Effects of adrenergic agonists on the extrahepatic expression of vitellogenin Ao1 in heart and brain of the Chinese rare minnow (Gobiocypris rarus)

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1. Introduction

Endocrine disrupters are compounds that alter normal hormone regulation in humans and wildlife. These could be either naturally occurring environmental chemicals and/or synthetic compounds from industrial products and wastes (Hutchinson et al., 2000). Some of these chemicals have biological activity similar to that of endogenous 17β-estradiol (E2) and are known to affect development, sexual maturation and reproduction of many wildlife species and humans. So far, a number of in vitro and in vivo biomarkers have been developed in fish to detect and assess estrogenic effects of xenobiotics and wastewater. Vitellogenin (VTG) is one of the most frequently used biomarkers for estrogenicity in oviparous vertebrates (Hutchinson et al., 2000; Van Den Belt et al., 2003; Waring and Harris, 2005; Iguchi et al., 2006). Traditionally, VTGs are considered as the estrogen responsive precursor proteins for egg yolk. They are synthesized in the liver of females in response to the endogenous estrogen E2, secreted into the bloodstream and finally taken up by the developing oocytes via endocytosis (Chen et al., 1997; Finn and Kristoffersen, 2007). Normally, VTGs are undetectable in male and immature female fish; however, their synthesis can be induced by exogenous estrogenic compounds. Since the production of VTGs is usually considered as oestrogen-dependent, the use of VTG as a biomarker has been well documented in both laboratory and field studies. Both techniques for quantification of plasma/hepatic VTG proteins and hepatic mRNA have been developed (Jensen and Ankley, 2006; Miller et al., 2007) and are valuable biomarkers for the monitoring of estrogenic contamination of aquatic environments (Hutchinson et al., 2000; Waring and Harris, 2005; Iguchi et al., 2006). However, the potential complexity of the physiological functions and expression regulation of VTGs in fish have not been explored in detail.

To this end, we decided to use the Chinese rare minnow (Gobiocypris rarus) to study VTGs. A freshwater cyprinid, the rare minnow is found mostly in the upstream waters of the Yangtze River in the Sichuan Province, China (Re and Fu, 1983). Similar to other more popular models for ecotoxicology such as the medaka (Oryzias latipes), zebrafish (Danio rerio) and fathead minnow (Pimephales promelas), rare minnow are small in size (30–80 mm in total length) and have a short life cycle (3–4 months). The fish possess a high fertilization and hatching rate (average 200–300 eggs per clutch and continuous batch spawner) and embryos develop quickly (72 h at 26 °C) (Wang, 1999; Zhong et al., 2005). Earlier studies have shown that rare minnow are sensitive to heavy metals, xenoestrogens and...
other aquatic pollutants (Lu and Shen, 2002; Zhou et al., 2002). All of these features make the Chinese rare minnow an attractive candidate for aquatic ecological risk assessment in China. The fish has been recommended to be a good native Chinese test species in toxicological tests (alongside more established models like medaka and zebrafish) by the Environmental Protection Agency of China since 2000.

For our study, we created a E2-treated rare minnow liver cDNA library from which several EST cDNA clones containing VTG coding region were found. By utilizing rapid amplification of cDNA ends (RACE) technique, we identified a full-length rare minnow VTG gene. Phylogenetic analyses reveal the highest identity and similarity between this rare minnow VTG and voga01 sequences available for other teleosts. The tissue distribution of the rare minnow voga01 expression under normal conditions and upon hormone administration was determined by RT-PCR and in situ hybridization. Our results indicated rare minnow voga01 was predominantly expressed in the liver, and also expressed significantly in heart. The expression levels of voga01 in liver and heart tissue are inducible by E2 treatment. Intriguingly, our studies also revealed that rare minnow voga01 expression in heart and liver can be repressed significantly by adrenergic agonist, suggesting that expression of this teleost VTG might be regulated by adrenergic hormone agents.

2. Materials and methods

2.1. Fish, exposure and sampling procedure

Four-month-aged Chinese rare minnow were kept in the laboratories in our institute. Fish were acclimated for at least 2 weeks in the system prior to the beginning of experiments to ensure that they were free of diseases. They were kept in an indoor aquaria system at a constant day/night rhythm (16 h in light:8 h in dark) and temperature (25 °C) with flowing dechlorinated water. Fish were fed with a commercial granule food (Tetra, Germany) at a rate of 0.1% body weight per day and dry brine shrimp (Artemia) was supplemented for newly hatched fry. Waste and uneaten food were removed daily. Before tissue samples collection, fish were anesthetized in buffered tricaine methanesulfonate (MS-222; 100 mg/l). The 17β-estradiol (E2, Merck) stock was prepared first by sonication in ethanol solution and then diluted with dechlorinated water to make the concentration of the stock at 1 mg/ml. In order to investigate the effect of E2 dosage on induction of voga01, serial dilutions of E2 stock was carried out in dechlorinated water to achieve 5, 1, 0.1 and 0.01 μg/l in working solutions. Groups of five Chinese rare minnow aged 4 months were exposed to different concentrations of E2 at 100, 10, 1 and 0.1 ng/l. The exposure solution was renewed once a day. The control group received vehicle only, which contains dechlorinated water with ethanol concentration at less than 0.1 ppm. Total RNA was extracted from liver tissue at the end of the 4-day treatment for voga01 mRNA detection.

Adrenergic agonists, phenylephrine (PE, Sigma) and isoproterenol hydrochloride (ISO, Sigma) stock solution were prepared first by dissolved in dimethyl sulfoxide (DMSO) and then diluted with dechlorinated water to make the final concentration of these adrenergic agonists stock at 1 mM. In order to test the response of voga01 mRNA in Chinese rare minnow, fish were exposed to 10 μM of adrenergic agonist solutions for 7 days (body weight, ~300 mg, 10 fish for each concentration group). The exposure solution containing DMSO less than 0.01% (v/v) was renewed once at day 4 during the exposure treatment. Rare minnow kept in dechlorinated tap water were used as the control group. Tissue samples of five fish from each treatment group were collected. All the treatment experiments have been done in duplication.

2.2. Construction of cDNA library and sequencing

Rare minnow was treated with 1 μg/l E2 to induce estrogen responsive mRNA. Four days following the treatment, liver tissue was collected. In order to help assure a sufficient yield of total RNA for use in reverse transcription, livers from six males treated with E2 were pooled and total RNA was extracted using Trizol reagent (Invitrogen). This was used for construction of a liver cDNA library using the ZAP Express CDNA Synthesis Kit and ZAP Express CDNA Gigapack III Gold Cloning Kit (Strategene, USA). The primary cDNA library was set up as described in the protocol provided by the manufacturer. The primary and amplified cDNA libraries were titred and the percentage of recombinant clones determined by blue and white screening. Lambda phages (1.0 × 107 pfu) of the amplified library were excited in vivo and recirculated to pBK-CMV phagemids. Fifty clones were randomly picked and single pass sequenced with T7 primer.

The 5' and 3' ends of voga01 expression were sequenced by SMART RACE Kit (Clontech). The PCR was conducted with an initial denaturation step at 94 °C for 2 min, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1.5 min, 10 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1.5 min, 15 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.5 min, and a final extension step at 72 °C for 10 min. Amplified PCR products were cloned into pGEM-T easy vector (Promega) and sequenced. Based on the assembled sequence information from the RACE reactions, a large single rare minnow voga01 fragment containing full-length coding region was later amplified from liver RNA sample by RT-PCR.

2.3. Analysis of Chinese rare minnow voga01 expression by RT-PCR

Various tissue samples, including liver, gill, brain, heart, muscle, gallbladder and intestine from adult male and female adult Chinese rare minnow were used for total RNA extraction with Trizol reagent (Invitrogen) following the procedure recommended by the manufacturer. The total RNA was digested with RNase free DNases to remove endogenous DNA contamination. One microgram of total RNA from each tissue samples was used to synthesis CDNA by utilizing the ReverAid kit (Fermentas). A 401 bp fragment of Chinese rare minnow voga01 was amplified with designed primers: RM VTG A9 primer (5'-caagagggcagagctggaag-3') and RM VTG B11 primer (5'-ttttgtgatcctggaag-3'). Chinese rare minnow β-actin gene expression was also analyzed with a 460 bp-amplified fragment as an internal control for calibration with their specific primers: forward primer 5'-gctcgttctgcctgctacccc-3' and reverse primer 5'-tgctgttgaattctgagcgcgc-3'. The 5'- and 3' RACE reactions using a SMART RACE Kit (Clontech). The PCR was conducted with an initial denaturation step at 94 °C for 2 min, followed by 33 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. Amplified PCR products were cloned into pGEM-T easy vector (Promega) and sequenced. Based on the assembled sequence information from the RACE reactions, a large single rare minnow voga01 fragment containing full-length coding region was later amplified from liver RNA sample by RT-PCR.

Real-time PCR was carried out on sequence detector (PRISM 7700; ABI, Foster City, CA, USA). Each reaction contained 2 μl cDNA and primers at a final concentration of 2 ng/μl. The samples were first heated to 50 °C for 2 min followed by 95 °C for 10 min. The PCR reaction was carried out for 30 cycles at 95 °C for 15 s and 60 °C for 1 min. SDS v1.7a software was used to define the cycle in which each sample attained the threshold value. All samples were run in triplicate, and the averages with standard deviations of two independent experiments are presented. Statistical analyses of the data were
exploited by performing an analysis of variances (ANOVA) using Statistica 6.0 (Statsoft. Inc.). For all statistical results, a probability of $p < 0.05$ was considered significant.

2.4. Sequence and phylogenetic analysis

The BLAST program was used for homology searches. Amino acid sequences were aligned using the CLUSTAL W multiple sequence alignment method and the phylogenetic tree was also constructed by using the program MegAlign with a nearest neighbor interchange method (DNASTAR). The sources and NCBI sequence identification numbers of these sequences are shown in Fig. 2.

2.5. Whole mount in situ analysis

Whole mount in situ hybridization was performed using single-stranded RNA probes labeled with digoxigenin-UTP of fluorescein-UTP (Roche) by slightly modification established protocols (Wan et al., 2006). A fragment flanking bp15–bp769 of the identified full-length Chinese rare minnow vtgAo1 (accession no. EU623081) amplified by RT-PCR from female rare minnow liver tissue was used as the probe for whole mount in situ hybridization experiments.

3. Results

3.1. VTG cDNAs from rare minnow

By sequencing 30 randomly selected cDNA clones from an E2-treated Chinese rare minnow adult liver cDNA library, five cDNA clones encoding VTGs were identified. Four of them were derived from the same gene and similar with teleost vtgAo1 while one was from a gene similar to vtgAo2 (clone 12, accession no. EU623081). Thus, the Chinese rare minnow has at least two distinct and functional vitellogenin genes. Primary sequencing data indicated that the four different vtgAo1 cDNA clones (clone 14, 16, 23 and 30) only contains the fragments near the 3′-ends of the predicted sequence of the vtgAo1 transcripts (ranging from bp 1921 to 4031) according to the full-length transcript of the Chinese rare minnow vtgAo1 with accession no. EU623080). To recover the 5′ end of the vtgAo1 cDNA, 5′ RACE-PCR was performed. The full-length sequence of vtgAo1 was assembled by combining the sequences from the original clones and the 5′RACE fragment to assemble a full-length of vtgAo1 cDNA. In order to confirm the existence of the transcripts, primers were designed to amplify a full-length vtgAo1 product from E2-treated Chinese rare minnow liver tissue via RT-PCR and subjected to sequencing. The full-length vtgAo1 contains 4031 nucleotides encoding a 1338 amino acid polypeptide, with an 14-bp 5′-untranslated region and a 182-bp 3′-untranslated region including a 27-bp poly(A) tail (accession no. EU623080). The molecular mass of the mature protein, based on its deduced amino acid sequence, was approximately 145 kDa.

3.2. Sequence analysis and comparison with classified teleost VTG members

According to the new VTG gene nomenclature of Finn and Kristoffersen (2007), our putative amino acid sequence has highest homology to fathead minnow vtgAo1 (88.1%), with some similarity with other teleost vtgA class members at 82.8–42.5%. To analyze the evolutionary relationship among members of the VTG and apolipoprotein B (apoB), a phylogenetic tree was made by using CLUSTAL analysis of the amino acid homology (Fig. 1). The tree shows clear grouping of the different vtg and apoB families and indicates that this Chinese rare minnow is most closely related to the vtgAo1 group. The tree also supports the existing teleost classification of VTGs as described by Finn and Kristoffersen (2007).

The overall primary structure of the identified provitellogenin is schematically represented in Fig. 2. Like all the other teleost vtgAo1, the Chinese rare minnow contains a 15 amino acid signal peptide, a lipovitellin heavy chain (LvH)/large lipid transfer module (LITM), phosvitin (Pv) and lipovitellin light chain (LvL). Other teleost vtgAo1 signatures, such as, a typical α-helical, a C-sheet, a α-sheet structure and a conserved vitelligenin receptor domain (VtgR) in its LvH/LITM region also present in the Chinese rare minnow vtgAo1. A Vitellogenin_N domain, a common domain found in the members of VTG, microsomal triglyceride transfer protein (MTP) and apolipoprotein B-100 (apoB) is, also identified at the first ~1000 amino acids region (Finn, 2007).

3.3. The inducibility of vtgAo1 transcript in Chinese rare minnow liver by E2

In order to confirm the inducibility of Chinese rare minnow vtgAo1 expression in liver, total RNAs were isolated from the liver tissue of un-treated control adult female fish or adult female fish treated with E2. The levels of vtgAo1 transcript in liver were then examined by RT-PCR. As evident in Fig. 3A, the expression level of vtgAo1 transcript is much lower in control female rare minnow as compared to E2-treated fish liver. β-Actin was used as a control to monitor the quality and quantity of the RNAs.

3.4. Tissue distribution of Chinese rare minnow vtgAo1

To examine the tissue distribution of Chinese rare minnow vtgAo1 mRNA in adult fish, RT-PCR was carried out. Total RNA samples were isolated from pooled various tissues from both female and male adult rare minnow. As shown in Fig. 3B, Chinese rare minnow vtgAo1 was expressed predominantly in the liver of adult rare minnow (presumably in the female liver tissue). Interestingly, a weak but detectable level of vtgAo1 transcripts was also detected in the heart and brain tissue. No vtgAo1 transcript was found in other tissues of adult rare minnow. To ensure the quality and relative equal amount of the RNA samples used, transcripts of rare minnow β-actin were also amplified from the same tissue samples (lower panel in Fig. 3B).

3.5. Effects of E2 on rare minnow vtgAo1 expression in heart and brain

Real-time PCR was used to examine the inducibility of the rare minnow vtgAo1 transcription in heart and brain. Total RNA samples were isolated from the brain and heart tissue samples from control and E2-treated adult female rare minnow. Results demonstrated that vtgAo1 transcript in female brain tissue did not exhibit significant differences between control and E2 treatment groups, while levels of vtgAo1 in female rare minnow heart increased significantly following E2 treatment compared with those from control female rare minnow group (Fig. 4).

3.6. Effects of adrenergic agonists on rare minnow vtgAo1 expression

In order to examine the adrenergic agonists on vtgAo1 expression in rare minnow, real-time quantitative PCR was used to analyze the transcription levels of the vtgAo1 presence in the liver, brain and heart tissue from adult female fish with or without adrenergic agonist treatment. As shown in Fig. 5A, the adrenergic agonists, PE and ISO can effectively inhibit the expression of the vtgAo1 in
Fig. 1. Phylogenetic analysis of deduced vtg proteins. The teleost vtgs were classified with the new vtg gene nomenclature of Finn and Kristoffersen (2007). Common names of fish species and the GenBank accession numbers of each vtg sequence is listed in the figure. The GenBank accession number of the newly identified full-length Chinese rare minnow vtg is EU623080 (shown as RMvtgprotein, arrowed in the figure).

heart and liver tissue (Fig. 5A). However, the effects of these two adrenergic agonists on brain vtgAo1 expression are not significant (Fig. 5A).

Using whole mount in situ hybridization analysis, a significant level of vtgAo1 transcript was also shown in adult male Chinese rare minnow hearts, especially, in atrial tissue (Fig. 5B.a and B.c, arrow). When Chinese rear minnow was treated with PE or ISO, a down-regulation of the levels of vtgAo1 transcript in the heart was shown in Fig. 5B.b and B.d (open arrow). The suppression effects on vtgAo1 expression of the adrenergic agonists have been observed in both male and female adult rare minnow hearts.

Fig. 2. Schematic structure of the Chinese rare minnow vtgAo1. Signal peptide, lipovitellin heavy chain/large lipid transfer module (LvH/LLTM), phosvitin (Pv) and lipovitellin light chain (LvL) are marked by ■, □, □, and □ boxes, respectively. The length of each domain (number of amino acid residues) and the positions of their boundaries are shown below the boxes and sketch lines.
Fig. 3. Chinese rare minnow vtgAo1 expression analyses with RT-PCR. Total RNA samples were collected from both male and female rare minnow adult tissues. β-Actin mRNA was used as an internal control. (A) E2 induction of the vtgAo1 expression in Chinese rare minnow liver and (B) tissue distribution of the Chinese rare minnow vtgAo1.

4. Discussion

Small fish species such as zebrafish (Danio rerio), Japanese medaka (Oryzias latipes) and fathead minnow (Pimephales promelas) have often been employed as model organisms for the screening of endocrine-disrupting chemicals (EDCs). There are clear regional preferences for the use of a particular species in toxicity tests. For example, regulatory programmes in North America often use the fathead minnow for toxicity tests while the zebrafish and fathead minnow is used in Europe and the medaka used in Japan.

The Chinese rare minnow has often been recommended as the “fathead minnow” in China for ecotoxicological model. While the fathead minnow has a ubiquitous distribution across North America, the Chinese rare minnow is native to China, living mostly in upstream waters in the Yangtze River, China. It shares similar attributes to the other small fish models such as the zebrafish and medaka in that it has a rapid life cycle, is easy to maintain and possesses active spawning capacity (Wang, 1999; Ankley and Johnson, 2004). Since 1990s, many physiological, embryonic developmental studies and some ecotoxicological works have been done with this species (Lu and Shen, 2002; Zhou et al., 2002; Zhong et al., 2005). However, many features concerning endocrinology and molecular background of the Chinese rare minnow remain...
largely unknown. Our present work is the first attempt for identification of its genetic feature regarding ecotoxicological work for the species.

Previous work by Liao et al. (2006) and Zha et al. (2007) have shown that plasma VTG level in Chinese rare minnow are likely to be responsive to estrogen treatment, no rare minnow VTG gene has been identified yet. We were interested to investigate this and thus created a E2-treated adult Chinese rare minnow liver cDNA library. Out of the 30 clones randomly selected from the library, we found four vtgAo1 and one vtgAo2 cDNA clones. Thus, Chinese rare minnow has at least two distinct VTG genes, both of which are estrogen responsive genes with vtgAo1 as the major form VTG gene in the fish (Finn, 2007). By comparison with available VTG sequences from other fish species, we found that this VTG gene is most similar to the other vtgAo1 genes in cyprinid fish (Fig. 1). Like all the other known cyprinid vtgAo1 genes, the rare minnow vtgAo1 contains a 15 amino acid signal peptide, a Lvh/LtmP, Pvl and Pvl domain. In addition, similar with the major VTG genes of fathead minnow, zebrafish and carp, rare minnow vtgAo1 also lacks the c-terminal von Willebrand factor type D domain (vWFD) domain (Fig. 2; Finn and Kristoffersen, 2007; Finn, 2007).

In teleosts, VTG genes have been suggested to be primarily expressed in the liver (Wang et al., 2000; Islinger et al., 2003; Tong et al., 2004; Wang et al., 2005; Mikawa et al., 2006). With this assumption, many studies reporting the cloning of new VTG transcripts have not examined or reported the tissue distributions of their newly cloned transcripts (Van der Ven et al., 2003; Jung et al., 2005; Sawaguchi et al., 2005). In addition, for studies that do examine the distribution of VTG mRNA, heart tissue has often been neglected (Wang et al., 2000, 2005; Islinger et al., 2003; Tong et al., 2004). One exception is perhaps the report by Mikawa et al. (2006) that vtgA1 expression is not expressed in the heart tissue of the Japanese conger.

The expression of vtgAo1 in normal adult heart tissue of the Chinese rare minnow was first detected by RT-PCR and further confirmed by whole mount in situ hybridization on heart tissue. Thus, it is clear that Chinese rare minnow vtgAo1 expresses in adult heart, particularly in atrial tissue. Our study is the first reporting the expression of VTGs in the heart of teleost fish and indicates a gap in the current knowledge of VTGs and their function. A similar observation of the presence of zebrafish vtgAo1 transcripts in adult zebrafish heart tissue has also been confirmed recently in our laboratory (data not shown here).

One possible function for the expression of VTGs in the heart could be for the protection from surplus intracellular lipids in cardiomyocytes. The heart utilizes a large amount of fatty acids for fuel and can convert fatty acids into triglycerides (Yokoyama et al., 2004). However, the capacity of cardiac myocytes to store triglycerides is limited, and abundant triglyceride accumulation in cardiac myocytes could lead to lipotoxic cardiomyopathy (Van der Vusse et al., 1992). Hence, animal hearts require strategies to unload surplus intracellular lipid.

Invertebrate and vertebrate VTGs, together with insect apolipophorin II, mammalian apoB and the large subunit of mammalian MTP are members of a large lipid transfer protein family, which have emerged from an ancestral molecule with a function in the intracellular and extracellular transfer of lipids and liposoluble substances. All of them are non-exchangeable apolipoproteins and intracellular lipid-exchange proteins involved in the assembly, secretion, and metabolism of lipoproteins (Babin et al., 1999; Finn, 2007). Previously, lipoprotein secretion had generally been thought to be limited to the liver and gut. However, Borén et al. (1998) found that apoB had robust expression in the heart. In addition, MTP, the protein required to add lipid to apoB, was also found in the heart.

Recently, cardiac apoB production has been demonstrated to play roles for improving cardiomyopathy by increasing lipid resorption from heart (Borén et al., 1998; Nielsen et al., 2002; Yokoyama et al., 2004). Therefore, the expression of VTG expression observed in the heart in the present study might suggest the existence of similar cardiomyocyte protection mechanisms in teleost for "reverse triglyceride transportation".

Alpha-adrenergic agonist, PE, and beta-adrenergic agonist, ISO treatment can decrease the secretion of triglyceride from perfused rat liver, causing the intracellular accumulation of triglyceride in the hepatic cells. It has been also suggested that the secretion of apoB can be suppressed probably via post-transcriptional level by these adrenergic agonists in the perfused rat liver tissue (Yamauchi et al., 1998). However, in our present studies, the transcript of Chinese rare minnow of vtgAo1, a teleost lipid transport protein, is suppressed in liver and heart with adrenergic agonist treatments (Fig. 5). Recently, a similar effect of the zebrafish vtgA1 caused by the adrenergic agonists have also been observed in this laboratory, and the zebrafish vtgAo1 probe used shares less than 5% similarity with the zebrafish apoB cDNA (data not shown here). In addition to that, no significant similarity was found between our rare minnow vtgAo1 probe for whole mount in situ hybridization and the zebrafish apoB cDNA, so it is unlikely that the transcript detected in heart of rare minnow by vtgA1 is the rare minnow apoB despite lack of a full-length apoB. As a lipid transport protein, at least some of the cyprinid vtgAo1s express in the heart and may play some physiological roles involved to the energy metabolism. This has provided strong evidence that the fish VTGs might actually be involved in some roles in vivo other than a major egg yolk protein. It has been shown that the estrogen administration could suppress the expression of beta-adrenoceptor in rat cardiomyocytes (Kam et al., 2004). Our results indicated that the heart vtgA1 expression could be regulated by both E2 and adrenergic agonist treatments. Further clarification of whether this phenomenon is due to the cross-talk between the estrogenic and adrenergic agents is needed. The reason for the failure of observation on the regulatory effects of the E2 and adrenergic agonist treatments on the minimal existing of the vtgA1 transcript in brain tissue also has to be explored in the future.

In our present study, we identify the full-length cDNA of Chinese rare minnow vtgAo1. Its inducibility to estrogen has been confirmed. Interestingly, for the first time the extrahepatic expression of a major VTG form has been observed in a much unexpected location—the heart. In addition, expression of the Chinese rare minnow vtgA1 can be suppressed by adrenergic agonists. Fish VTG mRNA is generally considered as a sensitive biomarker for monitoring oestrogenic effect. Our work provides the first evidence that the VTG expression can actually be regulated by adrenergic reagents. It would be interesting to further verify whether the circulating levels of vtg could be actually regulated by adrenergic signals. Thus, further characterization of the teleost VTG gene family may provide new insights into the mechanisms of the physiological functions of VTGs and their expression regulation.

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References


