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Do persistent organic pollutants interact with the stress response? Individual compounds, and their mixtures, interaction with the glucocorticoid receptor

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HIGHLIGHTS

- POP mixtures tested are chemically defined and are relevant to human exposure.
- High content analysis used to screen mixtures for GR translocation.
- Twenty-nine POPs and their mixtures were assessed using a GR reporter gene assay.
- *p,p′*-DDE decreased GR transcriptional activity to 72.5%.
- PFOS, PFDA and BDE-47 enhanced transcriptional activity in the presence of cortisol.

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ABSTRACT

Persistent organic pollutants (POPs) are toxic substances, highly resistant to environmental degradation, which can bio-accumulate and have long-range atmospheric transport potential (UNEP, 2001). The majority of studies on endocrine disruption have focused on interferences on the sexual steroid hormones and so have overlooked disruption to glucocorticoid hormones. Here the endocrine disrupting potential of individual POPs and their mixtures has been investigated *in vitro* to identify any disruption to glucocorticoid nuclear receptor transcriptional activity. POP mixtures were screened for glucocorticoid receptor (GR) translocation using a GR redistribution assay (RA) on a CellInsight™ NXT high content screening (HCS) platform. A mammalian reporter gene assay (RGA) was then used to assess the individual POPs, and their mixtures, for effects on glucocorticoid nuclear receptor transactivation. POP mixtures did not induce GR translocation in the GR RA or produce an agonist response in the GR RGA. However, in the antagonist test, in the presence of cortisol, an individual POP, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), was found to decrease glucocorticoid nuclear receptor transcriptional activity to 72.5% (in comparison to the positive cortisol control). Enhanced nuclear transcriptional activity, in the presence of cortisol, was evident for the two lowest concentrations of perfluorodecanoic acid (PFOS) potassium salt (0.0147 mg/ml and 0.0294 mg/ml), the two highest concentrations of perfluorodecanoic acid (PFDA) (0.0025 mg/ml and 0.005 mg/ml) and the highest concentration of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (0.0000858 mg/ml). It is important to gain a better understanding of how POPs can interact with GRs as the disruption of glucocorticoid action is thought to contribute to complex diseases.

1. Introduction

Persistent organic pollutants (POPs) are toxic organic substances that are highly resistant to environmental degradation, bio-accumulate and have long-range atmospheric transport potential (UNEP, 2001). This group of environmental chemicals have been detected in human adipose tissue, serum and breast milk samples collected in Asia, Europe, North America and the Arctic (Bi et al., 2006; Pereg et al., 2003; Sjödin et al., 1999, 2008) due to their lipophilic nature and resistance to degradation (de Wit et al., 2004). The high lipid solubility of POPs enables them to pass

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through biological barriers, such as the placental (Beesoon et al., 2011; Inoue et al., 2004; Ode et al., 2013) and blood–brain barriers. A large number of POPs have been shown to be endocrine disrupting chemicals (EDCs) in animals and humans which alters hormone-mediated responses (Birnbaum and Staskal, 2004; Boas et al., 2006; Darnerud, 2003; Schantz and Widholm, 2001; Zoeller, 2005). The majority of studies have focused on endocrine disruption of the sex steroid hormones and so have overlooked the disruption to glucocorticoid hormones.

Induction of the hypothalamic–pituitary–adrenal (HPA) axis occurs when individuals are faced with a stressful situation. The hypothalamus will secrete corticotropin-releasing hormone (CRH), which causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland in the brain to stimulate the release of cortisol from the adrenals. The glucocorticoids, cortisol in humans and corticosterone in rodents, are central to the regulation of many physiological processes including the control of energy metabolism and the modulation of the immune system (Charmandari et al., 2005; Sapolsky et al., 2000). The release of glucocorticoids alters the individuals physiological state in response to environmental conditions (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Physiological changes shift energy investment away from reproduction and redirect it towards survival (Wingfield and Sapolsky, 2003). Glucocorticoids are therefore extremely important to survival and have been strongly associated with fitness traits such as breeding success and individual quality (Angelier et al., 2009, 2010; Bókony et al., 2009; Goutte et al., 2011). Glucocorticoids, in addition, also play important roles in the process of immunomodulation (Jondal et al., 2004). Despite the importance of glucocorticoids for the regulation of physiological processes, the relationship between environmental chemicals and potential disruption of the HPA axis has not been extensively studied (Odermatt et al., 2006).

Glucocorticoids are lipophilic and can cross the blood–brain barrier where they bind to glucocorticoid receptors (GRs). In humans, the hippocampus and frontal lobes of the brain contain GRs. These are parts of the brain that are involved in cognitive functions such as memory and emotional maladjustments including impulsivity. Changes in the function of the HPA-axis may lead to altered stress responses and changes in cognitive functions. Glucocorticoids are responsible for maturation of tissues essential for neonatal survival (Langlois et al., 2002), therefore disruption of normal HPA axis activity may have widespread consequences. In humans, elevated cortisol and aldosterone levels are associated with low birth weight (Martinez-Aguayo et al., 2011). Lanoix and Plusquellec (2013) suggested that a disruption of the stress system could explain an association between environmental contaminants and mental health, especially in children and elderly people.

In contrast to the human estrogen and androgen receptors that are mainly expressed in the gonads, the human GR is expressed in every cell type (Akner et al., 1994). GR disruption has the potential to affect numerous processes. In stressful situations, when levels of glucocorticoids are high, GR activation is necessary for the HPA feedback regulation (de Kloet et al., 1998). GR deficient mice have a range of abnormalities including hyper activation of the HPA axis, impaired lung function and die shortly after birth (Cole et al., 1995). Hyper activation of the HPA axis is expected if GR signalling is disrupted as the HPA axis is subject to feedback inhibition from circulating glucocorticoids which act through GRs (Keller-Wood and Dallman, 1984). Hyper activation of the HPA axis is associated with psychiatric disorders including anorexia nervosa, obsessive-compulsive disorder and anxiety. Furthermore, glucocorticoid-mediated feedback inhibition is impaired in people who suffer from depression (Juruena et al., 2003). Hyperactivation of the HPA axis has also been associated with hyperthyroidism (Tsigos and Chrousos, 2002). Patients with excessive levels of corticosteroids are at a higher risk of developing cardiovascular disease (Pimenta et al., 2012). Disruption of glucocorticoid signalling could also have implications for obesity, as this system is central to adipocyte differentiation. EDCs have been found to promote adipogenesis in the 3T3-L1 cell line through the activation of the GR, thus leading to obesity (Sargis et al., 2009).

POPs have been linked to GR disruption. Methylsulfonyl metabolites from PCBs have been found to act as GR antagonists (Johansson et al., 1998). POPs can also disrupt regulation of adrenal hormone secretion and function at different levels of the HPA axis. The human H295R adrenal cell model highlighted that the adrenal cortex is a potential target for perfluorononanoic acid (PFNA) (Kraugerud et al., 2011), polychlorinated biphenyls (PCBs) (Li and Wang, 2005; Xu et al., 2006) and polybrominated diphenyl ethers (PBDEs) (Song et al., 2008). POPs can also decrease adrenal hormone production; as has been observed for the organohalogen pesticide γ-HCH (Lindane) (Oskarsson et al., 2006; Ulleras et al., 2008). Methylsulfonyl metabolites of dichlorodiphenyldichloroethylene (DDE) caused a decrease in H295R cell viability (Asp et al., 2010). Furthermore reduced plasma corticosterone levels were recorded *in vivo* in suckling mice following administration of these DDE metabolites to their lactating mothers (Jönsson et al., 1993). In arctic birds, high baseline corticosterone concentrations and a reduced stress response have been associated with high concentrations of organochlorines, PBDEs and their metabolites in blood plasma (Verboven et al., 2010). Reduced responsiveness of the HPA axis has been demonstrated in amphibians (Gendron et al., 1997) and birds (Mayne et al., 2004) and this has been associated with exposure to POPs.

This study aimed to assess the interaction of individual POPs and their mixtures at the GR level and to see if they disrupted this nuclear receptor's transcriptional activity. Two *in vitro* bioassays were used; a high content GR redistribution assay (RA) and a GR reporter gene assay (RGA). The GR RA was used as a screening method for the POP mixtures as it measures GR translocation and would therefore presumably detect any GR activity, agonism or antagonism. The GR RGA uses a human mammary gland cell line, with natural steroid hormone receptors for glucocorticoids and progestogens, which has been transformed with a luciferase gene (Willemsen et al., 2004), thereby allowing endocrine disruption at the level of nuclear receptor transcriptional activity to be identified. Disruption of GR activity is important and can have significant implications on health however the interaction of individual POPs and their mixtures with GRs has not been extensively studied.

2. Materials and methods

2.1. Chemicals

All PBDEs, PCBs and other organochlorines were originally purchased from Chiron As (Trondheim, Norway) and all perfluorinated compounds (PFCs) were obtained from Sigma–Aldrich, St. Louis, MO, USA except perfluorohexanesulfonic acid (PFHxS) which was obtained from Santa Cruz (Dallas, US). Hexabromocyclododecane (HBCD), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), thiazolyl blue tetrazolium bromide (MTT) and the steroid hormone cortisol were obtained from Sigma–Aldrich (Dorset, UK). Hoechst nuclear stain was purchased from Perbio (Northumberland, England). Cell culture reagents were supplied by Life Technologies (Paisley, UK) unless otherwise stated. All other reagents were standard laboratory grade.

2.2. Mixtures

Mixtures of the test POPs were designed and premade by the Norwegian University of Life Sciences, Oslo. Seven mixtures were