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A role of Histidine^{151} in the lamprey gonadotropin-releasing hormone receptor-1 (lGnRHR-1): Functional insight of diverse amino acid residues in the position of Tyr of the DRY motif in GnRHR from an ancestral type II receptor

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ABSTRACT

The highly conserved DRY motif located at the end of the third transmembrane of G-protein-coupled receptors has been described as a key motif for several aspects of GPCR functions. However, in the case of the vertebrate gonadotropin-releasing hormone receptor (GnRHR), the amino acid in the third position in the DRY motif is variable. In the lamprey, a most basal vertebrate, the third amino acid of the "DRY" in lamprey (lGnRHR-1) is His, while it is most often His/Gln in the type II GnRHR. To investigate the functional significance of the substitution of DRY to DRH in the GnRHR-1, second messenger signaling, ligand binding and internalization of the wild-type and mutant lGnRH receptors were characterized with site-directed mutagenesis. Treatment of the DRE^{151} and DRS^{151} mutant receptors with lamprey GnRH-I significantly reduced inositol phosphate compared to wild-type (DRH^{151}) and DRY^{151} receptors. The Log IC_{50} of wild-type receptor (-9.554 ± 0.049) was similar to the Log IC_{50} of DRE^{151}, DRS^{151} and DRX^{151} mutants, yet these same mutants were shown to significantly reduce cell-surface expression. However, the DRY^{151} mutant compared to the wild-type receptor increased cell-surface expression, suggesting that the reduction of IP production was due to the level of the cell-surface expression of the mutant receptors. The rate of internalization of DRX^{151} (35.60%) was reduced compared to wild-type and other mutant receptors. These results suggest that His^{151} of the lamprey GnRH receptor-1 may play a critical role in the retention of a certain level of cell-surface expression for subsequent cellular second messenger events.

1. Introduction

The hypothalamic–pituitary (HP) system is considered to be a vertebrate innovation and seminal event that emerged prior to or during the differentiation of the ancestral agnathans, reviewed in Sower et al. (2009). In spite of the very diverse patterns of life cycles and reproductive strategies and behaviors, this endocrine system is remarkably conserved throughout the gnathostome lineages. Lampreys as basal vertebrates are the earliest evolved vertebrates for which there are demonstrated functional roles for two possibly three gonadotropin-releasing hormones (GnRHs) that act via the hypothalamic–pituitary–gonadal axis controlling reproductive processes (Kavanaugh et al., 2008). To date, the biochemical, molecular, immunocytochemical and functional studies on the structure and function of the GnRHs in lamprey have established that similar to all other vertebrates, the lampreys have a hypothalamic–pituitary–gonadal axis and that there is a high conservation of the mechanisms of GnRH action (Sower et al., 2009). From recent data, we propose a modified paradigm in that the neuroendocrine control of reproduction and thyroid functions in an Agnathan, the sea lamprey, exhibits an overlapping, simplified organization represented by one and possibly two glycoprotein hormones putatively interacting with two glycoprotein receptors, a gonadotropin-like receptor and a thyroid stimulating hormone-like receptor (Sower et al., 2009). This modified paradigm now includes the agnathans and can serve as a model for analysis of the evolutionary mechanisms leading to emergence of the highly specialized gnathostome endocrine axes.

GnRH is a central regulator of reproductive function in vertebrates and acts via the hypothalamic–pituitary–gonadal axis. Its function is mediated through a pituitary GnRH receptor (GnRHR), a class A 7-transmembrane G-protein-coupled receptor (GPCR). One of the remarkable characteristics of vertebrate GnRHRs is the absence or presence of the intracellular C-terminal tail. The receptors are generally categorized as two different types: type I receptor, lacking the intracellular C-terminal tail, or type II receptor, retaining the C-terminal tail (Millar et al., 2004). The other notable characteristic of GnRHRs is the variation of a DRY motif of GPCRs that is a highly conserved amino acid triplet at the end or junction of the third transmembrane domain and the second

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intracellular loop. DRY has been described as a key motif for several aspects of GPCR functions including receptor activation, ligand binding and G-protein coupling (Rovati et al., 2007). There are variable substitutions of the third amino acid in the "DRY" motif of GnRHRs from different classes of vertebrates. This region potentially contributes to GnRHR function. In many cases, type I receptor DRY motif is substituted with DR'S', while type II has DR'H/Q' (Fig. 1). To date, there are few reports about the functional significance of the Ser in DRS of type I receptors (Arora et al., 1995, 1997; Byrne et al., 1999). Thus, the functional significance of this variation of the DRY motif, particularly the type II GnRHR, is not established.

In the sea lamprey, *Petromyzon marinus*, one of only two extant representatives of the oldest lineage of vertebrates, agnathans, a functional type II GnRH receptor was cloned from the pituitary (Silver et al., 2005). The lamprey GnRH receptor-1 (lGnRHR-1) was shown to activate both the cAMP and IP signaling systems; however, the IP system was activated at an approximately 10-fold lower concentration to both lamprey GnRH-I and lamprey GnRH-III, and was also activated to a greater magnitude of approximately 4.5-fold, compared to ~1.7-fold (lamprey GnRH-I) or ~2.1-fold (lamprey GnRH-III) (Silver and Sower, 2006). These responses of IP_3 and cAMP signaling systems are similar to the type I and type II GnRH receptors from different vertebrate species (Arora et al., 1998; Grosse et al., 2000; Liu et al., 2002; Oh et al., 2005; Stanislaus et al., 1998). In addition, the lGnRHR-1 retains conserved structural features and amino acid motifs of other known GnRH receptors. An HFRK motif in the membrane proximal region of the bullfrog type II GnRH receptor-1 was shown to be required for cAMP signaling, but not for IP signaling (Oh et al., 2005). Similarly a homologous motif, HVRR, was shown also to be required for cAMP signaling in the lGnRHR-1 (Silver and Sower, 2006). In the lamprey, the third amino acid residue of the DRY motif in GnRH receptor is "His" instead of "Tyr" and in the gnathostome type II GnRH receptors, the third amino acid residue is most often His/Gln instead of Tyr. These identified conserved motifs in the lamprey GnRH receptor are thought to be evolutionarily conserved throughout vertebrates. Thus, the lGnRHR-1, an ancestral form of vertebrate GnRHRs, is a critical model in our understanding of the structural and functional evolution of GnRHR. To investigate the functional significance of the substitution of DRY to DRH in the lGnRHR-1, second messenger signaling, ligand binding and internalization of the wild-type and mutant lGnRHRs were characterized with site-directed mutagenesis. The His^{151} of DRH was substituted with Tyrosine (DRY^{151} mutant), Serine (DRS^{151} mutant), Glutamate (DRE^{151} mutant), or point deletion (DRX^{151} mutant).

2. Materials and methods

2.1. Site-directed mutagenesis

The point mutations were introduced into the pcDNA3.1 vectors (Invitrogen, Carlsbad, CA, USA) including the full coding sequence of lGnRHR-1 by Quikchange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) using mutagenic primers as follows: DRY^{151} mutant was amplified using the sense primer, 5′-GCTTGGA CCGCTACTCGGCCATCCTC-3′ and the antisense primer, 5′-GAGGAT GGCCGAGTAGCGGTCCAAGC-3′. The sense primer, 5′-GCTTGGACC GCGAGTCGGCCATCCTC-3′ and the antisense primer, 5′-GAGGA TGGCCGACTCGCGGTCCAAGC-3′ were used for DRE^{151} mutant, the sense primer, 5′-GCTTGGACCGCTCATCGGCCATCCTC-3′ and the antisense primer, 5′-GAGGATGGCCGATGAGCGGTCCAAGC-3′ were for DRS^{151} mutant, and the sense primer, 5′-CAGCTTGGACCGCTCG GCCATCCTCA-3′ and the antisense primer, 5′-TGAGGATGGCCG AGCGGTCCAAGCTG-3′ were for DRX^{151} mutant. The underlined nucleotides indicate the sequences encoding the substituted amino acids (Fig. 2). PCR was performed with 2 ng of the pcDNA3.1 including the full coding sequence of lGnRHR-1 as the template

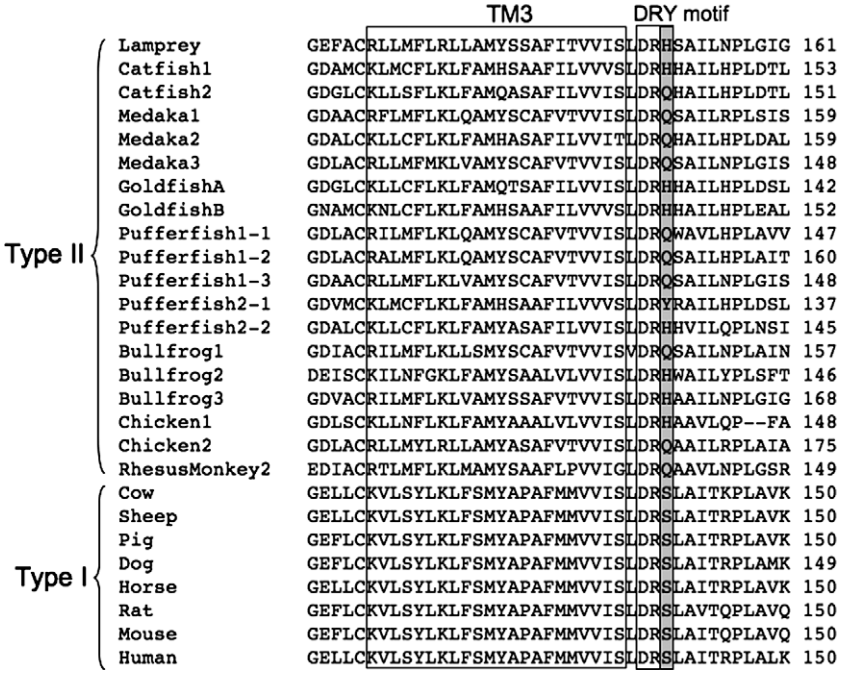

Fig. 1. Sequence alignment of the proximal region of TM3 and DRY motif in GnRHR from representative vertebrate species. The alignment was arranged with the entire amino acid sequences of the receptors based on Clustal W. The accession numbers for species are followed: Lamprey (AAQ04564), Catfish1 (O42329), Catfish2 (AAM95605), Medaka1 (NP_001098352), Medaka2 (NP_001098392), Medaka3 (NP_001098393), GoldfishA (AAD20001), GoldfishB (AAD20002), Pufferfish1-1 (BAE45695), Pufferfish1-2 (BAE45697), Pufferfish1-3 (BAE45699), Pufferfish2-1 (BAE45701), Pufferfish2-2 (BAE45704), Bullfrog1 (AAG42575), Bullfrog2 (AAG42949), Bullfrog3 (AAG42574), Chicken1 (NP_989984), Chicken2 (NP_001012627), RhesusMonkey2 (NP_001028014), Cow (NP_803480), Sheep (NP_001009397), Pig (NP_999438), Dog (NP_001003121), Horse (NP_001075305), Rat (NP_112300), Mouse (NP_034453), Human (NP_000397).