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Evolution of gonadotropin-inhibitory hormone receptor and its ligand

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ABSTRACT

Gonadotropin-inhibitory hormone (GnIH) is a neuropeptide inhibitor of gonadotropin secretion, which was first identified in the Japanese quail hypothalamus. GnIH peptides share a C-terminal LPXRFamide (X = L or Q) motif in most vertebrates. The receptor for GnIH (GnIHR) is the seven-transmembrane G protein-coupled receptor 147 (GPR147) that inhibits cAMP production. GPR147 is also named neuropeptide FF (NPFF) receptor 1 (NPFFR1), because it also binds NPFF that has a C-terminal PQRFamide motif. To understand the evolutionary history of the GnIH system in the animal kingdom, we searched for receptors structurally similar to GnIHR in the genome of six mammals (human, mouse, rat, cattle, cat, and rabbit), five birds (pigeon, chicken, turkey, budgerigar, and zebra finch), one reptile (green anole), one amphibian (Western clawed flog), six fishes (zebrafish, Nile tilapia, Fugu, coelacanth, spotted gar, and lamprey), one hemichordate (acorn worm), one echinoderm (purple sea urchin), one mollusk (California sea hare), seven insects (pea aphid, African malaria mosquito, honey bee, buff-tailed bumblebee, fruit fly, jewel wasp, and red flour beetle), one cnidarian (hydra), and constructed phylogenetic trees by neighbor joining (NJ) and maximum likelihood (ML) methods. A multiple sequence alignment of the receptors showed highly conserved seven-transmembrane domains as well as disulfide bridge sites between the first and second extracellular loops, including the receptor of hydra. Both NJ and ML analyses grouped the receptors of vertebrates into NPFFR1 and NPFFR2 (GPR74), and the receptors of insects into the receptor for SIFamide peptides that share a C-terminal YRKPPFNGSIFamide motif. Although human, quail and zebrafish GnIHR (NPFFR1) were most structurally similar to SIFamide receptor of fruit fly in the Famide peptide (FMRFamide, neuropeptide F, short neuropeptide F, drosulfakinin, myosuppressin, SIFamide) receptor families, the amino acid sequences and the peptide coding regions of GnIH precursors were most similar to FMRFamide precursor of fruit fly in the precursors of Famide peptide families. Chromosome synteny analysis of the precursor genes of human, quail and zebrafish GnIH and fruit fly Famide peptides further identified conserved synteny in vertebrate GnIH and fruit fly FMRFa precursor genes as well as other Famide peptide precursor genes. These results suggest that GnIH and its receptor pair and SIFamide and its receptor pair may have diverged and co-evolved independently in vertebrates and insects, respectively, from their ancestral Famide peptide and its receptor pair, during diversification and evolution of deuterostomian and protostomian species.

1. Introduction

In 2000, a novel hypothalamic neuropeptide was shown to inhibit gonadotropin release from the cultured quail anterior pituitary gland and the peptide was named gonadotropin-inhibitory hormone (GnIH) (Tsutsui et al., 2000). The receptor for GnIH (GnIHR) was identified to be a G protein-coupled receptor (GPCR) 147 (GPR147), which is thought to be coupled to $G_{\alpha i}$ protein (Yin et al., 2005; Son et al., 2012). GnIH neurons not only project to the median eminence to control anterior pituitary function (Tsutsui et al., 2000; Ubuka et al., 2003, 2009b, 2009c; Son et al., 2012; Ciccone et al., 2004; Clarke et al., 2008), but also project to gonadotropin-releasing hormone (GnRH) neurons that express GPR147 in birds and mammals (Ubuka et al., 2008a,b, 2009b, 2009c, 2012a; Kriegsfeld et al., 2006). Accordingly, GnIH can inhibit gonadal activity (Ubuka et al., 2006) by decreasing the activity of GnRH neurons or directly inhibiting gonadotropin secretion from the pituitary (for reviews, see Tsutsui 2009; Tsutsui et al., 2007, 2010a, 2010b, 2012, 2013; Tsutsui and Ubuka, 2012; Ubuka et al., 2008a,b, 2012c, 2013a, 2013c). GnIH expression is regulated by melatonin (Ubuka et al., 2005, 2012a) and glucocorticoids (Son et al., 2014; Kirby et al., 2009), and GnIH release is regulated by norepinephrine according to social context (Tobari

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et al., 2014). A recent study has further shown that GnIH inhibits socio-sexual behavior of birds by stimulating the activity of aromatase in the brain and increasing neuroestrogen synthesis (Ubuka et al., 2014). To understand the evolutionary history of the GnIH system in the animal kingdom, we searched for receptors structurally similar to GnIHR in the genome of 29 bilaterian and 1 cnidarian species. A multiple sequence alignment has shown conserved seven-transmembrane domains as well as disulfide bridge sites in the receptor. Both neighbor joining (NJ) and maximum likelihood (ML) methods have grouped the receptors of vertebrates into two receptor families, neuropeptide FF (NPFF) receptor 1 (NPFFR1) (GPR147) and NPFFR2 (GPR74). The receptors of insects were grouped into SIFamide receptor (SIFR) family. We first summarize the structure and functions of GnIH and GnIHR and discusses their evolutionary histories in terms of co-evolution of receptor–ligand pairs.

2. Structures of GnIH peptides

2.1. Discovery of GnIH peptides in birds

GnIH was first discovered in the brain of the Japanese quail, *Coturnix japonica*, while searching for a novel RFamide peptide. RFamide peptides are peptides that have an Arg-Phe-NH_2 motif at their C-terminus, which were first isolated in invertebrate species in the late 1970s. The structure of the first RFamide peptide was Phe-Met-Arg-Phe-NH_2 (FMRFamide), which was a cardioexcitatory molecule isolated from the ganglia of the venus clam *Macrocallista nimbosa* (Price and Greenberg, 1977). After the discovery of FMRFamide peptide, numerous RFamide peptides that act as neurotransmitters, neuromodulators and peripheral hormones have been identified in various invertebrates, including annelids, molluscs, nematodes, arthropods, and cnidarians. Because some FMRFamide-immunoreactive (-ir) neurons projected close to the pituitary gland in vertebrates, it was suggested that RFamide peptides may regulate pituitary function in vertebrates.

In 2000, Tsutsui et al. (2000) isolated a novel RFamide peptide from 500 quail brains by high-performance liquid chromatography (HPLC) and an enzyme-linked immunosorbent assay using an antibody raised against the dipeptide Arg-Phe-NH_2 (Tsutsui et al., 2000). Amino acid sequence analysis has identified the structure of the isolated peptide to be SIKPSAYLPLRFamide (Table 1). Its C-terminus was identical to chicken LPLRFamide, which was reported to be the first RFamide peptide in vertebrates (Dockray et al., 1983). Because the isolated peptide was localized in the hypothalamo-hypophysial system by immunohistochemistry, and it decreased gonadotropin release from cultured quail anterior pituitary glands, this RFamide peptide was named GnIH (Tsutsui et al., 2000).

A cDNA that encoded the GnIH precursor polypeptide was identified by a combination of 3′ and 5′ rapid amplification of cDNA ends (3′/5′ RACE) (Satake et al., 2001). GnIH precursor consisted of 173 amino acids that encoded one GnIH and two GnIH-related peptides (GnIH-RP-1 and GnIH-RP-2). These peptides all possessed an LPXRFamide (X = L or Q) sequence at their C-termini (for reviews, see Ukena and Tsutsui, 2005; Tsutsui and Ukena, 2006). GnIH-RP-2 was also identified as a mature peptide by mass spectrometry in quail (Table 1; Satake et al., 2001). Endogenous GnIH peptides were further identified in European starlings (Ubuka et al., 2008a,b) and zebra finch (Tobari et al., 2010) within the class of birds (Table 1; for reviews, see Tsutsui 2009; Tsutsui et al., 2007, 2010a, 2010b, 2012, 2013; Tsutsui and Ubuka, 2012; Ubuka et al., 2008a,b, 2012c, 2013a, 2013c).

2.2. Identification of GnIH peptides in mammals

In mammals, cDNAs encoding LPXRFamide peptides have been investigated by a gene database search (Hinuma et al., 2000). Hinuma et al. (2000) have named mammalian LPXRFamide peptides as RFamide-related peptides (RFRPs) from their structure. Mammalian LPXRFamide precursor cDNAs encode two LPXRFamide peptides (RFRP-1 and -3) and the RFRP-2 sequence was lost or different from LPXRFamide. Human RFRP-1 and -3 (Ubuka et al., 2009c), macaque RFRP-3 (Ubuka et al., 2009b), bovine RFRP-1 (Fukusumi et al., 2001) and -3 (Yoshida et al., 2003), rat RFRP-3 (Ukena et al., 2002), Siberian hamster RFRP-1 and -3

Table 1
Identified endogenous LPXRFa (X = L or Q) peptides in vertebrates.

	Animal	Name	Sequence	Reference
Mammals	Human	RFRP-1	MPHSFAN**LPLRFa**	Ubuka et al. (2009c)
		RFRP-3	VPN**LPQRFa**	Ubuka et al. (2009c)
	Macaque	RFRP-3	SGRNMEVSLVRQVLN**LPQRFa**	Ubuka et al. (2009b)
	Bovine	RFRP-1	SLTFEEVKDWAPKIKMNKPVVNKMPPSAAN**LPLRFa**	Fukusumi et al. (2001)
		RFRP-3	AMAHLPLRLGKNREDSLSRWVPN**LPQRFa**	Yoshida et al. (2003)
	Rat	RFRP-3	ANMEAGTMSHFPS**LPQRFa**	Ukena et al. (2002)
	Hamster	RFRP-1	SPAPANKVPHSAAN**LPLRFa**	Ubuka et al. (2012a)
		RFRP-3	TLSRVPS**LPQRFa**	Ubuka et al. (2012a)
Birds	Quail	GnIH	SIKPSAY**LPLRFa**	Tsutsui et al. (2000)
		GnIH-RP-2	SSIQSLLN**LPQRFa**	Satake et al. (2001)
	Starling	GnIH	SIKPFAN**LPLRFa**	Ubuka et al. (2008a)
	Zebra finch	GnIH	SIKPFSN**LPLRFa**	Tobari et al. (2010)
Amphibians	Frog	fGRP/R-RFa	SLKPAAN**LPLRFa**	Koda et al. (2002), Chartrel et al. (2002)
		fGRP-RP-1	SIPN**LPQRFa**	Ukena et al. (2003b)
		fGRP-RP-2	YLSGKTKVQSMAN**LPQRFa**	Ukena et al. (2003b)
		fGRP-RP-3	AQYTNHFVHSLDT**LPLRFa**	Ukena et al. (2003b)
	Newt	nLPXRFa-1	SVPN**LPQRFa**	Chowdhury et al. (2011)
		nLPXRFa-2	MPHASAN**LPLRFa**	Chowdhury et al. (2011)
		nLPXRFa-3	SIQPLAN**LPQRFa**	Chowdhury et al. (2011)
		nLPXRFa-4	APSAGQFIQTLAN**LPQRFa**	Chowdhury et al. (2011)
Fish	Goldfish	gfLPXRFa-3	SGTGLSAT**LPQRFa**	Sawada et al. (2002b)
	Lamprey	lLPXRFa-1a	SGVGQGRSSKTLFQ**PQRFa**	Osugi et al. (2012)
		lLPXRFa-1b	AALRSGVGQGRSSKTLFQ**PQRFa**	Osugi et al. (2012)
		lLPXRFa-2	SEPFWHRTR**PQRFa**	Osugi et al. (2012)

The C-terminal LPXRFamide (X = L or Q) motifs are shown in bold.