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Cellular distribution and induction of CYP1A following exposure of gilthead seabream, *Sparus aurata*, to waterborne and dietary benzo(a)pyrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: An immunohistochemical approach

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Abstract

The present study aimed to investigate cellular expression of cytochrome P4501A (CYP1A) protein in the seabream, *Sparus aurata*, exposed to one of two CYP1A-inducing contaminants, benzo(a)pyrene (B(a)P) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Male adult fish were exposed to several concentrations of TCDD or B(a)P either via water or via food. Fish were sampled after 0, 5, 10, 15 or 20 days of treatment and the time- and concentration-dependent induction of CYP1A protein in cells and tissues was studied using immunohistochemistry. A general site of CYP1A induction was the vascular endothelium. Aqueous exposures resulted in elevation of CYP1A immunoreactivity in gill pillar cells, heart endothelium, renal tubular epithelium, hepatocytes, and gut mucosal epithelium. In contrast, dietary exposure resulted in strong CYP1A immunostaining in gut epithelium but in only mild to moderate staining elsewhere. Both B(a)P and TCDD induced CYP1A in similar cellular response patterns in most organs examined, although TCDD generally led to higher staining intensity and frequency (i.e. the number of CYP1A-positive cells within an organ), an effect that is likely to be related to compound-specific differences in induction potency, metabolism and penetration. Contaminant-specific staining patterns were observed in the gills, where TCDD exposure evoked CYP1A immunostaining in the endothelial pillar cells, while B(a)P induced CYP1A staining in the branchial epithelial cells. This work points to the importance of immunohistochemical identification of cell-specific CYP1A responses in assessing the toxicology of CYP1A-inducing xenobiotics.

Keywords: CYP4501A; Immunohistochemistry; Digestive tract; Liver; Kidney; Spleen; Gills; Heart; *Sparus aurata*; B(a)P; TCDD

1. Introduction

In vertebrates, exposure to diverse xenobiotics can result in the induction of enzymes involved in

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xenobiotic metabolism (Black and Coon, 1987; Buhler and Williams, 1989). Cytochrome P4501A (CYP1A) is among the major oxidative enzymes induced in fish by congeners of polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aromatic hydrocarbons (PHAHs) (Stegeman and Hahn, 1994). CYP1A responds to environmental levels of these compounds in a dose-dependent manner and is commonly used in field and laboratory studies as a marker of PAH and PHAH exposure and effects.

The liver is considered to be the main site of CYP1A expression in fish, but CYP1A expression and induction has been observed in other tissues as well (for review see Sarasquete and Segner, 2000), including those in direct contact to the environment, such as gills (Miller et al., 1989), intestine (Van Veld et al., 1990) and olfactory epithelium (Smolowitz et al., 1992; Monod et al., 1996). Contaminant-related induction of CYP1A has been reported in multiple extrahepatic organs, tissues and cells of fish collected from polluted environments (Stegeman et al., 1991; Husoy et al., 1996), as well as in fish exposed to CYP1A-inducing chemicals in the laboratory (e.g., Arellano et al., 2001; Smolowitz et al., 1991; Lindström-Seppä et al., 1994; Husoy et al., 1994; Grinwis et al., 2000, 2001; Ortiz-Delgado, 2001; Ortiz-Delgado and Sarasquete, 2004; Ortiz-Delgado et al., 2002). The induction of CYP1A at extrahepatic sites may depend on tissue-specific factors or on the exposure route. Aquatic organisms can accumulate toxicants via the intestine from contaminated food and ingested sediments, or via gills and skin from contaminated water (Spacie and Hamelink, 1985; Knezovich et al., 1987; Bruggeman et al., 1984; Varanasi et al., 1986; Rubinstein et al., 1984). Although chemical toxicity depends not only on exposure dose and duration, but also on the route by which exposure occurs (Blomquist, 1992; Le Curieux et al., 1992; Xu et al., 1992), the relative importance of the branchial and intestinal uptake route to overall exposure remains poorly understood.

The objectives of the present study were to examine how cell and organ distribution of CYP1A protein expression varies (1) with the type of inducer, and (2) with exposure route. The first objective was studied by comparing the CYP1A response of seabream, *Sparus aurata*, to benzo(a)pyrene (B(a)P), a rapidly metabolisable PAH compound, with the CYP1A response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a poorly metabolisable PHAH compound. The second objective was addressed by studying cell and tissue expression of CYP1A following either waterborne or dietary exposure to B(a)P and TCDD. The fish species investigated in this study, the seabream *S. aurata*, is of great economic importance in the Atlantic and Mediterranean areas and has been suggested as a monitoring species for the coastal zones. In order to avoid sex-related variation of the CYP1A induction response, only male fish (1 year old; protandric development) were used. Methodologically, immunohistochemistry was used to study expression and cellular localisation of CYP1A at the level of the protein.

2. Materials and methods

2.1. Animals and treatments

Males of gilthead seabream, *S. aurata* (average weight: 250–300 g), were obtained from a commercial fish farm (CUPIMAR, S.A. San Fernando, Cádiz, Spain). Prior to the experiments, fish were kept in tanks during two weeks for acclimatization and were supplied with flow-through sea water at constant temperature ($19 \pm 1\,^{\circ}C$).

2.2. Xenobiotic exposure

After the acclimation period, fish were randomly distributed to the experimental tanks and were exposed to B(a)P (Sigma Aldrich, 97% analytical grade) or TCDD (Supelco, HPLC grade) either via water or via food. The water treatment included the following groups: (a) control (only vehicle added (20 μL acetone/L)); (b) 100 μg B(a)P/L; (c) 200 μg B(a)P/L; (d) 300 μg B(a)P/L; (e) 500 μg B(a)P/L; (f) 1 pg TCDD/L; (g) 3 pg TCDD/L; (h) 4 pg TCDD/L; (i) 6 pg TCDD/L. The dietary group included (a) control (1 mL corn oil/100 g dry food); (b) 100 μg B(a)P/g dry food; and (c) 1 pg TCDD/g dry food. All concentrations are nominal concentrations. In aquatic exposures, fish were maintained in 120 L tanks under semi-static condition for 20 days. Daily, the specimens were fed freely with *Loligo* spp. and the excess of food was removed from the tanks. Immediately after feeding, the water in the experiment tanks was exchanged every 24 h, which was followed by the addition of fresh B(a)P or TCDD solutions. Before the experiments were initiated, tanks