EFFECTS OF REPEATED *IN UTERO* AND LACTATIONAL TCDD EXPOSURE ON SEXUAL BEHAVIOR AND SEX RATIO OF OFFSPRING

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Introduction

High serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in parents from Seveso, Italy, were linked to their having more girls than boys after the parents were exposed to TCDD that was accidentally released into the environment in 1976[1]. A more intensive analysis on the Seveso data exhibited that an increased probability of female births is related to an increased TCDD concentration in the plasma from the fathers, and that the fathers exposed at ages younger than 19 sired significantly more girls than boys[2]. Earlier studies reported that the reproductive organs of male offspring are susceptible to a single exposure of TCDD on gestational day (GD) 15 in rats. Male offspring exhibited shortened length of ano-genital distance (AGD), reduced ventral prostate weight, daily sperm production, and cauda epididymal sperm numbers, decreased responsiveness of the adult prostate to androgenic stimulation, partially feminized and demasculinized sexual behavior, and feminized patterns of luteinizing hormone regulation. The underlying mechanisms by which TCDD exerts such effects, however, are still unclear. We have reported that *in utero* and the lactational TCDD exposure on GD 15 eliminated sex difference in brain aromatase activity during pre- and postnatal development; this change of brain aromatase activity during a sensitive window for sex differentiation might have induced demasculinized behavior in adult male offspring[7].

Kinetic variations of TCDD are known to exist among many species of animals during different stages of development. When rats were daily exposed to TCDD at a certain dose, the equilibrium in plasma and various organs is reached after several weeks. Thus the elimination half-life of TCDD in rats is calculated to be about 3 weeks. To keep the TCDD body burden as constant as possible, rats were given with an initial loading dose followed by weekly maintenance doses of TCDD[8].

In this study, TCDD were administered orally prior to mating with initial dose at 400 ng/kg followed by weekly maintenance dose at 80 ng/kg during mating, pregnancy, and the lactation period, and the effects of repeated TCDD-exposure on sexual behavior, reproductive organs (F_1), and sex ratio of offspring (F_2) were examined.

Materials and Methods

Materials

Labeled TCDD (2,3,7,8-tetrachloro [U-^{14}C] dibenzo-*p*-dioxin, 47.7 mCi/mmol) was obtained from ChemSyn Laboratories (Lenexa, KS) and dissolved in a corn oil.

Animals and treatment

Holtzman rats were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN) and maintained

in our laboratory under specific pathogen free conditions. Female rats were treated orally with TCDD (400 ng/kg) 2 weeks prior to mating followed weekly with 80 ng/kg during mating, pregnancy, and the lactation period. In control, corn oil was administered (2 ml/kg). The treated females were mated with untreated males. The day of plug positive was considered as Day 0 of pregnancy. Offspring (F_1) were weaned at postnatal day (PND) 28. Some of male F_1 were mated with normal females (see below).

Measurement TCDD concentration

Dams were killed on GD 20, and the concentration of TCDD in the liver, adipose tissue, the ovary and plasma was measured by a liquid scintillation counter following solubilization of samples with Solvable (Packard).

Saccharin preference test

Saccharin preference test was started at PND84. Two weeks prior to the experiment, two bottles filled with water were supplied to each cage for habituation. Three days prior to the experiment no difference in water intake between bottles was confirmed. During the first 3 days of testing, water in one bottle was replaced with 0.25 % saccharin solution. The saccharin concentration was then elevated to 0.5 % at a 3 day-interval. Bottle position was changed daily to exclude possible position preference. Saccharin preference has been expressed in two ways. First, it has been expressed as the amount of saccharin solution consumed per kg body weight (ml/kg). Second, it has been expressed as the ratio of saccharin solution consumed to total fluid consumed (%).

Reproductive organ weight

The male offspring (F_1) were killed on PND120. The testis, epididymis, seminal vesicle, and prostate were excised and weighed. Sperm number in the cauda epididymis was counted.

Effects of TCDD on offspring (F_2)

The male offspring (F_1) from TCDD-exposed or vehicle-exposed dams were mated with untreated females on PND98. TCDD-and control F_2 were killed on PND2, and pups weight, AGD, litter size and sex ratio (% of male) were examined.

Results and discussion

Maternal exposure to TCDD showed no significant effects on mortality, litter size, sex ratio and fetal weight on GD20 and PND2. TCDD was detected in maternal cerebrum (31 pg/g), cerebellum (55 pg/g), adipose tissue (1.8 ng/g), liver (1.2 ng/g), ovary (225 pg/g) and serum (58 pg/ml).

The body weight in control male offspring (F_1) at 12 weeks of age was significantly greater than that in females. TCDD exposure did not influence the body weight of offspring at 12 weeks of age in either sex. Saccharin (0.25 %) intake (ml/kg body weight) was significantly higher in female offspring than in males at 12 weeks of age in the control group while TCDD exposure significantly increased 0.25% saccharin intake at the first day in males compared with that in control males, but no effect of TCDD was observed in females. In the control group, saccharin preference (%) in males was significantly higher than that in females. Saccharin preference (%) in TCDD-treated males significantly decreased than that in control males. In contrast, the preference significantly increased in the TCDD-treated females than in control females. Since sweet preference is a sexually dimorphic behavior, these results suggest that TCDD-exposure in males induced behavioral feminization while it induced behavioral masculinization in females.

In male offspring (F_1) from TCDD-exposed dams, body weight and relative organ weight (% of body weight) of the testis, epididymis and seminal vesicle and sperm number in the cauda epididymis

were not significantly different from those in male offspring from vehicle-exposed dams. However, the relative prostate weight in male offspring from TCDD-exposed dams was significantly lower than that in the control.

Male offspring from TCDD- or vehicle-exposed dams were mated with untreated females, and pups (F_2) were delivered. There were no significant differences in litter size, pups body weight, and pups AGD of F_2 at PND2 between TCDD- and vehicle-exposed groups. However, sex ratio (% of male) was significantly lower in pups (F_2) born from maternally and lactaionally TCDD-exposed male F_1 than that in pups (F_2) from the control male F_1. Every female mated with TCDD exposed male F_1 delivered more females than males.

In conclusion, repeated *in utero* and lactational exposure to TCDD influenced sexual behavior, prostate weight (F_1), and sex ratio (F_2) of subsequent generations.

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