Isolation, characterization and expression of 11 β -hydroxysteroid dehydrogenase type 2 cDNAs from the testes of Japanese eel (*Anguilla japonica*) and Nile tilapia (*Oreochromis niloticus*)

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Abstract

The Japanese eel (*Anguilla japonica*) and Nile tilapia (*Oreochromis niloticus*) 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) cDNAs were isolated from their respective testes cDNA libraries. The cDNAs predict two peptides of 436 and 406 amino acid residues that share about 42% homology with mammalian 11 β -HSD type 2 proteins. Analysis of the tissue distribution pattern by RT-PCR reveals that 11 β -HSD2 is expressed in a wide variety of tissues in tilapia, with higher expression in kidney and gill of both sexes, and with the highest expression in testis. 11 β -Dehydrogenase activity of the eel 11 β -HSD2 was confirmed by demonstrating the conversion of cortisol to cortisone by the recombinant protein after transient expression of this cDNA clone in COS-1 cells. Bands of ~2.7 and ~3.8 Kb were detected in Northern blot of eel and tilapia testes respectively, which is consistent with the cloned cDNA sizes of the two species. Northern blot analysis also revealed that the expression of the eel testis 11 β -HSD2 gene could be induced by human chorionic gonadotropin (hCG) injection, implying a role of 11 β -HSD2 in hCG-induced 11-ketotestosterone production and spermatogenesis in the Japanese eel.

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Introduction

11-Ketotestosterone (11-KT) is a major androgen in fish. Using a testis organ culture system, we showed that hormonal induction of spermatogenesis in the testis of the Japanese eel (*Anguilla japonica*) involves gonadotropin stimulation of Leydig cells to produce 11-KT which, in turn, activates Sertoli cells to stimulate premitotic spermatogonia to complete spermatogenesis (Miura *et al.* 1991a,b,c, Nagahama 1994). 11-Ketotestosterone is synthesized from testosterone by the actions of two enzymes, 11 β -hydroxylase (P450(11 β)) and 11 β -hydroxysteroid dehydrogenase

(11 β -HSD). A cDNA encoding the P450(11 β) was isolated from the Japanese eel testis and its expression was investigated in testes during human chorionic gonadotropin (hCG)-induced spermatogenesis (Jiang *et al.* 1996). Recently, P450(11 β) cDNAs were also isolated from rainbow trout (Liu *et al.* 2000, Kusakabe *et al.* 2002) and the Nile tilapia (D S Wang, B Senthilkumaran, T Kobayashi and Y Nagahama, unpublished data). It is important to further investigate the role of 11 β -HSD (the final enzyme in the biosynthetic pathway of 11-KT) in hCG-induced 11-KT production and spermatogenesis in the Japanese eel and other fish species.

Until recently, cloning of 11 β -HSD cDNA has been reported only for several mammals. Two different 11 β -HSD enzymes have been described. The type 1 11 β -HSD (11 β -HSD1) was first isolated and cloned from liver microsomes of rat (Agarwal *et al.* 1989, Monder & Lakshmi 1989). This type of enzyme is an NADP-dependent enzyme with a relatively low affinity for cortisol or corticosterone. The type 1 11 β -HSD was also cloned from human (Tannin *et al.* 1991), mouse (Rajan *et al.* 1995), pig (AF414124), cow (AF548027), squirrel monkey (Moore *et al.* 1993) and sheep (Yang *et al.* 1992) tissues. The type 2 11 β -HSD (11 β -HSD2) has been cloned from human (Albiston *et al.* 1994), sheep (Agarwal *et al.* 1994), rat (Zhou *et al.* 1995), mouse (Cole 1995), pig (AF374414), cow (Romero *et al.* 2000) and horse (AF126744) kidney and rabbit collecting duct cells (Naray-Fejes-Toth & Fejes-Toth 1995). This type of 11 β -HSD is NAD-dependent and has a higher affinity in the nanomolar range for corticosterone and cortisol, and co-localizes with the mineralocorticoid receptor (Naray-Fejes-Toth *et al.* 1991, Rusvai & Naray-Fejes-Toth 1993, Krozowski *et al.* 1994, Tomlinson & Stewart 2001). These two types of 11 β -HSD belong to the short-chain dehydrogenase/reductase (SDR) superfamily. The homology in amino acid sequence is only about 18%, indicating that they are products of different genes (Naray-Fejes-Toth & Fejes-Toth 1995). More recently, the cloning and characterization of rainbow trout 11 β -HSD2 was reported (Kusakabe *et al.* 2003).

In this study, our aim is to investigate gonadotropin regulation of 11 β -HSD gene expression as well as to compare evolutionary and structural aspects of this enzyme. We report here the isolation of two full-length cDNAs encoding 11 β -HSD2 from eel and tilapia testes cDNA libraries. The enzyme activity of eel 11 β -HSD expressed in COS-1 cells was analyzed. In addition, eel 11 β -HSD gene expression in the testis during hCG-induced spermatogenesis and the distribution of 11 β -HSD mRNA in different tissues were studied.

Materials and methods

Isolation of eel and tilapia 11 β -HSD cDNAs

Based on 11 β -HSD sequences of mammals, a conserved five amino acid sequence, MEVNF,

was selected and its mixed degenerate oligonucleotides, 5'-ATGGA(AG)GT(ACGT)AA(CT)TT(CT)-3', were synthesized. The synthesized oligonucleotides were used as a probe by labeling the 5' ends with γ -³²P-ATP using T4 polynucleotide kinase (Takara, Otsu, Shiga, Japan). A cDNA library from testes of Japanese eels sampled 3 days after hCG injection was constructed in λ ZAP II. A total of 200 000 phages from this library were screened with low stringency. One positive clone was isolated and excised. Sequence analysis of the cDNA insert indicated that it was similar to mammalian 11 β -HSD type 2, but truncated at the 5' end. This cDNA insert was then labeled by ³²P-dCTP using the Random Extension Plus kit (Dupont, Wilmington, DE, USA) to screen the cDNA library again. Another 200 000 phages were screened. The hybridization and the subsequent wash were conducted this time at high stringency. Ten positive clones were isolated, excised and sequenced. Nest deletion was performed to delete the cDNA insert from both ends using the ExoIII/Mung bean nuclease deletion system.

In order to obtain tilapia 11 β -HSD2, based on eel and mammalian 11 β -HSD2 sequences a pair of degenerate primers, forward primer (*Fw*), 5'-GG(TC)CTGTGGGG(ACT)CT(GT)GT(GCT)AA CAA and reverse primer (*Rv*), 5'-GCTGC(TC)TT(GC)GAGG(TC)(TC)CCATA, were designed for RT-PCR. A 236 bp tilapia 11 β -HSD2 fragment was obtained, sub-cloned and sequenced. This fragment was labeled by ³²P-dCTP and used as a probe to screen the tilapia testis library using the procedures described above. Fifteen positive clones were isolated, excised and sequenced. All cDNA sequence analyses were carried out using either an ABI 373 or an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were submitted to GenBank under the accession nos AB097668 (eel 11 β -HSD2) and AY190043 (tilapia 11 β -HSD2).

Phylogenetic analysis

Homology analyses of 11 β -HSD2 protein were performed with Lasergene software. The multiple alignment software ClustalX (Thompson *et al.* 1997) was employed to calculate and depict trees by the N-J method using human 11 β -HSD1 as an outgroup. The fugu 11 β -HSD2 protein sequences used in the alignment were obtained from the fugu

(*Takifugu rubripes*) genome (<http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/fugu.html>). Initially, fugu 11 β -HSD2 genomic sequences were obtained by BLAST with tilapia 11 β -HSD2, and then the four intron sequences were deleted to deduce the protein sequence.

Analysis of 11 β -HSD2 mRNA tissue distribution by RT-PCR

Total RNA (5.0 μ g) was isolated from various tissues of adult tilapia containing either post-vitellogenic ovary or spermiating (sperm expressed when gentle pressure was applied to the abdomen) testis and treated with DNase I (Invitrogen, Carlsbad, CA, USA). The cDNAs were then synthesized and RT-PCR was employed for the analysis of tilapia 11 β -HSD2 expression. The PCR reaction consisted of 2 min at 94 °C, followed by 33 cycles of 94 °C (30 s), 55 °C (30 s), and 72 °C (1 min), ending with 10 min extension at 72 °C. The following two gene specific primers, which are located in exons 3 and 5 respectively were used to amplify 541 bp cDNA fragments by PCR: (*Fw*) 5'-AATAACGCCGGCGTGTGCGTGAAC-3' and (*Rv*) 5'-GGCTCAAGCCAGAGCCTGCA AAGT-3'.

A 342 bp tilapia β -actin fragment was amplified to test the quality of the cDNA used in the PCR reaction with a pair of β -actin primers specific for the Nile tilapia, (*Fw*) 5'-GGCATCACACCTTCT ACAACGA-3' and (*Rv*) 5'-ACGCTCTGTCAGG ATCTTCA-3'. All the PCR products were electrophoresed using 1.5% agarose gels and the gels were stained with ethidium bromide to visualize bands.

Transient expression and enzymatic activity

The eel 11 β -HSD2 cDNA insert was cut out of the multicloning sites of pBluescript by SpeI and HindIII and inserted into the same sites in the expression vector pBK-CMV. The constructed recombinant expression vector was named pBK-E11 HSD. The construct was transfected into COS-1 cells using LipofectAMINE reagent (Gibco-BRL, Gaithersburg, MD, USA) according to the supplier's instructions. Seventy-two hours after transfection, the COS-1 cells were incubated with 16 pmol ³H-labeled cortisol (2.33 TBq/mmol, Amersham, Buckinghamshire, UK) in triplicate.

After 2 h incubation, the medium was collected, extracted twice with equal volumes of diethyl ether and the extracts were dried. The samples were dissolved in acetone, mixed with authentic cortisol and cortisone, spotted onto a TLC plate (64 F254, Merck, Lindenplatz, Haar, Germany) and developed in chloroform and acetone (82:18, vol/vol). The cold steroids were detected under UV light and served as mobility markers. The plate was then exposed to ³H-Hyperfilm (Amersham). For calculating the percentage conversion of cortisol to cortisone, both cortisol and cortisone bands were scraped from the thin-layer chromatography plate, eluted from the silica with 0.5 ml isopropanol, and separated from the silica by centrifugation. The isopropanol solution was decanted into vials for scintillation counting.

RNA isolation and Northern blotting

Eels were injected with hCG (Teikoku Zoki, Tokyo, Japan) at a dosage of 5 IU/g. Total RNA was isolated from testes of eels killed from 1 to 18 days post hCG injection or from untreated eels. Ten micrograms total RNA from each sample were separated on a 1% formaldehyde-agarose denaturing gel, transferred to Hybond-N⁺ nylon membrane (Amersham) and baked at 80 °C for 2 h. The full cDNA insert of eel 11 β -HSD2 was labeled with ³²P-dCTP using the Random Extension Plus kit (Dupont). Hybridization was performed at 60 °C in a hybridization solution containing 6 $\times$ SSC, 5 $\times$ Denhardt's solution, 1% SDS and 200 μ g/ml denatured herring sperm DNA. After 20 h hybridization, the membrane was washed three times at 60 °C with a series of SSC-SDS buffer. The membrane was exposed to an imaging plate, and the hybridization signals were analyzed by a BAS 2000 Bio-Image Analyzer. To perform Northern blot analysis in tilapia, total RNA was extracted from post-vitellogenic ovary and spermiating testis using ISOGEN (Nippon Gene, Toyohama, Japan). Poly(A)⁺ RNAs were purified using Oligotex-DT30 (Takara), and subsequently separated by electrophoresis and hybridized with a fragment of tilapia 11 β -HSD2 cDNA following the method described above. The membrane was stripped and further hybridized with ³²P-labeled tilapia β -actin probe. Subsequently, densitometry measurements were carried out for each band detected. The eel and tilapia 11 β -HSD2 relative

mRNA levels were calculated as the ratio to β -actin and expressed as the percentage of respective controls.

Results

Nucleotide and amino acid sequence of eel and tilapia 11 β -HSD2

The cloned 11 β -HSD cDNAs from eel and tilapia are 2689 and 3804 bp respectively. The full length eel cDNA consists of a 69 bp 5'-untranslated region (UTR), a 1308 bp open reading frame (ORF) which predicts a protein of 436 amino acids, and a 1312 bp 3'-UTR with a 23 bp poly(A) tail. The tilapia cDNA consists of a 223 bp 5'-UTR, a 1221 bp ORF which predicts a protein of 406 amino acids, and a 2361 bp 3'-UTR with a 18 bp poly(A) tail. Both cDNAs possess polyadenylation signals.

Phylogenetic analysis

A BLAST search against the GenBank nucleotide and protein databases indicated that the amino acid sequences encoded by these cDNAs are similar to mammalian 11 β -HSD type 2, belonging to the SDR superfamily. Alignments of their amino acid sequences depicted in Fig. 1 revealed two motifs in both eel and tilapia 11 β -HSD2 which are characteristic of 11 β -HSD2. One (box I) near the amino terminus most likely represents the NAD-binding site. The other (box II) contains tyrosine and lysine residues. These two amino acids are highly conserved in all short-chain alcohol dehydrogenase enzymes. The conserved region in box II is probably important for catalytic activity. Alignments of these amino acid sequences also revealed a region consisting of 47 amino acids in eel 11 β -HSD2 that had no counterparts in the sequences of tilapia, rainbow trout, fugu and mammals 11 β -HSD2s. Eel 11 β -HSD2 is also 19 amino acids shorter than the other fish and mammalian 11 β -HSD2s in the N-terminus (Fig. 1). Amino acid sequence similarity among 11 β -HSD2 of eel, tilapia, other fish species and mammals are shown in Table 1. Generally, fish 11 β -HSD2s show 60–70% similarity among themselves and show about 40–45% similarity to those of mammals. However, the cloned eel and tilapia 11 β -HSDs only show around 20% similarity to the human and other mammalian 11 β -HSD1s.

Based on the alignments of the amino acid sequences, a phylogenetic tree of all the cloned 11 β -HSD2s from fish and mammals was constructed with human 11 β -HSD1 as an outgroup (Fig. 2). The cloned eel and tilapia 11 β -HSDs showed much longer phylogenetic distance to the human 11 β -HSD1 than to the mammalian 11 β -HSD2s. Fish 11 β -HSD2s formed a group which is also relatively far from the mammalian group in phylogenetic distance. Among the four fish species, tilapia and fugu 11 β -HSD2s constitute one clade different from that formed by the eel and trout proteins.

Tissue distribution of eel and tilapia 11 β -HSD mRNA

Analysis of the tissue distribution pattern of 11 β -HSD2 in tilapia revealed that the gene is expressed in a wide variety of tissues with higher expression in kidney, gill and intestine. The highest expression level was found in testis (Fig. 3). Further, positive and negative controls validated the distribution pattern. A similar pattern of expression was also found in Japanese eel by RT-PCR (data not shown).

Enzymatic activity in COS-1 cells

COS-1 cells transfected with pBK-CMV/E11 HSD converted large amounts of cortisol into cortisone after only 2 h incubation with ^3H -labeled cortisol. A clear ^3H -labeled band corresponding to cortisone was visualized by TLC of extracts of media from pBK-CMV/E11 HSD transfected COS-1 cells but not from extracts of media from COS-1 cells transfected with the pKB-CMV vector. After 2 h incubation, COS-1 cells transfected with pBK-CMV/E11 HSD had converted 50% of ^3H -labeled cortisol to cortisone (Fig. 4).

11 β -HSD2 mRNA levels during hCG-induced spermatogenesis in eel and in natural maturation in Nile tilapia

Northern blot analysis revealed a single transcript of about 2.7 kb present in eel testis. The hybridization signal could not be detected from eel testis on day 0 (before hCG injection), but appeared at day 1, and peaked in density at day 3 post hCG injection. The hybridization signals

Figure 1 Alignment of the amino acid sequences of fish and mammalian 11 β -HSD2s. BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html) was used to make this figure. The conserved five amino acid sequence, MEVNF, is marked by a solid line above. The four intron positions are numbered and marked by arrows. Boxes I and II are the putative NAD-binding domain and the catalytic site respectively. For GenBank accession numbers refer to the legend of Fig. 2.

Table 1 Amino acid similarities (shown as %) between eel and tilapia 11β-HSD2 and those of rainbow trout, fugu and mammals

	Eel	Tilapia	Trout	Fugu	Sheep	Mouse	Rat	Human	Human-1*
Eel	100.0	59.2	70.3	58.6	42.8	43.5	43.2	42.7	19.9
Tilapia	59.2	100.0	66.2	70.9	41.3	42.7	42.8	40.5	21.9

*Human 11β-HSD1 is listed in the table for comparison.

decreased rapidly from day 6, and were very weak after day 12 (Fig. 5). In adult tilapia, gonadal 11β-HSD2 showed a sexually dimorphic expression pattern. A single transcript of about 3.8 kb was detected in both sexes; however, the band was very intense in the testis but weaker in the ovary (Fig. 6). This is consistent with the tissue distribution revealed by RT-PCR. The ~2.7 and ~3.8 kb transcript sizes detected in Northern blots were also consistent with the cloned eel and tilapia cDNA size, 2689 and 3804 bp respectively.

Figure 2 Phylogenetic tree of cloned fish and mammalian 11β-HSD2s. Human 11β-HSD1 was used as an outgroup. GenBank accession numbers are: horse (AF126744), human (S62789), rabbit (P51976), rat (NP_058777), mouse (CAA62219), sheep (AAA93156), cow (O77667), pig (AF374414), rainbow trout (BAC76709), fugu (<http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/fugu.html>), tilapia (AY190043) and Japanese eel (AB097668).

Discussion

Because of the low homology between the two types of mammalian 11β-HSD and the lack of information about which type may exist in fish testis, it is difficult to select two conserved regions present in both types of 11β-HSD that are suitable for PCR primers. Therefore, a method using oligonucleotides as screening probes was employed. By using mixed degenerate oligonucleotides corresponding to the sequence of a five amino acid region that is conserved in the two types of mammalian 11β-HSDs as an initial probe, a cDNA clone encoding 11β-HSD was isolated from Japanese eel testis cDNA library. Later, based on the eel 11β-HSD sequence, we designed degenerate primers to obtain an 11β-HSD fragment from Nile tilapia which was subsequently used as a probe to screen the cDNA library and obtain the full length tilapia 11β-HSD cDNA. Sequence analyses confirmed that this five amino acid region is conserved in 11β-HSDs from fishes to mammals. Analysis of the predicted amino acid sequence indicated the proteins encoded by these two cDNAs are type 2-like 11β-HSDs, belonging to the short-chain alcohol dehydrogenase superfamily. Alignments of 11β-HSD2 amino acid sequences revealed two highly conserved motifs in both eel and tilapia 11β-HSD2 proteins which are characteristic of 11β-HSD2s. One is most likely the

Figure 3 RT-PCR analysis of 11β-HSD2 from various tissues of adult tilapia. B, brain; P, pituitary; G, gill; H, heart; S, spleen; L, liver; I, intestine; O, ovary; K, kidney; M, Muscle; T, testis; + and -, positive and negative controls; 1, 2 and 3, markers. Lower panel, β-actin as internal control.

Figure 4 Enzyme assay validations for eel 11 β -HSD. (a) Autoradiogram of thin-layer chromatography showing the conversion of cortisol to cortisone by COS-1 cells transfected by pBK-CMV/E11 HSD. Note COS-1 cells transfected by pBK-CMV (mock) did not catalyze the reaction. (b) Percentage conversion of ³H-labeled cortisol to cortisone by COS-1 cells.

NAD-binding site. The other is probably important for catalytic activity. The amino acid sequences of Japanese eel and Nile tilapia 11 β -HSD2s show about 40–45% similarity to mammalian 11 β -HSD2s, but only show 20–22% similarity to mammalian 11 β -HSD1s. In the phylogenetic tree with human 11 β -HSD1 as an outgroup, both eel and tilapia 11 β -HSDs were grouped into 11 β -HSD2s.

A BLAST search with tilapia 11 β -HSD2 nucleotide sequences in GenBank enabled us to isolate the fugu 11 β -HSD2 genomic sequences. Fugu 11 β -HSD2 protein sequences were obtained by locating the ORF and removing the intron sequences. The fugu 11 β -HSD2 gene consists of five exons interrupted by four introns, which is in accordance with the mammalian counterparts (Moore *et al.* 2000, Tomlinson & Stewart 2001). All four introns are located in the same position as those of mammalian 11 β -HSD2 (numbered and marked with arrows in Fig. 1), and have the typical intron characteristics, starting with GT ... and ending with ... AG. These data may indicate a conserved gene structure of 11 β -HSD2 among vertebrates.

Notably, the eel 11 β -HSD2 is 19 amino acids shorter than the other fish and mammalian 11 β -HSD2s in the N-terminus (Fig. 1). It is unlikely that the cloned eel 11 β -HSD2 is truncated because it contains 69 bp 5'-UTR sequences which show

no homology to the N-terminus of other fish 11 β -HSD2s. A region consisting of 47 amino acids in eel 11 β -HSD2 also cannot match those of other species including tilapia, fugu and rainbow trout. Thus, eel 11 β -HSD2 is quite different in amino acid sequence compared with those of the other vertebrate 11 β -HSD2s, but the significance of the changes to function, if any, remains unclear. Nevertheless, BLASTp of all fish 11 β -HSD2 sequences including eel to GenBank resulted in high alignment (from 81 to 96%) to the adh-short domain, the functional domain for the short-chain alcohol dehydrogenase superfamily. Unlike the fish 11 β -HSD2s, none of the mammalian 11 β -HSD2 sequences showed higher than 77% similarities to the 252 residues of the adh-short domain. This is also in contrast to the mammalian 11 β -HSD1s, which showed similarities higher than 80% to the 252 residues of the adh-short domain. Are these differences between fish and mammalian 11 β -HSD2 responsible for functional differences between the two? Both display 11 β -dehydrogenase activity. However, the fish 11 β -HSD2 is also likely to be responsible for converting 11 β -hydroxytestosterone to 11-KT.

Tilapia and eel 11 β -HSD2 genes were found to be expressed in a wide variety of tissues, which is more similar to the tissue distribution of mammalian 11 β -HSD1 than of 11 β -HSD2. A similar pattern of expression of 11 β -HSD2 was

Figure 5 Northern blot analysis of the expression of 11 β -HSD2 in eel testis after hCG induction. (a) The 32 P-labeled eel 11 β -HSD cDNA was hybridized with 10 μ g total RNA from 0, untreated eels and 1–18, eels 1–18 days after hCG injection (upper panel). The lower panel shows the same membrane after stripping and hybridization with eel β -actin probe. (b) The expression levels were normalized to β -actin and expressed as the percentage of respective controls.

found in rainbow trout (Kusakabe *et al.* 2003). In mammals, aldosterone acts via mineralocorticoid receptors (MRs) to control salt and water flux in epithelial organs such as the kidney and colon to maintain circulatory homeostasis. Inappropriate glucocorticoid-mediated activation of MRs in aldosterone target tissues is prevented by the glucocorticoid metabolizing enzyme 11 β -HSD2

(Moore *et al.* 2000). In tilapia, RT-PCR analysis of tissue distribution revealed a high level of expression of 11 β -HSD2 in the kidney (including the cortisol synthesizing interrenal cells), gill and intestine, the most important organs for maintenance of circulatory homeostasis. This seems to suggest that fish 11 β -HSD2 might function in the same way as the mammalian counterparts.

Figure 6 Northern blot analysis of the expression of 11 β -HSD2 in tilapia gonads. (a) The 32 P-labeled tilapia 11 β -HSD cDNA fragment was hybridized with 5 μ g mRNA from testis (T) and ovary (O). The lower panel shows the same membrane after stripping and hybridization with the tilapia β -actin probe. (b) The expression levels were normalized to β -actin and expressed as the percentage of respective controls.

However, although a mineralocorticoid receptor exists in rainbow trout (Colombe *et al.* 2000), its native ligand is unclear, since aldosterone is generally absent in teleosts.

Transient expression of pE11 HSD in COS-1 cells showed that the protein encoded by this cDNA has dehydrogenase activity typical of 11 β -HSD2. COS-1 cells transfected by the recombinant expression vector pBK-CMV/E11 HSD could efficiently convert cortisol to cortisone in only two hours of incubation (Fig. 4). In addition, this eel 11 β -HSD2 could also convert corticosterone to 11-dehydrocorticosterone when expressed in COS-1 cells (data not shown). In mammals, two isoforms of the enzyme 11 β -hydroxysteroid dehydrogenase interconvert the active glucocorticoid, cortisol, and inactive cortisone. Whether fish has both types or has only one type (11 β -HSD2) of 11 β -HSD is still unknown.

In tilapia, the highest expression level of 11 β -HSD2 was found in the testis. On the contrary, the expression level in the ovary was very

low (Fig. 3). This sex dimorphic expression pattern was also confirmed by Northern blotting (Fig. 6). Therefore, it seems likely that fish 11 β -HSD2 also functions to convert 11 β -hydroxytestosterone to 11-KT, the main androgen found in the majority of fish species. The expression of the 11 β -HSD gene in eel testis detected by Northern blotting appeared to be induced by hCG injection. In untreated eels, no obvious 11 β -HSD2 transcripts were detected. This is different from results on tilapia, in which 11 β -HSD2 transcripts were dominantly found in testis. This difference might be explained by the fact that tilapia mature naturally under laboratory and aquaculture conditions whereas eels cannot start spermatogenesis and reach maturation under these conditions without hormone treatment. One day after hCG injection, strong hybridization signals were detected from eel testicular RNA. The signal intensity peaked in samples from eels three days after hCG treatment. From day 6, the hybridization signal started to decrease. The time course of such expression was consistent with that

of hCG-induced changes in serum 11-KT levels of eels (Miura *et al.* 1991a), indicating the importance of 11 β -HSD activity in hCG-induced 11-KT synthesis in testis. From results of this study and the previous study on P450(11 β) (Jiang *et al.* 1998), it is obvious that the hCG-induced 11-KT production in Japanese eels involves enhanced testicular expression of both the P450(11 β) gene and the 11 β -HSD gene. Increased transcripts from both genes may contribute to an increase in enzyme proteins and activities, which lead to increased 11-KT synthesis in hCG-treated Japanese eels. Very recently, the ability of fish 11 β -HSD2 to convert 11 β -hydroxytestosterone to 11-KT was demonstrated using recombinant rainbow trout 11 β -HSD2 (Kusakabe *et al.* 2003). Unlike tilapia, both ovary and testis of rainbow trout show relatively high expression of 11 β -HSD2. Transcripts were localized to testis Leydig cells and theca and granulosa cells of the ovarian follicle. A strong seasonal pattern of expression of 11 β -HSD2 was found in rainbow trout gonads, but unlike this study, no strong relationship was found between circulating 11-KT levels and testis 11 β -HSD2 mRNA levels.

In summary, 11 β -HSD2 cDNAs were cloned from the testes of the Japanese eel and Nile tilapia. These 11 β -HSD2 genes were expressed in a wider range of tissues than mammalian 11 β -HSD2s. Similar to mammals, fish 11 β -HSD2 appeared to act exclusively as an NAD-dependent dehydrogenase, inactivating cortisol (or corticosterone) to cortisone (or 11-dehydrocorticosterone). Most importantly, like rainbow trout 11 β -HSD2, eel and tilapia 11 β -HSD2s may also have the ability to convert 11 β -hydroxytestosterone to 11-KT, the main androgen found in the majority of fish species, and therefore expression of the 11 β -HSD2 gene in testes may play an important role in spermatogenesis.

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