Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on brain development and function

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic isomer among a group of dioxin compounds, is a ubiquitous environmental contaminant created by manufacturing and incinerating processes , and exerts a variety of toxicities such as carcinogenicity, immunotoxicity and reproductive/developmental toxicities[1,2]. Because dioxins are transferred transplacentally and lactationally to a developing fetus or newborn from its mother, developmental neurotoxicity in the offspring is a matter of deep concern to society. Epidemiological studies suggested that dioxins might affect brain development and influence cognitive function in humans. Mental retardation and reduced learning abilities in children have been reported to be associated with certain levels of exposure of their mother to dioxins that exceed the currently acceptable environmental levels[3,4]. Exposure of rodents to TCDD leads to various biochemical alterations in central and peripheral nervous systems, such as changes in aromatase activity, plasma adrenocorticotropin to corticosterone ratio, and levels of pituitary luteinizing hormone, c-Fos expression and *glutamic acid decarboxylase* gene expression[5~9]. Furthermore, administration of TCDD to pregnant rats alters the patterns of schedule-controlled operant performances and cortical lateralization differentially in female and male offspring[10,11]. Since perinatal exposure to TCDD affected the manifestation of sexual behavior, it is speculated that TCDD affects sex differentiation of the brain[12]. However, the cellular target and mechanism of action regarding the neurotoxicity of TCDD in laboratory animals are largely unknown.

Thus, we attempted to study whether or not perinatal exposure to TCDD affected sex differentiation of brain of the offspring in the rat in terms of behavior and histology[13]. Next, we had an interest in N-methyl-D-aspartate (NMDA) receptor that have an important role in the advanced brain function, and found that exposure to TCDD during development had continued influence on the advanced brain function of the offspring[14]. Lastly, using mice, we investigated possible changes in gene expression in the brain after exposure to TCDD. Because the developing central nervous system is vulnerable to teratogenic insults, including TCDD, throughout the embryonic and fetal periods, we searched for up- and down-regulated transcripts in the fetal brain of rodents exposed to TCDD *in utero*. Possible mechanisms of how TCDD-induced changes in gene expression in the brain may contribute to the developmental neurotoxicity of TCDD suggested by not only our data but also other people's relevant studies will be discussed.

Methods and Materials

Laboratory animals used in the present study were handled with care according to the guidelines on animal experiments at NIES and University of Yamanashi.

Sexual Behavior: On day 15 of gestation, pregnant Long-Evans Hooded female rats were administered 0, 200, or 800 TCDD ng/kg by gavage. Male rat sexual behavior was assessed on PNDs 87

and 97. Animals were decapitated on PND 120, and brain samples were used to measure the volume of the SDN-POA and determine the TCDD concentration. A part of animals were used for *in situ* hybridization to examine expression of the brain-derived neurotrophic factor (BDNF) and c-Fos mRNA induced by copulation.

mRNA Quantification: On day 15 of gestation, pregnant Long-Evans Hooded female rats were administered 0, 200, or 800 TCDD ng/kg by gavage. On PNDs 5 and 49, the neocortex (NC) and hippocampus (HIP) of offspring were collected for the examination of NMDA receptor NR2A and NR2B subunit mRNA levels by competitive RT-PCR.

Differential display (DD) and localization of SFRP2 by *in situ* hybridization: On gestation day (GD) 12.5, TCDD (5 μg/kg body weight) was administered orally to pregnant C57BL/6N mice by gavage. Pregnant mice that received an equivalent volume of corn oil served as control.

1) DD; Total RNA from the brains of either TCDD-exposed or vehicle-control female fetuses killed on GD 18.5 was analyzed by differential mRNA display with the use of two hundreds and sixteen different combinations of primer. The cDNA clone with a marked difference in levels of corresponding mRNA between TCDD-exposed and vehicle-control fetuses was cloned and used as a probe of northern blot analysis to confirm the change in expression of its corresponding gene by exposure to TCDD.

2) *In Situ* Hybridization; TCDD-exposed and vehicle-control fetuses were collected on GD18.5. Non-radioactive *in situ* hybridization of tissue sections was done as described above with the use of secreted frizzled-related protein 2 (SFRP2) cDNA fragment as a probe.

Results and Discussion

In the sexual behavior test, perinatal exposure to TCDD significantly reduced the number of mounts and intromissions. The mRNA semi-quantification in *in situ* hybridization showed that the mating stimulus in control male rats increased the level of c-Fos and BDNF mRNAs in the frontal cortex and the POA, respectively (Figure 1). In contrast, perinatal exposure to TCDD suppressed the level of mating-induced BDNF mRNA upregulation in the frontal cortex, but not that of c-Fos mRNA in the POA. The volume of SDN-POA was not affected. The results suggested that TCDD affected the neocortical function independently from the brain sexual differentiation and altered the expression of sexual behavior, and that perinatal exposure to TCDD altered the brain function in adulthood.

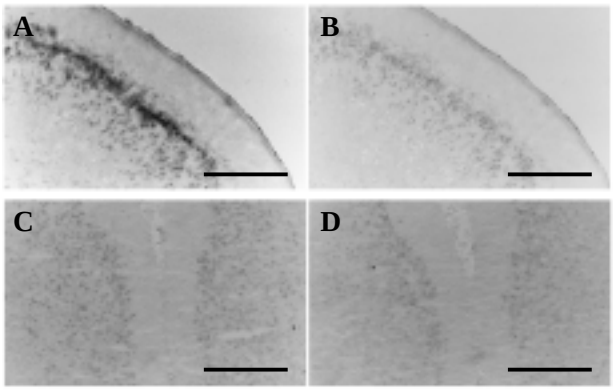
The mRNA quantification by competitive RT-PCR clearly revealed that TCDD inhibited NR2B mRNA expression and enhanced NR2A mRNA expression in the neocortex and hippocampus of the rats on PND 49. The results demonstrate that the perinatal exposure to TCDD can alter the molecular basis of brain of offspring in adulthood (Figure 2).

Next, we studied how TCDD exposure altered the developing brain in terms of the expression of genes by DD. In the search for TCDD-responsive genes in the mouse fetal brain we found for the first time, that *sfrp2* and c-*myc* genes that encode a Wnt modulator and a Wnt target, respectively, were up-regulated by perinatal exposure of mice to TCDD. *In situ* hybridization analysis revealed that the localization of SFRP2 mRNA was asymmetrical around the ventricular zone of the third ventricle in the brain from TCDD-exposed fetuses (Figure 3C) in contrast to the symmetrical localization in the brain from the vehicle-control fetuses (Figure 3B). We suggest that perinatal exposure to TCDD may cause defects in development and function of the mouse brain via the Wnt signal transduction pathway. To our knowledge, this is the first demonstration of the chemically-induced altered gene expression in terms of magnitude and localization in the brain.

Conclusions

The present results suggested that effects of TCDD on sex behavior are exerted not by hypothalamus-mediated mechanism but by neocortex-mediated, or advanced brain function-mediated mechanisms. Perinatal exposure to TCDD affected the level and histochemical localization of SFRP2 mRNA. In the adulthood of the TCDD-exposed offspring, mRNA levels of NMDA receptors were altered in the neorcortex and hippocampus. These changes at the molecular level might permanently affect the advanced brain function of the TCDD-exposed offspring.

Figure 1: *In situ* hybridization of BDNF mRNA in the frontal cortex (A, B) and c-Fos mRNA in the preoptic area (C, D)



BDNF mRNA expression in vehicle-exposed (A) and 800 ng TCDD/kg-exposed (B) males after mating.
c-Fos mRNA expression in vehicle-exposed (C) and 800 ng TCDD/kg-exposed (D) males after mating.

Scale bars, 500 μm.

Figure 2: Effects of perinatal exposure to TCDD on NR2A and NR2B mRNA levels in the neocortex and the hippocampus on PND 49

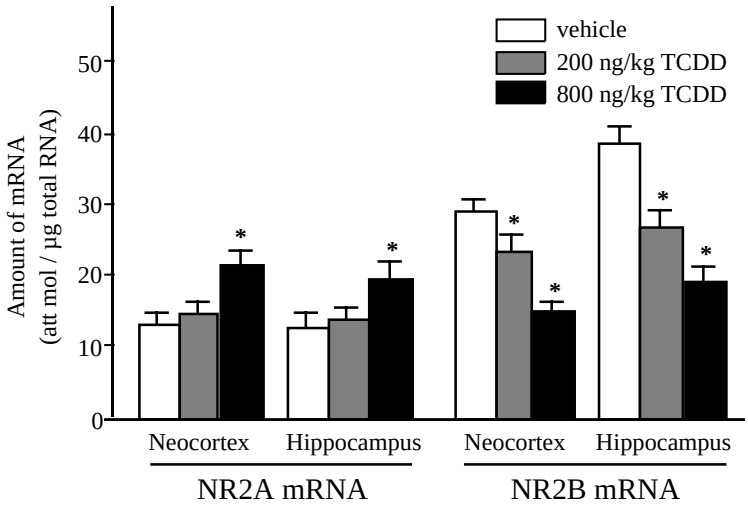
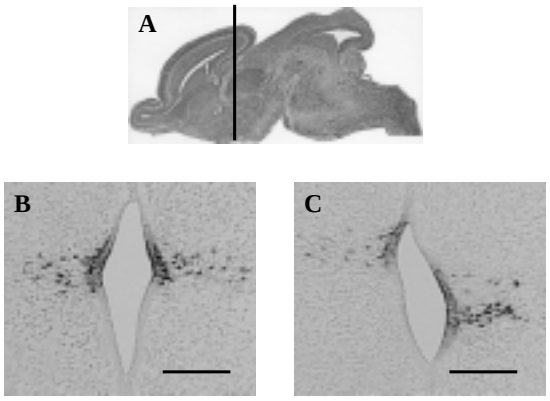


Figure 3: *In situ* hybridization of SFRP2 mRNA in the brain of fetal female mouse on GD 18.5



(A) A vertical bar shows the plane of section shown in B & C.

Scale bars, 100 μm.

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