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Lack of CYP1A responsiveness in species inhabiting chronically contaminated habitats: Two varieties of resistance?

Ben F. Brammell [a,*,1], David J. Price [b], Wesley J. Birge [a,2], Adria A. Elskus [a,c,3]

[a] Department of Biology, University of Kentucky, Lexington, KY 40506, USA
[b] Lexington-Fayette Urban County Government, Division of Water Quality, 301 Lisle Industrial Avenue, Lexington, KY 40511, USA
[c] Graduate School of Toxicology, University of Kentucky, Lexington, KY 40506, USA

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ABSTRACT

Organisms chronically exposed to organic pollutants such as polychlorinated biphenyls (PCBs) can develop resistance to these chemicals, a condition associated with reduced inducibility of the biomarker enzyme cytochrome P450 1A (CYP1A). This study addresses the CYP1A response of members of the families Ictaluridae and Centrarchidae, two fish families found throughout much of the United States. We measured CYP1A expression, PCB body burdens, and conducted CYP1A challenge experiments in species from these families residing in the Town Branch/Mud River system (Logan County, KY, USA), a stream system historically contaminated with high levels of PCBs. Despite PCB concentrations in muscle tissue typically associated with elevated CYP1A (16.7 to 75.2 μg PCB/g wet edible flesh), resident fish in the contaminated Town Branch/Mud River sites (yellow bullhead [*Ameiurus natalis*], green sunfish [*Lepomis cyanellus*], and spotted bass [*Micropterus punctulatus*]) had hepatic CYP1A activity levels similar to, rather than higher than, those in reference fish, suggesting reduced sensitivity to CYP1A induction. Lack of CYP1A expression following direct contaminant exposure has often been associated with resistance to those contaminants. To determine if CYP1A in resident populations was resistant to induction by PCBs, we exposed resident fish to a single, intraperitoneal injection with a potent CYP1A inducer, 3,4,3′,4′-tetrachlorobiphenyl (PCB 77). PCB 77 treatment significantly induced hepatic CYP1A activity and protein in yellow bullhead from reference, but not contaminated, sites and had no effect on CYP1A in green sunfish from either site. The low CYP1A expression levels in resident fish with elevated PCB body burdens, together with the failure of PCB injection to induce CYP1A in certain populations, indicate an acclimatory CYP1A response in yellow bullheads and likely an inherently resistant CYP1A in green sunfish. This work demonstrates for the first time acclimation of CYP1A to PCBs in a species within the family Ictaluridae and provides further support for our previous work indicating an apparent inherent lack of CYP1A sensitivity to chlorinated inducers in Centrarchids. These traits may explain, at least in part, the common association of these families with degraded habitats and indicate *Lepomis* members are likely to be excellent candidates for exploring the mechanistic basis of 'inherent' CYP1A resistance. This study also underlines to the need for thorough characterization of the CYP1A responsivity of a population and/or species prior to using CYP1A as a reliable biomonitoring tool.

1. Introduction

Numerous studies have demonstrated that populations living in chronically contaminated habitats can develop increased tolerance to those contaminants (Vinson et al., 1963; Klerks and Levinton, 1989; Weis and Weis, 1989). Increased tolerance to organic contaminants is typically associated with reduced inducibility of cytochrome P450 1A (CYP1A) (Elskus, 2001), a xenobiotic metabolizing enzyme widely used as a biomarker for organic contaminants (Whyte et al., 2000; Schlenk et al., 2008). Both reduced CYP1A inducibility and increased resistance to pollutant-induced developmental abnormalities have been reported in fish populations residing in chronically contaminated habitats (Nacci et al., 1999). Marsh minnows (*Fundulus heteroclitus*) from areas heavily contaminated with organic pollutants express lower levels of CYP1A activity in response to PCB exposure relative to reference populations (Cooper, 1996; Elskus et al., 1999). *F. heteroclitus* from a highly contaminated site in Newark, NJ (USA) exhibit reduced CYP1A inducibility and reduced developmental abnormalities in response to

* Corresponding author.
E-mail address: ben.brammell@asbury.edu (B.F. Brammell).
[1] Present address: Department of Natural Sciences, Asbury University, Wilmore, KY 40390, USA.
[2] Deceased.
[3] Present address: U.S. Geological Survey, S.O. Conte Anadromous Fish Research Laboratory, Leetown Science Center, Maine Field Office, 5751 Murray Hall, University of Maine, Orono, ME 04469-5751, USA.

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treatment with dioxin (Prince and Cooper, 1995a, 1995b) and dioxin-like PCBs (Arzuaga and Elskus, 2010). Attenuated CYP1A expression has also been reported in Atlantic tomcod (*Microgadus tomcod*) (Wirgin et al., 1992, 2011) and largemouth bass (*Micropterus salmoides*) (Zielinski et al., 2000; Zielinski, 2001) inhabiting PCB contaminated portions of the Hudson River (NY, USA). Yellow perch (*Perca fluviatilis*) inhabiting a lake chronically contaminated with PCBs likewise display increased tolerance (Förlin and Celander, 1995). An in-depth study of 24 populations of killifish (*F. heteroclitus*) in New England found intraspecific variation in PCB-tolerance spanning four orders of magnitude (Nacci et al., 2010). Such chemical tolerance can have both costs, such as increased susceptibility to disease (Frederick et al., 2007), and benefits, such as increased survival in highly contaminated environments (Nacci et al., 1999, 2010).

The Town Branch/Mud River system, located in southwestern Kentucky, has been heavily contaminated with PCBs from a local manufacturing plant since the 1960s (Commonwealth of Kentucky, F.C.C., 1997). The PCBs in this system are bioavailable to fish as demonstrated by induction of CYP1A in caged rainbow trout (Brammell et al., 2010a) and elevated PCB body burdens in resident longear sunfish (Brammell et al., 2010b). In the present study we hypothesized that fish resident in this system have likely developed a reduced CYP1A response following exposure to PCBs. To examine this, we 1) compared hepatic CYP1A activity in fish collected from a contaminated portion of this system to activity observed in reference site fish, 2) measured PCB body burdens in these fish, and 3) conducted CYP1A challenge experiments in 3 depurated fish species from this system.

2. Materials and methods

2.1. Materials

7-Ethoxyresorufin and resorufin were obtained from Molecular Probes (Eugene, OR, USA). The monoclonal antibody made against scup CYP1A protein, MAb 1-12-3, was a generous gift of Dr. John Stegeman (Woods Hole Oceanographic Institution). Cy™5-conjugated affinipure goat anti-mouse IgG was obtained from Jackson Immunoresearch Laboratories Inc. (West Grove, PA, USA) and precast polyacrylamide gradient gels were from Invitrogen (Carlsbad, CA, USA). Nitrocellulose membrane (0.45 μm) was obtained from Schleicher and Schull (Keene, NH, USA). The Bio-Dot SF Microfiltration Apparatus was obtained from Bio-Rad (Hercules, CA, USA). High purity pesticide grade solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other reagents were obtained from Sigma, Fisher Scientific, or Invitrogen.

2.2. Study sites

2.2.1. Town Branch/Mud River

The Town Branch/Mud River system (Fig. 1) has been contaminated since the 1960s with PCBs from a local manufacturing plant. For over 20 years PCBs were released into a lagoon behind the plant that leaked waste containing high concentrations of PCBs into Town Branch approximately 8 km (5 miles) upstream of its confluence with the Mud River (contaminant source, Fig. 1) (Commonwealth of Kentucky, F.C.C., 1997). Sediment PCB concentrations of 280 μg/g (dry sediment, clay–silt fraction) (Birge et al., 1988) were documented in Town Branch in 1986, precipitating remediation efforts that began in 1997. Removal of contaminated sediments from both the streambed and floodplain of Town Branch was completed in July 2001 (Michael Mills, KY Division of Water, personal comm.). Despite those efforts, we documented extremely high sediment levels of PCBs (45.7 μg/g dry mass) in 2002 in the remediated Town Branch section (Brammell et al., 2010a). Therefore in this study, we will refer to this section as Town Branch Contaminated (TB Cont, Fig. 1). No remediation has been conducted in the Mud River section downstream of the Town Branch confluence (MR Cont), and relatively high levels of PCBs continue to be found in sediments in this area (Price and Birge, 1999). Upstream sections were used as reference sites for Town Branch (TB Ref) and Mud River (MR Ref).

2.2.2. Fishing Creek

Some fish species of interest in this study were not available from MR Ref and fish from Fishing Creek (FC) were used instead. Fishing Creek is a relatively clean stream located in south central Kentucky (Pulaski, County) similar in size and habitat to the Mud River. Previous studies indicate that contaminants in fish tissue from this system are very low or below detection (KY DEP 2001) making it an ideal reference stream for the contaminated Mud River section.

2.3. Experimental design

2.3.1. Field study: fish PCB body burdens

Fish were collected from contaminated and reference areas using a backpack shocker (Smith-Root, Inc., Model 12-B) or by a larger portable floating electroshocking unit as described (Price et al., 2003) between October 21 and November 1, 2002. Although all fish were of sufficient size to have reached sexual maturity (Table 2) all fish species utilized in this study were collected between October 21 and November 1st placing them far outside the season (April–July) in which gravid females would have been observed (Etinier and Starnes, 1993). Fish were sacrificed in the field or within 24 h of returning to the laboratory, and livers flash frozen in liquid nitrogen. Carcasses, minus the liver, were wrapped in aluminum foil, tagged, and stored at −20 °C until fillets were taken for PCB extraction (within 2 months).

Yellow bullheads (*Ameiurus natalis*) were collected from MR Cont and from FC Ref, green sunfish (*Lepomis cyanellus*) were collected from TB Cont and TB Ref, and spotted bass (*Micropterus cumulatus*) were collected from MR Cont and FC Ref.

2.3.2. Laboratory study: CYP1A challenge experiment

Green sunfish and yellow bullhead used in the laboratory CYP1A challenge experiments were collected from TB Ref and TB Cont on April 22, 2003 using a backpack shocker and held in the laboratory for 14 weeks (98 days), a length of time exceeding the half-life of the predominant CYP1A inducing PCB congeners in fish tissue that are significant components of Aroclor 1248 (PCB 77, 47 days; PCB 105, 59 days; 118, 95 days) (Coristine et al., 1996; Frame et al., 1996). The vast majority of PCBs detected in this study (98.5%) were components of Aroclor 1248, the remainder was Aroclor 1260. Fish were held at 17–20 °C and fed mealworms *ad libitum* two times per week for eight weeks and then fed approximately 3% of their body weight two times per week for six weeks prior to the experiment to standardize the feeding regime between species.

All fish were fasted for seven days prior to treatment and injected intraperitoneally with either PCB 77 at 1 mg/kg in corn oil or corn oil as the vehicle control. Fish were sacrificed on day seven following injection, weighed (whole body weight), and livers removed and flash frozen in liquid nitrogen. Fillets from vehicle control yellow bullheads were analyzed for PCB content. Gonad weights were also taken.

2.4. Liver microsomal protein isolation

Livers were removed from liquid nitrogen and weighed. Livers were immediately homogenized in 10 volumes (weight:volume) of ice-cold 50 mM Tris buffer (pH 7.4). Microsomal fractions were obtained by differential centrifugation as previously described (Stegeman et al., 1979). The final 100,000 *g* microsomal pellets were resuspended in 50 mM Tris containing 1 mM EDTA, 1 mM DTT and 20% glycerol at a 1:1 ratio (liver weight:resuspension buffer volume). Microsomal samples were stored in liquid nitrogen until analyzed for catalytic activity and CYP1A protein content (within three weeks).