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Regulation of arcuate genes by developmental exposures to endocrine-disrupting compounds in female rats

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ABSTRACT

Developmental exposure to endocrine-disrupting compounds (EDCs) alters reproduction and energy homeostasis, both of which are regulated by the arcuate nucleus (ARC). Little is known about the effects of EDC on ARC gene expression. In Experiment #1, pregnant dams were treated with either two doses of bisphenol A (BPA) or oil from embryonic day (E)18-21. Neonates were injected from postnatal day (PND)0-7. Vaginal opening, body weights, and ARC gene expression were measured. *Chrm3* (muscarinic receptor 3) and *Adipor1* (adiponectin receptor 1) were decreased by BPA. *Bdnf* (brain-derived neurotropic factor), *Igf1* (insulin-like growth factor 1), *Htr2c* (5-hydroxytryptamine receptor), and *Cck2r* (cholescystokinin 2 receptor) were impacted. In Experiment #2, females were exposed to BPA, diethylstilbestrol (DES), di(2-ethylhexyl)phthalate, or methoxychlor (MXC) during E11-PND7. MXC and DES advanced the age of vaginal opening and ARC gene expression was impacted. These data indicate that EDCs alter ARC genes involved in reproduction and energy homeostasis in females.

1. Introduction

Recent research has begun to elucidate the perturbations of central nervous system regulators of homeostatic functions including reproduction and energy homeostasis by endocrine-disrupting compounds (EDCs). These two functions are controlled, in part, through the hypothalamus, which is regarded as a key brain region integrating hormonal control of energy homeostasis and reproduction [1,2]. Many hypothalamic nuclei are involved in this integration include the arcuate nucleus (ARC), the paraventricular nucleus (PVH), the lateral hypothalamus (LH), and the ventromedial nucleus (VMH) [3]. Of these, the ARC is near an area of local permeability in the blood brain barrier and is readily exposed to circulating steroids, hormones, and nutrients including insulin, leptin, and glucose [4]. ARC neurons, in particular proopiomelanocortin (POMC), neuropeptide Y (NPY), and KNDy (Kisspeptin-Neurokinin B-Dynorphin) neurons, respond to both peripheral signals, reflecting the energy status of the body, and to inputs from the hindbrain to integrate sensory attributes, emotional states, and motivated behaviors [4–6].

The effect on energy homeostasis from developmental exposures to common EDCs has potential relevance in the increase in childhood and adulthood obesity in the industrialized world. Evidence suggests that EDCs, especially Bisphenol A (BPA), may affect normal energy homeostasis by altering homeostatic functions, glucose homeostasis, and peripheral gene expression [7–9]. Recent evidence also suggests that perinatal exposure to BPA alters hypothalamic melanocortin neurocircuitry and ARC neuropeptide gene expression in response to high fat diets [10]. The mechanisms behind these effects are unknown but potentially involve organizational and epigenetic effects in the hypothalamus during development, which may be mediated by the classical estrogen receptors (ER) α/β. Little is known about the effects of perinatal exposure to other estrogenic and non-estrogenic EDC on expression of ARC genes involved in energy homeostasis.

Abbreviations: *Adipor1*, adiponectin receptor 1; ARC, arcuate nucleus of the hypothalamus; *Bdnf*, brain-derived neurotropic factor; BPA, bisphenol A; *Cck2r*, cholescystokinin 2 receptor; *Chrm3*, muscarinic receptor 3; DEHP, di(2-ethylhexyl)phthalate; DES, diethylstilbestrol; *Htr2c*, 5-hydroxytryptamine receptor; *Igf1*, insulin-like growth factor 1; MXC, methoxychlor; NPY, neuropeptide Y; POMC, proopiomelanocortin; TLDA, Taqman® Low-Density Array.

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Developmental exposures to estrogenic EDCs alter many neuroendocrine reproductive functions [11,12]. BPA and other estrogenic EDCs (diethylstilbestrol (DES), methoxychlor (MXC)) are reported to alter the timing of puberty onset [11–15], however not consistently [16,17]. Phthalates are typically considered anti-androgenic and are known to alter male reproduction including puberty [18,19], but there is limited data with mixed results on phthalates (e.g., di(2-ethylhexyl)phthalate, dibutyl phthalate) in females. A few studies showed that the exposure advanced pubertal onset [20,21], and other studies suggested the exposure delayed puberty [22]. Differences in the timing of exposure may explain, in part, the contradictory results with phthalates. Puberty and the control of reproduction are initiated by interactions between peripheral energy signals (leptin, insulin, adiponectin, etc.) with hypothalamic neurons, in particular, NPY/AgRP [23] and KNDy [24]. Activity of neurotransmitters [25–28] and expression of growth factors (IGF-1) [29] in the hypothalamus are associated with the onset of puberty and the reproductive cycle in females. However, little is known about the effects of perinatal exposure to EDCs on expression of the genes associated with these signals in the hypothalamus and especially in the ARC.

There are numerous ARC genes that have been characterized for their role in controlling reproduction and energy homeostasis. These genes include receptors for hormones and neurotransmitters, signaling molecules, transcription factors, and neuropeptides (reviewed in [6,30,31]. Peripheral hormonal receptors, including members of G-protein coupled receptor (GPCR) and cytokine receptor families, are vital for the hypothalamic sensing of the body's energy status, which is then communicated to other hypothalamic neurons to control reproduction, especially during puberty. GPCRs are common receptors for neurotransmitters involved in reproduction and energy homeostasis including serotonin receptors. ARC neuropeptides (e.g., POMC, NPY, AgRP, Kisspeptin) are integral to the hypothalamic control of feeding, energy expenditure, and puberty and are modulated by both peripheral and central signals of energy homeostasis. Therefore, to address the question of whether these genes are altered in the ARC by developmental exposures to EDCs, we designed a Taqman Low-Density Array (TLDA) to analyze relative gene expression.

In order to determine the organizational effects of EDCs, dams and neonates were treated during fetal and neonatal periods when steroid receptor expression and the development of the melanocortin neurocircuitry in the rodent hypothalamus occur (E10-P14) [32–37]. In addition, EDCs alters hypothalamic gene expression [38], including steroid hormone receptor expression (ERα/β and AR) [7,39] during similar developmental windows. Furthermore, this exposure window encompasses critical gonadal differentiation events that start mid-gestation (i.e., E11) and subsequent early ovarian developmental events, including oocyte nest breakdown and follicular assembly in rats (i.e., E18-PD7), which can influence reproductive health later in life [40].

Therefore, the current study sought to determine if developmental (gestational and neonatal) exposures to a selection of known EDCs will alter puberty (vaginal opening), body weight, and ARC gene expression in adult female rats. As an initial EDC screen, we exposed dams and neonate females to two doses of BPA, a known disruptor of reproduction and energy homeostasis [10,14] and analyzed for gene expression using the TLDA. In the subsequent experiment, dams and neonate females were exposed to either BPA, MXC, di(2-ethylhexyl)phthalate (DEHP), or DES. We hypothesize that important genes involved in reproduction and energy homeostasis would be differentially regulated in the ARC by developmental exposures to these compounds, which may contribute to the dysregulation of reproduction and energy homeostasis in juveniles and adults.

2. Materials and methods

2.1. Animals

Eight- to twelve-week-old Fischer CDF female rats (Charles River, Wilmington, MA) were maintained on a 14-h light/10-h dark cycle and fed, *ad libitum*. A reduced isoflavone diet was provided in order to minimize possible effects of phytoestrogens (Purina, 5V01, Brentwood, MO). The estrous cycles of the rats were followed daily, and individual females were mated with untreated males starting on the day of proestrus. A sperm-positive vaginal smear was designated embryonic day 0 (E0). All animal care and treatment protocols were carried out in accordance with institutional guidelines and were approved by Rutgers University Institutional Animal Care and Use Committee.

2.2. Chemicals

All chemicals (BPA, MXC, DES, DEHP, dimethyl sulfoxide (DMSO), sesame oil, and corn oil) were purchased from Sigma-Aldrich.

2.3. Experimental design and treatments

In Experiment #1, the timed-pregnant females received one of two different treatment dosages of BPA: 50 μg/kg/day (Lo-BPA; n = 3 litters; 10 pups) and 50 mg/kg/day (Hi-BPA; n = 4 litters; 8 pups) in 1 ml vehicle/kg BW. Control animals (n = 5 litters; 12 pups) received an emulsion of 10% absolute ethanol (EtOH) and 90% corn oil. Daily treatments were administered (i.p.) to the pregnant dams beginning on E18 and sub-cutaneously (in the folds of the neck) to the neonates within 8 h of birth (considered PND 0) and continued until PND 7. Starting on PND 28, females were followed for their vaginal opening as an indication of pubertal age to determine if developmental exposures to EDCs alters the age of puberty [41–43]. After vaginal opening, the stages of estrous cycles of all females were followed by vaginal cytology, and 8 randomly selected females from each treatment were weighed and euthanized between PND 50 and 60 on proestrous day after their third estrous cycle for hypothalamic tissue isolation and gene expression analysis.

In Experiment #2, the timed-pregnant females were divided into groups with each group receiving one of four different EDCs or two control vehicles. The EDCs and doses were as follows: BPA (50 mg/kg/day; n = 8 litters, 26 pups); DES (0.1 μg/kg/day; n = 6 litters, 20 pups); and DEHP (500 mg/kg/day; n = 5 litters, 18 pups) in a vehicle of EtOH:oil (1:9) (1 ml vehicle/kg BW volume). Another group received MXC (75 mg/kg/day; n = 8 litters, 16 pups) in a vehicle of dimethylsulfoxide (DMSO): sesame oil (1:2). There were two sub-groups of control animals receiving either the EtOH:corn oil vehicle (n = 5 litters, 15 pups) or the DMSO:sesame oil vehicle (n = 5 litters, 13 pups). Daily treatments were administered (i.p.) to the pregnant dams beginning on E11 and sub-cutaneously (in the folds of the neck) to the neonates within 8 h of birth (considered PND 0) and continued until PND 7. The vaginal opening of all female offspring and the stages of estrous cycle were followed, similar to Experiment #1. One to two females from each dam (*n* = litter) were randomly chosen, weighed, and euthanized on proestrous day between PND 70 and 90 for isolation of hypothalamic tissues and analysis of gene expression. The age of sacrifice was determined by estrous cyclicity and due to perturbations in estrous cycles by EDC treatments, the range in ages is ~20 days. See Table 1 for a list of the samples sizes (litters) and number of total pups for each experiment, treatment, and endpoint.

Doses of compounds were chosen from previous experimentation demonstrating reproductive effects or from the literature.