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The impact of neonatal bisphenol-A exposure on sexually dimorphic hypothalamic nuclei in the female rat

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ABSTRACT

Now under intense scrutiny, due to its endocrine disrupting properties, the potential threat the plastics component bisphenol-A (BPA) poses to human health remains unclear. Found in a multitude of polycarbonate plastics, food and beverage containers, and medical equipment, BPA is thought to bind to estrogen receptors (ERs), thereby interfering with estrogen-dependent processes. Our lab has previously shown that exposure to BPA (50 mg/kg bw or 50 μg/kg bw) during the neonatal critical period is associated with advancement of puberty, early reproductive senescence and ovarian malformations in female Long Evans rats. Here, using neural tissue obtained from the same animals, we explored the impact of neonatal BPA exposure on the development of sexually dimorphic hypothalamic regions critical for female reproductive physiology and behavior. Endpoints included quantification of oxytocin-immunoreactive neurons (OT-ir) in the paraventricular nucleus (PVN), serotonin (5-HT-ir) fiber density in the ventrolateral subdivision of the ventromedial nucleus (VMNvl) as well as ERα-ir neuron number in the medial preoptic area (MPOA), the VMNvl, and the arcuate nucleus (ARC). Both doses of BPA increased the number of OT-ir neurons within the PVN, but no significant effects were seen on 5-HT-ir fiber density or ERα-ir neuron number in any of the areas analyzed. In addition to hypothalamic development, we also assessed female sex behavior and body weight. No effect of BPA on sexual receptivity or proceptive behavior in females was observed. Females treated with BPA, however, weighed significantly more than control females by postnatal day 99. This effect of BPA on weight is critical because alterations in metabolism, are frequently associated with reproductive dysfunction. Collectively, the results of this and our prior study indicate that the impact of neonatal BPA exposure within the female rat hypothalamus is region specific and support the hypothesis that developmental BPA exposure may adversely affect reproductive development in females.

1. Introduction

It is well established that mammalian neuroendocrine development is sensitive to changes in steroid hormone levels (Simerly, 2002; Wallen, 2005). This sex specific organization of endocrine sensitive tissues and circuits is thought to be vulnerable to endocrine disruption by compounds that act as 'hormone mimics'. Such compounds can mimic or interfere with the natural organizational effects of hormones by interacting with hormone receptors or disrupting hormone-dependent signaling pathways (Gore, 2008; Welshons et al., 2006). Collectively referred to as endocrine disrupting compounds (EDCs), they are now ubiquitous in the human environment and their potential health effects are only beginning to be elucidated. The present study tested the hypothesis that exposure to the EDC bisphenol-A (BPA) during the neonatal critical period interferes with the sex specific organization of the female rat hypothalamus. Each of the hypothalamic areas selected for the present study is well known to be densely populated with estrogen receptor (ER) containing neurons and critical for some aspect of female reproduction, including regulation of the estrous cycle, mating or maternal behaviors. We also assessed the impact of neonatal BPA exposure on sexual proceptivity and body weight, two endpoints of emerging concern (Monje et al., 2009; Ryan et al., 2010b).

Apprehension over the long-term health effects of neonatal and childhood exposure to EDCs arises from animal data indicating that the critical periods of fetal, infant and pubertal development are more sensitive to low doses of hormones than adult tissues, and thus more vulnerable to endocrine disruption (Selevan et al., 2000; Vom Saal and Moyer, 1985). Research focused on neonatal critical windows of development in rodents (prior to and immediately after birth) has shown that perinatal exposure to BPA can alter

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reproductive development and influence reproductive potential later in life, a concept referred to as the 'Fetal Basis of Adult Disease' (FEBAD) hypothesis (Heindel, 2005; Heindel and Levin, 2005; Patisaul and Adewale, 2009; Vandenberg et al., 2009). In rodents, perinatal exposure to low, environmentally relevant doses of BPA (ranging between 2 and 250 μg/kg body weight) has been shown to advance puberty and reproductive senescence, alter estrous cyclicity, disrupt ovarian and mammary gland development and has been correlated with an increased incidence of mammary tumors (Adewale et al., 2009; Durando et al., 2007; Honma et al., 2002; Markey et al., 2001, 2003; Susiarjo et al., 2007). All of these reproductive and neuroendocrine endpoints are regulated by the hypothalamic–pituitary–gonadal (HPG) axis. Thus, disruption of hypothalamic sexual differentiation could be a unifying mechanism underlying this suite of effects induced by neonatal BPA exposure.

BPA entered commercial production in the 1950s after initially undergoing development as a possible synthetic estrogen (Dodds et al., 1938), and is now primarily used as a component of polycarbonate plastics, epoxy resins and thermal paper receipts (Biedermann et al., 2010). It can be found in a wide variety of food and beverage containers, medical equipment and plastic tubing, among other products (Calafat et al., 2009; Vandenberg et al., 2007, 2009; Welshons et al., 2006). The Centers for Disease Control (CDC) has estimated that an estimated 93% of the US population has detectable levels of BPA in their bodies (Calafat et al., 2005). Urine analysis has detected BPA in men and women across all age groups, but children and hospitalized infants have significantly higher circulating levels of BPA than adults, prompting concern about the long-term health effects that might result from developmental exposure (Calafat et al., 2009; Lakind and Naiman, 2010; Meerts et al., 2001; Welshons et al., 2006). Exposure can occur throughout life, including during fetal development because BPA fails to bind to α-fetoprotein and can thus jeopardize the developing fetus through placental transfer. Once the infant is born, further susceptibility can occur through lactational transfer from the mother, or by interacting with BPA-containing toys and products, thus exposing the child during multiple critical developmental periods (Ikezuki et al., 2002; Nagel et al., 1999; Tsutsumi, 2005). The lowest observed adverse effect level (LOAEL) for BPA is currently set in the US at 50 mg/kg body weight (bw) per day and the "safe" or reference dose is defined as 50 μg/kg bw per day.

Although evidence that BPA exposure to doses equivalent to or lower than the LOAEL can adversely affect the development of the female reproductive tract is relatively robust, whether or not it can affect neuroendocrine development remains unclear. We have previously shown, for example, that neonatal exposure to BPA can affect adversely affect ovarian development, pubertal timing, and the ability to maintain a regular estrous cycle in female rats (Adewale et al., 2009; Patisaul et al., 2009b). The goal of the present study was to determine, within this same group of animals, if sexually dimorphic hypothalamic development had also been disrupted. In rodents, estrogen is well recognized to masculinize hypothalamic regions which govern sex specific physiology and behavior, including ovarian cyclicity and sexual receptivity (Bachman, 1995; Baum, 1979; McCarthy, 2008; Simerly, 2002). In males, testosterone from the testis enters the bloodstream and is carried to the brain where it is aromatized to estrogen. It is this estrogen which then masculinizes the brain (Simerly, 1998, 2002). The female brain, on the other hand, develops largely in the absence of estradiol and is considered to be feminized. Thus, exposure to EDCs that mimic estrogen could potentially masculinize the female hypothalamus. In primates, the relative role steroid hormones play in hypothalamic differentiation are less well understood but androgens are now recognize to be important for masculinization (Wallen, 2005). To further elucidate potential mechanisms through which BPA might be acting within the female rat brain, we employed the ERα-selective agonist PPT as an additional positive control group to compare the effects of ERα agonism with BPA exposure. Estradiol benzoate (EB) and vehicle were also included as additional control groups (positive and negative respectively).

BPA is hypothesized to work through an ER mediated pathway and although the RBA of BPA for both ERα and ERβ is similarly low (Kuiper et al., 1998; Welshons et al., 2003), at least one study has shown it to be a more effective ligand for ERβ than ERα (Routledge et al., 2000). We therefore hypothesized that low dose exposure to BPA, during the neonatal critical window of postnatal days (PND) 0–4, would disrupt the organization of sexually dimorphic regions of the hypothalamus important in regulating female reproductive behavior and physiology, particularly those known to contain ERβ. It was further hypothesized that this disruption would result in a male-like pattern of development in neonatally exposed females. The neonatal window was selected as the exposure period because it is a well recognized critical window of sensitivity in the rat (Bachman, 1995; Baum, 1979; Simerly, 2002), approximately akin to the 2nd trimester in humans (Bayer et al., 1993) (www.endocrinedisruption.com).

We focused on four ER-rich hypothalamic regions involved in female reproduction and which exhibit sexually dimorphic differences in volume (size or cell number) or hormone receptor expression between males and females. The first area of interest, the paraventricular nucleus (PVN), is the primary site of oxytocin (OT) synthesis. OT is crucial for many aspects of maternal, social, sexual and cognitive behaviors and in many species its release is sensitive to circulating levels of estradiol (Amico et al., 1997; Lee et al., 2009; Neumann, 2008; Rosenblatt et al., 1988). Because OT neurons coexpress ERβ (also referred to as ESR2), but not the ERα (ESR1) subtype (Hrabovszky et al., 2004; Patisaul et al., 2003; Sharon and Allan, 1997), we hypothesized that any BPA-induced effects within this region are likely mediated via ERβ and not through ERα. The second area of interest was the ventrolateral (vl) subdivision of the ventromedial nucleus (VMN) an area critical for regulation of female sex behavior (McCarthy, 2008; Pfaff and Sakuma, 1979). It receives numerous serotonergic (5-HT) projections and is sexually dimorphic, with males having a significantly greater density of 5-HT fibers than females (Patisaul et al., 2008). The medial preoptic area (MPOA) was selected as the third area because it is another region critical for the regulation of the female reproductive cycle, as well as maternal and sexual behavior (Baskerville and Douglas, 2008; Charlton, 2008; Numan and Stolzenberg, 2009). The MPOA expresses both ER isoforms and exhibits sexually dimorphic expression of ERβ but not ERα, with males showing greater expression of ERβ than females (Kudwa et al., 2004; Weiser et al., 2008). The final area of interest was the arcuate nucleus (ARC), which, while involved in feedback regulation of the female reproductive cycle, is typically not considered to be sexually dimorphic in volume or cell number, thus we hypothesized that this area might be more resistant to endocrine disruption (Walsh et al., 1982). Body weight and sexual behavior were also assessed as part of this project. Elucidating the mechanisms by which BPA disrupts the ontogeny and function of the HPG axis will help establish whether or not this compound poses a potential threat to human health.

2. Materials and methods

2.1. Animals and neonatal exposure

Animals used for this study were also used for a prior study and detailed methods concerning housing conditions, diet, behavioral testing and surgical procedures can be found in Adewale et al.