Effects of in utero and Lactational Exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin on Sexual Differentiation in Rats

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Introduction

We have previously reported that *in utero* and lactational exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 200 ng/kg) to malignant Holtzman rats induced demasculinization of sexually-dimorphic behavior and inhibited the development of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in male offspring. However, these effects of TCDD were not observed in higher dose (800 ng/kg) of TCDD-exposed male offspring [1]. The shortening of anogenitgal distance and the decrease of the ventral prostate weight in male offspring by *in utero* and lactational TCDD exposure were reported and these effects of TCDD were observed in a dose-dependent manner [2,3]. This study was undertaken to examine the influence of the TCDD exposure at the varying dosage levels on sexually dimorphic behavior and the development of SDN-POA.

Methods and Materials

Animals and treatments:

Male and female Holtzman rats were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN) and bred in The National Institute for Environmental Studies (NIES). Female rats in proestrus were mated 1:1 overnight with males, and if females that had a vaginal plug in the following morning, that day was designated as gestation day (GD) 0. Dams were housed individually in clear plastic cages with heat-treated wood chips as bedding. TCDD solution, dissolved in nonane at a concentration of 20 g/ml, was diluted in corn oil so that the desired dose of TCDD was delivered at a dose volume of 2.5 ml/kg. The following administration and necropsies were all carried out in the hazardous chemical regulation area in NIES. Pregnant Holtzman rats were given a single oral dose of 50, 100, 200 or 400 ng TCDD/kg body weight or an equivalent volume of vehicle (control) on GD15. Pups number, pups weight and anogenital distances were measured on postnatal day 2 (PND 2). A part of pups were anesthetized with diethyl ether and blood was collected from abdominal aorta. Remaining pups (4~6 pups/dam) were maintained and weaned on PND 28. At weaning, pups were housed in unisexual groups with two

or three rats per cage. The female pups were examined daily for vaginal opening and maturation date. The presence of a vaginal thread was noted after the vagina began to open. Serum testosterone was extracted with five volume of diethyl ether and measured by the EIA kit (Cayman, Ann Arbor, MI).

Saccharin test:

A saccharin test was started at 12 weeks of age. One week prior to the experiment, offspring were housed individually and two bottles filled with water (Milli-Q) were supplied to each cage for habituation. Three days prior to the experiment no difference in daily water consumption between bottles was confirmed. During the first 3 days of testing, water in one bottle was replaced by a 0.25% saccharin solution. Water and saccharin consumption and body weight were measured daily. The saccharin concentration was then elevated to 0.5% then 0.75% at 3 day-intervals, respectively. The bottle position was changed daily to exclude possible position preference.

Measurement of the SDN-POA volume:

After a saccharin preference test, rats were anesthetized with diethyl ether, and blood was collected from abdominal aorta. The brain was dissected and fixed with a 10% neutral formalin solution for more than two weeks at room temperature. The brain was then immersed in a 30% sucrose solution until the brain settled. The brain was frozen by dry-ice powder and sectioned at 30 μm thickness. Each section was stained with cresyl fast violet (1A396, CHROMA-GESELLSCHAFT, Münster, Germany). After a magnified image of each section was captured by Axio Vison (Carl Zeiss co., Ltd, Germany), an area of SDN-POA was traced using Photoshop (Adobe Systems Incorporated, San Jose, CA) and calculated by the NIH image (NIH, Bethesda, MD).

Results and Discussion

PND2:

In utero TCDD (50-400 ng/kg) exposure had no significant effect on litter size, pup body weight and anogenital distance of either male or female on PND2. In male pups, there was large variation in serum testosterone concentration, which was not significantly changed by *in utero* TCDD (50-400 ng/kg) exposure in male pups. In females on PND2, however, the *in utero* exposures significantly decreased the serum testosterone concentration except 200 ng/kg exposure. In the control group, serum testosterone concentration of female pups was one fifth of that in male pups.

Vaginal opening:

In utero and lactational TCDD exposure at 50-200 ng/kg had no significant effect on the vaginal opening age. However, 400 ng/kg TCDD exposure significantly delayed vaginal opening age by 4.5 days. The vaginal thread was observed at 43% of female offspring from dams exposed to 400 ng/kg TCDD. Gray et al. reported that a significant delay of vaginal opening age was observed when Long-Evans Hooded rats were maternally exposed to TCDD at 800 ng/kg on GD15. The percentage of females with a vaginal thread dose-dependently increased to 97% in 800 ng TCDD/kg group from 15% in the control group [4]. These results coincided with our results.

Ventral prostate weight:

After the saccharin test, the ventral prostate was dissected from male offspring on PND98-112 and weighed. *In utero* and lactational TCDD exposure reduced ventral prostate weight (% of body weight) in a dose-dependent manner, and the decrease was significant at 400 ng/kg TCDD as compared with that of control. This result coincided with previously reports [2,3].

Saccharin test:

After the replacement of water to 0.25% saccharin solution, all rats drank only the saccharin solution. Saccharin solution consumption per body weight in the control female was two times greater than that in the control males. In TCDD-exposed males, 0.25% saccharin consumption increased dose-dependently and the increase in the consumption was significant at a dose of 200 ngTCDD/kg. However, there was no significant difference in 0.25% saccharin solution consumption between 400 ng/kg TCDD exposure and control. This difference of the saccharin consumption between the exposed group and the control group was the greatest at 0.25% saccharin, and it became less with the elevation of saccharin concentration. These results suggest that *in utero* and lactational TCDD exposure induced demasculinized behavior at particular concentrations in male offspring. Hany et al. have reported that maternal exposure to a reconstituted polychlorinated biphenyls (PCB) mixture reduced aromatase activity in hypothalamus/preoptic area of newborn male rats and exhibited a behavioral feminization in a saccharin preference test [5]. We have also reported that *in utero* TCDD exposure on GD15 eliminated sex difference in brain (preoptic area) aromatase activity on PND2 [6]. Both dioxins and PCB bind aryl hydrocarbon receptor. Therefore, the two compounds may exert these effects through similar mechanisms.

In female offspring, however, TCDD-exposure decreased saccharin consumption of the 3 concentrations at the doses of 50, 100 and 400 ng/kg, but 200 ng/kg-TCDD did not change the consumption at any saccharin concentrations. The decrease of saccharin consumption in female offspring due to *in utero* TCDD-exposure might suggest defeminization as TCDD-induced demasculinization in male offspring. The relationship of the decrease of saccharin consumption and the decrease of serum testosterone on PND2 was also unclear. It is to be noted that there are sex differences in the sensitivity to TCDD and saccharin concentrations in the saccharin test.

SDN-POA volume:

SDN-POA is differentiated by perinatal estrogen. The volume of SDN-POA in control males was more than 2 times larger than that in control females. In male offspring, the volume of SDN-POA decreased in a dose-dependent manner due to 100 and 200 ng/kg exposure of TCDD. In contrast, the volume was significantly larger than that in control at 400 ng/kg TCDD exposed group. These results coincided with our previously findings [1]. The responses to TCDD-exposure in the volume of SDN-POA was similar to the responses to saccharin consumption in male offspring. Bjerke et al. reported that TCDD exposure dosed at 700 μg/kg on GD15 in Holtzman rat had no effect on the SDN-POA volume in male offspring on PND152-156. But TCDD-exposure increased intromission latencies and a greater number of intromissions prior to ejaculation [7]. It must be elucidated how the sexually dimorphic behavior (saccharin consumption), sex behavior and the SDN-POA volume are related.

Conclusion

The two major results were obtained in this study. First, we confirmed *in utero* and lactational TCDD exposure increased saccharin consumption and decreased the SDN-POA volume in adult male offspring. Their responses to TCDD are varied by the doses with 'inverted U-shaped' curve. This dose-response manner was different from those of the decrease in ventral prostate weight and the delay of vaginal opening age. Second, *in utero* and lactational exposure to TCDD induced defeminization in sexually-dimorphic behavior (saccharin consumption) in female offspring with a t dose-dependent manner different from that of demasculinization in males.

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