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Effects of bisphenol A given neonatally on reproductive functions of male rats

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Abstract

Male Sprague–Dawley rats (Crj:CD (IGS) were treated neonatally with bisphenol A (BPA) to evaluate effects on reproductive parameters. Animals were given BPA subcutaneously in corn oil to dosages of 0.002–97 mg/kg body weight, or 0.9 mg/kg 17β-estradiol (E2) once a day from postnatal day (PND) 0 to PND 9. Preputial separation, copulatory rate, fertility rate, sperm analysis, serum testosterone levels, and gene expression in the testis were assessed. Males in the E2 group showed a decrease in testis weight and alterations of estrogen-mediated gene expression in the testis on PND 10, and by PND 150 incomplete preputial separation, decreases in the copulatory rate, testicular and accessory organ weights and number of sperm. In contrast, males in all BPA groups showed normal reproductive parameters. These results indicate that in male rats, BPA given during the neonatal period neither affected reproductive function nor evoked estrogen-mediated gene responses in the testis.
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Keywords: Bisphenol A; Low dose; Reproductive function; Neonatal period; Male rat; Gene expression; 17β-estradiol

1. Introduction

The relationship between chemical pollution and reproductive health is of major public health concern. Some etiological studies have disclosed regional declines in sperm count [1,2] and increases in hypospadias [3]; however, some studies have not reported similar findings [4–9]. The relation between chemical pollution and reproductive abnormalities has also been described in wildlife [10]. Such abnormalities have been attributed partly to estrogenic activity of the contaminants [3]. A potent synthetic estrogen, diethylstilestrol (DES) administered as a pharmaceutical agent to pregnant women between the late 1940s and early 1970s in the USA and Europe resulted in an increase in the risk of urogenital tract abnormalities, the so-called DES syndrome, in daughters and sons of DES treated women [11–13].

Bisphenol A (BPA) is used in the manufacture of polystyrene and epoxy resins. It is reported to have weak binding affinity to the estrogen receptors (ER)α and ERβ [14] and scant estrogenic activity when measured by the yeast two-hybrid assay [15]. Nevertheless, BPA has been detected in human urine [16], maternal blood samples [17], fetal placental units [17–20], and ovarian follicular fluids [20].

Oral administration of BPA at very low-dose levels (2 and 20 μg/kg) has been reported to cause reduced sperm production [21] and increased prostate weight [22]. These effects were reported in CF-1 mice exposed orally during prenatal development from gestation day (GD) 11 to GD 17, but comparable to the dose to which humans are usually exposed to in their daily lives. Results with BPA at low-dose levels given to mothers have been inconsistent. Some studies reported reproductive changes in male offspring including a reduction of sperm production

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[23–25], whereas others failed to demonstrate these changes [26–28]. The National Toxicology Program has evaluated the discrepancies in the scientific evidence on low-dose effects [29]. The preponderance of evidence favored the conclusion that there are no low-dose effects of BPA; however, the possibility of a low-dose effect could not be discounted because the effects being measured were subtle and can be influenced by large number of variables that are difficult to control.

Most studies were conducted to expose mothers, but not neonates, to low-dose levels of BPA. In male rats and mice, neonatal treatment of estrogen for 10–30 days results in a long-lasting suppression of spermatogenesis [30,31]; however, if the commencement of estrogen injection is delayed until the 10th postnatal day, then a permanent arrest of spermatogenesis takes place in rats and mice [32,33].

The present study has evaluated the effect of neonatal exposure of low dose of BPA in male rats. BPA treatment was by subcutaneous administration as the dosing route rather than oral administration to replicate exposure conditions of a former experiment that studied BPA in neonatal female rats [34]. Low-dose effects on male reproductive functions were analyzed using end points for general reproductive toxicity studies. Under the hypothesis that male reproductive parameters would be adversely affected by BPA exposure, we analyzed gene expression for the steroidogenic enzymes in the testis. Quantification of gene expression, which was identified by the subpanel of the National Toxicology Program as an additional research to clarify uncertainties [29], is sensitive and easily measured molecular end point.

2. Materials and methods

2.1. Animal rearing and treatment

Pregnant Sprague–Dawley rats (Crj:CD, IGS) were used in this study. The animals were housed individually in cages placed in an animal room on a 12-h light/12-h dark cycle (lighting: 06:00–18:00 h) with controlled temperature (21–25 °C), relative humidity (45–65%), and filter-sterilized fresh air changes. The animals were given free access to CRF-1 diet manufactured from natural raw materials (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. Estrogenic activity of CRF-1 diet is relatively low compared to the other commercially available certified rodent diets in Japan [35]. Twelve dams which spontaneously delivered offspring on the same day were used for each of three tests, in which the neonates were necropsied on PND 10, PND 35, or PND 150. The day of delivery was defined as PND 0. A total of 56 male neonates whose body weights were similar were selected from all male neonates delivered, and eight foster mothers nursed seven male neonates each. Seven males from each litter were allotted to seven groups. The remaining males and all female neonates were not used in this study. The male neonates were given the vehicle (ethanol) only, which served as the control, 24 ng, 120 ng, 600 ng, 3 μg, or 1 mg of bisphenol A [BPA; 2,2-bis (4-hydroxyphenol) propane, Kanto Chemical Co., Inc., Tokyo, Japan], or 10 μg of 17β-estradiol (E2, Sigma Chemical Co., St. Louis, MO) once a day from PNDs 0 to 9. BPA was dissolved in ethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to 500 mg/ml. The solution thus prepared was diluted with ethanol to BPA concentrations of 12, 60, 300, and 1500 μg/ml. Each dilution prepared was mixed with corn oil (Wako) to a concentration of 1/10. The control group was treated with a mixture of ethanol and alcohol at a volumetric ratio of 1:9. E2 was likewise dissolved at a dose of 500 μg/ml in the ethanol–oil solution. The animals were given a subcutaneous injection of 0.02 ml solution dorsally. To test effects of BPA on three different progressive stages, necropsy was performed on PNDs 10, 35, and 150. At all stages, eight male neonates of each group were given the vehicle, BPA, or E2.

Average doses of BPA in the present study were calculated on the basis of the body weight from PNDs 0 to 9 as reported previously [34]: the doses of BPA per kg body weight were 2 μg (1–3.5 μg) in the 24 ng group, 11 μg (4.8–18 μg) in the 120 ng group, 56 μg (24–87 μg) in the 600 ng group, 277 μg (124–429 μg) in the 3 μg group and 97 mg (43–152 mg/kg) in the 1 mg group. The dose of E2 per kg body weight was 0.9 mg (0.4–1.4 mg) in the 10 μg group.

For animals necropsied on PND 10, the serum testosterone levels and the testicular weight were measured, and histological changes and gene expression in the testis were examined. For animals necropsied on PND 35, in addition to the parameters on PND 10, the seminal vesicle, ventral prostate, and epididymis were weighed. For animals necropsied on PND 150, the males were weaned on PND 21, checked for preputial separation daily from PNDs 35 to 70, and bred to untreated females from PNDs 105 to 130 for confirmation of the fertility. On PND 150, all males were sacrificed to measure some sperm parameters in the left cauda epididymis, gene expression in the testis and serum testosterone levels, and to examine histology of the testis and ventral prostate. At the same time, the organs corresponding to those which were weighed on PND 35 were weighed.

The right testis on PNDs 10, 35, and 150, and ventral prostate on PND 150 were fixed in 10% neutral buffered formalin, cut in paraffin at 4 μm, and stained routinely with hematoxylin and eosin for histological examination. The left testis on PNDs 10, 35, and 150, and left cauda epididymis on PND 150 were frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Mating and cesarean section

The males were paired and mated with untreated females at proestrus on a one-to-one basis from the evening (about 17:00 h) to the next morning (about 09:00 h). Females which had sperm in the vaginal smear the next morning were regarded as having copulated. The day when the sperm was found was defined as Day 0 of gestation. Males which failed to copulate were separated once from the females and mated again with other untreated females at proestrus 3 or 4 days later. Thus, the males were mated with untreated females a maximum of four times until evidence of copulation was observed. Females in which copulation had been confirmed were subjected to Caesarean section on Day 13 of gestation and examined for the number of corpora lutea, embryonic mortality, and implantation sites.

2.3. Measurements of serum testosterone levels

The serum testosterone level was estimated by commercially available radioimmunoassay kit (rat testosterone RIA kit, IMMUNOTECH, Marseille, France). Regarding the serum samples obtained on PND 10, the serum from two to three animals in the same group were stored at −20 °C until use. Testosterone was extracted from the serum with diethyl ether in a volume 10 times as much as the volume of the serum in order to concentrate testosterone because the serum testosterone level on PND 10 was under the lower limit of quantitative analysis (5 ng/ml). The organic and aqueous phases were separated by the dry ice–ethanol method. The ether phase was evaporated by ventilation and reconstituted in a serum diluent provided with the RIA kit. In contrast, the serum samples obtained on PNDs 35 and 150 were subjected to the measurement for each animal without extraction.

2.4. Sperm analysis

To prepare semen samples, the right cauda epididymis was cut into strips in culture fluid for sperm (Medium 199 with 0.5% BSA) at 37 °C and left still for about 5 min. The prepared semen samples were examined for: (1) sperm motility and (2) sperm morphology as described below. Suspensions of sperm were prepared using the left cauda epididymis to examine (3) the number of sperm.

2.4.1. Sperm motility

The diluted semen sample was put into a sample chamber (MICROSLIDES, #HTR1099, VitroCom INC., NJ, USA) to calculate the motile sperm rate (%) and progressive sperm rate (%) using TOX IVOS (Hamilton Thorne Research,

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