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Bisphenol A regulation of testicular endocrine function in male rats is affected by diet☆

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HIGHLIGHTS

- Exposure of male rats to BPA with a high fat diet increased serum testosterone.
- BPA exposure or feeding a high fat diet decreased serum adiponectin concentration.
- Exposure of male rats to BPA and/or HFD consumption impaired antioxidant capacity in the testis.

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ABSTRACT

There is concern that early-life exposure to bisphenol A (BPA) may alter developmental programming and predispose individuals to obesity and reproductive anomalies. The present study was designed to determine if a high fat diet at sexual maturation moderates testicular toxicity occasioned by exposure to BPA during reproductive development. Therefore, male rats were exposed to BPA by maternal gavage (0, 2.5 or 25 μg/kg body weight/day) from gestational day 12 to postnatal day 21. At weaning, control and BPA-exposed animals were placed on a regular normal fat diet (NFD) until 70 days of age when they were continued on the NFD or were maintained on a high fat diet (HFD) until euthanasia at 98 days. Adult male rats maintained on HFD were generally heavier than NFD animals due to greater energy intake but energy intake per unit body weight gain was similar in all animals. However, perinatal exposure to BPA decreased ($P < 0.05$) serum adiponectin as well as adiponectin and AdipoR2 protein expression levels in Leydig cells. Importantly, the combination of BPA exposure and HFD consumption promoted lipid peroxidation evidenced by elevated serum thiobarbituric acid reactive substances and glutathione concentrations. These findings imply that interaction between BPA and HFD potentially causes testicular dysfunction to a greater degree than would be due to BPA exposure or HFD consumption. Given the relationship that exists between energy homeostasis and reproductive activity, additional studies are warranted to investigate the consequences of BPA-diet interactions on testicular function.

1. Introduction

Bisphenol A (BPA) is used extensively in the manufacture of consumer products, e.g., polycarbonate plastic bottles and as epoxy resin for coating of canned foods and beverages. Available estimates indicate that children between 6 and 12 years of age have greater tissue BPA concentrations compared to adults (Calafat et al., 2008). Thus, children represent the most vulnerable segment of the population to BPA exposure effects. This is concerning because developmental exposure to BPA is associated with reproductive toxicity in males (Aydoğan et al., 2010; Chitra et al., 2003; Salian et al., 2009) and predisposes to insulin resistance, weight

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gain and obesity (Alonso-Magdalena et al., 2006; Ben-Jonathan et al., 2009; Rubin and Soto, 2009). For example, data from biomonitoring studies showed that greater urinary BPA concentrations are present in individuals suffering from diabetes mellitus (Lang et al., 2008), cardiovascular disease (Lang et al., 2008), hypertension (Shankar and Teppala, 2012) and obesity (Trasande et al., 2012). On the other hand, 33% of total calories in a typical western diet is derived from fat, and high fat diet consumption is considered to be a major contributing factor to increased incidence of obesity in developed countries (Grotto and Zied, 2010; Ebbesson et al., 2007). Epidemiological observations were supported by results of animal studies showing that developmental exposure to BPA increased body weights in adult rodents (Akingbemi et al., 2004; Miyawaki et al., 2007; Rubin et al., 2001; Wei et al., 2011). In this regard, BPA promoted proliferation and differentiation of preadipocytes (Phrakonkham et al., 2008) and inhibited adiponectin secretion (Hugo et al., 2008), whereas a high fat diet (HFD) increased adipose tissue accretion and affected male fertility (Ghanayem et al., 2010; Olivares et al., 2010; Fernandez et al., 2011).

In addition to regulating gonadal steroid hormone secretion (Akingbemi et al., 2004; Nanjappa et al., 2012), developmental exposure to estrogenic agents such as BPA may induce epigenetic modifications that increase estrogen receptor (ESR) expression and estrogen metabolism in several tissues (Khurana et al., 2000; Wadia et al., 2007; Bromer et al., 2010). Estrogen degradation is regulated by the estrogen sulfotransferase (EST) enzyme, which deactivates estrogens through sulfo-conjugation (Leiter and Chapman, 1994). Interestingly, EST is highly expressed and physiologically relevant in Leydig cells (Kester et al., 2002; Tong et al., 2004) because mice lacking the EST enzyme show Leydig cell hyperplasia and hypertrophy, disrupted steroidogenesis and decreased fertility (Qian et al., 2001; Tong et al., 2004). In addition, perinatal BPA exposure caused oxidative stress in testis and brain of male mice (Kabuto et al., 2004) and human subjects (Hong et al., 2009). Because oxidative stress is linked to infertility (Aitken and Baker, 2004), diabetes and obesity (Spector, 2000), it is a likely mechanism by which BPA exerts toxicity in several tissues (Kovacic, 2010).

Several studies have described BPA effects on reproductive tract development and function (Chitra et al., 2003; Salian et al., 2009; Aydoğan et al., 2010) and its capacity to interfere with metabolic homeostasis (Alonso-Magdalena et al., 2006; Ben-Jonathan et al., 2009; Rubin and Soto, 2009). Interestingly, several cytokines produced by adipose tissue (adipokines), including leptin and adiponectin, can affect pubertal development and fertility in the male (Fernandez-Fernandez et al., 2006) and thereby contribute to reproductive dysfunction associated with metabolic derangements and energy imbalance (Fernandez-Fernandez et al., 2006). We consider that BPA-induced changes in adipokine secretion affects testicular endocrine function because adiponectin and its receptors (AdipoR2) are constitutively expressed in Leydig cells and have the capacity to regulate androgen biosynthesis (Bjursell et al., 2007; Caminos et al., 2008; Pfaehler et al., 2012). Still, there is little or no information on whether diet plays a role in BPA toxicity. Therefore, the present study was designed to determine if a high fat diet in adulthood affects testicular toxicity arising from developmental exposure of male rats to BPA.

2. Materials and methods

2.1. Animal studies

All experimental and euthanasia procedures were performed in accordance to a protocol approved by the Auburn University Institutional Animal Care and Use Committee. Timed-pregnant Long-Evans female rats were purchased from Harlan-Teklad (Indianapolis, IN) and allowed to acclimatize at the housing facility of the Division of Laboratory Animal Health, College of Veterinary Medicine, Auburn University. Pregnant and nursing dams were housed one per cage, whereas weanlings were kept in groups of two to four depending on age and size. Animals were kept on a 12L:12D cycle, with ambient temperature of 68–74 °F, and were provided feed and water *ad libitum*. Previous studies showed that environmental estrogenscan interfere with experimental results, e.g., phytoestrogens present in soy beans and BPA that leach from used polycarbonate cages and water bottles (Brown and Setchell, 2001; Howdeshell et al., 2003; Hunt et al., 2003). Therefore, soy-free rodent diets (X2020, Harlan-Teklad), polypropylene cages and glass water bottles were used in order to minimize background exposure to estrogens in the environment. Animals were assigned randomly to different treatment groups to achieve equal weight distribution.

Table 1
Composition of normal and high fat diets (NFD, HFD).

Parameters	2020X (NFD)	TD88137 (HFD)
Protein (%)	19.1	17.3
Carbohydrate (%)	47	48.5
Fat (%)	6.5	21.2
Total saturated fatty acids (%)	0.8	13.3
Total monounsaturated fatty acids (%)	1.1	5.9
Total polyunsaturated fatty acids (%)	2.9	0.9
Cholesterol (%)		0.2
Calories from fat (%)	16	42
Calories from protein (%)	24	15.2
Calories from carbohydrates (%)	60	42.7
Energy (kcal/g)	3.1	4.5

Pregnant Long-Evans dams ($n = 14$) were gavaged daily with BPA (2.5 or 25 μg/kg bw daily) from gestational day (GD) 12 through postnatal day (PND) 21. This route of exposure mirrors the pattern of human exposures to BPA and the exposure paradigm does not cause any toxicity to fetal development (Akingbemi et al., 2004). Furthermore, the potential confounding effect of animal handling associated with activation of the hypothalamus-adrenal axis (Vecsey et al., 2013) was mitigated by feeding the olive oil (vehicle) by gavage to control animals. During the exposure period, BPA dosage was adjusted in order to account for changes in body weights of dams, which were measured every other day. At five days post-partum, female pups were euthanized, whereas male pups were pooled and randomly distributed among dams (5–6 pups/dam) for cross-fostering to minimize litter effects related to xenobiotic capacity in individual dams. Subsequently, male rats were allowed to attain sexual maturity at 70 days of age after which they were randomly selected and distributed into two subgroups of 8–10 animals and fed either a NFD or HFD. The diets were determined by the manufacturers to be soy- and alfalfa-free (X2020, #88137; Harlan) and had 16% and 42% of their energy contents derived from fat, respectively (Table 1). NFD and HFD feeding was for a period of 28 days from PND 71-98; this period of HFD feeding was considered prolonged enough to cause chronic effects but not affect postnatal Leydig cell development (Sebokova et al., 1988; Benton et al., 1995; Gromadzka-Ostrowska et al., 2002). Food intake and body weight gains were recorded weekly during this period. Animals were fasted overnight prior to euthanasia by CO_2 asphyxiation and cervical dislocation. At the time of euthanasia, trunk blood was collected to obtain serum, which was stored at −20 °C for analysis of serum sex steroid hormone concentrations. Leydig cells were isolated to analyze androgen biosynthetic capacity or stored at −80 °C until processed for gene expression analysis.

2.2. Assessment of steroid hormone secretion capacity

Purified Leydig cells (95–97%) were obtained from testis of HFD- or NFD-fed male rats following collagenase digestion and percoll density gradient centrifugation according to a previously described protocol (Klinefelter and Ewing, 1988; Akingbemi et al., 2004). In all cases, aliquots of 0.3×10^6 Leydig cells were incubated in micro-centrifuge tubes containing DMEM/F-12 culture medium buffered with 14 mM $NaHCO_3$ and 15 mM HEPES (Sigma–Aldrich, St. Louis, MO, USA) and containing ovine luteinizing hormone (LH, 100 ng/ml), 0.1% bovine serum albumin (BSA) (MP Biomedicals, Solon, OH, USA) and 0.5 mg/ml bovine lipoprotein (Sigma) for 3 h at 34 °C.

Serum T and the amounts of Leydig cell T secretion into spent media were measured by a previously described tritium-based radioimmunoassay (RIA) (Cochran et al., 1979; Akingbemi et al., 2004). In order to localize sites of lesion in the androgen biosynthetic pathway, luteinizing hormone receptor (LHCGR), steroidogenic acute regulatory protein (STAR) and steroidogenic enzymes were analyzed in Western blots [i.e., cytochrome P450 side-chain cleavage enzyme (CYP11A1), HSD3B, cytochrome P45017α-hydroxylase/$_{C17/20}$-lyase (CYP17A1), and 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3)]. Because perinatal BPA exposure affected estrogen signaling in adult tissues (Khurana et al., 2000; Wadia et al., 2007), we determined if BPA exposure regulated estrogen metabolism in Leydig cells by assay of serum 17β-estradiol (E_2) concentrations and analysis of ESR1 and EST protein levels in Western blots.