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Developmental exposure to an organophosphate flame retardant alters later behavioral responses to dopamine antagonism in zebrafish larvae

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ABSTRACT

Human exposure to organophosphate flame retardants (OPFRs) is widespread, including pregnant women and young children with whom developmental neurotoxic risk is a concern. Given similarities of OPFRs to organophosphate (OP) pesticides, research into the possible neurotoxic impacts of developmental OPFR exposure has been growing. Building upon research implicating exposure to OP pesticides in dopaminergic (DA) dysfunction, we exposed developing zebrafish to the OPFR tris(1,3-dichloroisopropyl) phosphate (TDCIPP), during the first 5 days following fertilization. On day 6, larvae were challenged with acute administration of dopamine D_1 and D_2 receptor antagonists and then tested in a light-dark locomotor assay. We found that both developmental TDCIPP exposure and acute dopamine D_1 and D_2 antagonism decreased locomotor activity separately. The OPFR and DA effects were not additive; rather, TDCIPP blunted further D_1 and D_2 antagonist-induced decreases in activity. Our results suggest that TDCIPP exposure may be disrupting dopamine signaling. These findings support further research on the effects of OPFR exposure on the normal neurodevelopment of DA systems, whether these results might persist into adulthood, and whether they interact with OPFR effects on other neurotransmitter systems in producing the developmental neurobehavioral toxicity.

1. Introduction

Recently, growing concern regarding the safety of common flame retardants has led to phase outs of the older polybrominated flame retardants and replacement with several new categories of compounds. Among these replacements are organophosphate flame retardants, or OPFRs. It has become apparent, since then, that exposure to OPFRs is widespread. The common OPFR tris(1,3-dichloroisopropyl) phosphate (TDCIPP) has been found in over 96% of samples of dust and furniture foam, with levels as high as 1.8 mg/g (Stapleton et al., 2009; Meeker and Stapleton, 2010; Canbaz et al., 2016), in elementary schools at levels as high as 0.27 mg/g (Mizouchi et al., 2015), and in daycare centers at levels up to 0.33 mg/g (Wu et al., 2016). TDCIPP has been found in foam inside of infant products (Stapleton et al., 2011) and in hand wipe samples of children (Hoffman et al., 2015; Stapleton et al., 2014). Correspondingly, metabolites of TDCIPP and other OPFRs can be found in the urine of adults (Carignan et al., 2013; Meeker et al., 2013) and pregnant women and paired mothers and children (Butt et al., 2014; Hoffman et al., 2015), as well as in human hair and fingernails (Liu et al., 2016) and placentas (Ding et al., 2016).

It is clear from these studies that pregnant mothers, infants, and children all likely receive significant exposure to organophosphate pesticides. An emerging concern, then, is whether these organophosphate flame retardants pose a developmental neurotoxic risk on par with other organophosphate compounds, such as organophosphate pesticides. A variety of epidemiological studies have linked these pesticides to abnormal neurobehavioral development in human populations. Prenatal exposures have been linked to impairments in the development of normal reflexes (Engel et al., 2007) and social functions (Furlong et al., 2014), and in lower IQ scores (Rauh et al., 2011; Bouchard et al., 2011). Children exposed to OP pesticides prenatally also score lower on other indices of normal neurobehavioral development (Rauh et al., 2006), including indicators for attention deficit disorders (Marks et al., 2010). Studies in rodent models have replicated this epidemiological evidence. Rats exposed to chlorpyrifos, one of the most widely used OP pesticides, during gestation later display abnormal exploratory behavior and neuromuscular development (Chanda and Pope, 1996), behavioral effects generally replicated if the exposure is moved to the early postnatal period (Dam et al., 2000). Effects arising from exposures during multiple exposure periods, including exposures spanning gestation and early postnatal periods, have been shown to persist into adulthood in rodents, and expand into multiple behavioral

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domains including measures of cognitive performance and memory (Levin et al., 2001; Levin et al., 2002) and anxiety and reward-seeking (Aldridge et al., 2005a; Ricceri et al., 2006; Braquenier et al., 2010; Carr et al., 2015).

Recently, a growing number of studies investigating the behavioral effects of developmental OP pesticide exposure have been conducted in the zebrafish model. Developmental exposure to the OP pesticide chlorpyrifos has been shown to cause long-term effects on novel tank exploratory behavior (Sledge et al., 2011), spatial learning (Levin et al., 2003; Sledge et al., 2011) and the response to a startling stimulus (Eddins et al., 2010) in adult zebrafish after embryonic exposure. Behavioral effects of OP exposure have also been observed in larval motility shortly following the end of a developmental exposure (Levin et al., 2004; Richendrfer et al., 2012; Dishaw et al., 2014). As seen in rodents, the magnitude of some behavioral effects is sensitive to the developmental window during which the zebrafish are exposed (Sledge et al., 2011).

Zebrafish studies examining the developmental neurotoxicity of OPFRs have found that exposures to various OPFRS produce morphological abnormalities and behavioral abnormalities in a similar range of concentrations in which chlorpyrifos has effects on the same endpoints (Dishaw et al., 2014; Noyes et al., 2015; Oliveri et al., 2015; Sun et al., 2016). Additionally, exposing adult fish to TDCIPP produces behavioral abnormalities in the larval offspring, implicating OPFRs in trans-generations developmental neurotoxicity (Wang et al., 2015a). It is worth noting that several studies in zebrafish have shown that multiple OPFRs have no acetylcholinesterase inhibitory activity (Wang et al., 2015b; Sun et al., 2016), suggesting that if these compounds do share that neurotoxic mechanism with organophosphate pesticides, it is likely other mechanisms are involved. One investigation into neurochemical effects found no changes in levels of dopamine in larval zebrafish following a short developmental exposure to TDCIPP, but following a chronic exposure paradigm from the embryonic stage through adulthood did lead to reduced dopamine levels in female adult fish (Wang et al., 2015b). Another study, in the PC12 cell model, found OPFRs to be at least as potent as chlorpyrifos in promoting preferential neurodifferentiation into a dopaminergic phenotype (Dishaw et al., 2011).

The possible interaction of OPFRs such as TDCIPP with the development and functioning of dopaminergic systems mirrors effects seen following developmental exposure to organophosphate pesticides. Several studies in rodents have shown that developmental exposures to chlorpyrifos can alter dopamine content in a brain region- and age-specific fashion (Slotkin et al., 2002; Aldridge et al., 2005b; Chen et al., 2011). Similarly, several studies have identified increased synaptic release of dopamine following developmental organophosphate exposure, measured as an increase in the ratio of the dopamine metabolite DOPAC to dopamine itself, as dopamine is only metabolized into DOPAC following release (Dam et al., 1999; Slotkin et al., 2002; Aldridge et al., 2005b; Slotkin and Seidler, 2007b; Eells and Brown, 2009; Slotkin et al., 2009). The adenylyl cyclase signaling cascade, which is involved in signaling downstream from dopamine receptors, has also shown to be altered following developmental organophosphate exposure (Song et al., 1997; Aldridge et al., 2003; Meyer et al., 2003; Aldridge et al., 2004; Meyer et al., 2004; Adigun et al., 2010), linking developmental exposures to both pre- and postsynaptic elements of dopamine neurotransmission.

Given the evidence that developmental exposure to OP pesticides cause short and long-term disruption of dopamine systems, we hypothesized that the OP flame retardant TDCIPP would also have neurobehavioral effects mediated via dopamine systems. Dopamine antagonists were used as probes because we hypothesized that the TDCIPP effects on dopamine systems would be subtle and would be best detected with increased vulnerability to the behavioral effects of dopamine antagonist challenge.

. Zebrafish larvae exposed to TDCIPP for the first 5 days post-fertilization, at concentration shown previously to generate hypoactivity (Dishaw et al., 2014), were then challenged with either the D_1-receptor antagonist SCH-23390 or the D_2-receptor antagonist haloperidol immediately preceding a light-dark locomotor assay.

2. Methods

2.1. Animal care and husbandry

Zebrafish (AB* strain) were bred from a colony originating with progenitors obtained from the Zebrafish International Resource Center (ZIRC, Eugene, OR, USA). Breeding tanks of $N = 12–15$ were maintained with a male to female ratio of approximately 2:1. Eggs were collected via in-tank inserts approximately 1–2 h after the lights-on phase of a 14:10 h light:dark cycle. Eggs from approximately six such tanks were combined and rinsed with 10,000× diluted solution of bleach for 1 min, followed by three likewise rinses in fresh aquarium water. Eggs were inspected under a dissection microscope and unfertilized or otherwise abnormal eggs were discarded. Approximately five-h post fertilization, eggs were randomly distributed into glass Petri dishes corresponding to differing exposures, and placed in an incubator held at 28 °C and illuminated with an identical 14:10 light cycle (lights on at 8:00 AM) until 6 days post fertilization. All behavioral testing was run between 1:00 PM and 5:00 PM.

2.2. Chemical exposures

At 5 h post fertilization, zebrafish eggs were placed in separate glass Petri dishes in 40-ml of solutions of tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) (Sigma-Aldrich, St. Louis, MO, USA, purity > 98%) at either 3 or 6 μM. DMSO 0.03% in aquarium water served as a vehicle control. These solutions were renewed every 24 h, through 5 days post-fertilization. Hatched larvae were then placed into fresh aquarium water for 24 h, at which point they were examined under a dissecting microscope. Larvae exhibiting arrested development or malformations such as spinal deformity were discarded. The larval morphology assessment included spinal deformity, reduction in size for age and viability. The degree of deformity was not quantitated. The concentration range of TDCIPP tested was below the threshold for increases in lethality and dysmorphogenesis.

The larvae then underwent another solution change into 40-mL solutions of SCH-23390 (Sigma-Aldrich, St. Louis, MO, USA, purity ≥98%) or haloperidol (Abbott Labs, Abbott Park, Il, USA, purity > 98%) at 5.0 μM, or into aquarium water as a control for 2 h prior to behavioral testing. From here, larvae in these dosing solutions were divided individually into four glass 96-well plates (two for haloperidol and two for SCH 23390) with $n = 29–33$ per combination of TDCIPP exposure and antagonist dosing (14–16 per plate for each of the six exposure conditions in a 2 × 3 design: vehicle control, TDCIPP3 alone, TDCIPP6 alone × antagonist alone with three replicates, TDCIPP3 + antagonist and TDCIPP6 + antagonist). These 96-well plates were returned to the incubator for 2 h until the larval motility assay. The dopamine concentrations used were determined by pilot studies to not in themselves cause increased dysmorphogenesis or lethality.

2.3. Larval motility assay

After 6-day-old larvae were inspected, they were placed into 96-well plates with glass well inserts each with 0.5-ml of aquarium water (n = 29–33 per exposure condition, over two exposure replicates). Exposure conditions were all represented within each plate and across multiple plates. Plates were then returned to the incubator for 2 h before being placed into a DanioVision™ lightbox running EthoVision XT® tracking software (Noldus, Wageningen, The Netherlands). Locomotor activity was tracked during a paradigm in which an initial 10-min acclimation period in the dark (0% illumination) was followed by 2 cycles