Research paper

Expression of two glycoprotein hormone receptors in larval, parasitic phase, and adult sea lampreys

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1. Introduction

The hypothalamic-pituitary (HP) system is specific to the vertebrates, which are classified into two major groups: the gnathostomes (jawed vertebrates) and the agnathans (jawless vertebrates). Lampreys and hagfish are the only two extant members of the oldest lineage of vertebrates, the agnathans. In all vertebrates, reproduction is regulated by the hypothalamic-pituitary-gonadotropic (HPG) axis (Sower et al., 2015). The hypothalamic-pituitary-thyroid (HPT) axis generally regulates metabolism, development, and other related processes in gnathostomes (Sower et al., 2015).

Sea lampreys (Petromyzon marinus) are considered to have a more primitive HP axis that is representative of an evolutionarily intermediate during the development of the pituitary from the extinct agnathans to the later evolved gnathostomes (Sower et al., 2009; Sower, 2015). This is in part due to the evolutionary relationships of vertebrate gonadotropin releasing hormones (GnRHs), glycoprotein hormones (GpHs), and GpH receptors (GpH-Rs). Generally, jawed vertebrates have one or two types of hypothalamic GnRHs (with some exceptions in teleost fish), two gonadotropic pituitary GpHs with two corresponding gonadal receptors, and one thyrotropic pituitary GpH with one corresponding thyroid receptor. In comparison, lampreys have three hypothalamic type 2 or 3 GnRHs (GnRH-I and -II, Type 3; GnRH-II, Type 2), at least one, but likely two glycoprotein hormones (lGpH and l-thyrostimulin), and two functional glycoprotein receptors, lGpH-R I and II. It is not known at this time whether there is a specific receptor for lGpH and l-thyrostimulin, or if both GpHs can differentially activate the lGpH-Rs. In this report, we determined the RNA expression of lGpH-R I and II in the gonads and thyroids of larval, parasitic phase, and adult lampreys. A highly sensitive dual-label fluorescent in situ hybridization technique (RNAscope™) showed lGpH-R I expression in the ovaries of larval lamprey, and co-localization and co-expression of lGpH-R I and II in the ovaries of parasitic phase and adult lampreys. Both receptors were also highly co-localized and co-expressed in the endostyle of larval lamprey and thyroid follicles of parasitic and adult lampreys. In addition, we performed in vivo studies to determine the actions of lamprey gonadotropin releasing hormones (lGnRHs) on lGpH-R I and II expression by real time PCR, and determined plasma concentrations of estradiol and thyroxine. Administration of lGnRH-III significantly (p < 0.01) increased lGpHR II expression in the thyroid follicles of adult female lampreys but did not cause a significant increase in RNA expression of lGpH-R I and II in ovaries. Concomitantly, there was a significant increase (p < 0.01) of plasma estradiol without any significant changes of plasma thyroxine concentrations in response to treatment to lGnRH-I, -II, or -III. In summary, our results provide supporting evidence that the lamprey pituitary glycoprotein hormones may differentially activate the lamprey GpH-Rs in regulating both thyroid and gonadal activities during each of the three life stages of the sea lamprey.

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et al., 2006). Similarly, the lamprey lGpH-Rs formed a sister group with the gnathostome TSH-Rs following phylogenetic analysis (Freamat and Sower, 2008a). However, the two lamprey GpH-Rs cannot be ascribed to any one of the LH-R, FSH-R, or TSH-Rs based on extensive structural analyses that shows that each of the two lGpH-Rs have characteristics of each of the gnathostome GpH receptors (Freamat et al., 2006; Freamat and Sower, 2008a,b). Although there are some similarities, such as structural features of lGpH-R I that are closer to LH-R and FSH-R (gonadotropin) receptors, while lGpH-R II seems more similar with the TSH-Rs (Freamat and Sower, 2008a,b). lGpH-R II has the longest signal specificity domain (SSD) region similar to a TSH-R, while lGpH-R I has the shortest SSD of any GpH-R.

Current phylogenetic analyses suggest that a single LH-R and FSH-R, and two TSH-Rs may have been the GnR-R repertoire in gnathostome ancestors, followed by loss of one TSH-R prior to 2R and a local duplication of LH-R leading to the actinopterygian lineages (Buechi and Bridgham, 2017; Maugars and Dufour, 2015; Maugars et al., 2014). It is unknown if duplication of lamprey GpH-Rs occurred before or during the evolution of the lamprey lineage (Freamat and Sower, 2008a). The proposed evolution of these hormone/receptor systems supported in part by these data led to the hypothesis that lampreys have functionally overlapping HPG and HPT axes (Sower et al., 2015; Sower et al., 2009).

Recently, a novel pituitary cell type was identified in the lamprey pituitary, called a proto-glycotope, which showed triple co-localization and co-expression of the subunits for lGpH (lGpA2 and lGpH1) and I-thyrostimulin (lGpA2 and lGpB5) in the pituitaries of adult lampreys, while the proto-glycotropes of larval and parasitic phase lampreys only showed dual co-localization and co-expression for the subunits of I-thyrostimulin (Marquis et al., 2017). The presence or absence of lGpH in larval and parasitic phase lampreys will need further examination. The question remains on how these two pituitary GpHs differentially regulate gonadal and thyroidal activity via the two receptors. At this time, it is not known whether there are specific receptors for lGpH and I-thyrostimulin, or whether both GpHs can differentially activate each of the lGpH-Rs. To gain a better understanding of the distribution of GpH-Rs, we examined the RNA expression of lGpH-R I and II in the ovaries and thyroid in lampreys at each of the three major life stages (larval, parasitic phase, and adult).

Sea lampreys begin their lifecycle as freshwater, filter-feeding larvae (ammoctoetes), undergo a dramatic metamorphosis and transition to salt-water phase parasitic lampreys, mature into adults, and return to freshwater streams where they spawn and die (Hardisty and Potter, 1971). Lamprey metamorphosis involves extensive morphological and physiological changes in which the larval lampreys develop into parasitic phase ocean-going lampreys. A major feature of metamorphosis relevant to this study is the transformation of the larval endostyle (sub-pharyngeal gland) into functional, mature thyroid follicles. The endostyle is the invertebrate homolog of the vertebrate thyroid gland, and is only found in protochordates and larval lamprey. It is positioned ventrally to the esophagus, containing glandular tracts for mucus production to aid in nutrient breakdown and absorption, and cells with iodide concentrating capacity used for thyroid hormone synthesis (Marine, 1913; Manzon and Manzon, 2017). The larval lamprey endostyle consists of five cell types: types I, II, III, IV, and V (Fig.1) (Marine, 1913). The changes of the thyroidal morphology and function in larval, parasitic, and adult stages of the sea lamprey life cycle coincide with distinct, synchronous reproductive stages that are controlled by environmental cues and governed by the neuroendocrine axis (Sower et al., 2009). However, there are still many unknown questions on how the HPG and HPT axes differentially regulate reproduction and thyroid processes during these key life stages of lampreys.

Compared to gnathostomes, there is very little known of the HPT axis in its regulation of thyroid activities in either hagfish or lampreys (reviewed in Sower and Hausken, 2017). Like gnathostomes, lampreys produce thyroxine (T4) and triiodothyronine (T3) from thyroid follicles that are suggested to be involved in larval development, metamorphosis, and reproduction (Manzon and Manzon, 2017; Sower and Hausken, 2017). In a previous report, a partly purified salamon gonadotropin and an analog of mammalian GnRH each stimulated the elevation of T4 in adult female sea lampreys, suggesting that there may be functionally overlapping HPG and HPT axes (Sower et al., 1985). At this time, there is no demonstrated link between thyroid hormone production and the lamprey GpH receptors.

The specificity and temporal expression of the two lamprey glycoprotein hormone receptors are not known. Earlier studies using RT-PCR showed that lGpH-R I and II RNA were both expressed in adult lamprey brain, intestine, liver, gonads, and thyroid; however, lGpH-R I was more highly expressed in the testis, whereas lamprey lGpH-R II was more highly expressed in the thyroid (Freamat et al., 2006; Freamat and Sower, 2008b). The expression of the IGpH-Rs has not been studied in larval or parasitic lamprey.

To gain an understanding of the dynamics of the glycoprotein hormone receptors in each of the major life stages of lampreys, we examined the (co)-localization and (co)-expression of lGpH-R I and II in larval, parasitic phase, and adult sea lamprey by dual-label fluorescent in situ hybridization. We also performed in vivo experiments to examine the relationship between lGpH-R I and II expression in the ovaries and thyroids of sexually mature, adult female lampreys upon injection with lGnRH-I, -II, or -III. In these experiments, we measured lGpH-R I and II expression by real-time PCR and determined plasma concentrations of estradiol (E2) and thyroxine (T4). The resulting data provide further evidence of the potential overlap in interactions between the HPG and HPT axes in regulating reproduction and development/metabolism in sea lampreys.

2. Materials and methods

2.1. Animals

Adult female lampreys were collected from fish ladders in coastal New Hampshire rivers (Cocheco River, Dover, NH; Exeter River, Exeter, NH; Lamprey River, Newmarket, NH) in May 2013–2015, and transported to the Anadromous Fish and Aquatic Invertebrate Research (AFAIR) lab at the University of New Hampshire (UNH), Durham, NH. Lampreys were maintained in a spawning channel supplied with flow-through water from a stream-fed reservoir (ambient temperature: 13–20 °C; natural photoperiod), as previously described (Fahien and Sower, 1990; Sower et al., 1987). The larval and parasitic lampreys were collected in late October 2015 from the Conte Anadromous Fish Research Center (U.S.G.S., Turners Falls, MA) and transported to UNH. Larval lampreys were maintained in aerated freshwater tanks with sand for burrowing (ambient temperature: 15–17 °C; natural photoperiod) and fed weekly with Fleischmann’s active dry baker’s yeast (ACH Food Companies, Inc., Cordova, TN, USA). Parasitic lampreys were dissected immediately upon transfer to UNH. All procedures for animal use followed the UNH Institutional Animal Care and Use Committee (IACUC) guidelines.

2.2. Gonadal staging histology

Female larval, parasitic phase, and adult ovaries were dissected and fixed in Bouin-Holland solution, processed through a typical dehydration series, embedded in paraffin, stained with hema-
toxylin/eosin by the New Hampshire Veterinary Diagnostic Laboratory (NHVDL, UNH), and staged for reproductive maturation as previously described (Bolduc and Sower, 1992; Nozaki et al., 1999; Sower et al., 2015; Sower et al., 2006).

2.3. Dual-Label fluorescent in situ hybridization (FISH)

Ovaries and thyroid tissues from two lampreys of each lamprey life stage, larval (avg. 14 cm/5 g), parasitic phase (avg. 15.5 cm/6 g), and adult (avg. 67.5 cm/900 g), were dissected and fixed in paraformaldehyde, cryoprotected in sucrose, embedded in Tissue-Tek OCT (Sakura Finetek USA, Inc. Torrance, CA), and stored at −80°C until use, as previously described (Marquis et al., 2017). Target probes were designed using custom software (Wang et al., 2012). GenBank accession numbers and probe regions were: Pma-GPHR1, AY750688.2, region 156-1132; Pma-GPHR2, AY750689.2, region 19-948. Dual-label FISH was performed using an RNAscope Multiplex Fluorescent Reagent Kit (Advanced Cell Diagnostics, Hayward, CA) according to the manufacturer’s instructions and Marquis et al. (2017) with the following modifications: slides were air-dried at −20°C (20 min) and then at RT (15 min), gradually baked in a hybridization oven (RT to 60°C, 30 min), post-fixed in 4% formaldehyde/1X PBS (RT, 10 min), washed in 1X PBS (RT, 5 min), dehydrated at RT in a graded series of ethanol (50%, 1 min; 70%, 1 min; 100% 1 min), rehydrated at RT in a graded series of ethanol (100%, 1 min; 70%, 1 min; 50%, 1 min), rinsed in nuclease-free Milli-Q water, post-fixed in 4% formaldehyde/1X PBS (RT, 10 min), rinsed in nuclease-free Milli-Q water, dehydrated in 100% ethanol (room temperature, 5 dips), and treated with Protease III (Advanced Cell Diagnostics, Hayward, CA) (40°C, 30 min). The slides were hybridized (40°C, 2 h) in a HybEZ oven (Advanced Cell Diagnostics, Hayward, CA) with target probes for IgP-H-R I and IgP-H-R II (premixed) or a two-plex negative control probe (Advanced Cell Diagnostics, Hayward, CA), followed by a series of fluorescence signal amplification and washing steps. Following amplification, sections were counterstained with DAPI (Advanced Cell Diagnostics, Hayward, CA) and cover-slipped using ProLong Gold Antifade Reagent (Invitrogen, Carlsbad, CA) and VWR Micro Cover Glasses (No. 1.5) (VWR, Radnor, PA). Confocal images were acquired with visualization of the CI/C2 fluorescence by excitation with 488-nm argon multiline and 543-nm helium/neon, respectively, according to the following specifications: emitted fluorescence collected from 505 to 530 nm and 560 to 615 nm; 16X scan averaging; pixel time 1.60 ms; 1.0 and 1.4 zoom on a 20X objective for tiled and single images, respectively. RNA signal was identified as red (IgP-H-R I) and green (IgP-H-R II) punctate dots.

2.4. In vivo injections

In June of each year from 2013 to 2015, forty adult female lampreys (avg. 65 cm) were used for in vivo studies. Ten lampreys per group were intraperitoneally injected at 24 h intervals for 2 days with 100 μg/kg fish IgNHR-I, -II, or -III dissolved in 0.6% saline; controls were injected with 0.6% saline. At 48 h following the first injection, blood was collected by cardiac puncture, and ovaries and thyroids were dissected after decapitation as previously described (Fahien and Sower, 1990; Sower et al., 1987). Blood from each lamprey was only collected in 2014 and 2015 and processed and stored as plasma at −20°C until analyzed by RIA. Ovaries and thyroids were snap frozen in liquid nitrogen, then stored at −80°C until RNA extraction.

2.5. RNA extraction and cDNA synthesis

Ovaries and thyroids were homogenized in 1 mL of QIAzol (QIA- GEN, Hilden, Germany) RNA extraction reagent according to the manufacturer’s protocol. Total RNA was extracted and treated with 3U of RNase-free DNase (Promega, Madison, WI) for one hour at 37°C.
2.6. Real-time PCR

SYBR Select Master Mix (Applied Biosystems, Foster City, CA) was used to perform qPCR with cDNA diluted 1/10. Thermal cycling was performed according to the manufacturers recommendations on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Forward and reverse primers (Table 1) were designed against exon-exon boundaries of their respective transcripts, and used at a final concentration of 400 nM each for each reaction product. The reactions were initially denatured at 95°C/5 min, extended and annealed for 40 cycles at 95°C/15 s and 60°C/60 s, respectively, followed by a dissociation analysis (95°C/15 s, 60°C/60 s, 95°C/15 s). Each reaction product yielded a single dissociation peak and no primer dimers. A no reverse transcriptase pool was utilized to ensure the absence of genomic DNA carry-over. A series of 25-fold dilutions of cDNA pools per tissue were prepared as standard samples to determine efficiencies of the reactions (90–110%). The fold changes in expression of lGpH-R I and II transcripts, relative to EF1α, were determined by the Pfaffl method (Pfaffl, 2001), and are represented as mean fold-change ± SEM.

2.7. Radioimmunoassays

Plasma for RIAs was only collected in 2014 and 2015. Plasma T4 concentrations were determined as previously described and validated in sea lampreys (Plisetskaya et al., 1983; Sower et al., 1985). Briefly, T4 concentrations were determined by RIA using 10 μL of plasma, 125I-labeled hormone (PerkinElmer, Waltham, MA), and anti-T4 antibody (Fitzgerald, North Acton, MA) 20-TR40, 1:5000. In 2014 the intra-assay coefficient of variance (CV) was 5.97% with 25.7% antibody binding. In 2015, the intra-assay CV was 9.80% with 36% antibody binding.

Plasma E2 concentrations were determined using 100 μL of plasma and anti-estradiol antibody (1:34,000) as previously described (Sower et al., 1985; Sower and Schreck, 1982). Antibody binding was 22.9% in 2014, and 23% in 2015.

2.8. Statistical analyses

All RIA and qPCR data were analyzed by one-way ANOVA and the Holm-Sidak test in Graphpad Prism 6.

3. Results

3.1. Gonadal histology and staging

All lampreys were female. The gonads of larval lampreys had primary oocytes. The parasitic phase female lampreys were recently metamorphosed such that the gonads had primary oocytes undergoing growth but not yet undergoing vitellogenesis/yolk deposition. Adults were in the final stages (III-IV) of sexual maturation (Sower, 2003).

3.2. Fluorescent in situ hybridization (FISH)

IGpH-R I, but not IGpH-R II, was expressed in larval ovaries (Fig. 2: A, B: B1-B4). In parasitic phase ovaries, IGpH-R I and IGpH-R II were co-localized (Fig. 2: C, D: D1-D4). In adult female lampreys IGpH-R I and II were co-localized in the follicular layer of the oocyte (Fig. 2: E: F: F1-F4). In both parasitic phase and adult ovaries IGpH-R I was more highly expressed than IGpH-R II, qualified by the red signal being more intense than the green, merged as orange rather than yellow.

In larval endoderm (Fig. 3: A: B: B1-B4) and thyroids of parasitic phase (Fig. 3: C: D: D1-D4) and adults (Fig. 3: E: F: F1-F4), IGpH-R I and II were highly co-localized and highly co-expressed. The relative levels of expression of IGpH-R I and II differed depending on the life stage. In larval endoderm, both receptors are expressed at a similar level, noted by the equal red and green signal intensities, merged as bright yellow; there was significantly higher intensity of expression of the IGpH-Rs in the type II and III cells versus type I, IV, and V cells in the larval endoderm. In both parasitic phase and adult thyroids, IGpH-R I is more highly expressed than IGpH-R II, noted by the red signal being more intense than the green and merged as orange rather than yellow.

3.3. In vivo RNA expression and dynamics of adult female GpH-R I and II

Sexually mature, adult, female sea lampreys injected with 100 μg/kg fish Ig GnRH-I, -II, or -III showed an increased (1–2-fold) trend in ovarian expression of both GpH-Rs compared to controls. In thyroid, expression of IGpH-R I did not change with treatment of any Ig GnRH, whereas IGpH-R II expression increased 1- and 3-fold with Ig GnRH-I and -II injections. Injections with Ig GnRH-III significantly increased (p < 0.01) thyroidal IGpH-R II expression (Fig. 4).

3.4. Radioimmunoassays

Lamprey GnRH-I, -II, and -III increased plasma E2 significantly (p < 0.05) in 2014 (332.3 ± 77.0, 287.1 ± 57.2, and 323.7 ± 41.2 pg/0.1 ml, respectively) compared to controls (73.1 ± 15.4 pg/0.1 ml), and in 2015 (169.7 ± 25.4, 126.2 ± 16.8, 222.2 ± 14.3; control 71.2 ± 6.7 pg/0.1 ml) (Table 2).

Treatments with Ig GnRH-I, -II, or -III did not significantly change plasma T4 concentrations. In 2014, plasma T4 concentrations were slightly lowered (p < 0.059) when treated with Ig GnRH-I, -II, or -III (1.38 ± 0.22, 1.71 ± 0.30, or 1.36 ± 0.41 ng/ml, respectively) compared to controls (2.56 ± 0.35 ng/ml). This trend was only observed in 2015 for Ig GnRH-I treated lampreys (1.52 ± 0.16 ng/ml), whereas Ig GnRH-II (2.45 ± 0.39 ng/ml) and Ig GnRH-III (2.05 ± 0.23 ng/ml) injections were comparable with controls (2.13 ± 0.34 ng/ml) (Table 2).

4. Discussion

Prior to the current study, the function and expression of each of the two lamprey glycoprotein hormone receptors, IGpH-R I and II, had not been elucidated in lampreys at each major life stage: larval, parasitic phase, and adult. In this study, we showed individual localization/expression and co-localization/co-expression of lamprey IGpH-R I and/or II transcripts by dual-label fluorescent in situ hybridization (FISH) in the ovaries and endoderm/thyroid of larval, parasitic phase, and adult lampreys. Larval ovaries only expressed IGpH-R I, whereas parasitic phase and adult ovaries, as well as thyroids of all life stages, exhibited robust co-localization/co-expression of IGpH-R-I and II. We measured expression of IGpH-R-I and II by qPCR after injections with Ig GnRH-I, -II, or...
masses and lumen; however, type II and III are the considered (Fig. 1). Type II, III, and V cells line the chamber of the glandular consists of five cell types: types I, II, III, IV, and V (Marine, 1913). The expression of lGpH-Rs in ovaries was seen at each of the three different life stages, suggesting a role of each of the receptors in the regulation of reproductive and/or developmental activities. In larval ovaries, only lGpH-R I was expressed but not lGpH-R II. This may reflect the reproductive status during the larval phase in which larval lampreys all have primary oocytes followed by sexual differentiation shortly before metamorphosis in which the gonads convert to testes or continue as oocytes (Hardisty and Potter, 1971). During sexual differentiation of males, the primary oocytes will degenerate, and the remaining germ cells will change to spermatogonia (Hardisty and Potter, 1971). Since the larval lampreys that were used in this experiment were not undergoing metamorphosis or any major reproductive changes, the expression of only lGpH-R I suggests that lGpH-R II may not be involved at this particular stage in reproduction or larval development. However, only two lampreys were examined in this experiment. Therefore, it would be important to examine lGpH-R I and II expression during larval development, from early to late larvae, to gain a better understanding of the dynamics of receptor expression.

Both lGpH-R I and II were highly co-localized/co-expressed in the follicular layer of parasitic phase and adult ovaries with weak and moderate intensity, respectively. During the adult period, the final processes of sexual maturation are ongoing with migration and subsequent breakdown of the germinal vesicle, resulting in ovulation, oviposition, and spawning (Sower, 2003, 2015). The robust co-expression of lGpH-R I and II in the adult lamprey ovaries strongly suggests that both receptors are involved in mediating the final reproductive processes. Unlike adult lampreys that do not feed in the final maturational period, parasitic phase lampreys do feed and undergo somatic growth to facilitate upstream migration as adults, while the ovaries are undergoing oocyte growth and vitellogenesis (Sower, 2003), which could reflect the low co-expression of the lGpH-Rs. The parasitic phase lampreys that were used in this study were recently metamorphosed and still in the early stages of oocyte growth, which could also explain the low (co-)expression that was observed.

Lamprey metamorphosis involves the transformation of the endostyle in larval lampreys into functional thyroid follicles that persist through adulthood (Youson, 1980). The larval endostyle consists of five cell types: types I, II, III, IV, and V (Marine, 1913) (Fig. 1). Type II, III, and V cells line the chamber of the glandular masses and lumen; however, type II and III are the considered the main subset of endostyle cells that persist and develop into the mature thyroid follicle (Fig. 1) (Wright and Youson, 1980). We showed that there was much higher co-expression of the lGpH-Rs in the type II and III cells versus type I, IV and V cells in the larval endostyle, which is consistent with the cytological development of thyroid follicles in lampreys. In earlier studies, it was shown that injection with bovine TSH and I^{131} resulted in increased iodide binding and sequestration in type II and III cells, as well as increased concentrations of the thyroid hormone precursors, mono-iodinated tyrosine and di-iodinated tyrosine (Clements-Merlini, 1962). In later studies examining T4 and GnRH in metamorphosing lampreys, there was a decrease in serum T4 prior to and during early metamorphosis, with a corresponding increase in concentrations of lGPH-I and -III coinciding with oocyte growth (Youson and Sower, 1991). Therefore, consistent with previous findings (Clements-Merlini, 1960, 1962; Manzon and Manzon, 2017), our results suggest that the two lGpH-Rs may be involved in thyroid hormone signaling and reproductive activities in larval lamprey.

Lamprey GpH-R I and II were highly co-localized/co-expressed in the thyroid of parasitic phase lampreys. However, there was high expression of these receptors in the larval endostyle compared to a more moderate expression the thyroid in parasitic phase lampreys. In addition, lGpH-R I was more highly expressed compared to lGpH-R II expression in thyroid follicles of parasitic phase lampreys. Due to the degeneration of the endostyle and re-differentiation of the cells fated to become adult thyroid follicles that occurs during metamorphosis, there appears to be a population of transitional thyroid follicular cells, which may explain the higher co-expression in some cells compared to others. The high co-expression shown throughout the thyroid follicles during this stage of recently metamorphosed, parasitic phase lampreys suggests that the thyroid follicles are functional. Similar to parasitic phase lampreys, there was high co-expression of lGpH-R I and II in adult lamprey thyroids. lGpH-R I and II were more highly expressed in adults compared to parasitic phase lampreys, but not expressed as highly as the expression observed in the larval endostyle. These changes likely reflect the distinct reproductive and developmental stages of lampreys (larval, parasitic, phase, and adult). Given the co-expression of lGpH-R I and II in both ovary and thyroid during the parasitic and adult phases, we propose that these two receptors are likely involved in reproduction and developmental/metabolic changes in metamorphosing and maturing adult lampreys.

In gnathostomes, specific temporal tissue expression profiles have been examined, specifically TSH receptors in relation to thy-roidal activities and LH and FSH receptors in relation to gonadal activities. In mammals, there was specific temporal tissue expression profiles of LH-R, FSH-R, and TSH-R (Pierce and Parsons, 1981). For example, bovine LH-R was localized to ovarian granulosa and Leydig cells with expression increasing in response to pituitary LH stimulation during the course of development into mature ovulatory follicles. In contrast, FSH-R expression was exclusively localized to granulosa cells, and peaked during recruitment of the dominant follicle (reviewed in Bao and Garverick, 1998). In teleost

Table 1

<table>
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<th>Target Gene</th>
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Fig. 2. FISH: larval, parasitic phase, and adult female ovaries. Dual-label FISH of lGpH-Rs, lGpH-R I and lGpH-R II. lGpH-R I (red), but not lGpH-R II (no green) was moderately expressed in larval ovaries (B3, B4, respectively). lGpH-R I (red) and lGpH-R II (green) were minimally expressed in parasitic phase ovaries (D3, D4, respectively). lGpH-R I (red) and lGpH-R II (green) were moderately expressed in adult ovaries (F3, F4, respectively). lGpH-R I and II were minimally co-expressed (orange) in the parasitic phase ovaries (C, D1), and moderately co-expressed (orange) in the adult ovaries (E, F1). Probe specificity was confirmed by a negligible background signal in negative controls, in the larval (B2), parasitic phase (D2), and adult ovaries (F2). Arrowheads in merged and individual images indicate the regions of co-expression of the lGpH-Rs, lGpH-R I and II. Scale bars (a) 100 μm, (b–d) 20 μm. GpH lamprey glycoprotein hormone (lGpH), lGpH-R I lamprey GpH receptor one, lGpH-R 2 lamprey GpH receptor two.
Fig. 3. FISH: larval endostyle, parasitic phase, and adult female thyroids. Dual-label FISH of IgP≤H-Rs, IgP≤H-R I and IgP≤H-R II. IgP≤H-R I (red) and IgP≤H-R II (green) were highly expressed in all cells of the larval endostyle, particularly Type II and III cells (B3, B4, respectively), as well as in the parasitic phase thyroid (D3, D4, respectively) and in the adult thyroid (F3, F4, respectively). IgP≤H-R I and II were highly co-expressed (yellow) in all the cells of the larval endostyle, particularly Type II and III cells (A, B1), as well as moderately co-expressed (orange) in the parasitic phase thyroid (C, D1), and adult thyroid (E, F1). Probe specificity was confirmed by a negligible background signal in negative controls, in the larval endostyle (B2), parasitic phase thyroid (D2), and adult thyroid (F2). Arrowheads in merged and individual images indicate the regions of co-expression of the IgP≤H-Rs, IgP≤H-R I and II. Scale bars (a) 100 μm, (b–d) 20 μm. GpH lamprey glycoprotein hormone (IgP≤H), IgP≤H-R I lamprey GpH receptor one, IgP≤H-R II lamprey GpH receptor two.
Fig. 4. In vivo expression of IgpH-R I and II transcripts from A) ovaries, and B) thyroids in response to injections with 100 µg/kg fish IgGnR-I, -II, or -III. Gene expression was measured relative to EF1α by qPCR. Data are represented as mean fold-change ± SEM for treatment with each IgGnR; N = 10 animals per year for three years.

Table 2

<table>
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<tr>
<th>E2 (pg/0.1 ml)</th>
<th>T4 (ng/ml)</th>
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<td>IgGnR-III</td>
<td><strong>323.7 ± 41.2</strong></td>
</tr>
</tbody>
</table>

thyroids support the hypothesis that the lamprey HPG axis overlaps with the HPT axis. Further studies will be needed to understand the hypothalamic-pituitary control over the dynamics of glycoprotein hormone/glycoprotein hormone receptor signaling that can provide more in-depth insights into the evolution of reproductive, metabolic, and developmental regulation in this basal vertebrate.

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References


