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Aroclor 1254 and BDE-47 inhibit dopaminergic function manifesting as changes in locomotion behaviors in zebrafish embryos

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HIGHLIGHTS

- Aroclor 1254 and BDE-47 caused hyperactivities in zebrafish embryos.
- Inhibition of tyrosine hydroxylase and VMAT2 also induced hyperactivities.
- Hyperactivities by these treatments were inhibited by precursors of dopamine synthesis.
- Organohalogens reduced dopamine contents and increased the DOPAC/dopamine ratio.

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ABSTRACT

Contamination with polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the environment is a major concern due to their persistent bioaccumulative toxicity that can disturb neurobehavioral functions including movements. Recently, it was reported that some PBDE including BDE-47 stimulates locomotor activities of zebrafish embryos by unknown mechanism. In this study, motor movements of the zebrafish embryo were used as a model system to evaluate the neuronal toxicity of a non-coplanar PCB-dominant mixture (Aroclor 1254) and BDE-47. Both organohalogens increased tail shaking and rotation of embryos in a concentration-dependent manner. Chemical inhibition and gene knock-down of tyrosine hydroxylase and vesicular monoamine transporter 2 (VMAT2) also induced hyperactivities. Hyperactivities induced by these treatments were all inhibited by supplementation of L-tyrosine and L-dopa, precursors of dopamine synthesis. Both organohalogens reduced dopamine contents and increased the 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine ratio in whole embryos. The results suggest that functional inhibition of dopaminergic neurons is involved in hyperactivities of zebrafish embryos caused by Aroclor 1254 and BDE-47.

1. Introduction

Polychlorinated biphenyls (PCBs) had been widely used in various products including flame retardants. However, due to extensive accumulation in human and animal tissues through the food web, the use of PCBs was banned in the 1970s and early 1980s in many countries. Tragic incidences of massive food poisoning by PCBs such as the case in Yusho and Yuchen were also a trigger of the ban (Mitoma et al., 2015). Even after the ban, however, some studies showed that concentrations of PCBs in some marine animals remained high (Jepson et al., 2016). Extensive PCB pollution was reported not only in developing countries but also in developed countries in accidents (Sloan et al., 1983; Lavandier et al., 2016).

Polybrominated diphenyl ethers (PBDEs) are also halogenated compounds that have emerged as major environmental pollutants instead of PCBs (Linares et al., 2015). PBDEs, which are used in many products as flame retardants, coatings and textiles, are chemically

Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter 2.

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similar to PCBs and resist degradation in the environment. Some of the less-brominated PBDEs such as tetra- or penta brominated PBDEs are highly lipophilic and can accumulate in the bodies of animals and humans. Penta- and octaBDE have also been banned in the European Union and some other countries.

Exposure to PCBs (especially non-coplanar PCBs: ncPCBs) and PBDEs has been shown to modify certain behavioral traits in various animals and humans (Roegge and Schantz, 2006; Winneke, 2011). For example, modifications in the schooling and swimming behavior of adult fish as a result of acute exposure to ncPCBs and PBDEs have been reported (Nakayama et al., 2005; Schmidt et al., 2005). In humans, the highest levels of exposure to both types of halogenated compounds occurs during the first years of life in breast-fed infants (Guvenius et al., 2003), which coincides with the period of rapid brain growth and maturation. This period is also called the postnatal transition period, which is known to be a period in which rodents are vulnerable to organohalogen exposure (Eriksson, 1997). Exposure of perinatal mice to a low concentration of ncPCBs caused hyperactivity and altered spontaneous behavior with aging (Eriksson, 1997; Eriksson et al., 2001). Hence, the potential adverse effect of both organohalogens on neurodevelopment is still a major concern.

However, the mechanisms of the neurobehavioral effects of both organohalogens remain largely unclear. It has been reported that ncPCBs disrupt thyroid homeostasis, leading to neurotoxic effects (Brouwer et al., 1998; Dingemans et al., 2016). Others have proposed direct effects of organohalogens on the developing brain, including oxidative stress (Bellés et al., 2010) and interference with calcium signaling, including agonistic effects of ncPCB on ryanodine receptor (Pessah et al., 2006). Studies on ncPCB neurotoxicity have indicated that the dopaminergic system is a potential target because of accumulating evidence of the effects of ncPCBs on the dopaminergic system in rodents. For instance, ncPCBs inhibit the activity of dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2/SLC18A2), which transports dopamine into vesicles in the presynaptic terminal (Wigestrand et al., 2013). Inhibition of VMAT2 by ncPCBs seemed to play a greater role than the inhibition of DAT in decreasing tissue dopamine levels (Bemis and Seegal, 2004).

The zebrafish (*Danio rerio*) has emerged as an important model organism for the study of vertebrate biology and human medicine, including human neuropsychological diseases (Hill et al., 2005; Kalueff et al., 2014). PBDEs (BDE 47 and 28) increased spontaneous movement (tail shaking and coiling or rotation) of zebrafish embryos at 24 h post fertilization (hpf) in a concentration-dependent manner (Usenko et al., 2011). Hyper-spontaneous movement of larvae was also observed at 96 and 120 hpf as higher swimming rates but was not observed at 168 hpf (Usenko et al., 2011). While it is well known that locomotor activities of the larvae were increased by switching to dark field from light condition (light-dark challenges), PBDE reduced locomotor activity of 120 hpf larvae stimulated by light-dark challenges (Wang et al., 2015). At the same stage, PBDE reduced the content of whole-body dopamine and its metabolite dihydroxyphenylacetic acid (DOPAC) as well as the expression of tyrosine hydroxylase in the brain of zebrafish larvae (Wang et al., 2015). Importantly, swimming and turning behaviors were also affected in adult zebrafish and their offspring by long-term dietary exposure to ncPCBs (Péan et al., 2013). These observations indicate the possible involvement of the dopaminergic system in the effects of PBDE and PCBs on neurobehavior in developing zebrafish.

In this study, we investigated the effects of ncPCB-dominant mixture Aroclor 1254, one of the most commonly used PCB mixtures on early locomotor behavior of zebrafish embryo. As Aroclor 1254 caused hyperactivity in 1 dpf zebrafish embryos exactly like PBDE (Usenko et al., 2011), we studied the mechanism underlying this phenomenon induced by Aroclor 1254 together with BDE-47. As the earliest dopaminergic neurons of zebrafish embryos are detected between 18 and 19 hpf as a cluster of cells in the ventral diencephalon (Holzschuh et al., 2001), we focused on the relationship between locomotion behavior and dopaminergic pathway. Simple locomotion behavior in zebrafish embryos is a useful model that could reflect dopaminergic function. An understanding of zebrafish behavioral phenotypes will provide important insights into neural pathways, the genetic basis of normal and pathological brain functions and their development (Kalueff et al., 2014).

2. Materials and methods

2.1. Chemicals

Polychlorinated biphenyl (PCB: Aroclor 1254) and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) were obtained from AccuStandard (New Haven, CT). α-Methyl-p-tyrosine (metyrosine), L-dopa and reserpine were purchased from Sigma (St. Louis, MO). L-Tyrosine hydrochloride was obtained from Kanto Chemical (Tokyo, Japan). L-Thyroxine was obtained from Tokyo Kasei (Tokyo, Japan). All other chemicals were commercially available products of special reagent grade.

2.2. Zebrafish and chemical treatment

Adult zebrafish (long-fin) were obtained from local pet stores. Fertilized eggs were obtained by natural mating of adult zebrafish in our laboratory according to the Zebrafish Book (Westerfield, 1993). Adult fish were maintained at 28.5 °C with a 14 h light and 10 h dark lighting schedule. Eggs were collected within 1 h of spawning, rinsed, and placed into a clean petri dish. Embryos were manually dechorionized at 19 hpf. At 22 hpf, embryos were exposed to either a vehicle, dimethyl sulfoxide (DMSO, 0.1%) or an apparent concentration of waterborne organohalogens (Aroclor 1254 and BDE-47) (1.0 parts per million (ppm) except the experiments for concentration-dependency (0.04, 0.2, 1.0 and 5.0 ppm) dissolved in 0.1% DMSO in 3 ml of Zebrafish Ringer solution (38.7 mM NaCl, 1.0 mM KCl, 1.7 mM HEPES-NaOH pH 7.2, 2.4 mM $CaCl_2$) in 3.5 cm petri dishes (Asahi Techno Glass, Yoshida, Japan) (10 embryos/dish) (Teraoka et al., 2009). In some experiments, embryos were exposed to metyrosine (2.9 mM), reserpine (4.1 μM) and L-thyroxine (10, 30 and 100 nM) from 22 hpf. Adequate concentrations of these chemicals were determined by preliminary experiments. L-Dopa was also included from 19 hpf. Embryos were incubated in a dark condition throughout the experiments.

2.3. Gene knock-down with morpholino antisense oligos

Morpholino antisense oligonucleotides (MOs) against translation of tyrosine hydroxylase (TH) (TH-MO, 5′-GCGGAACAGATGGAACTATATTGTG) and against splicing of vesicular monoamine transporter 2 (VMAT2) (Wen et al., 2008) (VMAT2-MO, 5′-GTGAACAACACAGCACTCACCGACC) were synthesized by Gene Tools (Philomath, OR). Standard control morpholino (STD-KD), which was recommended by Gene Tools as a placebo control, was used as a universal control for injection in all morpholino studies unless otherwise indicated. Each MO was injected into the yolk of embryos at one cell to four cell stages with a fine glass needle connected to an automatic injector (IM-300: Narishige, Japan). Approximately 2 nL of 50 μM MOs in Ca^{2+}-free Zebrafish Ringer solution was injected.