Characterization of tilapia (*Oreochromis niloticus*) gonadotropins by modeling and immunoneutralization

Joseph Aizen, Noga Kowalsman, Masha Y. Niv, Berta Levavi-Sivan

**A R T I C L E   I N F O**

Article history: Available online 2 June 2014

Keywords: 11-ketotestosterone LH FSH GnRH Gonadotropin receptor

**A B S T R A C T**

In fish, both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play important roles in reproduction. Here we explored the structure and differential specificity of tilapia (†) gonadotropins (GTHs) to delineate their physiological relevance and the nature of their regulation. We generated structural models of tGTHs and GTH receptors (R) that enabled us to better understand the hormone–receptor interacting region. In tilapia, FSH release is under the control of the hypothalamic decapeptide GnRH, an effect that was abolished by specific bioneutralizing antisera (anti-recombinant (r) tFSH). These antisera also reduced the basal secretion and delayed GnRH-stimulated production of 11-ketotestosterone (11KT), and dramatically reduced LH levels. Immunoneutralization of tLH using anti-rtLH significantly reduced its GnRH-stimulated levels. Basal 11KT and FSH levels were also reduced. Taken together, these results suggest a feedback mechanism between FSH and LH release in tilapia.

© 2014 Elsevier Inc. All rights reserved.

**1. Introduction**

The general model of vertebrate reproduction involves the control of gonadal functions by two heterodimeric plasma glycoproteins (gonadotropins; GTHs) secreted from the gonadotrophs in the anterior pituitary. In fish, two distinct GTHs—GTH-I and GTH-II—have been purified, and the cDNAs of the corresponding specific subunits have been cloned from more than 56 fish species representing at least 14 teleost orders. Based on their molecular structures and physiological effects, a new nomenclature has emerged, with fish GTH-I and GTH-II being referred to as follicle-stimulating hormone (FSH) and luteinizing hormone (LH), respectively. FSH and LH are members of the glycoprotein hormone family, which also includes thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG) (reviewed by Levavi-Sivan et al., 2010).

In ovarian follicles, FSH regulates granulosa cell proliferation, the synthesis of cell-cycle-regulatory proteins and induction of differentiation-specific genes’ expression. LH, on the other hand, promotes follicular maturation, ovulation and the synthesis of ovarian steroid hormones. In the testes, FSH determines Sertoli cell proliferation and is involved in germ cell maturation, while LH supports Leydig cell functions and stimulates the synthesis of androgens, regulating the final stages of spermatogenesis (reviewed by Yaron and Sivan, 2006).

The presence of two distinct GTH receptors (GTHR) in a single fish species was confirmed by the molecular cloning of two different cDNAs in several fish species from different taxa (Levavi-Sivan et al., 2010). The FSH and LH receptors (FSHR and LHR, respectively) are G-protein-coupled receptors (GPCRs) from family A of the rhodopsin-like receptors (Gether, 2000). Despite their overall similarity to mammalian GTHRs, in vitro binding and functional studies have revealed promiscuous ligand recognition of fish GTHRs, in contrast to the strict ligand selectivity described in mammals. Studies on GTHRs from representatives of the piscine orders Siluriformes (Kumar et al., 2001a,b; Vischer et al., 2003) and Cypriniformes (So et al., 2005) have indicated that FSHRs show a preference for FSH but also respond to LH, whereas LHRs respond specifically to LH. Studies on receptors from a salmonid fish, the amago salmon (Oba et al., 1999a,b), concluded that LHR, but not FSHR, responds to both GTHs. However, in the sea bass, each GTHR is exclusively activated by its corresponding hormone (Rocha et al., 2009).
2007; Moles et al., 2011). The physiological significance of the promiscuity of the piscine GTHRs therefore remains unclear.

Structural information is crucial to deciphering the function of GPCRs (Katritch et al., 2013). Homology modeling can help elucidate the details of interactions between GPCRs and their ligands (Brockhoff et al., 2010; Levit et al., 2012, 2014; Born et al., 2013). In particular, in our previous work (Aizen et al., 2012), analysis of three-dimensional structural models suggested that residues outside of the currently established hormone–receptor interface region may be involved in GTH interactions with their receptors. Recently, a new crystal structure of human (h) FSHR in complex with hFSH has provided structural information on the extracellular region of the receptor (Jiang et al., 2012; Kreuchwig et al., 2013). This new structure was used here as a template for modeling GTH–GTHR interactions in tilapia.

The Nile tilapia is the second most farmed fish species in the world. However, the Nile tilapia is not only globally important as a cultured food fish, it is also a widely used model for studies in physiology, neurobiology, molecular genetics, endocrinology and toxicology. The advantages of working with tilapia in these areas are their medium size, short generation time and capacity to produce numerous eggs in each spawning. The recently published genomic resources for Nile tilapia and the existence of shared syn-teny between tilapia and other known model fish genomes will further improve the use of tilapia as a model fish. Our laboratory has been using tilapia as a model fish for a long time, and we have already developed several specific molecular tools, such as the synthesis of recombinant (r) GTHs (Kasuto and Levavi-Sivan, 2005; Aizen et al., 2007a), the development of specific ELISAs (Aizen et al., 2007b), and the establishment of transgenic fish (Golan and Levavi-Sivan, 2013). The current study aimed to gain more insight into the structure and differential specificity of tilapia GTHs toward understanding their physiological relevance and the nature of their regulation.

2. Materials and methods

2.1. Structural models of tilapia (t) LH and tFSH and their receptors

The tilapia sequences used for the modeling were extracted from the ExPASy server using the UniProtKB database (Gasteiger et al., 2003; Wu et al., 2006): Q7TT2Y4 (LH and FSH x-subunit), Q7TT2Y5 (LH β-subunit), Q7TT2Y6 (FSH β-subunit), Q9DGCS (LH) and Q9DGCC6 (FSHR). Prior to the modeling, the sequences were aligned to their human counterparts as described previously by Aizen et al. (2012).

Models of tLH and tFSH and their receptors were prepared using the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TAS-SER/) (Zhang, 2007; Roy et al., 2010). For the receptor model, we directed the I-TASSER server to use either the structure of the human FSHR–ectodomain (ECD) complex (PDB entry 1XWD; Fan and Hendrickson, 2005) or the new structure (Jiang et al., 2012). For the ligand models, we used the default parameters with no predefined template or with FSH template from the 4AY9 structure. Visualization and superposition of the models were performed using Discovery Studio 2.5 (Accelrys Inc.). To create the LH/LHR–ECD and FSH/FSHR–ECD complex models, sequence-align ment-based superposition of the models with the structure of the complex of hFSH with hFSHR ectodomain, 4AY9.pdb, using SPDBV4.10 software (Guex and Peitsch, 1997).

2.2. Specific ELISAs for tFSH and tLH

Levels of tFSH and tLH in the plasma were determined by specific competitive ELISAs developed for tilapia (Aizen et al., 2007b) based on tGTHs. For the ELISA, we used primary antibodies raised in rabbits against rtLHβ or rtFSHβ, and single-chain polypeptide rtLHβα or rtFSHβα (Kasuto and Levavi-Sivan, 2005; Aizen et al., 2007a) for the standard curves. Antiserum specificity was tested and validated with tilapia pituitary glands as described previously by Aizen et al. (2007b). Sensitivity for plasma determinations was 15.84 pg/ml for LH and 0.24 pg/ml for FSH. Interassay coefficient of variation (CV) was 14.8% and 12.5%, while intra-assay CV was 7.2% and 8% for LH and FSH, respectively.

2.3. ELISA for 11-ketotestosterone (11KT)

The levels of 11KT were determined by specific ELISA according to (Levavi-Sivan et al., 2004; Hurvitz et al., 2005), using acetylcholinesterase as the label. The anti-11KT was kindly donated by Dr. David E. Kime (University of Sheffield, England) and is described by (Cuisset et al., 1994). All samples were analyzed in duplicate, and for each ELISA plate, a separate standard curve was run. The lower limit of detection was 1.56 pg/ml for 11KT. The intra- and interassay CVs were less than 7% and 11%, respectively. Steroid levels in the plasma were validated by verifying that serial dilutions were parallel to the relevant standard curve.

2.4. Effect of immunoneutralization with anti-rtFSHβ or anti-rtLHβ on 11KT, LH and FSH levels

Tilapia (Oreochromis niloticus) were kept and bred in the fish facility unit at the Hebrew University in 500-L tanks at 26 °C under a 14 L:10 D photoperiod. Fish were fed every morning ad libitum with commercial pellets and flakes containing 50% protein, 6% fat, 5.6% ash, and 2.6% cellulose (Zemach Feed Mills, Zemach, Israel). All experimental procedures were in compliance with the Animal Care and Use Guidelines of the Hebrew University and were approved by the local Administrative Panel on Laboratory Animal Care. Mature male tilapia [mean ± SEM, 182.1 ± 7.65 g BW; GS1 (gonadal somatic index; the percentage of fish gonad weight to body weight), 0.72% ± 0.15%] were used for the in vivo experiment. For each GTH, fish were allocated to five groups of 15 fish each. The control group was injected intraperitoneally (i.p.) with normal goat serum. The second, third, and fourth groups were injected with salmon (s) GnRHa (15 μg/kg BW; Salmon GnRH analog; D-Arg6, Trp6, Leu9–Net–LHRH). The time of sGnRHa injection was defined as time 0 h. The second group was injected with sGnRHa only. The third group was injected with sGnRHa and concomitantly immunized with 0.2 ml rtFSHβ or rtLHβ antisera. The fourth group was immunized i.p. with 0.2 ml of rtFSHβ or rtLHβ antisera twice, 12 h before and together with the sGnRHa injection. The fifth group was only injected with anti-rtFSHβ or anti-rtLHβ once, 12 h before they were injected with sGnRHa. Blood was collected from the caudal vasculature into heparinized syringes from anesthetized fish at 4-h intervals until 24 h after treatment, and was centrifuged to obtain plasma which was stored at −20 °C until assay for 11KT, LH or FSH.

2.5. Statistical analysis

Data are presented as means ± SEM. The significance of the differences between group or treatment means of hormone or steroid levels was determined by one-way analysis of variance (ANOVA).
followed by Newman-Keuls test using Graph-Pad Prism 6 software (GraphPad, San Diego, CA).

3. Results

3.1. Structures and models of tLH, tFSH and their receptors

Superposing the individual structures onto the units in the hFSH/hFSHR–ECD complex, we found high similarity between the structures of tilapia LH \textit{a} and \textit{b} subunits and tLHR and the equivalent structures in 1XWD, with backbone RMSD (root mean square deviation) of 1.83 Å, 1.996 Å and 0.95 Å, respectively. The high similarity between the structures tLH and tLHR and the sequence similarity between tilapia sequences and sequences of the structural templates led us to conclude that for tLH/LHR–ECD, we could use the available crystal structures (in particular the hFSH/hFSHR–ECD complex and the hCG structures) as templates (Fig. 1). In tFSH\textit{b}, however, one of the Cys residues is missing, leading to structural changes in the area known as the “seatbelt region”, which is a major part of the binding site of the FSH\textit{b} subunit (Hearn and Gomme, 2000; Levavi-Sivan et al., 2010), resulting in a RMSD of 3.814 Å. In addition, tFSHR has a backbone RMSD (4.73 Å) when superposed on hFSHR (from the 1XWD structure) due to exon duplication which causes an insert in the leucine-rich repeat (LRR) domain (Chauvigne et al., 2010). Hence only a crude model could be made for tFSH and tFSHR, as well as the tFSH/ tFSHR–ECD complex (Fig. 1A and C).

3.2. The effect of immunoneutralization of tGTHs

We further aimed to study the differential effects of GTHs on the secretion of an androgen in male tilapia. We used immunoneutralization of endogenous FSH and LH by specific antibodies, and tested their effect on the secretion of 11KT and GTHs.

3.2.1. Effect of immunoneutralization with anti-rtFSH\textit{b} on 11KT and LH levels

As described previously by Aizen et al. (2007a), 11KT levels increased 4 h after sGnRHa injection. However, concomitant injections of sGnRHa and anti-rtFSH\textit{b} postponed the 11KT peak by 4 h (Fig. 2A). Fish that received two injections of anti-rtFSH\textit{b}, at 12 h before and together with sGnRHa, also showed a 4-h delayed 11KT peak, and 11KT level was suppressed. Injection of anti-rtFSH\textit{b} only once, 12 h before sGnRHa injection, resulted in a dramatic decrease in basal 11KT levels that lasted for 24 h (Fig. 2A).

Four hours after the sGnRHa injection, LH levels increased from 8.81 ± 1.04 ng/ml to 21.53 ± 1.44 ng/ml. However, concomitant injections of sGnRHa and anti-rtFSH\textit{b} reduced the levels of LH after 4 h to only 5.05 ± 1.22 ng/ml (Fig. 2B). Fish that received two injections of anti-rtFSH\textit{b}—12 h before and together with sGnRHa—had basal LH levels that were decreased to 4.361 ± 0.66 ng/ml. Injection of anti-rtFSH\textit{b} alone 12 h before sGnRHa administration resulted in a dramatic decrease in LH levels (8.90 ± 0.76 ng/ml vs. 1.984 ± 0.16 ng/ml; Fig. 2B). These results suggest that FSH is responsible not only for the GnRH-stimulated 11KT secretion but also has an effect on LH levels.

3.2.2. Effect of immunoneutralization with anti-rtLH\textit{b} on 11KT and FSH levels

Immunoneutralization of LH was used to determine the correlation between LH and the secretion of 11KT and FSH in male tilapia. sGnRHa injection was followed by a significant increase in 11KT levels 8 h after the injection. However, a concomitant injection of sGnRHa and anti-rtLH\textit{b} reduced the 11KT peak to only 2.49 ± 0.61 ng/ml (Fig. 2C). Fish that received two injections of anti-rtLH\textit{b}—12 h before and together with sGnRHa—had a similar level of 11KT as with the previous treatment (2.40 ± 0.17 ng/ml). Injection of anti-rtLH\textit{b} alone, 12 h before sGnRHa injection, resulted in a decrease in 11KT levels (from 0.89 ± 0.21 ng/ml to

![Fig. 1. Tilapia gonadotropin models. Structural three-dimensional models of tilapia FSH and tilapia LH (A and B, respectively). The gonadotropin subunits are in ribbon representation. Transparent surface of the subunits is shown. The \textit{a}-subunit is in magenta and the \textit{b}-subunit is in light blue and cyan for FSH and LH, respectively. Structural three-dimensional models of the tilapia LH/LHR–ECD (C) and FSH/FSHR–ECD (D) complexes. FSH and LH are colored as in A and B. The FSHR and LHR are shown in ribbon representation in gray and green, respectively.](image-url)
0.28 ± 0.14 ng/ml) that lasted for 24 h. These results indicate that LH is responsible not only for the GnRH-stimulated 11KT secretion but also for the basal steroid secretion.

FSH levels peaked 8 h after sGnRHa injection. A sGnRHa injection given concomitantly with the anti-rtLHβ resulted in similar elevation of FSH levels (Fig. 2D). However, fish that received two injections of anti-rtFSHβ at 12 h before and together with sGnRHa resulted in a decrease of basal FSH levels after 8 h reaching 2.60 ± 0.361 ng/ml. Injection of anti-rtLHβ alone, 12 h before the sGnRHa injection, yielded FSH levels that were very similar to those of the control throughout the 24 h of the experiment (Fig. 2D).

4. Discussion

Among the very large family of GPCRs, glycoprotein hormone receptors constitute a three-member subgroup made up of FSHR, LHR/CGR and TSHR. They are part of a subfamily of receptors characterized by an ectodomain containing LRR (leucine-rich repeat) motifs, in addition to the canonical heptahelical serpentine domain typical of GPCRs (Costagliola et al., 2005). The structure of the GTHRs carries a functional dichotomy: their LRR-containing ectodomain is responsible for the binding specificity of their specific cognate ligands, which activates the rhodopsin-like serpentine domain which is responsible for transducing the signal within the cell, mainly via the stimulatory G protein Gs (Vassart et al., 2004; Karges et al., 2005). Glycoprotein hormones are dimers with a common α-subunit and hormone-specific β-subunits encoded by paralogous genes. This explains why the β-subunits of FSH, LH and TSH share about 40% sequence identity (Moyle et al., 1994). The corresponding receptors, in both mammals and fish, are also encoded by paralogous genes and, accordingly, they also display about 40% sequence identity in their hormone-binding ectodomain (reviewed by (Moyle et al., 1994; Levavi-Sivan et al., 2010)). This fits nicely with the suggestion that the hormone ectodomain experienced coevolution, resulting in tight binding specificity and avoiding promiscuous cross-signaling between the three endocrine systems (Moyle et al., 1994).

The new human FSH/FSHR–ECD crystal structure, 4AY9, can provide a suitable template for model generation of the tilapia FSH/FSHR–ECD model, and is similar to our recently published model (Aizen et al., 2012). It has been shown that hFSH makes contacts with all LRRs, and different residues, buried at the receptor/ligand interface by the α- and/or β-subunit, have been identified. However, fish FSHRs seem to exhibit some remarkable differences in their ECDs. Different from LHRs and mammalian FSHRs, fish FSHRs do not contain a typical cysteine-rich domain (NCR). In the tilapia FSHR, like other perciform species, a 9-aa insertion exists in this region, and one of the Cys residues is missing (Levavi-Sivan et al., 2010). In addition, in the tilapia FSHRs, there is a 25-aa insertion in this area, which represents an extra LRR and predicts a different curvature and length for this region, probably affecting their ligand-binding mode. It is tempting to suggest that these insertions are responsible for the specificity of the GTHRs. Due to the lack of Cys in the tilapia FSHβ subunit and due to the insertion in the FSHR–ECD, the models would need further exploration and validation by mutagenesis.

Fig. 2. Immunoneutralization of tilapia gonadotropins. Male fish (mean ± SEM; 182.1 ± 7.65 g BW; GSI, 0.725% ± 0.150%) were injected i.p. with normal goat serum (control; squares), anti-rtFSHβ at the time indicated by arrow 1 (0.2 ml of anti-rtFSHβ; circles), sGnRHa at the time indicated by arrow 2 (15 μg/kg BW of sGnRHa; triangles), sGnRHa (15 μg/kg BW) and 0.2 ml of anti-rtFSHβ, both at the time indicated by arrow 2 (sGnRHa together with anti-rtFSHβ; inverse triangles), or 0.2 ml of anti-rtFSHβ at the times indicated by arrows 1 and 2 and sGnRHa (15 μg/kg of BW) only at the time indicated by arrow 2 (sGnRHa together with anti-rtFSHβ; diamonds). Levels of 11KT (A) Reprinted from (Aizen et al., 2007b) with permission and LH (B). The same experimental design as for panels A and B was performed using anti-rtLHβ (C and D); levels of 11KT (C) and FSH (D) were determined by specific ELISAs.
In contrast to the mammalian receptors, in some teleosts, FSHR and LHR can be cross-activated by their ligands and therefore, teleost receptor specificity is unclear. Previous studies on the specificity of piscine GTHRs have reported that in African catfish (Bogerd et al., 2001; Visher et al., 2003), zebrafish (So et al., 2005), amago salmon (Oba et al., 1999a,b) and Atlantic salmon (Andersson et al., 2009), LHRs are specific for their respective LHS, whereas the FSHRs, although showing a clear preference for FSH, respond to LH as well. However, in trout and eel, each recombinant GTH activates its cognate receptor in a dose-dependent manner (Kazeto et al., 2008; Aizen et al., 2012) with no crossreactivity. In tilapia, which belongs to the evolved teleost order Perciformes, each GTH is exclusively activated by its corresponding hormone (Aizen et al., 2012). A similar preference of GTHRs has been described in another perciform fish, the sea bass (Rocha et al., 2007; Moles et al., 2011).

One of the most effective approaches to determining the physiological role of a biological factor is its elimination from the system in question. If the physiological factor is a hormone that travels through the blood, it can be efficiently eliminated by immunoneutralization (i.e., the administration of specific antisera produced against the hormone). This method provides relatively high specificity in eliminating only the factor in question, if specific antisera are used. In the current study, we used specific antisera raised against tilapia LHβ and FSHβ. We previously confirmed the specificity of these antibodies in several ways: (1) The different antibodies stained different cell populations in the pituitary of tilapia: anti-rtFSHβ-stained cells that were localized in a more periventricular region of the PPD (Aizen et al., 2007a). (2) These specific localizations of the different GTHs were identical to their spatial distribution as determined by in situ hybridization using RNA probes (Yaron et al., 2003); (3) Since both anti-rtFSHβ and anti-rtLHβ were produced in rabbits, we could not perform double-staining experiments. However, we recently developed transgenic tilapia in which FSH gonadotrophs were fluorescein labeled with enhanced green fluorescent protein (EGFP). We used anti-rtLHβ to visualize the LH dispersion pattern relative to the location of the FSH (labeled with the anti-GFP antibodies) (Golan and Levavi-Sivan, 2013). We found that the localization of LH and FSH gonadotrophs was very similar to that obtained by immunohistochemistry. LH and FSH gonadotrophs were found to be situated in close proximity within the pituitary but their staining did not overlap (Golan and Levavi-Sivan, 2013); (4) When a homogenate of female tilapia pituitaries was subjected to SDS-PAGE under reducing conditions, the two antibodies recognized different proteins: those of approximately 17, 30, and 55 kDa reacted specifically with anti-rtFSHβ, whereas anti-rtLHβ reacted only with those of approximately 17 and 30 kDa (Aizen et al., 2007b); (5) Positive-staining bands were not observed in SDS-PAGE analysis when anti-rtFSHβ was replaced with serum with preabsorbed rtLHβ, or when anti-rtLHβ was replaced by serum that was preabsorbed with rtFSHβ (Aizen et al., 2007b). (6) Preabsorption of anti-rtFSHβ or anti-rtLHβ with the respective recombinant gonadotropin led to elimination of the staining from tilapia pituitary sections after immunohistochemistry (Biran et al., submitted for publication).

In tilapia, the release of LH in response to GnRH was more pronounced than that of FSH, a fact that can be explained by differences in the control of the two GTHs (Aizen et al., 2007a). Alternatively, it may be related to the larger quantity of LH in the pituitary than of FSH (Weitzien et al., 2004), as we showed also for tilapia (Aizen et al., 2007b). In mammals, FSH and LH interact with their membrane-associated receptors, FSHR and LHR, in a highly specific manner at physiological hormone concentrations. Leydig cells express the LHR gene, and accordingly LH regulates Leydig-cell sex-steroid production. Sertoli cells express the FSHR gene, and FSH regulates the structural, nutritional, and regulatory support that Sertoli cells provide to germ cells (Kierszenbaum, 1994). However, in fish, FSHR is not only expressed by Sertoli cells, but also by Leydig cells. Hence, in fish, Leydig-cell steroid production is regulated by both LH and FSH, whereas Sertoli cell functions are predominantly regulated by FSH (Schulz et al., 2010). We have shown that before both rGTHs used in the current study succeeded to release 11KT from the testes of mature and premature tilapia males (FSH: (Aizen et al., 2007a) and LH: (Kasuto and Levavi-Sivan, 2005). This is in agreement with the increased secretion of 11KT observed here in response to GnRH. This increase was delayed and attenuated when FSH was eliminated and only delayed when LH was eliminated. Injecting anti-rtFSHβ or rtLHβ sera into male tilapia reduced and delayed the 11KT response to sGnRH. Moreover, in the presence of anti-rtFSHβ or anti-rtLHβ, not only the GnRH-stimulated 11KT levels, but also the basal steroid levels were reduced. This strongly implies for the involvement of both FSH and LH in 11KT secretion in tilapia. Similar results were obtained when zebrafish testes were incubated with recombinant zebrafish (rzf) GTHs (i.e., rzfLH and rzfFSH) that stimulated androgen release both in vitro and in vivo, with rzfFSH being significantly more potent than rzfLH (Garcia-Lopez et al., 2010).

Here we found that eliminating one GTH causes not only a reduction in androgen levels but also a decrease in the other GTH’s levels, nevertheless, this interaction was asymmetric. When anti-rtFSHβ was injected into male tilapia, both the GnRH-stimulated and basal levels of tLH were strongly reduced. However, when anti-rtLHβ was injected, the GnRH-stimulated and basal levels of tFSH were reduced, albeit to a lesser extent. This phenomenon is probably due to the feedback mechanisms of gonadal steroids in both fish and mammals. In fish, gonadal steroids regulate the production and release of FSH and LH, with the type of influence varying with the gonadal stage of development. Positive or negative feedback mechanisms operate indirectly on certain hypothalamic nuclei, and/or directly on the pituitary cells. The classical negative effects of gonadal steroids on GTH secretion have been demonstrated in a variety of teleost species using gonadectomy and steroid-replacement protocols (reviewed by (Levavi-Sivan et al., 2010). Another route by which the negative feedback could operate is upregulation of dopamine receptor mRNA by E2 as seen in the pituitary of tilapia (Levavi-Sivan et al., 2006). Furthermore, in adult male rats, immunoneutralization of endogenous FSH also decreases serum hormone levels, testicular function, and fertility (Davies et al., 1979). This is in line with the results from the immunoneutralization of FSH in tilapia and can explain the reduction in LH levels due to a decrease in FSH levels that are mediated through steroids in fish.

As described, immunoneutralization of FSH has a dampening effect on LH levels; however, immunoneutralization of LH decreased FSH levels only after two injections and then only 8 h after the second injection of anti-rtLHβ. This seems to imply a feedback mechanism between FSH and LH release in tilapia through other mediators. Another possible explanation for this effect in the absence of LH on FSH levels might be related to the autocrine-paracrine regulation of pituitary function by activin and follistatin (Bilezjkian et al., 2004).

In summary, the results from this study describe the physiological consequences, and structural and receptor-binding characteristics of GTHs in tilapia.

Acknowledgments

This work was presented in a workshop that was supported by BARD, the United States – Israel Binational Agricultural Research and Development Fund, Workshop No. W-94-13. We thank Dr. Talia Yarnitzki for help with the modeling, and Prof. D. Kime for the anti-11-ketosterone.