EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ON SEXUAL DIFFERENTIATION –

INFLUENCE OF THE *IN UTERO* EXPOSURE ON FETUS BRAIN AROMATASE ACTIVITY AND SEXUAL DIMORPHISMS IN RATS–

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Introduction

Aromatase cytochrome P450 enzyme catalyzes intraneuronal conversion of androgens to estrogens in the hypothalamus-preoptic brain area during fetal development. At a specific time during development, local estrogen formation plays a central role in sexual differentiation. In rats, brain aromatase activity appears after day 16 of gestation (GD), increases sharply after GD 17, peaks on GD 19, then declines to low but detectable levels on the postnatal day (PND) 2 [1]. In general, males display slight-to-moderately higher levels of brain aromatase activity than females during early development. We have reported that *in utero* exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on GD 15 inhibits brain aromatase activity in male fetuses on GD 20, and the sex difference of aromatase activity disappears [2]. This result suggests that the inhibition of brain aromatase activity during peri- and neonatal periods in male rats may inhibit both defeminization and masculinization in adults.

Females tend to consume more sweet solutions than males in many species. A saccharin preference test is used to assess the sexually dimorphic behavior with females normally consuming relatively larger amounts of saccharin solution than males. A saccharin preference test is sensitive in assessing toxic effects of chemicals such as nicotine [3] and ethanol [4] on sexual differentiation in the brain.

The volume of sexually dimorphic nucleus in the preoptic area (SDN-POA) is approximately five times larger in adult males than in adult females [5]. The volume of SDN-POA is increased by perinatal estrogen derived from androgen by aromatase. Pre- and postnatal treatment of female rats with an aromatizable androgen or with estrogen increased the volume of SDN-POA. Pre- and postnatal treatment of male rats with an estrogen antagonist, tamoxifen, inhibited SDN-POA development [6]. These reports suggest that the inhibition of brain aromatase activity during peri- and neonatal periods influences the volume development of SDN-POA.

To examine the effects of TCDD exposure on brain sexual differentiation, changes in rat brain aromatase activity at prenatal and postnatal periods by *in utero* TCDD exposure, and the

consequent influence on sexual dimorphisms of saccharin preference and volume of SDN-POA in mature offsprings were investigated.

Materials and Methods

Materials

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from Cambridge Isotope Laboratories (Andover, MA). [1β-^{3}H]-androsto-4-ene-3, 17-dione ([^{3}H]-androstenedione) was obtained from NEN (Boston, MA) and a testosterone EIA kit from Cayman (Ann Arbor, MI).

Animals and treatments

Holtzman rats were obtained from Charles River Laboratories (Kingston, NY) and maintained in our laboratory under specific pathogen-free conditions. Holtzman rats at 12 to 14 weeks of age were mated, and the day of plug positive was considered GD 0. TCDD dissolved in corn oil was orally administered (200 ng/kg) to pregnant rats on GD 15. Weights and ano-genital (AG) distance of pups were measured on PND 2. Some pups on PND 2 were anesthetized with ether, and blood was collected from the abdominal aorta. Pup brains were dissected and immediately frozen with dry ice powder. Remaining pups were weaned at PND 28 and housed in unisexual groups on litter base.

Assay for brain aromatase activity in brain

Frozen fetal brains were cut into 2 mm thick sections at the anterior and posterior 1 mm each from optic chiasma. The section containing the preoptic area was homogenized in 250 μl of 10 mM potassium phosphate buffer (pH 7.4) containing 100 mM KCl, 1 mM EDTA, 10 mM dithiothreitol and protease inhibitors, and centrifuged for 10 min at 1800 g. Aromatase activity in the supernatant was assayed in terms of released ^3H_2O from ^{3}H-androstenedione as described by Lephart and Simpson[7] and Roselli and Resko[8]. Briefly, the enzyme solution was incubated with a substrate solution containing 10 mM potassium phosphate buffer (pH 7.4), 100 mM KCl, 1 mM EDTA, 10 mM dithiothreitol, 2 mM NADPH, 20 mM glucose-6-phsphate, 2 U/ml glucose-6-phosphate dehydrogenase, 0.3 μM [^{3}H]-androstenedione for 1 hour at 37°C. The reaction was terminated by an addition of $CHCl_3$ and H_2O. The mixture was then centrifuged for 20 min at 1500 g. An aliquot of the supernatant was placed into tubes containing 0.5% dextran T-70 and 5% charcoal, vortexed for 1 min, and centrifuged for 15 min at 9000 g. Radioactivity in the supernatant was counted by a liquid scintillation counter (LSC3100, Aloka, Tokyo, Japan).

Saccharine preference test

A saccharin preference test was started at 12 weeks of age. 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 by a 0.25% saccharin solution. The saccharin concentration was then elevated to 0.5% at 3 day-intervals. Bottle position was changed daily to exclude possible position preference.

Measurement of SDN-POA volume

After a saccharin preference test, rats were anesthetized with ether, and blood was collected from the abdominal aorta. The brain was dissected and fixed with a 10% neutral formalin solution

for 2 weeks at room temperature. The brain was then immersed in a 30% sucrose solution until the brain settled. The brain was frozen with dry-ice powder and sectioned at 30 μm thickness. Each section was stained with cresyl fast violet (CHROMA).

Other methods

Protein concentration was measured as described by Lowry et al [9]. Testosterone in fetal serum was extracted with ether and measured by EIA.

Results and Discussion

There was no significant difference in litter size or sex ratio on PND 2 between control and TCDD treated rats. TCDD exposure did not influence pup weights (litter means), brain weights (litter means) and AG distance at PND 2 in either sex.

Aromatase activity in brain sections was higher in males than in females on PND 2 in the control group. The female / male ratio was 0.75. TCDD exposure decreased brain aromatase activity only in males, and significantly increased the female / male ratio of brain aromatase activity to 0.86, leading to disappearance of sexual difference. Serum testosterone concentration of pups on PND 2 was significantly higher in males than in females in control group. These results coincided with our previous results. TCDD exposure did not change the serum testosterone concentration in either sex.

Body weight in control male offsprings at 12 weeks of age was significantly greater than that of females. TCDD exposure did not influence the body weight of offsprings at 12 weeks of age in either sex. Saccharin (0.25%) intake (ml/kg body weight) was 50% higher in female offsprings than in males at 12 weeks of age in the control group. TCDD exposure, however, increased saccharin intake significantly in males compared with males in the control group. Such preference change was not seen in the TCDD treated females. Since sweet preference is a sexually dimorphic behavior, these results suggest that TCDD exposure in males induced behavioral feminization. Hany et al. reported that maternal exposure of reconstituted PCB mixture to rats decreased aromatase activity in the hypothalamic/preoptic brain region and induced behavioral feminization in a saccharin test [10]. Ziegler et al. reported that nicotine treatment led to a reduction of perinatal brain aromatase activity [11]. These reports indicate that peri- and neonatal inhibition of aromatase activity by TCDD may cause behavioral feminization.

The volume of SDN-POA was significantly greater in males than in females at 14 weeks of age in the control group. TCDD exposure significantly decreased the volume of SDN-POA in males without significant influence in females. Döhler et al. reported that pre- and postnatal treatment of male rats with an estrogen antagonist, tamoxifen inhibited SDN-POA development [4]. They suggested that the development and differentiation of the SDN-POA was primarily under estrogenic control. Our results show that the reduction of brain aromatase activity in TCDD treated males appears to be related to the inhibition of the development of SDN-POA.

In conclusion, *in utero* TCDD exposure on GD 15 eliminated sex difference in brain aromatase activity during pre- and postnatal development, and this change of brain aromatase activity during

a sensitive window for sex differentiation might have induced demasculine behavior in adult male offsprings.

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