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Position of Hydroxyl Group Influences PCB Estrogenicity and Antiestrogenicity

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Abstract

Hydroxylated polychlorinated biphenyls (PCBs) can be estrogenic or antiestrogenic depending upon the position and number of chlorine substitutions. However, little is known regarding the importance of the position of the hydroxyl group since most published studies have used PCBs hydroxylated in the para position only. We examined the position of the hydroxyl group on the activity of 2,5-dichlorobiphenyl (DCBP) and 3,4-DCBP. Estrogenicity and antiestrogenicity were tested in a human breast cell assay, the MCF-7 Focus assay, in which cells respond to 17-β estradiol with post-confluent proliferation and formation of nodules or foci with an EC_{50} of 0.1 nM. Results showed that at 5μM 1) the parent congener 2,5-DCBP was weakly estrogenic, whereas the parent congener 3,4-DCBP was not estrogenic, 2) neither of the parent congeners were antiestrogenic, 3) the para hydroxylated metabolite exhibited the greatest estrogenicity for both DPCBs, 4) the meta hydroxylated metabolite showed activity for 2,5-DCBP and no estrogenic activity for 3,4-DCBP, 5) none of the hydroxylated 2,5-DCBPs were antiestrogenic, 6) 3,4-dichloro-3'-biphenylol was antiestrogenic.

Introduction

Possible alteration of endocrine function in humans and wildlife by background levels of various industrial and other environmental pollutants has emerged as a major public health concern. Various studies indicate or suggest that the multitude of effects observed are a consequence of alterations of the normal pleopotent nature of the endocrine system [1-2]. In these reports , the association between chronic or acute exposures to "endocrine disruptors" with reproductive and gestational deficits, sexual dysfunction, neurotoxicity, immunotoxicity and cancer etiology is debated.

Limited studies by others have shown that certain polychlorinated biphenyls (PCBs) and/or their metabolites are estrogenic in animals[3-7]. The consequences of inappropriate estrogenic activity due to environmental exposure to these compounds for humans is unknown. Studies are underway to aid in the determination of human risk, if any, from estrogenic activity associated with PCB or PCB metabolite exposure. The structural characteristics required for estrogenic activity are being determined and their relation to potency is being assessed.

Hydroxylated polychlorinated biphenyls can be estrogenic or antiestrogenic depending upon the position and number of chlorine substitutions[3,8]. Recent studies from this laboratory

and others suggest that para hydroxylation of PCBs is a requirement for this estrogen activity[7-10]. However, little is known regarding the importance of the position of the hydroxyl group since most published studies have used PCBs hydroxylated in the para position only. We examined the position of the hydroxyl group on the activity of the ortho substituted 2,5-dichlorobiphenyl (DCBP) and the non ortho substituted 3,4-DCBP.

Estrogenicity and antiestrogenicity were tested in a human breast cell assay, the MCF-7 Focus assay, in which cells respond to 17-β estradiol with post-confluent proliferation and formation of multicellular nodules or foci with an EC_{50} of 0.1 nM. Estrogenic compounds were challenged with an antiestrogen (LY156758) which binds to the estrogen receptor. Inhibition of the estrogenic activity by this compound indicates involvement of the estrogen receptor. This was further examined by testing the estrogen receptor (ER) binding affinity of the parent congener and hydroxylated metabolites.

Experimental Methods

Estrogen induction of MCF-7 foci was undertaken as previously described[10]. Briefly, MCF-7 cells were suspended in Dulbecco's Modified Eagles' Medium , without phenol red, supplemented with 5% bovine calf serum (DC_5) after treatment with trypsin (0.25%), seeded into 24-well plastic tissue culture plates at a density of 10^5 cells/ml/well and placed in a 37°C, humidified, CO_2 incubator. Cells were refed at 24 h and every 3-4 days thereafter with 2 ml of DC_5 with various concentrations of the experimental compounds in DMSO (0.1% maximum). After 14 days the cultures were fixed with formalin and stained with 1% Rhodamine B. The stained foci were then counted using a New Brunswick automated colony counter.

PCBs and hydroxylated PCBs were tested in the competitive estradiol binding assay. Different concentrations of test compounds competed with a 2.5 nM concentration of [^{3}H]labeled 17β-estradiol for the binding site on a 1.2 nM concentration of recombinant human estradiol receptor. Hydroxyapatite was used to separate receptor bound radio labeled estradiol. The ED_{50} for estradiol was 1 nM.

Results and Discussion

The MCF-7 focus assay was conducted to assess estrogen-like activity. Results of dose-response studies using the MCF-7 focus assay show that the parent congener 2,5-DCBP is weakly estrogenic at 5μM and the 3,4 -DCBP congener is not estrogenic at this concentration (Fig 1A, 1B). When challenged with 1 nM 17β-estradiol (E_2), neither the 2,5-DCBP nor the 3,4-DCBP displayed estrogen inhibitory activity at any concentration. (Fig 2A, 2B). Para-hydroxylation of both 2,5-DCBP and 3,4-DCBP to 2,5-DCBP-4'-OH and 3,4-DCBP-4'-OH elicited maximal or near maximal focus development, based on E_2 exposure, at 5μM (Fig 1A, 1B). Hydroxylation in the meta position of 2,5-DCBP to 2,5-DCBP-3'-OH resulted in activity similar to the parent congener, while meta hydroxylation of 3,4-DCBP to 3,4-DCBP-3'-OH was devoid of activity. Ortho hydroxylation to 2,5-DCBP-2'-OH and 3,4-DCBP-2'-OH resulted in essentially no activity for either congener. These results support the importance of para-hydroxyl groups on the unsubstituted ring in MCF-7 focus induction. In addition, the comparison of the ortho substituted 2,5-DCBP-4'-OH to the non-ortho substituted 3,4-DCBP-4'-OH indicates that ortho substitution is not an absolute requirement for MCF-7 focus induction. However, the

meta-hydroxylation of 3,4-DCBP-3'-OH at a non cytotoxic concentration of 5μM was the only congener in this group that clearly inhibited the E_2 induction of focus development (Fig 2B).

The role of the estrogen receptor in estrogen modulation of these compounds was demonstrated by the inhibition of the focus induction by the anti-estrogen LY156758, which acts by blocking the estrogen receptor. LY156758 at a concentration of 1-10 nM is non cytotoxic and completely blocked induction of foci by 1 nM E_2 and 5μM 2,5-DCBP-4'-OH and 3,4-DCBP-4'-OH (data not shown).

To further ascertain that the induction of foci in MCF-7 cultures was mediated through an estrogen receptor mechanism, the ability of the parent and hydroxylated congeners to compete with E_2 for the estrogen receptor was determined. The parent 2,5-DCBP showed no effect while 50 μM 2,5-DCBP-4'-OH completely suppressed binding. At this concentration, 2,5-DCBP-3'-OH caused a 50% reduction and 2,5-DCBP-2'-OH was without effect (Fig 3A). The 3,4-DCBP-2'-OH and 3,4-DCBP-3'-OH hydroxylated congeners demonstrated no clear dose response while the para-hydroxylated 3,4-DCBP-4'-OH suppressed ^{3}[H] E_2 binding (Fig 3B). These results are in general agreement with the MCF-7 focus assay results suggesting an estrogen receptor mediated E_2 mimicking mechanism for the active hydroxylated PCBs. This estrogen activity is most potent for the para hydroxylated congeners and the results suggest that ortho substitution is not an absolute requirement for estrogenic activity.

The role of endogenous metabolism of metabolism of the parent congener or the hydroxylated congener by the MCF-7 cultures has not been addressed in this study. It is possible that such metabolism may be responsible for the minimal activity of 2,5-DCBP (Fig 1A) in the absence of estrogen receptor binding activity (Fig 3A). The suppression of E_2 induced foci by 3,4-DCBP-3'-OH (Fig 3B) is also inconsistent with the lack of estrogen receptor binding and may be the result of further hydroxylation by the MCF-7 cell cultures or possible induction of E_2 and subsequent reductionof the intracellular E_2 levels as is the case with 2,3,7,8-tetrachlorodibenzo-p-dioxin[12]. These possibilities increase the complexity of estrogen modulatory activity by PCB hydroxylated metabolites and are currently being examined.

In conclusion, these results indicate that the estrogenicity of the hydroxylated PCB metabolites is through an estrogen receptor mediated mechanism and modulated by position of hydroxylation. The most active parahydroxylated congeners exhibit less than one thousand fold the potency of the endogenous human estrogen, 17β-estradiol.

Figure Legends

Figure 1 A and B- Test for Estrogenicity- 2,5-DCBP, 3,4-DCB, and the indicated hydroxylated metabolites were evaluated for Focus Induction using the MCF-7 Focus Assay as described in methods.

Figure 2 A and B- Test for Antiestrogenicity- 2,5-DCBP, 3,4-DCBP and the indicated hydroxylated metabolites were evaluated for suppression of 17β-estradiol induced Focus Induction using the MCF-7 Focus Assay as described in mehtods.

Figure 3 A and B- Test for Estrogen Receptor Binding- 2,5-DCBP, 3,4-DCBP and the indicated metabolites were tested for estrogen receptor binding as described in methods.

Figure 1. Estrogenicity

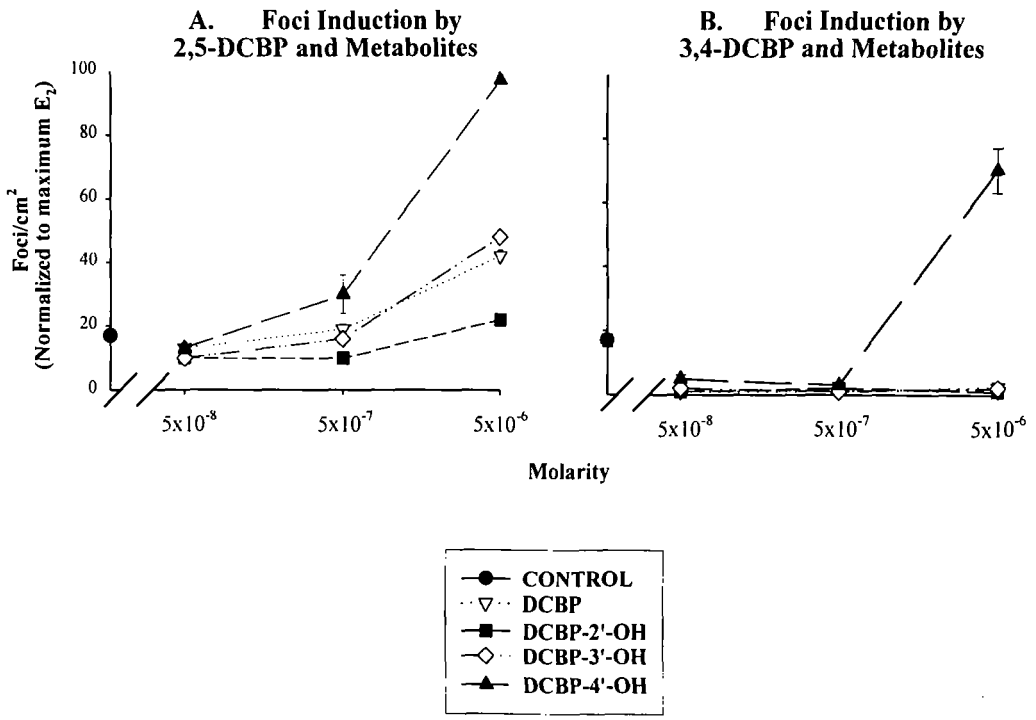


Figure 2. Antiestrogenicity

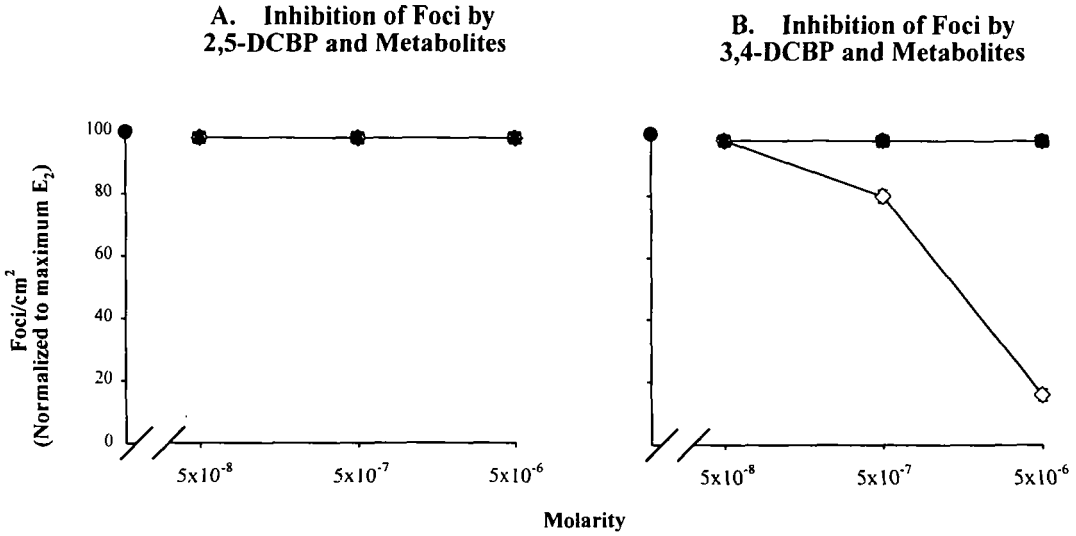
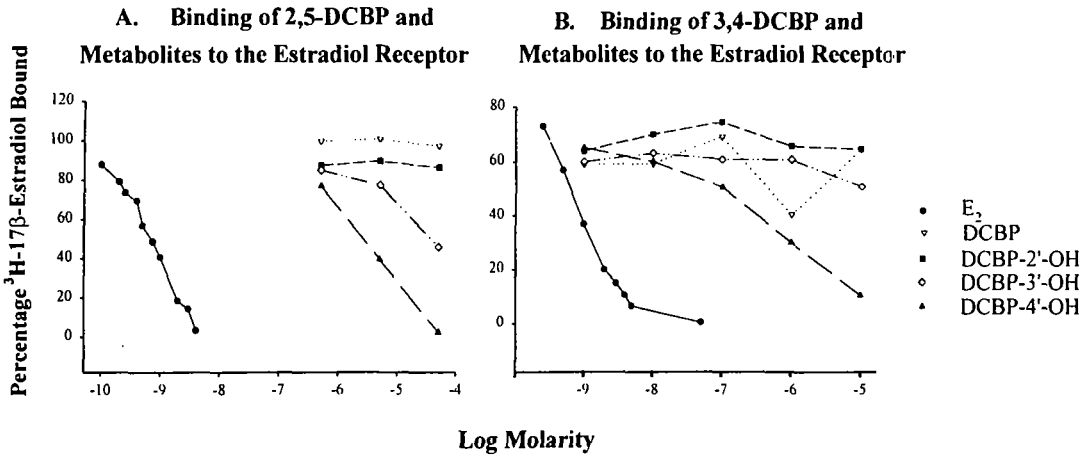


Figure 3. Estrogen Receptor Binding



Acknowledgments

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