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Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells

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Abstract

Many studies have shown that 2,2-bis 4-hydroxyphenyl propane (BPA), an estrogenic chemical, affects the reproductive health of wildlife and possibly of humans. In this study, we investigated the effects of BPA on steroid hormone production in rat ovarian theca-interstitial cells (T-I cells) and granulosa cells. In T-I cells, BPA increased testosterone synthesis and mRNA expression of 17-α hydroxylase (P450c17), cholesterol side chain cleavage enzyme (P450scc) and steroidogenic acute regulatory protein (StAR) at concentrations of 10^{-7} to 10^{-4} M after a 72 h incubation period. Treatment of granulosa cells with BPA at concentrations of 10^{-7} to 10^{-5} M caused an increase in progesterone levels and P450scc mRNA expression, with an unexpected decrease at 10^{-4} M. BPA (10^{-7} to 10^{-5} M) tended to elevate the expression of StAR mRNA with a significant increase at 10^{-4} M concentration. A significant concentration-dependent inhibitory effect of BPA (10^{-6} to 10^{-4} M) on estradiol levels and the expression of P450arom mRNA was observed. These results suggest that BPA may interrupt ovarian steroidogenesis by altering the steroidogenic enzymes.

Keywords: Bisphenol A; Theca-interstitial cells; Granulosa cells; Steroid hormone; Steroidogenic enzymes

1. Introduction

In the recent years, environmental toxicants have become a serious health concern, and studies on environmental endocrine disruptors are becoming more prevalent. 2,2-bis 4-hydroxyphenyl propane is widely used in the manufacture of polycarbonate plastics and epoxy resins, which are often used as a building block for surface lacquer coatings for reusable milk and food storage containers because of their high impact strength, hardness, toughness, transparency, resistance to temperatures between −40 and 145 °C and resistance to many acids and oils (Staples et al., 1998). When these containers are heated repeatedly, BPA and its derivatives may leach into foods due to incomplete polymerization (Bae et al., 2002; Yamamoto and Yasuhara, 1999; Yoshida et al., 2001; Takao et al., 2002). With the increasing use of products based on epoxy resins and polycarbonate plastics, exposure of humans to BPA has increased dramatically (Kang et al., 2006). A previous report demonstrated that concentrations of BPA could reach as high as 1.49 ± 0.11 and 0.64 ± 0.10 ng/ml in the serum of adult men and women, respectively (Takeuchi and Tsutsumi, 2002a). BPA has also been shown to be present in human serum and follicular fluid at concentrations of 2.0 ± 0.8 ng/ml (mean ± S.D.; non-pregnant), 1.5 ± 1.2 ng/ml (early pregnancy), 1.4 ± 0.9 ng/ml (late pregnancy) and 2.4 ± 0.8 ng/ml (follicular fluid), as well as in fetal serum (2.2 ± 1.8 ng/ml) and amniotic fluid (Ikezuki et al., 2002). BPA has also been detected in the milk of nursing mothers (Sun et al., 2004). Remarkably, the concentration of BPA in amniotic fluid obtained at 15–18 weeks of gestation was shown to be approximately a 5-fold higher (8.3 ± 8.9 ng/ml; $P < 0.0001$) than maternal plasma concentrations). (Ikezuki et al., 2002). A recent study with a reference human population (394 samples analyzed) reported that BPA was detectable in 95% of the samples examined at concentrations ≥0.1 μg/l urine; the geometric mean and median concentrations were 1.33 μg/l (1.36 μg/g creatinine) and 1.28 μg/l (1.32 μg/g creatinine), respectively and the 95th percentile concentration was 5.18 μg/l (7.95 μg/g creatinine) (Calafat et al., 2005). Concentrations of urinary bisphenol A have been shown to be higher in epoxy resin sprayers (median 1.06 μmol/mol creatinine) compared to control subjects (median 0.52 μmol/mol creatinine). Urinary levels of

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conjugated BPA have recently been examined by high-pressure liquid chromatography with fluorescence detection (HPLC/FD), and ranged from 0.03 to 62.4 μg/l (median, 7.86 μg/l) (Yang et al., 2006).

BPA is composed of two unsaturated phenolic rings and resembles diethylstilbestrol, and could leach from autoclaved polycarbonate flasks (Krishnan et al., 1993). Its estrogenic action has been demonstrated (Dodds and Lawson, 1936; Gaido et al., 1997). These findings suggest a role for BPA as a possible endocrine disruptor. Studies have shown that low, presumably environmentally relevant doses of BPA could increase estrous cycle length, and induce morphological and functional alterations in genital system, especially in the ovary. Prenatal exposure of CD-1 mice to either 25 or 250 ng BPA/(kg day) caused an increase in the percentage of ovarian tissue occupied by antral follicles, and a trend toward decreased percentages of corpora lutea in the 3-month-old BPA-treated groups compared to the controls (Markey et al., 2003). Development of polycystic ovaries (PCO, Kato et al., 2003) and the absence of corpora lutea also have been observed in CD-1 mice treated with BPA (Nikaido et al., 2004a, 2005b). Moreover, levels of plasma luteinizing hormone (LH) were decreased in rats exposed to BPA (Rubin et al., 2001). Taken together, these studies suggest BPA can influence the reproduction systerm of rodents animals.

Currently, data on the mechanism of reproductive toxicity of 2,2-bis 4-hydroxyphenyl propane are limited. In this study, we treated rat ovarian theca-interstitial cells and granulosa cells *in vitro* with different concentrations of BPA and observed the effects on steroid hormone synthesis and changes in the expression of selected steroidogenic enzymes.

2. Materials and methods

2.1. Chemicals

The following materials were purchased from Sigma Chemical Co. (St. Louis, MO): 17β-estradiol, bisphenol A, Medium 199 with Hanks' Balanced Salt Solution (HBSS) and $NaHCO_3$, Medium 199 with HBSS (10-strength), McCoy's 5a medium (modified, without serum), L-glutamine, 4-androstene-3,17-dione, Percoll and fetal bovine serum (FBS). Collagenase type I (Clostridium histolyticum, CLS1; 146 U/mg) and DNase I (bovine pancreas; 2298 U/mg) were obtained from Worthington Biochemical Co. (Freehold, NY). The following materials were purchased from Grand Island Biological Co. (Grand Island, NY): trypan blue stain (0.4%; w:v), antibiotic–antimycotic preparation (penicillin, 10,000 IU/ml; streptomycin, 10,000 mg/ml; amphotericin B, 25 mg/ml). Hepes was purchased from American Bioanalytical (Natick, MA).

2.2. Cell culture

2.2.1. Isolation of theca-interstitial cells

Ovarian T-I cells were isolated as described previously (Magoffin and Erickson, 1988; Duleba et al., 1997a). Briefly, ovaries were obtained from immature (25-day-old) female Sprague–Dawley rats (Animal Center of Nanjing Medical University, Nanjing, China) injected with 17β-estradiol (1 mg/0.3 ml sesame oil, s.c.) from 28 to 30 days of age to stimulate ovarian development. This treatment yields ovaries with medium-size follicles and actively dividing T-I cells (Duleba et al., 1999b). On the second day, animals were killed by CO_2 asphyxiation. All of the treatments and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and a protocol approved by the Nanjing Medical University Animal Care Committee. Ovaries were dissected, and the T-I cells were purified using discontinuous Percoll gradient centrifugation. The cells were counted and viability was determined to be between 85% and 95%. The purity of this cell preparation was demonstrated by immunochistochemical staining for vimentin (95.4% ± 0.7%), cytokeratin (7.6% ± 1.6%) and factor VIII (2.2% ± 0.7%) (Duleba et al., 1997a).

2.2.2. Isolation of granulosa cells

Ovaries from mature Sprague–Dawley rats were carefully excised from the surrounding fat pad. Follicles were punctured with a hypodermic needle to extrude granulosa cells into the medium bath. Cells in medium were pooled, centrifuged (5 min, 200 × *g*,), and resuspended in McCoy's 5a containing antibiotics and 10% FBS. The blood cells were separated by 50% Percoll and centrifuged at 600 × *g* for 5 min.

2.2.3. Cell culture

Cells were cultured in McCoy's 5a medium supplemented with L-glutamine (2 mM), 10% FBS, penicillin (10,000 IU/ml), streptomycin (10,000 mg/ml) and amphotericin B (25 mg/ml). Cultures were carried out at 37 °C in an atmosphere of 5% CO_2 in humidified air in 6-well plates (Corning Glass Works, Corning, NY) and 24-well plates (Corning Glass Works). The cultured cells in the 24-well plates were used for hormone analysis and those in the 6-well plates were used for isolation of mRNA.

2.2.4. BPA treatment

After an initial 72 h of culture in McCoy's 5a medium with 10% FBS, the culture medium was carefully removed and replaced with fresh medium. Next, the T-I cells and granulosa cells were simultaneously exposed to different concentrations of BPA. In addition, 4-androstene-3,17-dione (final concentration, 500 nM) was added into the granulosa cell medium as substrate for estrogen biosynthesis. BPA and 4-androstene-3, 17-dione were dissolved in ethanol. 17β-Estradiol (final concentration, 10^{-8} M) was used as a positive control. Ethanol was added

Table 1
The sequence of probe and primer

Gene	Probe	Primer	Concentration (nm)
β-Actin	5′-ACGCGCTCCCCCATGCCATCCTGCGT-3′	F: 5′-TCACCCACACTGTGCCCATCTATGA-3′	200
		R: 5′-CATCGGAACCGCTCATTGCCGATAG-3′	300
P450c17	5′-TTGTCGGACCAAGGGAAAGGCGTG-3′	F: 5′-CCCAGATGGTGACTCAAAGC-3′	350
		R: 5′-GGCTTCCTGACAGATTAGCTTC-3′	400
P450Scc	5′-TCCAGCCAAGACTTTGGTGCAGGTG-3′	F: 5′-TTGTGAACGACCTGGTGCTT-3′	400
		R: 5′-GGAAGTGCGTGGTGTTTTGG-3′	420
StAR	5′-ACGTGGCTGCTCAGTATTGACCTCAAG-3′	F: 5′-TGGCTGGAAGTCCCTCAAAG-3′	350
		R: 5′-GTGGCTGGCGAACTCTATCT-3′	400
P450c19	5′-AGCTCATCTTCCATACCAGGTCCTGGCT-3′	F: 5-TCCTCCTGATTCGGAATTGTG-3′	350
		R: 5-GGCCCGATTCCCAGACA-3′	250