Neurotoxicology and Teratology 30 (2008) 462–467

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Effects of methylmercury on postnatal neurobehavioral development in mice

Yu Gao [a,b], Yu Ding [a], Rong Shi [a], Ying Tian [a,b,*]

[a] Environmental Health Department, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Shanghai Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine, Shanghai Institute for Pediatric Research, Shanghai Key Laboratory of Children's Environmental Health, Shanghai, China

ARTICLE INFO

Article history:
Received 11 March 2008
Received in revised form 29 May 2008
Accepted 6 July 2008
Available online 20 July 2008

Keywords:
Methylmercury
Morris water maze
Neurobehavioral development
NMDA receptors

ABSTRACT

Eighty ICR mice were randomly assigned to one of four groups given daily intraperitoneal injections of 0, 0.1, 1 or 3 mg/kg MeHg chloride respectively from postnatal days (PD) 15–17, and then tested with the Morris water maze on PD45. After that the mice were sacrificed by cervical dislocation, and the protein levels of NMDA receptor subtypes in the hippocampus were measured by Western blot analysis. A significant increase in the latency (F=2.88, P<0.05) before finding the platform was observed in the 1 and 3 mg/kg MeHg exposure groups. Further, the 3 mg/kg MeHg exposure group also had a longer swim distance (F=2.97, P<0.05) for finding the platform. In the probe test, the MeHg exposure groups displayed a smaller number of platform crossings when the hidden platform was moved, but this did not reach statistical significance. Western blot analysis results showed significant increases in the levels of NR1, NR2A and NR2B proteins of the hippocampus in the 1 and 3 mg/kg MeHg exposure groups. Overall, the current study found that MeHg exposure at 1 and 3 mg/kg doses during the postnatal brain growth spurt induces subtle and persistent learning deficits, and the neurobehavioral abnormalities of MeHg-exposed mice might be ascribed to alteration of the gene expression of specific NMDA receptor subunits in the hippocampus.

1. Introduction

Mercury is a ubiquitous environmental toxin that causes a wide range of adverse health effects in humans. Inorganic Hg occurring naturally or from pollution can be converted to Methylmercury (MeHg) by microorganisms, which is then bioaccumulated through the food chain. MeHg is highly and selectively toxic to the central nervous system [1]. While it is well established that fetuses and neonates are high-risk groups for MeHg exposure [1], the evidence for developmental impairments associated with lower level exposure is less clear. Epidemiological studies focusing on communities with high MeHg-contaminated fish consumption have suggested that subtle alterations in child motor and cognitive behaviour are related to prenatal MeHg exposure [2]. More recently, the results of our human neonatal study showed that increased prenatal Hg exposure was associated with decreased behavioral ability for males, but not for females, and provide some support for the hypothesis that there is neurodevelopmental risk from prenatal MeHg exposure resulting from fish consumption [3]. However, another large cohort study conducted in the Seychelles found no apparent neurodevelopmental risk from prenatal MeHg exposure resulting solely from ocean fish consumption [4]. Therefore, results from studies of MeHg exposure and children's development are contradictory. From currently available data, it is difficult to say conclusively whether there is an association between prenatal exposure to low levels of MeHg and adverse effects on children's development.

In animal experiments, one of the most frequent findings related to prenatal MeHg exposure is represented by learning and memory deficit. The behavioral and spatial learning deficits have been observed in lactation and *in utero* animal models of MeHg exposure [5,6]. Recently, results of Coluccia et al. [7] show that low level exposure to MeHg during the postnatal brain growth spurt in mice induces subtle and persistent motor and learning deficits, further underlining the serious potential hazard for exposed children. The postnatal brain spurt in mice is a critical period for neurodevelopment since the maturation of astrocytes occurs during postnatal developmental stages, which correspond to the third trimester of pregnancy in humans [7]. Past research has shown that the critical postnatal brain spurt in rodent is around PD16 [8]. However, little information on the neurobehavioral effects of MeHg exposure during the postnatal brain growth spurt in mice is available in the literature.

Many studies on the potential mechanisms of MeHg neurotoxicity during prenatal development have been conducted. Several studies have demonstrated that MeHg-induced uptake inhibition of glutamate occurs [9], and causes hyperactivation of NMDA receptors [10]. It is now commonly accepted that activation of NMDA receptors plays a pivotal role in mediating learning and memory [11]. Taking these facts together, it is likely that NMDA

* Corresponding author. No 280 South Chongqing Road, Environmental Health Department, Shanghai Jiao Tong University School of Medicine, 200025 Shanghai, China. Tel.: +86 21 63846590x776197; fax: +86 21 64663944.
E-mail address: tianmiejp@yahoo.com.cn (Y. Tian).

0892-0362/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2008.07.004

receptors play a major role in MeHg neurotoxicity during development [12,13]. NR-1, NR-2, and their subtypes are the two most important families of NMDA receptors. However, available data on changes in gene expression of the NMDA receptor subtypes related to MeHg exposure are few.

Therefore, in the present experiment, we exposed mice to different levels of MeHg during the postnatal brain growth spurt and assayed the effects upon spatial learning and memory in the later life stages of offspring using the Morris swim maze, examined the gene expression of NMDA receptor subunits in the hippocampus, and tested the hypothesis that the cognitive dysfunctions observed after MeHg postnatal exposure correlate with changes in the levels of expression of various NMDA receptor subtypes.

2. Methods

2.1. Chemicals

Methylmercury chloride was obtained from Sigma-Aldrich Company (Ireland). Polyclonal antibodies for NMDAR1 (Catalog Number AB1516), NMDAR2A (Catalog Number AB1555), and NMDAR2B (Catalog Number AB1557) subunits were purchased from Chemicon Company (Chemicon/Millipore, Inc., Billerica, MA, USA). The secondary antibody, anti-rabbit IgG-HRP-linked whole antibody, ECL Western blotting detection reagent, and all other reagents were analytical grade from Sigma Company (Sigma-Aldrich Co., St. Louis, MO, USA).

2.2. Animals and treatments

The experimental protocol was conducted in accordance with the guide for the care and use of laboratory animals, provided by the Chinese Academy of Science, Shanghai, China. Primiparous 10-week-old ICR mice (Chinese Academy of Science, Shanghai, China) were used. The animals were allowed to acclimatize for one week, with free access to food and water. They were housed at constant room temperature (20–22 °C) and exposed to a light cycle of 12 hour days (08:00 h–20:00 h). In the late afternoon, pairs of females were placed with single male mice. The day on which a plug was present was designated day 0 of gestation (GD 0). Each litter was culled to 4 males and 4 females on postnatal day (PD) 4. In each litter, 4 males and 4 females were randomly assigned to four groups (one control and three MeHg exposure groups), with one male and one female for each group, in this way, totally eighty pups from ten litters were divided into four groups, consisting of 10 males and 10 females each, and maintained with their mothers before weaning. From PD15–17, the three MeHg exposure groups consisted of mice that were given a daily intraperitoneal injection of 0.1, 1 or 3 mg/kg MeHg chloride, respectively, while the control group was administered sterile saline. The MeHg dose was based on previous studies [14–16]. The general condition and body weight of the offspring were evaluated periodically.

2.3. Morris water maze

Spatial learning and memory were evaluated in 45-day-old mice using the Morris water maze test. The swim maze was a black circular pool (90 cm in diameter) filled with clear water (22–25 °C). A circular platform (7 cm in diameter) was placed 1 cm below the surface of the water in the middle of the northeast quadrant. Distal visual cues were placed on the walls around the pool. A video camera connected to an image analyzer recorded the following measures: the swim path, the time to reach the platform (escape latency), the swim distance to reach the platform, and the swim speed.

One day before training, the animals were habituated to swimming for 60 s in the pool without a platform. Visual cues were placed around the tank, and the platform remained in a fixed location. During acquisition training, four trials were conducted daily for four consecutive days. A trial consisted of gently placing the mouse by hand into the water, facing the wall of the pool. A different starting point was used on each of the four daily trials. The order of the starting point was pseudorandom, but the same for all animals. After finding the platform, the mice were allowed to remain there for 30 s, then held gently and dried for another 30 s until the next trial. If the mouse failed to locate the platform within 120 s, it was guided by hand to the platform. The 60-second retention probe test was performed 24 h after the last training session. This test was identical to the training sessions, with the exception that the platform was removed during a trial. All Morris water maze tests were performed between 08:00 and 12:00 h. The set-up and procedure are described in detail elsewhere [16,17].

2.4. Western blot analysis

Twenty-four hours after the probe trial test, three mice from each group were sacrificed by cervical dislocation, and the hippocampus was quickly removed and immediately cooled in liquid nitrogen, then kept at −80 °C for Western blot studies. Western blotting was performed as described elsewhere [18,19]. Hippocampi were homogenized in lysis buffer (5 mM Tris–HCl, 1 mM phenylmethylsulfonyl fluoride, 150 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS, and protease and phosphatase inhibitors; pH 7.5). The homogenates were centrifuged for 20 min at 12,000 g at 4 °C. The quantity of protein in the supernatants was determined using a BCA (bicinchoninic acid) protein assay kit (#23227, Pierce Co., Rockford IL, USA). Protein extracts (60 μg per sample) were denatured in Laemmli sample loading buffer (10% SDS, 0.1 M Tris pH 8.0, 50 mM DTT, 3 mM EDTA, 0.001% bromphenol blue) at 100 °C for 5 min, separated by 12% SDS-PAGE and then transferred to a nitrocellulose membrane. After blocking with 5% skim milk in TBS-T (0.1% Tween 20, 20 mM Tris–HCl, 137 mM NaCl, pH 7.6) for 2 h, the membrane was incubated with one of the following antibodies at the concentration of 1:200: rabbit NMDA NR1, NMDA2A, and NMDA NR2B in TBS-T overnight at 4 °C. The membranes were rinsed 4 times, for a total of 20 min in TBS-T, incubated in goat anti-rabbit HRP-conjugated secondary antibody diluted in blocking buffer for 2 h at room temperature, and rinsed 5 times for 35 min total in TBS-T. The immunoreactive bands were visualized using ECL (enhanced chemiluminescence) and Hyperfilm (Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA). Relative changes in the polypeptide levels were evaluated by measuring the intensity of the immunoreactive bands using a densitometer and MCDI software (Kodak Digital Science and 1D Image Analysis Software; Rochester, NY). The membrane blots were stripped and re-probed with polyclonal anti-β-actin. Relative expression levels of the different NMDAR subunit proteins were normalized by computing the ratio of NMDAR subunits toβ-actin, respectively.

2.5. Statistical analysis

SPSS (Statistical Package for the Social Sciences) 11.5 for Windows (SPSS Inc., Chicago, Illinois) was used for the statistical calculations. Descriptive statistics were calculated for each variable. The results obtained in the experiments were expressed as the mean ± SEM, unless otherwise stated. The comparison among control and MeHg-exposed groups was carried out by means of one-way ANOVA, followed by Tukey's posthoc test for body weight. Morris water maze data during the four days were analysed with repeated measures ANOVA, with the treatment as a between-subjects factor and that days as the repeated measures factor, followed by Tukey's posthoc test. We used the Kruskal–Wallis H test for comparing the number of platform crossings in the probe trial. $P<0.05$ was considered significant.