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Effects of perinatal bisphenol A exposure on the volume of sexually-dimorphic nuclei of juvenile rats: A CLARITY-BPA consortium study

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ABSTRACT

Bisphenol A (BPA) is a high volume endocrine disrupting chemical found in a wide variety of products including plastics and epoxy resins. Human exposure is nearly ubiquitous, and higher in children than adults. Because BPA has been reported to interfere with sex steroid hormone signaling, there is concern that developmental exposure, even at levels below the current FDA No Observed Adverse Effect Level (NOAEL) of 5 mg/kg body weight (bw)/day, can disrupt brain sexual differentiation. The current studies were conducted as part of the CLARITY-BPA (Consortium Linking Academic and Regulatory Insights on BPA Toxicity) program and tested the hypothesis that perinatal BPA exposure would induce morphological changes in hormone sensitive, sexually dimorphic brain regions. Sprague-Dawley rats were randomly assigned to 5 groups: BPA (2.5, 25, or 2500 μg/kg bw/day), a reference estrogen (0.5 μg ethinylestradiol (EE_2)/kg bw/day), or vehicle. Exposure occurred by gavage to the dam from gestational day 6 until parturition, and then to the offspring from birth through weaning. Unbiased stereology was used to quantify the volume of the sexually dimorphic nucleus (SDN), the anteroventral periventricular nucleus (AVPV), the posterodorsal portion of the medial amygdala (MePD), and the locus coeruleus (LC) at postnatal day 28. No appreciable effects of BPA were observed on the volume of the SDN or LC. However, AVPV volume was enlarged in both sexes, even at levels below the FDA NOAEL. Collectively, these data suggest the developing brain is vulnerable to endocrine disruption by BPA at exposure levels below previous estimates by regulatory agencies.

1. Introduction

Perhaps one of the best-known and most intensely studied endocrine disrupting chemicals (EDCs) is bisphenol A (BPA). A high production volume chemical, BPA is used as a monomer in the production of polyvinyl chloride and polycarbonate plastics, epoxy resins, and a multitude of other commercial and consumer products (FAO/WHO, 2011). Human exposure to BPA is virtually unavoidable and occurs primarily from contaminated food and beverages. In industrialized countries, well over 90% of individuals are estimated to have detectable amounts of BPA in their bodies, albeit in small amounts (serum levels are typically in the range of 4 ng/ml or lower) (Bushnik et al., 2010; Calafat et al., 2005, 2008; Casas et al., 2013; LaKind and Naiman, 2015). The most significant route of human exposure is thought to be ingestion, with dietary intake estimated to range from 0.1–1.4 μg/kg body weight (bw)/day, but exposure can also occur from other sources (FAO/WHO, 2011). BPA can cross the placenta and there is some evidence that it may accumulate in the fetus after repeated exposures (Ikezuki et al., 2002; Schonfelder et al., 2002; Taylor et al., 2008). In fetal rodents, BPA has been shown to preferentially accumulate in brain, in some cases to a greater degree in males than females (Negri-Cesi, 2015). In its 2014 updated safety assessment of Bisphenol A (BPA) for use in food contact applications, the US Food and Drug Administration defined the No Observed Adverse Effect Level (NOAEL) as 5 mg/kg bw/day based largely on two multigenerational rodent studies (documents available for download here: https://www.fda.gov/NewsEvents/PublicHealthFocus/ucm064437.htm).

BPA has been reported to interfere with the metabolism and signaling of endogenous steroid hormones, particularly estrogen,

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and numerous studies, including our own, have repeatedly shown in multiple species that developmental exposure to BPA can perturb sexually dimorphic brain development and behavior, even at exposures below the current FDA NOAEL (representative examples include (Braun et al., 2011; Jasarevic et al., 2013; Kinch et al., 2015; Patisaul et al., 2012b; Rebuli and Patisaul, 2016; Sullivan et al., 2014; Wolstenholme et al., 2011)). Although this compounding evidence is compelling, because few published studies are evaluated to be of high utility for human risk assessment, there remains a lack of consensus on the potential risks BPA pose to the developing brain in humans. The studies herein were specifically designed and conducted in response to that informational limitation under the CLARITY-BPA research program (Consortium Linking Academic and Regulatory Insights on BPA Toxicity) (Birnbaum et al., 2012; Heindel et al., 2015; Johnson et al., 2016; Schug et al., 2013), a multi-investigator effort coordinated and supported by the National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS), and U.S. Food and Drug Administration (FDA) to help provide clarifying evidence. The present study tested the hypothesis that early-life BPA exposure can alter the volume of sexually dimorphic structures in the brain, and serves as a follow-up to our prior CLARITY-BPA paper describing non-reproductive behavioral outcomes in the same animals (Rebuli et al., 2015).

Designed to draw upon the strengths of academic and guideline-compliant studies in order to address research gaps and confirm prior findings, this and all other CLARITY-BPA studies are uniquely powerful because they incorporate research recommendations published by the WHO and others (Beronius et al., 2010, 2009; Chapin et al., 2008; FAO/WHO, 2011; FDA, 2012; NTP, 2008) for enhancing robustness and reproducibility, including complete data blinding, use of only one animal per sex per litter (with the litter as the experimental unit), oral dosing, inclusion of a reference estrogen, and evaluation of multiple BPA doses, particularly levels at or below the FDA NOAEL. Additionally, as in our prior, published CLARITY-BPA studies (Arambula et al., 2016; Rebuli et al., 2015), sex was considered as a biological variable. Exposure levels and endpoints examined were established via consortium consensus and selected to maximize utility in risk assessment. The animals used for the present study were tested as juveniles for effects on sexually dimorphic, non-reproductive behaviors prior to sacrifice (Rebuli et al., 2015). Only limited and inconsistent evidence for heightened anxiety and exploratory behavior were observed, leading us to conclude that there were no systematic effects of BPA on the behavioral endpoints tested. Here we focused on exposure-related effects on the volume of sexually dimorphic brain nuclei in these animals with the hypothesis that perinatal exposure would abrogate volumetric sex differences. Sprague-Dawley rats from an existing colony at the National Center for Toxicological Research (NCTR-SD) were perinatally exposed to vehicle, BPA (2.5, 25, or 2500 μg/kg bw/day), or a reference estrogen (0.5 μg/kg bw/day 17α-ethinylestradiol (EE_2)). To ensure precise oral dosing, dams were gavaged from gestational day 6 (GD 6) until parturition and offspring were directly gavaged from postnatal day 1 (PND 1) to weaning (PND 21). PND 28 brains were coronally sectioned, thionin-stained for Nissl substance, and unbiased stereology was used to quantify the volume of sexually dimorphic brain regions.

Throughout the mammalian brain, several morphological and functional brain sex differences arise during the fetal and postnatal period in response to the organizational effects of steroid hormones (De Vries, 2004; McCarthy, 2008; Simerly, 2002). The two regions most classically associated with morphological sex differences in rodents are the aptly named sexually dimorphic nucleus (SDN) of the preoptic area and the anteroventral periventricular nucleus (AVPV). Both of these morphometric sex differences are mediated by estradiol but effects on apoptosis are opposite, resulting in the SDN being 5–7 times larger in males (Gorski, 1978) and the AVPV being nearly 1.6 times larger in females (Davis et al., 1996; Simerly et al., 1997). Volumetric sex differences of the SDN and AVPV emerge perinatally and during adolescence, respectively, and increase in magnitude until adulthood (Ahmed et al., 2008; Davis et al., 1996; Gorski, 1978; Simerly et al., 1997). The mechanisms by which the SDN and AVPV are sexually differentiated are well described, require estrogen receptor alpha (ERα), and can be predictably manipulated by exogenous hormones (Lenz and McCarthy, 2010; Schwarz and McCarthy, 2008; Simerly, 2002). Thus, they are considered particularly useful targets for assessing the endocrine disrupting properties of chemicals such as BPA.

The posterodorsal portion of the medial amygdala (MePD) was also selected for assessment because ERs are known to play a role in the sexual differentiation process (Cooke et al., 2003), and we have previously demonstrated that prenatal BPA exposure can alter sex-specific patterns of MePD ERβ expression (Cao et al., 2013). Although anatomical sex differences in the prepubescent rodent amygdala are not as great as in the preoptic area of the hypothalamus, the volume of the rat MePD is roughly 15–20% larger in prepubertal males than females (Cooke et al., 2007; Cooke and Woolley, 2005). Circulating levels of gonadal steroids maintain and enhance the MePD volumetric sex difference throughout puberty (Ahmed et al., 2008) and adulthood (Cooke et al., 2003), resulting in the adult male MePD being approximately 2 times larger than females (Cooke et al., 1999; Hines et al., 1992).

Lastly, we explored the effects of BPA on the volume of the locus coeruleus (LC), a nucleus located in the pons and selected because one laboratory has generated data suggesting perinatal BPA exposure can have sex-specific effects on LC volume in Wistar rats (Kubo et al., 2001, 2003). A female biased sexual dimorphism in rodent LC volume has been reported, however, this appears to be strain- and species- dependent (Babstock et al., 1997; Garcia-Falgueras et al., 2005). Thus, whether or not a sex difference exists, and might be vulnerable to BPA, was of interest in our animal model. As the principal site of norepinephrine synthesis in the central nervous system, the LC plays a critical role in modulating behavioral, autonomic, and endocrine responses to stress.

2. Materials and methods

The study is a component of the CLARITY-BPA program and used the same animals for which behavioral data are already published (Rebuli et al., 2015). Because the comprehensive study design details are described in that prior publication, only the most directly relevant methods are summarized here.

2.1. Animal husbandry

Sprague-Dawley rats from the National Center for Toxicological Research colony (NCTR-SD rats) were housed in an Association for Assessment and Accreditation of Laboratory Animal Care- (AALAC) accredited facility at NCTR (23 ± 3 °C, 50 ± 20% relative humidity, and 12:12 h light dark cycle, lights off at 0600 h). All aspects of this study were approved by the NCTR Institutional Animal Care and Use Committee (IACUC). Rats were housed in conditions designed to minimize unintentional exposure to BPA and other EDCs (use of glass water bottles with filtered water, thoroughly washed polysulfone caging and woodchip bedding) and a soy- and alfalfa-free diet (5K96 verified casein diet 10 IF, round pellets, γ-irradiated; Cat. 1810069, Purina Mills, Richmond IN) and Millipore-filtered water were provided ad libitum. Extracts of diet and other study materials were monitored for BPA and myco/phytoestrogens by liquid chromatography/mass spectrometry