General and Comparative Endocrinology 192 (2013) 222–236

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Evolutionary origin and divergence of the growth hormone receptor family: Insight from studies on sea lamprey

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ARTICLE INFO

Article history:
Available online 28 May 2013

Keywords:
Agnatha
Gnathostomata
Growth hormone receptor
Prolactin receptor
Molecular evolution
Ligand–receptor interactions

ABSTRACT

Sea lamprey, one of the oldest extant lineages of vertebrates, Agnatha, was used to clarify the evolutionary origin and divergence of the growth hormone receptor (GHR) family. A single full-length cDNA encoding a protein that shares amino acid identity with GHRs and prolactin receptors (PRLRs) previously characterized from teleost fish was identified. Expression of the GHR/PRLR-like transcript was widespread among tissues, including brain, pituitary, heart, liver, and skeletal muscle, which is consistent with the broad physiological roles of GH-family peptides. Phylogenetic analysis suggests that the lamprey possess an ancestral gene encoding a common GHR/PRLR that diverged to give rise to distinct GHRs and PRLRs later in the course of vertebrate evolution. After the divergence of the Actinopterygian and Sarcopterygian lineages, the GHR gene was duplicated in the Actinopterygian lineage during the fish-specific genome duplication event giving rise to two GHRs in teleosts, type 1 GHR and type 2 GHR. A single GHR gene orthologous to the teleost type 1 GHR persisted in the Sarcopterygian lineage, including the common ancestor of tetrapods. Within the teleosts, several subsequent independent duplication events occurred that led to several GHR subtypes. A revised nomenclature for vertebrate GHRs is proposed that represents the evolutionary history of the receptor family. Structural features of the receptor influence ligand binding, receptor dimerization, linkage to signal effector pathways, and, ultimately, hormone function.

1. Introduction

Growth hormone (GH), prolactin (PRL), and somatolactin (SL) are structurally-related hormones that have become well known for their multifunctional natures. GH, for example, regulates numerous aspects of growth, metabolism, reproduction, osmoregulation, immune function, and behavior (Forsyth and Wallis, 2002; Björnsson et al., 2004; Norrelund, 2005; Norbeck et al., 2007; Møller and Jørgensen, 2009). Approximately 300 actions have been reported for prolactin, with the most well-known being iono-osmoregulation and stimulation of milk production in mammals (Kaneko and Hirano, 1993; Wongdee and Charoenphandhu, 2012). Somatolactin, found only in teleost fish to date, appears to play a role in energy homeostasis, sexual maturation, and background color adaptation (Vega-Rubín de Celis et al., 2004; Porollo and Meller, 2007; Benedet et al., 2008; Cánepa et al., 2012). Although GH, PRL and SL are all produced by distinct cells in the pituitary, there is evidence of extra-pituitary production of all three proteins (Ben-Jonathan et al., 1996; Yang et al., 1997; Imaoka et al., 2000; Harvey, 2010). GH is a single-chain polypeptide roughly 21–22 kDa in size, and shares structural similarities with the 22–25 kDa PRL-protein and the roughly 23–24 kDa SL-protein (Rand-Weaver et al., 1992; Cavari et al., 1995; Law et al., 1996; Yang et al., 1997; Yang and Chen, 2003; Li et al., 2007; Benedet et al., 2008; Wang et al., 2010). Crystal structures of GH and PRL reveal that both have a four α-helical bundle conformation (Rowlinson et al., 2008; Agthoven et al., 2010), and although a crystal structure of SL has not been obtained, our sequence comparison suggests a structural model similar to that of GH and PRL. To date, GH but neither PRL nor SL have been detected in Agnathans, suggesting that GH is the ancestral hormone of the GH family and that the emergence of PRL and SL resulted from gene duplication events during the evolution of vertebrates (Kawauchi and Sower, 2006).

The biological actions of GH, PRL, and SL result from interactions with their receptors, which also appear to be structurally related and belong to the type-1 cytokine receptor superfamily. Like all members of the this superfamily, GH receptor (GHR) family proteins are single-spanning transmembrane proteins, which possess an extracellular domain involved in receptor–receptor dimerization and hormone-binding, a single transmembrane domain, and an intracellular domain that connects the receptor to downstream

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http://dx.doi.org/10.1016/j.ygcen.2013.05.008

effector pathways (Brooks et al., 2008). Based on studies in mammals, it appears that two receptors dimerize ($receptor_1$–$receptor_2$) to become a functional receptor complex which is then able to bind hormone (Biener et al., 2003; Brooks et al., 2008; Broutin et al., 2010). In the past decade, numerous GHR-family mRNAs have been characterized. A review of GenBank reveals some 1200 sequences for GHRs and 1700 sequences for prolactin receptors (PRLRs); although, over 50 sequences each for GHRs and PRLR sequences for teleosts have been reported, most sequences are for tetrapods, and no information has been reported from basal vertebrate groups (i.e., Agnathans, Chondrichtians, Holosteians, and basal teleosts such as Osteoglossomorhpha). The existence of multiple types of GHRs and PRLRs (and in some cases subtypes) in teleosts supports the notion that the GHR family arose through a series of gene duplication events (Fukamachi and Meyer, 2007); however, considerable confusion over GHR nomenclature also has emerged, particularly with regard to the classification of a distinct SL receptor (SLR). In this study we used sea lamprey (*Petromyzon marinus*), one of the oldest extant lineages of vertebrates, Agnatha, to clarify the evolutionary origin and divergence of the GHR family.

2. Materials and methods

2.1. Experimental animals

Adult sea run lamprey was collected from the Cocheco River fish ladder in Dover, NH from May 22–29, 2008 during their spawning migration from the sea to fresh water. The lamprey were then transported to the Anadramous Fish and Invertebrate Research Laboratory in Durham, NH, where they were maintained in an artificial spawning channel with flow-through fresh water at ambient temperatures (9–18 °C) and natural photoperiod following the protocol reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee.

2.2. RNA extraction

Total RNA was extracted using RNAzol RT as specified by the manufacturer (Molecular Research Center, Inc., Cincinnati, OH, USA). Isolated total RNA was re-hydrated in RNase-free water and eluted over oligo dT beads from the GenElute™ mRNA Miniprep kit (Sigma–Aldrich, St. Louis, MO, USA) to isolate mRNA. mRNA was quantified by UV spectrophotometry (A_{260}), diluted to 100 ng/μl, and stored at −80 °C for further analysis.

2.3. Oligonucleotide primers

Gene-specific primers used for isolation of cDNAs were designed by examining known GHR sequences using GeneTool software (BioTools, Inc., Edmonton, AB) and custom synthesized by Sigma–Genosys (The Woodlands, TX, USA). Additional primers for reverse transcription were provided in the SMARTER™ RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA, USA). Gene-specific oligonucleotide primers used for real-time PCR of GHR were designed using ABI Primer Express® Version 2 software, based upon the sequence of our determined GHR sequence. Primers and probes were used for reverse transcription and PCR without further purification.

2.4. Isolation and characterization of GHR-like mRNAs

A three-phase approach was adopted for the isolation of a GHR-encoding cDNA using reverse-transcription (RT)-PCR and rapid amplification of cDNA ends (RACE)-PCR. Isolation of the cDNA sequence was accomplished using the SMARTER™ RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA, USA) following the manufacturer's protocol. In Phase I, mRNA from sea lamprey liver was reverse transcribed into cDNA containing the sequence tags necessary for SMARTER™ chemistry, and remaining cycles of PCR were carried out using a gene-specific (5′-GCCCGGATTACGTCACTGC-3′) primer designed for 3′ RACE. After an initial denaturation at 95 °C for 3 min, a 35 cycle-PCR was performed with each cycle consisting of denaturation (95 °C for 30 s), annealing (65 °C for 30 s) and extension (72 °C for 30 s) steps; in the last cycle, the extension time was increased to 10 min. The PCR products were identified by electrophoresis on an agarose gel containing 1% of each OmniPur (EMD chemicals, Gibbstown, NF, USA) and NuSieve GTG agarose (Bio-Wittaker Molecular Applications, Rockland, ME, USA) in 1× Tris–borate-EDTA (TBE) buffer followed by ethidium bromide staining. The PCR products were then cloned into the pGEM-T Easy Vector (Promega, Madison, WI, USA) and inserted into JM109 cells. Purified plasmids (75 fmol) were sequenced using the CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's protocol. Initial attempts at 5′ RACE, immediately after obtaining sequence from Phase 1 were unsuccessful; therefore, we adopted RT-PCR (Phase 2) in order to obtain more sequence, extending off the known 3′ end of the mRNA, so that new gene-specific primers could be designed for 5′ RACE.

In phase 2, after determining that the initial PCR reaction resulted in a unique cDNA with reasonable sequence identity with known GHR-encoding cDNAs, a unique set of primers were designed for RT-PCR (forward, 5′-CTGCTGGCGTGATGACATT-3′; reverse, 5′-GGCCCCGCAGTGACGTAAT-3′). The forward primer was designed based on a review of coding regions of known GHR sequences together with ESTs that became available in the lamprey genome during the course of our characterization of the phase 1 product; the reverse primer for RT-PCR was designed based on the Phase 1 product. The resulting Phase 2 PCR product was visualized, cloned into the pGEM-T Easy Vector, and sequenced as described previously.

After obtaining the additional sequence in phase 2, new gene-specific primers were designed and used in Phase 3, 5′ RACE. 5′-RACE was performed using a SMARTER™ RACE cDNA Amplification Kit under manufacturer's suggested conditions with the GHR gene-specific primer (5′-GCAGACTCGTTCGCCAGGG-3′) designed for 5′ RACE. The resulting PCR products were visualized, cloned into the pGEM-T Easy Vector, and sequenced as described previously.

2.5. Quantification of GHR-encoding mRNA

2.5.1. Preparation of cDNA standards

Approximately 1 μg of known cDNA product was used as template for PCR with forward and reverse gene-specific primers (primers used in phase 2). Following an initial denaturation cycle of 94 °C for 5 min, 35 PCR cycles were performed; each consisting of 1 min denaturation (94 °C), 1 min of annealing (42 °C), and 1 min of extension (72 °C). In the last cycle, the extension time was increased to 10 min to ensure complete extension. The resulting PCR products were visualized under ultraviolet light, cloned into the pGEM-T easy vector, and their sequences verified as described previously. The cDNA standard (purified from cloned plasmid containing the cDNA) was used to generate the standard curve. With the sequence known, the cDNA (standard) was quantified by UV (A_{260}) spectrophotometry and converted to molecule number based upon molecular weight and was then serially diluted. The average threshold cycle number (C_T) value from each dilution was plotted against the absolute amount of standard in the sample to generate the standard curve.

2.5.2. Real-time reverse transcription PCR

The previously purified mRNA (ca. 100 ng), was reverse transcribed in a 10 μL reaction using AffinityScript QPCR cDNA Synthe-