NEONATAL EXPOSURE TO PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) AFFECT DEVELOPMENTAL MARKER PROTEINS, CaMKII, GAP-43 AND SYNAPTOPHYSIN LEVELS, IN THE MOUSE BRAIN

Johansson Niclas, Eriksson Per and Viberg Henrik

Department of Environmental Toxicology, Uppsala University, Norbyvägen 18 A, S-75236 Uppsala, Sweden.

Introduction

Perfluorinated compounds (PFCs) have been identified as an emerging class of persistent environmental contaminates and found to be present in humans as well as wildlife [1,2,3]. PFCs are found in numerous industrial and consumer applications such as stain resistant treatment coatings for clothing fabrics, carpets, and oil-resistant coatings for paper products in food contact. It is also used in fire extinguishers [4]. The PFCs are stable and found to accumulate in the environment due to their chemical stability and general lack of biodegradation [5]. The human half-lives for the most relevant PFCs, e.g. PFOS and PFOA are about 4-6 years [6].

A recent report from WWF, indicate a higher level of PFCs in the children's generation, compared to mother's and grandmother's generation [7]. This is the opposite of older, banned persistent chemicals as organochlorine pesticides and PCBs. Lau et al. have reported that PFOS can cause developmental neurotoxic effects in rats and mice following exposure throughout pregnancy. The pups were born alive, active, and pink, but 30-60 after birth, following exposure to 10 mg PFOS/kg body weight the neonates became pale, inactive, and moribund, and died soon afterward [8]. We have earlier reported that low-dose exposure to environmental contaminated like PCBs, DDT, and PBDEs during a critical period of neonatal brain development can lead to disruption of the adult brain function. The disruptions was manifested deranged spontaneous behaviour, lack of/or reduced habituation, defect learning and memory faculties and changes in the cholinergic neurotransmission [9,10,11,12,13,14,15]. Recently, we have reported that neonatal exposure to PFOS and PFOA cause the same effects on behaviour and cholinergic susceptibility as the PBDEs [16]. This neonatal period, known as the brain growth spurt (BGS), is a phase during the development when the maturational processes of CNS are at a stage of critical vulnerability. In humans, the BGS begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mice and rats, this period is neonatal, spanning the first 3-4 weeks of life. The BGS includes axonal and dendritic outgrowth, establishment of neuronal connections, synaptogenesis, and proliferation of glia cells [17].

Recently, we have reported that neonatal exposure to highly brominated PBDEs can result in changes in BDNF, CaMKII and GAP-43 levels in the neonatal mice brain [18,19]. These proteins are biochemical substrates for neuronal survival, growth, and synaptogenesis [20,21,22,23]. GAP-43 is frequently used as a marker for axonal sprouting [20]. CaMKII is involved in regulation of both synaptogenesis and synaptic plasticity [21,22]. Synaptophysin is an integral membrane protein on synaptic vesicles. The function of synaptophysin is unknown, but suggestions include calcium binding and exocytosis [24,25].

In regard to our earlier findings where several developmental marker proteins have been affected by neonatal exposure to higher brominated diphenyl ethers [18,19] and that neonatal exposure to PBDEs, PFOS and PFOA [11,12,13,14,15,16] causes similar developmental neurobehavioural deficits the present study was undertaken to investigate whether similar developmental neuroproteins also are affected by developmental exposure to PFOS and PFOA.

Material and Methods

Exposure to PFOS or PFOA. Perfluorooctane sulfonate (PFOS, potassium salt) purity ≥ 98%, and perfluorooctanoic acid (PFOA) purity = 96%, were purchased from Sigma-Aldrich. Neonatal NMRI male mice were given a single oral dose of either 21 μmol PFOS/kg body weight (11.3 mg/kg body weight), or 21 μmol PFOA/kg body weight (8.70 mg/kg body weight) via a metal gastric-tube at the age of 10 days. The control animals received in the same manner 10 ml/kg body weight of the 20% fat emulsion vehicle. Only male mice were used in order to compare with our earlier developmental neurotoxicological study on PFOS and PFOA and highly brominated PBDEs [11,12,16]. The animals were euthanized 24 hr after exposure (i.e. on PND 11) and the brains were dissected on an ice-cold glass plate. The cortex and hippocampus were collected and frozen at -80°C until assayed. Tissues from 7 to 8 animals per treatment group were used in the protein analysis.

Slot-blot analysis for CaMKII, GAP-43, and synaptophysin. The slot blot procedure was carried out in accordance to the previously described method by Viberg et al. [18] for cortex and hippocampus homogenates. The antibodies against GAP-43 (Chemicon AB5220, 1:5,000), CaMKII (Chemicon MAB8699 1:10,000) and synaptophysin (Chemicon 573822, 1:10,000) were used. The membranes were incubated overnight at 4°C with primary antibodies. Immunoreactivity was detected using a horseradish peroxidase-conjugate secondary antibody against mouse (074-1806, 1:20,000) or rabbit (KPL 074-1506, 1:20,000) depending on the primary antibody.

Statistical analysis. Differences between CaMKII, GAP-43, and synaptophysin protein levels in control, PFOS- and PFOA-treated animals were determined using a one-way ANOVA. Pairwise testing between the different treatment groups was performed with Newman-Keuls multiple comparison test.

Results and Discussion

The present study has shown that the administration of 21 μmol PFOS/kg body weight or 21 μmol PFOA/kg body weight to mice on PND 10, the peak of brain growth spurt, alter the amount of CaMKII, GAP-43 and synaptophysin in the brain 24 h after exposure. The present study also indicates the effects of PFOS and PFOA were different in the hippocampus and the cerebral cortex.

Exposure to PFOS resulted in a 22% increase in the amount GAP-43 in hippocampus, but no significant change in the level of GAP-43 protein in cortex. Exposure to PFOS resulted in a 57% increase in the amount of CaMKII protein in hippocampus, whereas no significant change in cortex. The levels of synaptophysin were altered by PFOS exposure in both the cortex and hippocampus. The increase in synaptophysin protein in hippocampus and cortex of PFOS-treated animals were 48% and 59% compared to the vehicle-treated control, respectively.

Exposure to PFOA resulted in a 17% increase in the amount GAP-43 in hippocampus, but no significant change in the level of GAP-43 protein in cortex. Exposure to PFOA resulted in a 58% increase in the amount of CaMKII protein in hippocampus, whereas no significant change in cortex. The levels of synaptophysin were altered by PFOA exposure in both the cortex and hippocampus. The increase in synaptophysin protein in hippocampus and cortex of PFOA-treated animals were 52% and 82% compared to the vehicle-treated control, respectively.

The present study explores possible mechanisms of the developmental neurotoxic effects of PFOS and PFOA. We have earlier reported that that neonatal exposure to PFOS and PFOA caused developmental neurotoxic effects, manifested as deranged spontaneous behaviour and lack of habituation, effects that worsen with age. We have also reported that PFOS and PFOA can affect the cholinergic system [16]. Similar changes in behaviour and altered susceptibility of the cholinergic system, as well as decreased amount cholinergic receptors in hippocampus has also been seen after exposure to PBDEs [9,11,12,13,14,15]. It is therefore interesting to compare the present changes of the studied developmental marker proteins with the changes observed after neonatal exposure to highly brominated PBDEs, where the levels of these proteins also were more pronounced in hippocampus compared to cortex.

In the present study the changes in GAP-43, CaMKII and synaptophysin protein levels were most pronounced in hippocampus after PFOS and PFOA exposure. Regional differences in the effects of neonatal exposure to PFOS and PFOA could result from both time differences in development of the brain and the amount of the compounds reaching the two brain regions. The interactions between the studied protein markers and development of the cholinergic system in the hippocampus might be a possible explanation for the effects on behaviour and habituation seen after neonatal exposure to PFOS and PFOA.

Acknowledgements

Financial support was provided by the Swedish Research Council for Environmental, Agricultural Sciences and Spatial Planning and the Foundation for Strategic Environmental Research.

References

1. Giesy JP, Kannan, K. *Eviron. Sci. Technol* 2001; 35:1339-42.

2. Giesy JP, Kannan, K. *Eviron. Sci. Technol* 2002; 146A-152A.

3. Olsen GW, Church TR, Hansen KJ, Burris JM, Butenhoff JL, Mandel JH. *Chemosphere* 2004; 54:1599-1611.

4. Renner BD. *Environ. Sci. Technol* 2001; 35:154A-160A.

5. Key BD, Howell RD, Criddles CS. *Environ Sci. Technol*, 1997; 31:2445-54.

6. Lau C., Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. *Toxicol Sci* 2007; 99:366-394.

7. WWF. Generations X, WWF DetoX Campaign, Brussels 2005; 59.

8. Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA. *Toxicol Sci* 2003; 74:382-92.

9. Eriksson P, Jakobsson E, Fredriksson A. *Environ Health Perspect* 2001;109(9):903-8.

10. Eriksson, P. In *PCBs: Human and Environmental Disposition and Toxicology* (L. G. Hansen, and L. W. Robertson, Eds.). University of Illinois Press, Urbana and Chicago 2007; 172-182.

11. Viberg H, Fredriksson A, Jakobsson E, Örn U, Eriksson P. *Toxicol Sci* 2003;76:112-20.

12. Viberg H, Fredriksson A, Eriksson P. *Neurotoxicology* 2007; 28:136-142.

13. Viberg H, Fredriksson A, Eriksson P. *Toxicology and Applied Pharmacology* 2003; 95-106.

14. Viberg H, Fredriksson A, Eriksson P. *Toxicol Sci* 2004; 81:344-353.

15. Viberg H, Fredriksson A, Eriksson P. *Environmental Toxicology and Pharmacology* 2005; 20:283-288.

16. Johansson N, Fredriksson A, Eriksson P. *Neurotoxicology* 2008; 29:160-69.

17. Davison, AN, Dobbing, J. *Applied Neurochemistry* Blackwell, Oxford, 1968; 178-253.

18. Viberg H, Mundy W, Eriksson P. *Neurotoxicology* 2008; 29:152-59.

19. Viberg H, Johansson N, Eriksson P. *The Toxicologist* 2008 vol. 102, 1:1708.

20. Oestreicher AB, De Graan PN, Gipsen WH, Verhagagen J, Schrama LH. *Prog Neurobiol* 1997; 53:627-86.

21. Frankland PW, O'Brien C, Ohno M, Kirkwood A, Silva AJ. *Nature* 2001; 411:309-13.

22. Rongo C, Kaplan, JM. *Nature* 1999; 402:195-9

23. Cui Q. *Mol Neurobiol* 2006; 33:155-79.

24. Hamos JE, DeGennarobLJ, Drachman DA. *Neurology* 1989; 39:355-61.

25. Masliah E, Terry RD, Alford M, DeTeresa R. *J Histochem Cytochem* 1990; 38:837-44.