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Tissue-specific induction of EROD activity and CYP1A protein in *Sparus aurata* exposed to B(*a*)P and TCDD ☆

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Abstract

The purpose of this study was to compare xenobiotic CYP1A induction in liver, gills, and excretory kidney of gilthead seabream, *Sparus aurata*. Fishes were exposed via water for 20 days to different concentrations of benzo(*a*)pyrene (B(*a*)P) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). CYP1A was measured at the enzyme activity level as 7-ethoxyresorufin-*O*-deethylase (EROD) activity, and at the protein level by means of ELISA. The liver displayed the highest absolute levels of EROD activity, both under non-exposed and exposed conditions. Organ- or inducer-related differences in the time course of CYP1A induction were moderate; however, the magnitude of the induction response varied between the organs and between B(*a*)P and TCDD. In the case of TCDD, liver, and kidney yielded a comparable induction response, whereas in the case of B(*a*)P, the kidney showed a substantially higher maximum induction factor than the liver. In the gills, the two xenobiotics resulted in similar maximum induction factors. In B(*a*)P-exposed seabream, EROD activities and CYP1A protein levels showed a good correlation in all three organs, whereas with TCDD as inducer the correlation was poor, what was mainly due to a decrease of EROD activities at the higher concentrations of TCDD, while CYP1A protein levels showed no concomitant decline. Overall, the study revealed both similarities and differences in the time-, concentration-, and inducer-dependent CYP1A responses of the three target organs, liver, kidney, and gills. Although, the findings of this study principally confirm the notion of the liver as the major metabolic organ in fish, they also provide evidence for substantial metabolic potential in gills and particularly in the kidney.

Keywords: B(*a*)P; TCDD; CYP1A; EROD; *Sparus aurata*

1. Introduction

The cytochrome P450 monooxygenase system comprises several families of structurally and functionally related haemo-proteins (Nebert et al., 1989; Nelson et al., 1996). Many of the cytochrome P450 proteins metabolize lipophilic organic substances, including a variety of environmental pollutants, into more water-soluble compounds (Bernhoft et al., 1994). The biotransformation of polyhalogenated aromatic hydrocarbons (PHAHs), such as dioxins or polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) is catalyzed by the cytochrome P4501A (CYP1A) subfamily.

In mammals this subfamily contains two different genes, CYP1A1 and CYP1A2 which are thought to originate from a gene duplication event during early vertebrate evolution (Nebert and Gonzalez, 1987). In fish, originally only a single CYP1A gene has been detected (Nebert and Gonzalez, 1987, Heilman et al., 1998), but recent studies indicated the existence of more piscine CYP1A genes although the functional significance of this finding is yet unknown (Berndson and Chen, 1994; Gooneratne et al., 1997).

A characteristic property of the CYP1A genes is the inducibility by xenobiotics. Pollutants such as PAHs and

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PHAHs lead to an activation of CYP1A gene expression through binding to a cytosolic receptor, the aryl hydrocarbon receptor (AhR) (Stegeman and Hahn, 1994; Hankinson, 1995; Hahn, 1998). The ligand–receptor complex binds to responsive elements on the DNA and thereby activates CYP1A transcription and translation (Cousinou et al., 2000). Due to its inducibility by xenobiotics, CYP1A is used as a biomarker of human and wildlife exposure to environmental organic contaminants (Levine and Oris, 1999).

Studies on the induction of CYP1A in fish have focused primarily in the liver (Stegeman and Hahn, 1994; Sarasquete and Segner, 2000). The relative role of different organs in xenobiotic metabolism and the induction response of CYP1A in extrahepatic tissues is less known (Ortiz-Delgado et al., 2005). Therefore, the aim of the present study was to perform a comparative examination of CYP1A induction (catalytic activity and protein contents) in liver, gill, and kidney. All three organs play key roles in the toxicokinetics of waterborne xenobiotics in fish, and all three organs express CYP1A. Still, little is known on the comparative induction response of CYP1A protein and catalytic activity in these organs. Therefore, in the present study we examined time- and concentration-dependent CYP1A induction in gills, liver, and kidney of seabream exposed to AhR ligands. CYP1A expression was measured at the level of catalytic activity as 7-ethoxyresorufin-*O*-deethylase (EROD) activity, and at the protein level by means of enzyme-linked immunosorbent assay (ELISA). As inducing compounds we used two prototype AhR ligands, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the polyaromatic hydrocarbon (PAH) benzo(*a*)pyrene (B(*a*)P) which differ in their rate of biotransformation and therefore may evoke different time-dependency of CY1A induction. The tested concentrations were chosen in order to simulate a high dose of exposure which may occurs in the case of spills and not long term, low dose of exposure what is usually the case under field conditions. The fish species investigated in this study is the seabream, *Sparus aurata*, which is of great commercial and economic importance in the Atlantic and Mediterranean areas and which has been suggested as a monitoring species for the coastal zones.

2. Material and methods

Immature male specimens of gilthead seabream, *Sparus aurata* (average weight: 250–300 g) were obtained from a commercial fish farm (CUPIMAR, S.A. San Fernando, Cádiz, Spain). Seabream is a protandric species, which develops as male during the first year of life and later undergoes sex conversion into females. The use of exclusively male fish has the advantage to exclude possible sex influence on the CYP1A response. Prior to the experiments, fish were kept in tanks during two weeks for their acclimatization. Fish were maintained under flow trough conditions (at 33‰) at constant temperature (19±1 °C) and under 12 h light/12 h dark cycle and fed with dried pellets during the experimental period.

2.1. Xenobiotic exposure

After the acclimatization period, fish were randomly distributed in the experimental tanks and submitted to the following treatments: (a) controls, (b) exposure to 1 pg TCDD/L, (c) 3 pg TCDD/L, (d) 4 pg TCDD/L, (e) 6 pg TCDD/L, (f) 100 μg B(*a*)P/L, (g) 200 μg B(*a*)P/L, (h) 300 μg B(*a*)P/L, and (i) 500 μg B(*a*)P/L nominal concentration. Stock solutions of both contaminants were prepared in toluene and added to the water.

Fish were maintained in 120 L tanks under semi-static conditions for 20 days. Each treatment was done in triplicate with 12 fish each per tank. The water in the experiment was changed every 24 h; afterwards, fresh B(*a*)P or TCDD solutions were added. No mortalities were recorded during experimental period. The contaminants were diluted in 20 μl toluene carrier/liter. Control fish received carrier—toluene—only. Preliminary experiments indicated no toluene effects on CYP1A induction.

Samples were taken after 5, 10, 15, and 20 days during experimental period. At sampling, fish were dissected and liver, gills, and excretory kidney (caudal portion) samples were immediately shock-frozen in liquid nitrogen for immunochemical (ELISA) and biochemical (EROD) analysis.

2.2. CYP1A catalytic activity/EROD

EROD activity was measured as described by Scholz et al. (1997). For this purpose samples of gills, kidney, and liver were homogenized in Tris-based homogenization buffer (4 ml/g tissue) and microsomes were prepared by means of a ultracentrifugation. The pellet containing the microsomal fraction was resuspended in 200 μl homogenization buffer and microsomal proteins determined using the Lowry Kit (Biorad). EROD activities were determined by adding NADPH and ethoxyresorufin (47 and 0.4 μM final concentration, respectively) in phosphate buffer saline to each well of special microtiter plates (SLT Fluostat; SLT-TECAN) and measured in a SLT fluorimeter at a wavelength of 344 nm with a reference filter set at 590 nm at room temperature. Each sample was analyzed in triplicate and the mean value was used for statistical analysis.

2.3. CYP1A protein content/ELISA analysis

An indirect ELISA method was performed according to Goksøyr (1991) and Scholz et al. (1997). The microsomal samples were adjusted to a protein content of 10 ng microsomal protein/mL. The primary antibody used for the ELISA and validated for *S. aurata* (Ortiz-Delgado et al., 2005) was the monoclonal antibody C10-7 diluted at 1:500. The primary reaction was followed by a horseradish-peroxidase-conjugated goat IgG anti-mouse (Dako) as secondary antibody. Staining was performed using 3,3′-diaminobenzidine as substrate. The peroxidase reaction product was measured as optical density in a spectrophotometer (Pharmacia, Freiburg, Germany) at 405 nm; controls (non-specific binding, blank) were included.

2.4. Statistical analysis

Statistica software (SigmaStat 2.03) was used for Statistical analyses. All the data were first tested for normality and homogeneity of variance to meet statistical demands. Variance analysis was used to compare results between time and dose of exposure and interactions between them, followed by the Tukey–Kramer test. The significance level adopted throughout the study was $P<0.05$.

Linear regression analysis was applied to evaluate the relationship between catalytic EROD activity and CYP1A protein (ELISA). This comparison was conduced separately for each treatment group (B(*a*)P and TCDD treatments) and for the combination of all treatment groups (for each studied organ).

3. Results

3.1. B(a)P exposure

3.1.1. EROD activities (Fig. 1a–c)

EROD induction response varied between liver, gills, and kidney and between B(*a*)P and TCDD. The liver