EFFECTS OF 2,2'4,4'-TETRABROMODIPHENYL ETHER ON NUCLEAR RECEPTOR REGULATED GENES: IMPLICATIONS FOR THYROID HORMONE DISRUPTION

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Introduction

2,2',4,4'-Tetrabromodiphenyl ether (BDE 47) is usually the most common polybrominated diphenyl ether (PBDE) congener found in human tissues and wildlife. Several studies demonstrate that PBDEs may act as endocrine disruptors through interference with thyroid hormone homeostasis. Although exposure risks to humans have not been determined, studies in rodents show that PBDE-mediated decreases in thyroid hormone most profoundly affect the developing animal[1-3]. Decreased concentrations of thyroid hormones following exposures to PBDEs have previously been linked to the induction of hepatic uridinediphosphate-glucuronosyltransferases (UDPGTs). While UGTs glucuronidate thyroid hormones and play a role in thyroid hormone homeostasis, it is not certain that metabolism alone is responsible for the effects of PBDEs on thyroid hormone concentrations. For example, studies exposing UGT1A-deficient Gunn rats to phenobarbital (PB) or polychlorinated biphenyl (PCB) demonstrated that serum total T4 decreases were not necessarily glucuronidation dependent[4,5]. This suggests that other mechanisms must be involved in the thyroid hormone decreases. UGTs are also important in the metabolism of xenobiotics and like other xenobiotic metabolism enzymes (XMEs) they are regulated by nuclear receptors such as aryl-hydrocarbon receptor (AhR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). Not only do these nuclear receptors regulate the expression of XMEs they have also been implicated in the regulation of xenobiotic transporters [6,7]. The ability of PBDEs to induce UGTs indicates that PBDEs may also activate nuclear receptors that regulate other genes involved in xenobiotic metabolism and transport. This study examined the ability of BDE 47 to activate genes in the AhR, CAR, and PXR pathways which may also be involved in thyroid hormone decreases due to metabolism, transport, and elimination.

Methods

Animals and treatment. Female C57BL/6 mice (65 days old) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Animals were maintained on a 12-hour light/dark cycle and were provided with rodent chow and tap water *ad libitum.* Mice (n=10/group) were administered daily doses of BDE 47 in a corn oil vehicle (0, 3, 10 or 100 mg/kg/day for 4 days) by oral gavage (10 ml/kg) using a curved ball-tipped animal feeding needle. 24 hours after the 4th day of dosing, the mice were euthanized and blood and liver were collected. Blood was centrifuged to obtain serum and then stored at -20°C. Livers were weighed and stored at -80°C until use.

Thyroid Hormone Analysis. Serum total thyroxine (T4) concentrations levels were measured using the Coat-a-Count radioimmunoassay (RIA) kit (Diagnostic Products Corporation, Los Angeles, CA). Each sample was measured in duplicate.

EROD, PROD and T4-UDPGT assays. Liver microsomal fractions were prepared and protein concentrations were measured using a protein assay kit[3]. Enzymatic activities for ethoxyresorufin O-deethylase (EROD), a marker for CYP1A1 and pentoxyresorufin O-deethylase (PROD), a marker for CYP2B were measured using a spectrofluorimetric assay[8]. Hepatic T4-UDPGT activity was determined by using the method of Zhou *et al*[3].

RNA isolation and relative real-time RT-PCR. Total RNA was isolated using the RNeasy Midi Kit with DNase I digestion performed during column purification (Qiagen, Hilden, Germany). To assess the integrity of the RNA

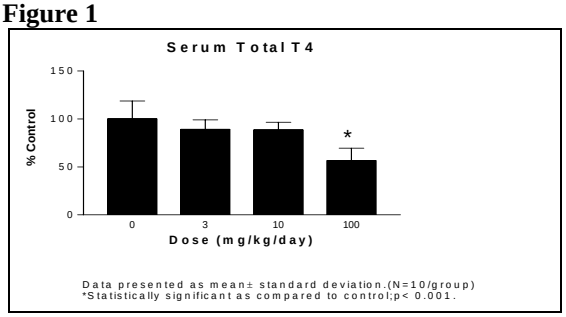
samples, the 2100 Bioanalyzer was used (Agilent Technologies, Palo Alto, CA). Real-time RT-PCR was performed using the ABI Prism 5700 Sequence Detection System (ABI, Foster City, CA). Data were analyzed using the Sequence Detection Systems software (ABI, Foster City, CA). All RT-PCR data were quantified by the $\Delta\Delta C_t$ method (Applied Biosystems User Bulletin 2) relative to a calibrator sample and an endogenous control (18S).

Data Analysis. Data were normalized by transforming to reciprocals (1/y). All data are represented as the mean ± standard deviation. The statistical intergroup comparisons were determined by using a one-way analysis of variance (ANOVA) followed by Dunnet's Multiple Comparison post test (GraphPad Prism 3.0, GraphPad Software, San Diego, CA). The levels of probability of statistical significance are $p < 0.05$.

Results and Discussion

Total serum T4 (Figure 1). Serum samples were collected 24 hours after the 4th day of dosing. Exposures to 3 and 10 mg/kg/day BDE 47 decreased T4 concentrations by 11 and 12%, respectively. Total T4 concentrations were significantly decreased by 43% as compared to control at the highest dose observed (100 mg/kg/day).

Figure 1



Hepatic P450 enzyme activity and mRNA expression (Figures 2 and 3). BDE 47 significantly increased EROD activity only 40% at the highest dose (100 mg/kg/day), whereas PROD activity increased significantly at the 10 and 100 mg/kg/day doses, 200 and 500% respectively. Hepatic CYP1A1 and CYP3A11 mRNA expressions were not significantly changed with treatment. CYP2B10 mRNA expression was significantly increased at 10 and 100 mg/kg/day, 250 and 1980% respectively. These data show that increases in hepatic EROD enzymatic activity did not correspond with increases in CYP1A1 mRNA expression. In contrast, hepatic PROD enzymatic activity and CYP2B10 mRNA expression both increased at 10 and 100 mg/kg/day. These data demonstrate that BDE 47 is more effective at inducing hepatic CYP2B activity and mRNA expression than other XME pathways evaluated in this study. The slight induction of CYP1A1 activity at the highest dose may be due to crossover of enzymatic activity resulting from CYP2B induction. The lack of induction in CYP1A1, regulated predominately by AhR, suggests that BDE 47 may not be an agonist for AhR. The dose-dependent induction of CYP2B10 mRNA expression and enzymatic activity in conjunction with lack of CYP3A11 mRNA induction, suggest that the BDE 47 response may be CAR-mediated.

Figure 2

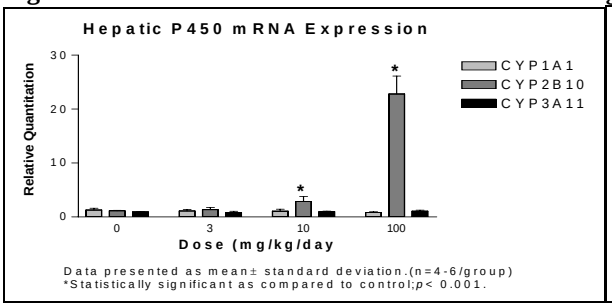
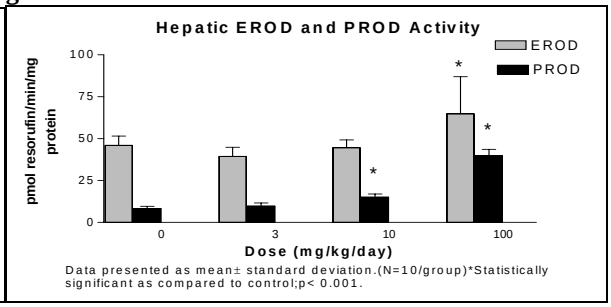


Figure 3



T4-UDPGT enzyme activity and mRNA expression (Figures 4 and 5). Hepatic T4-UDPGT enzyme activity was unchanged with BDE 47 treatment. UGT1A1 and UGT2B5 mRNA expression were significantly increased 20 and 30% at 100 mg/kg/day, respectively. UGT1A7 mRNA expression was significantly increased 30% at 10 mg/kg/day and 70% at 100 mg/kg/day BDE 47. While it is uncertain which isoforms of UDPGT were measured in the assay, results show that the T4-UDPGT activity does not parallel the changes observed in UGT mRNA expression.

Figure 4

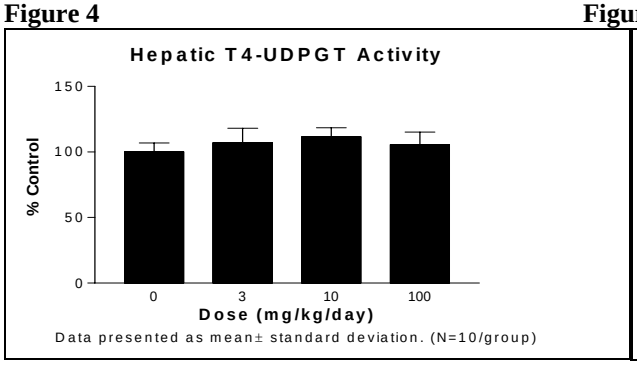
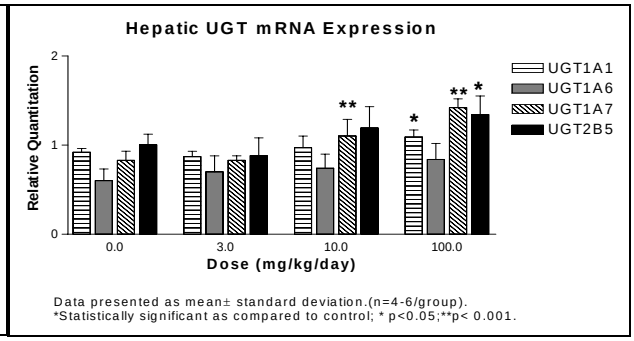
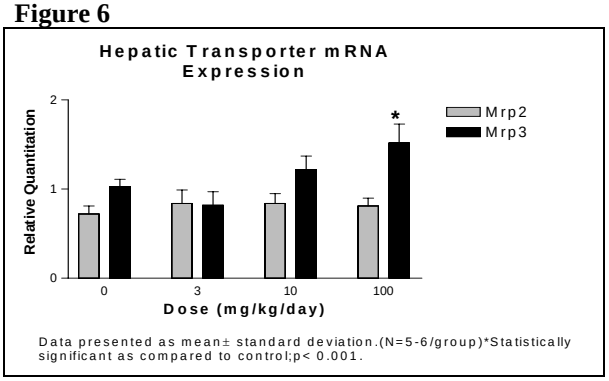


Figure 5



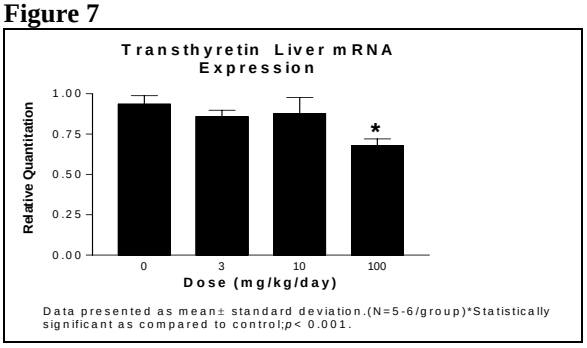
Efflux Transporter mRNA expression (Figure 6). Transporters known to transport thyroid hormones or glucuronides where chosen for analysis of effects following BDE 47 treatment. The transporters examined are also regulated by CAR and PXR[6,7]. Hepatic Mrp2, one of the major canalicular efflux transporters of organic anions into the bile, was unchanged with BDE 47 treatments. Hepatic Mrp3, a major efflux transporter of glucuronides was increased 20% at 10 mg/kg/day and significantly increased 50% at 100 mg/kg/day BDE 47. These data suggest that BDE 47 may not affect Mrp2-mediated transport into the bile, but may increase transport of glucuronide out of the liver and into the blood stream through the induction of Mrp3 mRNA expression.

Figure 6



Transthyretin mRNA expression (Figure 7). Transthyretin(Ttr), the major thyroid hormone transporter in rodents was also measured following exposure to BDE 47. Studies by Hallgren *et al.* demonstrate that PBDE exposures are associated with decreases in thyroid hormone concentrations and reductions in *ex vivo* binding of T4 to plasma transthyretin; together these data suggest that PBDEs or their metabolites may competitively bind to Ttr[1]. The results of this study showed a decreasing trend in Ttr mRNA expression in which Ttr mRNA decreased 10% at 3 and 10 mg/kg/day BDE 47. Ttr mRNA expression was significantly decreased 30% at the highest dose tested (100mg/kg/day). Decreased transthyretin (Ttr) mRNA expression, particularly at the high dose, suggests that BDE 47 may alter Ttr-mediated T4 transport.

Figure 7



Conclusions

Previous rodent studies have reported decreases in circulating T4 concentrations following exposure to PBDEs, though the mechanism by which T4 is reduced is unclear. Several studies suggest that the T4 decreases are related to increased metabolism by UGTs; however, the results of this study demonstrate that multiple mechanistic pathways may be involved. The data from this study show that BDE 47 does not activate the AhR regulated CYP1A1 genes, however some of the CAR and PXR regulated genes were induced with BDE 47 exposure. Increases in hepatic UGT1A7, Mrp3, and decreases in Ttr mRNA expression were associated with decreases in total serum T4; r^2= 0.46, 0.47, and 0.61, respectively. Although the mRNA expressions of several isoforms of UGT were induced, a parallel induction of T4-UDPGT activity was not observed. Induction in mRNA of the glucuronide transporter Mrp3 suggests a BDE 47-mediated increase in glucuronide transport into the blood stream from the liver. One of the major thyroid hormone transporters in humans is thyroid-binding globulin, so it is unclear if the effects seen on Ttr mRNA expression would be relevant to humans. The doses used in this study are outside the range of linear kinetic behavior and may be as much as 100 times the amounts seen in the U.S. population[9,10]. Although induction of hepatic transporter mRNA expression was seen in this study using adult animals, it is not known if the same effects would be observed in the potentially susceptible developing animal.

Acknowledgements

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This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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