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Receptor interactions by polybrominated diphenyl ethers *versus* polychlorinated biphenyls: a theoretical Structure-activity assessment

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Abstract

The extensive body of literature regarding the interaction of polychlorinated biphenyls (PCBs) with transcription factors (receptors) has great value to understand similarities and distinctions and in formulating hypotheses regarding the activity of polybrominated diphenyl ethers (PBDEs) toward those same receptors. Our goal is to present the most comprehensive overview of PBDE effects on AhR, CAR, PXR, ER, AR, PR, DHT, TH, T3, T4 and IGF, as well as hypothetical biological activities of PPAR, RyR, GR and GABA. Aside the influence of the conformation of the ligand, we discuss its constitution influencing the binding affinity: size and polarizability, hydrophilicity, Gibbs free energy of solvation, inductive and mesomeric effects. We evaluate the techniques to determine the biologically relevant conformation of these halogenated hydrocarbons, including computation methods, X-ray and microwave spectroscopy. A novel fluoro-tagged ligand approach holds promise as tools for illuminating the steric and electronic effects in ligand-receptor interaction. Based on our assessment, we predict that PBDEs do not exhibit AhR activity themselves, but impurities are responsible for these effects.

Keywords

polybrominated diphenyl ethers; PBDEs; polychlorinated biphenyls; PCBs; AhR; CAR; PXR; ER; AR; PR; DHT; TH; T3; T4; IGF; PPAR; RyR; GR; GABA; Conformation; Constitution; Binding affinity; Polarizability; Hydrophilicity; Gibbs free solvation energy; Inductive effects; Mesomeric effects

1 Introduction

The ‘chemical links’ between Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), the ‘PCBs of 21st century’ and Polybrominated biphenyls (PBBs) are: each one consists of 209 individual congeners, distinguishable by the number and position of halogen substituents in two connected aromatic rings. The substitution by bromine or chlorine and the connection by a direct carbon-carbon bond or an ether function distinguish PBDEs, PCBs, and PBBs. These factors have strong influence on the physico-chemical properties,

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e.g. solubility, boiling point, vapor pressure, dipole momentum, London force, stability during light radiation and combustion, molecular volume, and constitution and conformation.

An analysis of transcription factor activation by PBDEs, in comparison to that of PCBs, is based on a three dimensional (3D) quantitative structure activity relationship (QSAR) method that can be either receptor or ligand based. Receptor-based 3D QSAR requires knowledge of protein quaternary structure. Alternatively, based on the QSAR of various ligands, an idealized receptor structure can be developed. Subsequently, the contour map generated from the model can provide some information about the molecular binding domain of the receptor (Oprea *et al.*, 1994). Despite decades of scientific inquiry employing a variety of approaches, the quaternary structure of most receptors remains an elusive target. This review will focus on the comparison of the structural, steric, electrostatic and hydrophobic similarities and distinctions of PCBs and PBDEs to understand their biological activity as receptor agonists and antagonists, generally called ligands.

Receptor interaction initiates a cellular response, *e.g.* the transcription of specific genes, among them genes encoding proteins responsible for the metabolism of xenobiotics. The interaction of the ligand and receptor is not a covalent or long-lived interaction; but in fact a dynamic and often transient effect. Ligands that bind but do not activate the receptor are called antagonists, in contrast to the agonists. Inverse agonists have the ability to reduce the constitutive activity of a receptor and ability to preferentially bind to an inactive form of the receptor. Orphan receptors share structural features found in the nuclear receptor family, but lack known physiologic ligands (Kretschmer and Baldwin, 2005). The extensive literature on PCBs is worthwhile to be extrapolated onto PBDEs, due to their structural similarities, for the purpose of formulating hypotheses on ligand activity with several well-known receptors. Our exploration of receptor activity begins through careful analysis of known ligands, *i.e.* constitution, substitution pattern, and configuration. We will also discuss the limitations of the applied techniques to determine the biologically relevant conformation. Our goal is to present, for the first time, a complete overview of known and hypothetical receptor activities of PBDEs.

2 Structural characteristics of PCBs and PBDEs

2.1 Planarity and the influence of dihedral and torsion angles

The fit of the ligand into the receptor depends on its constitution and conformation, which is determined by the substitution patterns on the aromatic rings. Distinct from the constitution, the conformation of the molecule is in most cases dynamic. For PCBs and PBBs the conformation is described by the dihedral angle Θ between the benzene rings of the biphenyl system (see Figure 1). The conformation of the PBDEs on the other hand is described by two torsion angles φ_1 ($C1'-O-C1-C2(6)$) and φ_2 ($C2'(6')-C1'-O-C1$) between the $C1-O-C1'$ plane and planes of the phenyl rings and the angle ν of the ether bond $C1-O-C1'$. The angles are defined as positive when the rotation is clockwise looking down the $C4-C1$ and $C4'-C1'$ axes toward the oxygen (Figure 1). Theoretically, there are four possible types of conformations of diphenyl ethers: planar ($\varphi_1 = \varphi_2 = 0^\circ$), butterfly ($\varphi_1 = \varphi_2 = 90^\circ$), skew ($\varphi_1 = 0^\circ, \varphi_2 = 90^\circ$) and twist ($\varphi_1, \varphi_2 > 0^\circ$).

For PCBs, the dihedral angle Θ is highly dependent on substitution in the *ortho*-positions (2, 2', 6 and 6'). According to our review of the literature, the experimental dihedral angles determined for non-*ortho*-chloro substituted PCBs are 35–45°, *mono-ortho* 47–51°, *di-* 58–68° and *tetra* 86–87° (Luthe *et al.*, 2007). Based on these findings, the descriptor of PCBs as '*coplanar*' is in fact a misnomer. Even for PCBs with no *ortho*-chloro substitutions there is only a weak approximation of planarity.

A variety of experimental and theoretical studies have shown that the unsubstituted diphenyl ether itself has a twist conformation in that ϕ_1 and ϕ_2 lie in the vicinity of 25–50° (Schaefer *et al.*, 1988). In general, PBDE congeners prefer a skew or twist conformation depending on the number of the *ortho*-bromo substituents (Klösener *et al.*, 2008). ‘Planar’ or ‘co-planar’ PBDEs do not exist. It is crucial to understand that the values for angles describe energy minima of rotation barriers, dependent on intra- and intermolecular forces. The weak point of contemporary models is their emphasis on the lowest energy conformation of both the receptor and the ligand. This assumption may not always be valid. Depending on the energy of the rotation barrier, the molecule can be temporally or episodically changed to another conformation. Intramolecular factors affecting this are defined by the general substitution pattern. Greater *ortho*-substitution increases the energy barrier for conformational change, such that unsubstituted *ortho*- is the lowest and *tetra-ortho*-chloro biphenyl the highest.

Furthermore, the size of the substituent is important as well, since an increased molecular volume determined on the C_(aromatic)-substituent bond length of the substituent, *i.e.* chloro (1.747 Å) versus bromo (1.899 Å), results in an increased steric interaction and therefore an increased energy barrier. But the substituents in *meta*- and *para*-positions influence the rotation barrier as well by a buttressing effect (Luthe *et al.*, 2007). In addition, a stacking interaction operating in the molecular recognition event is hypothesized. Here, the most highly halogenated ring would be considered to be the preferred stacking plane for asymmetrically substituted molecules (McKinney *et al.*, 1985). Conformational properties of variously substituted diphenyl ethers have been studied using semi-empirical or *ab initio* calculations (Nevalainen and Rissanen, 1994; Luthe *et al.* 2007) and for PBDEs as well (Klösener *et al.*, 2008).

2.2 The search for the molecular conformation in the liquid state: X-ray versus semi empirical and *ab initio* computation and microwave spectroscopy

Intermolecular influences are dependent on the environment with the exception of the determination of conformation in a vacuum by microwave spectroscopy or computation. X-ray determinations, computations with semi-empirical self consistent field molecular orbital calculations (SCF-MO), *ab initio* calculations and comparative molecular field analysis (CoMFA) give different results, since the environment and the assumptions are different. X-ray determination of the conformation in a crystal is influenced by $\pi \dots \pi$ stacking and C-H... π phenyl interactions, halogen-halogen interactions, hydrogen bonds and van der Waals interactions. All these factors deform the energetic optimal conformation (Luthe *et al.* 2007; Klösener *et al.*, 2008). The programs used in computational chemistry are based on many different quantum-chemical methods that solve the molecular Schrödinger equation associated with the molecular Hamiltonian (Dewar *et al.*, 1985). *Ab initio* methods that do not include any empirical or semi-empirical parameters in their equations are being derived directly from theoretical principles, with no inclusion of experimental data. None of these methods can stand alone; they are all approximate quantum mechanical calculations. Therefore, a comparison of the various methods needs to be carried out and the results evaluated on the basis of the environment of the ligand. Rotational or microwave spectroscopy studies the absorption and emission of electromagnetic radiation, typically in the microwave region associated with a corresponding change in the rotational quantum number of the molecule. Rotational spectroscopy (Townes and Schawlow, 1975) is only really practical in the gas phase where the rotation motion is quantized. In solids or liquids the rotational motion is usually quenched due to collisions. However, none of these techniques determines the true conformation of the molecule in a liquid matrix, which is of greatest biological relevance. Even the possibility of determining the conformation in liquid state by nuclear magnetic resonance spectrometry will not result in a true picture of the conformation of the guest molecule ligand in the receptor host. Only co-crystallization and X-ray determination of the guest-host agonist/antagonist-receptor

complex will reveal the biologically relevant active confirmation. And even then, the binding site may be expected to be more complex, *i.e.* constituted of amino acids with more or less flexible side chains, which may impose more or less strict steric restrictions in various positions on the ligand for their high affinity binding.

2.3 Molecular constitution and receptor affinity

The constitution of the ligand is defined by its backbone and substitution pattern. In general, the xenobiotic receptor activity is thought to depend on the three dimensional fit of the ligand to its endogenous receptor. But often the constitution of the ligand itself is less important than the steric factors influencing its conformation (see 1.2). There are some intriguing findings in PCB receptor activity indicating that the constitution plays not just an indirect role keeping the larger molecule at the correct dihedral angle, but has a direct influence on the receptor activities. 2,3',4,4',5-Pentachlorobiphenyl (PCB 118) has a mixed functionality, inducing both aryl hydrocarbon (AhR) and constitutive androstane (CAR) receptor activities. The substitution pattern for the non-prime ring is identical to the symmetric 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), a typical CAR receptor agonist (Denomme *et al.*, 1983), while the prime ring is identical with the symmetric 3,3',4,4'-tetrachlorobiphenyl (PCB 77) isomer, an AhR agonist (Bandiera, *et al.*, 1982). Nearly all AhR inducers are more co-planar, but PCB 118 does not fit this description. Depending on the energy of the rotation barrier, the molecule might temporally achieve more co-planar properties. There are some other examples of mixed CAR and pregnane X (PXR) ligand activities for 2,4,4'-trichlorobiphenyl (PCB 28) and 2,2',4,4'-tetrachlorobiphenyl (PCB 47) (Ludewig *et al.*, 2007). However, this finding for PCBs 28 and 47 is anticipated; since these ligands exhibit structure characteristics for both receptors and do not radically differ in conformation.

As the constitution seems vital for the portability of findings to PBDEs, one must take the increased size and molecular volume of the bromo substituent into consideration. Communication between ligand and receptor is based solely on electrostatic interaction. The bromo substituent may be larger ($C_{(aromatic)}-Cl$ 1.747 Å versus $C_{(aromatic)}-Br$ 1.899 Å), but it exhibits an increased 'chemical weakness' according to the Hard and Soft Acid and Basis (HSAB) model (Pearson, 1963) compared to the chloro substituent. Therefore, the strength of the electrostatic interaction must be evaluated on both the orbital size and the strength of the electrostatic interaction between the interacting areas of ligand and receptor. Studies have shown that the most important explanation for the observed difference in binding affinity is the decrease in relative free energy of binding ($\Delta\Delta G_{(binding)}$), compared with the Gibbs free energy of solvation ($\Delta G_{(solv.)}$) in the unbound state. Receptor-ligand binding in solution must take solvation into consideration, which can be calculated using Monte-Carlo simulations (see Figure 2). Recent developments in and applications of free energy perturbation methods have emphasized the fact that if the relative difference in free energy in receptor binding of ligands are small relative to the differences in free energy of solvation, the latter determines the strength of the ligand-receptor interactions. In other words, if the binding site is conceived as being delineated by predominantly hydrophobic amino acids, then less water-soluble ligands will bind more strongly to the receptor.

These different factors may all discretely influence ligand-receptor communication and must be examined alone and in concert to see synergistic effects. A promising tool for illuminating these effects lies in our novel concept of fluorine-tagging (F-tagging) PCBs and PBDEs.

3 PCBs and PBDEs as receptor ligands

3.1 AhR

The aryl hydrocarbon receptor (Ah receptor or AhR) is a cytosolic (Ashek *et al.*, 2006) ligand-activated transcription factor involved in the regulation of several genes. These include xenobiotic-metabolizing enzymes such as cytochrome P450 (CYP) 1A and 1B. Ligands for the AhR include a variety of aromatic hydrocarbons, including chlorinated dioxins and co-planar PCBs (Hahn, 1998). An endogenous ligand for the AhR has not been identified and it is possible that its natural ligand is of exogenous nature. As no receptor structural information is available for the AhR, ligand based QSAR model have been developed (Ashek *et al.*, 2006). The activity of various halogenated aromatics is structure dependent (Poland and Knutson, 1982; Safe, 1990). On the other hand, it is known that halogens are required for receptor activation by halogenated dioxins, dibenzofurans and PCBs. It is *not only the planarity* of the molecule that determines affinity for the receptor. Moreover, examples have been found, *e.g.* chloro-methyl-dibenzo-*p*-dioxin, that can be fitted into both mentioned patterns but have virtually no activity. This gives clear indication that neither of these patterns describes sufficiently the requirements for high-affinity binding. Other factors, such as hydrophobicity of the ligand, are also important determinants for AhR binding (Maitland *et al.*, 1987). AhR binding of PCBs and dioxins has also been correlated with polarizability and stacking parameters (McKinney *et al.*, 1985).

The findings for AhR active PCBs are the so called 'co-planar' *meta*-, and/or *para*-substituted which are not *ortho*-substituted (Bandeira *et al.*, 1982). This is in agreement with the conformational finding, that 'co-planar' PCBs give a better steric fit, and that bulky groups in the 3 and 4 positions increase activity. There are reports in literature regarding weak to moderate AhR activity of the PBDEs 3, 15, 17, 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 126, 152, 153, 154, 183 and the metabolite 6-OH-PBDE 47 (Wang *et al.*, 2006; Chen and Bruce, 2003; Peters *et al.*, 2006a; Peters *et al.*, 2006b; Germer *et al.*, 2006). PBDE 47 and PBDE mixture (D-71) were found to be a weak AhR antagonist (Kuiper *et al.*, 2004).

These findings have been questioned. Polybrominated dibenzofurans (PBDFs) and polybrominated dibenzodioxins (PBDDs) are found as impurities in individual PBDEs components and the mixture DE71 (Sanders *et al.*, 2005). These impurities of PBDFs and PBDDs have been suggested as responsible for most, if not all, AhR receptor activity observed by PBDEs (Sanders *et al.*, 2005). That AhR activity of 6-OH-PBDE 47 might as well be based on impurities by the formation of the corresponding dioxin due to ring closure *via* the hydroxyl group or to weak electrostatic interactions between the hydroxyl and bromo substituents.

Correlations of AhR affinity also seem to stress the importance of steric factors. This finding would be supported by the hypothesis that the conformation plays the main role in the activity and the theoretical studies of Ashek *et al.* (2006). Their CoMFA studies on TCDD analogues show that the biological activity is diminished by a sterically disfavored non-planarity between the benzene rings. Since PBDEs are all out-of-plane (non *co-planar*) they should not exhibit AhR activity. It can be hypothesized based on mixed receptor activities like in PCB 118, that the ether bond in PBDEs allows an interaction between AhR and one phenyl ring, while the other ring does not interact. In our considerations mixed activities are just found due to low rotational barriers between the rings. However, PBDEs do not approach a co-planar conformation, even though this seems probable due to the large distance between the two aromatic rings compared to PCBs, and the ether bond possesses more effective degrees of freedom.

3.2 Constitutive androstane (CAR) and pregnane X receptors (PXR)

The constitutive androstane receptor (CAR) received its name from the androstanes, which act as inverse agonists. CAR is a member of the orphan receptor subfamily, like the pregnane X receptor (PXR). Both PXR and CAR are activated by xenobiotics, controlling regulators of phase I through III enzymes involved in the detoxification and elimination of steroids, bile acids, and xenobiotics (Udea *et al.*, 2002; Rosenfeld *et al.*, 2003; Kretschmer and Baldwin, 2005; Waxman, 1999). Activated detoxification genes induced include several phase I CYPs (Lemaire, de Sousa and Rahmani, 2004), phase II enzymes such as uridine diphosphoglycoronosyltransferases (UDPGT), glutathione-S-transferases (GSTs) and sulfotransferases (SULTs) (Sugatani *et al.*, 2001), and the phase III transporters such as bile salt export pump (BSEP) and the multidrug resistance-associated protein (MRP2) (Kast *et al.*, 2002).

Initially, CAR was thought to regulate CYP 2B, while PXR controlled up regulation for CYP 3A. More recent investigations have shown that there is 'cross-talk' between CAR and PXR (Wei *et al.*, 2002) which results in overlapping functions. *Ortho*- and *para*-substituted PCBs are known to induce CAR activity (Denomme *et al.*, 1983), as well as *poly-ortho*-substituted PCBs (Hurst and Waxman, 2005; Schuetz *et al.*, 1998). PBDE 71 and 79 are both probable activators of CAR and PXR (Kretschmer and Baldwin, 2005; Sanders *et al.* 2005; Germer *et al.*, 2006). However, only PBDE 71 shows two substitutions in the *ortho*-positions, PBDE 79 none. The conformation of both PBDEs is very different, but the patterns in one ring of both isomers are identical. If PBDE 71 and 79 show CAR and PXR activity it must be due to the constitution, not the conformation.

3.3 Thyroid function (TH), thyroxin (T4) and triiodothyronine (T3)

The thyroid hormone thyroxin (T4) is produced by the thyroid gland. The main part of the T4 is bound to transport proteins, *e.g.* globulin (70%) and albumin (15-20%), circulating in the blood. Only the unbound form is biologically active. Thyroid hormones are essential to proper development and differentiation of all cells of the human body (Bianco *et al.*, 2002). These hormones also regulate protein, fat and carbohydrate metabolism (Nussey and Whitehead, 2001). Numerous physiological and pathological stimuli influence thyroid hormone synthesis. In addition, T4 plays a role in the poorly understood mechanism resulting in inhibition of neuronal activity, *e.g.* hibernation cycles (Dratman and Gordon, 1996). To our knowledge, PCBs have only an indirect influence on the thyroid hormone (Gauger *et al.*, 2004). However, a structural comparison of T3 and T4 with PBDEs reveal remarkable similarities, such that receptor activity seems likely. The common diphenyl ether forms the backbone of both molecules, while halogenation is also a common trait. In addition, the radii of iodo ($C_{(aromatic)}-I$ 2.095 Å) and bromo ($C_{(aromatic)}-Br$ 1.899 Å) substituents are of similar size. 2,3',6-Tribromodiphenyl ether (PBDE 27) with triiodothyronine (T3), and 2,3',5',6-tetrabromodiphenyl ether (PBDE 73) with T4 exhibit the same backbone and substitution pattern (see Figure 3). It was determined that OH-PBDEs and PBDE 47 function as agonists (Kretschmer and Baldwin, 2005; Eslami *et al.*, 2006; Hallgren and Darnerud, 2002; Zhou *et al.*, 2002) and PBDE 71 and 79 as disruptors (Kretschmer and Baldwin, 2005). Based on structural similarity, the PBDE metabolite 4'-hydroxyl-2,3',6-tribromodiphenyl ether (4'-OH-PBDE 27), 4-hydroxyl-2,3',5',6-tetrabromodiphenyl ether (4OH-PBDE 73) are of initial interest for investigation. Furthermore, it has been determined that they reduce the serum level of T4 (Kretschmer and Baldwin, 2005; Stoker *et al.*, 2004; Hooper and McDonald, 2000; Zhou *et al.*, 2002; Eslami *et al.*, 2006).

3.4 Other activated receptors

For peroxisome proliferator activated receptors (PPARs) Ariyoshi *et al.* (1998) and Robertson *et al.* (2007) found that the highly toxic more co-planar PCB 126 acts as inverse agonist and

reduces liver peroxisomal enzyme activity in the rat model. Other co-planar *meta*- and *para*-substituted PCBs should have the same potential (Ludewig *et al.*, 2007). For PBDEs, PPAR activity has not been reported. Based on the here presented theoretical assessment, it is our hypothesis that PBDEs do not have potential for PPAR interaction. Pessah *et al.* (2006) established a structure-activity relationship for non co-planar PCBs toward the ryanodine receptor (RyR). It was shown that *ortho*-substituted PCBs, *e.g.* 2,2',3,5',6-pentachlorobiphenyl (PCB 95), alter microsomal calcium transport by direct interaction with the RyR of mammalian brain (Wong *et al.*, 1997). Some phase I metabolites of PCBs demonstrated RyR interaction (Pessah *et al.*, 2006). Nothing is reported in literature regarding the RyR activity of PBDEs or their metabolites. Based on the structure-activity relations by Pessah *et al.* (2006) it can be hypothesized that PBDEs exhibit some RyR activity, since PBDEs are all non co-planar and the molecular size of a hydroxyl substituent ($C_{(aromatic)}-OH$ 1.364 Å) is still comparable in size compared with the bromo substituent ($C_{(aromatic)}-Br$ 1.899 Å).

Recent studies have found that poly-*ortho*-substituted PCBs like 2,2',6,6'-tetrachlorobiphenyl (PCB 54) are estrogenic (ER) (Arcaro *et al.*, 1999; Bonefeld-Jorgensen *et al.*, 2001). Hydroxylated phase I metabolites of PCBs were shown to have an indirect estrogenic effect by inhibiting estrogen sulfotransferase (Kester *et al.*, 2000). For 2,2',4,4',5-pentabromodiphenyl ether (Ceccatelli *et al.*, 2006) and mono- and dihydroxyl PBDEs (Kretschmer and Baldwin, 2005; Stoker *et al.*, 2004; Meerts *et al.*, 2001) an estrogenic activity was found as well. As for the RyR, a possible activation could be postulated to occur on the basis of the non co-planarity of PBDEs in general, especially *ortho*-substituted ones. The poly-*ortho*-substituted PCBs are active as antagonists for the AhR, just as with ER (Fang *et al.*, 2003; Potigal *et al.*, 2002; Schrader and Cooke, 2003).

This contrasts with the findings for PBDEs. The poly-*ortho*-substituted PBDEs 71 and 100 have shown androgen receptor (AR) suppression and inhibition and poly-*ortho*-substituted PBDEs 47, 99, 100, 153, 154 demonstrated competitive anti-androgenic (Stoker *et al.*, 2005). Conner *et al.* (1997) reported that phase I metabolites of PCBs act as antagonists for progesterone based on structure-activity relationships. Ceccatelli *et al.*, (2006) measured that progesterone receptor (PR) was down-regulated by poly-*ortho* substituted PBDE 99. Based on this finding, it can be assumed that more poly-*ortho* substituted PBDEs and their phase I metabolites could affect PR. Johansen *et al.* (1998) observed an interaction between methylsulfonyl PCBs ($MeSO_2$ -PCBs) and the glucocorticoid receptor (GR), where 4-substituted $MeSO_2$ -PCBs displayed only antagonistic effects. In an extensive study by Johansson *et al.* (2005), it was shown that substances with a structural resemblance *via* a methyl sulfonyl group, *e.g.* 4- $MeSO_2$ -2,5,6,2',4'-pentachlorobiphenyl (4- $MeSO_2$ -CB91), 4- $MeSO_2$ -2,3,6,2',4',5'-hexachlorobiphenyl (4- $MeSO_2$ -CB149) and the fungicide tolylfluanid inhibit glucocorticoid receptor-regulated gene transcription. We suggest that 4-substituted $MeSO_2$ -PBDEs may exhibit antagonistic GR activity as well. Thus far, $MeSO_2$ -PBDEs have not been detected, which may be due to their specific physico-chemical properties. Some studies on γ -Aminobutyric acid (GABA) have been carried out to determine the interactions of PCBs with GABA receptors (Mariussen and Fonnum, 2001; Wong *et al.*, 1997; Wong *et al.*, 1997). It was found that the non co-planar PCBs interact with the GABA receptor. We want to address the hypothesis that this might be the case as well for PBDEs, especially for the non *meta*- and *para*-substituted *ortho*-brominated PBDEs. Studies by Ceccatelli *et al.* (2006) have reported a weak up-regulation of insulin-like growth factor (IGF), especially IGF-I by 2,2',4,4',5-pentabromobiphenyl (PBDE 99).

4 Conclusions

In this review we present for the first time an overview of biological transcription factors activated by PBDEs. These include AhR, CAR, PXR, ER, AR, PR, DHT, TH, T3, T4 and IGF.

In addition, we hypothesized regarding presently unknown activities of PBDEs based on comparisons with PCB receptor activities. Since all PBDEs and OH-PBDEs are not co-planar, we suggest that they are agonists for RyR and affect the Ca^{2+} channel function in analogy to non co-planar PCBs and OH-PCBs (Pessah *et al.*, 2006). We addressed the suggestion that PBDEs can be metabolized to MeSO_2 -PBDEs like PCBs and repress by competitive antagonism GR function (Johansen, *et al.*, 1998). By analogy to the non-*meta*- and *para*- but *ortho*-substituted PCBs (Wong *et al.*, 1997), we hypothesize that analog substituted PBDEs are GABA agonists.

Second, we focused on the factors determining biological activity. While several models exclusively take the most energetically stable conformer into consideration, we discussed the influence of rotation barriers and dihedral versus torsion angles, along with the size of the substituents. A brief overview was given of the advantages and disadvantages of several techniques to determine the true molecular conformation of a ligand in solvation. Especially, conformation dynamics and 3D changes of ligand and receptor are extensively neglected.

Based on the assessment done in this review, we strongly support the conclusion that PBDEs are unlikely to exhibit AhR activity by themselves. The electronic interaction might be possible, but to do so the conformation of all PBDEs must be drastically changed to give the right fit for AhR, leading to an electrostatic interaction or receptor-ligand binding. The determination of purity is of critical nature in biological studies. Purity of the PBDEs is not easy to determine by gas chromatography coupled mass spectrometry (GC-MS), especially their discrimination from furan analogues. Both PBDEs and furan analogues form oxonium ion fragments indistinguishable in GC-MS. Bromine loss in the injector can also confound analytical determinations. Only clear carbon-hydrogen correlated nuclear magnetic resonance spectra (^{13}C ^1H COSY NMR) can definitively characterize a given PBDE.

It is evident that the 3D conformational fit of ligand and receptor does not exclusively lead to transcription. The molecular constitution influences the binding affinity by size and chemical hardness (polarizability) of the substituents, hydrophilicity, relative Gibbs free energy of solvation, inductive and mesomeric effects. The mesomeric effect of a given substituent seems to be critical compared with an inductive effect, either negative or positive. Only substituents with positive mesomeric effects seem to interact with the AhR. This finding might be explained on the basis of the extension of the aromatic system *via* hyperconjugation or mesomerism. This would explain why phase I and II metabolites of xenobiotics – which do not have mesomeric effects – do not exhibit agonist activity, in fact demonstrating feedback inhibition against overstimulation. Ligand-receptor relationships must be examined in concert with various synergistic influences and not on a basis of a competition of various models.

A novel fluoro-tagged ligand approach holds promise as a tool for illuminating the steric and electronic effects in ligand-receptor interaction. With the F-tagging approach it is possible to have several analogues of one specific PCB and PBDE congener available for receptor investigations. The fluoro-substituent alters the electronic distribution in the substrate and changes the conformation without affecting the main values (Luthe *et al.*, 2007). These finely tuned changes in the electron distribution can provide detailed information of the electrostatic interaction with the receptor.

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Figure 1.

Definition of bond angles in diethyl ether (left) $\phi 1$ ($\text{C1}'\text{-O-C1-C2(6)}$), $\phi 2$ ($\text{C2}'(6')\text{-C1}'\text{-O-C1}$) between the $\text{C1-O-C1}'$ plane and planes of the phenyl rings and the angle ν of the ether bond $\text{C1-O-C1}'$, and of biphenyl (right) with the dihedral angle Θ ($\text{C1-C1}'\text{-C2(6)}$) between the benzene rings of the biphenyl system.

$$\Delta\Delta G_{(\text{binding})} = \Delta G_{\text{lig}(solv.)} - \Delta G_{\text{lig}} = \Delta G_{(\text{binding})} - \Delta G_{(solv.)}$$

Figure 2.

Thermodynamic cycle describing the determination of the relative free Gibbs energy ($\Delta\Delta G_{(\text{binding})}$) of receptor (R) ligand (L) binding in solution by solvation (solv.) *via* separate solvation of R and L ($\Delta G_{(solv.)}$) followed by the binding in solvation ($\Delta G_{\text{lig}(solv.)}$) and the theoretical pathway of the formation of a transition state ($\ddagger$) binding ($R \dots L^\ddagger$) followed by the binding in solvation at ground state ($R \dots L_{(solv.)}$). Adapted from Gillner *et al.* (1993).

Figure 3. Structural formulas of thyroxine (T4) and triiodothyronine (T3), with highlighted diphenyl ether backbone and substitution patterns of PBDEs 27 and 73.

Table 1

Receptor and function interactions of polychlorinated biphenyls (PCBs), diphenyl ethers (PBDEs) and their metabolites.

Receptor abbreviation	Major Gene or function affected	PCBs		PBDEs	
		Ligands	Response and references	Ligands	Response and references
Aryl hydrocarbon (AhR)	CYP 1A	co-planar, <i>meta</i> -, <i>para</i> - PCBs	>20–30 increase (Bandeira, Safe and Okey, 1992)	PBDEs 3, 15, 17, 28, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 126, 152, 153, 154, 183, 6-OH-PBDE 47	agonists, weak to moderate (Wang <i>et al.</i> , 2006; Chen and Bruce, 2003; Peters <i>et al.</i> , 2006a; Peters <i>et al.</i> , 2006b; Germer <i>et al.</i> , 2006;)
				47, PBDE mixture (D-71)	antagonist in fish, weak (Kuiper <i>et al.</i> , 2004)
				PBDF impurities	no Ah activity by PBDEs, but from PBDFs impurity (Sanders <i>et al.</i> , 2005)
Constitutive androstane (CAR)	CYP 2B	<i>ortho</i> -, <i>para</i> - PCBs	>20–30 increase (Denomme <i>et al.</i> , 1983)	PBDE mixtures DE-71, PBDE 79	weak (Kretschmer and Baldwin, 2005; Sanders <i>et al.</i> , 2005; Germer <i>et al.</i> , 2006)
Pregnane X (PXR)	CYP 3A	poly- <i>ortho</i> - PCBs, PCBs 47, 184	5–10 increase (Hurst and Waxman, 2005; Schuetz, Brimer and Schuetz, 1998)	PBDE mixture D-71	(Germer <i>et al.</i> , 2006)
Peroxisome proliferator (PPAR)	CYP 4A	co-planar, <i>meta</i> -, <i>para</i> -PCBs	repression (Ariyoshi <i>et al.</i> , 1998; Robertson <i>et al.</i> , 2007)	Ah active PBDEs and potentially PBDFs	suggested repression in analogy to PCBs (Ariyoshi <i>et al.</i> , 1998)
Ryanodine (RyR)	Ca ²⁺ channel	PCB 95, OH- PCBs, PCB-catechols	2–50 increased (Pessah <i>et al.</i> , 2006; Pessah and Wong, 2001; Wong, Brackney and Pessah, 1997)	Non co-planar PBDEs and OH- PBDEs	suggested agonist in analogy to the findings in PCBs (Pessah <i>et al.</i> , 2006)
Estrogen (ER)	Estrogen	poly- <i>ortho</i> - PCBs, OH-PCBs	agonist and antagonist (Arcaro <i>et al.</i> , 1999; Bonefeld-Jorgensen <i>et al.</i> , 2001; Connor <i>et al.</i> , 1997)	OH-PBDE Di-OH-PBDE PBDE 99	(Kretschmer and Baldwin, 2005; Stoker <i>et al.</i> , 2004; Meerts <i>et al.</i> , 2001; Ceccatelli <i>et al.</i> , 2006)
Androgen (AR)	Androgen	poly- <i>ortho</i> -PCBs	antagonist (Fang <i>et al.</i> , 2003; Portugal <i>et al.</i> , 2002; Schrader and Cooke, 2003)	PBDE mixture DE-71, PBDEs 100 PBDEs 47, 99, 100	Suppression and inhibition (Stoker <i>et al.</i> , 2005)
				153, 154	competitive anti-androgenic (Stoker <i>et al.</i> , 2005)

Receptor abbreviation	Major Gene or function affected	PCBs		PBDEs	
		Ligands	Response and references	Ligands	Response and references
Progesterone (PR)	Progesterone	OH-PCB	antagonist (Conner <i>et al.</i> , 1997)	PBDE 99	suppression (Ceccatelli <i>et al.</i> , 2006)
Dihydro-testosterone (DHT)	Dihydrotestosterone			PBDE 47, 100	Inhibitor transcriptional activation (Stoker <i>et al.</i> , 2005)
Thyroid function (TH)	Thyroid hormone	PCBs, OH-PCBs	indirect (Gauger <i>et al.</i> , 2004)	OH-PBDEs PBDE 47	Agonist (Kretschmer and Baldwin, 2005; Eslami <i>et al.</i> , 2006; Hallgren, Darnerud, 2002; Zhou <i>et al.</i> , 2002)
Thyroxine (T4) Triiodothyronine (T3)	Thyroxine			PBDE mixtures DE-71, 79 PBDEs 71, 73, 125 4'OH-PBDE 27	disruptor (Kretschmer and Baldwin, 2005) reducing serum T4 level (Kretschmer and Baldwin, 2005; Stoker <i>et al.</i> , 2004; Hooper and McDonald, 2000; Zhou <i>et al.</i> , 2002; Eslami <i>et al.</i> , 2006)
Glucocorticoid (GR)	Glucocorticoid	MeSO ₂ -PCBs	Competitive antagonist (Johansen, Nielsson and Lund, 1998)	MeSO ₂ -PBDE	suggested repression by competitive antagonism in analogy to MeSO ₂ -PCBs (Johansson <i>et al.</i> , 1998)
γ-aminobutyric acid (GABA)	Neurotransmitter	non co-planar PCBs	(Mariussen and Fonnum, 2001; Wong, Brackney and Pessah, 1997; Wong <i>et al.</i> , 1997).	non- <i>meta</i> - and <i>para</i> - but <i>ortho</i> - substituted PBDEs	Suggested agonist in analogy to findings (Wong <i>et al.</i> , 1997).
Insulin-like growth factor (IGF)	Insulin-like growth factor	Xenobiotics in general	Scarth, 2006	PBDE 99	agonist, weak (Ceccatelli <i>et al.</i> , 2006)