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Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A

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Abstract

Changes in the volumes of sexually dimorphic brain nuclei are often used as a biomarker for developmental disruption by endocrine-active compounds (EACs). However, these gross, morphological analyses do not reliably predict disruption of cell phenotype or neuronal function. In the present experiments, we used a more comprehensive approach to assess whether postnatal exposure to the EACs genistein (GEN) or bisphenol-A (BIS) affected the development of two sexually dimorphic brain regions in male rats: the anteroventral periventricular nucleus of the hypothalamus (AVPV) and the sexually dimorphic nucleus of the preoptic area (SDN). In addition to nuclear volumes, we also measured the number of immunopositive calbindin neurons in the SDN and the activational patterns of gonadotropin-releasing hormone (GnRH) neurons, a neuronal population that is functionally linked to the AVPV. In rats, exposure of the neonatal male brain to endogenous estrogen, aromatized from testicular testosterone, is essential for the proper sexual differentiation of these endpoints. Thus, we hypothesized that exposure to BIS and GEN during this critical period could disrupt brain sexual differentiation. Animals were given four subcutaneous injections of sesame oil (control), 250 μg GEN, or 250 μg BIS at 12 h intervals over postnatal days (PND) 1 and 2, gonadectomized on PND 85, and treated sequentially with estrogen and progesterone to stimulate Fos expression in GnRH neurons, a marker for their activation. A cohort of age-matched ovariectomized (OVX) females that were given the same hormone treatment in adulthood served as a positive control group. SDN volume was unchanged by treatment, but the number of calbindin neurons in the SDN was significantly increased by both BIS and GEN. GEN, but not BIS, demasculinized male AVPV volume, but patterns of GnRH neuronal activation were not affected by either compound. These results suggest that acute exposure to EACs during a critical developmental period can independently alter nuclear volumes of sexually dimorphic nuclei and their phenotypic profiles in a region specific manner.

Keywords: AVPV; SDN; Calbindin; Estrogen; Development; Endocrine disruptor

1. Introduction

The volumes of sexually dimorphic nuclei within the brain are often used as a biomarker for neurodevelopmental disruption by endocrine-active compounds (EACs) because their anatomical development is hormonally regulated. However, results from these gross, morphological analyses are often inconsistent between studies, and do not reliably predict changes in neuronal function or disruption of cell phenotype within these nuclei. Thus, a more comprehensive approach is necessary to assess whether exposure to EACs during the organizational period, when sexually dimorphic systems differentiate, ultimately alters the sex specific function of these systems in adulthood. In addition, most studies of EAC action focus on synthetic compounds. However, not all EACs are man made, and one class of plant-produced EACs, the soy isoflavone phytoestrogens, are becoming more prevalent in the diets of humans, research animals, and other captive animals (Degen et al., 2002; Setchell et al., 1987; Thompson et al., 2006). In the present study, we assessed how the plastics component bisphenol-A (BIS) and the phytoestrogen genistein (GEN) affected the development of two sexually dimorphic brain regions in adult male rats: the sexually dimorphic nucleus of the preoptic area (SDN) and the anteroventral periventricular

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nucleus of the hypothalamus (AVPV). In addition to nuclear volume, we also measured the activational patterns of gonadotropin-releasing hormone (GnRH) neurons surrounding the vascular organ of the lamina terminalis (OVLT), a neuronal population functionally linked to the AVPV, and the number of immunopositive calbindin neurons in the SDN.

BIS entered commercial development in the 1950s and is an industrial chemical used primarily to make polycarbonate plastic and epoxy resins. GEN is an isoflavone phytoestrogen found in legumes and soy-based foods. BIS and GEN have been shown to bind to both major forms of the estrogen receptor (ERα and ERβ) *in vitro*, albeit with low relative binding affinities (RBAs) compared to 17β-estradiol (Kuiper et al., 1997; Kuiper et al., 1998). Despite their low RBAs for estrogen receptors, both compounds have been found to influence adult reproductive physiology and behavior when administered during development. For example, exposure to BIS during the pre- or postnatal period can increase ERα mRNA expression in the female mediobasal hypothalamus and in the male anterior pituitary (Khurana et al., 2000), disrupt the sexual differentiation of the locus coeruleus (Kubo et al., 2003), decrease the expression of tyrosine hydroxylase (TH) in the female AVPV (Rubin et al., 2006) and result in early, persistent estrus in females (Rubin et al., 2001). Perinatal exposure to GEN can impair male reproductive behavior and function in adulthood (Wisniewski et al., 2003), demasculinize tyrosine hydroxylase immunoreactivity in the AVPV (Patisaul et al., 2006) and ultimately result in early persistent estrus in mature females (Lewis et al., 2003). These data suggest that both compounds are capable of disrupting estrogen-dependent processes in the developing brain, including sexual differentiation and the organization of other behavioral and neuroendocrine circuits.

The neural circuits that coordinate reproductive function in mammals are sexually differentiated by exposure to gonadal hormones during a perinatal critical period (Cooke et al., 1998). In rodents, the male brain is exposed perinatally to high levels of estrogen, synthesized locally from the aromatization of testicular testosterone. In contrast, the ovaries of developing females are generally quiescent, so normal development of the female brain occurs in the relative absence of estrogen. These sex differences in hormone synthesis and exposure result in the development of distinct male and female neuroanatomical circuits, neuroendocrine functions, and reproductive behaviors.

The SDN is sexually differentiated both anatomically and functionally. In rats, it is 2–4 times larger in males than females and is thought to play a role in the display of male sex behaviors (Gorski, 1985). The SDN is sensitive to the developmental effects of gonadal steroids from embryonic day 18 until postnatal day 4 (PND 4) (Bleier et al., 1982; Bloch and Gorski, 1988; Davis et al., 1996a,b; Dohler et al., 1984). Following this phase of development, a period of sexually dimorphic apoptosis occurs from PND 5 through PND 12 (Davis et al., 1996a,b). Early exposure to estrogen appears to protect the male SDN from this programmed cell death, possibly through the induction of nerve growth factors and their tyrosine kinase receptors (Gibbs, 1998; Toran-Allerand, 1996). In females, because endogenous estrogen levels are low during this neonatal critical period, SDN apoptosis is unabated and SDN volume is significantly reduced compared to that of the male (Davis et al., 1996a,b).

Previous toxicological studies have attempted to utilize measurements of SDN volume as a biomarker for the developmental disruption of brain differentiation by EACs, but results have been inconsistent. For example, female SDN volume was significantly increased by daily injections of 500 or 1000 μg/day genistein during postnatal days 1–10 but SDN volume in males was unaffected (Faber and Hughes, 1993; Faber and Hughes, 1991). In contrast, prenatal exposure to genistein through the maternal diet beginning on (GD 7), followed by postnatal exposure until PND 50 through lactation and diet has been shown to decrease SDN volumes in males, but not females (Ferguson et al., 2000; Slikker et al., 2001). These conflicting findings may result from methodological differences in SDN volumetric measurements, as well as in the timing and route of administration of the compounds. Measurements of nuclear volume in the brain have traditionally been achieved using two-dimensional approaches (Meredith et al., 2001), a method that yields highly variable results. For the present studies we employed a stereological approach, a methodology that produces accurate and replicable estimates of nuclear volume, even for nuclei that have an irregular shape (Glaser and Glaser, 2000; Schmitz and Hof, 2005).

Recently, a sexually dimorphic subpopulation of neurons within the SDN that expresses calbindin-d28k was described (Brager et al., 2000; Lephart et al., 1998; Scallet et al., 2004; Sickel and McCarthy, 2000). Calbindin is a calcium binding protein thought to protect against apoptosis (Dowd et al., 1992; McMahon et al., 1998) and, accordingly, the volume of the SDN that contains calbindin expressing neurons (CALB-SDN) is significantly larger in males than females. While lifelong exposure to GEN has been shown to further increase CALB-SDN volume in males (Scallet et al., 2004), the effects of exposure restricted to the neonatal critical period for sexual differentiation remain uninvestigated. Thus, in the present studies we sought to determine whether neonatal exposure to BIS and GEN affected the volume of the SDN overall, as well as the volume of the CALB-SDN and the number of SDN neurons containing calbindin.

The anteroventral periventricular nucleus of the hypothalamus is a sexually dimorphic nucleus thought to convey hormonal and environmental signals to the gonadotropin-releasing hormone neurons that regulate ovulation. The female AVPV is nearly twice the size of the male AVPV (Davis et al., 1996a,b). As with the SDN, the organization of this sex difference in volume appears to take place in the first few days of life, as castration before PND 5 can sex reverse AVPV volume in males (Davis et al., 1996a,b). Neurons in the AVPV provide direct projections to GnRH neurons (Gu and Simerly, 1997; Le et al., 1999; Polston and Simerly, 2006) and in adult females, stimulation of the AVPV by estrogen and progesterone coincides with GnRH neuronal activation and the preovulatory surge of luteinizing hormone (LH) (Gu and Simerly, 1997; Lee et al., 1990; Petersen et al., 2003). In males, however, this