PERINATAL EXPOSURE TO A LOW-DOSE OF DIOXIN INDUCES DEFICITS IN FEAR CONDITIONING IN MICE

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Abstract

In utero and lactational exposure to dioxins have been reported to affect brain functions of the offspring even when the exposure level is too low to affect their dams. We investigated the effects of the perinatal exposure to a low-dose of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) on the fear memory in mouse offspring. Pregnant C57BL/6J mice were given by gavages TCDD at a dose of 0, 0.6, 3.0 μg/kg, and their male offspring were used for the behavioral test. In the fear conditioning, three tone-footshock pairings were presented. Retention tests for the contextual and auditory fear memory were carried out 1 h after conditioning. The offspring born to dams exposed to 0.6 μg/kg of TCDD showed a significant decrease in freezing response in the contextual, but not the auditory, retention test. On the other hand, the perinatal 3.0 μg/kg of TCDD-exposed group showed deficits in both the contextual and auditory retention tests. The results indicate the perinatal exposure to a low-dose of dioxin disrupted the functions of the memory and emotion in the male mouse offspring.

Introduction

The developing brain is vulnerable to the external influences from the fetal to juvenile period. It has been reported that the maternal exposure to the environmental pollutants such as dioxins affect the brain function of the offspring even when the exposure level is too low to affect their dams[1, 2]. Previous studies reported that *in utero* and lactational exposure to a low dose of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) reduces the mRNA level of the hippocampal glutamate NMDA receptor subunit in the adult rat offspring [3, 4, 5]. Since the hippocampal NMDA receptors are thought to be important for the memory function[6, 7], this finding may be one of the molecular basis of toxic effects of TCDD on the cognitive function of the brain. Regarding the behavioral toxicity, we have reported that the maternal TCDD exposure results in alterations of the learning of the schedule-controlled operant behavioral task[8] and the paired-associate learning task[9] in rat offspring. In addition, we found that the maternal exposure to

TCDD also caused a learning impairment in the adult male mouse offspring[9]. However, in the mice, the toxic effects of the perinatal exposure to TCDD on the cognitive and emotional functions have not yet been fully investigated. Here, we investigated the effects of the perinatal exposure to a low-dose of TCDD on the contextual and auditory fear conditioning in the mouse offspring.

Materials and Methods

Animals and exposure to chemicals

Time-mated pregnant C57BL/6J mice were obtained from CLEA Japan (Tokyo, Japan). They were housed in a temperature- and humidity-controlled room (22-24 ℃, 30-40 % humidity), and were maintained on a 12 h light-dark cycle. Behavioral testing was conducted during the light cycle (08:00–20:00). Food and water were available *ad libitum*. On gestation day 12.5, TCDD (0.6 or 3.0 μg/kg b.w.) in corn oil was administered by gavages to pregnant mice. Pregnant mice which served as Control group were given an equivalent volume of corn oil. Pups were housed with their biological mothers until weaning at 21 days of age. At their 25-28 weeks of age, one male offspring per litter (7 animals in each group) was randomly selected for the behavioral experiment. Animal experiments and handling of hazardous chemicals were performed, according to the guidelines for animal experiments and environmental safety, respectively, at the University of Tokyo.

Apparatus and stimulus

Experiments were conducted in a windowless room containing two conditioning chambers (Med Associates, St. Albans, USA). Each conditioning chamber (32 cm wide, 25 cm high, 25 cm deep) containing a stainless steel shock-grid floor (36 rods, each rod 3 mm diameter, 6 mm apart) and a stainless steel drop pan located within a sound attenuating box (55 cm wide, 50 cm high, 50 cm deep) equipped with a speaker in the side wall. The front, top, and back of the chamber were made of clear acrylic and the two sides made of modular aluminum.

A tone (80 dB, 1800 Hz, 30 s) was presented as a conditioned stimulus (CS) via a speaker connected with a computer running MED Associates software (FreezeFrame) which controlled presentation of the stimuli. An electrical footshock (0.75 mA, 2 s) via a shock generator (Med Associates, St. Albans, USA) was delivered as an unconditioned stimulus (US). Background noise (65 dB) was provided by internal fans.

Behavioral procedure

The mice were habituated to the conditioning chamber for 10 min, 1 day before the conditioning and retention tests were conducted. On the conditioning session, the mice were placed in the conditioning chamber. After a 2 min acclimation period in the chamber, they received three co-terminating CS-US pairings. At 120, 210

and 300 s, the CS sounded for 30 s, and the US occurred during the last 2 s of the CS. The mice remained in the chamber for 60 s after the third CS–US presentation. Retention tests for the contextual and auditory fear memory were carried out 1 h after conditioning session. In the contextual test, the mice were placed back into the same chamber for 3 min in the absence of the tone and the footshock. One hour after the contextual test, the auditory test began. The mice were placed in an alternative chamber (of different shape with white polyvinyl chloride floors and walls) without any stimuli for 2 min, and then subjected to 2 min of the CS tone exposure without the footshock.

Results and Discussion

The mean percent freezing (±SEM) after footshock deliveries during the conditioning session was shown in Fig. 1. In the conditioning session, all groups of animals increased percent freezing as the electrical footshocks were repeated. However, the percent freezing of both TCDD-exposed groups were lower than that of Control group. A two-way analysis of variance (ANOVA) showed statistically significant main effects of group ($F_{(2, 18)}$=4.01, $p< 0.05$) and footshock delivery ($F_{(3, 54)}$=111.18, $p< 0.01$), and a significant interaction of group × footshock delivery ($F_{(2, 54)}$=3.13, $p< 0.05$). Further analyses of simple main effects at each footshock delivery revealed that there were significant main effects of group at the first ($F_{(2, 56)}$=3.03, $p< 0.05$), second ($F_{(2, 56)}$=3.31, $p< 0.05$), and third ($F_{(2, 56)}$=4.39, $p< 0.05$) footshock deliveries. Post-hoc comparison by a Tukey's HSD test for each footshock delivery indicated that TCDD 0.6μg/kg-exposed group (TC0.6) in the first and second footshock deliveries were lower than Control group ($p< 0.05$), and TCDD 3.0 μg/kg-exposed group (TC3.0) in the second and third footshock deliveries were lower than Control group ($p< 0.05$).

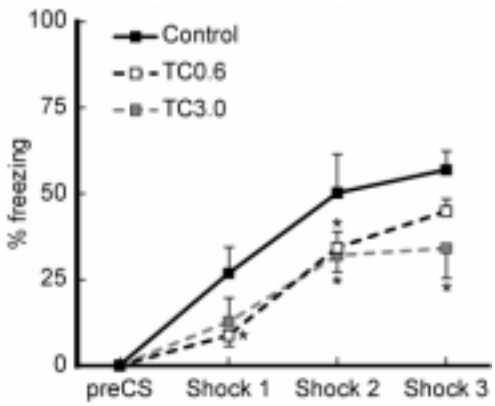

Fig. 1 Mean percent freezing (±SEM) after shock deliveries during conditioning session. Asterisks show significant differences from Control group (*$p< 0.05$). TC0.6 and TC3.0 indicate the offspring born to dams exposed to TCDD at a dose of 0.6μg/kg and 3.0 μg/kg.

The mean percent freezing (±SEM) in the contextual test 1 h after the conditioning session is shown in Fig. 2. Both TC0.6 and TC3.0 groups showed less freezing responses than Control group. A one-way ANOVA showed a significant main effect of group ($F_{(2, 18)}$=11.14, $p< 0.01$). Post-hoc analysis by a Tukey's HSD test indicated that the percent freezing of both TC0.6 and TC3.0 groups were significantly lower than that of Control group ($p< 0.01$).

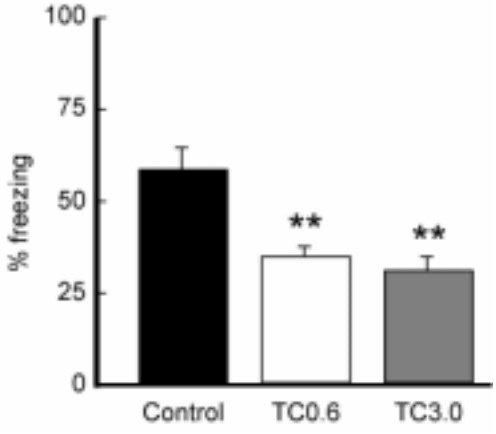

Fig. 2 Mean percent freezing (±SEM) in the contextual test 1 h after the conditioning session. Asterisks show significant differences from Control group (**p< 0.01).

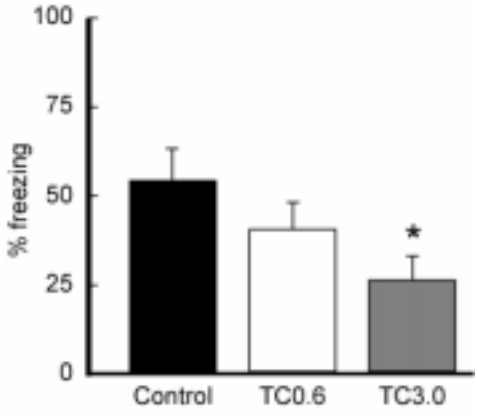

Fig. 3 Mean percent freezing (±SEM) in the auditory test 1 h after the conditioning session. Asterisk shows a significant difference from Control group (*p< 0.05).

The mean percent freezing (±SEM) in the auditory test 1 h after the conditioning session is shown in Fig. 3. Both TCDD-exposed groups again displayed a decrease in freezing response, although TC0.6 group showed a higher freezing response than TC3.0 group. A one-way ANOVA showed a significant main effect of group ($F_{(2, 18)}$=3.55, p< 0.05). Post-hoc analysis by a Tukey's HSD test indicated that the percent freezing of TC3.0 group was significantly lower than that of Control group (p< 0.05).

The present study indicates that even in a low-dose of the perinatal TCDD exposure (0.6 μg/kg b.w.) can affect the memory and emotional functions in mice. Since TCDD-exposed group altered the immediate learning during conditioning and 1 h retention tests of the contextual and auditory fear memory, the present study suggests that the perinatal exposure to a low-dose of TCDD disrupts the formation of the short-term memory and/or the emotional associative learning in the male mouse offspring in adulthood. It has been considered that the hippocampus is responsible for the contextual fear memory[10]. Since previous studies reported that TCDD exposure decreases the hippocampal excitatory postsynaptic potentials in vitro[11], and reduces the mRNA levels of the hippocampal glutamate NMDA receptor in vivo[3, 4, 5], the perinatal TCDD exposure might cause the hippocampal dysfunction. In addition, since the amygdala has critical roles in the contextual and auditory fear conditionings[10], the perinatal TCDD exposure might also alter the function of the amygdala, although TC0.6 group did not show an impairment in the auditory test. To confirm and link between behavioral abnormalities as shown in the present study and brain dysfunction, further studies are needed.

In conclusion, the perinatal exposure to TCDD, even in a low-dose exposure, affected the memory and/or emotional functions, and may alter the function of the hippocampus and/or the amygdala.

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References

1. Kakeyama M., Tohyama C. *Ind Health* 2003; 41: 215.

2. Hauser P., McMillin J.M., Bhatara V.S. *Toxicol Ind Health* 1998; 14: 85.

3. Kakeyama M., Sone H., Tohyama C. *Neuroreport* 2001; 12: 4009.

4. Nayyar T., Wu J., Hood D.B. *Cell Mol Biol (Noisy-le-grand)* 2003; 49: 1357.

5. Hood D.B., Woods L., Brown L., Johnson S., Ebner F.F. *Neurotoxicology* 2006; 27:1032.

6. Morris R.G. *Eur J Neurosci* 2006; 23: 2829.

7. Haberny K.A., Paule M.G., Scallet A.C., Sistare F.D., Lester D.S., Hanig J.P., Slikker W. *Toxicol Sci* 2002; 68: 9.

8. Hojo R., Kakeyama M., Yonemoto J., Tohyama C. *Organohalogen Comp* 2004; 50:194.

9. Kakeyama M., Endo T., Zhang Y., Miyazaki W., Tohyama C. *Dioxin 2008.*

10. Maren S. *Eur J Neurosci* 2008; 28: 1661.

11. Hong S.J., Grover C.A., Safe S.H., Tiffany-Castiglioni E., Frye G.D. *Toxicol Appl Pharmacol* 1998; 148: 7.