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Driving reproduction: RFamide peptides behind the wheel

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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–NH_2 (RFamide) in regulating the reproductive axis will be highlighted. Neuropeptides containing the C-terminal Phe–Met–Arg–Phe–NH_2 (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 opiatergic 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; Kiss1; Metastin; RFRP; GnIH; Reproductive; Sex; Mating

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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.

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Individual neuropeptides containing the C-terminal Arg–Phe–NH_2 (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 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 30 years ago, the cardioexcitatory neuropeptide containing the C-terminal Phe–Met–Arg–Phe–NH_2 (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 neurochemicals 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–NH_2 (LPLRFamide), was identified in chicken (Dockray et al., 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 (Tables 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 et al., 1996; Perry et al., 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 the endogenous opioid system (Jhamandas et al., 2006; Yang et al., 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 et al., 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 et al., 2002; Jarry et al., 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 et al., 2000; Samson et al., 2003; Seal et al., 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 et al., 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 et al., 2000).

Table 1
Primary sequences for RFamide peptides across species

Peptide	Species	Sequence	Reference
FMRFamide	Clam	FMRF-NH2	(Price and Greenberg, 1977)
LPLRFamide	Chicken	LPLRF-NH2	(Dockray et al., 1983)
GnIH	Quail	SIKPSAYLPLRF-NH2	(Tsutsui et al., 2000)
NPFF	Human	FLFQPQRF-NH2	(Perry et al., 1997)
NPAF	Human	AGEGLSSPFWSLAAPQRF-NH2	(Perry et al., 1997)
PrRP20	Human	TPDINPAWYASRGIRPVGRF-NH2	(Hinuma et al., 1998)
RFRP-1	Bovine	SLTFEEVKDWAPKIKMNKPVVNKMPPSAANLPLRF-NH2	(Fukusumi et al., 2001)
RFRP-3	Bovine	AMAHLPLRLGKNREDSLSRWVPNLPQRF-NH2	(Yoshida et al., 2003)
Kisspeptin	Human	GTSLSPPPESSGSPQQPGLSAPHSRQIPAPQGAVLVQREKDLPNYNWNSFGLRF-NH2	(Ohtaki et al., 2001)