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Ahr and *Cyp1a2* genotypes both affect susceptibility to motor deficits following gestational and lactational exposure to polychlorinated biphenyls

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants known to cause adverse health effects and linked to neurological deficits in both human and animal studies. Children born to exposed mothers are at highest risk of learning and memory and motor deficits. We developed a mouse model that mimics human variation in the aryl hydrocarbon receptor and cytochrome P450 1A2 (CYP1A2) to determine if genetic variation increases susceptibility to developmental PCB exposure. In our previous studies, we found that high-affinity *Ahr*^{b}*Cyp1a2(−/−)* and poor-affinity *Ahr*^{d}*Cyp1a2(−/−)* knockout mice were most susceptible to learning and memory deficits following developmental PCB exposure compared with *Ahr*^{b}*Cyp1a2(+/+)* wild type mice (C57BL/6J strain). Our follow-up studies focused on motor deficits, because human studies have identified PCBs as a potential risk factor for Parkinson's disease. Dams were treated with an environmentally relevant PCB mixture at gestational day 10 and postnatal day 5. We used a motor battery that included tests of nigrostriatal function as well as cerebellar function, because PCBs deplete thyroid hormone, which is essential to normal cerebellar development. There was a significant effect of PCB treatment in the rotarod test with impaired performance in all three genotypes, but decreased motor learning as well in the two *Cyp1a2(−/−)* knockout lines. Interestingly, we found a main effect of genotype with corn oil-treated control *Cyp1a2(−/−)* mice performing significantly worse than *Cyp1a2(+/+)* wild type mice. In contrast, we found that PCB-treated high-affinity *Ahr*^{b} mice were most susceptible to disruption of nigrostriatal function with the greatest deficits in *Ahr*^{b}*Cyp1a2(−/−)* mice. We conclude that differences in AHR affinity combined with the absence of CYP1A2 protein affect susceptibility to motor deficits following developmental PCB exposure.

1. Introduction

Polychlorinated biphenyls (PCBs) are widespread persistent organic pollutants linked to numerous human health problems, with the most serious effects seen in children of exposed mothers (Ross, 2004; Schantz et al., 2003; Jacobson and Jacobson, 2003). They are number 5 on the U.S. government's list of priority pollutants (ATSDR, 2015). Worldwide, an estimated 200 billion kg remain in the environment (WHO World Health Organization, 2003). The primary route of exposure is contaminated food, especially fatty fish, meat and dairy products (Malisch and Kotz,

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2014; Langer et al., 2007; Gomara et al., 2005). New sources of PCB exposure have been reported with the inadvertent production of highly toxic PCB congeners (e.g. PCB 77 and PCB 153) during the synthesis of paint pigments (Anezaki et al., 2015; Hu and Hornbuckle, 2010) and the discovery of airborne PCBs near rural and urban schools (Marek et al., 2017). PCBs will remain a problem for generations because highly exposed cohorts are now reaching reproductive age (Bányiová et al., 2017; Quinn et al., 2011).

Multiple human studies found deficits in motor function in children exposed to high levels of PCBs (Boucher et al., 2016; Wilhelm et al., 2008; Vreugdenhil et al., 2002; Stewart et al., 2000). The hydroxylated metabolite 4-OH-CB 107 can also cause motor deficits in highly exposed children (Berghuis et al., 2013). In adults, an increased risk of Parkinson's disease (PD) was reported in women with high workplace exposures (Steenland et al., 2006) and in adults who consumed contaminated whale meat and blubber (Petersen et al., 2008). Rodent studies found adverse PCB effects in both the striatum (Caudle et al., 2006; Chishti et al., 1996) and cerebellum (Nguon et al., 2005). PCB effects on dopamine, the major neurotransmitter associated with motor function, are well known (Seegal et al., 1986, 1994, 1997, 2005).

PCBs occur as mixtures of coplanar congeners which can bind and activate the aryl hydrocarbon receptor and non-coplanar congeners which do not. Human studies clearly show differential responses to PCBs and related AHR agonists (Marek et al., 2014; Novotna et al., 2007; van Duursen et al., 2005; Tsuchiya et al., 2003). The AHR regulates three members of the cytochrome P450 family: CYP1A1, CYP1A2 and CYP1B1.The level of CYP1A2 found in human livers varies about 60-fold (Nebert and Dalton, 2006), and maternal CYP1A2 can sequester planar pollutants to prevent transfer to offspring (Curran et al., 2011a; Dragin et al., 2006,). In humans, there is a greater than 12-fold difference in the inducibility of CYP1A1, although the polymorphism responsible has not been identified (Nebert, 2017; Nebert et al., 2013).

We developed a mouse model to mimic human variation in the AHR and CYP1A2 to better understand genetic susceptibility to PCBs and similar pollutants. High-affinity *Ahr*^{b} mice will respond to low levels of xenobiotics such as dioxin and coplanar PCBs whereas poor-affinity *Ahr*^{d} mice are considered non-responders. To model the wide variation in CYP1A2, we used wild type *Cyp1a2(+/+)* mice and *Cyp1a2(−/−)* knockout mice (Denison and Faber 2017; Nebert, 2017; Nebert and Dalton, 2006). We previously showed that both high-affinity *Ahr*^{b}*Cyp1a2(−/−)* knockout mice and poor-affinity *Ahr*^{d}*Cyp1a2(−/−)* mice were more susceptible to learning and memory deficits when exposed to an environmentally relevant mixture of PCBs during gestation and lactation compared with *Ahr*^{b}*Cyp1a2(+/+)* wild type mice (Curran et al., 2011a,b, 2012). The studies described here extend those findings by testing the hypothesis that there is similar genetic susceptibility to PCB-induced motor deficits. Our motor battery was also designed to help clarify if PCBs affect motor function by disruption of nigrostriatal pathways or primarily target the cerebellum.

2. Materials and methods

2.1. Animals

Three genotypes of mice were included. High-affinity *Ahr*^{b}-*Cyp1a2(+/+)* wild type mice were purchased from The Jackson Laboratory (Bar Harbor, ME) as C57BL/6J mice, which was the background strain for the two knockout lines used: *Ahr*^{b}*Cyp1a2 (−/−)* and poor-affinity *Ahr*^{d}*Cyp1a2(−/−)*. All animals were housed in standard shoebox polysulfone cages with corncob bedding and one 5.1 cm^2 nestlet per week as enrichment. Water and Lab Diet 5015 chow were provided ad libitum.

Animals were kept on a 12 h/12 h light-dark cycle with all experiments conducted during the light cycle. Genotype was confirmed at the end of the behavior experiments. All experiments were approved by the Northern Kentucky University Institutional Animal Care and Use Committee. All husbandry and handling was in accordance with the Eighth Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.

2.2. Breeding

Nulliparous females between 2.5 and 4 months of age were mated on a four-day breeding cycle with males of the same genotype. Females were separated from males the morning when a vaginal plug was found. Litters were culled or cross-fostered to balance litter size at 6 pups per dam, matching pups with dams of the same genotype and treatment. Pups were weaned at postnatal day 25, group housed by genotype, sex and treatment, and behavioral testing began at P60.

2.3. Treatments

We used the same dosing regimen (Table 1) described in our prior studies (Curran et al., 2011a,b), which was based on the toxic equivalency factors for coplanar PCBs 77, 126 and 169. Noncoplanar (PCB 105, 118, 138, 153, 180) congeners were selected based on reports of their high toxicity and prevalence in the human food supply (Van den berg et al., 2006; Costabeber et al., 2006; Langer et al., 2007). Controls were treated with the corn oil vehicle (Kroger, unstripped). Dams were randomly assigned to treatment groups and treated by gavage at a volume of 10 μl/g body weight. at gestational day 10 (GD 10) and postnatal day 5 (PND 5). The PCB doses used in the current study were higher than typical human exposures, but consistent with risk assessment principles requiring higher doses of a toxicant to observe toxicity in the short timeframe of a typical animal study (Doull, 2003) and at the low end of cumulative exposures used in other rodent studies of PCB neurotoxicity (Lein et al., 2007; Lee et al., 2012).

2.4. Chemicals

Polychlorinated biphenyl congeners were ordered from Ultra-Scientific (N. Kingstown, RI). Unless otherwise noted, all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

2.5. Western blot

CYP1A1 induction was confirmed in high-affinity *Ahr*^{b} mice using livers collected from P30 littermates of animals used in behavior. The liver was removed, rinsed in ice-cold phosphate buffered saline, blotted and stored at −80 °C until processing. Approximately 500 mg of tissue per animal was homogenized using a polytron homogenizer and a buffer of 0.25 M sucrose, 10 mM HEPES, 1 mM Na2EDTA, and 1 mM EGTA with 0.1% bovine

Table 1
PCB congeners and concentrations used in dosing mixture.

PCB congener	Planarity	IUPAC #	Dose
2,3,3′,4,4′-Pentachlorobiphenyl	non-coplanar	105	10 mg/kg
2,3′,4,4′,5-Pentachlorobiphenyl	non-coplanar	118	10 mg/kg
2,2′,3,4,4′,5′-Hexachlorobiphenyl	non-coplanar	138	10 mg/kg
2,2′,4,4′,5,5′-Hexachlorobiphenyl	non-coplanar	153	10 mg/kg
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	non-coplanar	180	10 mg/kg
3,3′4,4′-Tetrachlorobiphenyl	coplanar	77	5 mg/kg
3,3′,4,4′,5-Pentachlorobiphenyl	coplanar	126	25 μg/kg
3,3′,4,4′,5,5′-Hexachlorobiphenyl	coplanar	169	250 μg/kg