Molecular characterization of three forms of putative membrane-bound progestin receptors and their tissue-distribution in channel catfish, *Ictalurus punctatus*

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Abstract

Membrane-bound progestin receptors (mPR) were recently cloned and characterized as a new class of steroid receptors that transduce cell-signals through alteration of MAP kinase- and cAMP-dependent pathways. To further develop our understanding of this new class of steroid receptors, we characterized the cDNAs and genes of the \(\alpha\), \(\beta\) and \(\gamma\) forms of the channel catfish mPRs (IpmPR). The predicted \(\alpha\) and \(\beta\) proteins have 49% sequence identity, whereas they only have 30% and 27% identities, respectively, with the \(\gamma\) form. Furthermore, IpmPR\(\alpha\) and IpmPR\(\beta\) genes have similar structures featuring intronless coding regions, while IpmPR\(\gamma\) gene is composed of 8 exons and 7 introns. The 5'-flanking region of each IpmPR gene differs, but each contains putative transcriptional regulatory elements of factors known to influence reproductive physiology and endocrine disruption, for example, responsive elements for cAMP and steroids and the recognition sites for steroidogenic factor-1 and for the aryl hydrocarbon receptor. The IpmPR\(\gamma\) gene was detected in all the tissues tested with relatively greater expression in brain, pituitary, muscle and testis. The expression of IpmPR\(\beta\) was much lower than that of IpmPR\(\alpha\) and the transcript was predominantly observed in brain, pituitary, ovary and testis. In contrast, the IpmPR\(\gamma\) transcript was mainly detected in gill, ventral aorta, intestine, and trunk kidney. In conclusion, all the structural features of the IpmPRs strongly suggest that the closely related \(\alpha\) and \(\beta\) forms are distantly related to the \(\gamma\) form. Additionally, regulatory features of the 5'-flanking regions and the differences in tissue-specific expression of each IpmPR gene suggest that they are involved in different endocrine functions in catfish.

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Introduction

Steroid hormones play important roles in many physiological processes, including reproduction. The classic mechanism of steroid action involves alteration of gene transcription, which typically is a relative slowly process, by diffusing into the cell and binding to ligand-activated transcription factors belonging to the nuclear steroid receptor superfamily (Beato 1989, Tsai & O'Malley 1994). Steroids have also been shown to exert rapid effects in many target tissues that are initiated at the cell surface by binding to steroid membrane receptors and frequently occur via nongenomic mechanisms (Falkenstein et al. 2000, Losel & Wehling 2003, Simoncini & Genazzani 2003). However, the identities of the steroid membrane receptors mediating most of these nonclassical actions remain unknown, and there is evidence for the existence of both unique receptors and nuclear receptor-like forms on the plasma membranes of steroid target cells (Bagowski et al. 2001, Losel & Wehling 2003, Xu et al. 2003). Meiotic maturation of fish and amphibian oocytes is one of the most well-characterized nongenomic steroid actions mediated by membrane-bound steroid receptors at both the biochemical and cytological levels (Yamashita et al. 2000). The precise mechanism for the activation and/or stabilization of the maturation-promoting factor by the maturation-inducing steroid (MIS) appears to differ among species. For example, the MIS-receptor (MIS-R), which in fish is a membrane-bound progestin receptor (mPR), appears to be coupled to an inhibitory G-protein (\(G\i\)) in three fish species: rainbow trout, Atlantic croaker and spotted scat trout, (Yoshikuni & Nagahama 1994, Thomas et al. 2002, Zhu et al. 2003a), whereas in zebrafish and an amphibian, *Xenopus*, it is likely that progestins activate a stimulatory G-protein (Gallo et al. 1995, Kalinowski et al. 2004). However, many other processes initiated through activation of the membrane-bound MIS-R, such as a decrease in intracellular cAMP levels, have been shown to be conserved (Yamashita et al. 2000).

Until recently, despite intensive research in several laboratories, efforts to characterize MIS-Rs in vertebrates at the molecular level have been unsuccessful (Maller 1998). For the first time in any species, a novel
mPR cDNA was recently isolated and characterized in a teleost fish, the spotted seatrout, and several lines of evidence suggest it is the MIS-R in this species (Zhu et al. 2003a). Subsequently, three forms of mPR (α, β and γ) have been identified in several other species of vertebrates, from fish to mammals (Zhu et al. 2003b). The finding that antisense oligonucleotides for both mPRα and β blocked MIS induction of oocyte maturation in zebrafish provided an initial indication that both mPRs may serve as the MIS-Rs in this species (Zhu et al. 2003a, Thomas et al. 2004). However, there are still many unanswered questions concerning the precise physiological roles of the three members of this new class of steroid receptors in fish and other vertebrates.

In this paper, we report the characterization of the channel catfish, Ictalurus punctatus, cDNAs encoding three forms of mPR (mPRα, β and γ), their genomic organization, the structures of their 5′-flanking regions, and their tissue distributions. Our findings clearly suggest that the catfish mPRs are related to the seatrout mPR and that the highly related α and β forms are involved in catfish reproductive physiology, but their roles as MIS-Rs remain to be demonstrated.

Materials and methods

Animals

All fish were maintained and tissue samples were taken in accordance with protocols approved by the University’s Institutional Animal Care and Use Committee.

Oligonucleotides and DNA sequencing

Oligonucleotide PCR primer synthesis and nucleotide sequence analysis were provided by the BioAnalytical Services Laboratory at the Center of Marine Biotechnology. Nucleotide sequences were determined with the PRISM Cycle sequencing kit using an ABI Model 377 DNA Sequencer (PE Applied Biosciences, Foster City, CA, USA). Oligonucleotide PCR primer synthesis and nucleotide sequence analysis were provided by the BioAnalytical Services Laboratory at the Center of Marine Biotechnology. Nucleotide sequences were determined with the PRISM Cycle sequencing kit using an ABI Model 377 DNA Sequencer (PE Applied Biosciences, Foster City, CA, USA). The resultant amplicons were cloned and sequenced. Subsequently, 5′- and 3′-RACE of testis cDNA, amplified the sequences of the 5′ and 3′ ends of each cDNA encoding IpmPRα and β using the SMART RACE cDNA Amplification kit (BD Bioscience) according to the manufacturer’s instructions. Posterior kidney cDNA was used for the RACE PCR cloning of the cDNA for the γ form. Gene-specific primers, mPRA4 and S4, were used for 5′- and 3′-RACE to obtain full length cDNA of the α form. Due to low transcript abundance, nested RACE PCRs were required for the isolation of the β form cDNA. The first 5′- and 3′-RACE PCRs were conducted with mPRA5 and S5 and were followed by a second set of RACE PCRs using mPRA6 and S6, respectively. RACE PCRs for the γ form were carried out using mPRA7 and S7.

Characterization of genomic organization and the 5′-flanking regions of the mPRs

Genomic DNA was extracted from catfish brain using a Blood & Cell Culture DNA Starter Kit (Qiagen). A catfish GenomeWalker library was constructed with a Universal GenomeWalker Kit (BD Bioscience) according to the manufacturer’s protocol. ‘Genome walking’ PCRs using the GenomeWalker library was carried out using Advantage-GC genomic polymerase and the gene-specific primers (mPRA11-A16) listed in Table 1 in order to characterize the 5′-flanking region of the three types of IpmPR genes. Typical PCRs using genomic DNA Sequencer (PE Applied Biosciences, Foster City, CA, USA).

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Table 1 Oligonucleotides and fluorescent probes used for PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotide sequence</th>
<th>Use and its target DNA</th>
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<tr>
<td>mPRS1</td>
<td>5′-GTVTACCARTWYGNGAGYGC-3′</td>
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<td>IpmPRβ cDNA fragment</td>
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<td>IpmPRβ cDNA fragment</td>
</tr>
<tr>
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<td>IpmPRγ cDNA fragment</td>
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<td>3′-RACE for IpmPRβ cDNA fragment</td>
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<td>5′-GAGCTATCCATTTGCCAGATAGTTGC-3′</td>
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<td>TamPR of IpmPRγ form</td>
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<td>mPBS5</td>
<td>5′-GAGCGAGTCTTTGCCAGATAGTTGC-3′</td>
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<tr>
<td>mPBS6</td>
<td>5′-AGGAGCTGCTTTGCCAGATAGTTGC-3′</td>
<td>TamPR of IpmPRγ form</td>
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DNA or ‘genome walking’ PCRs were also conducted using proof-reading DNA polymerases (PfuTurbo DNA polymerase (Stratagene), Advantage-GC genomic polymerase or Advantage 2-GC polymerase (BD Biosciences)) and appropriate primers based on the cDNA sequences for each mPR under the thermal cycling conditions recommended by the manufacturer. Intron/exon boundary sites were determined by comparing the genomic sequence with the respective cDNA sequence.

Putative transcriptional factor binding sites located on the 5′-flanking region of each IpmPR gene were identified with the aid of analyses posted on the web site, http://motif.genome.ad.jp/ with an 80% cut off score. Additionally, consensus core sequences for transcriptional factor binding sites potentially related to reproductive physiology or endocrine disruption, i.e., cAMP responsive element (CRE: TKACGTMA), estrogen responsive element (ERE: AGGTCANNTGACCT), glucocorticoid/progestin/androgen responsive element (GRE/PRE/ARE: AGAAGANNNTGTCTT), steroidal chronic factor-1 binding site (SF-1: TCAAGGTYA) and the responsive element for aryl hydrocarbon receptor and its nuclear transfer (AhR/Arnt: TYGCGT) were manually searched using software, DNASIS-Mac v3.5. All potential elements are more than 80% identical to their corresponding core sequences.
Gene-specific expression of catfish and zebrafish mPRs

Total RNA (1 µg) prepared from various tissues (i.e., parts of the brain (telenencephalon, cerebellum, diencephalon, hypothalamus and hindbrain), pituitary, gill, ventral aorta, intestine, muscle, heart, spleen, liver, posterior kidney, head kidney and ovary) from a female adult catfish and from testis from a male adult catfish was primed with a clamped oligo(dT) primer and reverse-transcribed using M-MLV reverse transcriptase (Life Technologies Inc). Real-time, quantitative RT-PCR, (rtqRT-PCR; PE Applied BioSystems) was employed to measure transcript abundance of each IpmPR gene. The primers and probes used for each gene are listed in Table 1. The primer/probe sets for IpmPRα and IpmPRβ were mPRS11, A17 and mPRP1, and mPRS12, A18 and mPRP2, respectively. The set of primer/probes for the γ form was mPRS13, A19 and mPRP3. Transcript abundance of each mPR was normalized to the abundance of catfish β-actin (GenBank accession number AY555575), Kazeto et al. (2003). The same type of analysis was conducted in zebrafish to examine the tissue-specific distribution of the zebrafish mPR (DrmPR) α and β genes. The primer/probe sets to measure transcript abundance of DrmPRα and DrmPRβ by TaqMan were mPRS14, A20 and mPRP4, and mPRS15, A21 and mPRP5, respectively (Table 1). The detailed procedure to design and synthesize the primer/probe sets, and to validate similar rtqRT-PCR assays has been described elsewhere (Trant et al. 2001).

Identification of putative mPRs and their genomic organization in human and Fugu

BLAST searches of the human and Fugu genome databases were conducted with each form of catfish mPRs using the analyses posted on the web site of the Wellcome Trust Sanger Institute (www.sanger.ac.uk) in order to identify cognate cDNAs and their genomic organization, and to compare them with the corresponding IpmPRs characterized in this report.

Results

Isolation of the cDNAs encoding IpmPRs

PCR-based isolation of cDNAs encoding putative IpmPRα (GenBank accession number AY533540), β (GenBank accession number AY533541) and γ (GenBank accession number AY533542) were completed with the aid of 5′ and 3′RACE PCRs. The length of the nucleotide sequences of IpmPRα, β and γ are 2303, 3487 and 1186 bp, respectively. Coding regions of α, β and γ forms consist of 1062, 1068 and 1017 bp encoding 354, 356 and 339 amino acid residues, respectively. The degree of amino acid sequence identity between the α form and the β and γ forms is 49% and 30%, respectively, while the β and γ forms have 27% sequence identity.

Identification of potential cognate cDNAs of mPRs from human and Fugu genome databases

A BLAST search of the human and Fugu genome databases was carried out using the deduced amino acid sequences of catfish mPRs to identify the homologous genes. Six putative mPRs, including α (Ensembl ID. SINFRUP00000149401) and γ (ID. SINFRUP0000127469), forms reported previously (Zhu et al. 2003b) were retrieved from the Fugu database. Fugu mPRβ (ID. SINFRUP0000160848) was also newly identified. Three other cognate forms named Fugu mPR-like protein (FmPRLP 1–3) appear to be novel forms. FmPRLP 1 (SINFRUP00000145330) and FmPRLP 2 (SINFRUP0000014528) are structurally closely related and share 78% identity. The predicted amino acid sequences of FmPRLP 1 and 2 show approximately 50%, 40% and 30% identities to Fugu mPR α, β and γ, respectively. The degree of identity between FmPRLP 3 (SINFRUP0000156015) and FmPRLP 1 and 2 is 44% and 38%. FmPRLP 3 shares 39%, 46% and 25% homology with Fugu mPRα, β and γ, respectively. Three forms of mPR α (ENSP00000329071), β (ENSP00000302785) and γ (ENSP00000260380) were identified by this search in the human genome as reported previously (Zhu et al. 2003b).

Structural and phylogenetic analysis of IpmPRs

Phylogenetic analysis of the mPR-related proteins clearly shows that the catfish mPRs are clustered together with their respective mPR types amongst the fish representatives and that the γ forms appear to be highly diversified from the other two groups (Fig. 1). FmPRLP 1 and 2 appear to be phylogenetically derived from mPRα, although they are not tightly clustered with fish mPRβs. In contrast, FmPRLP 3 is clearly placed together with fish mPRβ, especially the Fugu form.

The amino acid sequences of each IpmPR and their related forms from the other species are aligned in Figures 2–4. The deduced amino acid sequence of IpmPRα showed high homology with other fish forms (higher than 70%) and 49–54% sequence identity with mammalian forms and FmPRLP 1 and 2. IpmPRβ has 72% sequence identity to zebrafish mPRβ and 48–55% identity to other mPRβ-related forms, including FmPRLP 3. Fugu mPRγ shares the closest identity to IpmPRγ (71%). Transmembrane and lipophilicity analyses identified seven potential transmembrane domains in all mPRα- and β-related forms except for FmPRLP 1 and zebrafish mPRβ that appeared to lack the sixth and second transmembrane domains.
respectively. IpmPRγ possesses seven potential transmembrane domains like mPRα and β, but other γ forms only have five or six potential transmembrane domains by this analysis.

One potential N-linked glycosylation site \( (N \times S/T) \) located in the N-terminal extra-cellular region was identified in the α and γ forms, but not in the β forms. Several phosphorylation sites that are conserved among the fish forms and among all forms of each type of mPR were identified, however all sites are located in extra-cellular regions or transmembrane domains. Two highly conserved regions were identified in all forms of mPRα, β and γ (Figs 2–4). One region, composed of sixteen amino acid residues that span the N-terminal region and first transmembrane domain and includes a potential N-linked glycosylation site, is highly conserved and the identity between this region of IpmPRα and those of all other mPR forms is over 70%. The second conserved region (fifteen amino acids) is located on the putative boundary of the third cytoplasmic loop and sixth transmembrane domain in the α and β forms, while this region corresponds to the fourth extracellular region of IpmPRγ. This conserved region in IpmPRγ is over 60% identical to the other forms of mPR.

**Comparison of IpmPRα, β and γ gene structure to human and Fugu mPRs**

The genomic organizations of each IpmPR, as determined by PCR-based strategy, are illustrated in Figure 5. Intron/exon junctions of each IpmPR gene were identified by comparison with the corresponding IpmPR cDNA sequence. Interestingly, both the IpmPRα and β genes do not possess any introns on their coding regions. The IpmPRα gene is over 4331 bp with a large single intron (only 2061 bp has been sequenced) interrupting the 5'-UTR. The untranslated exon I is 577 bp in length, whereas exon II contains 33 nucleotides of the 5'-UTR as well as the entire coding region (1062 bp) and 3'-UTR (598 bp). The genomic organization of the IpmPRβ gene is comparable to that of the IpmPRα gene, except for an additional intron in the 5'-UTR. The entire sequence of this gene has been determined to be 5118 bp in length. First and second exons of the IpmPRβ gene encode 582 bp and 125 bp of the 5'-UTR, respectively. Exon III consists of the remaining 9 bp of the 5'-UTR, the whole coding region (1068 bp) and the 3'-UTR (1681 bp). Intron I and II are 756 bp and 897 bp, respectively. In contrast, the structure of IpmPRγ gene spanning approximately 15.7 kbp is quite different from the other IpmPR forms and includes 8 exons (with a non-coding exon I) and 7 introns. The sizes of introns are 263, 1727, 7585, 243, 1883, 1397 and 1431 bp, respectively. All the nucleotide sequences of the intron/exon boundary sites are consistent with the classical GT/AG rule (Breathnach & Chambon 1981) involved in the recognition for intron splice site.

The structures of the mPRα and β genes of human and Fugu share the basic characteristics of their respective IpmPR genes, i.e., these genes do not contain introns in their open reading frames. Furthermore, human and Fugu mPRγ genes possess 6 introns in the coding region like the IpmPRγ gene and the position for all intron/exon junctions are identical to those in the IpmPRγ gene. Additionally, there are no introns in the coding regions of FmPRLP 1 and 2 genes that are structurally cognate to mPRα at the phylogenetic level. In contrast, the FmPRLP β gene is structurally different from all three IpmPRs (i.e., 4 intron/exon junctions that are not identical to those in mPRγ genes) even though the predicted protein is highly similar to mPRβ.
Structure of 5'-flanking regions of the IpmPR genes

Sequence analysis of the GeneWalker amplicons generated 3045 bp (GenBank Accession number AY587768), 3701 bp (GenBank accession number AY587769) and 3382 bp (GenBank accession number AY587770) of unique sequences representing the 5'-flanking regions of IpmPR genes, respectively. Figure 6 is a schematic diagram showing potential transcription regulatory elements located within the 3·0 kbp upstream of the 5'-UTR for each IpmPR gene. The full lengths of the 5'-UTRs were determined by sequence analysis of 5'-RACE amplicons. A TATA box was identified within 90 bp of the end of the 5'-UTR of each IpmPR. The 5'-flanking region of the IpmPR gene contained the consensus sequences of two CREs, two binding sites for SF-1, five former-half sequences of GRE/PRE/AREs and two latter-half sequences of EREs. The regulatory 5'-flanking region of the IpmPR gene showed the consensus sequences of three binding sites for SF-1, four half-GRE/PRE/AREs, three half-EREs, a binding site for AhR/Arnt and a binding site for early growth response-4 (Egr-4). Four half palindromic sequences for GRE/PRE/AREs, a binding site for SF-1, a binding site for AhR/Arnt, and an AhR/Arnt binding site overlapped with a recognition site for Egr-2 and Egr-3 were located on the 5'-flanking region of IpmPR gene.

Tissue distribution of transcripts of mPRs in catfish and zebrafish

Differential tissue-distribution of the transcripts for mPRs in catfish (Fig. 7A) and for zebrafish (Fig. 7B) was determined by sensitive real-time, quantitative RT-PCR
Figure 3 Alignment of the deduced amino acid sequence of IpmPRβ with the mPRβ related proteins of other animal species. The key for the symbols is shown in Figure 2 legend.

Figure 4 Alignment of the deduced amino acid sequence of IpmPRγ with the mPrγ related proteins of other animal species. The key for the symbols is shown in Figure 2 legend.
assays. Expression of the IpmPRα gene was detected in all tissues analyzed. However, relatively high expression was evident in all parts of the brain, pituitary, muscle and the testis. The IpmPRβ transcript was generally lower in abundance than IpmPRα and was observed primarily in parts of the brain (especially the hypothalamus and hindbrain), the pituitary, the ovary and the testis. Even though the IpmPRβ transcript was detected in other parts of the brain, gill, ventral aorta (including the surrounding thyroid follicles), and head kidney, abundance was very low. On the other hand, the IpmPRγ transcript was mainly detected in the gill, ventral aorta, intestine, and trunk kidney with lower levels of expression in the cerebellum, hypothalamus and testis. The tissue expression of the mPRα gene in zebrafish (DrmPRα) and catfish differed. DrmPRα mRNA was detected primarily in zebrafish gonadal tissues and was less abundant than the transcript of DrmPRβ. DrmPRβ was predominantly detected in the same tissues that the mPRβ transcript was found in catfish (i.e., brain, pituitary and gonadal tissues).

Discussion

In the present study, the cDNAs encoding three catfish putative mPRs, IpmPRα, β and γ, were isolated, and their secondary structures phylogenetic relationship to other mPR forms were determined. IpmPRα and β showed relatively high identity at the amino acid sequence level, approximately 50%, however, the γ form shared less than 30% identity with the other IpmPRs. Furthermore, phylogenetic analysis clearly placed each form of IpmPR together with their respective mPR subtype groups and showed greater divergence between the clusters of mPRγs compared with those of the α and β forms. These findings strongly suggest that mPRα and mPRβ are closely related, whereas it is likely that

Figure 5 Schematic structures of the genes encoding IpmPR α, β and γ. Open boxes indicate untranslated regions of exons. Closed boxes indicate translated regions of exons. Bars represent introns.

Figure 6 Diagram of structures and potential transcription regulatory elements located in the 5'-flanking regions of the catfish mPR genes. AhR/Arnt, responsive element for aryl hydrocarbon receptor and its nuclear translocation factor; CRE, cAMP responsive element; Egr, binding site for early growth response genes; SF-1, steroidogenic factor-1 binding site; ERE, estrogen responsive element; GRE/PRE/ARE, glucocorticoid/progestin/androgen responsive element.
mPRγ evolutionally diversified from other groups of mPRs.

Interestingly, six mPR-related sequences including mPRα, β and γ, and three uncharacterized forms (FmPRLP 1–3) were retrieved from the Fugu genome database. Structure analysis of these novel forms demonstrated that two of them, FmPRLP 1 and 2, are closely related to mPRα, while the predicted amino acid residue of FmPRLP 3 shares high identity with the β form. These conclusions are supported by the phylogenetic analysis showing their relationships to other mPRs. These findings suggest that FmPRLPs are potential members of mPR family, although further characterization of them is required, including the demonstration that their mRNAs are expressed in fish tissues and are functional genes. The mPRLPs are possibly species-specific or teleost-specific forms of mPR-related genes, since the human genome appears to contain only three forms of mPRs from this phylogenetic analysis. Further information on the number of mPR-related proteins in other animal species would provide valuable insight into the evolution of this new steroid receptor family.

The deduced amino acid sequences of each IpmPR showed high homology to other animal forms; in two regions in particular. The amino acid residues 69–84 and 273–287 in IpmPRα are quite highly conserved among all mPR forms, including FmPRLPs. The former conserved region contains a portion of the N-terminal extracellular region and a potential N-linked glycosylation site identified in mPRα and γ. These data may imply that these conserved regions are involved in fundamental functions of mPR, for example, the ligand-binding, coupling with G-proteins.

Interestingly, IpmPRα and β show comparable genomic organizations without any introns in their coding regions. In contrast, the structure of the mPRγ gene is quite different in that it includes six intron
junctons within the coding region. This difference in the gene structure between IpmPRγ and those of the α and β forms appears to coincide with the secondary structural and phylogenetic divergence of the γ from the α and β forms. Furthermore, these features of genomic structure are completely conserved in the corresponding human and Fugu mPR genes. This finding strongly suggests that the mPR genes are phylogenetically conserved throughout the vertebrates based on a high degree of conservation of their genomic organization as well as their coding sequences.

The putative transcriptional regulatory elements located on the unique 5′-flanking regions of each IpmPR gene were also identified. 5′-flanking regions of each IpmPR show a TATA box within 90 bp of the end of the 5′-UTR, which suggests that these 5′-termini are close to the transcription start sites for each IpmPR gene. The 5′-flanking region of each IpmPR gene differentially exhibits the consensus regulatory elements for transcription factors related to reproductive physiology (CRE, SF-1 binding site; Morohashi et al. 1992), steroid hormone regulation, and cellular growth and differentiation (Egr; Tourtellotte et al. 1999 and AhR/Arnt; Poland & Knutson 1982). Therefore, these transcription factors may be partially responsible for the direct transcriptional modulation of IpmPR genes. In fact, we recently have shown that the catfish mPRα gene is down-regulated by a progestin, 17α, 20β-dihydroxy-4-pregnene-3-one (the MIS in this species) while estradiol up-regulates its expression (Kazeto et al. 2005). Furthermore, it was reported that the protein abundance of mPRα was enhanced in seaturt ovary by hCG (Zhu et al. 2003a) whose effects are generally mediated by cAMP (Ascoli et al. 2002), although recent studies have shown that the catfish and zebrafish mPRs are not regulated by gonadotropins (Kazeto et al. 2005). The transcriptional regulatory elements identified in this study adds significantly to the paucity of information that is currently available concerning the gene regulation of mPRs and it provides valuable insights into the potential mechanisms of mPR gene regulation.

The different tissue distributions of the three IpmPR genes was expected based on the significant structural differences in their 5′-flanking regions. The transcript for IpmPRα was detected in all tissues examined whereas the β form, although poorly expressed, was in greatest abundance in the brain, pituitary and gonads. The γ form was primarily expressed in the posterior kidney, gill and intestine. The more sensitive RT-PCR procedure used in the present study demonstrates that the distribution of IpmPRα transcript is ubiquitous in catfish whereas Northern blot analysis only showed significant expression of the mPRα gene in the brain, pituitary and gonads in seaturt (Zhu et al. 2003a), and in various reproductive tissues and the kidney in humans (Zhu et al. 2003b). Similarly, the RT-PCR procedure reveals that the mPRβ gene is also expressed in the gonads in catfish, whereas only neural expression of the gene was detected in human tissues by Northern blot analysis (Zhu et al. 2003b). However, these two methods showed similar tissue expression of mPRγ genes in catfish and humans, with expression primarily in kidney and intestinal tissue (Zhu et al. 2003b). Interestingly, catfish gill also expresses IpmPRγ, which suggests that IpmPRγ may be involved in ion regulation.

In order to determine if the apparent differences seen in tissue-specific expression of mPRα and β in catfish are specific to this species, the tissue distribution of mPRα and β expression was examined in a second teleost species, the zebrafish. The tissue-specific gene expression of mPRα differed in zebrafish, where the mPRβ transcript was expressed almost exclusively in gonadal tissues. The apparent differences in tissue expression of the mPRα gene among the different fish species investigated to date may indicate possible species-specific differences in the extra-gonadal functions of the mPRα in fish. In contrast, the tissue-specific distribution of the mPRβ transcript in zebrafish is essentially the same as in the catfish, that is in brain, pituitary and gonadal tissues, and potentially could reflect some conserved functions of mPRβ among these teleost species.

In conclusion, the cDNAs and the genes encoding three putative mPRs, the α, β and γ forms, were isolated from catfish and structural analyses strongly suggest that the highly related α and β forms of the catfish mPR are distantly related to the γ form. Furthermore, structural analysis of the 5′-flanking regions of the mPR genes and the differences in the tissue-specific distributions of their transcripts suggest that expression of these genes is differentially modulated by multiple regulators in catfish. Currently, only a few studies have been published on this new class of steroid receptors. The molecular characteristics of the mPRs described in this study provide valuable insights into the possible functions and physiology of these novel steroid receptors.

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