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Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals

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ABSTRACT

Humans are increasingly and consistently exposed to a variety of endocrine disrupting chemicals (EDCs), chemicals that have been linked to neurobehavioral disorders such as ADHD and autism. Many of such EDCs have been shown to adversely influence brain mesocorticolimbic systems raising the potential for cumulative toxicity. As such, understanding the effects of developmental exposure to mixtures of EDCs is critical to public health protection. Consequently, this study compared the effects of a mixture of four EDCs to their effects alone to examine potential for enhanced toxicity, using behavioral domains and paradigms known to be mediated by mesocorticolimbic circuits (fixed interval (FI) schedule controlled behavior, novel object recognition memory and locomotor activity) in offspring of pregnant mice that had been exposed to vehicle or relatively low doses of four EDCs, atrazine (ATR – 10 mg/kg), perfluorooctanoic acid (PFOA – 0.1 mg/kg), bisphenol-A (BPA – 50 μg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD – 0.25 μg/kg) alone or combined in a mixture (MIX), from gestational day 7 until weaning. EDC-treated males maintained significantly higher horizontal activity levels across three testing sessions, indicative of delayed habituation, whereas no effects were found in females. Statistically significant effects of MIX were seen in males, but not females, in the form of increased FI response rates, in contrast to reductions in response rate with ATR, BPA and TCDD, and reduced short term memory in the novel object recognition paradigm. MIX also reversed the typically lower neophobia levels of males compared to females. With respect to individual EDCs, TCDD produced notable increases in FI response rates in females, and PFOA significantly increased ambulatory locomotor activity in males. Collectively, these findings show the potential for enhanced behavioral effects of EDC mixtures in males and underscore the need for animal studies to fully investigate mixtures, including chemicals that converge on common physiological substrates to examine potential mechanisms of toxicity with full dose effect curves to assist in interpretations of relevant mechanisms.

1. Introduction

Multiple classes of chemicals, e.g., fertilizers and herbicides, plastics, organic pollutants, metals, flame retardants and heat stabilizers, have been shown to have endocrine disrupting characteristics. Given the chemical heterogeneity of EDCs, a broad range of physiological targets have been identified. EDCs may interfere with the production, secretion, transportation, metabolism, binding action and/or excretion of natural hormones (Diamanti-Kandarakis et al., 2009). There is mounting evidence that developmental exposures to EDCs impact neurochemical pathways leading to lifelong disease susceptibility and behavioral deficits into adulthood (de Cock et al., 2012; Schantz and Widholm, 2001; Schug et al., 2011). Developmental EDC exposures can cause physiological reprogramming of hormonal homeostasis with impacts on peripheral and neurological hormone relationships, e.g., glucocorticoids and glutamate, estrogen and dopamine function (Patisaul and Adewale, 2009; Vandenberg et al., 2012). EDCs have been implicated in the etiopathogenesis of ADHD, autism, and other neurodevelopmental and behavioral disorders

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(de Cock et al., 2012; Schug et al., 2011); thus, understanding the consequences of developmental exposure to low dose EDC mixtures for neurological disease etiology is vital (Colborn, 2004b; de Cock et al., 2012).

Studies in animal models indicate that monoaminergic neural pathways are specifically altered as a result of developmental exposure to EDCs, particularly mesocorticolimbic dopaminergic systems (Palanza et al., 2008). For instance, in vivo and in vitro studies indicate atrazine (ATR) causes a reduction in striatal dopamine (Coban and Filipov, 2007; Hossain and Filipov, 2008). In rats, prenatal exposure to ATR decreased striatal dopamine and decreased locomotor activity (Bardullas et al., 2011; Lin et al., 2013; Rodríguez et al., 2012). Prenatal exposures to perfluorooctanoic acids (PFOAs), as fire retardants, increased home-cage activity in male mice, and enhanced astrogliosis and pro-inflammatory cytokines in the hippocampus and cortex of rats (Onishchenko et al., 2011; Zeng et al., 2011). Mice exposed to low dose TCDD in the perinatal period exhibited hypo-activation of the prefrontal cortex, increased brain monoamines and increased social behavior abnormalities (Ahmed and Perinatal, 2011; Endo et al., 2012). In mice, prenatal bisphenol A (BPA) exposure altered the development of central dopaminergic systems and resulted in hyperactivity and increased reward-seeking behavior (Mizuo et al., 2004a,b; Narita et al., 2006; Suzuki et al., 2003). Also, prenatal and developmental exposure to BPA has been shown to elicit multiple sex-specific behavioral deficits including increase impulsivity, neophobia and exploratory behavior, altered maternal behavior and adult social behavior (Adriani et al., 2003; Gioiosa et al., 2013; Palanza et al., 2002; Patisaul et al., 2012; Spulber et al., 2014; Wolstenholme et al., 2011).

This is notable given that mesocorticolimbic dopamine systems mediate multiple behavioral domains particularly related to cognitive and executive functions as well as memory consolidation, temporal discrimination, exploratory and food-reinforced reward behaviors (Cory-Slechta et al., 1997; Rossato et al., 2013; Sy et al., 2010). Further, there is growing appreciation that maternal and early life exposure to environmental toxicants can potentially have a profound lifelong impact on the central nervous system (CNS). Evidence is accumulating that these alterations occur at low doses, as many EDCs show non-monotonic dose–response relationships (Vandenberg et al., 2012). However, most of this information has been obtained on a chemical-by-chemical basis. The neuroendocrine and behavioral deficits associated with EDC mixtures, which is more representative of human exposures, have not been evaluated (Diamanti-Kandarakis et al., 2009).

There has been significant controversy as to whether low levels of EDCs can act together, particularly when present at lower than threshold concentrations, particularly if they have different mechanisms of action. However, as we have previously pointed out, multiple insults occurring concurrently at multiple sites within the e.g., dopamine system, may constrict the range and flexibility of compensatory mechanisms, thereby compromising integrity and viability of the system, and ultimately be more damaging than multiple insults at the same molecular target sites (Cory-Slechta, 2005). Indeed, recent studies of low dose mixtures of chemicals reducing androgens via different mechanisms resulted in additive male reproductive dysfunction effects (Howdeshell et al., 2008; Rider et al., 2009).

Based on this presumption, this study sought to determine whether the impact of multiple EDCs, all known to impact brain mesocorticolimbic systems but by different mechanisms, would yield enhanced effects in combination, as manifest in behaviors known to be mediated by these dopamine/glutamate circuits and correspondingly whether observed effects would, as expected, differ by sex. To this end, we assessed performance under the fixed-interval schedule of reinforcement, object exploration, novel object recognition, and spontaneous locomotor activity, all behaviors in which mesocorticolimbic system function is important (Cory-Slechta et al., 1997; Rossato et al., 2013; Sequeira-Cordero et al., 2013), in offspring exposed developmentally to four EDCs alone and combined in a mixture. As a first such study pursuing this hypothesis, it did not include full concentration–effect curves for all EDCs but was intended to provide critical information that could be used to more specifically formulate subsequent studies.

2. Methods

2.1. Developmental exposure

C57BL/6 mice (age 9 weeks) were obtained from the Jackson Laboratory (Bar Harbor, ME). Nulliparous females were housed with males, and checked daily for presence of a vaginal plug. The day a vaginal plug was found was designated as gestational day (GD) 0. Pregnant mice were then individually housed for the remainder of the study. Pregnant mice were exposed orally to either the single EDC dose or the combination of all four doses: atrazine (ATR – 10 mg/kg), perfluorooctanoic acid (PFOA – 0.1 mg/kg), bisphenol-A (BPA – 50 μg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD – 0.25 μg/kg) and their mixture (MIX), from GD 7, a time point chosen because it represents the period shortly after embryonic implantation, until weaning (Wang and Dey, 2006). Vehicle (VEH) control dams were gavaged with peanut oil containing an equivalent concentration of anisole and given control treats daily. These compounds have all been shown to influence mesocorticolimbic and neuroendocrine systems (see above), and doses were defined as relatively low doses either based on current human reference doses or were below levels typically shown to cause effects in animal studies (Fenton et al., 2009; Rowe et al., 2008; Vom Saal and Hughes, 2005; Vorderstrasse et al., 2006). ATR and BPA (Sigma–Aldrich, St. Louis, MO) were dissolved in peanut oil and administered to pregnant mice daily via puffed wheat cereal. PFOA (Sigma–Aldrich, St. Louis, MO) was dissolved in water and administered daily via puffed wheat cereal. TCDD (Cambridge Isotopes, Cambridge, MA) was dissolved in anisole and diluted in peanut oil. Female mice were administered TCDD via oral gavage on GD 7 and 14, and postnatal day (PND) 2, as TCDD has a relatively longer half-life (11 days), this schedule facilitates an even dosing throughout the pregnancy (Gasiewicz et al., 1983). MIX exposed mice were given puffed wheat cereal containing the same dose of each EDC and gavaged on the same schedule as each singly treated group. Random observations of dams indicated total consumption of the treated puffed wheat cereal used for dosing.

Offspring were weaned at PND 21. In order to minimize litter specific effects, no more than two mice per sex per dam were used. All mice were pair-housed in microisolator cages after weaning in a specific pathogen-free facility under a 12 h light–dark cycle maintained at $22 \pm 2\,^{\circ}C$ at the University of Rochester Medical Center. All animal treatments were conducted with approval of the Institutional Animal Care and Use Committees at the University of Rochester.

2.2. Maternal health and weight

Pregnancies were monitored daily to evaluate whether there were differences in time to parturition, litter size, or sex ratio of all the offspring born to each dam. As adults, all offspring were weighed to ensure that there were no significant body weight differences before behavioral testing began. In preparation for behavioral testing, mice were food-restricted to 85% free feed