Perinatal Exposure to BDE-99 Causes Decreased Protein Levels of Cyclin D1 via GSK3β Activation and Increased ROS Production in Rat Pup Livers

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We here examined the potential liver toxicity in rat pups from dams exposed during the gestational and lactation periods to 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99). Dams were exposed to 0, 1, and 2 mg/kg/day of BDE-99 from gestation day 6 to postnatal day 21. When the pups were weaning, the liver from 1 pup of each litter was excised to evaluate oxidative stress markers and the messenger RNA (mRNA) expression of multiple cytochrome P450 (CYP) isoforms. To determine whether thyroid hormone (TH) was disrupted, the protein and mRNA expressions of several TH receptor (TR) isoforms, as well as the protein levels of cyclin D1 and the phosphorylated protein kinases Akt and glycogen synthase kinase 3 beta (GSK3β), were evaluated. Perinatal exposure to BDE-99 produced decreased levels of cyclin D1 in rat pup livers. A decrease in the active form of Akt and an increase in the active form of GSK3β were observed. The decreased Akt pathway may be due to a potential disruption of the nongenomic actions of TH by BDE-99 and its metabolites. This possible TH disruption was noted as a decrease in TR isoforms expression. By contrast, we observed an upregulation of CYP2B1 gene expression, which is correlated with an increase in reactive oxygen species production. This outcome indicates activation of the nuclear constitutive androstane receptor, which could induce the expression of other enzymes capable of metabolizing TH. The present findings support the hypothesis that perinatal exposure to PBDEs, at levels found in humans, may have serious implications for metabolic processes in rat pup livers.

Key Words: BDE-99; reactive oxygen species; CYP enzyme system; thyroid hormone receptors; Akt; GSK3β; cyclin D1.

Among the organic pollutants identified in biota samples, there is a family of substances used as flame retardants classified as polybrominated diphenyl ethers (PBDEs). The lipophilic nature and resistance to degradation of PBDEs have allowed their biomagnification in trophic chains, being these compounds currently present in all ecosystems and human food (Costa et al., 2008; Darnerud, 2003; Domingo, 2004, 2012; Klosterhaus et al., 2012; Na et al., 2013). Despite the bans and restrictions on the use and production of PBDEs (COP4, 2009), high amounts of these compounds have accumulated in humans (Costa et al., 2008). These levels are particularly harmful in women because the biodisponibility of PBDEs is increased during pregnancy and lactation, being potentially transferred to the offspring (Gomara et al., 2007; Schuhmacher et al., 2009; Shen et al., 2012). Epidemiological studies have correlated higher concentrations of PBDEs in cord blood and maternal milk with worse neurophysiological development and increased activity/impulsivity behaviors in toddlers (Eskenazi et al., 2013; Gascon et al., 2012; Herbstman et al., 2010; Hoffman et al., 2012; Roze et al., 2009; Shy et al., 2011). Moreover, various animal studies have attributed the primary neurotoxic action of PBDEs to their ability to act as disruptors of the thyroid hormone (TH) signaling (Blanco et al., 2013; Kuriyama et al., 2005, 2007; Ta et al., 2011; Talsness et al., 2005; Zhou et al., 2002).

The liver is a key organ implicated in the TH disruption caused by PBDE exposure. PBDEs induce increased hepatic gene expression of enzymes involved in the detoxification of xenobiotic compounds (Blanco et al., 2012; Sanders et al., 2005; Szabo et al., 2009). These enzymes catalyze the addition of hydrophilic groups to the lipophilic structures of PBDEs, resulting in metabolites that are easily removed from the body. However, these metabolites may act as stronger endocrine disrupters than the parent compounds. In vitro studies have identified the mechanism by which several hydroxylated PBDEs bind antagonistically to TH transport proteins (Yang et al., 2011) and nuclear TH receptors (TRs) (Kitamura et al., 2008; Kojima et al., 2009), thereby deregulating the expression of genes that are fundamental for normal cell growth and survival.
Triiodothyronine (T3), the biologically active form of TH, has been shown to be a powerful inducer of cell proliferation. Among other mechanisms, T3 increases the levels of cyclin D1 via nongenomic actions, leading to cell cycle transition from G1 to S phase. T3 stimulates activation of Akt via phosphatidylinositol-3-kinase (PI3K) nongenomic signaling (Cao et al., 2005; Hiroi et al., 2006; Pibiri et al., 2001; Radenne et al., 2008). The active protein kinase Akt restrains the activity of glyco- gen synthase kinase 3 beta (GSK3β), avoiding the exclusion of cyclin D1 from the nucleus and its proteasomal degradation (Takahashi-Yanaga and Sasaguri, 2008). Dunnick and Nyska (2009) and Lee et al. (2010) reported that long-term exposure to PBDEs produced hepatocyte hypertrophy, necrosis, and increased vacuolization in rodents, which suggests deregulation of the cell cycle. Recently, we observed liver enlargement, increased hepatic production of reactive oxygen species (ROS), and gene induction of detoxifying enzymes in the livers from rat fetuses whose dams were orally exposed to PBDEs on gestation days 6–19 (Blanco et al., 2012). High levels of ROS are known to produce DNA damage, programmed cell death, or can activate specific signaling pathways (Finkel and Holbrook, 2000).

The main goal of this study was to determine whether liver toxicity occurred in rat pups from dams exposed, during the gestational and lactation periods, to one of the most prevalent PBDE congeners in human samples, 2,2',4,4',5-pentabromodiphenyl ether (BDE-99). Pup livers were excised when pups were weaned. We assessed the hepatic messenger RNA (mRNA) expression of several detoxifying isoforms of the cytochrome P450 (CYP) family enzymes and their correlation with the antioxidant enzyme activity of catalase (CAT), superoxide dismutase (SOD), and the total levels of thiobarbituric acid reactive substances (TBARS). To evaluate possible TH disruption, the protein and mRNA expressions of several TR isoforms, as well as the protein levels of cyclin D1 and phosphorylated protein kinases Akt and GSK3β, were evaluated.

**MATERIALS AND METHODS**

**Animals and chemicals.** Male and female Sprague Dawley rats purchased from Charles River (Barcelona, Spain) were used. Animals were acclimated to the new environment for at least 1 week before starting the experiments. Rats were maintained under controlled conditions of temperature (22 ± 2°C), relative humidity (50 ± 10%), and light (12-h light/12-h darkness). After acclimation, virgin female rats were cohabitated with breeder male rats, 1 male rat per 2 female rats. The presence of sperm in the vaginal smear of the female was considered to be day 0 of gestation (GD 0). Pregnant rats were singly housed in plastic cages, with ad libitum access to food (Panlab rodent chow, Barcelona, Spain) and water. All experiments were approved by the Animal Care and Use Committee of the Rovira i Virgili University (Catalonia, Spain). BDE-99 (99% pure) was purchased from LGC Standards S.L.U. (Barcelona). It was diluted in corn oil (vehicle) and administered by gavage.

**Experimental procedures.** After sperm-positive smears were observed, female rats were placed in plastic cages and randomly divided into 3 groups. From GD 6 to the postnatal day (PND) 21, animals in the 3 groups were orally (gavage) given a daily dose of BDE-99 of 0 mg/kg (control group, n = 8), 1 mg/kg (BDE 1 group, n = 8), and 2 mg/kg (BDE 2 group, n = 8) body weight. Rats in the control group were treated with the same dose volume of vehicle (corn oil) during the same period. The BDE-99 doses used in this experiment were chosen based on the estimated lowest observed adverse effect level of 1 mg/kg/day, mainly from pentaBDE data (Damerud et al., 2001), as well as from a recent report on redox responses and tissue distribution of BDE-99 in rats (Cheng et al., 2009).

The size of the litters was adjusted to 4 rats within the first 48h after birth, by the killing of excess pups. On PND 21, pups were weaned. One pup from each litter was sacrificed by decapitation, and the liver was immediately dissected, frozen in liquid nitrogen, and stored at −80°C.

**Oxidative stress markers.** A fraction of the rat pup liver was used to assess the activity of the antioxidant enzymes SOD, and CAT, the level of TBARS, and the protein content. Tissue samples were thawed and washed in 0.9% saline, and homogenized in 0.2M sodium phosphate buffer (pH 6.25, 1:20 wt/vol) in a Potter-Elvehjem homogenizer fitted with a Teflon pestle (Braun, Melsungen, Germany). The supernatant was collected after centrifugation at 105000 × g for 1 h and used for biochemical analyses. The protein content was measured by the Bradford spectrophotometric method (Sigma Chemical Co, St Louis, Missouri) using bovine serum albumin as a standard (Merck, Darmstadt, Germany). The activities of SOD and CAT were determined according to Mulero et al. (2006), whereas the total TBARS levels were determined according to Zapan et al. (2008).

**RNA isolation and complementary DNA synthesis.** Tissue samples of rat pup livers were homogenized and total mRNA was isolated using a Qiagen RNeasy Kit according to the manufacturer’s protocol. The concentration and purity of total RNA were detected by spectrophotometry using UV absorbance at 260 and 280 nm. The quality of the RNA was assessed by electrophoresis on 1% denaturing agarose gel. The RNA was reversed transcribed from 1 μg of total RNA from each sample using a QuantiTect Reverse Transcription Kit (Qiagen Inc, Hilden, Germany) according to the manufacturer’s protocol. An identical reaction, without reverse transcriptase, was performed to verify the absence of genomic DNA. The complementary DNA (cDNA) was subsequently amplified by PCR using rat-specific primers for CYP1A1 (NM_012540; forward: 5′-CTG CAG AAA ACA TGC CAG GA-3′; reverse: 5′-CAG GAG GCT GGA CGA GAA TGC-3′), CYP1A2 (NM_012541; forward: 5′-CCA AGC CTTCCA GCA GAC TT-3′; reverse: 5′-GAG GGA TGA CAC CAC CGT TG-3′), CYP2B1 (NM_001134844; forward: 5′-CCA AGC CGT CCA CGA GAC TT-3′; reverse: 5′-TTG GGA AGC AGG TAC CCT C-3′), CYP3A1 (NM_013105; forward: 5′-CCG CGT GGA TTG GCA GA-3′; reverse: 5′-TTG GAG GTG CCT TAT TGC-3′), CYP3A2 (NM_153312; forward: 5′-TTG ACG ATC GCT TGC TGT CA-3′; reverse: 5′-GCC GAC GTA AAA CAA GAC AA-3′), Trx1 (NM_00107960); forward: 5′-TGC CCT TAC TCACC CTA CA-3′; reverse: 5′-AGG CCA AGC CAA GCT GTG CT-3′), Trp1 (NM_012672; forward: 5′-AGC CAG CCA CAG CAC AGT GA-3′; reverse: 5′-CCG CAG CAG ACT GAA GCT TGC-3′), and β-actin (NM_031144; forward: 5′-TGT CAC CAA CTG GGA GA-3′; reverse: 5′-GGG GTG TTG AAG GTG TCA AA-3′) with a PyroStart Fast PCR Master mix (2×) kit (Fermentas, Burlington, Canada) according to the manufacturer’s protocol. The PCR products were separated on 1% agarose gel, and only specific bands were detected. The nonreactivity of the primers with contaminant genomic DNA was tested by the inclusion of controls that omitted reverse transcriptase from the cDNA synthesis reaction.

**Real-time reverse-transcription-PCR.** Quantitative PCR for CYP1A1, CYP1A2, CYP2B1, CYP3A1, CYP3A2, Trx1, and β-actin was completed using the QuantiTect SYBR Green PCR kit (Qiagen Inc) according to the manufacturer’s protocol, and a Rotor-Gen Q Real-Time PCR cycler (Qiagen Inc). The thermal cycling comprised an initial step at 50°C for 2 min, followed by a polymerase activation step at 95°C for 15 min and a cycling step with the following conditions: 40 cycles of denaturing at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. As oligonucleotides of varying lengths produce dissociation peaks at different melting temperatures, at the end of the PCR cycles, the PCR products were analyzed using a heat dissociation protocol.
to confirm that one single PCR product was detected by SYBR Green dye. Fluorescence data were acquired at the 72°C step. The threshold cycle (Ct) was calculated by Rotor-Gene Q 2.0 software to identify significant fluorescence signals above noise during the early cycles of amplification. The software calculated copy numbers for the target samples from the Ct by interpolating from a standard curve. The relative levels of the expression of the target genes were measured using β-actin mRNA as an internal control according to the 2−ΔΔCt method.

Western blot analysis. Aliquots containing 30 μg of protein per sample of liver lysate were analyzed by Western blot analysis. Briefly, samples were placed in sample buffer (0.5M Tris-HCl pH 6.8, 10% glycerol, 2% [wt/vol] SDS, 5% [vol/vol] 2-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95°C–100°C for 5 min. Samples were then separated by electrophoresis on 10% acrylamide gels. Proteins were subsequently transferred to Immobilon-P PVDF sheets (Millipore Corp, Bedford, Massachusetts) using a transblot apparatus (Bio-Rad). The membranes were blocked for 1 h with 5% nonfat milk dissolved in TBS-T buffer (50mM Tris, 1.5% NaCl, 0.05% Tween 20, pH 7.5). They were then incubated overnight with primary monoclonal antibodies against TRα1 (molecular weight [MW]: 55kDa), TRβ1 (MW: 53kDa), Akt-total (MW: 60kDa), Akt-phospho Ser473 (MW: 60kDa), GSK3β-total (MW: 46kDa), GSK3β-phospho Ser4 (MW: 46kDa), β-actin (MW: 45kDa), or β-tubulin (MW: 55kDa). The blots were washed thoroughly in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated immunoglobulin G antibody. Immunoreactive proteins were visualized using an Immun-Star Chemiluminescence Kit (Bio-Rad) according to the manufacturer’s instructions. Digital images were taken with a Versadoc (Bio-Rad), which permits semiquantification of the band intensity. The protein load was periodically monitored via the immunodetection of β-actin, with the exception of GSK3β-total, which was monitored via immunodetection of β-tubulin.

Statistics. Levene’s test of homogeneity was applied to test the variance of the data. After testing the normality and homogeneity of variances, statistical significance was assessed by 1-way ANOVA, followed by a Bonferroni’s post hoc test. When assumptions of homogeneity of variance were not found, data were analyzed using the Kruskal-Wallis nonparametric test, followed by individual post hoc group comparisons using the Mann-Whitney U test adjusted for multiple comparisons. Statistical analyses were carried out using the version 15 of SPSS (SPSS Sciences, Chicago, Illinois). Statistical significance was defined as a p value less than .05. The relationships among the examined endpoints were assessed using Pearson correlations.

RESULTS

BDE-99 Increases the Body Weight of Rat Pup

Body weight of the pups was significantly increased (p = .033) in the BDE 2 group compared with the control group (Table 1). Although the pup liver weight was increased in a dose-dependent manner, no significant differences were observed (Table 1). However, this trend disappeared when the liver weight was corrected for the pup body weight.

BDE-99 Increases Oxidative Stress Markers in the Rat Pup Liver

The production of ROS in the rat pup liver was indirectly evaluated by measuring different oxidative stress markers. The activity of the antioxidant enzymes SOD and CAT and the total level of TBARS were measured in the rat pup liver (Table 1). A BDE-99 dose-dependent increase was observed in the levels of the evaluated oxidative stress markers. The CAT activity was significantly increased in the BDE 2 group (p = .003) compared with the control group. The SOD activity was also significantly increased in both the BDE 1 (p = .014) and BDE 2 (p < .001) groups compared with the control group. These results suggest that ROS production in pup liver is proportional to the level of BDE-99 exposure in pregnant dams.

The BDE-99-Induced Production of ROS in the Rat Pup Liver is Related to Increased mRNA Expression of the CYP2B1 Isoform

The CYP enzyme family plays a key role in the detoxification of xenobiotics, such as PBDEs. In rat pup livers, we evaluated the mRNA expression of 5 principal CYP isofoms involved in the biotransformation and detoxification of PBDEs (Fig. 1). Compared with the control group, a significant increase was found in the expression of the CYP2B1 isofom (714±332%, p = .019) in pup livers from the BDE 2 group. Because the production of ROS may be related to the increased gene expression of the CYP2B1 isofoms, we calculated the relationship between multiple oxidative stress markers and the upregulated CYP2B1 isofom. A significant correlation between the increased CAT activity and CYP2B1 gene expression (R = .445, p = .029) was observed. A similar correlation was also noted for the total level of SOD and CYP2B1 expression (R = .650, p = .001).

BDE-99 Deregulates Gene and Protein Expression of the TRs in the Rat Pup Liver

The TRα and TRβ genes encode 2 functional isofoms of TR, TRα1, and TRβ1, which are expressed at similar levels in the liver. Because little information exists regarding the effects

| Table 1 |

<table>
<thead>
<tr>
<th>Exposure Group</th>
<th>Control (n = 10)</th>
<th>BDE 1 (mg/kg/day) (n = 10)</th>
<th>BDE 2 (mg/kg/day) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>180.96 ± 10.25a</td>
<td>194.64 ± 20.67ab</td>
<td>200.96 ± 13.42a</td>
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<td>Liver weight (g)</td>
<td>8.24 ± 0.70</td>
<td>8.76 ± 0.87</td>
<td>9.12 ± 0.97</td>
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<tr>
<td>Liver weight/body weight (%)</td>
<td>4.55 ± 0.03</td>
<td>4.50 ± 0.02</td>
<td>4.53 ± 0.03</td>
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<tr>
<td>Catalase (mol/min/mg protein)</td>
<td>13.47 ± 1.80a</td>
<td>16.49 ± 1.72b</td>
<td>18.38 ± 1.80a</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>359.25 ± 81.35a</td>
<td>477.3 ± 46.42ab</td>
<td>542.95 ± 119.37b</td>
</tr>
<tr>
<td>TBARS (nmol/mg protein)</td>
<td>19.84 ± 3.02</td>
<td>22.64 ± 3.82</td>
<td>24.26 ± 4.74</td>
</tr>
</tbody>
</table>

Notes. The results are expressed as the mean values ± SD. The statistical differences were analyzed by ANOVA followed by Bonferroni’s post hoc test. The values in the same row with a common superscript (a and b) are not significantly different at p < .05.
of PBDEs on TR expression in the liver, changes in the relative mRNA and protein levels of these 2 isoforms were examined by real-time qPCR (Fig. 2A) and Western blot (Fig. 2B), respectively. The mRNA expression of TRα1 was significantly downregulated in the BDE 1 (44 ± 13%, p = .001) and the BDE 2 (42 ± 4%, p = .001) groups compared with the control group. Similarly, the transcript levels of TRβ1 were also significantly downregulated (31 ± 14%, p = .033) in the BDE 2 group compared with the control group. In contrast to the mRNA expression, the relative protein levels of the TRs were significantly decreased (36 ± 15%, p = .018) only for the TRα1 isoform in the BDE 2 group compared with the control group.

BDE-99 Decreases the Protein Expression of Cyclin D1 and Phosphorylation of the Proteins Kinases AKT and GSK3β

The phosphorylation levels of the protein kinases Akt (Fig. 3A) and GSK3β (Fig. 3B) were reduced in a dose-dependent manner, with high perinatal exposure of the pups to BDE-99. The ratios of Akt-p/Akt-total and GSK3β-p/GSK3β-total were significantly decreased (28 ± 7%, p = .025; 49 ± 9%, p = .001, respectively) in the BDE 2 group compared with the control group. The same effect was also observed for the relative protein expression of cyclin D1. Cyclin D1 was significantly decreased in the BDE 1 (22 ± 7%, p = .032) and BDE 2 groups (41 ± 9%, p = .001) compared with the control group (Fig. 3C).

DISCUSSION

In the current study, perinatal exposure to BDE-99 caused clear signs of toxicity in rat pup livers. The decreased levels of the cell survival PIP3K/Akt pathway and cyclin D1, and the increased ROS production, which are related to high expression of CYP2B1, indicate a possible alteration in cell cycle progression and hepatocyte functions.
Thyroid hormone disruption may be partially responsible for the decreased levels of cyclin D1. Several PBDE metabolites are antagonistic of TH actions. We hypothesized that these metabolites might also inhibit the nongenomic actions of TH related to activation of the PI3K/Akt pathway (Cao et al., 2005; Hiroi et al., 2006; Pibiri et al., 2001; Radenne et al., 2008). Downregulation of the active form of Akt, leading to decreased phosphorylation and activation of GSK3β, was observed. Phosphorylation of cyclin D1 on Thr286 by GSK-3β facilitates its association with CRM1, a nuclear protein that mediates the nuclear export of proteins, resulting in the exclusion of cyclin D1 from the nucleus to initiate its proteasomal degradation (Takahashi-Yanaga and Sasaguri, 2008). Another possible reason for the decrease in cyclin D1 is the increased metabolism of TH. One of the earlier events in hepatocyte proliferation induced by T3 is the induction of cyclin D1 (Pibiri et al., 2001). Alisi et al. (2005) showed that rats treated with propylthiouracil, a drug that inhibits TH synthesis in the thyroid gland, exhibited downregulation of cyclin D1 levels in hepatocytes. A number of studies have shown that the high expression of detoxifying enzymes induced by PBDEs can promote high elimination of TH in the liver, which reduces TH levels in the serum in the long term (Kuriyama et al., 2007; Tseng et al., 2008; Zhou et al., 2001, 2002). In the present investigation, we noted that the CYP2B1 isoform was upregulated after perinatal exposure to BDE-99. The mRNA expression of CYP2B genes is mediated by activation of the constitutive androstane receptor (CAR). This nuclear receptor also encodes enzymes, such as sulfotransferases and uridine diphosphate glucuronosyltransferases, which are capable of metabolizing TH in the liver (Szabo et al., 2009). Maglich et al. (2004) showed that TH concentrations in the serum decreased in wild-type mice treated with the CAR agonist TCPOBOPF; whereas in Car<sup>−/−</sup> mice, the TH levels remained significantly higher. In a recent study, we found an increase in CYP1A, CYP2B, and CYP3A gene expressions in rat fetus livers exposed to the same conditions than those of the current study (Blanco et al., 2012). A possible difference in the expression of these detoxifying enzymes might be the different concentrations of BDE-99 and their metabolites or impurities transmitted during the gestation and/or lactation periods. Differences between the concentrations of PBDE congeners in maternal serum and breast milk samples have been recently reported by Jakobsson et al. (2012). On the other hand, we also found a positive correlation between the increase in

![Graph showing protein expression](image_url)

**FIG. 3.** Perinatal exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) decreases the protein expression of cyclin D1 and phosphorylation of the protein kinases Akt and glycogen synthase kinase 3 beta (GSK3β). Total protein was extracted of rat pup livers from dams orally exposed to 0, 1, and 2 mg/kg/day of BDE-99 from gestation day 6 to postnatal day 21. The relative protein expression levels of the ratio of Akt-p Ser473/Akt-total (A), of the ratio of GSK3β-p Ser9/GSK3β-total (B), and of cyclin D1 (C) were measured by Western blot analysis. The intensity of the bands was determined by densitometric analysis. Data were normalized using β-actin (A and C) and β-tubulin (B) as internal controls. Data from the experiments are expressed as means ± SD in the lower panel. Significant differences relative to the control group were analyzed by 1-way ANOVA followed by Bonferroni’s post hoc test: *p < .05 and **p < .001.
the enzymatic activity of SOD and CAT, with a higher mRNA expression of CYP2B. Several CYP enzymes synthesized ROS as secondary products in their detoxifying reactions. A higher activity of CYP2B could be, in part, responsible for a higher activity of SOD and CAT. Furthermore, the decrease in the active form of Akt could be also related with the increase in the intracellular ROS levels. The active form of Akt blocks the action of the proteins Bad and Bax. These 2 apoptotic proteins alter the permeability of the mitochondria membrane, allowing the exhaust of ROS to the cell.

Other signs of possible TH disruption by BDE-99 are the decreased expression of TR isoforms, the gene regulation of which is mediated by the concentration of TH (Samuels et al., 1977). An increase in TH levels leads to a decrease in the predominant isoforms of TR expressed in cells, whereas a decrease in TH levels increases the predominant TR isoforms as an adaptive mechanism to maximize the TH response (Monden et al., 2006; Sadow et al., 2003; Samuels et al., 1977). PBDEs and OH-PBDEs have a similar structure to TH and may activate this autoregulatory mechanism by creating a state that mimics hyperthyroidism (Blanco et al., 2011). The liver is the primary target organ for PBDEs in rats and mice, and a large hepatic concentration of these organobrominated pollutants would lead to a decrease in the expression of the TR isoforms.

Furthermore, the decreased activity of the PIP3K/Akt pathway may also affect the regulation of other proteins involved in cell survival functions or glucose and lipid metabolism. Similar to the results of previous studies, we also observed an increased body weight for rat pups exposed to BDE-99 (Suvorov et al., 2009). Recently, it has been postulated that PBDEs may contribute to the onset of diabetes in humans. It is well known that the PIP3K/Akt pathway is an obligate mediator of many of metabolic actions of insulin. Zhang et al. (2013) and Nash et al. (2013) have reported dose-related hyperglycemia, decreased insulin in the serum, increased percentage of lipids, and changes in the expression of genes related to type I diabetes mellitus in livers of rodents exposed to a commercial mixture of PBDEs or to the congener 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE (BDE-209). The decreased activity of the PIP3K/Akt pathway in other tissues may explain the negative effects of PBDEs for neurobehavioural or reproductive toxicity.

In this study, we found a clear alteration of the Akt/GSK3β pathway and a decrease in the protein levels of cyclin D1 in rat pup liver after perinatal exposure to BDE-99. However, our hypothesis about a possible disruption of the nongenomic actions of TH must be confirmed by further studies. The precise regulating mechanisms by which PBDEs can disrupt metabolic pathways would help to improve the knowledge of the toxic action of PBDEs.

In summary, perinatal exposure to BDE-99 through gestation and ingestion of maternal breast milk may produce decreased levels of cyclin D1 in liver. In the present investigation, we also observed a decrease in the active form of Akt and a concomitant increase in the active form of GSK3β. The decrease in the PIP3K/Akt pathway may be due to disruption of the nongenomic actions of TH by BDE-99 and its metabolites. This TH disruption is noted as the decreased expression of TR isoforms. In contrast, we observed upregulation of CYP2B1 gene expression, which is correlated with an increase in ROS production. It indicates the activation of CAR and the possible expression of other enzymes capable of metabolizing TH and decreasing its serum levels. These findings support the hypothesis that perinatal exposure to PBDEs, at levels found in humans, might have serious implications on metabolic and body weight programming. Further studies on the potential health risks of these environmental pollutants are still necessary for a better understanding of these effects.

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