Review

The origins of the vertebrate hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–thyroid (HPT) endocrine systems: New insights from lampreys

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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. Reproduction in vertebrates is controlled by a hierarchically organized endocrine system. 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. To date, our biochemical, molecular, immunocytochemical and functional studies on the structure and function of the GnRHs in lampreys 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. 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 receptors. In 1977, Fontaine and Burzawa-Gerard proposed that a common ancestral molecule in gnathostomes gave rise to two subunits α and β via gene duplication (Fontaine and Burzawa-Gerard, 1977). The first glycoprotein hormone (α – β) was gonadotropic but later had both gonadotropic and thyrotropic functions. The identity of any glycoprotein hormone in an agnathan was unknown at that time (Fontaine and Burzawa-Gerard, 1977). Thus, our 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.

2. Background: lamprey genome project

As an agnathan, the oldest extant lineage of vertebrates, the sea lamprey has become a model system for analysis of the evolution
of many genes and systems including the evolution of the neuroendocrine regulation of reproduction (Sower, 2003; Freamat et al., 2006; Kawauchi and Sower, 2006; Silver and Sower, 2006) and the evolution of development (EvoDev) (Kuratani et al., 2002). Lampreys as basal vertebrates were identified in a key position such that the mapping of the lamprey genome started in Jan 2005 (http://www.genome.gov/12511858). Nine non-mammalian organisms were chosen by NIH for mapping the genome, each of which represents a position on the evolutionary timeline marked by important changes in animal anatomy, physiology, development or behavior (NIH News Release August 4, 2004). Based on the proposed size of the lamprey genome approximately 1/3 the size of the human genome, it is estimated that the current coverage of the genome is about 5.9××, which infers that much of the genome has been sequenced and available for analysis using the trace archives and the partially assembled genome by Ensemble. The phylogenetic position of lampreys as a basal vertebrate allows lampreys to be a basis for understanding the molecular evolution of the genes encoding receptors and hormones that arose in the vertebrates.

3. Background: identified components of the reproductive neuroendocrine axis in lampreys

Lampreys are the earliest evolved vertebrates for which there are demonstrated functional roles for two possibly three GnRHs that act as neurohormones controlling the reproductive processes. To date, biochemical, molecular, immunocytochemical and functional studies on the structure and function of the GnRHs in lampreys have established that similar to all other vertebrates, the lamprey has a hypothalamic–pituitary–gonadal axis and that there is a high conservation of the mechanisms of GnRH action (Sower, 2003; Kavanaugh et al., 2008). Generally, gnathostomes have one or two GnRHs that act as hypothalamic hormones, two pituitary gonadotrophins luteinizing hormone, LH, and follicle-stimulating hormone, FSH, and one gonadal FSH receptor and one LH receptor compared to the lamprey that have two, possibly three, hypothalamic GnRHs, only one known pituitary gonadotropin and one gonadal glycoprotein receptor (Fig. 1). To date, the identification of the primary amino acid and cDNA sequences of two forms of GnRH, lamprey GnRH-I and –III, a novel GnRH (lamprey GnRH-II), the cDNA of one GnRH receptor as well as the identification of one pituitary gonadotropin-β have been completed (Sherwood et al., 1986; Sower et al., 1993, 2006; Suzuki et al., 2000; Silver et al., 2004, 2005; Kavanaugh et al., 2008). The lamprey GnRH receptor identified shares several characteristics of both type-I and type-II vertebrate GnRH receptors (Silver et al., 2005; Silver and Sower, 2006). The high conservation of the GnRH and its receptor throughout vertebrate species makes the lamprey model highly appropriate for examining the GnRH system in terms of its ligands and novel receptors. Similarly, the recent identification of a novel GnRH, lamprey gonadotropin (GTH) β (Sower et al., 2006) and two glycoprotein receptor, a GTH-like receptor and a TSH-like receptor (Freamat et al., 2006; Freamat and Sower, 2008), provides an opportunity for comparative and evolutionary analysis of the neuroendocrine system in vertebrates.

4. Vertebrate phylogeny

The phylogenetic relationship between hagfish, lamprey and the jawed vertebrates is an unresolved issue (Kuraku et al., 1999; Delarbre et al., 2002). In this paper, agnathans are considered to

Fig. 1. Schematic representation of the hypothalamic–pituitary–gonadal axes in jawless vertebrates, lampreys compared to jawed vertebrates, the gnathostomes. This diagram emphasizes that lampreys have two, possibly three, hypothalamic GnRHs (lamprey GnRH-II (Type 2) and Type 4 GnRH: GnRH-I, GnRH-III); one pituitary glycoprotein (GTH-like) hormone and one gonadal glycoprotein receptor (LH, FSH like receptor) compared to one GnRH (type I) and/or two GnRHs (Type II and/or III); two pituitary gonadotropsins (LH, FSH) and two glycoprotein receptors.
be monophyletic in origin with the modern agnathans classified into two groups, myxinoids (hagfish) and petromyzonids (lamprey); while the gnathostomes constitute all the other living vertebrates, including the bony and cartilaginous fishes and the tetrapods. In 2002, Janvier and his collaborators based on analysis of the complete mitochondrial DNA suggested that lamprey and hagfish form a clade (Delarbre et al., 2002). Most recently, Ota et al. (2002) of the complete mitochondrial DNA suggested that lamprey and tetrapods. In 2002, Janvier and his collaborators based on analysis of the complete mitochondrial DNA suggested that lamprey and hagfish form a clade (Delarbre et al., 2002). Most recently, Ota et al. (2002) of the complete mitochondrial DNA suggested that lamprey and hagfish form a clade (Delarbre et al., 2002). It is generally believed that two large-scale genome duplications (2R) occurred during the evolution of early vertebrates, although there is controversy on whether the 2R duplications occurred before the divergence of the hagfish and lampreys or whether there was one round prior to the jawless vertebrates and one round after the divergence of the jawless vertebrates (Ohno, 1970; Holland et al., 1994; Irvine et al., 2002; Larhammar et al., 2002; Fried et al., 2003; Vandepoele et al., 2004; Kuraku et al., 2009). Further information on the evolution of vertebrate brain/pituitary hormones and their genes in lamprey and hagfish can contribute to the ongoing phylogenetic analysis that may help in resolving the phylogenetic relationships between hagfish, lamprey and jawed vertebrates.

5. Reproductive and metamorphic cycle of the lampreys

There are approximately 40 species of lamprey that are classified as parasitic or non-parasitic (Sower and Gorbman, 1999). Lamprey spawns only once in their lifetime, after which they die, thus sexual maturation is a synchronized process coordinated with the life stages of the lamprey. The sea lamprey begin their lives in freshwater as blind, filter-feeding larvae. After approximately five to seven years in freshwater streams, metamorphosis occurs and the ammocoetes become free-swimming, sexually immature lamprey. Lamprey metamorphosis is a highly synchronized and programmed process that involves major physiological and morphological changes, reviewed in Youson and Sower (2001). Following metamorphosis, lamprey migrates to the sea or lakes for an approximately 15 to 24 month-long parasitic sea phase. After this period at sea, lamprey return to freshwater streams and undergo the final maturational processes resulting in mature eggs and sperm, spawning and then death.

6. Gonadotropin-releasing hormone (GnRH)

The synthesis and secretion of GnRH is the key neuroendocrine function in the hypothalamic regulation of the hypothalamic–pituitary–gonadal axis. In response to GnRH, gonadotropins (GTH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are released from the pituitary gland, which in turn regulate gametogenesis and steroidogenesis. The GnRH family currently now includes 28 isoforms, 15 and 13 from representative vertebrate and invertebrate species, respectively (Kavanaugh et al., 2008; Tsai and Zhang, 2008; Zhang et al., 2008). Sixteen GnRH and/or their respective cDNAs have been determined by Professor Nancy Sherwood along with her collaborators and students (Sherwood et al., 1983, 1986; Lovejoy et al., 1992; Ngamwongchon et al., 1992; Powell et al., 1994, 1996; Carolsfeld et al., 2000; Adams et al., 2002, 2003). To date, two to three isoforms have been identified in representative species of all classes of vertebrates (Gorbman and Sower, 2003; Giguur et al., 2006; Kah et al., 2007; Kavanaugh et al., 2008; Okubo and Nagahama, 2008). Growing evidence reveals almost all vertebrates synthesize at least two isoforms of GnRH in the brain (Dubois et al., 2002; Silver et al., 2004; Giguur et al., 2006; Kah et al., 2007; Kavanaugh et al., 2008; Okubo and Nagahama, 2008). During the past few years, as more GnRH primary sequences and their respective cDNAs have been identified, more phylogenetic and functional studies have been done leading to proposed phylogenetic relationships of the GnRHs (Grober et al., 1995; Fernald and White, 1999; Parhar, 2002; Morgan and Millar, 2004; Silver et al., 2004; Giguur et al., 2006; Kah et al., 2007; Zhang et al., 2008). Early analyses by Grober et al. (1995) and Fernald and White (1999), suggested that there are three paralogous groups of GnRH in gnathostome brains. In recent reviews of GnRH and respective receptors, various scenarios on the phylogenetic relationships among just the gnathostomes GnRHs and receptors have been suggested (Giguur et al., 2006; Kah et al., 2007; Okubo and Nagahama, 2008). We had shown from an extensive analysis available cdna GnRH sequences including the eight cloned lamprey GnRH-I and -III cDNAs, that the vertebrate GnRHs are grouped into four paralogous lineages: GnRH I, 2, 3 and 4 (Silver et al., 2004). We based our analysis on phylogenetic analysis, function, neural distribution and developmental origin (Silver et al., 2004). This phylogenetic analysis confirmed the earlier model of three GnRH lineages and in addition showed that lamprey GnRH-I and -III form a fourth group. The identification of two more invertebrate mollusc GnRHs and one in Annelid has suggested a fifth grouping of GnRHs (Aplysia, octopus, Limpet, Annelid GnRH) in invertebrates and supports the fourth group of lamprey GnRH-I and -III (Tsai and Zhang, 2008; Zhang et al., 2008) (Fig. 2A). However, the phylogenetic relationships of the lamprey type 4 (lamprey GnRH-I and -III) to the gnathostome GnRHs was unclear from these studies. From the information to date on the identification of the GnRHs there is likely an ancestral GnRH that gave rise to the GnRH lineages in the deuterostome and proteomes (Tsai and Zhang, 2008) (Fig. 2A). Much further information and identification of GnRH will be required across the metazoans to elucidate the evolution of GnRH.

Recently, a cDNA encoding a preproGnRH was cloned from the sea lamprey (Kavanaugh et al., 2008). The deduced amino acid sequence of the newly identified lamprey GnRH-II in lamprey is QHWSHGWFPG. As with all other vertebrate GnRHs, the lamprey GnRH-II is highly conserved with 10 amino acids and conserved C- and N-termini. Similar to GnRH-II expression in gnathostomes, lamprey GnRH-II was shown to be widely distributed and expressed in a number of tissues. In the brain, lamprey GnRH-II was shown to be located in POA/hypothalamus by in situ hybridization and immunocytochemistry. In contrast to the type 2 GnRH generally found in the midbrain of gnathostomes and generally acting as a non-hypothalamic hormone in gnathostomes, lamprey GnRH-II may have a role as a third hypothalamic form in lampreys. In summary, the newly discovered lamprey GnRH-II offers a new paradigm of the origin of the vertebrate GnRH family. Likely due to a genome/gene duplication event, an ancestral gene gave rise to two lineages of GnRHs—the gnathostome GnRH and lamprey GnRH-II (Fig. 2B). The gene duplication events that generated the different fish and tetrapod paralogous groups likely took place within the gnathostome lineage, after its divergence from the ancestral agnathans. Lamprey GnRH-I and -II (type 4) can be identified now as paralogous homologs of gnathostome GnRH and lamprey GnRH-II group (type 2), resulting from a duplication within the lamprey lineage. This implies that there probably was a genome/gene duplication event that gave rise to all forms of vertebrate GnRH affecting the common ancestor of lamprey and gnathostome isoforms. The gnathostome branch of the lamprey GnRH-I and -III orthologous group was probably lost during evolution. Future studies on the identification of GnRH and its cDNA as well as the functions and distribution in hagfish, chondrichthyes and amphioxus will be necessary to have a fuller understanding of the evolution of chordate GnRH.
Fig. 2. A diagram representing the evolution of GnRH in metazoans (A) and chordates (B). (A) Distinct GnRH lineages have been identified in metazoans including GnRH in Tunicates; Mollusks and Annelids; and Vertebrates. From the information to date on the identification of the GnRHs there is likely an ancestral GnRH that gave rise to the GnRH lineages in the deuterostome and protostomes. (B) In the vertebrates and likely due to a genome/gene duplication event, an ancestral gene gave rise to two lineages of GnRHs—the gnathostome GnRH and lamprey GnRH-II. The gene duplication events that generated the different fish and tetrapod paralogous groups likely took place within the Gnathostome lineage, after its divergence from the ancestral agnathans. Lamprey GnRH-I and -III (Type 4) can be identified now as paralogous homologs of Gnathostome GnRH and lamprey GnRH-II group, resulting from a duplication within the lamprey lineage. This implies that there probably was a genome/gene duplication event that gave rise to all forms of vertebrate GnRH affecting the common ancestor of lamprey and Gnathostome isoforms. The Gnathostome branch of the lamprey GnRH-I and -III orthologous group was probably lost during evolution. Distinct GnRH lineages are depicted as follows: tunicates (purple circle); mollusks and annelids, Type 5 GnRH (orange circle); mammalian GnRH and orthologs, Type 1 GnRH (brown circle); chicken GnRH-II and lamprey GnRH-II, Type 2 GnRH (green circle); salmon GnRH, Type 3 GnRH (light blue circle); and lamprey GnRH-I and -III, Type 4 GnRH (dark blue circle). 1R, 2R and 3R indicate whole genome duplications.
7. GnRH receptor (GnRH-R)

At the anterior pituitary, GnRH action is mediated through high-affinity binding with the GnRH receptor, a class A or rhodopsin-like seven transmembrane G-protein coupled receptor (GPCR). GnRH receptors have been classified typically as type-I (without a C-terminal tail) and type-II (with a C-terminal tail) (Silver et al., 2005; Guilgur et al., 2006). The type-I GnRH receptor is unique among all GPCRs in that these receptors lack the highly conserved intracellular carboxy-terminal (C-terminal) tail, which has been shown to be a vital structural element required for several key functions, such as G-protein coupling, second messenger activation, ligand binding, cell surface expression and ligand-dependant (Koenig and Edwards, 1997; Heding et al., 1998; Blomenrohr et al., 1999; Bockaert et al., 2003; Ronacher et al., 2004). Since the first successful cloning of a GnRH receptor transcript from the mouse (Tsutsumi et al., 1992), over 83 GnRH receptor cDNAs have been cloned (Silver et al., 2005). A full-length transcript was isolated and cloned encoding a functional type-II GnRH receptor from the pituitary of the sea lamprey (Silver et al., 2005). This study was the first to identify a pituitary GnRH receptor transcript in an agnathan, which is the oldest vertebrate lineage and considered basal to all vertebrates. The cloned receptor retains the conserved structural features and amino acid motifs of other known GnRH receptors and notably includes a C-terminal intracellular tail of ~120 amino acids, the longest C-terminal tail of any vertebrate GnRH receptor identified to date. The presence of a C-terminal tail has been suggested to be an ancestral feature since the identified GnRH receptors in protochordate, Ciona intestinalis, and amphioxus have C-terminal tails (Kusakabe et al., 2003; Tello et al., 2005; Sherwood et al., 2007).

The lamprey GnRH receptor was initially shown to activate the inositol phosphate (IP) signaling system; stimulation with either lamprey GnRH-I or lamprey GnRH-III led to dose dependent responses in transiently transfected COS-7 cells. Expression of the receptor transcript was demonstrated in the pituitary and testes using RT-PCR, while in situ hybridization showed expression and localization of the transcript in the proximal pars distalis of the pituitary (Silver et al., 2005). The phylogenetic placement, structural and functional features of this GnRH receptor suggested that it is representative of an ancestral GnRH receptor. The extensive C-terminal tail of this lamprey GnRH receptor may have great significance for understanding the evolutionary change of this vital structural feature within the GnRH receptor family.

In recent functional studies, the lamprey GnRH receptor was also shown to activate the cAMP signaling system, which required the first 40 amino acids of the C-terminal tail (Silver and Sower, 2006). Pharmacological profiling provided evidence that the lamprey GnRH receptor is lamprey GnRH-III selective, which supports the hypothesis that lamprey express a second, lamprey GnRH-I selective receptor. Truncations of the lamprey GnRH receptor’s C-terminal tail were shown to reduce binding affinity. Two addition partial/complete clones of two more GnRH receptors have been identified in lamprey (Accession Nos.: DQ915103, DQ915102/ EF166083, EF166082). Once these GnRH receptors have been cloned, structure–function activities of the lamprey GnRHs can be performed with the transfected GnRH receptors and distribution studies can be done to better understand the role of each of the receptors in relation to the GnRH in the lamprey neuroendocrine axis. Comparisons and phylogenetic analyses then can be done on the molecular evolution of GnRH receptors and their respective ligands.

8. Thyrotropin-releasing hormone (TRH)

In mammals, thyrotropin releasing hormone (TRH) is considered a major hypothalamic hormone that acts on the pituitary to stimulate the synthesis and release of thyrotropin hormone (a member of the pituitary glycoprotein family) that in turn acts on the thyroid gland to stimulate the synthesis and/or release of the thyroid hormones, thyroxine and triiodothyronine. TRH has also been shown to release pituitary growth hormone (GH) (Guillemin, 1978; Schally, 1978) and prolactin (PRL) (Jackson and Reichlin, 1977). The role of TRH in non-mammalian vertebrates is less established although it has been shown to activate PRL, TSH, GH and α-melanocyte-stimulating hormone (α-MSH) from the pituitary in amphibians and frogs, reviewed in Del Carmen de Andres et al. (2002). In addition and as reviewed in De Groef et al. (2006), corticotropin-releasing hormone (CRH) has also been shown to active pituitary TSH release in non-mammalian vertebrates and is suggested that CRH might be a common regulator of both the thyroidal and adrenal/interrenal axes (De Groef et al., 2006). A potential role of CRH as a pituitary GpH regulator in lampreys has yet to be investigated.

To date, there are only two reports on the presence of TRH in lampreys. Youngs et al. (1985) showed that TRH content was present in the pituitary, brain and spinal cord of larval and adult sea lampreys and adult river lampreys as determined by radioimmunoassay. A more extensive immunocytochemistry study was done in which the distribution of TRH mainly occurred in the preoptic region and the hypothalamus in large larvae and adult upstream migrating sea lamprey (Del Carmen de Andres et al., 2002). There are no reports that have examined the biological activity of TRH in lampreys. In 1985, Sower et al. (1985) reported that adult lampreys treated with a partly purified salmon gonadotropin or a GnRH analog significantly elevated plasma thyroxine. It was hypothesized from these studies, that one hypothalamic GnRH stimulated both the pituitary–thyroid and pituitary gonadal axes. In later studies, lamprey GnRH-I and –III were shown to be significantly correlated with the seven stages of lamprey metamorphosis (Younson and Sower, 2001). Unlike the induction of frog metamorphosis and even amphioxus metamorphosis by thyroid hormones (Denver, 2008), thyroid hormones do not stimulate the process of metamorphosis in lamprey. Metamorphosis in sea lampreys is characterized by a significant decline in thyroid hormones, changes in lipid metabolism and elevated GnRH, reviewed in Youson and Sower, 2001. Subsequently, to date, we have determined that lampreys likely have only one pituitary glycoprotein (GTH) and one gonadal glycoprotein receptor and one thyroid glycoprotein receptor. Therefore, our working hypothesis is that the glycoprotein hormone/glycoprotein hormone receptor systems emerged as a link between the neuro–hormonal and peripheral control levels during the early stages of gnathostome divergence.

9. Gonadotropin (GTH)

The glycoprotein hormone family consists of GTHs, LH and FSH and one thyroid-stimulating hormone (TSH). The α subunit is common within a single species (Kawauchi, 1989). The β subunits are homologous and convey hormone specificity (Kawauchi, 1989; Swanson, 1991; Huhtaniemi, 2005). Two GTHs have been identified in all taxonomic groups of gnathostomes (Suzuki et al., 1988; Kawauchi, 1989; Querat et al., 2000, 2004; Huhtaniemi, 2005). To date, only one GTH has been identified in jawless vertebrates (agnaths) from a collaboration with Professor Kawauchi, his laboratory and my laboratory (Sower et al., 2006). We have identified the first and perhaps only gonadotropin β-like protein by cDNA cloning in sea lamprey (Sower et al., 2006). Because the sea lamprey GTH β protein is a clear out-group compared to those of the LH and FSH family based on phylogenetic analysis, we had proposed that an ancestral glycoprotein hormone gave rise to only one GTH in lampreys and to the glycoprotein hormone family that gave rise to LH, FSH and TSH during the early evolution of gnathostomes. A
fifth heterodimeric glycoprotein hormone (after FSH, LH, TSH and chorionic gonadotropin, CG) was discovered in 2002 and termed “thyrostimulin” due to its thyroid-stimulating activity (Nakabayashi et al., 2002). However, the thyrostimulin α subunit, called GPA2, is homologous but not identical to the common α subunit (GPA1 or α) in the other glycoprotein hormones. With the discovery of GPA2 (Thyrostimulin-α) and GPB5 (Thyrostimulin-β) homologs in invertebrates (including Drosophila melanogaster), it is proposed by Sudo et al. (2005) that an ancestral heterodimeric glycoprotein hormone existed before the divergence of vertebrates/invertebrates, and that a later gene duplication event in vertebrates produced the thyrostimulin (GPA2 & GPB5) and gonadotropin/thyrotropin (GPA1 & LHβ/FSHβ/TSHβ). Our preliminary evidence to date shows only one glycoprotein α subunit in lampreys that has higher similarity with mammalian GPA2 compared to the GPA1 (α) subunits. Despite extensive research by both molecular cloning and blasting the lamprey genome, we have found no evidence of a GPA2 in vertebrates yet we now have preliminary data for a GPB5. Thus, it appears that lampreys may have an ancestral GPA2, GPβ and GPB5, but this will have to be confirmed by cloning.

Our current working hypothesis is that the glycoprotein hormone subunit A2 identified is the ancestral α subunit; after the gnathostome–agnathan divergence, gene duplications produced the two α (GPA1 and GPA2) subunits and four β subunits (FSHβ, LHβ, TSHβ) (Fig. 3) and potentially lampreys may have two GpHs: GPA2/β and/or GPA2/GPB5.

10. Glycoprotein receptors (GpH-R)

Gonadotropin and thyrotropin hormone actions are mediated through a subfamily of G-protein coupled receptors (GPCR), namely the glycoprotein hormone receptors (GpH-Rs) (Combarnous, 1992). Known GpH-Rs share a number of unique features. They are composed of two of functionally distinct modules of similar size, an extracellular N-terminal domain followed by a prototypical GPCR segment. The extracellular N-terminal domain is primarily responsible for high-affinity hormone binding and contains a central portion of nine Leu-rich repeat (LRR) motifs, flanked by N- and C-terminal Cys-rich clusters. The C-terminal half of the receptor contains a transmembrane region with seven α-helices, connected by intra and extracellular loops and an intracellular C-terminal domain (Grossmann et al., 1997; Dufau, 1998; Ascoli et al., 2002; Moyle et al., 2005). To date, approximately 79 GpH-Rs have been identified and described in 36 different species, mostly in mammals and also in three species of birds, two species of reptiles, one amphibian, and ten species of fish (Hovergen Database, http://pbl.univ-lyon1.fr).

Until recently, there had been no GpH-Rs described in any species of agnathans. We have identified one functional glycoprotein hormone receptor (Igph-R I) (Freamat et al., 2006) from lamprey testis and second functional Igph-R (Igph-R II) expressed mainly in the thyroid tissue (Freamat and Sower, 2008). We hypothesize that Igph-R I and Igph-R II are the only members of the glycoprotein hormone receptor subfamily in lamprey (Freamat and Sower, 2008). They are descendents of the thyrotropin receptor-like molecular ancestors of the GpH-Rs in Gnathostomes and are likely the result of the genome duplication event hypothesized to have taken place before the divergence of lamprey lineage (Sidow, 1996; Kuratani et al., 2002).

The 719-amino acid full-length cDNA encoding Igph-R I is highly similar and likely a homolog of the vertebrate GpH-Rs (including LH, FSH and TSH receptors) (Freamat et al., 2006). The key motifs, sequence comparisons and characteristics of the identified GpH-R reveal a mosaic of features common to all other classes of GpH-Rs in vertebrates. The Igph-R I was shown to activate the cAMP signaling system using hCG in transiently transfected COS-7 cells. The highest expression of the receptor transcript was demonstrated in the testes using reverse transcriptase-PCR. The high expression of Igph-R I in the testis and the high similarity with gnathostome gonadotropin hormone receptors suggest that Igph-R I functions as a receptor for lamprey gonadotropin hormones.

The second glycoprotein hormone receptor (Igph-R II) in the agnathan sea lamprey has 781 residues protein and was approximately 43% identical with mammalian TSH–R and FSH–R representative sequences (Freamat and Sower, 2008). Similar to these two classes of mammalian receptors, Igph-R II is assembled from 10 exons. A synthetic ligand containing the lamprey glycoprotein hormone β chain upstream of a mammalian α chain activated the Igph-R II expressed in COS-7 cells but to a lesser extent than Igph-R I. The most obvious feature of the coding sequence of Igph-R II is the presence of a long linker fragment (SSD or ‘hinge’) located in between the Leu Rich Domain (LRD) of the extracellular segment (ED) and the transmembrane domain (TMD). This is one of the longest linker fragments described so far in all vertebrate glycoprotein hormone receptors. This is in contrast with the similar region of the Igph-R I which is the shortest SSD/hinge segment amongst all vertebrate GpH-Rs (Freamat et al., 2006). Molecular phylogenetic analysis of vertebrate GpH–R protein sequences suggests a closer relationship between Igph-R II and Gnathostome thyrotropin receptors.

Therefore, at this point, a comparative perspective on this endocrine compartment in lamprey relative to the gnathostome well established paradigm suggests the involvement of one pituitary glycoprotein hormone (only β subunit was found) and two glycoprotein hormone receptors as opposed to three or four dimeric hormones and three receptors in gnathostomes. This role of the GpH/GpH-R system in lamprey has yet to be confirmed experimentally. This requires identification and characterization of a GpH α chain homolog. The existence of a second GpH ligand in lamprey with a distinct binding specificity to GpH-R I and II can not be excluded but if it exists it is likely even less similar with gnathostome sequences than the one already described (Freamat and Sower, 2008). From the studies completed to date, we hypothesize that there is lower specificity of gonadotropin and its receptor in agnathans and that during co-evolution of the ligand and its receptor in gnathostomes, there were increased specificities of interactions between each GpH (TSH, LH and FSH) and its receptor (Fig. 3).

11. Summary

Reproduction in vertebrates is controlled by a hierarchically organized endocrine system. In spite of the very diverse patterns of life cycles, reproductive strategies and behaviors, this endocrine system is remarkably conserved throughout the vertebrates. The reproductive physiology of vertebrates is controlled ultimately by the tissue and temporal profiles of gonadotropin receptor genes expression, by the specificity of this expression as well as by the selectivity of interactions of gonadotropin receptors with the glycoprotein hormone ligands. Other factors which have been shown to affect the functional parameters of the gonadotropin receptors and of glycoprotein hormone receptors in general are the interactions between receptors on the cell membrane, their constitutive activity in relationship with receptor activation by the cognate ligands and the regulation of receptor biogenesis by mechanisms acting at the post-transcriptional level. Most of these processes are well understood in more
evolved vertebrates, particularly in mammals or are under intense study. In lamprey, however, little is known about these mechanisms and their understanding has a particularly important significance, especially from an evolutionary point of view. The hypothalamic–pituitary–gonadal axis in particular has an interesting position due to its closeness, in terms of molecular factors involved to hypothalamic–pituitary–thyroid axis. This similarity is remarkably strong at the pituitary-peripheral gland level, where both pathways involve protein hormones from the glycoprotein hormone family, acting on similar glycoprotein hormone receptors. Therefore, the reproductive and thyroid endocrinology of lamprey can be considered a picture of the situation at the time when these two systems were emerging, a picture, however, modified during the hundreds of millions of years of independent evolution of the modern agnathans (Fig. 4). Understanding the exquisite architecture and functional precision of these two systems from an evolutionary point of view are important not only in the study of the mechanisms of evolutionary change of the sequences of the protein components of these pathways but also in the regulatory mechanisms that lead to the spatial and temporal specificity of their expression as well as their relationships with other endocrine systems. Further comparative studies on the GnRH–GTH proteins and respective receptors will help provide clues on the evolution of reproductive mechanisms and insights into our understanding of gene duplication, structure–activity relations and the molecular evolution and functional diversity of these hypothalamic–pituitary systems.

Fig. 3. This figure depicts a proposed evolution of the glycoprotein subunits and glycoprotein receptor in vertebrates. (Bottom right) We hypothesize that IGpH-R I and IGpH-R II are the only members of the glycoprotein hormone receptor subfamily in lamprey (Freamat and Sower, 2008). They are descendants of the thyrotopin receptor-like molecular ancestors of the GpH-Rs in Gnathostomes and are likely the result of the genome duplication event hypothesized to have taken place before the divergence of lamprey lineage. (Bottom left) Ancestral glycoprotein subunits (GPA, α) and (GPB5, β) likely existed in a common ancestor of invertebrates and vertebrates (Sudo et al., 2005). We propose that an ancestral glycoprotein hormone gave rise to only one, possibly two glycoproteins (GpH) in lampreys and to the glycoprotein hormone family that gave rise to LH, FSH and TSH during the early evolution of gnathostomes. Lampreys may have one ancestral GPA2 and one, possibly two β subunits: GPA1 and/or GPB5 (unpublished data). From the studies completed to date, we hypothesize that there is lower specificity of gonadotropin and its receptor in agnathans and that during co-evolution of the ligand and its receptor in gnathostomes, there were increased specificities of interactions between each GpH (TSH, LH and FSH) and its receptor, depicted by the increase of shading from left to right. Thyrostimulin, another glycoprotein, is depicted by B5/A2 GpH. To date, thyrostimulin has only been shown to interact with TSH-R in gnathostomes. The question marks indicate the possible interactions of the subunits of the lamprey GpHs that are currently under investigation.
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References


Fig. 4. We hypothesize that the HPG and HPT endocrine systems evolved from an ancestral, pre-vertebrate exclusively neuroendocrine mechanism by gradual emergence of the components of a new control level (glycoprotein hormones/glycoprotein hormone receptors) concomitantly with the development of the corresponding anatomical structure (pituitary). The endocrine control of reproductive and thyroid functions in lamprey may reflect an intermediary stage on the evolutionary pathway to the highly specialized Gnathostome HPG and HPT axes.


