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Identification of a group of brominated flame retardants as novel androgen receptor antagonists and potential neuronal and endocrine disrupters

Joubert Banjop Kharlyngdoh [a,1], Ajay Pradhan [a,1], Solomon Asnake [a,1], Anders Walstad [a,2], Per Ivarsson [b], Per-Erik Olsson [a,*]

[a] Biology, Örebro Life Science Center, School of Science and Technology, Örebro University, SE-701 82 Örebro, Sweden
[b] ALS Laboratory Group, Analytical Chemistry & Testing Services, Stockholm, Sweden

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ABSTRACT

Brominated flame-retardants (BFRs) are used in industrial products to reduce the risk of fire. However, their continuous release into the environment is a concern as they are often persistent, bioaccumulating and toxic. Information on the impact these compounds have on human health and wildlife is limited and only a few of them have been identified to disrupt hormone receptor functions. In the present study we used *in silico* modeling to determine the interactions of selected BFRs with the human androgen receptor (AR). Three compounds were found to dock into the ligand-binding domain of the human AR and these were further tested using *in vitro* analysis. Allyl 2,4,6-tribromophenyl ether (ATE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE) and 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) were observed to act as AR antagonists. These BFRs have recently been detected in the environment, in house dust and in aquatic animals. The compounds have been detected at high concentrations in both blubber and brain of seals and we therefore also assessed their impact on the expression of L-type amino acid transporter system (LAT) genes, that are needed for amino acid uptake across the blood–brain barrier, as disruption of LAT gene function has been implicated in several brain disorders. The three BFRs down-regulated the expression of AR target genes that encode for prostate specific antigen (PSA), 5α-reductases and β-microseminoprotein. The potency of *PSA* inhibition was of the same magnitude as the common prostate cancer drugs, demonstrating that these compounds are strong AR antagonists. Western blot analysis of AR protein showed that ATE, BATE and DPTE decreased the 5α-dihydrotestosterone-induced AR protein levels, further confirming that these BFRs act as AR antagonists. The transcription of the *LAT* genes was altered by the three BFRs, indicating an effect on amino-acid uptake across cellular membranes and blood–brain barrier. This study demonstrated that ATE, BATE and DPTE are potent AR antagonists and the alterations in *LAT* gene transcription suggest that these compounds can affect neuronal functions and should be considered as potential neurotoxic and endocrine disrupting compounds.

1. Introduction

Flame retardants (FRs) are chemicals used in combustible materials such as plastics, wood, paper, textiles and electronic goods to increase their fire resistance (Alaee et al., 2003; Chen et al., 2012). Brominated FRs (BFRs) are abundantly used due to their low cost and high performance efficiency (Alaee et al., 2003; Birnbaum and Staskal, 2004). The usage of BFRs increased from 311,000 metric tons in 2005 to 410,000 metric tons in 2008, resulting in elevated levels in the environment (Covaci et al., 2011; Fink et al., 2008). Although the presence of BFRs in humans, the environment and wildlife have been reported (Covaci et al., 2011), the biological effects of many BFRs are not well studied.

Due to the negative impact of the classical BFRs, alternative BFRs are being introduced. However, these alternative compounds are now also detected in air, water, soil and sediments as well as in aquatic and terrestrial biota, from zooplanktons to polar bears and humans (Covaci et al., 2011). We recently identified an alternative BFR (1, 2-dibromo-4-(1, 2-dibromoethyl) cyclohexane; TBECH) as a potent androgen receptor (AR) agonist (Khalaf et al., 2009; Larsson et al., 2006). This discovery prompted us to screen for additional BFRs that could interact with the human AR (hAR). Using *in silico* screening we identified a new group of BFRs that showed strong interaction

* Corresponding author. Tel.: +46 19 301244; fax: +46 19 303566.
E-mail address: per-erik.olsson@oru.se (P.-E. Olsson).
[1] These authors have contributed equally.
[2] Present address: Toxicon AB, SE-26192 Härslöv, Sweden.

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with the human AR. These were allyl 2,4,6-tribromophenyl ether (ATE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE) and 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE). These compounds have recently been found to be present at high concentrations in the environment (Covaci et al., 2011; Ma et al., 2012). Thus the presence of these alternative BFRs in the environment is a concern both to human health and wildlife (Covaci et al., 2011).

ATE, BATE and DPTE are present at high concentration in the environment as well as in wildlife (Covaci et al., 2011; Ma et al., 2012; von der Recke and Vetter, 2007). The three BFRs have been detected in harp and hooded seals from Barents and Greenland seas (von der Recke and Vetter, 2007). Recent studies have also shown that DPTE is present in European and American eels (Suhring et al., 2013a,b). In addition, ATE and DPTE have been detected in a sewer slime from German urban residential zones (Sauer et al., 1997) and recently the presence of ATE, BATE and DPTE in house dust from Vancouver, Canada was reported (Shoeib et al., 2012). DPTE has also been reported to be present in house dust in Flanders, Belgium (Geens et al., 2010). ATE is the only one of the three BFRs that is presently produced and it is distributed as BFR PHE-65 (Great Lakes Corporation). Nonetheless, all three BFRs are commonly detected together in both animals and environmental samples since ATE and BATE are metabolites of DPTE (von der Recke and Vetter, 2007). ATE, the major DPTE metabolite, constituted nearly 68% of the initial pool of DPTE in an anaerobic transformation study (von der Recke and Vetter, 2007). ATE, BATE and DPTE have been detected in harp and hooded seals with equal concentrations (50%–50%) in the brain tissue and blubber (von der Recke and Vetter, 2007). This indicates that these BFRs have a high ability to penetrate the blood–brain barrier and thereby could affect neuronal functions. DPTE is found at comparable concentrations to BDE-47 and BDE-99 in the Atlantic and Southern Ocean (Xie et al., 2011). The DPTE and polybrominated diphenyl ethers (PBDE) concentrations in sea water from East of Greenland are also comparable (Moller et al., 2011). It has been reported that DPTE can be 5–30 times more enriched than polychlorinated biphenyls (PCBs) and PBDEs in the brain of harp and hooded seals (von der Recke and Vetter, 2007) suggesting that DPTE may be of high concern for neurological toxicity. In a study on the distribution of BDE-153 in rat it was observed that only 0.2% of the body burden of BDE-153 or 2% of the levels observed for the adipose tissue was localized to the brain (Sanders et al., 2006). In another study it was observed that the brain tissue accumulated less than 0.5% of the ingested tetrabromobisphenol A (TBBPA) and BDE-99 (Viberg and Eriksson, 2011). These compounds show a drastically lower brain uptake than ATE, BATE and DPTE but nevertheless they caused severe neuronal dysfunctions in laboratory animals (Viberg and Eriksson, 2011; von der Recke and Vetter, 2007; Zhang et al., 2013). Thus, it may be that ATE, BATE and DPTE not only pass across the blood–brain barrier to a higher degree than the other BFRs but that they are also likely to affect neuronal functions.

Several BFRs along with their metabolites have been shown to disrupt endocrine and reproductive systems (Khalaf et al., 2009; Legler and Brouwer, 2003; Porter et al., 2013; Wang et al., 2013) acting as either agonist or antagonist to different hormone receptors. A number of hydroxylated and methoxylated polybrominated diphenyl ethers (HO-/MeO-PBDEs) have recently been shown to possess anti-androgenic properties (Wang et al., 2013). BFRs have also been shown to have neurotoxic effects (Fonnum and Mariussen, 2009). In the present study, we used *in silico* analysis to determine the interaction potential of several BFRs and discovered that ATE, BATE and DPTE were able to bind to hAR. As dihydrotestosterone (DHT) is a more potent androgen than testosterone, it was used as a positive control in the present study (Askew et al., 2007; Deslypere et al., 1992; Khalaf et al., 2009). Using *in vitro* analysis we then identified the three BFRs as AR antagonists. Analysis of *AR* target genes such as prostate specific antigen (*PSA*), 5α-reductases (*SRD5A1* and *SRD5A3*) and β-microseminoprotein (*MSMB*) was performed to determine the effect of ATE, BATE and DPTE at the transcript level. The effect of the prostate cancer drugs bicalutamide, flutamide and hydroxyflutamide was observed to be comparable to the antiandrogenic potency of these three studied BFRs. Further, the effect of these BFRs on L-type amino acid transporter (LAT) encoding genes was analyzed to determine if they affected amino acid uptake mechanisms. The present study demonstrates that ATE, BATE and DPTE interact with AR as antagonists, are equally potent as the common prostate cancer drugs at inhibiting PSA and that they alter the expression of LAT genes indicating effect on amino acid uptake in the brain. The results indicate that ATE, BATE and DPTE are AR antagonists and can be regarded as potential neurotoxic and endocrine disrupting compounds.

2. Materials and methods

2.1. Chemicals

All the tested brominated compounds were synthesized at 98% purity by Wellington Laboratories (Guelph, Canada) whereas DHT, bicalutamide, flutamide and hydroxyflutamide were purchased (Sigma Aldrich, USA). All compounds were dissolved in DMSO (Sigma Aldrich, USA). The DMSO concentration was maintained at 0.1% (v/v) in the exposure studies.

2.2. Computational modeling

Molecular modeling was performed using the Molecular Operating Environment (MOE 2008.10) software as previously reported (Khalaf et al., 2009). Briefly, the crystal structure of human wildtype AR (AR_{wt}) was obtained from the Protein Data Bank entry 1e3g (PDB 2009) then protonated and energy minimized using the AMBER99 forcefield. AR carrying a T877A mutation (AR_{T877A}) was modeled using the crystal structure of the AR_{wt} as a template, as previously described (Larsson et al., 2006). Using the site finder protocol a binding site was chosen that interacts with the key amino acids. Docking and calculation of interaction energy were performed as described previously (Khalaf et al., 2009). Compounds modeled included DHT, ATE, BATE, DPTE, OBIND (4,5,6,7-tetrabromo-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)lindane), TBBPA-R1 to R4 [(2,2-bis(3,5-dibromo-4-allyloxy)phenyl)propane (R1); 2,2-bis(3,5-dibromo-4-(2,3-dibromopropyloxy)phenyl)propane (R2); 2,2-bis(3,5-dibromo-4-(2-bromoallyloxy)phenyl)propane (R3); 2,2-bis(3,5-dibromo-4-(2-hydroxyethoxy)phenyl)propane (R4)], TBPIC (tris(2,3-dibromopropyl)isocyanurate), bicalutamide, flutamide and hydroxyflutamide.

2.3. Polarizability and K_{ow}

Calculations of polarizability and K_{ow} for the different compounds were performed using the MarvinSketch 6.0.5 software (Chemaxon, Hungary). The K_{ow} and polarizability values for selected compounds were compared to acetaminophen and acetylsalicylic acid, which are non-steroidal anti-inflammatory drugs known to permeate the blood–brain barrier (Kumpulainen et al., 2007; Lundquist et al., 2002).

2.4. Cell culture conditions

Human cervical carcinoma (HeLa), ductal breast cancer (T-47D) and prostate carcinoma (LNCaP) cell lines were obtained from ATCC and maintained as per instructions. HeLa cells were grown and maintained in DMEM medium (Hyclone, UK) containing 4 mM L-glutamine and supplemented with 10% FBS (Hyclone, UK). T-47D and LNCaP cell lines were maintained in RPMI-1640 medium (Hyclone, UK) containing 2 mM L-glutamine and supplemented with 1 mM sodium pyruvate (Invitrogen, USA), 10 mM HEPES (Invitrogen, USA), 1× non-essential amino acids (Invitrogen, USA), and 10% FBS and additionally 0.01 mg/ml bovine insulin (Invitrogen, USA) was provided for T-47D cells. All cell lines were grown in an incubator under a stable environment of 95% humidity, 5% CO_2, and 37 °C.