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Prenatal melamine exposure induces impairments of spatial cognition and hippocampal synaptic plasticity in female adolescent rats

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ABSTRACT

Developmental exposure to melamine induces long-term effects on brain and behavior in male rodents. To examine whether this prenatal event damages cognitive function in female offspring, we evaluated the behavioral effects and further attempted to investigate synaptic function. Prenatal melamine exposure (PME) was given by oral treatment to pregnant females through the whole gestational days with 400 mg/day/kg bodyweight. On postnatal day (PD) 36, female offspring were assessed for spatial cognition in the Morris Water Maze (MWM) test. Simultaneously, the alterations in hippocampal synaptic plasticity in Schaffer collaterals-CA1 pathway *in vivo* were measured. The results of behavioral test showed that PME lead serious deficits of memory and re-acquisition abilities. PME depressed depotentiation in the hippocampal CA1 area of the PME group, but no alteration in LTP. Furthermore, variations of post-tetanic potentiation (PTP) and paired-pulse facilitation (PPF) were also observed in this pathway. This finding indicated that the PME affected spatial cognition in adolescent females, and the impairment of hippocampal synaptic functions may partly play a significant role in these effects.

1. Introduction

The addition of melamine to infant formula in the 2008 Chinese milk scandal has had consequences on human health, resulting in the hospitalization of over 294,000 infants diagnosed with urinary tract stones and sand-like calculi, which included a number of associated deaths (Puschner and Reimschuessel, 2011; Sharma and Paradakar, 2010). The incidents raised global concerns about food and feed safety related to the melamine contamination (Gossner et al., 2009; Levinson and Gilbride, 2011). Currently, melamine has not only been detected from many food sources including eggs, chicken, pork, fish, and vegetable protein products but also could be released from daily-use melamine-made tableware in a wide range of conditions (Chien et al., 2011; Dorne et al., 2013; Gossner et al., 2009). Furthermore, infants and children might be exposed to melamine due to the persistent accumulation of melamine in drinking water and daily uses such as dishware and kitchenware (Mattarozzi et al., 2012).

The apparent non-toxic dose of prolonged prenatal exposure to environmental contaminants might represent a major risk factor for offspring health. In this respect, melamine, one of the most widely used industrial uses, represented a paradigmatic example, as it elicited developmental toxicity, such that adverse effects could occur in pregnant women and children (Chu et al., 2013; Kim et al., 2011). The evidence of the selective vulnerability of fetuses, neonates, and adolescents to melamine has been well documented (Gossner et al., 2009; Hau et al., 2009; Jingbin et al., 2010; Lee et al., 2011; Melnick et al., 1984). Furthermore, male children were at about twofold increased melamine-associated kidney stone risk compared with female children (Lu et al., 2011). Both the sub-chronic and chronic studies have shown that there was a greater likelihood of male rats fed diets containing melamine to develop urinary bladder stones than females (Gamboa da Costa et al., 2012; Melnick et al., 1984). Recently, the results showed that RPA-1, which was distal tubule and collecting duct injury biomarker), was significant increased starting at the 120 ppm group in male rats while starting at the 180 ppm dose in female rats (Zhang et al., 2012). In treated female rats, alpha-GST (proximal tubular injury) and GST-Yb1 (distal tubular injury) levels in urine were an order of magnitude lower than those in male rats (Zhang et al., 2012).

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Additionally, the up-regulation of genes in response to co-exposure to melamine and cyanuric acid was consistently higher in males than in female rats (Camacho et al., 2011). They also found that there were differences in constitutive gene expression levels between males and females. The most reliable support for the effects of gestational melamine on the development of the offspring comes from nephrotoxicity studies in rats, and some results have been reported in patients.

Hippocampal synaptic plasticity may be a substrate for certain types of learning and memory (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). Chronic melamine administration and prenatal melamine exposure can interact with synaptic plasticity in hippocampal pathways such as Schaffer collateral-CA1 synapses (An et al., 2011; An and Zhang, 2014a) possibly by altering the probability of presynaptic neurotransmitters release (An and Zhang, 2014a; Yang et al., 2011) as well as this treatment can affect cognition (An et al., 2014; An and Sun, 2017a; An and Zhang, 2014b). More importantly, the different uric acid levels induced by chronic melamine exposure between male and female children may be due to sex steroid (Lu et al., 2011), which play a significant role in uric acid regulation in biological fluids (Adamopoulos et al., 1977). In addition, oestrogen may results in lower urinary calcium excretion and calcium oxalate saturation (Heller et al., 2002). Although research on neurotoxicity of melamine is scarce relative to prenatal exposure, the available research indicated that sex differences in the hypothalamic-pituitary-adrenal (HPA) axis function emerge. For hippocampus-derived sex hormones, one of the essential functions may be the rapid and repetitive modulation of synaptic plasticity and cognitive functions, since circulating sex hormones can penetrate into the hippocampus by crossing the blood-brain barrier (Ooishi et al., 2012). Indeed, in male rats, the PME depressed the excitatory neurotransmitter release probability and further damaged hippocampal LTP, which was contributed to the impaired spatial cognitive function (An and Sun, 2017c; An and Zhang, 2014a). Considering that female seems to have a higher HPA axis stress reactivity than that of male (Hulshof et al., 2012), which means the PME may have a stronger effect on brain function in females.

However, the sex-specific effect of melamine in the CNS has not yet been well defined. Here, we determined the neurotoxic effect of PME on female offspring, which exposed to melamine during the whole gestation days. To test whether PME insult may produce disruptive effects on spatial learning and memory function, we have compared the water maze performance. Meanwhile, long-term potentiation (LTP) and paired-pulse ratio (PPR) from Schaffer collaterals to CA1 region were recorded *in vivo*. Additionally, the levels of estrogen in the serum and hippocampus were detected to investigate the potential action of PME on female offspring.

2. Materials and methods

2.1. Reagents

Melamine (purity > 99.5%) was purchased from Yingda Sparseness & Nobel Reagent Chemical Factory, Tianjin, PR China. Melamine assay kit was purchased from Huaan Magnech Bio-Tech Co., Ltd. Beijing, PR China. Other reagents were of A.R. grade.

2.2. 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 maintained on a 12 h light/dark cycle (9:00–21:00 h) with free access to food and water. All procedures adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Committee for Animal Care at Nankai University.

After an adaptation period of 1 week in the facilities, one or two female rats were housed with one male for mating. Vaginal plug check and 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 males, while the body weight (BW) was recorded daily. Pregnant dams were randomly divided into two groups (seven rats per group). They were the melamine (M) group, in which rats were given 400 mg/kg/day (40 mg/mL in saline) melamine by intragastric administration, and the control (C) group, in which animals were received the same dose of saline. The doses were based on our previous study (An and Zhang, 2014a, 2016) and the results of other labs (Dobson et al., 2008; Jingbin et al., 2010; Kim et al., 2011). This selected dose was converted from human dose to rat dose by the online FDA Dose Calculator, including height, weight and surface area (FDA, 2011), which was approximate 10 fold of the equivalent dose of human tolerable daily intake (TDI) (FDA, 2008). In this animal model, maternal body weight (Dalal and Goldfarb, 2011; Hau et al., 2009), clinical signs of offspring (An et al., 2011), and smaller litter size (An and Zhang, 2014a) were found previously. Our lab has also found the spatial memory defects in adolescent offspring (An and Sun, 2017c; An and Zhang, 2014a). Gavage was performed and melamine or saline was given once a day throughout the whole gestational period. The day of birth was identified as postnatal day (PD) 0.

Female offspring of each group were selected and used in the current study. They were weaned on PD 21 and housed in groups with free access to food and water. Experiments were performed in prenatal melamine exposure (PME) and prenatal saline (control) exposure (PCE) adolescent offspring (~PD 36). Rats in the PME group were selected from dams that were administered with melamine throughout their gestational period. The offspring rats were randomly divided into two groups, which were (1) the PME group for the MWM task (n = 7) and (2) the PME group for electrophysiological recordings (n = 7). Rats in the PCE group were selected from dams administered with saline. The offspring rats were randomly divided into two groups as well, which were (1) control group for MWM task (n = 7) and (2) control group for electrophysiological recordings (n = 7). One rat from each litter was used in each test to exclude the possibility of confounds resulting from specific litter effects. The weight of each offspring rat was recorded every day at the same time. In addition, both dams and pups were examined for neurological deficits and physical changes during the experimental days.

2.3. Morris water maze experiment

Beginning around PD 36 (range PD 34–39), female offspring rats were trained and tested in the Morris water maze (MWM) which is a model widely used for studying learning and memory of rodents. The protocol was as same as previous studies (An et al., 2012b; Han et al., 2014). Briefly, the maze was consisted of a circular pool (60 cm deep, 150 cm diameter) filled with water (maintained at $25 \pm 1\,^{\circ}C$ using a built-in heater) to a depth of 20 cm and made opaque by addition of black nontoxic ink. The tank was divided into four equal quadrants (I, II, III, and IV), and a circular escape platform (diameter 10 cm) was located away from the wall of the pool and in the center of quadrant III. The task consisted of three consecutive stages: initial training (IT) stage, space exploring test (SET) stage and re-acquisition (RT) stage. In IT stage, all offspring were tested for 5 consecutive days. During this phase, the hidden escape platform remained in the same location in the pool. On each test day, the animals received four trials, with a 5 min rest period