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Brain region-specific perfluoroalkylated sulfonate (PFSA) and carboxylic acid (PFCA) accumulation and neurochemical biomarker Responses in east Greenland polar Bears (*Ursus maritimus*)

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ABSTRACT

Perfluoroalkyl substances (PFASs) is a growing class of contaminants in the Arctic environment, and include the established perfluorinated sulfonates (PFSAs; especially perfluorooctane sulfonate (PFOS)) and carboxylic acids (PFCAs). PFSAs and PFCAs of varying chain length have been reported to bioaccumulate in lipid rich tissues of the brain among other tissues such as liver, and can reach high concentrations in top predators including the polar bear. PFCA and PFSA bioaccummulation in the brain has the potential to pose neurotoxic effects and therefore we conducted a study to investigate if variations in neurochemical transmitter systems i.e. the cholinergic, glutaminergic, dopaminergic and GABAergic, could be related to brain-specific bioaccumulation of PFASs in East Greenland polar bears. Nine brain regions from nine polar bears were analyzed for enzyme activity (monoamine oxidase (MAO), acetylcholinesterase (AChE) and glutamine synthetase (GS)) and receptor density (dopamine-2 (D2), muscarinic cholinergic (mAChR) and gamma-butyric acid type A (GABA-A)) along with PFSA and PFCA concentrations. Average brain ∑PFSA concentration was 25 ng/g ww where PFOS accounted for 91%. Average ∑PFCA concentration was 88 ng/g ww where PFUnDA, PFDoDA and PFTrDA combined accounted for 79%. The highest concentrations of PFASs were measured in brain stem, cerebellum and hippocampus. Correlative analyses were performed both across and within brain regions. Significant positive correlations were found between PFASs and MAO activity in occipital lobe (e.g. ∑PFCA; $r_p=0.83$, $p=0.041$, $n=6$) and across brain regions (e.g. ∑PFCA; $r_p=0.47$, $p=0.001$, ∑PFSA; $r_p=0.44$, $p>0.001$; $n=50$). GABA-A receptor density was positively correlated with two PFASs across brain regions (PFOS; $r_p=0.33$, $p=0.02$ and PFDoDA; $r_p=0.34$, $p=0.014$; $n=52$). Significant negative correlations were found between mAChR density and PFASs in cerebellum (e.g. ∑PFCA; $r_p=-0.95$, $p=0.013$, $n=5$) and across brain regions (e.g. ∑PFCA; $r_p=-0.40$, $p=0.003$, ∑PFSA; $r_p=-0.37$, $p=0.007$; $n=52$). AChE activity and D2 density were negatively correlated with single PFCAs in several brain regions, whereas GS activity was positively correlated with PFASs primarily in occipital lobe. Results from the present study support the hypothesis that PFAS concentrations in polar bears from East Greenland have exceeded the threshold limits for neurochemical alterations. It is not known whether the observed alterations in neurochemical signaling are currently having negative effects on neurochemistry in East Greenland polar bears. However given the importance of these systems in cognitive processes and motor function, the present results indicate an urgent need for a better understanding of neurochemical effects of PFAS exposure to wildlife.

1. Introduction

The polar bear (*Ursus maritimus*) is an apex predator in the Arctic. Due to its trophic position, long life span and preference for a lipid-rich diet, the polar bear accumulates high levels of

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persistent organic pollutants (POPs) (Letcher et al., 2010, 2009, Ramsay and Stirling, 1988). The legacy POP polychlorinated biphenyls (PCBs) have long been the dominant contaminant group in polar bears (AMAP, 2004). However, studies have shown that during the early 2000s perfluoroalkyl substances (PFASs), including perfluorinated carboxylates (PFCAs) and perfluorinated sulfonates (PFSAs), especially the highly bioaccummulative perflurooctane sulfonate (PFOS), reached comparable liver levels ($\sum PCB_{51}$ = 3125 ng/g ww, $\sum PFAS_{11}$ = 2636 ng/g ww) (Gebbink et al. 2008, Smithwick et al., 2005). Among circumpolar populations of polar bears, those from East Greenland accumulate the highest levels of PFASs (Butt et al., 2010; Dietz et al., 2008; Greaves et al., 2012; Houde et al., 2011; Rigét et al., 2013).

PFASs and their precursors contain carbon chains where all hydrogen atoms are substituted with fluorine. Due to their oil- and water repellent nature, PFASs are incorporated into products such as Teflon and Gore-Tex (Lindstrom et al., 2011). PFASs end up in the Arctic environment though direct release of PFAS (Paul et al., 2009) as well as through release and subsequent degradation of PFAS precursors such as perfluorooctane sulfonamide metabolism of PFOS (Z. Wang et al., 2013.).

PFSAs and PFCAs have been shown to affect a number of physiological systems, including the nervous system. For example, there are limited studies from laboratory experiments showing alterations in behavior, motor function, memory and learning in rodents after exposure to PFOS or PFOA. Effects were most pronounced for individuals exposed during development (Johansson et al., 2009, 2008). At the molecular level, correlations have been found between exposure to PFOS or PFOA and changes in the cholinergic system in rodents (Johansson et al., 2008) and protein kinase C (involved in neurotransmission by G protein-coupled receptors) in hatchlings (Pinkas et al., 2010). Taken together, these findings suggest that PFASs may disrupt the function of the nervous system.

It is not known if PFSAs and PFCAs exposure is causing neurological stress in polar bears (or other arctic marine mammals), although previous studies of polar bears demonstrated that PFASs, especially the long chained PFASs (C_{10}–C_{15}), cross the blood brain barrier (BBB) and reach the brain at concentrations up to 116 ng/g ww ($\sum PFAS_{17}$) (Greaves et al., 2013). PFASs are mainly bound to blood-proteins (Greaves et al., 2012) and the crossing of the BBB resembles the transport of free fatty acids (Greaves et al., 2013, 2012). Due to the importance of the nervous system in mediating critical aspects of animal health, behavior, reproduction, and survival (Gore, 2008), resolving whether contaminants such as PFASs may cause neurotoxicity is of pressing concern. Prior to affecting the structure and/or function of the nervous system, neurotoxic contaminants will first cause a range of sub-clinical effects (Basu, 2014, Basu and Head, 2010), and one of the earliest indicators of neurotoxic disease is disruption of brain neurochemistry. Neurochemical biomarkers have been used previously in wildlife, including polar bears, to assess the risk of mercury exposure (Basu et al., 2009', 2007a', 2005).

Although PFASs have been increasing in the Arctic, and in particular in East Greenland polar bears, over the past three decades, several of the most dominant PFASs have started to decline around 2007 (Dietz et al., 2008; Rigét et al., 2013). However relatively high levels have recently been found in polar bear brains (Greaves et al., 2013, 2012), and it is not known whether these exposures are related to any neurological effects. In the present study activities of neurochemical enzymes (monoamine oxidase, MAO; acetylcholinesterase, AChE and glutamine synthetase, GS) and densities of central neuro-receptors (γ-aminobutyric acid type A receptor, GABA-A; dopamine-2 receptor, D2 and muscarinic acetylcholine receptor, mAChR) were determined in nine brain regions from nine East Greenland polar bears. Furthermore concentrations of several PFSAs and PFCAs were measured in each of the brain regions. Correlative association between neurochemical biomarkers and brain region-specific levels of PFSA and PFCA were investigated to test the hypothesis that PFSA and PFCA exposure is correlated with neurochemical changes.

2. Materials and methods

2.1. Sampling of animals

Brain samples were obtained from nine polar bears legally hunted within the established Greenlandic quota system for subsistence hunting in the Scoresby Sound area, East Greenland (69°00'N to 74°00'N) during February and March 2011 and 2012. For further information, including biological data see Supplementary material, Table 1. Briefly, the entire brain was rapidly removed, dissected into nine regions (brain stem (n=6), cerebellum (n=6), frontal cortex (n=6), occipital lobe (n=7), temporal cortex (n=9), striatum (n=7), thalamus (n=7), hypothalamus (n=7) and hippocampus (n=5)) as illustrated in Fig. 1, and stored frozen in liquid nitrogen at −196 °C (contaminant analysis) or −80 °C (neurochemical marker analysis) until analyses.

2.2. PFAS analyses

PFASs were analyzed in brain regions from 10 individuals. Chemical standards (all > 98% purity) of the four C_4, C_6, C_8 and C_{10} PFSAs (perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorodecane sulfonate (PFDS)) and ten C_6–C_{15} PFCAs (perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluoropentadecanoic acid (PFPeDA)) were purchased from Wellington Laboratories (Guelph, ON, Canada). Internal standards (all > 98% purity) consisted of ^{18}O-PFHxS, ^{13}C-PFOS and ^{13}C-PFCAs (C_6, C_8–C_{12}) (Wellington Laboratories, Guelph, ON, Canada). Based on retention times ^{13}C-PFHxA was chosen as the surrogate for PFBS and PFHpA, ^{13}C-PFUnDA for PFDS, and ^{13}C-PFDoDA for PFTrDA, PFTeDA, and PFPeDA. Chemicals used for extraction of PFASs included formic acid (98-100%, Sigma-Aldrich, Oakville, ON, Canada), ammonium hydroxide (28–30% w/v, EMD Chemicals Canada), ammonium acetate (Fisher Scientific, Ottawa, ON. Canada), acetonitrile (Caledon Laboratories, Georgetown, ON, Canada) and diatomaceous earth (VWR International). All were of analytical grade or higher. All solvents used were high-performance liquid chromatography (HPLC)-grade, including methanol (Caledon Laboratories, Georgetown, ON, Canada), and Milli-Q water (treated on-site).

All PFCA and PFSA sample analyses were performed at NWRC (Carleton University, Ottawa, Canada) in the Letcher/ Organic Contaminants Research Laboratory (OCRL). The sample extraction and cleanup steps followed a previously used method for analysis of PFASs from brain tissue (Greaves et al., 2013). In brief, 1 g of tissue was spiked with 10 ng of each isotopically labeled internal standard, followed by homogenization with a 10 mM potassium hydroxide acetonitrile:water (80:20, v/v) solution to extract the PFASs. Samples were subjected to cleanup by solid-phase extraction (Waters Oasis WAX cartridges). PFSAs and PFCAs were eluted by 2 ml of 1% ammonium hydroxide in methanol, evaporated to dryness, reconstituted in methanol, filtrated, and transferred to a LC–MS vial for analysis.

The analysis of the final clean-up fractions for PFCAs and PFSAs was performed using a Waters 2695 HPLC coupled with a Waters