Distribution and induction of cytochrome P450 1A1 in the rainbow trout brain

Tommy Andersson* and Anders Goksøyr

1 Department of Zoophysiology, University of Göteborg, Box 25059, 400 31 Göteborg, Sweden; 2 Laboratory of Marine Molecular Biology, University of Bergen, HIB, N-5020 Bergen, Norway

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Abstract

Cytochrome P450 (CYP) 1A1 participates in the activation as well as detoxification of environmental pollutants such as aromatic hydrocarbons. This CYP form is also efficiently induced by aromatic hydrocarbons. The presence of CYP 1A1 in the brain might thus be of physiological and toxicological importance. In the present investigation on rainbow trout, the distribution of 7-ethoxyresorufin-O-deethylase (EROD) activity, a cytochrome CYP 1A1 catalyzed reaction, was measured in whole tissue homogenates from brain parts. In control fish, a relatively high activity was found in the rainbow trout olfactory bulb compared to the other brain parts. Although an EROD induction (3 to 7-fold) by β-naphthoflavone (BNF) was recorded in all brain parts from the rainbow trout, the highest induced activity was measured in the olfactory bulbs. To ascertain the distribution of EROD activity in cells, whole brain tissue was subfractionated by differential centrifugation. The fractionation scheme separated mitochondria (P2 fraction) and microsomes (P3 fraction) as determined by marker enzymes and electron microscopy. In control rainbow trout, a low EROD activity could be measured in the P2 fraction. BNF induced the EROD activity in both P2 and P3 fractions. Western blotting showed the induction by BNF of a protein band in the P2 and P3 fractions with a molecular mass around 58,000 when highly specific anti-cod CYP 1A1 antibodies were used. ELISA measurements confirmed the induction of CYP 1A1 protein in the rainbow trout brain subcellular fractions.

Introduction

The cytochrome P450 (CYP) 1A1 enzyme system is involved in bioactivation and/or detoxification of many organic compounds. Much attention has been directed towards members in the CYP 1A subfamily because of their role in metabolism and activation of aromatic hydrocarbons to cytotoxic intermediates (Guengerich 1988). Xenobiotic metabolizing CYPs are most abundant in the liver but also present in many extrahepatic tissues. In mammals, the level of CYP in the brain is considerably lower than in the liver (Guengerich and Mason 1979; Marietta et al. 1979). In spite of the low cerebral

3 Abbreviations: CYP, cytochrome P450; EROD, 7-ethoxyresorufin-O-deethylase; BNF, β-naphthoflavone; ELISA, enzyme-linked immunosorbent assay; PAH, polyaromatic hydrocarbon, PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-p-dioxin; PCDF polychlorinated dibenzo-furan; P450 c and P450 d are trivial names for CYP 1A1 and CYP 1A2, respectively.

* Correspondence to: Tommy B. Andersson, Department of Pharmacokinetics and Drug Metabolism, Astra Hässlé AB, S-431 83 Mölndal, Sweden, Fax 46 - 31 776 3701
CYP levels several CYP activities have been detected and immunohistochemical studies have located individual forms to specific areas and cells (Mesnil and Testa 1984; Köhler et al. 1988; Warner et al. 1988; Hansson et al. 1990). Immunohistochemical studies also suggested that CYP 1A or immunologically similar forms are constitutively expressed in rat brain (Köhler et al. 1988). Moreover, treatment of rats with polyaromatic hydrocarbon (PAH)-type inducers increased brain 7-ethoxyresorufin-O-deethylase (EROD) activity, which indicate induction of a CYP 1A form (Walther et al. 1987; Unkila et al. 1993). The brain may thus be an important target organ for environmental pollutants, contributing to neurotoxicological or endocrinological effects of such compounds.

In all fish species studied only one member of the CYP 1A subfamily has been identified. So far the biochemical and regulatory properties of CYP 1A in rainbow trout (Oncorhynchus mykiss), scup (Stenotomus chrysops) and cod (Gadus morhua) indicate that this form is related to the mammalian CYP 1A1 form (for review see Goksoyr and Förlin 1992). As in mammals, the basal levels of CYP 1A1 in fish are often low or non-detectable. However, the expression of CYP 1A1 is highly inducible by several PAHs such as polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). Several studies have shown that fish living in areas polluted with PAHs have induced hepatic CYP 1A1 levels. Recently CYP 1A1 immunoreactivity in brain vascular endothelial cells was detected in PCDF-treated scup (Smolowitz et al. 1991). The presence of CYP 1A1 in brain endothelial cells was also demonstrated in scup from PAH-polluted areas (Stegeman et al. 1991). Furthermore, male rainbow trout pituitary CYP 1A1 was found to be elevated by a PAH-type inducer (β-naphthoflavone, BNF) (Andersson et al. 1993). Pituitary cells containing inducible CYP 1A1 were identified as gonadotrophs containing gonadotropin II, indicating one possible link between reproductive disturbances and PAH exposure.

The brain of several fish species accumulates relatively high levels of PAHs (Varanasi et al. 1989). Moreover, experimental exposure of teleost fish to several aromatic hydrocarbons such as hexachlorobenzene, PCBs and PCDDs has shown a great species difference in their accumulation in the brain (Ingebrigtsen and Solbakken 1985; Ingebrigtsen et al. 1992; Hektoen et al. 1992). Tape section autoradiography has revealed high concentrations of these compounds in the cod brain whereas considerably lower levels were found in the rainbow trout brain. In teleosts, the toxicological significance of brain CYP could therefore be species specific. Cod and rainbow trout were recently reported to exhibit CYP-dependent 7-ethoxycoumarin-O-deethylase activity and UDP glucuronosyltransferase activity using p-nitrophenol as substrate (Ingebrigtsen et al. 1992). In this study, we have investigated the regional and subcellular distribution as well as the inducibility of CYP in the rainbow trout brain.

Materials and methods

Animals

Rainbow trout (Oncorhynchus mykiss) were obtained from a local fish farm. Fish were kept in aquaria with circulating fresh water. BNF (50 mg kg\(^{-1}\) body weight) was given intraperitoneally 3 times in two day intervals. Two days after the last injection the fish were killed and the brain was removed and rinsed in ice-cold 0.1 M phosphate buffer (pH 7.4).

Homogenization and subcellular fractionation

The whole brain or the various parts (defined as described by Harder (1975)) were homogenized in a Potter-Elvehjem glass-teflon homogenizer by 4 strokes in 4 volumes of 0.1 M phosphate buffer containing 0.15 M potassium chloride, 0.2 mM butylhydroxytoluene, 1 mM dithiotreitol, 0.1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride. Thereafter the homogenate was ultrasonicated for 15 s.

To study the distribution of enzyme activities in subcellular fractions the following procedure was used. The tissue homogenate was centrifuged at