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Effects of perfluorooctane sulfonate and alternatives on long-term potentiation in hippocampus CA1 region of adult rats in vivo

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With limited but ongoing usage of perfluorooctane sulfonate (PFOS), the health effects of both PFOS and its alternatives are far from being understood. Long-term potentiation (LTP) was evaluated in rats after exposure to PFOS and alternatives, aiming to provide some evidence about their potential to affect cognitive ability. Different dosages of PFOS and alternative chemicals, including perfluorohexane sulfonate (PFHxS), perfluorobutane sulfonate (PFBS) and chlorinated polyfluorinated ether sulfonate (Cl-PFAES), were given to rats via acute intracerebroventricular injection. The field excitatory postsynaptic potential (fEPSP) amplitude of the input/output functions, paired-pulse facilitations, and LTP in vivo were recorded. PFOS and alternatives inhibited LTP in varying degrees, without significant effects on the normal synaptic transmission. In addition, PFHxS and Cl-PFAES exhibited comparable potential to PFOS in disturbing LTP. The results suggested that acute exposure to PFOS and alternatives impaired the synaptic plasticity by a postsynaptic rather than presynaptic mechanism. Besides, the fEPSP amplitude of baseline was reduced by CI-PFAES but not by other compounds, indicating that CI-PFAES might act in a different mode. Providing some electrophysiological evidence and potential mechanism of the neurotoxicity induced by PFOS and alternatives, the present study addresses further evaluation of their safety and health risk.

Introduction

Perfluorooctane sulfonate (PFOS) is an eight-carbon fully fluorinated organic chemical, which is extremely stable and resistant to be degraded by biological metabolism and other physicochemical processes. Due to its physicochemical stability and oil- and water-resistance, PFOS has been extensively used in a variety of industrial processes and consumer applications, leading to its ubiquitous presence in various environmental matrices, even in human and wildlife. In 2009, PFOS was listed into Annex B of the Stockholm Convention on Persistent Organic Pollutants. According to the Stockholm Convention, although the ultimate goal is the elimination of PFOS-related substances, production of these chemicals may continue for limited expectation of lower toxicity and health risk. Therefore, perfluorohexane sulfonate (PFHxS) and perfluorobutane sulfonate (PFBS), with six and four perfluorinated carbon atoms, respectively, were regarded as the appropriate alternatives of PFOS. Correspondingly, increasing temporal trends of PFHxS levels have been observed in primiparous women from Sweden during 1996-2010. And PFHxS was also extensively found in the breast milk collected from seven countries in Asia, at concentrations comparable to the report from Sweden. However, limited information is available about the toxicity of PFHxS and PFBS. Lower bioaccumulation and toxicity of the short carbon chain perfluorinated compounds were reported that C4-based chemicals are neither bioaccumulative nor toxic in a battery of environmental and safety tests. However, recent studies showed that neonatal PFHxS exposure exhibited similar potency to PFOS in altering both spontaneous behavior and neuroprotein levels. Moreover, chlorinated polyfluorinated ether sulfonate (Cl-PFAES, C4ClF26O4SK, locally called F-358) has been used as the only available mist suppressant in Chinese electroplating industry before the emergence of PFOS related products. After phasing out of firefighting foams and pesticides.
Toxicology Research Accepted Manuscript

Toxicity and neurotoxicity of PFOS and its alternatives have been studied extensively, with increasing evidence suggesting that these contaminants can cause severe adverse effects on the nervous system. PFOS, a perfluorooctane sulfonate, is a widely used surfactant that has been associated with cognitive dysfunction and neurological disorders, including Alzheimer's and Parkinson's disease. Several pieces of evidence suggest that PFOS can cross the blood-brain barrier, enabling it to interact with glutamate receptors including N-methyl-D-aspartic acid (NMDA) receptors.

Recent studies have shown that PFOS exposure can significantly impair long-term potentiation (LTP), a fundamental cellular mechanism that supports learning and memory. LTP is a form of synaptic plasticity that involves the strengthening of synapses, which are the sites of communication between neurons. This process is crucial for learning and memory, and its impairment can lead to cognitive decline.

In a recent study, researchers investigated the effects of PFOS and its alternatives on LTP in hippocampus CA1 region of rats. They found that PFOS and its congeners, such as PFHxS and PFBS, reduced the fEPSP amplitude, a key indicator of synaptic transmission, as well as the LTP amplitude. These findings are consistent with previous studies indicating that PFOS can cross the blood-brain barrier and affect brain function.

Moreover, the chronic lead (Pb) and aluminum (Al) exposure also impaired LTP in rats, which has been associated with cognitive dysfunction and neuronal diseases. These results suggest that PFOS and its congeners could be valuable for a better understanding of the neurotoxicity of PFOS and its congeners, as well as for developing strategies to mitigate exposure to these contaminants.
After exposure to PFOS, PFHxS and PFBS by i.c.v. injection, no significant impacts on the fEPSP amplitude before HFS were observed (Fig.1B). But Cl-PFAES injection decreased the fEPSP amplitude of the baseline, especially the high dose treatment. To further testify the observed effect of Cl-PFAES on baseline, the baseline recording was prolonged to 90 min after Cl-PFAES injection. As shown in Fig.2A, the inhibition on fEPSP amplitude induced by Cl-PFAES was irreversible and was still observed 90 min after injection. A slight but statistically significant decrease in baseline fEPSP was observed in 10 µM Cl-PFAES group and a further depression was apparent in 100 µM group (Fig.2B).

Effects of PFOS and Alternatives on I/O curves and PPF

To test the effects of PFOS and alternatives on basic synaptic transmission and short-term synaptic plasticity in CA1 region, I/O curves and PPF were measured before induction of LTP. Fig.3A illustrated the relationship between stimulus current and fEPSP amplitude in rats from control and treatment groups. There were no remarkable changes in fEPSP amplitude at stimulus current of 0.1-1.0 mA in 10 µM treatment groups compared with control, with significant differences observed in few scattered points in 100 µM groups. As shown in Fig.3B, all the groups exhibited a maximal facilitation at inter-pulse interval of 60 ms, but neither 10 µM nor 100 µM of PFOS and alternatives posed significant effects on the average peak facilitation compared with control group.

Discussion

The present study evaluated and compared the neurotoxic effects of PFOS, PFHxS, PFBS and Cl-PFAES in vivo on synaptic
PFHxS were similar to that observed for PFOS. And these neurotoxic effects of CaMKII, GAP-43, synaptophysin and tau, essential for normal behavior and cognitive function. Further, Lee and Viberg that a single neonatal PFHxS dosage altered adults spontaneous and neuroproteins levels of mammals. Consistent with previous study that PFHxS exposure posed alterations reported in previous studies. Fuentes et al. reported that shortened retention in water maze probe task was induced by administration of 3 mg PFOS/kg/day via gavage for four consecutive weeks in adult mice. In the study of Johansson et al., hyperactivity and the deficits in spontaneous behavior and habituation were observed in mice treated with a single-oral dose of PFOS on PND10. And our previous study further demonstrated that prenatal and postnatal PFOS exposure to PFOS caused the prolonged escape latency in water maze test of the rat pups, suggesting the decline in spatial learning and memory abilities. Although the relevance of LTP to some of these behavioral alterations is still unclear, our observations at minimum provide a possible cellular substrate for some of these alterations. Up to now, little information is available about the toxicity of PFOS alternatives. The present study found that PFHxS exhibited comparable potency to PFOS in affecting LTP, consistent with previous study that PFHxS exposure posed similar neurotoxic effects with PFOS in both behavior indicators and neuroproteins levels of mammals. Viberg et al. reported that a single neonatal PFHxS dosage altered adult spontaneous behavior and cognitive function. Further, Lee and Viberg found that neonatal PFHxS exposure altered neuroprotein levels, e.g. CaMKII, GAP-43, synaptophysin and tau, essential for normal brain development in mice. And these neurotoxic effects of PFHxS were similar to that observed for PFOS. These support the results from the present study and suggest that PFHxS and PFOS have similar neurotoxic potency and mechanism of action. In contrast, the present electrophysiological examination found that PFBS exhibited relatively lower potency to impair LTP than the other three target compounds. Similarly, only mild reduction in red blood cell counts, hematocrit, and hemoglobin were observed in male rats given 600 mg/kg PFBS 90-day via oral gavage, and no abnormal behaviors in motor activity and functional observation battery were noted. PFBS has a much lower potential for accumulation in human serum, and the minimal doses to elicit the same degree of hepatotoxicity was approximately 600 times lower than that of PFOS. The elimination kinetics has been regarded as a decisive factor leading to the difference of PFCs homologues in their toxicity potency, where the rate of elimination is related to carbon chain length. Olsen et al. reported that in human serum geometric elimination half-life of PFOS was 1751 days, with 2662 days for PFHxS and 25.8 days for PFBS. Kudo et al. observed a tendency that perfluoroalkyl carboxylates (PFCAs) with longer carbon chain length were less eliminated in urine in both male and female rats. Although the elimination in itself may control less to the difference among target compounds in LTP inhibition after acute exposure in the present study, similar mechanism underlies the bioaccumulation potency and toxicity. The difference in the hydrophobicity of the PFCs compounds and the corresponding bioavailability to the target cells may be an important reason. It had been demonstrated that C4-C6 PFCs is less hazardous than C7-C8 PFCs both in mammals and in aquatic organisms. Together with the findings in the present study that PFOS and PFHxS posed higher potency to affect LTP, the concern is raised about the neurotoxicological potential of long carbon chain PFCs. Recently, Route et al. found that perfluorodecane sulfonate (PFDS) was the second abundant analyte, taking up 23% of the PFCs amount in the blood plasma of the wild bald eagle in the upper Midwestern United States. Therefore, further toxicological evaluation of the long carbon chain PFCs is necessary. The present study is the first about the neurotoxicity of Cl-PFAs. Different from PFOS, PFHxS and PFBS, Cl-PFAs showed the potency to inhibit the fEPSP amplitude of baseline, indicating that Cl-PFAs might act in a different mode on synaptic transmission from perfluoroalkyl acids. Similar phenomena were observed when PC113 is administered to hippocampal slices, when both the amplitude of the fEPSP of baseline and LTP were decreased. PC113 has widely been considered lacking in significant toxicity due to its poor activity with Ah receptor. However, the findings about its effects on LTP suggest that it may not be the case. VPA was considered as an excitotoxicant which induced apoptotic neurodegeneration in the developing rat brain, lowered excitatory neurotransmission might be the reason for the inhibition of baseline. Comparing the chemical structure with PFOS, Cl-PFAs with a larger molecular volume and contained an ether group inside the carbon chain, which characterized an increasing hydrophobicity and better flexibility of the fluorinated chain making Cl-PFAs easier to be
incorporated into the lipid bilayer of the cell membrane.\(^{45}\) As Wang et al. \(^{21}\) reported, the acute LC\(_{50}\) of Cl-PFAES is similar to that of PFOS, where the slope of the dose-response curve of Cl-PFAES was even higher than that of PFOS. Without human exposure assessment and the toxicokinetic data of CI-PFAES in mammals and humans, it is impossible to estimate the health risk of Cl-PFAES. Therefore, the toxicity of Cl-PFAES needs further characterization, when the present study provides preliminary evidence of its potential effects on the nervous system.

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I/O curves reflect the basal synaptic transmission competency. Thus, no effects of acute exposure to PFOS and alternatives on IO functions implied that the normal synaptic transmission at Schaffer Collateral-CA1 synapse was not interrupted. PPF is a short-term synaptic plasticity which is a sensitive indicator of the change in the transmitter release amount, or presynaptic connections.\(^{46,47}\) Neither PFOS nor alternatives led to significant changes in PPF, hinting that PFOS and alternatives might not play effects on presynaptic cells after acute exposure. Besides, the quantity of PSD95 in dendrites decreased significantly when neurons were continuously treated with PFOS, clarify the effects of PFOS mainly focus on postsynaptic cells.\(^{23}\) In the research of Xing et al. \(^{31}\), lactational PBBDE 209 exposure from mother milk did not affect I/O functions and PPF but decreased LTP, suggesting a weaker inhibition on synaptic plasticity compared with intragastric lactational exposure and exposure after weaning. Together with the findings in the present study that PFOS and alternatives significantly affected the fEPSP amplitude of LTP, it is suggested that acute exposure to these compounds mainly acted in a postsynaptic rather than a presynaptic mechanism. In another hand, acute exposure to the target compounds may pose relatively weak neural inhibitory effects. However, the chronic exposure to PFOS and its bioaccumulative alternatives, as well as the long carbon chain PFCs possibly pose stronger effects on the nervous system considering the bioaccumulation potency.

Different from the present study, Liao et al. \(^{21}\) reported that 400 \(\mu\)M of PFOS could affect synaptic transmission in brain slices in rats. Besides the difference in the administration dose, the in vitro electrophysiological status also differs from the in vivo status, while the in vitro hippocampus slice is a valuable tool to elucidate the effects of pollutants on ion channel functions in central nervous system neurons. The mechanisms underlying the impairment in LTP caused by PFOS and its alternatives might be related to several aspects. Firstly, the high concentrations of Ca\(^{2+}\) are necessary to induce LTP, with a number of Ca\(^{2+}\) sources available. The calcium imbalance induced by PFOS may cause the LTP deficit.\(^{48,49}\) Secondly, PFOS affected the Ca\(^{2+}\) /calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), which play dominant roles in the induction and maintenance of LTP.\(^{27,48}\) Thirdly, N-methyl-D-aspartic acid (NMDA) receptors were impaired by PFOS, while the activation of NMDA receptors and the consequent calcium flooding into postsynaptic cell is necessary for LTP induction.\(^{18,25}\) Moreover, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptor, might also be involved in the mechanism of the effects of PFOS and its alternatives, which is an important regulator of both LTP maintenance and the raise of intracellular Ca\(^{2+}\) level.\(^{50}\)

However, no information is available about the effects of PFOS on AMPA receptor regulation. Lastly, PFOS might act indirectly on learning and memory through disruption of thyroid function. LTP is known to be depressed in hypothyroid conditions in both animals and humans,\(^{51}\) while PFOS exposure significantly reduced serum levels of free thyroxine in rats.\(^{52}\)

### 388 Experimental

#### 389 Animals and Chemicals

All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, China. Animals were acclimated in the lab for at least 7 days before experiments, with free access to water and food. All experiments were performed at room temperature (25 ± 2 °C), with a 12:12 light/dark cycle.

PFOS, PFHXs, and PFBS were purchased from Sigma (USA) and Cl-PFAES was obtained from Shanghai Synica Co. (China), with a purity of higher than 98% (Table 1). The target chemicals dissolved in 2% dimethyl sulfoxide (DMSO) and then diluted to 10 and 100 µM with physiological saline. Physiological saline with DMSO was administered at the same proportion both in treated and control groups. It was found that PFOS can accumulate up to 2-20 µM in some animal tissues.\(^{53}\) The doses (10, 100 µM) were administrated according to previous literatures,\(^{11,23}\) which representing the actual environmental relevant and potential accumulated concentrations.

#### 411 Animal Treatment and Electrophysiological Recordings in Vivo

Six animals were used for recording LTP in each group. The rats were deeply anesthetized with urethane (15 g/kg bw, Sigma) via intraperitoneal (i.p.) injection placed in a stereotoxic head holder (DMA-1511, Narishige, Japan) for surgery and recording. Skin and fascia were removed to expose the skull, and bregma and posterior fontanelle were kept at the same height. Small holes were drilled in the ipsilateral skull for the insertion of cannula, stimulating and recording electrode. A stainless steel cylindrical cannula (0.7 mm outer diameter) was inserted into the lateral ventricle (0.8 mm posterior to bregma, 1.3 mm lateral to midline, and 4.1 mm below skull) and fixed using acrylic dental cement for intracerebroventricular (i.c.v.) injection of chemicals. A concentric bipolar stimulating electrode (FHC, USA) was positioned at the Schaffer Collateral (4.2 mm posterior to bregma, 3.8 mm lateral to the midline) for LTP inducing, and a monopolar recording electrode (FHC, USA) was inserted at the Schaffer Collateral-CA1 synapse (4.2 mm posterior to bregma, 3.8 mm lateral to the midline) for field excitatory postsynaptic potential (fEPSP) recording.
The amplitude of fEPSP was transferred through the amplifier (CED 1401, UK), and filtered at each ISI. PPF values were standardized at each ISI by fEPSP 2/250, 300, 350 and 400 ms.

PFHxS: Potassium perfluorohexane sulfonate
PFBS: Potassium perfluorobutane sulfonate
Cl-PFAES: 2-[(6-Chloro-1,1,2,2,3,4,4,5,6,6-dodecafluorohexyl)oxy]-1,1,2,2,3,3,4,4,5,5,6,6-tetrafluoroethanesulfonic acid potassium salt

The electrodes were slowly lowered with single test stimuli (0.033 Hz, interval of 30 s) until a stable and maximal fEPSP was monitored. The stimulus current was adjusted to yield about 50% of maximum amplitude of fEPSP, and then began to record the baseline for 30 min. Targeted compounds solution of 5 µL was slowly administered to the rats via i.c.v. injection in 5 min by micro-syringe. Thirty minutes of contacting with target compounds in the brain tissues were remained after i.c.v. injection. Then the baseline was recorded for another 30 min, followed by IO and PPF test. LTP was induced by a high-frequency stimulus (HFS) protocol composed of 3 trains of 20 pulses at 200 Hz at an interval of 30 s. After HFS, the amplitude of fEPSP was recorded for at least 60 min.

The Input/Output (I/O) curves reflect the relationship between amplitude of fEPSP and stimulus intensity, which were employed to evaluate synaptic potency. I/O curves were generated by systematic variation of the stimulus current by steps of 0.1 mA (0.1-1.0 mA). Three responses were averaged at each current level. Paired-pulse facilitation (PPF), a form of short-lasting plasticity, was examined before HFS. The current was adjusted to yield about 50% of maximum amplitude of fEPSP, and pairs of stimuli were delivered with inter-stimulus intervals (ISI) of 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 250, 300, 350 and 400 ms. Three responses were averaged at each ISI. PPF values were standardized at each ISI by fEPSP 2/fEPSP 1, comparing at the peak facilitation with control group.

Conclusions

In summary, the present study provides electrophysiological evidence and potential mechanism of the neurotoxicity of PFOS and its alternatives. PFOS and alternatives exposure repressed LTP, and PFHxS and Cl-PFAES even exhibited comparable potency to PFOS. Higher potency of PFHxS and PFBS than PFBS to inhibit LTP point to the possibly higher neurotoxicity potential of the long carbon chain perfluoroalkyl compounds. Absent disruption of normal synaptic transmission suggested that acute exposure to the target compounds mainly acted in a postsynaptic rather than a presynaptic mechanism. Besides affecting LTP, Cl-PFAES also affected the baseline fEPSP, indicating a different action mode with the perfluoroalkyl acids. It should be noted that the present study is limited in the performance of acute exposure, and stronger effects on synaptic plasticity may occur when chronically exposed to PFOS, its bioaccumulative alternatives, as well as the long carbon chain perfluoroalkyl compounds. These findings present fact that PFOS alternatives could impair synaptic plasticity, explore primarily neurotoxic mechanism of PFOS alternatives with neuroelectrophysiological method, and address the necessity of...
further toxicological evaluation of PFOS alternatives, to improve
their safety and health risk assessment.

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