Nuclear Hormone Receptor Activity of Polybrominated Diphenyl Ethers and Their Hydroxylated and Methoxylated Metabolites Based on Transactivation Assays

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used in large quantities worldwide as a flame retardant additive in plastic materials, paints, textile fabrics, and so forth (Darnerud et al. 2001). Because of their leachability from various materials and persistence, PBDEs have been identified in various environmental sectors, including sediment and fish from rivers (Sellström et al. 1998), indoor air (Saito et al. 2007), and house dust (Stapleton et al. 2005). Moreover, PBDEs have been identified in human tissue samples, such as blood and breast milk, due to their bioaccumulative nature (Mazdai et al. 2003; Schecter et al. 2003). In addition, more recent studies have shown that hydroxylated and methoxylated PBDEs are formed as metabolites from parent PBDE congeners by oxidizing enzymes, and are also present in the plasma of wild animals (Verreault et al. 2005) and humans (Athanasiadou et al. 2008; Qiu et al. 2009), and in human breast milk (Lacorte and Ikonomou 2009).

Nuclear hormone receptors are ligand-dependent transcription factors that regulate a variety of important physiological processes (McKenna et al. 1999). A number of studies have reported that nuclear hormone receptors such as estrogen receptor (ER) and androgen receptor (AR) interact with xenobiotic chemicals, for example, persistent pesticides (Kelce et al. 1995; Kojima et al. 2004) and polychlorinated biphenyls (PCBs) (Bonefeld-Jørgensen et al. 2001). This implies that these chemicals may disrupt the endocrine system via a variety of nuclear hormone receptors, and PBDEs may display similar toxicity to PCBs due to their structural resemblance. In the present study, to elucidate the endocrine disrupting property of PBDEs and their hydroxylated and methoxylated metabolites, we have characterized the agonistic and antagonistic activity of principle PBDE congeners together with several hydroxylated and methoxylated PBDEs against six hormone nuclear receptors of ERα/β, AR, glucocorticoid receptor (GR) and thyroid hormone receptor (TR) α_1/β_1.

Materials and Methods

Chemicals: 17β-Estradiol (E_2), 5α-dihydrotestosterone (DHT), hydrocortisone (HC), 3,3',5-triiodo-*L*-thyronine (T_3), tamoxifen citrate (TAM), hydroxyflutamide (HF), mifepristone (RU-486), and tetrabrominated bisphenol A (TBBPA) were prepared as an hormone receptor agonist or antagonist. The PBDE congeners and HO- and MeO-PBDEs tested in this study (Table 1) were synthesized according to the methods previously reported, or purchased from AccuStandard, Inc.

Plasmids: The expression plasmids pcDNAERα, pcDNAERβ, pZeoSV2AR, pSG5-GR, pZeo-TRα_1 and pZeo-TRβ_1 as well as the reporter plasmids, pGL3-tkERE, pIND-ARE, pGRE-tk-Luc and pIND-TREpal, were prepared as previously described (Kojima et al. 2004; Kitamura et al. 2005). The internal control plasmid, pCMVβ-Gal, was purchased from Clontech.

Transactivation assays: Chinese hamster ovary (CHO-K1) cells were transiently transfected with hormone receptor expression plasmid, the reporter plasmid, and the control plasmid using the FuGENE 6 Transfection Reagent. After a 3-hr transfection period, cells were dosed with various concentrations ($\leq 1 \times 10^{-5}$ M) of the test compounds or with 0.1% DMSO (vehicle control) in the presence (for detecting antagonism) and absence (for detecting agonism) of E_2, DHT, HC, and T_3 for ERα/β, AR, GR, and $TR\alpha_1/\beta_1$ activity, respectively. We measured the *firefly* luciferase activity and the β-galactosidase activity in the cell lysate. The luciferase activity was normalized against the β-galactosidase activity for each treatment. Results were expressed as means ± SD from at least three independent experiments performed in triplicates.

Results and Discussion

Figure 1 shows the dose-response curve of E_2, DHT, HC, and T_3 from the ERα/β, AR, GR, and $TR\alpha_1/\beta_1$ assays, respectively. This indicates that these assay systems are highly sensitive and specific to the endogenous hormones. In this study, we characterized the potential hormone receptor activity of 16 PBDEs and their HO and MeO metabolites on the basis of these transactivation assays. The results summarized in Table 1 reveal that these compounds, excluding BDE-15 and BDE-209, exhibited not only ERα/β agonistic and AR antagonistic activities but also antagonistic activities via ERα/β, GR, and $TR\alpha_1/\beta_1$ (Kojima et al. 2009).

In the ERα assay, six of 16 compounds tested, showed agonistic activities. The relative potencies of their ERα agonistic activities descended in the following order: 4'-HO-BDE-17 >> 4'-MeO-BDE-17, 4-HO-BDE-42 > BDE-100 > BDE-47 > BDE-28. These estrogenic properties of the PBDE congeners were in good accordance with those from the studies of Hamers et al. (2006) and Meerts et al. (2001). In addition, we found that six compounds had an inhibitory effect on the estrogenic activity induced by 1×10^{-11} M of E_2. The order of relative potencies for ERα antagonistic activity was 4'-HO-BDE-49, 4-MeO-BDE-90 > BDE-153 > 4'-MeO-BDE-49 > BDE-99 > 4'-HO-BDE-17. In the ERβ assay, we found that of the 16 compounds tested, only 4'-HO-BDE-17 and 4-HO-BDE-42 induced estrogenic activity. In addition, we found that six compounds had an inhibitory effect on the estrogenic activity induced by 1×10^{-10} M of E_2. The order of relative potencies for ERα antagonistic activity was 4'-HO-BDE-49 > BDE-100, BDE-153 > 4'-MeO-BDE-49 > 4-MeO-BDE-90 > BDE-99. These results suggested that 4'-HO-BDE-17 showed most potent estrogenic activity via ERα/β, and 4'-HO-BDE-49 showed most potent anti-estrogenic activity via ERα/β. In the AR assay, although none of the compounds showed any AR agonistic activity, 12 compounds inhibited agonistic activity induced by 1×10^{-10} M of DHT. In particular, 4'-HO-BDE-17 inhibited at low concentrations in the order of 10^{-8} M. The order of relative potencies for AR antagonistic activity was 4'-HO-BDE-17 > BDE-100 > 4-HO-BDE-42, BDE-47 > BDE-85 > 4'-HO-BDE-49 > BDE-28 > 4'-MeO-BDE-17, BDE-99 > 4'-MeO-BDE-49, 4-MeO-BDE-42, 4-MeO-BDE90. The anti-androgenic activities of 4'-HO-BDE-17 and BDE-100 were about 5- and 10-fold lower than that of HF, respectively. In the GR assay, although none of the compounds showed any GR agonistic activity, seven compounds, including two HO-PBDEs and two MeO-PBDEs, showed weak antagonistic activity. The order of relative potencies for GR antagonistic activity was 4-MeO-BDE-90 > 4'-HO-BDE-49 > 4'-MeO-BDE-49 > 4'-HO-BDE-17, BDE-85 > BDE-99 > BDE-100. In the $TR\alpha_1$ and $TR\beta_1$ assays, although none of 16 compounds tested showed any $TR\alpha_1/\beta_1$ agonistic activity, we found that only 4-HO-BDE-90 inhibited both $TR\alpha_1$- and $TR\beta_1$-mediated

transcriptional activity induced by 1 x 10^{-8} M of T_3 whereas its corresponding methoxylated 4-MeO-BDE-90 did not. The anti-thyroid hormone activities of 4-HO-BDE-90 were estimated to be similar to those of TBBPA, previously reported to be a TR antagonist by Kitamura et al. (2005).

Taken together, these results suggest that PBDEs and their metabolites might have multiple endocrine-disrupting effects via nuclear hormone receptors, and *para*-HO-PBDEs, in particular, possess more potent receptor activity compared to that of the parent PBDEs and corresponding *para*-MeO-PBDEs. Further experiments are needed to elucidate endocrine-disrupting effects of accumulative PBDE metabolites such as 6-HO-BDE-47, 5-HO-BDE-47 and 5'-HO-BDE-99 recently found in human blood (Athanasiadou et al. 2008; Qiu et al. 2009).

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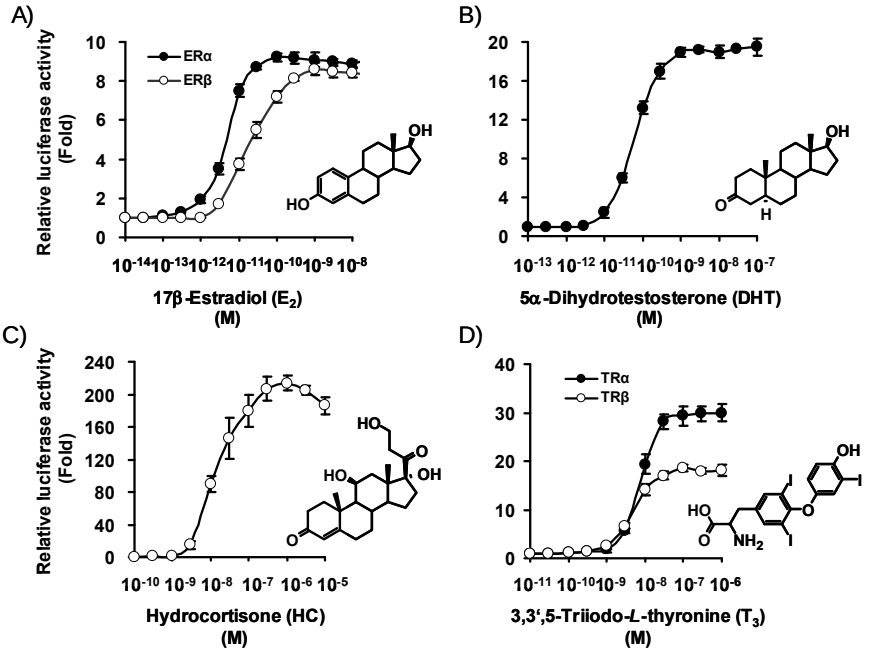

Figure 1. Dose-response curves for E2 (A), DHT (B), HC (C), and T3 (D) obtained from the ERα/β, AR, GR, and TRα$_1$/β$_1$ transactivation assays.

Table 1 Comparison of agonistic and antagonistic activities of PBDEs and their hydroxylated and methoxylated metabolites agoinst ERα, ERβ, AR, GR, TRα$_1$, and TRβ$_1$.

Compounds	Agonistic activity; REC_{20}[a] (M)		Antagonistic activity; RIC_{20}[b] (M)					
	ERα	ERβ	ERα	ERβ	AR	GR	TRα$_1$	TRβ$_1$
E_2	2.5×10^{-12}	5.3×10^{-12}	-	-	-	-	-	-
TAM	-	-	6.0×10^{-9}	7.0×10^{-9}	-	-	-	-
HF	-	-	-	-	1.8×10^{-8}	-	-	-
RU-486	-	-	-	-	-	5.7×10^{-9}	-	-
TBBPA	-	-	-	-	-	-	4.1×10^{-6}	9.6×10^{-6}
BDE-15	N.E.[c]	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
BDE-28	6.7×10^{-6}	N.E.	N.E.	N.E.	1.3×10^{-6}	N.E.	N.E.	N.E.
BDE-47	5.0×10^{-6}	N.E.	N.E.	N.E.	8.1×10^{-7}	N.E.	N.E.	N.E.
BDE-85	N.E.	N.E.	N.E.	N.E.	1.1×10^{-6}	7.0×10^{-6}	N.E.	N.E.
BDE-99	N.E.	N.E.	5.7×10^{-6}	9.2×10^{-6}	1.4×10^{-6}	7.7×10^{-6}	N.E.	N.E.
BDE-100	4.0×10^{-6}	N.E.	N.E.	4.3×10^{-6}	2.1×10^{-7}	8.5×10^{-6}	N.E.	N.E.
BDE-153	N.E.	N.E.	3.3×10^{-6}	4.4×10^{-6}	N.E.	N.E.	N.E.	N.E.
BDE-209	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
4'-HO-BDE-17	1.9×10^{-7}	2.1×10^{-7}	8.8×10^{-6}	N.E.	8.6×10^{-8}	6.7×10^{-6}	N.E.	N.E.
4'-MeO-BDE-17	2.5×10^{-6}	N.E.	N.E.	N.E.	8.2×10^{-7}	N.E.	N.E.	N.E.
4-HO-BDE-42	2.9×10^{-6}	3.6×10^{-6}	N.E.	N.E.	1.1×10^{-6}	N.E.	N.E.	N.E.
4-MeO-BDE-42	N.E.	N.E.	N.E.	N.E.	1.7×10^{-6}	N.E.	N.E.	N.E.
4'-HO-BDE-49	N.E.	N.E.	2.3×10^{-6}	3.6×10^{-6}	1.5×10^{-6}	5.5×10^{-6}	N.E.	N.E.
4'-MeO-BDE-49	N.E.	N.E.	4.4×10^{-6}	6.1×10^{-6}	8.5×10^{-7}	6.2×10^{-6}	N.E.	N.E.
4-HO-BDE-90	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.	8.1×10^{-6}	7.3×10^{-6}
4-MeO-BDE-90	N.E.	N.E.	2.6×10^{-6}	7.6×10^{-6}	2.5×10^{-6}	2.5×10^{-6}	N.E.	N.E.

[a] 20% relative effective concentration; the concentration of the test compound showing 20% of the agonistic activity of 10^{-9} M E_2.
[b] 20% relative inhibitory concentration; the concentration of the test compound showing 20% of the antagonistic activity of 1×10^{-11} M E_2 via ERα, 1×10^{-10}M E_2 via ERβ, 1×10^{-10}M DHT via AR, 3×10^{-8} M via GR, 1×10^{-8} M via TRα, or 1×10^{-8} M via TRβ, respectively.
[c] No effect (REC_{20} or $RIC_{20} > 1 \times 10^{-5}$M).