PRENATAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) DISTURBED DEVELOPMENT OF TASTE PREFERENCE IN RAT OFFSPRING

Nishijo M[1,5], Kuriwaki J[2,5], Hashimoto A[3], Fukunaga K[3], Nakagawa H[1], Pham T[1], Hori E[2,5], Torii K[4], Nishijo H[2,5]

[1]Department of Public Health, Kanazawa Medical University, Uchinada, 920-0293, Japan; [2]System Emotional Science, Graduate School of Medicine, University of Toyama, Toyama, 930-0194, Japan; [3]Department of Pharmacology, Graduate School of Pharmaceutical Science, Tohoku University, Sendai, Japan. [4]Basic Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan, [5]CREST, JST, Japan

Abstract

The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on higher brain functions remains unclear. In the present study, we investigated effects of TCDD (oral administration 1.0 μg/kg on gestational day 15) on development of taste preference in rat offspring, and the level and/or activity of Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα), which plays an important role in learning and memory.

Rats were free access to six amino acid solutions (Threonine, Glycine, Monosodium glutamate (MSG), Lysine HCl, Histidine, Arginine), saline and distilled water in a choice paradigm. Averaged 24 hours intake of each solution for 5 days, postnatal day (P.D.) 24-28 and P.D.29-33 were compared between exposed and control groups. MSG consumption during P.D.29-33 was significantly lower, and lysine consumption was higher in the female exposed group than those in female controls. A phosphorylated/total CaMKIIα ratio in the amygdala was significantly decreased in the exposed female group, suggesting that TCDD might affect intra-cellular Ca^{2+} and Ca^{2+} transport signaling pathways. The level of phosphorylated CaMKIIα in the orbital cortex was increased in the exposed group. These results suggested that TCDD exposure during pregnancy and lactation disturbs development of amino acid taste preference in female rat offspring through functional changes in the amygdala and orbital cortex.

Introduction

TCDD exposure during pregnancy induced deficits in higher brain functions such as working memory in a radial arm maze,[1] operant responding in running wheels or two-lever chamber,[2,3] and discrimination-reversal learning.[4] We have also reported that TCDD exposure during pregnancy and lactation significantly delayed motor development and affected active avoidance behavior of rat offspring during growth period.[5] These animals displayed a developmental delay in the forebrain, and TCDD was suggested to affect the limbic system that plays an important role in avoidance behaviors.

The amygdala and orbital cortex, main structures of the limbic system, which are implicated in avoidance behaviors, are also involved in various motivated behaviors such as feeding behaviors, taste preference, etc. A previous neurophysiological study reported that the amygdala plays a role in the evaluation of taste palatability.[6] Bilateral lesions of the amygdala and those of the orbital cortex induced changes in food preference and disturbed food choice behaviors based on reward expectancy, respectively.[7,8] Since our previous study suggests that prenatal exposure to TCDD affects development of the limbic system, it might also affect various motivated behaviors. However, effects of prenatal TCDD exposure on taste preference, especially amino acid taste, have not been studied.

In the present study, we investigated an influence of prenatal TCDD exposure on taste preference of amino acids in rat offspring, which were free access to six amino acid solutions, saline and distilled water in a choice paradigm. Furthermore, it has been suggested that taste preference develops based on learning such as conditioned taste preference and aversion.[9] To investigate effects of TCDD on learning processes for development of taste preference in the limbic system, the level and/or activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which plays an important role in learning and memory, [10] were analyzed in various parts of the limbic system including the amygdala, hippocampus, and orbital cortex.

Materials and Methods

TCDD exposure protocol

Ten virgin Wister female rats aged 10 weeks old (body weight, 190-224g) were mated with male rats of same species. On the gestational day (GD) 15, pregnant rats were assigned to the TCDD-exposed group (n=5) and control (n=5) group. The appropriate volume (2-3ml) of TCDD solution dissolved in corn-oil (1.0μg/ml) was administrated to the dams of the TCDD-exposed group based on body weight (1.0μg/kg) by single intragastric injection using an oral cannula. The control group received only corn-oil in same way.

Amino acid preference

After weaning at the 21 post-natal day after birth (P.D.21), each offspring rat of the TCDD-exposed group (16 males and 14 females) and control group (18 males and 11 females) was housed individually in a plastic cage and free access to food as well as seven chemical solutions (0.15M NaCl, 0.4M Threonine, 0.5M Glycine, 0.15M Monosodium glutamate (MSG), 0.2M Lysine HCl, 0.05M Histidine, 0.05M Arginine) and distilled water in a choice paradigm for 2 weeks. Previous studies reported that these solutions were useful to test taste preference in rats.[11,12] The amounts of 24 hours intake of each solution were measured at 5-6 p.m. every day, and the difference in average intakes of various solutions for 5 days from P.D. 24 to P.D.28, and from P.D.29 to P.D.33 between the two groups were examined using student *t*-test.

CaMKII measurement

Since disturbance of taste preference development of amino acids was observed in female rat offspring, CaMKII activity were investigated only in female off-springs. After decapitation, the amygdala, hippocampus, and orbital cortex were quickly dissected and immediately frozen in liquid nitrogen.

Frozen samples were homogenized in a buffer, containing 0.5 % Triton X-100, and insoluble material was removed by centrifugation after sonication. Samples of the supernatant, containing equivalent amounts of protein, were applied to SDS-polyacrylamide gel electrophoresis. Western blotting analysis were carried out using polyclonal antibodies directed against CaMKII and phosphorylated CaMKII as described by Fukunaga et al.[13,14]

Results and Discussion

During P.D.24-28, consumption of distilled water, NaCl, Threonine, Lysine HCl and Histidine was low, whereas that of Glycine was the highest and MSG was second. This pattern of preference was similar in both the TCDD-exposed and control groups. During P.D.29-33, MSG consumption was increased to be the highest among these fluids in both sexes of the control groups. However, MSG consumption during P.D.29-33 in the female TCDD-exposed group was lower than that in the female controls. This indicated that taste preference development was disturbed by prenatal TCDD exposure in female offspring.

Lysine consumption in the female exposed group was significantly higher than that in the female controls. Previously, it has been reported that rats prefer bitter taste under stressful circumstance.[12] Therefore, TCDD exposure, a kind of chemical stress, might let rats prefer lysine, because lysine tastes bitter. There was no significant difference in fluid intakes between the exposed and control groups in the male offspring. Only the female rats showed deviant development of taste preference.

Sexually demographic behaviors such as preference of sweet solution are organized under hormonal influence, and TCDD exposure altered adult expression of saccharin preference behavior in female rat offspring exposed in prenatal and during lactation period.[15] Taste of amino acids, especially glutamate anion, activate taste neurons sensitive to sweetness in the brainstem taste area.[16] This taste area sends taste information to the amygdala that palys a role in evaluation of taste preference.[6] Disturbance of development of amino acid preference in the present study suggested changes in neuronal activity in the limbic system, especially in the amygdala.

Although there was no difference in levels of phosphorylated CaMKIIα in the amigdala between the exposed and control groups, a ratio of phosphorylated CaMKIIα to total CaMKIIα was significantly decreased in the exposed female group, as compared with the controls. These findings suggest that TCDD might affect intra-cellular Ca^{2+} and Ca^{2+} transport signaling pathways. Mitsui et al. reported that prenatal TCDD exposure impaired activation of cyclic AMP response element-binding protein (CREB) in the hippocampal CA1 region in rat offspring that showed deficits in learning of fear conditioning.[17] Since CREB activity is regulated by CaMKII, these results are consistent with our results and suggest that learning processes for taste preference in the amygdala are disturbed in the TCDD-exposed female group.

The level of phosphorylated CaMKIIα in the orbital cortex was increased in the TCDD-exposed group, but

there was no significant difference in the ratio of phosphorylated CaMKIIα to total CaMKIIα between the TCDD-exposed and control female groups. These results suggest that TCDD may increase neuronal activity in the orbial cortex without affecting intra-cellular Ca^{2+} circumstance. Therefore, hyperactivity of the orbital neurons might affect taste preference. In conclusion, the present results suggest that TCDD exposure during pregnancy and lactation disturbs development of amino acid taste preference in female rat offspring through functional changes in the amygdala and orbital cortex.

References

1. Seo BW, Sparks AJ, Medra K, Amin S, Schantz SL. *Neurotoxicol Teratol* 1999; 21:231-239.
2. Markowski VP, Zareba G, Stern S, Cox C, Weiss B. *Environ. Health Perspect* 2001;109:621-627.
3. Markowski VP, Cox C, Preston R, Weiss B. *Neurotoxicol Teratol* 2002; 24:209-218.
4. Schantz SL, Bowman RE. *Neurotoxicol Teratol* 1989; 11:13-19.
5. Nishijo M, Kuriwaki J, Hori E, Tawara K, Nakagawa H, Nishijo H. 2007 (in submittion)
6. Nishijo H, Uwano T, Tamura R, Ono T. *J Neurophysiol* 1998; 79:21-36.
7. Murray EA, Gaffan EA, Flint RWJr. *Behav Neurosci* 1996; 110:30-42.
8. Izquierdo A, Suda RK, Murray EA. *J Neurosci* 2004; 24:7540-8.
9. Myers KP, Sclafani A. *Develop Psycobiol* 2006; 48:380-8
10. Kaitsuka T, Fukunaga, Soeda F, Shirasaki T, Miyamoto E, Takahama K. *Neurosci* 2007; 144:1415-1424.
11. Tabuchi E, Ono T, Nishijo H, Torii K. *Physiol behav*, 1991; 49: 951-964.
12. Kondoh T, Nishijo H, Takamura Y, Torii K, Ono T. *Behav Neurosci*, 1996; 110:1187-1192.
13. Fukunaga K, Goto S, Miyamoto E. *J Neurochem* 1988; 51:1070-1078.
14. Fukunaga K, Horikawa K, Shibata S, Takeushi Y, Miyamoto E. *J Neurosci Res* 2002; 70:799-807.
15. Amin H, Moore RW, Peterson RE, Schantz SL. *Neurotoxicol Teratol* 2000;22:675-682.
16. Nishijo H, Ono T, Norgren R. *Physiol Behav* 1991; 49:965-971.
17. Mitsui T, Sugiyama N, Maeda S, Tohyama C, Arita J. *Neurosci Lett* 2006; 398:206-210.

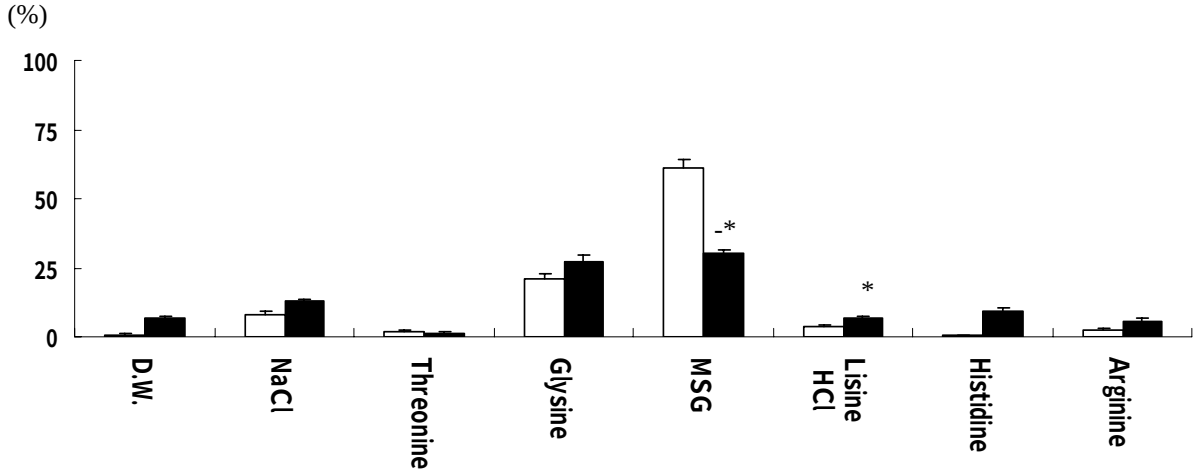

Figure 1 Comparisons of average intakes of various solutions in female offspring for 5 days from P.D.29 to P.D.33 between prenatal TCDD exposed (N=14) and control (N=11) groups. Intake of each solution was expressed as percentage of each solution / total fluid intake.
D.W., distilled water; MSG, monosodium glutamate
¶ -*, significant decrease ($P<0.05$); *, significant increase ($P<0.05$) as compared with the controls

Table 1 Comparisons of Ca2+/calmodulin-dependent protein kinase IIα (CaMKIIα) activity in various parts of limbic system.

	N	phosphorylated mean	SD	total mean	SD	p/t mean	SD	
Amygdala								
control	3	1798.7	428.8	2122	432.4	0.8437	0.0348	
Dioxn	5	2232	449.4	3172.2	376.3 *	0.6988	0.0721	-*
Hyppocampus								
control	3	570.7	394.3	3275	509.9	0.1817	0.1431	
Dioxn	5	1033.6	270.4	3169.2	506.3	0.3328	0.1005	
Orbital cortex								
control	3	3371.3	71.1	3902.7	396.5	0.8687	0.0741	
Dioxn	5	4126.6	349.8 *	4166.4	336.2	0.9992	0.1585	

N: number, SD: standard deviation, p/t: a ratio of phosphorylated / total CaMKIIα
¶ -*, significant decrease (P<0.05); *, significant increase (P<0.05) as compared with the controls