Published in final edited form as:

Toxicol Appl Pharmacol. 2008 March 1; 227(2): 196–206.

Ligand-Dependent Interactions Of The Ah Receptor With Coactivators In A Mammalian Two-Hybrid Assay

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Abstract

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a high affinity ligand for the aryl hydrocarbon receptor (AhR). In this study, we investigated structure-dependent differences in activation of the AhR by a series of halogenated aromatic hydrocarbons. TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB126) induced CYP1A1-dependent activities in HEK293 human embryonic kidney, Panc1 pancreatic cancer, and Hepa1c1c7 mouse hepatoma cell lines. There was a structure-dependent difference in the efficacy of TCDF and PCB126 in HEK293 and Panc1 cells since induced CYP1A1 mRNA levels were lower than observed for the other congeners. A mammalian two hybrid assay in cells transfected with GAL4-coactivator and AhR-VP16 chimeras was used to investigate structure-dependent interactions of these chimeras in Panc1, HEK293 and Hepa1c1c7 cells. The reporter construct, pGAL4-luc contains five tandem GAL4 response elements linked to several coactivators including steroid receptor coactivator 1 (SRC-1), SRC-2 and SRC-3, the mediator coactivator TRAP220, coactivator associated arginine methyl transferase 1 (CARM1), and peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1). Results of the mammalian two hybrid studies clearly demonstrate that activation of pGAL4-luc in cells transfected with VP-AhR and GAL4-coactivator chimeras is dependent on the structure of the HAH congener, cell context and coactivator, suggesting that the prototypical HAH congeners used in this study exhibit selective AhR modulator activity.

Keywords

AhR; agonist; coactivator interactions

INTRODUCTION

The aryl hydrocarbon receptor (AhR) was initially identified by Poland and coworkers as an intracellular receptor (Poland *et al.*, 1976) that binds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbon (HAH) industrial compounds and

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combustion by-products (Poland *et al.*, 1976; Poland and Knutson, 1982; Safe, 1986). The AhR is a ligand-activated transcription factor that forms a transcriptionally-active heterodimeric nuclear complex with the AhR nuclear translocator (Arnt) (Gu *et al.*, 2000; Hankinson, 1995). Both the AhR and Arnt are members of the basic helix-loop-helix/Per-Arnt-Sim (bHLH-PAS) gene family and among the bHLH family the AhR is the only protein that binds ligands and Arnt serves as a heterodimeric protein for other bHLH transcription factors (Gu *et al.*, 2000; Hankinson, 1995). Results of gene ablation studies in mice demonstrate that the AhR is important for diverse physiological processes (Gonzalez and Fernandez-Salguero, 1998); however, the endogenous ligand that mediates these AhR-mediated responses is unknown. Arnt knockdown is embryolethal in mice (Maltepe *et al.*, 1997), and this protein is also known as hypoxia-inducible factor-1b and is required for critical hypoxia-dependent developmental processes.

The AhR binds with high affinity to 2,3,7,8-TCDD and early studies showed a correlation between the rank order AhR binding affinities of 2,3,7,8-TCDD and related HAHs and their relative biochemical and toxic potencies in a limited number of assays (Poland *et al.*, 1976; Poland and Knutson, 1982; Safe, 1986). Subsequent studies have determined the biochemical and toxic potencies of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and selected non-ortho substituted polychlorinated biphenyls (PCBs) in multiple AhR-responsive *in vitro* and *in vivo* assays (Ahlborg *et al.*, 1994; Van den Berg *et al.*, 2006; Van den Berg *et al.*, 1998). For a selected number of environmentally relevant halogenated aromatic hydrocarbon (HAH) congeners, their toxic equivalency factors (TEFs) or relative potency factors (RPFs) compared to 2,3,7,8-TCDD (RPF = 1.0) have been assigned. These TEF/RPF values are routinely used to monitor and quantitate the toxic equivalency (TEQs) of various HAH mixtures in the environment, food and biota where the TEQ for a sample extract is the sum of the HAH concentrations times their respective TEF/RPF value. Like many other ligand-activated receptors, the AhR binds structurally diverse compounds including polynuclear aromatic hydrocarbons (PAHs), synthetic aromatic amines, drugs, endogenous biochemicals, pesticides and phytochemicals (flavonoids and indoles) (Bjeldanes *et al.*, 1991; Chen *et al.*, 1998; Ciolino *et al.*, 1998; Ciolino and Yeh, 1999; Denison and Nagy, 2003; Gasiewicz *et al.*, 1996), and interactions between HAH AhR agonists and dietary/endogenous biochemical AhR creates difficulties for risk assessment of HAHs (Ashida *et al.*, 2000; Zhang *et al.*, 2003).

Ligand-dependent activation of the AhR and members of the nuclear receptor (NR) superfamily is complex, and studies on NRs have defined some of the elements which contribute to this complexity (Katzenellenbogen *et al.*, 1996; Smith and O'Malley, 2004). Activation of NRs depends on the structure of the NR agonist, tissue/cell-specific expression of nuclear cofactors including coactivator proteins and gene promoter context which can also influence modulation of gene expression. Because of the multiple factors that are required for ligand-activation or repression of a nuclear receptor, structurally diverse compounds that activate NRs can be referred to as selective receptor modulators (SRMs), which exhibit tissue-specific NR agonist or antagonist activity (Klinge, 2000; Krishnan *et al.*, 2001). For example, the chemotherapeutic drug tamoxifen is a selective estrogen receptor (ER) modulator (SERM) which induces or represses many of the same genes modulated by 17 β -estradiol; however, tamoxifen is an ER antagonist in breast tumors and an ER agonist in the endometrium whereas 17 β -estradiol is an ER agonist in both tissues (Jordan, 2003).

DIM and 6-methyl-1,3,8-trichlorodibenzofuran (6-MCDF) have been characterized as selective AhR modulators (SAhRMs) (Chen *et al.*, 1998; Hestermann and Brown, 2003; McDougal *et al.*, 2001; Safe *et al.*, 2001), and this paper investigates the possibility that even structurally similar HAHs may also exhibit SAhRM-like activity. The five HAHs selected for this study include two PCDDs [2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin

(PeCDD)], two PCDFs [2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorochbenzofuran (PeCDF)] and 3,3',4,4'-pentachlorobiphenyl (PCB126). All five compounds induce CYP1A1 mRNA/protein expression in human Panc1 pancreatic, HEK293 human embryonic kidney, and Hepalclc1 mouse hepatoma cells. However, their effects on transactivation in a mammalian two-hybrid assay using several GAL4-coactivator chimeras and a chimera containing the AhR fused to the acidic activator VP16 (VP-AhR) were dependent on HAH structure, coactivator chimera and cell context, suggesting that the five HAHs also exhibit SAhRM-like activity.

MATERIALS AND METHODS

Cell lines, constructs and antibodies

Panc1 and HEK293 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Hepalclc7 cells were kindly provided by Dr. Yanan Tian (Texas A&M University, TX). The pDRE₃-luciferase reporter plasmid was constructed in this laboratory and contains three tandem consensus dioxin response elements (DRE) (TCT TCT CAC GCA ACT CCG A — a single DRE sequence). The Gal4 reporter containing 5X Gal4DBD (pGAL4-luc) was kindly provided by Dr. Marty Mayo (University of North Carolina, Chapel Hill, NC). Gal4-coactivator fusion plasmids pM-SRC-1, pM-AIB1, pM-TIFII, pM-DRIP205, pM-TRAP220, and pM-CARM-1 were obtained from by Dr. Shigeaki Kato (University of Tokyo, Tokyo, Japan). VP-AhR was provided by Dr. Gary Perdew (Penn State University, PA). Antibodies for CYP1A1, AhR and Arnt proteins were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody for β -actin was obtained from Sigma (St. Louis, MO).

Transient transfection assays

Cells were cultured in 12-well plates in 1 ml of DME/F12 medium supplemented with 2.5% fetal bovine serum. After 16–20 h when cells were 30–50% confluent, the pDRE-luc (0.4 μ g) or GAL4-coactivator/GAL4-luc (0.4 μ g), β -galactosidase (0.1 μ g) constructs were transfected using lipfectamine 2000 Reagent (Invitrogen, Carlsbad, CA). Cells were harvested 36–44 h after transfection by manual scraping in 1X lysis buffer (Promega, Madison, WI). For whole cell lysates, cells were frozen and thawed in liquid nitrogen, vortexed for 30 s, and centrifuged at 12,000 g for 1 min. Lysates were assayed for luciferase activity using luciferase assay reagent (Promega, Madison, WI). β -Galactosidase activity was measured using Tropix Galacto-Light Plus assay system (Tropix, Bedford, MA) in a Lumicount microwell plate reader (Packard Instrument Co.).

Western immunoblot analysis

Cells were seeded into 35-mm six-well tissue culture plates in phenol red-free DME/F12 medium supplemented with 2.5% dextran/charcoal-stripped fetal bovine serum. After 24 h, cells were treated with five HAHs and DMSO control for 24 h, respectively, and harvested in ice-cold high salt lysis buffer (50 mM HEPES, 500 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EGTA, pH 7.5) supplemented with protease inhibitor cocktail (Sigma). An aliquot of whole cell lysates containing 30 μ g protein was diluted with loading buffer, boiled, and loaded on a 10% SDS-polyacrylamide gel. Samples were electrophoresed at 150–180 V for 3–4 h, and separated proteins were transferred to polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Proteins were detected by incubation with polyclonal primary antibodies CYP1A1, AhR, Arnt and β -actin (1:1000 dilution), respectively, followed by blotting with horseradish peroxidase-conjugated anti-rabbit (for CYP1A1, AhR and Arnt) or anti-mouse (for β -actin) secondary antibody (1:5000 dilution).

Quantitative real-time PCR

Cells were seeded into 35-mm six-well tissue culture plates in phenol red-free DME/F12 medium supplemented with 2.5% dextran/charcoal-stripped fetal bovine serum. After 24 h, cells were treated with five HAHs and DMSO control, respectively, for 24 h, and then cells were collected and total RNA was isolated using the RNeasy Protect Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. RNA was eluted with 30 μ l of RNasefree water and stored at -80°C . RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. cDNA obtained from reverse transcription using a combination of oligodeoxythymidylic acid and dNTP mix (Applied Biosystems, Foster City, CA) and Superscript II (Invitrogen). Each PCR was carried out in triplicate in a 25- μ l volume using SYBR Green Master mix (Applied Biosystems) for 15 min at 95°C for initial denaturing, followed by 40 cycles of 95°C for 30 s and 60°C for 1 min in the ABI Prism 7700 sequence detection system (Applied Biosystems). The ABI Dissociation Curves software was used after a brief thermal protocol (95°C 15 s and 60°C 20 s, followed by a slow ramp to 95°C to control for multiple species in each PCR amplification. Primers used in the experiment are human CYP1A1 (forward: 5'-TGG TCT CCC TTC TCT ACA CTC TTG T - 3'; reverse: 5'-ATT TTC CCT ATT ACA TTA AAT CAA TGG - 3'), mouse CYP1A1 (forward: 5'-CAC CAT CCC CCA CAG CAC - 3'; reverse: 5'-ACA AAG ACA CAG CAC CCC TT - 3') and TBP (forward: 5'-TGC ACA GGA GCC AAG AGT GAA - 3'; reverse: 5'-CAC ATC ACA GCT CCC CAC CA - 3'). Primers were purchased from Integrated DNA Technologies (Coralville, IA).

Statistical analysis

Statistical significance was determined by analysis of variance and Scheffe's test, and the levels of probability are noted. The results are expressed as means $\pm$ SD for at least three separate (replicate) experiments for each treatment group.

RESULTS

2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and PCB126 are structurally-related HAHs that binds the AhR and induce AhR-mediated biochemical and toxic responses in different tissues/cell lines. Relative potency factors for all five compounds have been established; however, for each compound, there is a range of potency values (relative to 2,3,7,8-TCDD) which is species, age, sex and tissue/cell-specific (Van den Berg *et al.*, 2006; Van den Berg *et al.*, 1998). Results in Figure 1 summarize the concentration dependent induction of CYP1A1 protein by the five HAHs in human Panc1 pancreatic cancer cells, human HEK293 embryonic kidney cells, and Hepa1c1c7 mouse hepatoma cells. Similar patterns of CYP1A1 protein induction were observed in all three cell lines, and these results are consistent with expression of both AhR and Arnt in these cells. In addition, we also observed ligand-dependent repression of AhR in Panc1 cells, whereas this response was less obvious in Hepa1c1c7 and HEK293 cells. Using concentrations that maximally induced CYP1A1 and correlated with relative potency factors, we also examined ligand-dependent induction of CYP1A1 mRNA levels by quantitative real-time PCR (Figs. 2A–2C). In Panc1 and HEK293 cells a similar fold-induction response was observed for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF, whereas $\leq 50\%$ of this response was induced by 2,3,7,8-TCDF and PCB126. This pattern of results was replicated in both cell lines and similar results were observed for lower concentrations of both compounds (data not shown). Higher concentrations of 2,3,7,8-TCDF and PCB126 were cytotoxic to these cells. In contrast, all five compounds induced maximal CYP1A1 mRNA levels in Hepa1c1c7 cells (Fig. 2C) demonstrating some cell context dependent differences in the efficacy of these HAHs as inducers of CYP1A1 mRNA.

We also used a third “induction” response to compare the five HAHs and cells were transiently transfected with a pDRE3-luc construct which contains three tandem consensus human DREs linked to luciferase gene. The concentration-dependent induction of luciferase activity by the five HAH congeners in Panc1 and HEK293 cells (Fig. 3A and 3B) showed that the fold-induction response for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF was higher than observed for 2,3,7,8-TCDF and PCB126. In contrast, comparable fold-induction responses were observed for all five HAHs in Hepa1c1c7 cells (Fig. 3C), and the ligand structure- and cell context-dependent differences observed for induction of luciferase activity was similar to that observed for induction of CYP1A1 mRNA levels (Fig. 2).

A mammalian two hybrid assay in cells transfected with GAL4-coactivator and AhR-VP16 chimeras was used to investigate structure-dependent interactions of these chimeras in Panc1, HEK293 and Hepa1c1c7 cells. The reporter construct, pGAL4-luc contains five tandem GAL4 response elements linked to a luciferase gene and the GAL4-coactivator chimeras contain the DNA binding domain of the yeast GAL4 gene linked to the full length coactivator. The VP16-AhR chimera contains the powerful C-terminal 80 amino acid transactivator from herpes simplex virus protein 16 (VP16) and the ligand binding domain of the AhR (Ko *et al.*, 1997). Results in Figure 4 illustrate the concentration-dependent induction responses in HEK293 cells treated with the five HAHs and transfected with the six different GAL4-coactivator chimeras used in this study. The range of HAH concentrations used in this assay was similar to those used for the studies illustrated in Figure 1 – 3.

The approach illustrated in Figure 4 was employed for all compounds in the three cell lines and Figure 5 summarizes the maximal ligand structure-dependent induction responses observed in these cells transfected with VP16-AhR and GAL4-SRC-1, GAL4-CARM-1, GAL4-TRAP220 or GAL4-TIFII. The HAH congeners did not induce transactivation in any of the cells line transfected with the GAL4-PGC-1 or GAL4-SRC3 chimeras. In Panc1 cells, increased activity (with variable significance) was observed for both 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in cells transfected with all four GAL4-chimeras, whereas among the two PCDF congeners and PCB126, only the latter compound induced transactivation, and this was observed for GAL4-TRAP220 and not the other GAL4-coactivation chimeras. A different pattern of ligand-dependent transactivation was observed in HEK293 cells (Fig. 5B). 2,3,7,8-TCDD induced luciferase activity in cells transfected with GAL4-SRC-1, GAL4-CARM-1, GAL4-TRAP220 but not GAL4-TIFII. 2,3,4,7,8-PeCDF activated only GAL4-SRC-1 and GAL4-TIFII, and the remaining compounds were inactive in cells transfected with all four GAL4-coactivator chimeras. Structure-dependent transactivation in Hepa1c1c7 was also highly variable. 2,3,7,8-TCDD, 2,3,7,8-TCDF and PCB126 activated GAL4-SRC-1. Only PCB126 activated GAL4-CARM-1. 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF activated GAL4-TIFII, whereas none of the HAHs activated GAL4-TRAP220. These results clearly demonstrate that activation of pGAL4-luc in cells transfected with VP-AhR and GAL4-coactivator chimeras is dependent on the structure of the HAH congener, cell context and coactivator, suggesting that the prototypical HAH congeners used in this study exhibit selective AhR modulator activity.

DISCUSSION

Coactivators and other nuclear cofactors play a critical role in activation of gene expression by serving as part of a budding complex between specific transcription factors and the basal transcription machinery and DNA polymerase II. Many coactivator interactions with NRs are ligand-dependent, and the interactions of steroid receptor coactivators (SRCs) through their LXXLL (NR-box) motifs with the ER and other steroid hormone receptors have been extensively investigated (Hall *et al.*, 2002; Roeder, 2005). Several assay methods have been used to demonstrate differences and similarities between diverse structural classes of estrogenic

compounds. Some of these assays include activation of wild-type and variant forms of ER α and ER β , activation of different E2-responsive promoter constructs, and the differential ligand-dependent effects of various LXXLL peptides on ER-coactivator interactions (Hall *et al.*, 2002; Matthews *et al.*, 2002; Routledge *et al.*, 2000; Schaufele *et al.*, 2000). In addition, a mammalian two-hybrid assay using GAL4-coactivator and VP-ER chimeras, has also shown that ER-coactivator interactions were dependent on the structure of the ligand (Mueller *et al.*, 2004; Nishikawa *et al.*, 1999).

The advantages of a mammalian two-hybrid assay is that this system is highly flexible, can be transfected into different cell lines, and is not dependent on over- or under-expression of an endogenous coactivator since the GAL4-coactivator chimera is transfected directly into the cells. In addition, promoter complexities are minimized since the GAL4-coactivator activates a yeast pGAL4-luc promoter construct in a mammalian cell context. However, an important feature of this assay is that the effects of cell context on transactivation can be determined since ligand-dependent GAL4-coactivator-VP-receptor interactions also rely on endogenous cofactors for transactivation.

Several studies report the NR receptor coactivators and corepressors and other nuclear cofactors also interact with and modulate ligand-dependent AhR-mediated transactivation (Antenos *et al.*, 2002; Beischlag *et al.*, 2002; Beischlag *et al.*, 2004; Chen *et al.*, 2006; Kim *et al.*, 2006; Kim and Stallcup, 2004; Kobayashi *et al.*, 1997; Kumar *et al.*, 1999; Kumar and Perdew, 1999; Nguyen *et al.*, 1999; Rowlands *et al.*, 1996; Wang *et al.*, 2004; Wang and Hankinson, 2002). For example, SRC-1, SRC-2 (TIFII) and SRC-3 (AIB1) enhance TCDD-induced transactivation in Hepa-1 cells (Beischlag *et al.*, 2002); knockdown of TRAP220 by RNA interference decreases TCDD-induced transactivation in human HepG2 cells (Wang *et al.*, 2004) and CARM-1 also enhances AhR-mediated gene expression (Yanan Tian, personal communication).

In this study, we used a mammalian two-hybrid assay in Panc1, HEK293 and Hepa1c1c7 cells to investigate possible ligand structure- and cell context-dependent differences in GAL4-coactivator-VP-AhR interactions using coactivators (SRC-1, SRC-2, SRC-3, TRAP220 and CARM-1) known to enhance AhR-mediated gene expression. We also selected PGC-1 for these studies since PGC-1 coactivates gene expression mediated by several NRs.

The five HAH ligands induced CYP1A1 protein and mRNA levels in all these cell lines and also induced luciferase activity in cells transfected with pDRE3 (Figs. 1–3). Inducibility of CYP1A1 by HAHs and other AhR agonists is probably the most characteristic response observed for these compounds in multiple tissues/cell-types. The only major differences among these compounds was observed between 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF which induced maximal CYP1A1 mRNA/luciferase activity and 2,3,7,8-TCDF and PCB126 which induced submaximal responses in Panc1 and HEK293 cells (Figs. 2 and 3). Higher concentrations of the latter two congeners were cytotoxic (i.e. floating cells) and did not enhance activity, and lower concentrations also did not induce CYP1A1 mRNA or luciferase activity in any of the cell lines. Previous studies with HAHs and PAHs have also observed structure-dependent differences in maximal induction of CYP1A1 mRNA and/or CYP1A1-dependent activity; however, these effects are variable and cell context-dependent. For example, both 2,3,7,8-TCDD and PCB126 induce similar maximal-induction responses in rat H4IIE cells, whereas in human HepG2 cells, differences are similar to those illustrated in Figures 2 and 3 (Zeiger *et al.*, 2001). Moreover, in COS7 cells transfected with a DRE promoter and AhR/Arnt from different species, maximal induction activity was highly variable and depended on the source (species) of the AhR (Abnet *et al.*, 1999; Yang *et al.*, 2006).

Results of the mammalian two-hybrid assay with the five HAHs (Figs. 4 and 5) clearly show that HAH-induced GAL4-coactivators-VP-AhR interactions were both structure- and cell context-independent. One common feature for all five HAHs in the three cell lines was that interactions of VP-AhR with GAL4-PGC-1 or GAL4-SRC-3 were not observed. This was somewhat surprising for GAL4-SRC3 since this coactivator interacts with AhR, and 2,3,7,8-TCDD induces recruitment of SRC-3 to the DRE enhancer region of the mouse CYP1A1 promoter in Hepa1c1c7 cells (Kumar *et al.*, 1999). Nevertheless, in this study, significant HAH-induced GAL4-SRC-3-AhR-VP interactions were not observed in Hepa1c1c7, HEK293 or Panc1 cells. Examination of the transactivation results (Fig. 5) clearly illustrates marked structure-dependent differences of the HAHs as inducers of transactivation in the three cell lines. 2,3,7,8-TCDD, which is the most avid ligand for the AhR, does not uniformly exhibit the highest potency for inducing GAL4-coactivator-AhR-VP interactions in the various cell lines and in no cell line does TCDD induce transactivation in cells transfected with all four GAL4-coactivator chimeras. Figure 6 illustrates some of the cell context-dependent differences and similarities between the five HAHs in activation of specific coactivator-AhR interactions. Results of the mammalian two-hybrid assay using GAL4-TRAP220 and VP-AhR in HEK293 and Hepa1c1c7 cells showed that TCDD induced transactivation in HEK293 but not Hepa1c1c7 cells, whereas the four other congeners were inactive (Fig. 6A). In Panc1 cells, TCDD, PCDD and PCB126, but not the chlorinated dibenzofuran congeners, induced transactivation (Fig. 5A). Figure 6B highlights differences in CARM1-AhR interactions which are induced only by TCDD in HEK293 cells and only by PCB126 in Hepa1c1c7 cells. Both TCDD and PCDD, but not the other congeners, induced transactivation in Panc1 cells (Fig. 5A). In contrast, a similar structure-dependent induction of transactivation by the HAHs was observed in Hepa1c1c7 and Panc1 cells transfected with GAL4-TIFII (Fig. 6C), whereas only PCDF induced transactivation in HEK293 cells (Fig. 5B).

In summary, results of this study demonstrate that five HAHs induce CYP1A1 or DRE-dependent gene expression in HEK293, Panc1 and Hepa1c1c7 cells with some cell context-dependent differences observed only in maximal-induced activity. However, in a mammalian two-hybrid assay in the same cell lines, induction of transactivation was ligand structure-, coactivator- and cell context-dependent. These results suggest that the five structurally-related HAHs exhibit selective receptor modulator activity, and this could result in tissue/cell-specific differences in their AhR agonist or antagonist activity at the gene or response level. Current studies are focused on several aspects of this intriguing problem including the identity of HAH-specific nuclear coactivators/cofactors in cell lines/tissues that may dictate structure-dependent differences in HAH action.

Acknowledgements

The financial assistance of the Dow Chemical Company, the National Institutes of Health (ES09106 and ES04917), and the Texas Agricultural Experiment Station is gratefully acknowledged.

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Fig. 1.

Effects of HAHs on induction of CYP1A1 protein. Panc1 (A), HEK293 (B) and Hepa1c1c7 (C) cells were treated with different concentrations of the five compounds for 24 h, and CYP1A1 protein levels were determined by Western blot analysis of whole cell lysates as described in the *Materials and Methods*. Similar results were observed in duplicate experiments.

Fig. 2. TCDD and structurally related four HAHs induce CYP1A1 transcription. Panc1 (A), HEK293 (B) and Hepa1c1c7 (C) cells were treated with the five compounds for 24 h, respectively, and mRNA levels were determined by real-time PCR as described in the *Materials and Methods*. Results are given as means $\pm$ S.E. for three replicate determinations for each treatment group, and all of the five compounds significantly ($p < 0.05$) increased activities (compared to solvent control) and are indicated by an *asterisk*. The concentration for each compound used in this study was not cytotoxic and maximally induced CYP1A1 (Fig. 1).

Fig. 3. Effects of TCDD and other HAHs on activation of the Ah-responsive pDRE3-Luc. Panc1(A), HEK293(B) and Hepa1c1c7(C) cells were transfected with Ah-responsive pDRE3-Luc construct and treated with DMSO, TCDD and four other HAHs at different concentration for 24 h, and luciferase activity was determined as described in the *Materials and Methods*. Results summarized in (A), (B) and (C) are means $\pm$ SD for three replicate determinations for each treatment group, and all the five compounds significantly ($p < 0.05$) increased luciferase activity (compared to solvent control) and are indicated by an *asterisk*.

Fig. 4.

Structure dependent induction of luciferase activity in a mammalian two-hybrid assay in HEK293 cells. Cells were transfected with pGAL4-luc, GAL4-coactivators and AhR-VP16 constructs, and treated with DMSO and different concentrations of TCDD (A), PCDD (B), PCDF (C), TCDF (D) or PCB (E) for 24 h. Luciferase activity was determined as described under the *Materials and Methods*. Results are expressed as means $\pm$ SD for three separate determinations for each treatment group and significant ($p < 0.05$) induction compared to solvent control is indicated (*).

Fig. 5.

Structure dependent activation of luciferase activity in a mammalian two-hybrid assay. Panc1 (A), HEK293 (B) and Hepa1c1c7 (C) cells were transfected with pGAL4-luc, GAL4-coactivators and AhR-VP16 constructs, and treated with DMSO, different concentrations of TCDD and four other HAHs for 24 h. Luciferase activity was determined as described under the *Materials and Methods*. Significant ($p < 0.05$) induction (*) was only observed at the maximal concentration of TCDD (10 nM), PCDD (20 nM), PCDF (20 nM), TCDF (20 nM) and PCB (50 nM) used in this study.

A Cell Context - DIFFERENCE

B Cell Context - DIFFERENCE

C Cell Context - SIMILARITIES

Fig. 6.

Cell context-dependent differences and similarities between the five HAHs in activation of specific coactivator-AhR interactions in a mammalian two-hybrid assay. Cell context-dependent differences in activation of GAL4-TRAP220 in HEK293 and Hepa1c1c7 cells (A) and activation of GAL4-CARM1 in HEK293 and Hepa1c1c7 cells (B) are derived from data in Figure 5. Similarities in the structure-dependent induction in Hepa1c1c7 and Panc1 cells transfected with GAL4-TIFII (C) are also derived from data in Figure 5. Significant ($p < 0.05$) induction (*) was only observed at the maximal concentration of TCDD (10 nM), PCDD (20 nM), PCDF (20 nM), TCDF (20 nM) and PCB (50 nM) used in this study.