Abstract

20β-Hydroxysteroid dehydrogenase (20β-HSD) is the enzyme that reduces C20 carbonyl group of C21 steroids, which has broad substrate specificity and is structurally similar to mammalian carbonyl reductase1. Here we report a 2.0 kb upstream sequence of 20β-HSD from the air-breathing catfish, Clarias gariepinus. Several putative transcription factor binding sites more importantly, cAMP, xenobiotic, glucocorticoid and progesterone responsive elements, AP-1, SP1, sterol regulatory element binding protein, SF-1, OCT-1, OCT-6, GATA and several other transcription factor binding sites were identified by in silico analysis. Luciferase reporter assays with progressive PCR based deletion mutants in Chinese hamster ovary and human embryonic kidney cell lines demonstrated that -562 region harboring CAAT box flanked by cAMP responsive element (CRE) is important for basal promoter activity. Further, increase in luciferase activity with cAMP altering drugs such as forskolin and 3-isobutyl-1-methylxanthine and specific electrophoretic mobility shift with oligonucleotides corresponding to CRE indicate the regulatory influence of cAMP on 20β-HSD promoter activity. A cDNA encoding CRE binding protein (CREB) isolated from the ovarian follicles was highly homologous to vertebrate CREB1. Real-time RT-PCR analysis demonstrated synergistic expression pattern of CREB with that of 20β-HSD during gonadotropin induced final oocyte maturation, in vitro and in vivo. Results from this study provide insights on the functional characteristics of 20β-HSD promoter.
Introduction

20β-hydroxysteroid dehydrogenase (20β-HSD) was first reported to be present in the prokaryote *Streptomyces hydrogenans* as an enzyme reducing C20 carbonyl group of C21 steroids (Hubener and Lehman, 1958) and subsequently found in teleosts and mammals (Wermuth, 1981; Tanaka *et al*., 1991; Senthilkumaran *et al*., 2004). cDNA cloning studies have demonstrated a striking similarity in structure and function of teleost 20β-HSD with that of mammalian carbonyl reductase1 (CBR1; Tanaka *et al*., 1991) and denoted as CBR1-like 20β-HSD. Enzymes from both the groups of animals have a broad spectrum of substrate specificity over a wide range of xenobiotic carbonyls, endogenous steroids and prostaglandins (Wermuth, 1981; Iwata *et al*., 1990; Senthilkumaran *et al*., 2004). Fish 20β-HSD is known to be involved in the production of 17α, 20β-dihydroxy-4-pregnen-3-one, the maturation inducing hormone (MIH; Nagahama, 1997). Up-regulation of 20β-HSD activity and transcript by gonadotropin during final oocyte maturation (FOM) has been well documented (Senthilkumaran *et al*., 2002 & 2004; Nagahama and Yamashita, 2008; Chapters 1 & 2). Besides the gonadotropin regulation, FOM is influenced by several other factors including activin (Ge, 2000), melatonin (Chatteraj *et al*., 2005), insulin like growth factor (Dilip *et al*., 2006) etc.

Although high levels of 20β-HSD was shown to be present in neonatal pig testis, its physiological significance remains unclear (Tanaka *et al*., 1991). In rodents, both inducible and non-inducible CRs have been identified (Aoki *et al*., 1997) and the
inducible form is implicated in ovulation (Espey et al., 2000). In humans, CR functions in phase-I xenobiotic metabolism, reduces several carbonyl compounds and CRs are involved in both physiological processes and pathological conditions (Ismail et al., 2000; Maser, 2006; Oppermann, 2007).

CRs are either appear to be house keeping genes or regulated via endogenous hormones and growth factors (Oppermann and Maser, 2000). Rodent CRs and teleost $20\beta$-HSDs are inducible by gonadotropins (Inazu et al., 1992; Senthilkumaran et al., 2002; Chapters 1 & 2) while human gene responds to classical xenobiotic inducers e.g. naphthoflavone, phenobarbital etc. and functional xenobiotic response elements were identified in human CBR1 gene promoter (Lakhman et al., 2007). However, factors that regulate rodent CRs and other vertebrate $20\beta$-HSD expression apart from gonadotropin are yet to be identified. Hence, functional characterization of $20\beta$-HSD promoter may shed light on our understanding of regulation of CR/$20\beta$-HSD expression in view of their roles in both reproductive and non-reproductive processes.

In the present study, we isolated upstream sequence of the air-breathing catfish, *Clarias gariepinus* $20\beta$-HSD. The putative cis-acting elements were identified by *in silico* analysis and functional analysis of the promoter was carried out by PCR based progressive deletion mutants. In addition, we present the evidences for the regulation of catfish $20\beta$-HSD gene expression by cAMP.
**Materials and Methods**

**Cell culture and reagents**

HEK-293 (human embryonic kidney) and CHO (Chinese hamster ovary) cell lines were obtained from the National Centre for Cell Science (Pune, India). Minimum essential medium (α-MEM), fetal bovine serum and other cell culture reagents were purchased from Gibco-BRL (Invitrogen, Carlsbad, CA, USA). Cells were routinely cultured in 75 cm$^2$ vented flasks using α-MEM supplemented with 10% fetal bovine serum. Cultures were grown in an incubator at 37°C, 5% CO$_2$ and 95% relative humidity. Cultures were maintained at low passage numbers (n<10). Forskolin and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Construction of Genome walking library and isolation of upstream elements**

Genomic DNA was isolated from the ovarian follicles of catfish using genomic DNA isolation kit (Bangalore Genei, Bangalore, India) following manufacturer’s protocol. Genome walking library was constructed using universal genome walker kit (Clontech, Mountainview, CA, USA) following manufacturer’s instructions. Twenty five µg of genomic DNA was digested overnight each with EcoRV, PvuII, DraI and StuI. Digested DNA was purified using phenol chloroform extraction method. Genomic DNA digested with each restriction enzyme was ligated to adaptors separately. $20\beta$-HSD upstream region was isolated using gene specific primers (Table 1) designed in the first exon of the $20\beta$-HSD and the adapter primer-1. Touchdown PCR was used for the
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amplification. Cycling conditions were 94°C 30sec, 72°C 3 min, 5 cycles, 94°C 30sec, 68°C 30sec, 72°C 3 min for 30 cycles. A nested PCR was carried out using gene specific primers (Table 1) and adaptor primer-2 (both the adapter primers were given in the genome walker kit), all the amplicons were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and sequence was determined bi-directionally.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’ – 3’</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>GSP-R1</td>
<td>CGTCGCAGCCCAATAGGTACAGTC</td>
<td>Genome walking, PCR</td>
</tr>
<tr>
<td>GSP-R2</td>
<td>CGTGACCTGGAAGCACAAGACGC</td>
<td>Genome walking, PCR</td>
</tr>
<tr>
<td>F1</td>
<td>GGTACCATGACCTCTAAACTTTAGCG</td>
<td>-1971/+42 (deletion mutant)</td>
</tr>
<tr>
<td>F2</td>
<td>GGTACACTTCACTTCACCCTCTCACTTC</td>
<td>-1474/+42 (deletion mutant)</td>
</tr>
<tr>
<td>F3</td>
<td>GGTACACTTCATATATTCACATTCTG</td>
<td>-803/+42 (deletion mutant)</td>
</tr>
<tr>
<td>F4</td>
<td>GGTACCTCGAGTAGGTACTCCTTCACAG</td>
<td>-562/+42 (deletion mutant)</td>
</tr>
<tr>
<td>F5</td>
<td>GGTACACTCCGTTTCTCCACAG</td>
<td>-384/+42 (deletion mutant)</td>
</tr>
<tr>
<td>F6</td>
<td>GGTACCTCCACCCTACGGATGGCTGCTG</td>
<td>-210/+42 (deletion mutant)</td>
</tr>
<tr>
<td>R</td>
<td>AAGCTTTGACTGCGAGCAAGACACGC</td>
<td>Common reverse primer (deletion mutants)</td>
</tr>
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Table 1. List of primers used for catfish 20β-HSD upstream sequence cloning and promoter analysis.
**In silico analysis**

Core promoter prediction was carried out using the software neural network promoter prediction program. Putative transcription factor binding sites were predicted using the programs such as MatInspector and TRANSFAC.

**Cloning of 20β-HSD promoter deletion constructs**

Progressive 5' deletion constructs were made by PCR using the primers listed in table 1. The resulted deletion mutants were cloned in KpnI and HindIII sites of pGL2 basic firefly luciferase vector (Promega). The identity of each construct was verified by double digestion and the absence of cloning artifacts was determined by nucleotide sequencing.

**Transient transfections and luciferase reporter activity assays**

Cells were plated 24-48 hours before transfections in 24 well plates. Reporter gene constructs (firefly luciferase), and the *Renilla* luciferase pRL-TK plasmid were co-transfected into 60 - 70% confluent cell cultures by using Tfx20 (Promega). Forty-eight hours after co-transfections, cultures were washed once with phosphate buffered saline solution and the cells were lysed with 100 µl per well passive lysis buffer (Promega). Cell lysates were incubated at room temperature (15 min), mixed with a vortex blender (10 sec), and centrifuged at 4°C (1500 rpm, 30 sec). Luciferase reporter activities were determined with the dual-luciferase reporter assay system (Promega) according to the
manufacturer’s instructions. Light intensity was measured using Turner Design 20/20 luminometer. Luciferase reporter activities were expressed as relative with respect to the values obtained for *Renilla* luciferase.

**Molecular cloning of CREB from catfish ovary**

A set of degenerate primers designed (Table 2) by aligning other vertebrate CREB cDNA sequences were used to amplify a partial cDNA fragment by RT-PCR. Gene specific primers (Table 2) for 5' and 3' rapid amplification of cDNA ends (RACE) were designed from partial cDNA sequence and RACE was performed to isolate 5' and 3' ends of catfish CREB. Methodologies used for obtaining partial cDNA and RACE were described in chapter 2.

**Northern blotting**

Twenty five micrograms of total RNA prepared from different stages of ovarian follicles was analyzed by Northern blotting. Catfish CREB open reading frame (ORF) cDNA was used to probe the membranes and method followed was described in detail in chapter 2.

**Real-time RT-PCR**

Transcript abundance of CREB during human chorionic gonadotropin (hCG)-induced oocyte maturation, *in vitro* and *in vivo* as well as ovary at different stages of gonadal
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cycle was done by real-time RT-PCR using gene specific primers (Table 2) following the methodology as described in chapter 2.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5' – 3'</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF1</td>
<td>CATMTATCAGACGACYGCASSGGA</td>
<td>Degenerate RT-PCR</td>
</tr>
<tr>
<td>DRI</td>
<td>CYTTCTCTCTTTTGGCAGACACTC</td>
<td>Degenerate RT-PCR</td>
</tr>
<tr>
<td>GSP-R1</td>
<td>AGTTTGCAGCCCTTTGGCACGCCTC</td>
<td>5' RACE</td>
</tr>
<tr>
<td>GSP-R2</td>
<td>CTGGATGGCTCCACCTGTGTGAT</td>
<td>5' RACE</td>
</tr>
<tr>
<td>GSP-F1</td>
<td>CGCCTCATGAAGAAGGGGGAAGC</td>
<td>3' RACE</td>
</tr>
<tr>
<td>GSP-F2</td>
<td>AGGGAGCGGCGCCCGAGGATGTCGC</td>
<td>3' RACE</td>
</tr>
<tr>
<td>ORF-F</td>
<td>GCTAGCATGACCATGGAGGCGGAGGAGGC</td>
<td>ORF cloning</td>
</tr>
<tr>
<td>ORF-R</td>
<td>CTCGAGTTACTCGGATTTATGGCGAGTACAG</td>
<td>ORF cloning</td>
</tr>
<tr>
<td>qRT-F</td>
<td>CGTCCTTCTTACAGGAAGATCC</td>
<td>Real-time RT-PCR</td>
</tr>
<tr>
<td>qRT-R</td>
<td>TCTCTGAAGCTGTATTTGGCAAGC</td>
<td>Real-time RT-PCR</td>
</tr>
</tbody>
</table>

Table 2. Primers used for CREB cloning and expression.

Electrophoretic mobility shift assay (EMSA)

Gel shift assay was performed as per the method described previously (Yoshiura et al., 2003). Oligomeric sequence of catfish 20β-HSD region harboring a cAMP responsive element (CRE) and a mutated CRE were synthesized and labeled by [32P]dATP using polynucleotide kinase. The labeled oligonucleotide probe was added to 20 µl binding
reaction with 5 µg protein of catfish ovarian follicular nuclear extract. Specificity of binding was assessed using un-labeled double stranded DNA oligos as competitors. Binding reactions were incubated on ice for 45 min and DNA-protein complexes were separated on 8% non denaturing polyacrylamide gel at 10 mA for an hour. Gels were dried and visualized using phosphorimager (Typhoon, GE Healthcare).

<table>
<thead>
<tr>
<th>oligo</th>
<th>Sequence 5’ – 3’</th>
</tr>
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<tbody>
<tr>
<td>CRE</td>
<td>CTGTCGTTTTGGCTGACGTCACTGTCCCCCAGAGGACGTC</td>
</tr>
<tr>
<td>CRE</td>
<td>GACGTCCTCTGGGGGACAGTGACGTCAGCCAAAAGGACAG</td>
</tr>
<tr>
<td>CRE-mutant</td>
<td>CTGTCGTTTTGGCTAAAGTCACTGTCCCCCAGGGACGTC</td>
</tr>
<tr>
<td>CRE-mutant</td>
<td>GACGTCCTCTGGGGGACAGTGACTTTAGCCAAAAGGACAG</td>
</tr>
</tbody>
</table>

Table 3. Native and mutant CRE oligonucleotide sequences used in EMSA. CRE region is underlined.

Data analysis

All the real-time RT-PCR and luciferase reporter activity assays were done three times independently in triplicates and data was expressed as ±SEM. Statistical analysis was done using Sigmastat 3.1 software. Significance between different groups was tested by ANOVA followed by Student’s-Neuman-Keul’s test. Difference between groups were considered at $P<0.05$. 

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Results

Cloning and in silico analysis of 20β-HSD promoter

A 2.0 kb upstream sequence of catfish 20β-HSD was obtained by genome walking approach (Fig. 1). Transcriptional start point (TSP) was determined by 5’ RACE (chapter 2) and found to be at 59 nucleotides upstream of ATG start codon. Analysis of TSP by neural network promoter prediction program also identified TSP at the same location. Analysis of core promoter motifs identified a non-canonical TATA box (TAATAAA). CpG islands in the proximal promoter region were also predicted with an observed/expected ratio greater than 0.6 but %C+G was marginal (Fig. 2). Computer assisted searches using the programs MatInspector and TRANSFAC for additional cis-acting elements indicated putative consensus motifs for several transcription factors. Those with maximum core and matrix similarity include activator protein1 (AP-1), specificity protein1 (SP1), steroidogenic factor1 (SF-1), octamer binding proteins 1 & 6, (OCT-1 & OCT-6), GATA binding factor (GATA), CAAT binding factor (CBF), TATA box binding protein (TBP), hepatic nuclear factor (HNF) glucocorticoid, progesterone responsive elements (GRE & PRE). More importantly, cAMP and xenobiotic responsive elements (CRE & XRE) were observed in both proximal and distal ends of the promoter (Fig. 2).
**Functional analysis of 20β-HSD promoter constructs**

We generated a series of progressive PCR based deletion mutants (Figs. 3 & 4) and performed gene reporter assays to identify the regions important for regulation of 20β-HSD expression. Results from the reporter assays indicated the presence of negative regulatory elements in the region of -1474 to -803 as there was a significant decrease in reporter activity from -1971/+42 construct. A considerably high reporter activity was found with -562/+42 construct that has a CAAT box flanked by CRE. Deletion of CRE decreased promoter activity significantly. Luciferase reporter activities were more or less similar in both HEK293 and CHO cell lines (Fig. 5).

**Effect of forskolin and IBMX on 20β-HSD promoter activity**

To implicate the functionality CRE, we used different doses of forskolin, drug that increase cellular cAMP with a fixed concentration of IBMX (phosphodiesterase inhibitor) in gene reporter assays. Forskolin at 0.5 µM concentration strongly induced the promoter activity with full-upstream region as well as with -562/+42 promoter construct while the deletion of CRE did not show significant difference in promoter activity (Fig. 6).

**Identification of CREB binding site**

Gel shift assay using ovarian follicular nuclear extracts demonstrated a complex formation with $^{32}$P labeled oligomeric sequence containing CRE motif while no
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complex was observed with mutated oligo. Inability of nuclear extracts in the presence of cold competitor oligo indicates the specificity of binding (Fig. 7).

Fig. 1. 1% Agarose gel showing the nested PCR amplifications using 20β-HSD gene specific primers in catfish genome walking library and right side panel is schematic representation of genome-walking strategy.
Fig. 2. Nucleotide sequence from the 5' flanking region of catfish 20β-HSD. The translational start site is double underlined and the transcriptional start site is indicated with an arrow and bold face letter. The different fragments corresponding to the series of progressive deletion promoter constructs are indicated with downward arrow. The putative transcription factor binding sites are underlined and shown in different colors.
Fig. 3. Schematic representation of catfish 20β-HSD promoter and PCR based deletion mutants. CDS stands for coding sequence.
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Fig. 4. Map of luciferase reporter vector pGL2 (A). A 1% agarose gel showing the PCR amplicons of deletion mutants (B). Numbers over each band represents the size of band in base pairs.
Fig. 5. Functional analysis of different 20β-HSD promoter constructs (indicated on the left side) co-transfected with pRL-TK into HEK293 (A) and CHO (B) cells. Luciferase activity is presented as relative to the activities measured for Renilla luciferase (* indicates significance ANOVA, P<0.05).
Fig. 6. Effect of cAMP altering drugs on the luciferase reporter activity of catfish 20β-HSD promoter in CHO cells with -1971/+42 (A) and -562/+42 (B) promoter constructs (* indicates significance, ANOVA, $P<0.05$).
Fig. 7. Autoradiogram showing the electrophoretic mobility shift of follicular nuclear extracts incubated with oligonucleotide sequence corresponding to predicted CRE. Same oligonucleotide with mutated sequence was used as negative control. Un-labeled oligonucleotides were used in indicated fold excess for competition.
Molecular cloning of CREB from catfish ovary

A partial cDNA of 405 bp was obtained from the ovarian follicles using a set of degenerate primers by RT-PCR. The identity of cloned cDNA was confirmed by BLAST search. Full-length cDNA of CREB was isolated from the catfish ovarian follicles by 5' and 3' RACE strategies (Fig. 8) using gene specific primers designed from partial cDNA clone (Table 3). Full-length catfish CREB cDNA was 1.398 kb with an ORF of 975 bp encoding a protein of 375 amino acids. The 5' UTR was 91 bp while 3' UTR was 332 bp (Fig. 9). The putative protein is highly conserved encompassing signature domains, kinase inducible and DNA binding domain (bZIP). Catfish ovarian CREB shares highest homology with that of zebrafish (79%). Phylogenetic analysis by ClustalW method demonstrated that catfish CREB segregated with zebrafish into a separate clade, while as tilapia CREBs formed into distinct clade (Fig. 10).

Expression of CREB in ovary

Real-time RT-PCR analysis demonstrated that transcript abundance of CREB was high in the preparatory and spawning phases while it was low in pre-spawning and regressed phases of ovarian cycle (Fig. 11). Northern blot analysis of CREB identified a single transcript of about 1.4 kb and the expression in different stages of ovary by Northern blot analysis was in accordance with real-time RT-PCR results (Fig.11). In hCG-induced oocyte maturation, in vitro, CREB mRNA levels increased by four hours after treatment with hCG, reached a peak level by 6 hours and there by decreased (Fig. 12).
Fig. 8. 1% Agarose gels showing RT-PCR products using degenerate primers for amplification of partial CREB cDNA fragment (A), 5' and 3' RACE (B & C) products using gene specific primers to obtain CREB full-length cDNA.
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Fig. 9. Nucleotide (blue) and deduced amino acid (red) sequence of catfish ovarian CREB. UTRs are shown in black letters and polyadenylation signal is shown in boldface letters with underline.
Fig. 10. Phylogenetic tree showing the evolutionary status of catfish CREB. (Accession no.: Human A NM_004379; Human B NM_134442; Mouse C NM_001037726; Xenopus NM_001086603; Mouse A NM_009952; Mouse B NM_133828; Rat A NM_134443; Rat B NM_031017; Chicken NM_204450; Zebrafish NM_200909; Pig NM_001099929; Cattle NM_174285; Songbird NM_001048256).
Fig. 11. Expression of *CREB* in different stages of catfish ovary by Northern blot (A; representative, n=3) and real-time RT-PCR analysis (B). P, preparatory; PS, pre-spawning; R, regressed (* indicates significance, n=3, ANOVA, *P*<0.05).
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Fig. 12. Real-time RT-PCR analysis of CREB expression during hCG-induced oocyte maturation, *in vitro* (A) and *in vivo* (B). * Indicates significance (n=3, ANOVA, $P<0.05$).
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Discussion

In the present study, we report a 2.0 kb up stream region of 20β-HSD from catfish. 20β-HSD has been identified both in mammals and teleosts, but reports on transcriptional regulation of 20β-HSD are very much limited (Senthilkumaran et al., 2001). However, promoter of CBR1 from rat and humans has been isolated (Aoki et al., 1997; Lakhman et al., 2007). Though these enzymes shares very high similarity in the structure and enzymatic activity, but they involve in different physiological processes and consistently their promoter organizations are also different. Human and mouse carbonyl reductase promoters have features of a prototypical CpG promoter with GC boxes and either TATA box or down stream promoter elements are absent (Aoki et al., 1997; Lakhman et al., 2007). In contrast, 20β-HSD promoter has a TATA box while CpG islands were predicted in the proximal region marginally. Our results with gene reporter experiments in HEK293 and CHO cells demonstrated the presence of regulatory elements in -562 region that appear to relevant to promote transcription at basal condition.

The orphan nuclear receptor Ad4BP/SF-1 is important for development and function of steroidogenic organs. Many studies have highlighted important roles for Ad4BP/SF-1 in transcriptional regulation of several steroidogenic enzymes both in teleosts and higher vertebrates (Ikeda et al., 1994; Yoshiura et al., 2003; Achermann, 2005). Presence of Ad4BP/SF-1 sites at -1519/-1525 region in catfish 20β-HSD promoter indicates possibility for the regulation of this enzyme by Ad4BP/SF-1. However, studies from
tilapia 20β-HSD promoter activity indicated no regulatory role for Ad4BP/SF-1 (Senthilkumaran et al., 2001 & 2004). GATA are members of zinc finger transcription factor family that regulate target gene transcription through a common DNA binding motif (A/T)GATA(A/G). GATA-4 and 6 are known to be important for development as supported by the deficient mice die during early development. An important role for GATA-4 in gonadal expression of steroidogenic enzyme genes has been suggested by several studies and GATA-4 is shown to work synergistically with Ad4BP/SF-1 (LaVoie, 2003). Since 20β-HSD expression is known to be associated with differentiation of spermatogonia (Miura et al., 2006), presence of GATA binding site on 20β-HSD promoter indicates that this also may be one of the pathways in gonadal development in fish. Expression of 20β-HSD is ubiquitous in teleosts, though their role is not well defined in tissues except for gonads. Observation of low expression of 20β-HSD in liver (Senthilkumaran et al., 2002; Chapter 2) and its induction by sewage effluents in liver of trout (Albertson et al., 2007) together with high specific activity of E. coli expressed recombinant 20β-HSD proteins on xenobiotics (Senthilkumaran et al., 2002; Chapter 2) provides impetus to the hypothesis that 20β-HSD may also be involved in xenobiotic metabolism like that of mammalian CBR1s. Further identification of XRE both in catfish 20β-HSD promoter as well as human CR gene potentiates the role of these enzymes in xenobiotic metabolism.
Transcriptional regulation of 20β-HSD

We observed GRE and PRE in the promoter of 20β-HSD. Though we did not perform assays relating to the modulation of 20β-HSD expression by steroids, few studies are available on this line. Similar to the one that occurs in teleost ovarian follicles, a shift in steroidogenesis from androgens to progestins was also demonstrated in spermiating male fishes (Barry et al., 1990) and it is believed that estradiol has a role to play in increasing the levels of MIH with the onset of spermiation (Vizziano et al., 1996), probably through feedback regulation. Imamura et al. (2001) demonstrated regulation of 20β-HSD activity by testosterone in rat and observation of sex steroid hormone responsive elements on 20β-HSD promoter provides the direct evidence for hormonal regulation of 20β-HSD.

cAMP responsive genes are known to be regulated by cues such as extracellular stresses or hormonal signals (Sands and Palmer, 2008). We identified a CRE element in the -328 region of 20β-HSD promoter. Luciferase reporter assays have demonstrated that promoter construct of this region is important for 20β-HSD promoter activity. Inducibility of the promoter constructs with cAMP enhancing drugs support the notion of 20β-HSD expression during oocyte maturation in teleost species (Senthilkumaran et al., 2004; Chapters 1 & 2).

CREB members represent a large family of bZIP transcription factors rather with diverse physiological functions. CREB members are both ubiquitously expressed and/or tissue specifically expressed, with latter controlling cell specific pattern of gene transcription (Sands and Palmer, 2008). Though the presence of multiple forms of
CREBs within a particular cell type is very common (Senthilkumaran et al., 2004) owing to its diverse array of functions, we could able to isolate a single type of CREB from the ovarian follicles of catfish. Synergistic expression pattern of CREB with that of 20β-HSD in catfish might support the observation of 20β-HSD mRNA surge upon stimulating with hCG. Although our finding that CRE regulates 20β-HSD promoter is not surprising, given its extensive role as transcriptional regulator, our results define that 20β-HSD gene is responsive to cAMP.
References


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Nagahama, Y., 1997. 17α-20β-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanism of synthesis and action. Steroids 62, 190-196.


