary function. In quail, GnIH inhibits gonadal activity by decreasing synthesis and release of gonadotropin. The widespread distribution of GnIH/RFRP immunoreactive fibers in all animals tested suggests various actions within the brain. In accordance, GnIH/RFRP receptor mRNA is also expressed widely in the brain and the pituitary. GnIH/RFRP immunoreactive axon terminals are in probable contact with GnRH neurons in birds and mammals, and we recently demonstrated expression of GnIH receptor mRNA in GnRH-I and GnRH-II neurons in European starlings. Thus, GnIH/RFRP may also inhibit gonadotropin synthesis and release by inhibiting GnRH neurons in addition to having direct actions on the pituitary gland. Intracerebroventricular administration of GnIH/RFRP further inhibits reproductive behaviors in songbirds and rodents, possibly via direct actions on the GnRH system. The expression of GnIH/RFRP is regulated by melatonin which is an internal indicator of day length in vertebrates. Stress stimuli also regulate the expression of GnIH/RFRP in songbirds and rodents. Accordingly, GnIH/RFRP may serve as a transducer of environmental information and social interactions into endogenous physiology and behavior of the animal. Recently, it was shown that GnIH/RFRP and its receptor are also expressed in the gonads of birds, rodents and primates. In sum, the existing data suggest that GnIH/RFRP is an important mediator of reproductive function acting at the level of the brain, pituitary, and the gonad in birds and mammals.

## Introduction

The decapeptide gonadotropin-releasing hormone (GnRH) is the primary factor responsible for the hypothalamic control of gonadotropin secretion. GnRH was originally isolated from mammals (Matsuo et al. 1971; Burgus et al. 1972) and subsequently from birds (King and Millar 1982; Miyamoto et al. 1982, 1984) and other vertebrates (Millar 2005). Gonadal sex steroids and inhibin can also modulate secretion of gonadotropin via feedback from the gonads, but a hypothalamic inhibitor of gonadotropin secretion was, until 2000, unknown in vertebrates. In 2000,

a previously unidentified hypothalamic neuropeptide was reported to inhibit release of gonadotropin from the cultured quail anterior pituitary and it was named gonadotropin-inhibitory hormone (GnIH; Tsutsui et al. 2000). GnIH immunoreactive neurons are located in the paraventricular nucleus (PVN) in quail (Ubuka et al. 2003; Ukena et al. 2003). These neurons project to the median eminence, thus providing a functional neuroanatomical infrastructure that regulates anterior pituitary function. A cDNA encoding GnIH precursor polypeptide was cloned from the brains of quail (*Coturnix japonica*), white-crowned sparrows

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<i>Coturnix japonica</i>	MEIISTQKFILLTLATVAFLTPHGACLEDELMKSSLESRED-DDDKYYETK
<i>Gallus gallus</i>	MEIISTQKFILLTLATVAFLTPHGMCLDELMKSSLESREE-DDDKYYEIK
<i>Sturnus vulgaris</i>	MKVILTQKFIIFALAAVLLTSNSMCLNEPMKPRQLQSRED-NDDKYEIK
<i>Zonotrichia leucophrys</i>	MKVIISTKFIIFALATVVFLLTSNSMCLNEAMKSRQLQSRED-NDDKYEIK
<i>Pan troglodytes</i>	MEIISSKLVLLTLATSSLLTSNIFCADELMMSNLHSKE--NYDKYSEPR
<i>Homo sapiens</i>	MEIISSKLVLLTLATSSLLTSNIFCADELVMNSNLHSKE--NYDKYSEPR
<i>Equus caballus</i>	MDFISAKRLILLTLATSSLLTSNIFCGDELMMSNLYSKD--NYDKYSKPR
<i>Bos taurus</i>	MEIISLKRIFLLMLATSSLLTSNIFCTDESMPNLYSKK--NYDKYSEPR
<i>Mus musculus</i>	MEIISLKRIFLLTVATSSFLTSNTFCTDEFMMPHFSKE--GDGKYSQLR
<i>Rattus norvegicus</i>	MEIISSKRFILLTLATSSFLTSNTLCSDELMMPHFSKE--GYGKYQLR
<i>Carassius auratus</i>	MSYFTLVFLALGLTSSFMLREVTALRWPLPDDSDPDRFTWGQFLENAQEI
<i>Danio rerio</i>	MSYFALLSLALGILSSFMLEVTALRPLSGERDLNGFTWGQFSENAQEI
	<b>GnIH-RP-1/RFRP-1</b>
<i>Coturnix japonica</i>	DSILEEKQRSLNFEEMKDWGSKNFMKVNTPTVNVKVPNSVANLPLRFGRSN
<i>Gallus gallus</i>	DSILEEEQRSLNFEEMKDWGSKNFKLVNTPTVNVKVPNSVANLPLRFGRSN
<i>Sturnus vulgaris</i>	DNILEEKQRSLNFDDEMEDWGSKDIKMNPFVTSKMPNSVANLPLRFGRNY
<i>Zonotrichia leucophrys</i>	DNILEEKQRSLNFEEMEDWGSKDIKMNPFVTSKMPNSVANLPLRFGRNY
<i>Pan troglodytes</i>	GYPKGE--RSLNFEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRFGRNV
<i>Homo sapiens</i>	GYPKGE--RSLNFEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRFGRNV
<i>Equus caballus</i>	GDPKGEKERSLNFEEDKDWGPKNVTIKIT--PAVNKMPHSAANLPLRFGRMT
<i>Bos taurus</i>	GDLGWEKERSLTFEEVKDWAPK--IKMNKPVVNMPPSAANLPLRFGRNM
<i>Mus musculus</i>	GIPKGEKERSVTFQELKDWGAKNVIKMSAPANKVPHSAANLPLRFGRTI
<i>Rattus norvegicus</i>	GIPKGVKERSVTFQELKDWGAKNVIKMSAPANKVPHSAANLPLRFGRNI
<i>Carassius auratus</i>	PRSLEIEDFTLNVAPTSGRVSSPTILRLHPKIKPHTLHANLPLRFGRDT
<i>Danio rerio</i>	PRSLEIQDFTLNVAPTSGLGASSPTILRLHPKIKPAHLHANLPLRFGRDA
	<b>GnIH/RFRP-2</b>
<i>Coturnix japonica</i>	PE---ERSIKPSAYLPLRFGRFAGFESLSRRAPN---LSNRSGRSPLARSS
<i>Gallus gallus</i>	PE---ERSIRPSAYLPLRFGRFAGFESLSRRAPN---LSYRLGRSPLARSS
<i>Sturnus vulgaris</i>	PE---ERSIKPFANLPLRFGRFAGFENIPNHAPK---VLHRLGRSPLLKGS
<i>Zonotrichia leucophrys</i>	PE---ERSIKPFSNLPLRFGRFAGFENTPNHSPR---VSHRLERSPLVKGS
<i>Pan troglodytes</i>	QE---ERSAGATANLPLRSGRNMEVSLVRRVVPN---LPQRFGRTTTAKSV
<i>Homo sapiens</i>	QE---ERSAGATANLPLRSGRNMEVSLVRRVVPN---LPQRFGRTTTAKSV
<i>Equus caballus</i>	EE---ERGPAMANLPLRFGRNTEESISRRIPN---LPQRFGR--TTAKSV
<i>Bos taurus</i>	EE---ERSTRAMAHPLPLRLGKNREDSLSRWVVPN---LPQRFGRTTTAKSI
<i>Mus musculus</i>	DE---KRSPAARVNMEAG-----TRSHFPS---LPQRFGR--TTAR-S
<i>Rattus norvegicus</i>	ED---RRSPRARANMEAG-----TMSHFPS---LPQRFGR--TTARI-S
<i>Carassius auratus</i>	EN--TPRERAKSNINLPLRFGRSCTMCARSCTGLSATLPLRFGRRNIFPLD
<i>Danio rerio</i>	QPGTGDRAPKSTINLPLRFGRSCTMCARSCTGPSATLPLRFGRRNIFALD
	<b>GnIH-RP-3</b>
<i>Coturnix japonica</i>	IQSLLNLPQRFGKSVPIISLSQGVQSESEPGM-----
<i>Gallus gallus</i>	IQSLLNLPQRFGKSVPIINLSQGVQSESEPGM-----
<i>Sturnus vulgaris</i>	SQSLLNLPQRFGKSLVFNLPQDIQSESEPGI-----
<i>Zonotrichia leucophrys</i>	SQSLLNLPQRFGKSLAVNLPQDIQEFEPGI-----
<i>Pan troglodytes</i>	CRMLSDLCQGSMRSPCANDLFYSMTQHQEIQNPQDQKQSRRLLFKKIDDA
<i>Homo sapiens</i>	CRMLSDLCQGSMSHSPCANDLFYSMTQHQEIQNPQDQKQSRRLLFKKIDDA
<i>Equus caballus</i>	AKTLDLQGSMSHSPANELLYSMTCPQEQEIQNSHQHPRRGLGFQKRDDT
<i>Bos taurus</i>	TKTLSNLLQGSMSHSPSTNGLLYSMACQPQEQEIQNPQKQNLRRRGFQKIDDA
<i>Mus musculus</i>	PKTPADLPQKPLHSLGSSELYVMICQHQEQEIQSPGGKTRRGAFFVETDDA
<i>Rattus norvegicus</i>	TKTLAGLPQKSLHSLASSELYAMTRQHQEQEIQSPGQEQPKRVRVFTETDDA
<i>Carassius auratus</i>	PFRALTYKRTPESE--FPKERTQVHDYMLETVEDSVEETVKNKDYTVLVD-
<i>Danio rerio</i>	PLRALALYTRTPESPSFPKERTQVHDYMFETVED--SEETVKNKDYTVLVD-
<i>Coturnix japonica</i>	-----
<i>Gallus gallus</i>	-----
<i>Sturnus vulgaris</i>	-----
<i>Zonotrichia leucophrys</i>	-----
<i>Pan troglodytes</i>	ELKQEK-----
<i>Homo sapiens</i>	ELKQEK-----
<i>Equus caballus</i>	ELKQEK-----
<i>Bos taurus</i>	ELKQEK-----
<i>Mus musculus</i>	ERKPEK-----
<i>Rattus norvegicus</i>	ERKQEKIGNLQPVLQGAMKL
<i>Carassius auratus</i>	-----
<i>Danio rerio</i>	-----

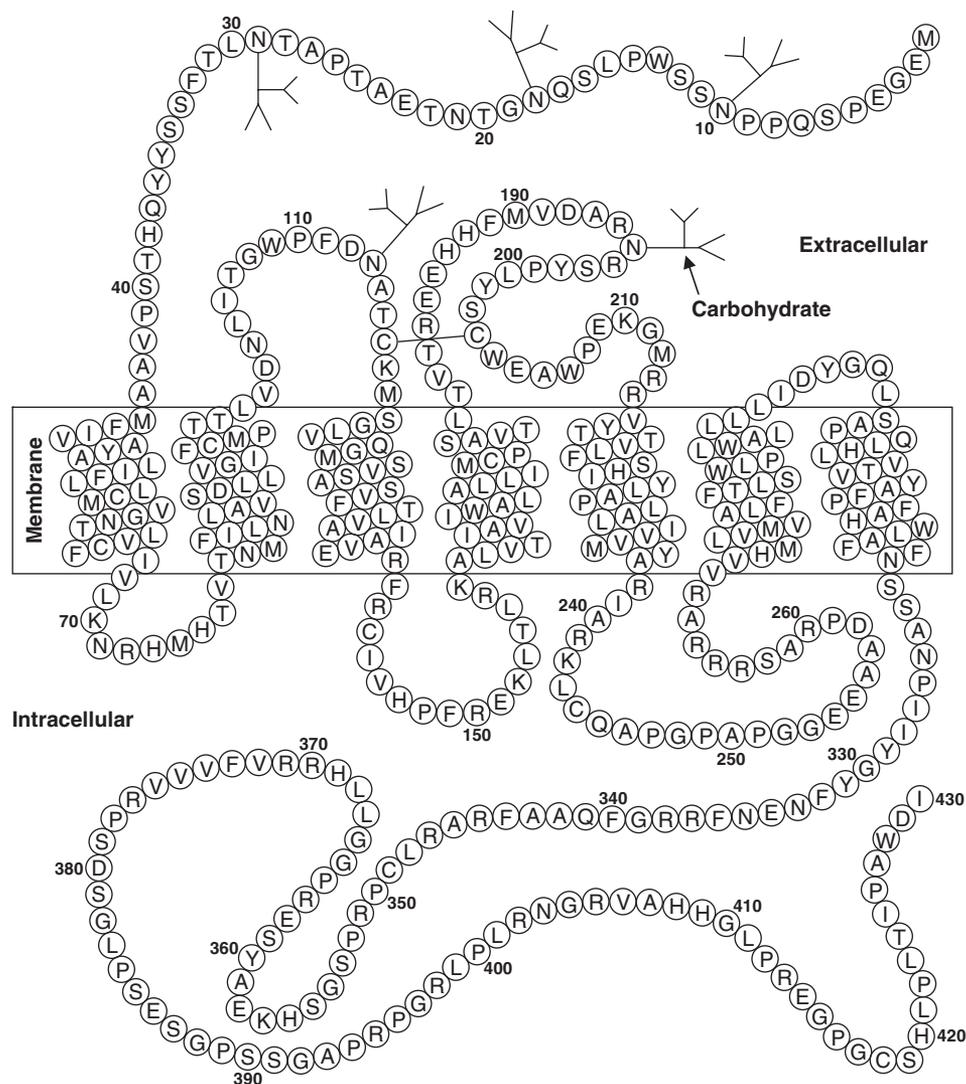
**Fig. 2** Alignment of GnIH precursor polypeptides in several birds, mammals, and fish. Characteristic LPXRF (X=L or Q) motif at the C-termini for RFRPs followed by glycine as an amidation signal and arginine or lysine as endoproteolytic basic amino acids are shown in bold. LPLRS-amide or LPLRL-amide sequences encoded in the GnIH/RFRP-2 position of the precursor polypeptide of chimpanzee (*Pan troglodytes*), human (*Homo sapiens*), and cattle (*Bos Taurus*) are also shown in bold. *Coturnix japonica*: Japanese quail; *Gallus gallus*: red jungle fowl; *Sturnus vulgaris*: European starling; *Zonotrichia leucophrys*: white-crowned sparrow; *Equus caballus*: horse; *Mus musculus*: house mouse; *Rattus norvegicus*: Norway rat; *Carassius auratus*: goldfish; and *Danio rerio*: zebrafish.

fowl (AAR14159), European starling (ABO86716), white-crowned sparrow (BAD21301)], mammals [chimpanzee (XP\_001160762), human (BAB17674), horse (XP\_001498898), cattle (NP\_776593), house mouse (BAB17673), Norway rat (NP\_076442)] and fish [goldfish (BAC06473), zebrafish (BAF34890)] for comparison.

### GnIH/RFRP actions in the hypothalamic-pituitary-gonadal axis

To study the biological actions of GnIH/RFRP peptide, identification of its receptor is critical. The receptor for quail GnIH was identified and its binding activities have been investigated (Yin et al. 2005). Structural analysis of the quail GnIH receptor revealed that it belongs to the G protein-coupled receptor (GPCR) superfamily. A crude membrane fraction of COS-7

cells transfected with the quail GnIH receptor cDNA specifically bound GnIH, GnIH-RP-1, and GnIH-RP-2 in a concentration-dependent manner. Interestingly, the GnIH receptor also bound with high affinities to various RFRP peptides which have LPXRF-amide (X=L or Q) motif at their C-termini. In contrast, C-terminal non-amidated GnIH failed to bind the receptor. Accordingly, it is thought that the C-terminal LPXRF-amide (X=L or Q) motif is critical for its binding to GnIH receptor. The identified quail GnIH receptor mRNA was expressed in the pituitary as well as in various parts of the brain. The mammalian homolog of GnIH receptor is GPR147 (OT7T022, NPFF-1) (Fukusumi et al. 2006). Figure 3 shows the predicted 2D structure of GPR147 from its nucleotide sequence (AB040104). RFRP peptides suppress the production of cAMP in ovarian cells of Chinese hamsters transfected with GPR147, suggesting that the receptor



**Fig. 3** 2D representation of the human RFRP receptor (GPR147). The transmembrane region was predicted using SOSUI (Hirokawa et al. 1998). Glycosylation and disulfide bridge sites were predicted by GPCRDB (Horn et al. 1998).

couples to  $G_{\alpha i}$ . GPR147 mRNA is also expressed in various parts of the brain as well as in the pituitary, suggesting that there are multiple actions within the central nervous system (Hinuma et al. 2000).

The actual release of GnIH or RFRP peptides into the hypothalamo-hypophyseal portal system has not been studied in any vertebrate. However, the dense population of GnIH immunoreactive (GnIH-ir) fibers at the median eminence in quail (Tsutsui et al. 2000; Ubuka et al. 2003; Ukena et al. 2003) as well as in house sparrows (*Passer domesticus*), song sparrows (*Melospiza melodia*) (Bentley et al. 2003), and European starlings (Ubuka et al. 2008) suggests a role for GnIH in the regulation of pituitary function at least in these birds. The fact that GnIH inhibits release of gonadotropin from cultured quail anterior pituitary provides strong support for this function (Tsutsui et al. 2000). GnIH administration to cultured chicken anterior pituitary further inhibits not only the release of gonadotropins but also the synthesis of gonadotropin subunit mRNAs (Ciccone et al. 2004). Nevertheless, direct regulation of pituitary function by GnIH may be regulated in a different way in some bird species (either developmentally or temporally) because there is no apparent GnIH-ir material in the median eminence in adult male Rufous-winged sparrows (*Aimophila carpalis*) (Small et al. 2007). In rodents, RFRP-ir fibers are present but sparse in the median eminence (Kriegsfeld et al. 2006). Accordingly, the physiological significance of the action of GnIH/RFRP in the direct control of pituitary function may differ among species. Our understanding of these differences is limited at present.

To clarify the functional significance of GnIH in the control of avian reproduction, Ubuka et al. (2006) investigated the action of GnIH in the pituitary-gonadal axis in male quail. It is generally accepted that in avian species luteinizing hormone (LH) stimulates the formation of testosterone in Leydig cells. Follicle-stimulating hormone (FSH) and testosterone stimulate growth, differentiation, and spermatogenic activity of the testis (Johnson 1986; Follett 1984). Peripheral administration of GnIH to mature quail via osmotic pumps for two weeks decreased the expressions of gonadotropin common  $\alpha$  and LH $\beta$  subunit mRNAs in the pituitary. Concentrations of plasma LH and testosterone were also decreased dose dependently. Furthermore, administration of GnIH to mature birds induced testicular apoptosis and decreased spermatogenic activity in the testis. In immature birds, daily administration of GnIH for two weeks suppressed testicular growth and the rise in concentration of plasma testosterone. An inhibition of molting by juveniles also occurred after GnIH administration.

These results show that GnIH can inhibit gonadal development and maintenance and also sexual development by decreasing the synthesis and release of gonadotropin (Ubuka et al. 2006).

### GnIH/RFRP actions in the brain

Although a dense population of GnIH neuronal cell bodies was only found in the PVN, GnIH-ir fibers were widely distributed in the diencephalic and mesencephalic regions in quail (Ukena et al. 2003). Dense networks of GnIH-ir fibers were found in the ventral paleostriatum, septal area, preoptic area, median eminence, optic tectum, and the dorsal motor nucleus of the vagus. Thus, it was hypothesized that GnIH may participate not only in the regulation of pituitary function, but also in behavioral and autonomic mechanisms. RFRP-ir neuronal fibers and GPR147 mRNA are also widely distributed in the rat brain (Fukusumi et al. 2006). Thus, the RFRP-GPR147 system may also function as a regulator of behavioral and autonomic mechanisms in the mammalian brain.

Immunohistochemical studies using light and confocal microscopy indicate that GnIH/RFRP-ir axon terminals are in probable contact with GnRH neurons in birds (Bentley et al. 2003) and rodents (Kriegsfeld et al. 2006). Thus, there is potential for the direct regulation of GnRH neuronal activity by GnIH neurons. Recently, Ubuka et al. (2008) investigated the interaction of GnIH neurons and GnRH neurons in the European starling brain. It is generally accepted that birds possess at least two forms of GnRH in their brains. One form is GnRH-I which is thought to be released at the median eminence to stimulate secretion of gonadotropin from the anterior pituitary (King and Millar 1982; Miyamoto et al. 1982; Sharp et al. 1990). The second form of GnRH is GnRH-II (Miyamoto et al. 1984; Millar 2003), which is thought to influence reproductive behaviors in birds (Maney et al. 1997) and mammals (Temple et al. 2003; Barnett et al. 2006). Double-label immunocytochemistry showed GnIH axon terminals on GnRH-I and GnRH-II neurons in the songbird brain (Bentley et al. 2003; Ubuka et al. 2008). Further, *in situ* hybridization of starling GnIH receptor mRNA combined with GnRH immunocytochemistry showed the expression of GnIH receptor mRNA in GnRH-I and GnRH-II neurons (Ubuka et al. 2008). Central administration of GnIH/RFRP inhibits the release of gonadotropin in white-crowned sparrows (Bentley et al. 2006), Syrian hamsters (Kriegsfeld et al. 2006) and rats (Johnson et al. 2007) in a manner similar to peripheral administration of GnIH (Osugi et al. 2004; Kriegsfeld et al. 2006; Ubuka et al. 2006). Accordingly, GnIH may inhibit the secretion of

gonadotropin by decreasing GnRH neuronal activity in addition to regulating the release of pituitary gonadotropin directly.

Central administration of GnIH/RFRP also inhibits reproductive behavior of females in white-crowned sparrows (Bentley et al. 2006) and of males in rats (Johnson et al. 2007). It was already known that GnRH-II enhances copulation solicitation in estrogen-primed female white-crowned sparrows exposed to the song of males (Maney et al. 1997). Because of the putative contact of GnIH neurons with GnRH-II neurons in white-crowned sparrows (Bentley et al. 2003), Bentley et al. (2006) investigated the effect of GnIH on copulation solicitation in females of this species. A centrally administered physiological dose of GnIH inhibited copulation solicitation in estrogen-primed female white-crowned sparrows exposed to the song of males without affecting locomotor activity. Johnson et al. (2007) investigated the effect of central administration of RFRP-3 on the reproductive behaviors of male rats. Behavioral tests indicated that RFRP-3 dose dependently suppressed all facets of male sexual behavior while not having any observable effects on males' ability to ambulate. In contrast, immunoneutralization of RFRP in the rat brain increased male sexual behaviors. These results suggest that GnIH/RFRP inhibits reproductive physiology and behavior not only by inhibiting the secretion of gonadotropin from the pituitary gland but also by inhibiting GnRH neuronal activities or by acting directly within the brain. The precise physiological mechanisms of GnIH/RFRP action in the brain and the differences between sexes will be the focus of future studies.

### Regulation of GnIH/RFRP expression

Identification of the regulatory mechanisms governing expression of GnIH/RFRP is also important in understanding the physiological role of the GnIH/RFRP system. Many bird species are photoperiodic, as are many mammals. Photoperiodic mammals rely on the annual cycle of changes in nocturnal secretion of melatonin to drive their reproductive responses (Bronson 1990). In contrast, a dogma has existed that birds do not use seasonal changes in melatonin secretion to time their reproductive effort, and a role for melatonin in birds has remained enigmatic (Wilson 1991; Juss et al. 1993). Despite the accepted dogma, there is strong evidence that melatonin is involved in the regulation of several seasonal processes, including gonadal activity and gonadotropin secretion (Ohta et al. 1989; Guyomarc'h et al. 2001; Rozenboim et al. 2002). In light of these reports and considering GnIH's inhibitory effects on the secretion of gonadotropin,

Ubuka et al. (2005) hypothesized that melatonin may be involved in the induction of GnIH expression, thus influencing gonadal activity. The action of melatonin on the expression of GnIH was studied in quail, a highly photoperiodic bird species. Because the pineal gland and eyes are the major sources of melatonin in the quail (Underwood et al. 1984), Ubuka et al. (2005) analyzed the effects of pinealectomy (Px) combined with orbital enucleation (Ex) (Px plus Ex) on the expression of GnIH precursor mRNA and GnIH peptide. Subsequently, melatonin was administered to Px plus Ex birds. Px plus Ex decreased the expression of GnIH precursor mRNA and the content of mature GnIH peptide in the hypothalamus. Further, melatonin administration to Px plus Ex birds caused a dose-dependent increase in the expression of GnIH precursor mRNA and the production of mature peptide. The expression of GnIH was photoperiodically controlled and increased under short-day photoperiods, when the duration of melatonin secretion increases. They also investigated the expression of melatonin receptor in GnIH neurons. *In situ* hybridization combined with immunocytochemistry for GnIH revealed that the mRNA of Mel1c, an avian melatonin receptor subtype, was expressed in GnIH-ir neurons in the PVN. Autoradiography of melatonin receptors further revealed specific binding of melatonin in the PVN. Accordingly, melatonin appears to act directly on GnIH neurons through its receptor to induce expression of GnIH. Recently, a similar, but opposite, action of melatonin on the inhibition of RFRP expression was shown in Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*) hamsters, both photoperiodic mammals (Revel et al. 2008). The level of RFRP mRNA and the number of RFRP-ir cell bodies were reduced in sexually quiescent Syrian and Siberian hamsters acclimated to short-day photoperiod (SD), compared to sexually active animals maintained under long-day photoperiod (LD). These effects of photoperiods were not observed in the laboratory rat, which is a nonphotoperiodic breeder. The photoperiodic variation of RFRP expression was abolished in Px hamsters and injections of LD hamsters with melatonin for 60 days reduced the expression of RFRP down to SD levels, indicating a dependence upon melatonin. These results in quail and hamsters demonstrate that GnIH/RFRP expression is photoperiodically modulated via a melatonin-dependent process. It has been proposed that high levels of melatonin in the prepubertal child are necessary for the suppression of sexual development (Macchi and Bruce 2004). Investigating GnIH/RFRP in the hypothalamus and its receptor in the pituitary and in GnRH neurons in prepubertal, pubertal, and adult humans will be an area of research

that could potentially identify a role for GnIH/RFRP in the initiation of puberty.

Stress can inhibit reproduction in birds and mammals. Because GnIH has an inhibitory effect on reproductive functions in birds, Calisi et al. (2008) hypothesized that inhibitory effects of stress on reproductive function may be mediated via the hypothalamic GnIH system. They examined the effects of capture-handling stress on the numbers of GnIH neurons in the hypothalamus of adult male and female house sparrows. Preliminary data indicated increases in the numbers of GnIH-ir neurons after capture-handling stress. Kirby et al. (2007) investigated how acute immobilization stress alters RFRP mRNA and protein levels in adult male rats. RFRP mRNA and protein levels increased immediately following stress but returned to a lower level 24 h after stress. Further, confocal microscopic analysis of *in situ* hybridization for RFRP precursor mRNA combined with immunocytochemical staining for the glucocorticoid receptor (GR) demonstrated co-localization of both signals within a single cell, confirming that RFRP neurons also express GR. They concluded that acute stress increases the level of rat RFRP, possibly by a direct glucocorticoid effect on RFRP neurons. These data from birds and rodents imply an influence of stress on the GnIH/RFRP system. GnIH/RFRP may therefore be a mediator of stress-induced reproductive disruption in birds and mammals.

### GnIH/RFRP in the gonad

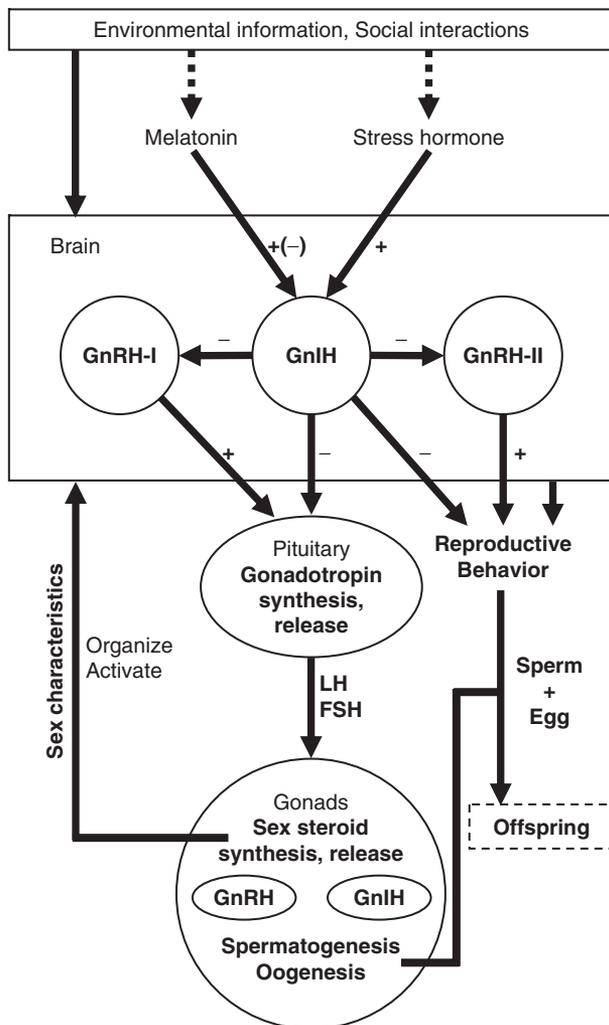
Peripheral administration of GnIH induces gonadal apoptosis with decreased gonadal activity in quail (Ubuka et al. 2006). Although this action may be mediated through the decreased secretion of gonadotropin from the anterior pituitary, the results do not preclude a direct action of GnIH on the gonad. Recently, Bentley et al. (2008) showed that GnIH and its receptor are synthesized and present in the testis and ovary of starlings and quail. Immunocytochemistry identified GnIH-ir material in the theca and granulosa cells of the ovary and in the interstitial cells and spermatogonia of the testis in both species. PCR analysis identified transcripts of GnIH from these tissues, and *in situ* hybridization in quail further confirmed the presence of GnIH precursor mRNA in the interstitial and germ cells of the testis. *In vitro* and *in vivo* receptor fluorography indicated that the testes and ovaries of starlings and white-crowned sparrows contained functional binding sites for GnIH. Rhodaminated-GnIH bound to the granulosa cells of the ovaries and to the interstitial cells and seminiferous tubules of the testes of

both species. The sequencing of PCR products from starling and quail tissues confirmed the presence of GnIH receptor transcripts in the testis and ovary. *In situ* hybridization of quail testis produced mRNA signals in the interstitial and germ cells. The expressions of RFRP precursor mRNA and GPR147 mRNA were also identified in rat gonads by RT-PCR analyses (Hinuma et al. 2000). These results suggest the potential for GnIH/RFRP to act as an autocrine or paracrine regulator within the gonads of birds and mammals.

Interestingly, evidence suggests that GnRH is also expressed in the gonads of humans, other mammals, birds, reptiles, amphibians, and fishes [for reviews see Kim et al. (2007) and Singh et al. (2007)]. Transcripts for GnRH receptor have been identified in gonadal tissues of several of these species as well. *In situ* hybridization indicates the expression of GnRH and its receptor in ovarian tissues: in granulosa and thecal cells in nonmammalian vertebrates (Singh et al. 2007), rat granulosa and luteal cells (Kogo et al. 1999) and human granulosa-luteal cells and corpora lutea cells (Clayton et al. 1979; Leung et al. 2003). In testicular tissues, *in situ* hybridization indicates the expression of GnRH mRNA in Sertoli and spermatogenic cells and the expression of GnRH receptor mRNA in interstitial cells in rats and humans (Bahk et al. 1995). It is hypothesized that GnRH stimulates basal steroidogenesis, but inhibits gonadotropin-stimulated androgen and progesterone biosynthesis in an autocrine/paracrine fashion. Because of the differential expression within follicles and seminiferous tubules, gonadal GnRH is also hypothesized as a regulator of follicular development and spermatogenesis (Ramakrishnappa et al. 2005). As we now know that GnIH/RFRP is also expressed in the gonads of birds and mammals, interactions of GnIH/RFRP and GnRH may also exist in the gonads, as it does in the brain.

### Summary

GnIH, an homologous peptide to mammalian RFRP, was originally identified in quail as a hypothalamic inhibitor for the secretion of gonadotropin. Homologous peptides and their receptors were also identified in birds and mammals. The action of GnIH/RFRP on the pituitary gland was studied as well as its function within the brain. Although there is a general agreement that GnIH/RFRP inhibits the reproductive physiology and behavior of birds and mammals, this inhibitory effect of GnIH/RFRP may be accomplished by one or all of three ways: (1) directly inhibiting synthesis and release of gonadotropin in the pituitary gland,



**Fig. 4** Possible control mechanisms of reproductive physiology and behavior by GnIH (RFRP) and GnRH in birds and mammals. Stimulatory and inhibitory control mechanisms of the reproductive physiology and behavior regulate the production of their offspring. GnRH: gonadotropin-releasing hormone; GnIH: gonadotropin-inhibitory hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

(2) decreasing the activities of GnRH neurons, or (3) by acting directly on the gonads. GnIH/RFRP may also directly inhibit reproductive behaviors by controlling various neurons within the brain. Figure 4 summarizes the possible action of GnIH/RFRP in the control of reproductive physiology and behavior in birds and mammals. The significance of each action may vary among species and developmental processes and between the sexes.

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