Published in final edited form as:

Horm Behav. 2006 December ; 50(5): 655–666. doi:10.1016/j.yhbeh.2006.06.004.

Driving Reproduction: RFamide Peptides Behind the Wheel

Lance J. Kriegsfeld

Department of Psychology and Helen Wills Neuroscience Institute, University of California, Berkeley, CA 94720-1650

Abstract

The availability of tools for probing the genome and proteome more efficiently has allowed for the rapid discovery of novel genes and peptides that play important, previously-uncharacterized roles in neuroendocrine regulation. In this review, the role of a class of neuropeptides containing the C-terminal Arg-Phe-NH₂ (RFamide) in regulating the reproductive axis will be highlighted. Neuropeptides containing the C-terminal Phe- Met-Arg-Phe-NH₂ (FMRFamide) were first identified as cardioregulatory elements in the bi-valve mollusk, *Macrocallista nimbosa*. During the past two decades, numerous studies have shown the presence of structurally-similar peptides sharing the RFamide motif across taxa. In vertebrates, RFamide peptides have pronounced influences on opiateergic regulation and neuroendocrine function. Two key peptides in this family are emerging as important regulators of the reproductive axis, kisspeptin and gonadotropin-inhibitory hormone (GnIH). Kisspeptin acts as the accelerator, directly driving gonadotropin-releasing hormone (GnRH) neurons, whereas GnIH acts as the restraint. Recent evidence suggests that both peptides play a role in mediating the negative feedback effects of sex steroids. This review presents the hypothesis that these peptides share complementary roles by responding to internal and external stimuli with opposing actions to precisely regulate the reproductive axis.

Keywords

neurosecretion; Kiss; Metastin; RFRP; GnIH; reproductive; sex; mating

The reproductive axis integrates information from a wide range of systems via a number of direct and indirect neurochemical inputs (Clarke and Pompolo, 2005; Gore, 2004; Smith and Grove, 2002). These neurochemical modulators allow the GnRH system to monitor the internal and external environments and adjust reproductive function according to current conditions. Whereas many regulatory inputs have been well-characterized, these systems cannot fully account for the exquisite precision by which the reproductive axis is regulated.

Individual neuropeptides containing the C-terminal Arg-Phe-NH₂ (RFamide) are emerging as prominent regulators of neuroendocrine function. All RFamide peptides characterized to date are in a position to directly or indirectly act on the reproductive neuroendocrine axis. This review will focus on the role of kisspeptin and gonadotropin-inhibitory hormone (GnIH), both shown to have marked direct, but opposing actions, on the GnRH system. In contrast to the effects of these peptides on GnRH, the means by which environmental, social, and internal stimuli impinge on these peptidergic systems to regulate reproductive function remain largely unspecified. The goal of this review is to highlight the identified roles played by these RFamide peptides and propose a model whereby interactions between

*Corresponding Author: Department of Psychology and Helen Wills Neuroscience Institute, 3210 Tolman Hall, # 1650, University of California, Berkeley, CA 94720-1650, Phone: 510-642-5148, Fax: 510-642-5293, Kriegsfeld@berkeley.edu.

these systems place these neurochemicals in a position to be chief 'yin and yang' regulators of the reproductive axis.

Discovery and Functional Significance of RFamide Peptides

Frank Beach borrowed the words of Ebbinghaus when he stated that behavioral endocrinology has a long history but a short past (Beach, 1974). The same can be said for RFamide peptides and the regulation of neuroendocrine function. Almost thirty years ago, the cardioexcitatory neuropeptide containing the C-terminal Phe-Met-Arg-Phe-NH₂ (FMRFamide) was identified in the ganglia of the clam, *Macrocallista nimbosa* (Price and Greenberg, 1977). Following this discovery, antibodies to FMRFamide peptides were applied as a tool for labeling structurally-similar peptides across taxa. Despite these antibodies recognizing and labeling cells in the CNS in several species, the identity of these labeled peptides remained unknown. In 1983, the first vertebrate RFamide peptide, Leu-Pro-Leu-Arg-Phe-NH₂ (LPLRFamide), was identified in chicken (Dockray, Reeve, Shively, Gayton, and Barnard, 1983). When LPLRFamide peptide was injected into rats, arterial blood pressure was grossly attenuated. Likewise, application of this peptide to rat brain stem neurons altered their firing activity (Price and Greenberg, 1977). These data provided the first evidence for the vertebrate expression of RFamide peptides and indicated a potential neuromodulatory role in mammalian brain (Table 1 and 2).

The Discovery of RFamide Peptides in Mammalian Brain

Over two decades following the discovery of an avian RFamide peptide, the first mammalian RFamide-related peptides (RFRPs) were cloned and characterized by identifying appropriate extracts for further investigation based on immunoreactivity with antibodies directed against FMRFamide peptides characterized in other species (Panula, Aarnisalo, and Wasowicz, 1996; Perry, Yi-Kung Huang, Cronk, Bagust, Sharma, Walker, Wilson, and Burke, 1997). These two peptides, called neuropeptide FF (NPFF) and neuropeptide AF (NPAF), are highly localized to the posterior pituitary, spinal cord, hypothalamus, and medulla (Panula et al., 1996). Intracerebroventricular injections of NPAF or NPFF reverse the analgesic actions of morphine suggesting interactions with endogenous opioid systems (Jhamandas, Milne, Sutak, Gouarderes, Zajac, and Yang, 2006; Yang, Fratta, Majane, and Costa, 1985). It was initially suspected that NPFF might be important for vasopressin secretion, as NPFF was absent from the pituitary of Brattleboro rats, but this role has not been confirmed.

One strategy for discovering RFRPs that has proven quite effective is a reverse pharmacological approach to identifying ligands for orphan G-protein-coupled receptors (GPCRs). Orphan receptors are so named because they do not have a known ligand. By monitoring downstream responses (e.g., calcium influx) of cells expressing a targeted GPCR, one could identify putative ligands without even knowing the biological actions or cellular targets of the peptide examined. The first mammalian RFamide peptide to be identified using this strategy was named prolactin-releasing peptide (PrRP). This nomenclature was based on the fact that the GPCR used to identify PrRP was expressed solely in pituitary and peptide administration led to potent prolactin release from pituitary cells (Hinuma, Habata, Fujii, Kawamata, Hosoya, Fukusumi, Kitada, Masuo, Asano, Matsumoto, Sekiguchi, Kurokawa, Nishimura, Onda, and Fujino, 1998). Since the time of its discovery, studies on the role of this peptide as a stimulator of prolactin release have been equivocal, and its role as a PrRP requires re-examination (Curlewis, Kusters, Barclay, and Anderson, 2002; Jarry, Heuer, Schomburg, and Bauer, 2000). As a result, this peptide is now more commonly referred to as RFRP-1.

RFRP-1 is also a potent stimulator of corticotrophin-releasing hormone (CRH) and, in turn, the glucocorticoids indicating a key role in mediating stress responsiveness and metabolism (Table 2) (Matsumoto, Maruyama, Noguchi, Horikoshi, Fujiwara, Kitada, Hinuma, Onda, Nishimura, Inoue, and Fujino, 2000; Samson, Keown, Samson, Samson, Lane, Baker, and Taylor, 2003; Seal, Small, Dhillo, Kennedy, Ghatei, and Bloom, 2002). In rats, retrograde tract tracing studies uncovered projections from RFRP-1 cells to the supraoptic nucleus (SON), with injections of the peptide mediating oxytocin release (Table 2) (Maruyama, Matsumoto, Fujiwara, Noguchi, Kitada, Hinuma, Onda, Nishimura, Fujino, Higuchi, and Inoue, 1999; Zhu and Onaka, 2003). Finally, a role for this RFRP in feeding regulation, through interactions with the dorsomedial nucleus of the hypothalamus (DMH), has been uncovered (Table 2) (Lawrence, Celsi, Brennand, and Luckman, 2000).

The Discovery of an RFamide Peptide Negatively Regulating Avian Reproductive Function

Following the discovery of RFRP-1, an RFamide peptide was isolated in quail brain by first probing with a competitive enzyme-linked immunosorbant assay (ELISA) using an antibody directed against the RF motif on the C-terminus. Using this approach, a novel hypothalamo-hypophysial RFamide peptide was identified that rapidly and dose-dependently inhibits gonadotropin release from cultured quail pituitaries (Tsutsui, Saigoh, Ukena, Teranishi, Fujisawa, Kikuchi, Ishii, and Sharp, 2000). Based on these initial findings, Tsutsui and colleagues named this peptide gonadotropin-inhibiting hormone (GnIH). In avian species, GnIH neurons are found only in the paraventricular nucleus of the hypothalamus (PVN) with extensive fibers projecting rostrally to the ventral paleostriatum, lateral and medial septum, preoptic area and caudally to the median eminence, optic tectum and brainstem (Bentley, Perfito, Ukena, Tsutsui, and Wingfield, 2003; Ukena, Ubuka, and Tsutsui, 2003). The receptor for GnIH, a novel GPCR, has recently been cloned and characterized in Japanese quail (Yin, Ukena, Ubuka, and Tsutsui, 2005). In collaboration with Tsutsui and colleagues, we have recently established an analogous role for the mammalian homologue of GnIH in reproductive axis regulation, in addition to a role for this peptide in mediating the negative feedback effects of sex steroids (Kriegsfeld, Mei, Bentley, Ubuka, Mason, Inoue, Ukena, Tsutsui, and Silver, 2006). A detailed examination of the role of GnIH in avian and mammalian reproduction and its mode of action follows in the next section.

The Discovery of Kisspeptin as a Positive Regulator of Mammalian Reproduction

In 2001, the RFamide peptide now referred to as kisspeptin was shown to be the endogenous ligand for the orphan GPCR, GPR54 (Kotani, Detheux, Vandenbogaerde, Communi, Vanderwinden, Le Poul, Brezillon, Tyldesley, Suarez-Huerta, Vandeput, Blanpain, Schiffmann, Vassart, and Parmentier, 2001; Ohtaki, Shintani, Honda, Matsumoto, Hori, Kanehashi, Terao, Kumano, Takatsu, Masuda, Ishibashi, Watanabe, Asada, Yamada, Suenaga, Kitada, Usuki, Kurokawa, Onda, Nishimura, and Fujino, 2001). Interestingly, the gene encoding this peptide was discovered years earlier by a group in Hershey, PA (Lee, Miele, Hicks, Phillips, Trent, Weissman, and Welch, 1996; Lee and Welch, 1997). To pay homage to another major 'discovery' in Hershey, PA the researchers named the gene *Kiss1*. Because the gene was originally recognized as a tumor metastasis suppressor, the product of the *Kiss1* gene was originally referred to as metastin (Lee et al., 1996; Lee and Welch, 1997). For the sake of this review, the gene product of the *Kiss1* gene will be referred to as *KISS-1* mRNA and its peptide product as kisspeptin.

A role for kisspeptin in regulating reproductive function was first identified in 2003 when individuals with hypogonadism were found to have a mutation in the *GPR54* gene (de Roux, Genin, Carel, Matsuda, Chaussain, and Milgrom, 2003; Seminara, Messager, Chatzidaki, Thresher, Acierno, Shagoury, Bo-Abbas, Kuohung, Schwinof, Hendrick, Zahn, Dixon, Kaiser, Slaugenhaupt, Gusella, O'Rahilly, Carlton, Crowley, Aparicio, and Colledge, 2003).

In humans and mice lacking GPR54, the hypothalamus fails to drive adequate secretion of the gonadotropins, while pituitary responsiveness to exogenous GnRH is unaffected. Since this initial discovery, several laboratories have systematically examined the neural pathways and cellular and molecular mechanisms by which kisspeptin regulates GnRH. These investigations have revealed a compelling role for kisspeptins in GnRH regulation that will be highlighted in this review.

GnIH: Structure and Function

Since the dodecapeptide GnIH (Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH₂) was identified 6 years ago by Tsutsui and colleagues (Satake, Hisada, Kawada, Minakata, Ukena, and Tsutsui, 2001; Tsutsui et al., 2000), research has focused on the pathways and mechanisms by which this RFamide peptide inhibits the reproductive axis. As mentioned previously, GnIH was first discovered in birds and initial studies focused on its role in avian reproduction using quail and sparrow models. GnIH has marked and rapid suppressive effects on gonadotropin release both *in vivo* and *in vitro* (Osugi, Ukena, Bentley, O'Brien, Moore, Wingfield, and Tsutsui, 2004; Tsutsui et al., 2000). Chronic delivery of GnIH to male quail decreases luteinizing hormone (LH) α and β subunit mRNA and leads to testicular apoptosis and reduced spermatogenesis (Ubuka, Ukena, Sharp, Bentley, and Tsutsui, 2006). In addition to these neuroendocrine effects, central injections of GnIH rapidly suppress female sexual behavior in white-crowned sparrows, as assayed by monitoring copulation-solicitation displays in response to male song (Bentley, Jensen, Kaur, Wacker, Tsutsui, and Wingfield, 2006). Cell bodies for GnIH are confined to the PVN in birds, with pronounced projections to hypothalamic areas, including contacts upon GnRH somata and processes (Bentley et al., 2003; Ukena et al., 2003) as well as terminals in the median eminence (Tsutsui et al., 2000). These findings suggest a role for GnIH at multiple levels of the hypothalamo-pituitary-gonadal (HPG) axis.

The GnIH precursor encodes three related peptides (GnIH-RP1, GnIH, and GnIH-RP2) (Osugi et al., 2004; Satake et al., 2001), all including the Leu-Pro-Xaa-Arg-Phe-NH₂ (where Xaa = Leu or Gln). In addition, Tsutsui and colleagues demonstrated that the chicken LPLRFamide peptide identified by Dockray and colleagues (Dockray et al., 1983) (and see above) is a fragment of the GnIH precursor (Dockray et al., 1983; Osugi et al., 2004; Satake et al., 2001; Tsutsui et al., 2000). Through gene database searches, mammalian homologues similar in structure to GnIH were identified (Hinuma, Shintani, Fukusumi, Iijima, Matsumoto, Hosoya, Fujii, Watanabe, Kikuchi, Terao, Yano, Yamamoto, Kawamata, Habata, Asada, Kitada, Kurokawa, Onda, Nishimura, Tanaka, Ibata, and Fujino, 2000). Initially, it was thought that the mammalian gene precursor encoded three peptides, termed RFRP-1, RFRP-2, and RFRP-3. It was later determined that the mammalian RFRP gene encodes only 2 RFamide peptides, RFRP-1 and -3, and the amino acid sequence thought to encode the C-terminus of RFRP-2 was, instead, included at the N-terminus of RFRP-3 (Fukusumi, Habata, Yoshida, Iijima, Kawamata, Hosoya, Fujii, Hinuma, Kitada, Shintani, Suenaga, Onda, Nishimura, Tanaka, Ibata, and Fujino, 2001; Fukusumi, Yoshida, Fujii, Maruyama, Komatsu, Habata, Shintani, Hinuma, and Fujino, 2003; Ukena, Iwakoshi, Minakata, and Tsutsui, 2002; Ukena and Tsutsui, 2005; Yoshida, Habata, Hosoya, Kawamata, Kitada, and Hinuma, 2003). NPFF and RFRP-3 are closely related, with four amino acids on the C-terminal being identical (Tsutsui and Ukena, 2006). Despite this high homology, these peptides have independent receptors (Tsutsui and Ukena, 2006), further suggesting that NPFF and the RFRP-3 are not the same mature peptide with two different names.

The putative receptor for GnIH has recently been cloned and characterized as a novel GPCR that shares high homology with the human and rat RFRP receptor (Yin et al., 2005). The

receptor binds avian GnIH and GnIH-RPs with high affinity. The GnIH receptor is expressed in pituitary, hypothalamus, and spinal cord, further suggesting a neuromodulatory role of GnIH in addition to its functioning as an inhibitor of pituitary gonadotropin release. Using a novel approach to receptor autoradiography, Bentley and colleagues sought to further characterize the location of GnIH binding sites in birds (Bentley et al., 2006). By applying a rhodaminated form of GnIH *in vivo*, putative binding sites were identified on chicken GnRH-II cells (cGnRH-II) and the median eminence in close proximity to GnIH-I terminals (Bentley et al., 2006). These data suggest that GnIH may act directly on cGnRH-II cells to mediate behavior in addition to modifying gonadotropin secretion by acting at GnRH-I terminal fibers.

RFRPs: A Functional Mammalian GnIH Homologue?

Whereas RFamide peptides similar to GnIH have been identified in mammals (Fukusumi et al., 2003; Ukena et al., 2002; Ukena and Tsutsui, 2005; Yoshida et al., 2003), the potential role of these peptides in mammalian reproductive function remained unspecified. As mentioned previously, a role for RFRP-1 in regulation of prolactin, oxytocin, CRH, and feeding behavior was known (Hinuma et al., 1998; Lawrence et al., 2000; Maruyama et al., 1999; Matsumoto et al., 2000; Samson et al., 2003; Seal et al., 2002; Zhu and Onaka, 2003), but the role of RFRPs as regulators of GnRH or gonadotropin secretion had not been explored. In collaboration with Tsutsui and colleagues, we sought to characterize the distribution and functional significance of the mammalian GnIH homologue (a precursor polypeptide encoding RFRP-1 and -3; hereafter referred to as RFRP) in rodents by investigating rats (*Rattus norvegicus*), mice (*Mus musculus*), and Syrian hamsters (*Mesocricetus auratus*).

We first characterized the distribution of mammalian RFRP to gain insight into the potential functional significance of this peptide in mammalian brain. In all three rodent species, RFRP-ir cell bodies were concentrated in the dorsomedial nucleus of the hypothalamus (DMH) (Fig. 1 and 2), with no other brain regions showing evidence of cell body staining (Kriegsfeld et al., 2006). As in birds, RFRP fibers and terminal fields were omnipresent in midline brain regions that concentrate GnRH neurons and fibers (Fig. 1); the medial septum, diagonal band of Broca, preoptic area, and anterior hypothalamus were all major targets for GnIH. The median eminence was sparsely innervated, although terminals were present. Given this projection pattern, we examined whether or not RFRP-ir cells might project upon GnRH perikarya as a mechanism of regulation. A marked percentage of GnRH cells (>40%) received projections from RFRP-ir fibers suggesting the potential for direct inhibitory control (Fig. 3 and 4). To confirm a role for mammalian RFRP as a regulator of gonadotropin secretion in mammals, we injected the peptide intracerebroventricularly (ICV) or peripherally in hamsters to determine its effects on LH. Both routes of administration rapidly and markedly inhibit LH secretion (Kriegsfeld et al., 2006). Analogous results have been obtained in rats (Anderson, Steyn, Tsutsui, Ukena, Bentley, and Herbison, 2005; Johnson, Bentley, Ukena, Tsutsui, and Fraley, 2005).

Given the pronounced inhibitory actions of RFRP in mammalian brain, we examined the possibility that this peptide participates in mediating the negative feedback effects of sex steroids. We found that RFRP-ir cells express estrogen receptor- α and respond to estrogen with increased FOS expression, suggesting activation by gonadal steroids (Kriegsfeld et al., 2006) (Figure 5). Whether this effect is mediated by projections to GnRH cells or actions on the anterior pituitary requires further examination. In rats, RFRP inhibits GnRH-stimulated LH release suggesting actions, at least in part, on the adenohypophysis (Anderson et al., 2005). Combined with our results indicating direct projections upon GnRH cells, these findings suggest that mammalian RFRP may mediate negative feedback effects of gonadal steroids at both the hypothalamic and pituitary levels.

To provide converging evidence that our antibody was labeling RFRP and not a related RFamide peptide, we cloned the avian precursor polypeptide in Syrian hamsters and stained hamster brains for RFRP mRNA using *in situ* hybridization (Kriegsfeld et al., 2006) (Figure 6). Cells expressing RFRP precursor mRNA were confined to the DMH and localized in a pattern identical to RFRP-ir labeling. As in other mammalian species (Fukusumi et al., 2003; Hinuma et al., 2000; Ukena et al., 2002; Ukena and Tsutsui, 2001; Yoshida et al., 2003), the precursor peptide encoded homologues to RFRP-1 and -3. Together, these recent findings point to a previously unidentified neurochemical pathway by which sex steroids act on the brain to regulate the reproductive axis. The extensive fiber projections of GnIH throughout the brain point to an opportunity for investigating the role of this peptide in an array of motivated behaviors.

Mammalian RFRPs and Avian GnIH: Conclusions and Caveats

Reports from our group and others suggest that mammalian RFRP has actions analogous to those of avian GnIH. RFRP-ir cells form close contacts with GnRH cells, and central and peripheral injections of RFRP markedly and rapidly inhibit LH. However, whether the inhibitory effects of RFRP are through GnRH, direct actions on the pituitary, or a combination of both mechanisms requires further exploration. In our initial studies, we observed most RFRP terminal fiber labeling in the inner layer of the median eminence (Kriegsfeld et al., 2006). Using immunohistochemical amplification strategies more recently, sparse fiber labeling in the outer layer of the median eminence was seen (Kriegsfeld, unpublished data). Whether failure to see significant RFRP-ir terminal labeling in the median eminence of rodents is the result of the specificity of the antiserum, labeling procedures, or differences in RFRP mode of action across taxa remains to be empirically determined.

Given the widespread fiber distribution of RFRP-ir cells, it would be prudent to confirm that all cell bodies for these peptides originate in the DMH. It is possible that other regions of the brain produce RFRPs that get rapidly released or transported down axons, therefore limiting cell body labeling via immunohistochemistry. *In situ* hybridization for RFRP precursor mRNA labels cells confined to the DMH (Kriegsfeld et al., 2006) suggesting this possibility is unlikely. However, injections of anterograde tracer into the DMH, combined with double-label immunohistochemistry for RFRP peptide, would allow verification of this conjecture.

As in birds, RFRP-ir fibers contact an impressive percentage of GnRH cells (Kriegsfeld et al., 2006). These findings suggest direct suppressive actions on GnRH production and/or release by mammalian RFRP. If RFRP acts, at least in part, on GnRH cells, its administration should lead to the suppression of both LH and FSH. To date, the role of mammalian RFRPs in FSH regulation has not been examined. Likewise, further work using mammalian GnRH cells lines, tissue culture preparations, and electrophysiological studies are necessary to begin to clarify the effects of RFRP on the mammalian GnRH system. Although research on avian GnIH and mammalian RFRP is in its relative infancy, these peptides are emerging as important regulators of the reproductive axis, underscoring the importance of further investigations into the neural, cellular, and molecular mechanisms by which these peptides act.

Kisspeptin (Mestatin): Structure and Function

As mentioned previously, a role for kisspeptin as a regulator of the reproductive axis was uncovered when hypogonadotropic hypogonadism was shown to be associated with a mutation of the GPR54 receptor in humans and other animals (de Roux et al., 2003; Seminara et al., 2003). The GPR54 receptor shares high homology with galanin receptors, although residues essential for high-affinity galanin binding are lacking (Kaiser and

Kuohung, 2005). Despite this homology, this receptor does not respond to galanin or galanin-like peptide administration. Instead, the GPR54 receptor is selective for kisspeptin (Clements, McDonald, Wang, Xie, O'Dowd, George, Austin, and Liu, 2001; Kotani et al., 2001; Muir, Chamberlain, Elshourbagy, Michalovich, Moore, Calamari, Szekeres, Sarau, Chambers, Murdock, Stepkowski, Shabon, Miller, Middleton, Darker, Larminie, Wilson, Bergsma, Emson, Faull, Philpott, and Harrison, 2001; Ohtaki et al., 2001).

Kisspeptin: A Potent Stimulator of the Reproductive Axis

Kisspeptin administration leads to marked, dose-dependent increases in LH and FSH across species (Gottsch, Cunningham, Smith, Popa, Acohido, Crowley, Seminara, Clifton, and Steiner, 2004; Kaiser and Kuohung, 2005; Navarro, Castellano, Fernandez-Fernandez, Barreiro, Roa, Sanchez-Criado, Aguilar, Dieguez, Pinilla, and Tena-Sempere, 2004; Navarro, Castellano, Fernandez-Fernandez, Tovar, Roa, Mayen, Nogueiras, Vazquez, Barreiro, Magni, Aguilar, Dieguez, Pinilla, and Tena-Sempere, 2005; Shahab, Mastronardi, Seminara, Crowley, Ojeda, and Plant, 2005; Thompson, Patterson, Murphy, Smith, Dhillon, Todd, Ghatei, and Bloom, 2004), including humans (Dhillon, Chaudhri, Patterson, Thompson, Murphy, Badman, McGowan, Amber, Patel, Ghatei, and Bloom, 2005). The effects of kisspeptin are principally mediated through its actions on the GnRH system; administration of kisspeptin leads to immediate early gene expression (i.e., FOS) in GnRH cells (Irwig, Fraley, Smith, Acohido, Popa, Cunningham, Gottsch, Clifton, and Steiner, 2004; Matsui, Takatsu, Kumano, Matsumoto, and Ohtaki, 2004). Additionally, kisspeptin-induced gonadotropin secretion is blocked across species by the GnRH receptor antagonist, acyline (Gottsch et al., 2004; Irwig et al., 2004; Shahab et al., 2005). In hypothalamic explants, kisspeptin increases GnRH cell firing and stimulates the release of GnRH (Han, Gottsch, Lee, Popa, Smith, Jakawich, Clifton, Steiner, and Herbison, 2005; Thompson et al., 2004). Application of tetrodotoxin to suppress sodium-dependent action potentials, does not affect this response, suggesting direct effects on GnRH cells (Han et al., 2005). Consistent with a direct action of kisspeptin on GnRH cells, a large proportion of GnRH cells co-express GPR54 mRNA in mice and rats (Han et al., 2005; Irwig et al., 2004; Messenger, Chatzidakis, Ma, Hendrick, Zahn, Dixon, Thresher, Malinger, Lomet, Carlton, Colledge, Caraty, and Aparicio, 2005).

In addition direct actions on GnRH neurons, kisspeptin may also act on pituitary gonadotrophs; kisspeptin can induce LH secretion in pituitary explants maintained in culture (Navarro et al., 2005). Given that acyline treatment blocks the effects of kisspeptin on gonadotropin secretion *in vivo*, kisspeptin is unlikely to act directly on the pituitary as its principal mechanism of action (Matsui et al., 2004). Although findings on kisspeptin influences on pituitary function are equivocal, the GPR54 receptor is, in fact, expressed in pituitary (Kotani et al., 2001; Muir et al., 2001). Whether kisspeptin regulates pituitary hormones other than the gonadotropins or whether GPR54 binds ligands other than kisspeptin requires further study.

Kisspeptin Expression and Functional Implications

Kisspeptin cell bodies identified by *in situ* hybridization are concentrated in the anteroventral-periventricular nucleus (AVPV) and arcuate nucleus (Arc), with scattered cells in the periventricular and anterodorsal preoptic nuclei (Gottsch et al., 2004; Smith, Clifton, and Steiner, 2006; Smith, Dungan, Stoll, Gottsch, Braun, Eacker, Clifton, and Steiner, 2005b). The availability of satisfactory antibodies against kisspeptin has precluded the ability to trace projections from kisspeptin cells (Brailoiu, Dun, Ohsawa, Yin, Yang, Chang, Brailoiu, and Dun, 2005; Kinoshita, Tsukamura, Adachi, Matsui, Uenoyama, Iwata, Yamada, Inoue, Ohtaki, Matsumoto, and Maeda, 2005). In reports of kisspeptin peptide expression, immunohistochemical labeling does not overlap with that of *Kiss-1* mRNA

expression patterns. Whereas *Kiss-1* expression occurs in the Arc and AVPV, cell bodies expressing kisspeptin peptide are typically seen in the DMH with some reports of labeling in the Arc. Because several amino acids on the C-terminus of RFamide peptides are often identical, cross-reactivity among antisera with related RFamide peptides is likely. RFRP-1 and RFRP-3 stain robustly in the DMH with marked fiber projections in the Arc nucleus (Fukusumi et al., 2001; Hinuma et al., 2000; Ukena et al., 2002; Ukena and Tsutsui, 2001; Yano, Iijima, Kakihara, Hinuma, Tanaka, and Ibata, 2003; Yoshida et al., 2003), suggesting that previous kisspeptin antibodies may cross-react with these related RFRPs.

As with mammalian RFRP, kisspeptin may mediate the negative feedback effects of sex steroids. Kisspeptin cells co-localize sex-steroid receptors, making them a direct target for sex steroid actions (Smith, Cunningham, Rissman, Clifton, and Steiner, 2005a; Smith et al., 2005b). In the Arc, castration/replacement has predictable effects; kisspeptin mRNA is increased following castration and decreased following sex steroid replacement (Smith et al., 2005a; Smith et al., 2005b). The opposite pattern is seen in the AVPV following the same manipulations. These findings suggest a possible role of the Arc nucleus in mediating negative feedback. The response of kisspeptin cells in the AVPV to castration and steroid replacement is incompatible with a role in sex steroid negative feedback, but consistent with a role in positive feedback effects. Further experiments are necessary to tease apart the role of both cell populations in regulating reproductive function. The availability of specific antibodies to allow tracing work to progress will provide important insight into the contribution of each kisspeptin cell population to GnRH function.

Integration and Dual Control by Kisspeptin/GnIH: A Working Model

Kisspeptin and GnIH act as pronounced opposing regulators of the reproductive axis, and the potential exists for these peptides to work in harmony to achieve precise control over reproductive function (Figure 7). The internal milieu and external environments likely impinge on both systems to allow stimulation of kisspeptin and inhibition of GnIH when conditions are favorable for reproduction and the opposite response when conditions are unfavorable. By applying this dual level of control, precision can be gained beyond that which can be achieved by either system acting alone.

In a recent insightful review, Steiner and colleagues propose that the kisspeptin system acts as an integration point for GnRH secretagogues (e.g., glutamate and vasopressin) and metabolic stimuli (e.g., leptin and glucocorticoids) (Dungan, Clifton, and Steiner, 2006). The potential exists for GnIH to integrate inhibitory signals (e.g., GABA and opiates) to regulate the negative arm of control. It also remains possible that kisspeptin and GnIH respond to both excitatory and inhibitory signals with appropriate actions, summate the contribution of each, and then produce the appropriate response. By each system responding to both positive and negative stimuli in addition to crosstalk between the systems, a more accurate depiction of current conditions can be relayed to the GnRH system for final integration and analysis (Figure 7). In summary, both of these RFamide peptides are in a position to play important, previously-unspecified roles in reproductive function. Enormous potential exists for exploring the relationship between these peptides and their interactions with other known regulators of the reproductive axis.

Acknowledgments

I am indebted to my graduate mentor, Randy Nelson, and my postdoctoral advisor, Rae Silver, for training and continuing support. I am also thankful to a host of friends and colleagues for valuable discussions and assistance in performing the research presented in this review and throughout my scientific career, including: Michael Antle, George Bentley, David Crews, Gregory Demas, Sean Duffy, Deborah Drazen, Darlene Francis, Andrea Gore, Toshi Hamada, Sabra Klein, Ruslan Korets, Joseph LeSauter, Alex Mason, Dan Feng Mei, Ilia Karatsoreos, Solomon

Snyder, Kazuyoshi Tsutsui, Takayoshi Ubuka, Kazuyoshi Ukena, Lily Yan and Irving Zucker. The social and intellectual support extended to me by many SBN members has been invaluable ever since I first attended the Conference on Reproductive Behavior (CRB) in 1995. I feel privileged and honored to have been selected for the Frank Beach Award. The work presented herein was supported by NIH Grant NS-37919 (RS) and training grant MH-12408 (LJK).

References

- Anderson GM, Steyn FJ, Tsutsui K, Ukena K, Bentley GE, Herbison AE. Is there a gonadotrophin-inhibitory hormone system in mammals? Society for Neuroscience. 2005:HH12.
- Beach F. Behavioral endocrinology and the study of reproduction. *Biol Reprod*. 1974; 10:2–18. [PubMed: 4462814]
- Bentley GE, Jensen JP, Kaur GJ, Wacker DW, Tsutsui K, Wingfield JC. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). *Horm Behav*. 2006; 49(4):550–5. [PubMed: 16460739]
- Bentley GE, Perfito N, Ukena K, Tsutsui K, Wingfield JC. Gonadotropin-inhibitory peptide in song sparrows (*Melospiza melodia*) in different reproductive conditions, and in house sparrows (*Passer domesticus*) relative to chicken-gonadotropin-releasing hormone. *J Neuroendocrinol*. 2003; 15(8): 794–802. [PubMed: 12834441]
- Brailoiu GC, Dun SL, Ohsawa M, Yin D, Yang J, Chang JK, Brailoiu E, Dun NJ. KiSS-1 expression and metastin-like immunoreactivity in the rat brain. *J Comp Neurol*. 2005; 481(3):314–29. [PubMed: 15593369]
- Clarke IJ, Pompolo S. Synthesis and secretion of GnRH. *Anim Reprod Sci*. 2005; 88 (1–2):29–55. [PubMed: 15993013]
- Clements MK, McDonald TP, Wang R, Xie G, O'Dowd BF, George SR, Austin CP, Liu Q. FMRFamide-related neuropeptides are agonists of the orphan G-protein-coupled receptor GPR54. *Biochem Biophys Res Commun*. 2001; 284(5):1189–93. [PubMed: 11414709]
- Curlewis JD, Kusters DH, Barclay JL, Anderson ST. Prolactin-releasing peptide in the ewe: cDNA cloning, mRNA distribution and effects on prolactin secretion in vitro and in vivo. *J Endocrinol*. 2002; 174(1):45–53. [PubMed: 12098662]
- de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A*. 2003; 100(19):10972–6. [PubMed: 12944565]
- Dhillon WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA, Bloom SR. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab*. 2005; 90(12):6609–15. [PubMed: 16174713]
- Dockray GJ, Reeve JR Jr, Shively J, Gayton RJ, Barnard CS. A novel active pentapeptide from chicken brain identified by antibodies to FMRFamide. *Nature*. 1983; 305(5932):328–30. [PubMed: 6137771]
- Dungan HM, Clifton DK, Steiner RA. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. *Endocrinology*. 2006; 147(3):1154–8. [PubMed: 16373418]
- Fukusumi S, Habata Y, Yoshida H, Iijima N, Kawamata Y, Hosoya M, Fujii R, Hinuma S, Kitada C, Shintani Y, Suenaga M, Onda H, Nishimura O, Tanaka M, Ibata Y, Fujino M. Characteristics and distribution of endogenous RFamide-related peptide-1. *Biochim Biophys Acta*. 2001; 1540(3): 221–32. [PubMed: 11583817]
- Fukusumi S, Yoshida H, Fujii R, Maruyama M, Komatsu H, Habata Y, Shintani Y, Hinuma S, Fujino M. A new peptidic ligand and its receptor regulating adrenal function in rats. *J Biol Chem*. 2003; 278(47):46387–95. [PubMed: 12960173]
- Gore AC. Gonadotropin-releasing hormone neurons: multiple inputs, multiple outputs. *Endocrinology*. 2004; 145(9):4016–7. [PubMed: 15321947]
- Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*. 2004; 145(9):4073–7. [PubMed: 15217982]

- Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci*. 2005; 25(49):11349–56. [PubMed: 16339030]
- Hinuma S, Habata Y, Fujii R, Kawamata Y, Hosoya M, Fukusumi S, Kitada C, Masuo Y, Asano T, Matsumoto H, Sekiguchi M, Kurokawa T, Nishimura O, Onda H, Fujino M. A prolactin-releasing peptide in the brain. *Nature*. 1998; 393(6682):272–6. [PubMed: 9607765]
- Hinuma S, Shintani Y, Fukusumi S, Iijima N, Matsumoto Y, Hosoya M, Fujii R, Watanabe T, Kikuchi K, Terao Y, Yano T, Yamamoto T, Kawamata Y, Habata Y, Asada M, Kitada C, Kurokawa T, Onda H, Nishimura O, Tanaka M, Iyata Y, Fujino M. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. *Nat Cell Biol*. 2000; 2(10):703–8. [PubMed: 11025660]
- Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology*. 2004; 80(4):264–72. [PubMed: 15665556]
- Jarry H, Heuer H, Schomburg L, Bauer K. Prolactin-releasing peptides do not stimulate prolactin release in vivo. *Neuroendocrinology*. 2000; 71(4):262–7. [PubMed: 10773746]
- Jhamandas K, Milne B, Sutak M, Gouarderes C, Zajac JM, Yang HY. Facilitation of spinal morphine analgesia in normal and morphine tolerant animals by neuropeptide SF and related peptides. *Peptides*. 2006; 27(5):953–63. [PubMed: 16515821]
- Johnson MA, Bentley GE, Ukena K, Tsutsui K, Fraley GS. DIURNAL DIFFERENCES IN THE EFFECTS OF GONADOTROPIN-INHIBITORY HORMONE ON REPRODUCTION IN THE MALE RAT. *Society for Neuroscience*. 2005:GG28.
- Kaiser UB, Kuohung W. KiSS-1 and GPR54 as new players in gonadotropin regulation and puberty. *Endocrine*. 2005; 26(3):277–84. [PubMed: 16034182]
- Kinoshita M, Tsukamura H, Adachi S, Matsui H, Uenoyama Y, Iwata K, Yamada S, Inoue K, Ohtaki T, Matsumoto H, Maeda K. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. *Endocrinology*. 2005; 146(10):4431–6. [PubMed: 15976058]
- Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*. 2001; 276(37):34631–6. [PubMed: 11457843]
- Kriegsfeld LJ, Mei DF, Bentley GE, Ubuka T, Mason AO, Inoue K, Ukena K, Tsutsui K, Silver R. Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proc Natl Acad Sci U S A*. 2006; 103(7):2410–5. [PubMed: 16467147]
- Lawrence CB, Celsi F, Brennand J, Luckman SM. Alternative role for prolactin-releasing peptide in the regulation of food intake. *Nat Neurosci*. 2000; 3(7):645–6. [PubMed: 10862694]
- Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst*. 1996; 88(23):1731–7. [PubMed: 8944003]
- Lee JH, Welch DR. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. *Cancer Res*. 1997; 57(12):2384–7. [PubMed: 9192814]
- Maruyama M, Matsumoto H, Fujiwara K, Noguchi J, Kitada C, Hinuma S, Onda H, Nishimura O, Fujino M, Higuchi T, Inoue K. Central administration of prolactin-releasing peptide stimulates oxytocin release in rats. *Neurosci Lett*. 1999; 276(3):193–6. [PubMed: 10612638]
- Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. *Biochem Biophys Res Commun*. 2004; 320(2):383–8. [PubMed: 15219839]
- Matsumoto H, Maruyama M, Noguchi J, Horikoshi Y, Fujiwara K, Kitada C, Hinuma S, Onda H, Nishimura O, Inoue K, Fujino M. Stimulation of corticotropin-releasing hormone-mediated adrenocorticotropin secretion by central administration of prolactin-releasing peptide in rats. *Neurosci Lett*. 2000; 285(3):234–8. [PubMed: 10806329]

- Messenger S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci U S A*. 2005; 102(5):1761–6. [PubMed: 15665093]
- Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem*. 2001; 276(31):28969–75. [PubMed: 11387329]
- Navarro VM, Castellano JM, Fernandez-Fernandez R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. *Endocrinology*. 2004; 145(10):4565–74. [PubMed: 15242985]
- Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology*. 2005; 146(1):156–63. [PubMed: 15375028]
- Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*. 2001; 411(6837):613–7. [PubMed: 11385580]
- Osugi T, Ukena K, Bentley GE, O'Brien S, Moore IT, Wingfield JC, Tsutsui K. Gonadotropin-inhibitory hormone in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*): cDNA identification, transcript localization and functional effects in laboratory and field experiments. *J Endocrinol*. 2004; 182(1):33–42. [PubMed: 15225129]
- Panula P, Aarnisalo AA, Wasowicz K. Neuropeptide FF, a mammalian neuropeptide with multiple functions. *Prog Neurobiol*. 1996; 48(4–5):461–87. [PubMed: 8804117]
- Perry SJ, Yi-Kung Huang E, Cronk D, Bagust J, Sharma R, Walker RJ, Wilson S, Burke JF. A human gene encoding morphine modulating peptides related to NPFF and FMRFamide. *FEBS Lett*. 1997; 409(3):426–30. [PubMed: 9224703]
- Price DA, Greenberg MJ. Structure of a molluscan cardioexcitatory neuropeptide. *Science*. 1977; 197(4304):670–1. [PubMed: 877582]
- Samson WK, Keown C, Samson CK, Samson HW, Lane B, Baker JR, Taylor MM. Prolactin-releasing peptide and its homolog RFRP-1 act in hypothalamus but not in anterior pituitary gland to stimulate stress hormone secretion. *Endocrine*. 2003; 20(1–2):59–66. [PubMed: 12668869]
- Satake H, Hisada M, Kawada T, Minakata H, Ukena K, Tsutsui K. Characterization of a cDNA encoding a novel avian hypothalamic neuropeptide exerting an inhibitory effect on gonadotropin release. *Biochem J*. 2001; 354(Pt 2):379–85. [PubMed: 11171117]
- Seal LJ, Small CJ, Dhillon WS, Kennedy AR, Ghatei MA, Bloom SR. Prolactin-releasing peptide releases corticotropin-releasing hormone and increases plasma adrenocorticotropin via the paraventricular nucleus of the hypothalamus. *Neuroendocrinology*. 2002; 76(2):70–8. [PubMed: 12169768]
- Seminara SB, Messenger S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. *N Engl J Med*. 2003; 349(17):1614–27. [PubMed: 14573733]
- Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A*. 2005; 102(6):2129–34. [PubMed: 15684075]
- Smith JT, Clifton DK, Steiner RA. Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling. *Reproduction*. 2006; 131(4):623–30. [PubMed: 16595713]

- Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*. 2005a; 146(9):3686–92. [PubMed: 15919741]
- Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK, Steiner RA. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology*. 2005b; 146(7):2976–84. [PubMed: 15831567]
- Smith MS, Grove KL. Integration of the regulation of reproductive function and energy balance: lactation as a model. *Front Neuroendocrinol*. 2002; 23(3):225–56. [PubMed: 12127305]
- Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF, Ghatei MA, Bloom SR. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. *J Neuroendocrinol*. 2004; 16(10):850–8. [PubMed: 15500545]
- Tsutsui K, Saigoh E, Ukena K, Teranishi H, Fujisawa Y, Kikuchi M, Ishii S, Sharp PJ. A novel avian hypothalamic peptide inhibiting gonadotropin release. *Biochem Biophys Res Commun*. 2000; 275(2):661–7. [PubMed: 10964719]
- Tsutsui K, Ukena K. Hypothalamic LPXRF-amide peptides in vertebrates: identification, localization and hypophysiotropic activity. *Peptides*. 2006; 27(5):1121–9. [PubMed: 16517011]
- Ubuka T, Ukena K, Sharp PJ, Bentley GE, Tsutsui K. Gonadotropin-inhibitory hormone inhibits gonadal development and maintenance by decreasing gonadotropin synthesis and release in male quail. *Endocrinology*. 2006; 147(3):1187–94. [PubMed: 16293662]
- Ukena K, Iwakoshi E, Minakata H, Tsutsui K. A novel rat hypothalamic RFamide-related peptide identified by immunoaffinity chromatography and mass spectrometry. *FEBS Lett*. 2002; 512(1–3): 255–8. [PubMed: 11852091]
- Ukena K, Tsutsui K. Distribution of novel RFamide-related peptide-like immunoreactivity in the mouse central nervous system. *Neurosci Lett*. 2001; 300(3):153–6. [PubMed: 11226634]
- Ukena K, Tsutsui K. A new member of the hypothalamic RF-amide peptide family, LPXRF-amide peptides: structure, localization, and function. *Mass Spectrom Rev*. 2005; 24(4):469–86. [PubMed: 15389843]
- Ukena K, Ubuka T, Tsutsui K. Distribution of a novel avian gonadotropin-inhibitory hormone in the quail brain. *Cell Tissue Res*. 2003; 312(1):73–9. [PubMed: 12712318]
- Yang HY, Fratta W, Majane EA, Costa E. Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. *Proc Natl Acad Sci U S A*. 1985; 82(22):7757–61. [PubMed: 3865193]
- Yano T, Iijima N, Kakiyama K, Hinuma S, Tanaka M, Ibata Y. Localization and neuronal response of RFamide related peptides in the rat central nervous system. *Brain Res*. 2003; 982(2):156–67. [PubMed: 12915251]
- Yin H, Ukena K, Ubuka T, Tsutsui K. A novel G protein-coupled receptor for gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*): identification, expression and binding activity. *J Endocrinol*. 2005; 184(1):257–66. [PubMed: 15642802]
- Yoshida H, Habata Y, Hosoya M, Kawamata Y, Kitada C, Hinuma S. Molecular properties of endogenous RFamide-related peptide-3 and its interaction with receptors. *Biochim Biophys Acta*. 2003; 1593(2–3):151–7. [PubMed: 12581859]
- Zhu LL, Onaka T. Facilitative role of prolactin-releasing peptide neurons in oxytocin cell activation after conditioned-fear stimuli. *Neuroscience*. 2003; 118(4):1045–53. [PubMed: 12732249]

Figure 1.

A schematic in the sagittal plane depicts the location of RFRP-ir cell bodies and their projections (**top**). Beneath the sagittal schematic is a tracing of the rostral-caudal extent of RFRP-ir fiber projections and cell bodies (**bottom**). Note that RFRP-ir cell bodies are clustered in the dorsomedial region of the hypothalamus with diffuse projections throughout most of the brain, with a concentration of terminals in midline brain regions. ac=anterior commissure; AH=anterior hypothalamus; AMG=amygdale; Arc=arcuate nucleaus; BNST=bed nucleus of the stris terminalis; DMH=dorsomedial hypothalamusl; LS=lateral septum; oc=optic chiasm; PAG=periaquaductal grey; POA=preoptic area; PVT=paraventricular nucleus of the thalamus.

Figure 2. Medium-power photomicrograph depicting RFRP-ir cell bodies clustered in the dorsal and ventral regions of the DMH; bar=200 μ m (**A**). The box in the top image outlines the cells bodies shown at high power; bar=100 μ m (**B**).

Figure 3. High-power wide-field (**A**) and confocal (**B**) images from the brains of Syrian hamsters depicting RFRP fiber contacts upon GnRH cells bodies and processes. Both images depict RFRP fibers ‘tracking’ the GnRH fiber and cell body, presumptive boutons are evident.

Figure 4.

As in hamsters, RFRP-ir fibers target GnRH cells in rats (A) and mice (B). Note how RFRP fibers 'track' the GnRH fiber following it to the cell body, with several presumptive boutons along the path. For visibility, images are shown as GnRH (red) alone, RFRP fibers (green) alone, followed by their respective overlays. Images were taken at 1000x at the light level.

Figure 5.

RFRP-ir cells are activated by sex steroid exposure. We pursued the role of mammalian RFRP in modulating estrogen negative feedback because of the importance of this process in regulation of ovulation and the coordination of receptivity. Ovariectomized hamsters were injected with either estradiol (**C, D**) or oil vehicle (**A, B**) and sacrificed 3 h following injection. The percentage of double-labeled RFRP and FOS neurons were counted (see Kriegsfeld et al., 2006). In oil-treated controls, few RFRP cells expressed FOS (**A, B**), while robust expression of FOS was evident in RFRP cells following estradiol treatment (**C, D**).

Figure 6.

The amino acid sequence of the preproprotein that encodes the Syrian hamster GnIH homolog exhibits high homology with other mammalian LPXRFamide peptides and avian GnIH. Alignment of preproproteins that encode RF-amide-related peptides (RFRP-1 and -3) and GnIH-related peptides (GnIH, -RP-1, and -RP-2) in mammals (hamster, rat, and mouse) and birds (quail, white-crowned sparrow). The predicted amino acid sequence for the Syrian hamster GnIH preproprotein homolog exhibits high homology to previously-identified RFRPs in mouse and rat (**A**). The hamster amino acid sequence is highly homologous to the avian GnIH-RP-1 (**A**). Comparison of immunocytochemical staining (**B**) with RNA labeling (**C**) at similar levels of the DMH. Note that both labels exhibit a dorsal and ventral population in the same general pattern of expression. References: Mouse and rat (Hinuma et al., 2000); white-crown sparrow (Osugi et al., 2004); Quail (Satake et al., 2001). Magnification bar=100μm

Figure 7.

A proposed model by which kisspeptin and GnIH/RFRP may respond to internal and external stimuli important for reproduction and subsequently regulate the HPG axis. At the top of the figure, local conditions, nutritional state, social cues, and other internal states signal systems upstream of the GnRH system, including kisspeptin and GnIH/RFRP. These stimuli are then integrated and weighed according to their relative magnitude and immediate importance. The output at this level is determined by the net effect on both systems, with favorable conditions stimulating kisspeptin and inhibiting GnIH/RFRP and unfavorable conditions having the opposite effect. Identified and unexplored (indicated by question marks) mechanisms by which GnIH/RFRP and kisspeptin do/may interact and directly and indirectly influence the reproductive axis are depicted.

Table 1

Primary sequences for RFamide peptides across species.

Peptide	Species	Sequence	Reference
FMRFamide	Clam	FMRF-NH ₂	(Price and Greenberg, 1977)
LPLRFamide	Chicken	LPLRF-NH ₂	(Dockray et al., 1983)
GnIH	Quail	SIKPSAYLPLRF-NH ₂	(Tatsui et al., 2000)
NPF	Human	FLFQPQRF-NH ₂	(Perry et al., 1997)
NPAF	Human	AGEGLSSPFWSLAAPQRF-NH ₂	(Perry et al., 1997)
PrRP20	Human	TPDINPAWYASRGIRPVGRF-NH ₂	(Hinuma et al., 1998)
RFRP-1	Bovine	SLTFEEVKDWAPKIKMNKPVVNKMPPSAANLPLRF-NH ₂	(Fukusumi et al., 2001)
RFRP-3	Bovine	AMAHPLRLGKNREDSLSRWVPNLPQRF-NH ₂	(Yoshida et al., 2003)
Kisspeptin	Human	GTSLSPPPSSGSPQPGLSAPHSRQIPAPQGAFLVQREKDLPNYNWNSFGLRF-NH ₂	(Ohnaki et al., 2001)

Table 2

Functions of Identified RFamide Peptides.

Peptide	Function	Reference
FMRFamide	Cardiac regulation	(Price and Greenberg, 1977)
LPLRFamide	Atrial blood pressure regulation, spinal neuromodulation	(Dockray et al., 1983; Price and Greenberg, 1977)
GnIH	Suppression of LH and FSH, actions on GnRH	(Bentley et al., 2003; Tsutsui et al., 2000)
NPEF	Attenuates morphine-induced analgesia	(Jhamandas et al., 2006; Perry et al., 1997; Yang et al., 1985)
NPAF	Attenuates morphine-induced analgesia	(Jhamandas et al., 2006; Perry et al., 1997; Yang et al., 1985)
RFRP-1(pRP)	Prolactin, CRH, glucocorticoid, oxytocin, and feeding regulation	(Fukusumi et al., 2001; Hinuma et al., 1998; Hinuma et al., 2000; Lawrence et al., 2000; Maruyama et al., 1999; Matsumoto et al., 2000; Samson et al., 2003; Yoshida et al., 2003)
RFRP 1 and/or 3	Suppression of LH, actions on GnRH?	(Kriegsfeld et al., 2006)
Kisspeptin	Stimulation of GnRH, tumor metastasis suppression	(Gottsch et al., 2004; Kotani et al., 2001; Ohtaki et al., 2001; Thompson et al., 2004)