NeuroToxicology 46 (2015) 125–136

Contents lists available at ScienceDirect

NeuroToxicology

The effect of the aquatic contaminants bisphenol-A and PCB-95 on the zebrafish lateral line

Lauren Hayashi [a,1], Meghal Sheth [a,1], Alexander Young [a], Matthew Kruger [a], Gary A. Wayman [b], Allison B. Coffin [a,c,*]

[a] College of Arts and Sciences, Washington State University, Vancouver, WA, USA
[b] Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, USA
[c] Department of Integrative Physiology and Neuroscience, Washington State University, Vancouver, WA, USA

ARTICLE INFO

Article history:
Received 28 September 2014
Accepted 22 December 2014
Available online 31 December 2014

Keywords:
Bisphenol-A
Polychlorinated biphenyl
Lateral line
Hair cell
Hearing

ABSTRACT

Environmental toxicants such as bisphenol-A (BPA) and polychlorinated biphenyls (PCBs) are prevalent in our water supply, soil, and many food products and can profoundly affect the central nervous system. Both BPA and PCBs can disrupt endocrine signaling, which is important for auditory development and function, but the effect of these toxicants on the auditory periphery is not understood. In this study we investigated the effect of PCB-95 and BPA on lateral line development, function, and regeneration in larval zebrafish. The lateral line is a system of mechanosensory hair cells on the exterior of the fish that are homologous to the hair cells located in the mammalian inner ear. We found that PCB-95 had no effect on lateral line development or hair cell survival. BPA also did not affect lateral line development, but instead had a significant effect on both hair cell survival and regeneration. BPA-induced hair cell loss is both dose- and time-dependent, with concentrations of 1 μM or higher killing lateral line hair cells during a 24 h exposure period. Pharmacologic manipulation experiments suggest that BPA kills hair cells *via* activation of oxidative stress pathways, similar to prior reports of BPA toxicity in other tissues. We also observed that hair cells killed with neomycin, a known ototoxin, failed to regenerate normally when BPA was present, suggesting that BPA in aquatic environments could impede innate regenerative responses in fishes. Collectively, these data demonstrate that BPA can have detrimental effects on sensory systems, both in aquatic life and perhaps in terrestrial organisms, including humans.

1. Introduction

Released into the environment through run-off and effluent, environmental contaminants such as polychlorinated biphenyls (PCBs) and bisphenol-A (BPA) are prevalent in our water supply, soil and food (reviewed in Halling-Sørensen et al., 1998). For example, a survey of over 100 streams across the US identified contaminants ranging from flame retardants to plasticizers, including BPA, in U.S. watersheds (Kolpin et al., 2002). BPA is also known to leach into canned foods through the breakdown of polycarbonate container linings (reviewed in Halling-Sørensen et al., 1998; Geens et al., 2012). Despite their widespread prevalence, we still do not fully understand how environmental contaminants impact human and animal health.

Banned since 1979, PCBs are the most pervasive industrial pollutants found in U.S. waterways (Stahl et al., 2009). In rats, developmental exposure *in vivo* is correlated with changes in synaptic development in the central nervous system and with inner ear dysfunction, while acute exposure promotes dendritic spine formation *in vitro* and can alter synaptic transmission *in vivo* (Gilbert and Liang, 1998; Crofton et al., 2000a,b; Carpenter et al., 2002; Lein et al., 2007; Powers et al., 2009; Yang et al., 2009; Wayman et al., 2012a,b; Lesiak et al., 2014). PCBs can also disrupt thyroid hormone signaling in rats and some toxic effects of PCB exposure may be attributed to altered thyroid hormone responses, particularly during development (Collins et al., 1997; Ness et al., 1993; reviewed in Kodavanti, 2006). Developmental exposure in fishes leads to muscle dysfunction and corresponding swimming

* Corresponding author at: 14204 NE Salmon Creek Ave, Vancouver, WA 98686, USA. Tel.: +1 360 546 9748.
E-mail addresses: lauren.hayashi@wsu.edu (L. Hayashi), meghal.sheth23@gmail.com (M. Sheth), a.young1204@gmail.com (A. Young), matthew.kruger@email.wsu.edu (M. Kruger), waymang@vetmed.wsu.edu (G.A. Wayman), Allison.coffin@wsu.edu (A.B. Coffin).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuro.2014.12.010
0161-813X/

defects, while acute treatment causes changes in liver metabolism and reproductive fitness (Wiseman and Vijayan, 2011; Farwell et al., 2012; Fritsch and Pessah, 2013).

BPA is a ubiquitous environmental toxicant used in polycarbonate plastics and epoxy resins. A known endocrine disruptor, it is linked to a myriad of problems ranging from adiponectin inhibition and diabetes in humans to decreased reproductive viability and motor coordination in fishes (Sohoni et al., 2001; Lahnsteiner et al., 2005; Hugo et al., 2008; Lang et al., 2008; Wang et al., 2013). Acute BPA exposure also appears to impact hippocampal synapses in both adult and developing rodents *in vivo*, leading to learning and memory impairment (Eilam-Stock et al., 2012; Inagaki et al., 2012; Viberg and Lee, 2012; Kuwahara et al., 2013).

Despite demonstrated effects on the central nervous system, little is known about the influence of either PCBs or BPA on the peripheral nervous system, specifically on sensory systems. As sensory reception and processing is critical to organismal survival, environmental contaminant-induced sensory dysfunction could have profound consequences. In this study, we asked if either PCB-95 or BPA are toxic to sensory hair cells in the zebrafish lateral line.

Mechanosensory hair cells in the vertebrate inner ear transduce vibrational stimuli received by the auditory periphery into action potentials that are transmitted to the central nervous system (reviewed in Hudspeth, 2005). The lateral line is an external sensory system containing hair cells that are structurally and functionally similar to those in the mammalian ear and respond similarly to many toxins (Harris et al., 2003; Ou et al., 2007; reviewed in Coffin et al., 2010, 2014). In humans, hair cell damage results in permanent hearing loss; contaminants that damage hair cells may therefore have serious consequences for human auditory function. Unlike mammals, fish naturally regenerate hair cells (Lombarte et al., 1993; Ma et al., 2008; reviewed in Brignull et al., 2009). Therefore, hair cell damage does not have the same repercussions in fish, unless regenerative capacity is also affected, as seen after copper exposure (Hernández et al., 2006; Linbo et al., 2006). In this study we show that BPA is significantly toxic to mature hair cells in the lateral line and that exposure reduces hair cell regeneration in an innately regenerative system. PCB-95 had no detectable effect on hair cell development, survival, or regeneration. Our findings fit within the broader context of recent evidence demonstrating the prevalence of environmental contaminants and the dangers these compounds pose to the nervous system (Kolpin et al., 2002; Wayman et al., 2012a,b; Elsworth et al., 2013).

2. Methods

2.1. Animals

We used 5–6 days post-fertilization (dpf) fish for all acute toxicity experiments because their hair cells exhibit mature sensitivity to known hair cell toxins (ototoxins) (Murakami et al., 2003; Santos et al., 2006). Developmental studies used animals beginning at 1 dpf, while regeneration studies were initiated in 5–6 dpf animals and concluded by 7 dpf. *AB wildtype or Brn3C:mGFP transgenic fish were used for all experiments. Hair cells of Brn3c:mGFP animals express membrane-bound GFP in all hair cells and the transgene is turned on early in hair cell development (Xiao et al., 2005), making this fish line ideal for developmental and regeneration studies. All procedures were approved by the Institutional Animal Care and Use Committee at Washington State University.

2.2. Environmental toxicant exposure

We performed three distinct series of experiments using BPA or PCB-95 to determine if either compound was toxic in the context of (1) lateral line development, (2) acute exposure of mature hair cells, or (3) regenerating hair cells. BPA concentrations were based on reported effective values in prior zebrafish studies (Lam et al., 2011; Wang et al., 2013) and on our own preliminary data that determined the range that yielded minimal mortality. We accomplished this by examining concentrations spanning several orders of magnitude across multiple time points. As PCB-95 has not, to our knowledge, been previously used in zebrafish studies, we selected our PCB-95 concentrations based on published literature for other fishes and *in vitro* studies in mammalian neurons (Wayman et al., 2012a; Fritsch and Pessah, 2013). Again, we corroborated these concentration ranges with empirical testing of several orders of magnitude across multiple time points. As both BPA and PCB-95 were dissolved in DMSO, control animals received the same volume of DMSO only for the same treatment duration ($\leq$0.2%). Unless specified below, all animals were assessed immediately following treatment.

All experiments were performed in defined E2 embryo medium (EM) containing 994 μM $MgSO_4$, 150 μM KH_2PO_4, 42 μM Na_2HPO_4, 986 μM $CaCl_2$, 503 μM KCl, 14.9 mM NaCl, and 714 μM $NaHCO_3$, with the pH adjusted to 7.2 (Westerfield, 2000). Fish were divided into treatment groups of 8–12 fish per well in a 6-well plate, with each treatment group housed in a custom transfer device constructed of PVC pipe and mesh netting that fit inside the well. Experiments were conducted in a VWR benchtop incubator set at 28 °C.

2.2.1. Development

1 dpf Brn3c:mGFP transgenic zebrafish were treated for 48 or 96 h with BPA (2, 10, 20, or 40 μM) or PCB-95 (0.1, 0.25, 0.50, or 2 μM). Controls were treated with DMSO (0–0.2%, matched to the volume of toxicant in DMSO), the solvent used to dissolve PCB or BPA. Counts of GFP + hair cells were performed on fish fixed in 4% paraformaldehyde and rinsed in PBS. Hair cells of both anterior (IO1, IO2, IO3) and posterior (P1, P2) neuromasts were counted in order to assess neuromasts that develop at different time points (Raible and Kruse, 2000; Van Trump and McHenry, 2008). Hair cell counts were summed to arrive at one value per fish.

2.2.2. Acute toxicity

5–6 dpf *AB zebrafish were treated for 4–24 h with BPA (0.1–80 μM), 24 h with PCB-95 (0.5–20 μM), or with DMSO only for control animals. Fish were rinsed in EM, anesthetized with MS-222, and hair cells were assessed using DASPEI as described in Section 2.3.1. A subset of BPA-treated fish were euthanized and hair cells were labeled with anti-parvalbumin and quantified as described in Section 2.3.2.

2.2.3. Regeneration

To assess how an environmental contaminant affects hair cell regeneration, we used neomycin to kill hair cells in 5 dpf zebrafish (Harris et al., 2003; Ma et al., 2008), then allowed the fish to recover for 24 or 48 h in either PCB-95 or BPA. Fish were treated with 300 μM neomycin for 30 min, then rinsed four times in embryo medium and incubated in fresh EM for 1 hr to allow for complete loss of hair cells (Coffin et al., 2009; Owens et al., 2009). Fish were then incubated in PCB-95 (0.25–2 μM) or BPA (1–20 μM) and assessed with DASPEI labeling in live fish or by counts of anti-parvalbumin-labeled hair cells.

Regeneration proceeds more slowly after hair cell death due to toxins such as copper or cisplatin (Hernández et al., 2006; Linbo et al., 2006; Mackenzie and Raible, 2012). In order to look for a similar phenomenon following BPA damage, we next examined hair cell regeneration after BPA-induced hair cell death. 5 dpf zebrafish were exposed to 1–20 μM BPA for 24 h, then rinsed four times in embryo medium and allowed to recover for 24 or 48 h. Hair cell regeneration was assessed as described above.