Effect of Tributyltin Compound on *N*-methyl-*D*-aspartate (NMDA) Receptors in Brain of Preweanling Mice

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Abstract

Objective: The aim of this study was to investigate the effect of tributyltin (TBT) compound on *N*-methyl-*D*-aspartate (NMDA) receptors in the brains of preweanling mice.

Methods: Pregnant ICR mice were exposed to TBT chloride at concentrations of 0, 15, and 50 ppm in water. Male offspring were sacrificed at 1, 2 and 3 weeks after birth. Mouse brain membranes were prepared from cerebral cortices, and the specific binding of [³H]MK-801 to an NMDA receptor was determined by radioligand binding assay.

Results: The mean body weight of preweanling mice of the 50 ppm dose group decreased by 17–25% ($p < 0.01$) at 1, 2 and 3 weeks of age, compared with that of preweanling mice of the corresponding control group. The [³H]MK-801 binding level significantly decreased ($p < 0.05$) in the 15 ppm F1 group at 1 week and in the 15 ppm and 50 ppm F1 groups at 3 weeks of age, compared with that in the corresponding control F1 group.

Conclusions: The exposure to TBT via placenta and dam's milk seriously affected not only the growth of preweanling mice, but also the F1 cerebral NMDA receptors involved in memory and learning.

Key words: tributyltin, neurotoxicity, mouse, NMDA receptor, [³H]MK-801

Introduction

Tributyltin (TBT) compounds have been widely used as biocide and antifouling agents. Restrictions on the application of TBT compounds as vessel antifouling agents have been introduced lately. However, pollution in fish and shellfish, marine water and sediment has been reported worldwide (1). Many organotin compounds, such as trimethyltin and triethyltin, have already been studied extensively for their neurotoxic effects (2). In a previous study, we showed that subacute dietary exposure of mice to 125 ppm TBT chloride inhibited the binding of a ligand to a *N*-methyl-*D*-aspartate (NMDA) receptor, and we suggested that the modulation induced neurotoxicity affecting learning and causing memory deficits (3). A single acute exposure to a nonlethal dose of TBT chloride could cause

a significant change in rat behavior (4). Recently, Tsunoda et al. (5) have reported the altered metabolism of dopamine in the midbrain of mice treated with TBT chloride due to subacute oral exposure. These findings indicate that these TBT derivatives are behaviorally toxic and/or neurotoxic. In the present study, the neurotoxicity of TBT in F1 offspring exposed to TBT via placenta and dam's milk was examined by determining the specific binding of [³H]MK-801 to the NMDA receptors in the mouse brain.

Materials and Methods

Animals

ICR mice that were pregnant for the first time were purchased from Nihon SLC (Shizuoka, Japan). The crossing started at 10 weeks of age, and mice were shipped at 12 weeks of age. Pregnant mice were randomly assigned to control or treatment groups. The care and treatment of mice were in accordance with the guidelines established by the National Institute of Health Sciences and were approved by the ethics committee. The mice were decapitated without anesthetic to avoid possible alteration in receptor levels.

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Treatment

Dams were administered 15 or 50 ppm TBT chloride in drinking water. The number of dams per group was 4 for the control and 15 ppm groups, and 5 for the 50 ppm group. The mice ate food and drank water *ad libitum*. The preliminary experiment showed that there were no significant differences between the control, 5, and 50 ppm dose groups. F1 offspring were exposed to TBT via placenta and dam's milk. The exposure was continued until the time when the offspring were weaned.

Sampling

Five male offspring from each group were used at the ages of 1, 2, 3 weeks. One offspring per dam of each group was used at each time of sampling. After checking the body weight, each offspring was decapitated, and its brain was removed and weighed. The cerebrum was dissected on ice according to the method of Glowinski and Iversen (6).

Binding assays

Mouse brain membranes were prepared from cerebral cortices according to the procedure described by Ransom and Stec (7). A noncompetitive antagonist [3 H]MK-801 was used as a radioligand. The membranes were incubated with 5.0 nM [3 H]MK-801 at room temperature for 60 min. The reaction was terminated by rapid filtration over a glass-fiber filter (Whatman GF/B filter) using a cell harvester. The filters were placed in counting vials, 2 ml scintillation fluid was added and radioactivity was counted in a liquid scintillation analyzer (Aloka model 3500). Specific binding was defined as the difference between total binding and nonspecific binding.

Protein assay

Protein measurements of the membrane preparations were performed using the method of Bradford (8) with a Bio-Rad Protein Assay kit (Nippon Bio-Rad Laboratories, Tokyo).

Statistics

Control and treatment group data were compared by one-way analysis of variance (ANOVA) using StatView for Macintosh, version 5.0 (SAS Institute Inc. Cary, N.C., USA). Fisher's PLSD test was used as the post hoc test. The level of $p < 0.05$ was considered statistically significant.

Results and Discussion

The body weight of preweanling mice was recorded for 3 weeks from their birth, and the mean values of the control F1 and TBT treated mice are shown in Fig. 1. The body weight of the 50 ppm dose group decreased by 17–25% ($p < 0.01$) at 1, 2 and 3 weeks of age, compared with that of the corresponding control group. The weight of the mice decreased in a dose-dependent manner, but there were no significant differences between the control and 15 ppm dose groups. These results clearly demonstrate that the exposure to TBT via placenta and dam's milk severely affects the growth of preweanling mice. On the other hand, the body weights of dams exposed to 15 and 50 ppm TBT were not significantly different, from that of

Fig. 1 Effect of TBT on weight of preweanling mice exposed via placenta and dam's milk. Mean $\pm$ SEM ($n=5$) was indicated for each treatment group. **: $p < 0.01$.

Fig. 2 Effect of TBT on NMDA receptor binding sites in brain of preweanling mice exposed via placenta and dam's milk. Mean $\pm$ SEM ($n=5$) was indicated for each treatment group. *: $p < 0.05$.

control mice at 3 weeks after delivery (data not shown). These results suggest that the adverse effects of TBT exposure of pregnant mice appear stronger in preweanling mice than in dams. In our previous studies (3), the mean body weight of adult male mice fed 125 ppm TBT for a month was significantly lower than that of the control for about the first 2 weeks, but was not significantly different during the last 2 weeks. Compared with the results of adult male mice, the adverse effects of TBT also appeared stronger in preweanling mice.

The binding of [^3H]MK-801 to the cortical membrane preparations from each mouse was measured at a final concentration of 5 nM, using 10 μM MK-801 to define nonspecific binding. The [^3H]MK-801 binding level significantly decreased ($p < 0.05$) in the 15 ppm F1 group at 1 week and in the 15 ppm F1 and 50 ppm F1 groups at 3 weeks of age, compared with that in the corresponding control F1 (Fig. 2). According to these results, it is highly possible that TBT exposure at 3 weeks of age via placenta and dam's milk has an adverse effect on the F1 cerebral NMDA receptors involved in memory and learning. However, the dose-response relationship at 1 or 2 weeks of age was unclear; thus, further study is required to clarify the neurotoxic effect of TBT on these age groups. In a previous study, we pointed out that subacute dietary exposure to 125 ppm TBT chloride inhibited the binding of a ligand to an NMDA receptor in the brain (3). In the current study, lower doses inhibited the binding of a ligand to a cerebral NMDA receptor of the F1 mice. It is suggested that the neurotoxic

effects of TBT appear stronger in preweanling mice exposed via placenta and dam's milk than in adult mice exposed to TBT via food. Aou et al. (9) examined the neurotoxic effects of TBT in F1 rats by the dietary administration of TBT chloride and showed that the toxic effects on behavior were induced in offspring exposed to 5 ppm (0.4 mg/kg/day) TBT.

From the results of this study, it is suggested that TBT adversely affects both the growth and central nervous system of F1 offspring mice more than those of adult mice. Further studies are required to determine the neurotoxic effects induced by long-term exposure and the developmental neurotoxicity of TBT.

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