CYP27A1 expression in gilthead sea bream (*Sparus auratus*, L.): effects of calcitriol and parathyroid hormone-related protein

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**Abstract**

Little is known about vitamin D metabolism in fishes. Several reports have shown hydroxylase activities in various organs to produce vitamin D metabolites, but the enzymes involved have not been isolated or characterized. We isolated and characterized a renal mitochondrial hydroxylase, CYP27A1, that governs vitamin D metabolism in gilthead sea bream, *Sparus auratus*. The enzyme is highly expressed in kidney and to a far lesser extent in liver. When treated with 25-hydroxy vitamin D or calcitriol, the kidney responded differentially and time dependently with CYP27A1 mRNA expression levels. This response substantiates a role for CYP27A1 in fish vitamin D metabolism. This notion is strengthened by upregulation of CYP27A1 in sea bream treated with parathyroid hormone-related protein (PTHrP), and suggests an original role for PTHrP in calcitriol-regulated processes in fish similar to the role of PTH in mammalian vitamin D-dependent processes.


**Introduction**

Vitamin D₃ requires transformations to become bioactive. These transformations involve cytochrome P450 (CYP) enzymes that hydroxylate the steroid. In mammals, the most abundant source of CYP enzymes is the liver, where a first hydroxylation takes place: the 25-carbon is hydroxylated to produce the intermediate 25-hydroxycholecalciferol (*Jones et al.* 1998). The proper enzyme activity responsible for this 25-hydroxylation is still rather enigmatic, even in mammals; several candidate enzymes with 25-hydroxylase ability and broad substrate specificity have been proposed (*Aiba et al.* 2006). One example is the sterol 27-hydroxylase (CYP27A1), which carries out the 25-hydroxylation of vitamin D₃ as well as the 27-hydroxylation of cholesterol in bile acid biosynthesis (*Ibarra et al.* 2004). In mammalian kidney, 25-hydroxycholecalciferol is once more hydroxylated into more potent metabolites, viz. 1α,25-hydroxyvitamin D₃ (calcitriol) and 24R,25-dihydroxycholecalciferol; CYP27B1 makes calcitriol and CYP24 produces 24R,25-dihydroxycholecalciferol. It is noteworthy that, CYP24 also uses calcitriol to produce 1α,24R,25-trihydroxycholecalciferol, the first product in the C-24 oxidation pathway responsible for degradation of calcitriol.

Comparatively little is known in teleosts concerning ‘vitamin D₃ physiology’. Indeed, classical genome-mediated responses to calcitriol have been demonstrated: calcitriol treatments in *vivo* elevate plasma calcium and/or phosphate levels (*Larsson et al.* 2003). Also, differential responses in non-genomic effects of vitamin D₃ metabolites have been reported, in euryhaline trout kept in either fresh water or seawater (*Larsson et al.* 2003) or between stenohaline species (freshwater carp and seawater cod; *Larsson et al.* 2001). Calcitriol increases active calcium transport in gut of freshwater (FW) fish, but not so in saltwater (SW) fish. The metabolite 24R,25-dihydroxycholecalciferol was reported to decrease intestinal calcium transport in freshwater fish (*Larsson et al.* 2001, 2003).

In mammalian calcium homeostasis, parathyroid hormone (PTH) and calcitriol act synergistically through a strict regulation of CYP27B1 and CYP24 activities. Calcitriol controls its own degradation by induction of CYP24 expression and suppression of CYP27B1 expression; PTH induces CYP27B1 expression and downregulates CYP24 (*Jones et al.* 1998).

The array of endocrines involved in the calcemic control in fish includes PTH-related protein (PTHrP), a key in calcium balance in fish (*Abbink & Flik* 2007, *Guerreiro et al.* 2007); more recently, PTH genes have been demonstrated in zebrafish (*Danski et al.* 2003) and pufferfish (*Gensure et al.* 2004), but our understanding of the role of the peptides coded by these genes in the calcium physiology of fishes awaits further study (*Abbink & Flik* 2007). A relationship between PTHrP and calcitriol in calcemic control was recently established for sea bream (*Abbink et al.* 2007); it was shown that a decrease of plasma calcitriol levels, due to dietary vitamin D₃ deficiency, result in an adjustment of (hypercalcemic) PTHrP activities in gills and pituitary cells. Apparently, PTHrP and calcitriol fulfill concerted...
hypercalcemic functions in fish, reminiscent of those of PTH and calcitriol in higher vertebrates.

Remarkably, little is known about the way vitamin D3 metabolism takes place in fish. A 25-OHD-1-hydroxylase activity has been detected in several fish species (Henry & Norman 1975) and the production of vitamin D3 metabolites has been demonstrated in vivo and in vitro in kidney and liver of trout, carp, halibut species, Atlantic salmon, Atlantic mackerel, and Atlantic cod (Hayes et al. 1986, Takeuchi et al. 1991, Graff et al. 1999). However, even though a 25-OHD-1-hydroxylase activity has been detected, the enzyme itself has never been isolated.

We report here the isolation and characterization of a gilthead sea bream CYP27A1 cDNA, the tissue distribution of gene expression and the effects of vitamin D3 metabolites and PTHrP on its expression.

Materials and Methods

Fish

Juvenile, male gilthead sea bream (Sparus auratus L.) of ~160–180 g were obtained from a commercial source (TiMar, Culturas em Água, Lda., Portugal). The fish were kept in 1000 l stock tanks with running seawater (flow, 250 l/h); the salinity was 36‰ and the water temperature was 22 °C. The fish were kept under natural photoperiod (14 h light:10 h darkness); the fish stock was fed daily commercial pellets (PROVIMI, Alverca, Portugal) at a ratio of 1% of the total estimated body mass per tank. Experiments were carried out in Portugal and approved by the local ethical committee.

Cloning and characterization of CYP27A cDNA from S. auratus

A 300 bp cDNA fragment was obtained when attempting to isolate a cDNA for 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1) from sea bream kidney using degenerate primers (1α.fw.3: 5’-CT (AGC) CT (AG) G (GC) (GCT) GG (AC) GT (GCT) GAC-3’; 1α.rev.3: 5’-(ACT) GA (ACT) GT (GT) G (AC) TAGTG (AG) CA-3’) giving the highest BLASTX score to vitamin D3-25-hydroxylase (CYP27A) when searching against the GenBank database (Altschul et al. 1990). The complete coding sequence of CYP27A was obtained by screening at high stringency (in Church–Gilbert buffer at 55 °C) with the radiolabeled cDNA probe (Rediprime II random labeling kit, Amersham Pharmacia Biosciences) a sea bream kidney cDNA library constructed in Lambda Zap using the UNIZAP vector (Stratagene, La Jolla, CA, USA). After screening 6×10⁵ recombinants, six independent clones were autoexcised in phagemid using helper phage and sequenced. Two clones were smaller and not further analyzed; the other four were identical to the sequence described here. The putative CYP27A cDNA was identified by searching the GenBank database with BLASTN and BLASTX. The sequence is deposited in EMBL with accession number AM885865.

Tissue distribution of CYP27A

Fish were deeply anesthetized with 2-phenoxyethanol (0–1%v/v; Sigma–Aldrich) and the whole pituitary gland, gill, liver, kidney, head kidney, muscle, foregut, midgut, and rectum were sampled. For mRNA extraction, tissues were flash-frozen on dry ice and stored at −80 °C until further use.

Phylogenetic analysis CYP27A1

Available vertebrate CYP27A1 sequences were taken from the NCBI site (www.ncbi.nlm.gov) via the GenBank database. Multiple sequence alignments were accomplished with the ClustalW program from the European Bioinformatics Institute (www.ebi.ac.uk/clustalw/). With the neighboring-joining method, and Mega version 3.1 (Kumar et al. 2004), a phylogenetic tree was constructed on the basis of amino acid difference (P distance) with 1000 bootstrap replications.

Physiology

Fish (n=8) randomly selected from their stock tank, one group per time point, were placed in cylindrical 500 l tanks (diameter of tank, 0.85 m). Fish were left to acclimatize for at least 1 week; feeding was discontinued 24 h before the start of the experiments.

Vitamin D metabolites

1α,25-dihydroxyvitamin D3 (calcitriol) or 25-hydroxycholecalciferol (25-OHD) were dissolved in a minimal amount of absolute ethanol and diluted in coconut oil. Fish received a single i.p. injection of coconut oil containing calcitriol (25 µg/kg bodyweight (BW)) or 25-hydroxycholecalciferol (50 µg/kg BW) or vehicle only. Fish were sampled 3, 6, and 9 days after injection. In pilots, no effects of these treatments were seen in the first 48 h following injection; therefore 3, 6, and 9 days were considered, in line with similar slow responses to, for example, estrogen in this fish (Bevelander et al. 2006).

PTHRP

Fish were injected intraperitoneally with saline (0–9% NaCl) or either Fugu PTHrP (1–34; Danks et al. 2003) or the recombinant full length sea bream PTHrP (sbPTHrP 1–125). For both the forms, the dose was 10 ng/g BW. Controls and treated groups were sampled 6 and 24 h after injection. The choice for these sampling times was based on pilots where maximum effects were seen within 48 h.

Sampling procedure

Fish were deeply anesthetized with 2-phenoxyethanol (0–1% v/v; Sigma–Aldrich), and blood was taken by puncturing the caudal vessels with a 1 ml tuberculin syringe, rinsed with ammonium heparin (Sigma (H-6279) dissolved in 0–9% NaCl (v/v), at 300 units/ml). The fish were then killed by spinal transection and blood was collected. The collected blood was centrifuged at 13 600 g for 10 min and the plasma stored

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at $-20^\circ$C until analysis (see below). Next, liver and kidney samples were quickly collected and flash-frozen in liquid nitrogen and stored at $-80^\circ$C for later analysis of mRNA expression (see below).

**Plasma analyses**

The 25-OHD concentration in the plasma was measured by a 25-OHD RIA method (IDS Ltd, Boldon, UK). Briefly, two extraction reagents were added to 50 μl standards and samples resulting in the precipitation of serum proteins and extraction of 25-OHD. After centrifugation (10 min at 2000 g), 50 μl extract supernatant were taken from standards and samples. For each sample, supernatant was incubated with 125I-labeled 25-OHD and a specific sheep antiserum (90 min at 18–25 °C). The bound fraction was separated from the free fraction by a short incubation with Sac-Cel (20 min at 18–25 °C) and followed by centrifugation (10 min at 2000 g). The supernatant was discarded and the precipitate reflecting the bound fraction was counted in a γ-counter. The bound radioactivity is inversely proportional to the concentration of 25-OHD. The intra- and inter-assay coefficients of variation were 5 and 8% respectively. Stripped plasma spiked with defined levels of 25-OHD gave highly predictable results (data not shown).

**CYP27A mRNA expression**

Relative expression of CYP27A mRNA was assessed using quantitative RT-PCR. Tissue was homogenized in TRIzol reagent (Invitrogen) and total RNA was extracted according to the manufacturer’s instructions. RNA (1 μg) was incubated with 1U DNase I (amplification grade; Invitrogen) for 15 min at room temperature to remove genomic DNA. To inactivate DNase activity and linearize RNA, 1 μl of 25 mM EDTA was added and the sample incubated for 10 min at 65 °C. Next, 1 μg RNA was reverse transcribed (RT) with 300 ng random primers, 0.5 mM dNTPs, 10 units RNase inhibitor (Invitrogen), 10 mM dithiothreitol, and 200 U Superscript II RT (Invitrogen) for 50 min at 37 °C.

For quantitative PCR analysis, five times diluted 5 μg RNA was used as a template in 25 μl amplification mixture, containing 12.5 μl SYBR Green Master Mix (Applied Biosystems Benelux, Nieuwerkerk aan den IJssel, The Netherlands), 3 μl each primer (final concentration 600 nM). The primer sets used in the RT-PCR for elongation factor-1α (EF-1α) were: qFWEF-1α and qRVEF-1α, for β-actin: qFWACTIN and qRVACTIN, and for CYP27A: qFWCYP27 and qRV CYP27 (Table 1).

All primer sets had an efficiency over 98% and all products showed a single melting transition. After an initial step at 95 °C for 10 min, a real-time quantitative PCR of 40 cycles was performed (GeneAmp 7500, Applied Biosystems) with each cycle consisted of 15 s denaturation at 95 °C and 1-min annealing and extension at 60 °C. The cycle threshold values were determined and the expression was calculated as a percentage of EF-1α or β-actin (Pfafl et al. 2002). All samples were assayed in duplicate. Both house-keeping genes gave similar results. For clarity, results are presented here only relative to EF-1α. Non-reverse transcriptase controls were consistently negative.

**Chemicals**

Calcitriol and 25-OHD were purchased from Sigma (Sigma–Aldrich). Pufferfish (Takifugu rubripes) PTHrP (1–34) was synthesized by Genemed Synthesis Inc. (San Francisco, CA, USA). Homologous recombinant parathyroid hormone-related protein sbPTHrP (1–125) was produced in bacteria (Anjos et al. 2005).

**Statistical analysis**

Data are presented as mean ± s.d. ANOVA was used to assess differences among groups, followed by the Bonferroni test to determine the level of significance (SPSS Windows version 14.0, Chicago, IL, USA). Significance was accepted when $P<0.05$.

**Results**

**Cloning and characterization of CYP27A cDNA from S. auratus**

The full length 2793 bp cDNA CYP27A1 sequence containing an open reading frame encoding a protein of 471 amino acids is shown in Fig. 1. The amino acid identity of the sea bream sequence compared with *Xenopus laevis*, human, rat, and mouse is 45, 40, 41, and 39% respectively.

### Table 1 Primer sequences for real-time quantitative PCR

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<th>Primer Sequence Accession number</th>
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<td>qFWEF-1α gtt gag atg cag cac gac gat tct AF184170</td>
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<tr>
<td>β-actin</td>
<td>qFWACTIN cg ttc atc cac gac gga ttt cc AF384096</td>
</tr>
<tr>
<td>CYP27A</td>
<td>qFWCYP27 aag cta cag cgg cgc gct tga AM885865</td>
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G S BEVELANDER and others.
CYP27A1 in Sparus auratus


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To compare the sequence of sea bream with that of other vertebrate species, a multiple alignment was made (Fig. 2). The three conserved residues (Lys_336, Lys_340, and Arg_403) essential for ferredoxin binding are indicated. Furthermore, two other sites are indicated: a well-conserved cysteine 429 residue, where heme is covalently bound to the enzyme and a conserved site which is the putative oxygen-binding site.

A phylogenetic tree was constructed using the neighbor-joining method (Fig. 3). Human, mouse, and xenopus CYP27B1 served as an outgroup. Mammalian CYP27A1 sequences are separated from the sea bream CYP27A1 sequence by chicken and xenopus CYP27A1, conform phylogeny. The predicted sequence of zebrafish clusters with the sequence of sea bream, but we do not include these data as the sequence is only predicted. No other fish sequences are known to us at this moment.

**Tissue distribution**

CYP27A1 gene expression was detected in pituitary gland, gills, intestine, head kidney, and muscle (Fig. 4) with an apparent absence in liver and highest abundance in kidney. The preparations of cDNA were of constant quality judged by consistent and highly reproducible amplification of the house-keeping gene EF-1α in all samples.

**Expression of CYP27A1**

In the injection experiments (vitamin D metabolites and PTHrP), only two tissues were studied: liver and kidney. Neither treatment induced CYP27A1 expression to detectable levels in liver (data not shown); however, renal mRNA levels of CYP27A1 were markedly affected by the hormone treatments. The vitamin D metabolite 25-OHD (Table 2) induced a significant downregulation of CYP27A1 expression 3 days following injection; calcitriol did not induce apparent effects at this time point (Fig. 5A). However, 6 days post-injection, both calcitriol and 25-OHD significantly increased CYP27A1 expression (Fig. 5B). No differences in expression were observed 9 days of post-injection.

CYP27A1 mRNA levels were significantly upregulated following injection of PTHrP (1–125) as readily as after 6 h (Fig. 6A). PTHrP (1–34) had a mild but not statistically significant effect. At 24-h post-injection, neither PTHrP (1–34) nor PTHrP (1–125) resulted in changes in CYP27A1 expression (Fig. 6B).

**Discussion**

This study describes the complete cDNA sequence of CYP27A1 from gilthead sea bream. Quantitative analysis of mRNA expression shows abundant expression of CYP27A1 in the kidney, while liver expression levels are very low, an essentially reversed situation compared with the mRNA expression profiles in mammals. Injection of two different vitamin D metabolites, viz. 25-OHD and calcitriol, had differential and time-dependent effects on the expression of renal CYP27A1, 25-OHD being inhibitory on the shorter term (at 3 days), calcitriol and 25-OHD being stimulatory on the longer term (at 6 days). PTHrP can upregulate CYP27A1 mRNA levels, which suggests a similar role for PTHrP in fish as that of PTH (and PTHrP) in mammalian vitamin D metabolism. Indeed, consensus exists that PTHrP and PTH share common receptors in fish and mammals alike and thus such a finding for PTHrP in fish is not surprising as the PTHrP receptor is expressed in sea bream kidney and many other tissues (Hang et al. 2005, Rotllant et al. 2006).

**Molecular considerations**

Mitochondrial cytochrome P450s are monooxygenases receiving their electrons from NADPH via a two-protein redox chain consisting of ferredoxin reductase and ferredoxin. It has been shown that all mitochondrial P450s possess a conserved binding domain involved in the interaction of P450s with their redox partner ferredoxin. There are two lysine residues considered crucial for this binding, and in human P450c27 (= CYP27A1) a third residue, the positively charged arginine at position 403, was found to participate in
Figure 2 (continued)
Three conserved residues are found in all vertebrate CYP27A1 sequences (Fig. 2) and indeed must be highly relevant for the functioning of the enzyme. The conserved cysteine \(\text{C}^{429}\) responsible for heme binding and a conserved site for oxygen binding are found not only in all known CYP27A1s but also in other vitamin D-related cytochromes (Jones et al. 1998). This taken together with

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession Number</th>
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<tr>
<td>Human</td>
<td>NP_000775</td>
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<tr>
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<td>P17177</td>
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<td>Xenopus</td>
<td>AAH77308</td>
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<tr>
<td>Sparus</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 Alignment of CYP27A1s for eight vertebrate species. Hyphens indicate gaps that were introduced to achieve maximum identity. Identical amino acids are indicated in black boxes, conservative substitutions in gray. The two arrows close to each other indicate the two lysine residues and the third arrow indicates the arginine residue. A conserved cysteine residue, the ligand for the heme group, is indicated in an underlined block and a putative oxygen-binding site is indicated (doubly underlined). Accession numbers: human (*Homo sapiens*), NP_000775; rabbit (*Oryctolagus cuniculus*), P17177; cow (*Bos taurus*), XP_883724; mouse (*Mus musculus*), NP_077226; rat (*Rattus norvegicus*), NP_849178; chicken (*Gallus gallus*), XP_422056; and Xenopus (*Xenopus laevis*), AAH77308.
significant yet moderate overall amino acid homology (homology for consensus sequences within the protein such as the heme- and the oxygen-binding regions are very much higher indeed) and the clustering in phylogenetic analyses, confirms the identity of the sea bream enzyme presented here as a mitochondrial CYP27A1.

25-Hydroxylation

The mammalian liver and several extrahepatic tissues possess mitochondrial enzyme activities that hydroxylate the 25-carbon of vitamin D₃ and other steroids (Bhattacharyya & DeLuca 1974, Bjorkhem et al. 1980). The presumed enzyme was first identified as a broad spectrum sterol 27-hydroxylase with a designated role in hepatic bile acid biosynthesis, catalyzing the first step in the oxidation of the cholesterol side chain in the 'acidic' bile biosynthesis pathway (Cali & Russell 1991). Consensus has been reached now that an extrahepatic CYP27A1 serves this role in cholesterol homeostasis and efficient clearance of cholesterol (Babiker et al. 1997). Further support for extrahepatic 25-hydroxylase activity came from studies that show expression of CYP27A1 in the brain, vascular endothelium, kidney, and liver (Andersson et al. 1989, Reiss et al. 1997); a situation that, with the exception of what we describe here for the liver, is comparable with that in sea bream tissues.

### Figure 3
Phylogenetic tree of CYP27A1 sequences constructed using the neighbor-joining method. Numbers at the branch nodes represent the confidence levels of 1000 bootstrap replications. Human, mouse, and *Xenopus tropicalis* (*Xenopus CYP27B1*) CYP27B1 served as an outgroup. Accession numbers as in Fig. 2. Additional accession numbers: human (*Homo sapiens*), NP_000776; mouse (*Mus musculus*), NP_034139; and *Xenopus* (*Xenopus tropicalis*), NP_001006907.

### Table 2
25-OHD plasma levels (nM) following 25-OHD and calcitriol treatment of seawater sea bream

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Vehicle</th>
<th>25-OHD</th>
<th>Calcitriol</th>
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<tr>
<td>3</td>
<td>31·2±2·20</td>
<td>51·0±9·96*</td>
<td>36·6±4·09</td>
</tr>
<tr>
<td>6</td>
<td>33·8±4·72</td>
<td>59·9±11·75*</td>
<td>36·4±3·29</td>
</tr>
<tr>
<td>9</td>
<td>33·4±3·01</td>
<td>47·7±9·11*</td>
<td>31·6±3·11</td>
</tr>
</tbody>
</table>

Seawater sea bream were injected with vehicle or hormones (see Materials and Methods) and sampled for blood 3, 6, and 9 days later. Plasma was analyzed for 25-OHD; vehicle-treated fish did not differ in time nor did calcitriol affect 25-OHD levels compared with vehicle-treated fish; 25-OHD-injected fish had elevated plasma levels of 25-OHD (*P<0·05; n=9*) for the duration of the experiment.
Henry & Norman (1975) were the first to demonstrate a vitamin D3 hydroxylase in vitro in renal tissue of a full series of vertebrates including fresh- and seawater fishes. Subsequent studies showed hydroxylation of vitamin D3 and 25-OHD into their polar metabolites in liver and kidney (Hayes et al. 1986, Bailly du Bois et al. 1988, Takeuchi et al. 1991). The demonstration of 1α-hydroxylase activity in liver in carp and bastard halibut (Takeuchi et al. 1991), and Atlantic cod (Sundell et al. 1992) led to the postulation that also in these species the liver is a site for the first hydroxylation of vitamin D3. However, hydroxylase activity was demonstrated in extrahepatic (and extrarenal) sites in a variety of fishes, and this suggests both that hydroxylations at the 1 and 25 positions outside the liver may occur to produce calcitriol (Graff et al. 1999). Considering the situation in mammals with a possible involvement of CYP27B1 in calcitriol metabolism, a further search for analogous hydroxylation pathways in fish seems warranted.

In the mammalian kidney, CYP27B1 hydroxylates 25-OHD to produce circulating calcitriol; remarkably, renal CYP27A1 may also catalyze 1α-hydroxylation (Postlind et al. 2000, Araya et al. 2003), and this implies that CYP27A1 may convert vitamin D3 into calcitriol.

In sea bream, just as in higher vertebrates, the expression of CYP27A1 is seen in a variety of tissues; remarkably,
the highest expression is observed in kidney and, from our quantitative mRNA analysis, little or none in liver. Also, and in contrast to reports on mammals, we could not alter liver CYP27A1 expression by ‘classical’ hormonal treatments, while renal CYP27A1 expression markedly responded to both calcitriol and 25-hydroxycholecalciferol. We conclude that the fish kidney may be the prime and possibly unique site for calcitriol formation, a situation clearly different from the two-step process in liver and kidney observed in mammals.

Our findings contrast with those of Takeuchi et al. (1991), who demonstrated that in carp and bastard halibut 25-hydroxylase activity is essentially confined to a hepatic microsomal fraction, and 1α-hydroxylase activity to renal and hepatic mitochondrial fractions (Takeuchi et al. 1991), which favors either a liver–renal or liver–liver pathway for dihydroxylation of vitamin D₃. Very low-expression levels of the mitochondrial CYP27A1 were found in sea bream liver and no effects could be established of vitamin D metabolite treatments and, therefore, we doubt whether in sea bream the liver plays a role in calcitriol formation; this would imply a renal–renal pathway for dihydroxylation in this species. Clearly, in fish, calcitriol production pathways may differ.

Responses to hormone treatments

The 25-OHD treatment predictably elevated plasma level of this metabolite reaching its maximum 6 days post-injection. Under this 25-OHD regimen, CYP27A1 expression showed an initial downregulation at day 3 followed by a transient upregulation at day 6. A plethora of receptors and pathways may be involved in regulation following 25-OHD treatment; to establish the factor responsible for the upregulation seen here requires more research (Langmann et al. 2005, Tang 2007, personal communication). We speculate that injection of 25-OHD elevated calcitriol levels (unfortunately, we were not able to assess plasma calcitriol levels) and by doing so enhanced expression of CYP27A1 (for calcitriol effects, see below).

Calcitriol injection was without effect after 3 days; however, after 6 days a twofold upregulation could be seen as in the 25-OHD-treated animals. In mammals, downregulation by calcitriol on CYP27A1 has been reported (Theodoropoulos et al. 2003), but the pattern we show here parallels data on Atlantic salmon: it was shown that calcitriol injections result initially in a drop of plasma calcitriol 3 days after the injection and then return to normal levels (on 5 and 7 days of post-injection); these findings suggest a clearance of the exogenous calcitriol and a suppression of the endogenous production of calcitriol in the first days of post-injection by downregulation of its hydroxylase, followed by an upregulation restoring basal levels. Such effects were also observed in salmon treated with calcitriol (Flik et al. unpublished).

The involvement of CYP27A1 in sea bream vitamin D metabolism is further supported by the upregulation of the gene when fish are subjected to PTHrP. Interactions of the vitamin D system and PTHrP in fishes are especially unknown, with the exception of one study in which the calcium regulation in vitamin D-deficient sea bream was studied and a relationship was found between PTHrP and calcitriol (Abbink et al. 2007). In humans, when exogenous PTHrP or PTH is administered, calcitriol levels tend to rise (Frahére et al. 1992, Everhart-Caye et al. 1996). Moreover, in a study looking at the vitamin D metabolism in a rat model for humoral hypercalcemia of malignancy, it was established that PTHrP enhanced 1α-hydroxylase expression in bisphosphonate-treated rats (Michigami et al. 2001). PTHrP is a well-established hypercalcemic hormone in sea bream and thus we tentatively conclude that PTHrP may well be involved in CYP27A1 regulation, may be to evoke hypercalcemia via CYP27A1-dependent calcitriol production to control calcium uptake. Interestingly, a similar interrelationship between PTHrP and hypercalcemic actions of estrogen was established in this fish (Guerreiro 2002).

Acknowledgements

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