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Effects of developmental perfluorooctane sulfonate exposure on spatial learning and memory ability of rats and mechanism associated with synaptic plasticity

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ABSTRACT

The present study aims to explore the effects of perfluorooctane sulfonate (PFOS) on cognitive function in developing rats and the underlying mechanism associated with synaptic plasticity. Pregnant Wistar rats were fed with 0, 5, and 15 mg/L of PFOS via drinking water during gestation and lactation. Offspring were exposed to PFOS on prenatal and/or postnatal days by cross-fostering. Spatial learning and memory abilities were tested from postnatal day (PND) 35. We also analyzed the expression pattern of the synaptic plasticity-related genes and proteins in the hippocampus on PND7 and PND35. Results revealed that PFOS exposure reduced the spatial learning and memory abilities of the offspring, particularly of those with prenatal exposure. Meanwhile, protein levels of growth-associated protein-43, neural cell adhesion molecule 1, nerve growth factor, and brain-derived neurotrophic factor decreased on PND35, which are involved in the formation of synaptic plasticity. In contrast, significant increase in *gap-43*, *ncam1*, and *bdnf* genes on the mRNA level was observed on PND7, possibly due to the post-transcriptional mechanism. Results of both behavioral effects and molecular endpoints suggested the high risk of prenatal PFOS exposure. The decline of spatial learning and memory abilities induced by developmental PFOS exposure was closely related to synaptic plasticity.

1. Introduction

Perfluorooctane sulfonate (PFOS), a lipid- and water-repellent surfactant, is extensively used in both household and industrial applications, such as carpets, leather, paper, and fabric, for more than 50 years (Thompson et al., 2011). Given the bioaccumulation and biomagnification characteristics of PFOS, this surfactant has been detected in various environmental media and is transferred through the food chain to high trophic levels and to humans (Giesy and Kannan, 2001; Jin et al., 2009; Lau et al., 2007). In May 2009, the Stockholm Convention listed PFOS as a persistent organic pollutant.

Toxicological studies in rodents have proven that PFOS can cross the placental and blood–brain barriers (Borg et al., 2011; Chang et al., 2009), thereby causing a potential damage to the central nervous system (CNS) (Austin et al., 2003; Liu et al., 2010a, 2010b) and resulting in developmental delay of neonatal growth (Fuentes et al., 2007a; Lau et al., 2003). However, limited information is available on the behavioral effects of PFOS. No effects on learning and memory abilities were observed in some studies (Lau et al., 2003; Luebker et al., 2005), and slight activity and exploratory behavioral effects occurred when rats and mice were prenatally exposed to PFOS (Fuentes et al., 2007a). PFOS exposure on postnatal day (PND) 10 induced deranged spontaneous behavior in adult mice (Johansson et al., 2008). Regarding the rapid growth of the developing brain, the dose–response relationship of the behavioral effects could be complicated by the corresponding sensitive periods.

Spatial learning and memory are fundamentally dependent on synaptic plasticity, which is the ability of individual synaptic junctions to use or disuse transmission over synaptic pathways. Our previous study, employing a genome-wide DNA analysis, showed that the differentially expressed genes induced by PFOS in rat cerebral cortex are involved in synaptic plasticity (Wang et al., 2010). Moreover, several microRNAs critical in synaptic transmission were also significantly altered by PFOS (Wang et al., 2012). Therefore, further research is required to evaluate the effects of PFOS on synaptic plasticity and its role in the PFOS-induced interference of cognitive ability.

The paper is to commemorate late Prof. Dr. Yihe Jin (1959–2013), who has devoted his whole life to the scientific research, and contributed greatly to the present research.

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During the neurodevelopmental period, several critical proteins are involved in synaptic plasticity. Both GAP-43 and NCAM1 are necessary membrane proteins for the neuronal development (Fields and Itoh, 1996; Oestreicher et al., 1997), while nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) are important members of the neurotrophin family, which regulate neuron survival, differentiation, growth, and hippocampal LTP (Fischer et al., 1991; Patterson et al., 1992). These synaptic plasticity related proteins play important roles in regulation and repair of the nervous system, whose alteration could be another or contributing mechanism behind the observed cognitive ability impairments after exposure to PFOS. But few studies reported that they were associated with the developmental neurotoxic effects of PFOS. Single neonatal exposure (PND10) or exposure from gestational day 1 (GD1) to PND14 caused up regulation of GAP-43, and NCAM1, respectively (Johansson et al., 2009; Wang et al., 2011). The evaluation of key molecular factors in synaptic plasticity would favor a better understanding of the potential neurotoxicological mechanism of PFOS.

We aimed to identify the effects of PFOS exposure during prenatal and postnatal periods on cognitive ability, as well as to elucidate the mechanism underlying PFOS-induced neurodevelopmental damage associated with synaptic plasticity. Critical proteins and genes (GAP-43, NCAM1, NGF, and BDNF in the hippocampus) involved in synaptic plasticity were evaluated in the hippocampus, an important functional area related to learning and memory abilities.

2. Materials and methods

2.1. Ethical approval of the study protocol

The study protocol was approved by the School of Environmental Science and Technology, Dalian University of Technology (Dalian, China).

2.2. Reagents

PFOS (CAS number 2795-39-3, purity ≥ 98%, Sigma-Aldrich, USA) solution was prepared using 2% Tween 20 (purity ≥ 97%, Amresco, USA) in deionized water at concentrations of 2.5 and 7.5 g/L. The stock PFOS solution was diluted 500-fold by sterile tap water and used as the drinking water in the 5 and 15 mg/L exposure groups. The control group was fed drinking water containing 0.004% Tween 20.

2.3. Animal treatment and sample collection

Wistar rats weighing 180 g to 220 g were fed with standardized rodents feed particles and tap water under a 12:12 hours of light:dark pattern at a temperature of 24 ± 2 °C and a relative humidity of 50–60%. Pregnant rats were randomly divided into control group (CC), low and high dose treatment group, fed with drinking water containing 0, 5, 15 mg/L PFOS, respectively. Details are presented in the Supplementary Information (SI). The PFOS concentrations in the serum of dams and in the hippocampus of rat offspring were analyzed to further confirm the internal exposure dose (SI, Tables S1 and S2). On PND1, litter parameters were recorded (SI, Table S3), pups in the control and exposed groups were cross-fostered to further establish the following groups: CC without exposure; continuous exposure to 5 and 15 mg/L of PFOS (TT5, TT15); only postnatal exposure at 5 and 15 mg/L of PFOS (CT5, CT15); and only prenatal exposure at 5 and 15 mg/L PFOS (TC5, TC15).

Pups were sacrificed on PND7 and PND35. In each rat, the hippocampus was dissected from the brain and immediately frozen at –80 °C before gene and protein analyses. Spatial learning and memory abilities were evaluated by Morris water maze (MWM) starting from PND35.

2.4. Morris water maze (MWM)

MWM was used to evaluate the spatial learning and memory abilities of pups (Morris et al., 1982). The MWM tests comprised 1 day (d) of visible platform tests, 7 days of hidden platform tests, and a probe trial 24 h after the last hidden platform test. Details are shown in SI.

2.5. Quantification of protein levels of GAP-43, NCAM1, NGF, and BDNF

Each hippocampal sample from individual rat was weighed and homogenized in ice-cold lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, 10 mg/mL of aprotinin, 1 mg/mL of leupeptin, 0.5 mM sodium vanadate, 10% glycerol, and 1% nonyl phenoxypolyethoxylethanol. Homogenized samples were then centrifuged at 14,000 *g* for 15 min at 4 °C. Supernatant was removed and assayed immediately. The total protein amounts were determined using the total protein assay kit (Nanjing Jiancheng Bioengineering Institute, China). The GAP-43, NCAM1, NGF, and BDNF protein levels were measured by rat ELISA kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. Concentrations of all the samples were higher than the detection limit of these four proteins.

2.6. Real-time quantitative RT-PCR analyses

RNA was isolated from the hippocampus of individual rats. The RNA was then reverse-transcribed to cDNA using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR kit (Transgene, China). Rat-specific primers were designed for the *gap-43, ncam1, ngf*, and *bdnf* genes. The sequences of the primers were designed using Primer Premier 5.0 software (Premier Biosoft International, USA). The housekeeping gene β-actin was assayed as an internal control as described by Liu et al. (2010b) (SI, Table S4). β-actin was chosen as a control because it was widely used and did not vary significantly between the brain areas and ages in the developing brain of rat compared with other housekeeping genes (Polesskaya et al., 2007). The PCR amplification protocol was 30 s at 94 °C, followed by 40 cycles at 94 °C for 5 s, and 60 °C for 30 s. Each sample analysis was performed in triplicate. The expression level of the target gene was normalized to that of β-actin. The fold change of the target genes was analyzed by $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

2.7. Statistical analyses

Data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA) and expressed as mean ± standard error. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) was used to determine the differences among groups of the gene and protein expression levels. For the hidden platform test, the latencies and distances were averaged and analyzed using repeated measures and multivariate ANOVA followed by LSD post-hoc test of the general linear mode. For the visible platform test and probe trials, the values were averaged and analyzed using one-way ANOVA followed by Duncan's multiple-range test. Statistical significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Effects of PFOS on spatial learning and memory abilities of pups

In the visible platform tests, no significant statistical difference was observed in the swimming speeds and the time to reach the visible platform (SI, Fig. S1). This finding indicated that PFOS exposure did not affect the visual and motor functions of the rat offspring.

The learning ability of the offspring was reflected by the hidden platform test in MWM. The results showed that the escape latency of the rat pups from the exposure groups was longer than that of the CC group, where the highest increase was observed in the prenatal high-dose group, TC15. Besides, significant difference was observed between TC15 and CC groups from PND36 to PND41. The escape latency also significantly increased in the prenatal low-dose group, TC5, on PND37 and PND38. For the continuous exposure group, decreased escape latency was observed in the high-dose group, TT15, on PND37 and PND38. No significant change in the escape latency occurred in the continuous low-dose group, TT5. For the postnatal exposure group, moderate increase in escape latency appeared in the low and high-dose groups, CT5 and CT15 (Table 1). Similarly trends appeared in the results of the escape distances (Fig. 1). The memory ability of the offspring in the original platform was reflected by the probe trial test. The results showed that the time spent in the target quadrant by pups in the exposure groups was shorter than that spent by the CC group, with a significant difference in the TT15 group (Fig. 2A). Meanwhile, the numbers of platform crossings in the TT15 group were also significantly smaller than that in the CC group (Fig. 2B).

Although the hippocampus PFOS concentrations in the TC15 group were much lower than those in the continuous exposure and postnatal high-dose groups (SI, Table S3), the mean escape distances were significantly longer in the TC15 group than in the CC group from PND36 to PND41. These findings suggested that strong neurotoxic effects occurred in the developing nervous system with