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Prenatal melamine exposure impairs spatial cognition and hippocampal synaptic plasticity by presynaptic and postsynaptic inhibition of glutamatergic transmission in adolescent offspring

Lei An[a,b,c,*], Wei Sun[a,b,*]

[a] South China Research Center for Acupuncture & Moxibustion, Guangzhou University of Chinese Medicine, 510006 Guangzhou, China
[b] Medical College of Acupuncture-Moxibustion and Rehabilitation, Guangzhou University of Chinese Medicine, 510006 Guangzhou, China
[c] Department of Physiology, University of Saskatchewan, S7N 5E5 Saskatoon, Canada

HIGHLIGHTS

- Study focuses on the neurotoxic effects of prenatal melamine exposure (PME).
- Behavioral test, electrophysiological recordings and ELISA method have been used.
- PME has neurotoxic effect on hippocampus, induced cognitive defects.
- PME inhibits hippocampal function by altering pre- and post-synaptic transmission.

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ABSTRACT

Our previous studies showed that prenatal melamine exposure (PME) could impair spatial cognition and hippocampal long-term potentiation (LTP). More importantly, the synaptic dysfunction induced by PME was associated with the probability of presynaptic glutamate release. Considering the crucial role of the other form of synaptic plasticity, long-term depression (LTD), in some types of learning and memory process, the aim of present study was to investigate if the hippocampal LTD and cognitive flexibility were affected. And then we attempted to explore the underlying mechanism. The animal model was produced by melamine exposure throughout gestational period with 400 mg/kg bodyweight, the male offspring rats were used in the study. Morris water maze (MWM) test was performed, and then LTD was recorded from Schaffer collaterals to CA1 region in the hippocampus. Behavioral test showed that learning, reference memory and re-acquisition learning abilities were impaired significantly by PME. The field excitatory postsynaptic potentials (fEPSPs) slopes of LTD were significantly higher after PME. Furthermore, the data of whole-cell patch-clamp experiments showed that PME markedly diminished the frequencies of spontaneous EPSCs (sEPSCs) and simultaneously reduced the amplitude of sEPSCs. In conclusion, PME inhibited glutamate transmission presynaptically and postsynaptically which could contribute importantly to the depressed hippocampal synaptic plasticity and further induced cognitive deficits in MWM tests.

1. Introduction

Melamine, an intermediate chemical, was extensively used in the preparation of polymers for manufacturing of fertilizers, laminates, paints and adhesives (Hau et al., 2009). Due to its high nitrogen by molecular weight, melamine was illegal to add into milk and other products to increase the apparent protein concentration readings (Langman et al., 2009; Puschner and

* Corresponding authors at: South China Research Center for Acupuncture & Moxibustion, Guangzhou University of Chinese Medicine, 510006 Guangzhou, China.
E-mail addresses: al_totti@sina.com, lei.an@gzucm.edu.cn (L. An), mercury1984@126.com (W. Sun).

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Reimschuessel, 2011). In the past, nephrotoxicity of melamine was best documented (Jacob et al., 2011; Melnick et al., 1984). However, several studies have shown that melamine induced neurobehavioral (An et al., 2012a) and neurotoxic effects in organisms (Yang et al., 2011). Chronic melamine exposure induced cognition deficits, particular in re-acquisition learning, which were extremely associated with inhibited synaptic transmission (An and Zhang, 2014b), oxidative damage (An et al., 2015, 2014) and dysfunctional cholinergic system (An et al., 2013). Furthermore, melamine decreased the frequency, but not the amplitude, of spontaneous EPSCs in the hippocampal CA1 area of infant rats, indicating that melamine was able to presynaptically reduce the release of glutamate in synaptic transmission of hippocampus (Yang et al., 2011).

Recently, study from our lab reported that prenatal melamine exposure (PME) was able to impair hippocampal function and cause spatial cognition impairment associating with synaptic dysfunction (An and Zhang, 2014a). It is interesting that paired-pulse facilitation (PPF), a phenomenon of short-term plasticity, was significantly facilitated by PME. A previous study reported that a significantly increased Ca^{2+} fluorescence was observed in hippocampal neurons 12 h following 312 mg/mL melamine treatment (Wang et al., 2011). It suggested that the toxicity of melamine was through disturbing the Ca^{2+} homeostasis, which played pivotal roles in modulating and controlling neuronal excitability (Toescu and Vreugdenhil, 2010). Therefore, the enhanced of PPF by melamine exposure prenatally may be associated with the initial probability of presynaptic glutamate release, and then affected synaptic efficiency.

The mechanism underlying hippocampus-dependent memory is extensively believed to be long-term potentiation (LTP) and long-term depression (LTD), which are characterized by a long-lasting increase or decrease in synaptic strength, respectively (Collingridge et al., 2010; Okada et al., 2003). Recent studies indicated that hippocampal LTD has been implicated in cognitive flexibility other than spatial learning and memory (Kim et al., 2011a; Mills et al., 2014; Nicholls et al., 2008). These findings suggested a correlation between LTD and novelty detection during learning procedure. Increasing evidences indicated that LTD may act by weakening previously encoded memory traces when new information was learned (Ge et al., 2010; Malleret et al., 2010). Furthermore, it has been well known that abnormal hippocampal LTD in depression, schizophrenia and bipolar disorder lead to deficits in attention, cognition and working memory (Hall et al., 2015; Maggio and Segal, 2011; Pittenger and Duman, 2008; Salavati et al., 2015). Considering the cellular mechanism that LTD served in ensuring proper behavioral response to novel environments (Martinowich et al., 2012), it cannot be neglected that PME might have adverse effect on this type of synaptic plasticity. In the present study, we used a rat model of prenatal melamine exposure (PME) to identify the PME-related effects on behavioral flexibility in Morris water maze (MWM) tests and assessed LTD in the CA1 region of hippocampus, which played a critical role in spatial memory (Morris, 2003; Okada et al., 2003; Vorhees and Williams, 2006). Furthermore, it has been well known that the basis of synaptic plasticity was synaptic transmission in hippocampus (Bliss and Collingridge, 1993; Whitlock et al., 2006). Hence, whole-cell patch-clamp measurements were also carried out to investigate a possible mechanism which involved in the effect of PME on synaptic transmission and process of neurotransmitter release by recording excitatory postsynaptic currents (EPSCs) in PME-treated rat hippocampus slices.

2. Materials and methods

2.1. Reagents

Melamine (purity > 99.5%) was purchased from Yingda Sparseness & Nobel Reagent Chemical Factory, Tianjin, PR China. Other reagents were of A.R. grade.

2.2. Experimental animals and treatment

Male and nulliparous female Wistar rats aged 10 weeks were obtained from the Laboratory Animal Center, Academy of Military Medical Science of People's Liberation Army, and reared in the animal house of Medical College of Acupuncture-Moxibustion and Rehabilitation in Guangzhou University of Chinese Medicine. All experiments were performed at light phase between 1300 and 1700, and approved by Local Animal Care Committee. Food and water were freely available during all phases of the experiment.

After an adaptation period of 1 week in our facilities, one or two female rats were housed with one male for mating. Vaginal plug check and vaginal smear observation by microscope were carried out each morning. Day 0 of gestation (GD 0) was determined by the presence of a vaginal plug and/or spermatozoids in the vaginal smear. The female rat was then housed separately from the male and body weight (BW) was recorded daily. Pregnant dams were randomly divided into two groups (seven rats per group): melamine (M) group in which animals intragastric received 400 mg/kg/day (40 mg/mL) and control (C) group in which animals received the same dose of distilled water. The doses and concentrations of melamine were based on our previous study (An and Zhang, 2014a) and the results of other labs (Dobson et al., 2008; Jingbin et al., 2010; Kim et al., 2011b). This dose was approximate 10 fold of the equivalent dose of human tolerable daily intake (TDI) referred by FDA (Chu et al., 2013). The dosage conversion from human dose to rat dose included height, weight and surface area and was corrected by conversion factor as previously described (FDA, 2008). The selected dose was also equivalent to the melamine levels detected in the contaminated dairy food products in the local area (CFS, 2008). By using this model, our and other labs have found maternal body weight changes (Dalal and Goldfarb, 2011; Hau et al., 2009), clinical signs of offspring (An et al., 2011), smaller litter size, and abnormal enhanced male/female sex ratio (An and Zhang, 2014a). In our previous report, we have also found the learning deficits in male animals on postnatal day (PD) 36 (An and Zhang, 2014a). Gavage was performed and melamine or distilled water was given once a day throughout the whole gestational period. The day of birth was identified as PD 0.

As there may be genders differences in the sensitivity of developing brain areas to melamine prenatally, only male offspring of each group were selected and used in the present study. The litter size was randomly culled to two male pups on PD 1 to assure uniformity of litter size between C and M groups. They were weaned on PD 21. There were two groups of animals, and each of them had fourteen 35-day-old male rats. The prenatal melamine exposure (PME) group rats were selected from dams that were administered with melamine throughout their gestational period. The offspring rats were randomly divided into two groups: (1) PME group for MWM test (PMEM) (n = 7); (2) PME group for electrophysiological experiment (PMEE) (n = 7). The prenatal control (PCE) group rats were selected from dams administered with distilled water. The offspring rats were randomly divided into two groups as well: (1) control group for MWM test (PCEM) (n = 7); (2) control group for electrophysiological experiment (PCEE) (n = 7).