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Perinatal exposure to low-dose 2,2′,4,4′-tetrabromodiphenyl ether affects growth in rat offspring: What is the role of IGF-1?

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ABSTRACT

Background: Polybrominated diphenyl ethers (PBDEs) are a group of environmental contaminants increasing in North America. Few data are available on their growth effects at low doses exposure.
Objectives: Our goal in the present study was to evaluate whether low doses of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), which is the most abundant PBDE found in human samples, affects growth and insulin-like growth factor 1 (IGF-1).
Methods: Dams were exposed either to the vehicle or low doses of BDE-47 (0.002 and 0.2 mg/kg body weight) every fifth day from gestation day 15 to postnatal day (PND) 20 by intravenous injections. Developmental landmarks, body length and body weight of pups were assessed during the first months of life. A glucose tolerance test was performed on PND 40 and 75. Plasma IGF-1 was analysed in trunk blood on PND 27.
Results: Exposure to BDE-47 increased plasma IGF-1 and glucose uptake in male but not in female pups. Developmental landmarks were not influenced by BDE-47 while body weight and body length was increased in both exposed male and female offspring during the first months of development.
Conclusion: These results demonstrate alteration of growth in rat offspring induced by BDE-47 at levels found in human population. The possible involvement of the growth hormone–IGF axis is discussed.

1. Introduction

A number of studies have raised concerns about polybrominated diphenyl ethers (PBDEs)—flame retardants used as additives in polymers that are found in some textiles, electronics (e.g., computers, televisions), plastics, and furniture. PBDE are potentially toxic, persistent, bioaccumulative and ubiquitous chemicals that have been found at rapidly increasing levels in humans during the past few decades (Birnbaum and Staskal, 2004; Hites, 2004; Schecter et al., 2005; Sjodin et al., 2004). The most prevalent PBDE congeners found in maternal and cord blood are BDE-47, BDE-99, BDE-100, and BDE-153 (Guvenius et al., 2003; Mazdai et al., 2003).

Blood PBDE concentrations in children are 2–5-fold higher than that of their parents exposed to the same indoor concentrations mainly due to higher rates of dust ingestion (Fischer et al., 2006), higher PBDE dietary intake due to higher food intake per kilogram of body weight in children and high levels of PBDEs in human milk (Schecter et al., 2006). The early stages of mammalian organism development are therefore at particular risk from increasing exposure to PBDE.

In spite of the well established abilities of PBDE congeners to affect the endocrine status of organism, only thyroid disruption has been addressed thoroughly (Darnerud, 2008; Kuriyama et al., 2007; Suvorov et al., 2008; Zhou et al., 2001). The susceptibility of other endocrine endpoints to PBDE exposure remains unknown (Suvorov and Takser, 2008).

In a recent study of American kestrels (*Falco sparverius*) exposed perinatally to environmentally relevant mixtures of PBDE congeners, it was shown that nestlings were larger (weight, bones, feathers) as they gained weight more quickly and ate more food (Fernie et al., 2006). Gee and Moser (2008) reported an increase in the weight of mice offspring after acute postnatal exposure to BDE-47. In other study (Ceccatelli et al., 2006), uterine expression of insulin-like growth factor 1 (IGF-1) was upregulated in adult rat offspring exposed prenatally to 1 mg/(kg day) (but not to 10 mg/(kg day)) of BDE-99. This evidence suggests that PBDE induced perturbations of growth and development could be among the most sensitive endpoints of toxicity and that IGF-1 may be involved.

IGF-1 is a polypeptide hormone similar in molecular structure to insulin, which is regulated by the growth hormone (GH)–IGF axis. In short, the physiological details of the axis based on a review

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by Scarth (2006) are as follows: the glycoprotein GH is secreted into the bloodstream by the anterior pituitary gland. It binds to a membrane bound GH receptors, inducing a second messenger cascade including Janus kinase (JAK) and signal transducers and activators of transcription (STAT) phosphorylation signalling pathways. In many tissues activation of the JAK-STAT pathways leads to IGF-1 gene induction and IGF-1 protein release which binds and activates the membrane-bound IGF-1 receptor. Activation of this receptor induces a second messenger cascade that increases mitogen activated protein kinase (MAPK) activity, leading to altered gene transcription and ultimately the anabolic effects of IGF-1. Many of GH's actions are mediated indirectly through the up-regulation of systemic IGF release by the liver. Both GH and IGF-1 stimulate childhood growth. Although both GH and IGF-1 have net anabolizing effects, GH promotes a rise in blood glucose level, whereas IGF-1 promotes a reduction in blood glucose levels (Mauras and Haymond, 2005).

A large number of environmental xenobiotics have been found to modulate the GH–IGF axis (Scarth, 2006). Epidemiologic studies indicate that higher IGF-I levels may predict risk for certain cancers, while lower IGF-I levels increase risk for ischemic heart disease (Hoffman, 2005). These data, taken together, rise serious public health concerns with respect to environmental exposures to substances suspected of endocrine disruption.

In our recent study, we employed levels of exposure to BDE-47 in our rat developmental model similar to those found in the human general population (Suvorov et al., 2008). In this study we demonstrate using the same exposure protocol that BDE-47 alters growth in rat offspring. The possible involvement of the growth hormone–IGF axis is discussed.

2. Materials and methods

2.1. Animals and treatment

We obtained 21 timed pregnant Wistar rats (250–300 g; Charles River Laboratories, St. Constant, PQ, Canada) on gestational day (GD) 14 and housed them in single plastic cages with a bedding of sawdust under regulated temperature (21 ± 2 °C), relative humidity (50 ± 10%), and a 12-h light/dark cycle. Food (Charles River Rodent chow 5075) and water were provided *ad libitum*. All animals received care in compliance with *The Guide to the Care and Use of Experimental Animals* from the Canadian Council of Animal Care, and the protocol was approved by our institutional animal research ethics review board.

Dams were randomly assigned among four groups (7 dams per group) and housed individually. Neutral standard of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47, 100% GC/MS purity) was purchased from Chromatographic Specialties Inc. (Brockville, Canada). We used the same protocol of exposure as in our previous study (Suvorov et al., 2008). In short, dams received intravenous injections of BDE-47 at doses of 0.002 or 0.2 mg/kg body weight (BW) in 0.3 ml/kg BW of vehicle: ethanol 95%, Cremophor EL, and sterile water for injections (1:1:8, v/v) every fifth day from GD15 to postnatal day (PND) 20 (a total of 6 injections/dam). The vehicle was administered to control dams according to the same protocol.

Dams were allowed to deliver, and the litter size was not artificially altered, to keep constancy of dose distribution among same number of pups at pre- and postnatal periods. Dams and pups were kept together until weaning on PND 21, after which dams, male pups and female pups were separated. Dams were sacrificed on PND 27.

2.2. Growth parameters and developmental landmarks

At delivery, all pups were identified (n total = 273). Their weight and body length (BL) (nose to rump) were measured each 5 days from delivery until PND 35 and then on PND 47. Body-mass index (BMI) was calculated for each pup on each day of measurement, as BW/BL^3. We use the cubic value of BL in order to avoid the influence of the absolute size of the pup on the relative value of BMI. This parameter was measured to estimate the contribution of the longitudinal growth and transversal increase in tissue volume to the BW increase. Dams were weighed each day of injection.

Developmental landmarks (fur development, eye opening, ear unfolding, and incisor eruption) were evaluated in all pups. Vaginal opening was checked daily from PND 30 in females. Descent of testes and preputial separation were checked daily in male pups from PND 20 and 30 respectively.

2.3. Plasma IGF-1 analysis

One male and one female pup were randomly selected from each litter and killed by decapitation on PND 27 between 9 AM and 10 AM. Trunk blood was collected in lithium heparin coated tubes (Vacutainer PST, Becton-Dickinson). Plasma was collected *via* centrifugation of samples (4000 rpm for 40 min at 4 °C) and stored at −20 °C for subsequent analyses of IGF-1. Total plasma IGF-1 was determined using TranDSL-2900 Mouse/Rat IGF-I Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., USA).

2.4. Glucose tolerance test

One male and one female pup were randomly selected from each litter for a glucose tolerance test on PND 40-41 and PND 75-76. These pups were starved for 8 h and were then subjected to gavage with 1 g/kg body weight of glucose (Glucose oral solution, Ratiopharm Inc., Canada) between 9 AM and 9.30 AM. The drop of blood was obtained from the tail vein by puncture before gavage and at different time points after gavage. Glucose levels were measured using an Accu-Check blood glucose monitoring system (Roche Diagnostics GmbH, Germany) as described elsewhere (Lin et al., 2007). The following time points were chosen based on our previous experiments (data not shown): 15, 30, 60, 90, 120 and 150 min for the test on PND 40-41 and 30, 60, 90, 120 and 150 min for the test on PND 75-76. Quality control check was performed when opening a new pack of test strips according to the manufacturer's instruction. According to manufacturer's information, the system was calibrated using the hexokinase method and has following performance parameters: the mean, systematic deviation from hexokinase method with deproteination using an automatic analyzer is max 4%; the within-series mean imprecision is 1%; the day-to-day mean imprecision is 1.8%; the method is linear within the range 0.6–33.3 mmol/l.

2.5. Statistical analyses

All statistical analyses were performed using SAS/STAT software (version 9.2). Variance (ANOVA) and covariance (ANCOVA) analyses were applied using PROC GLM modelling.

Body weight female and male data were pooled because no interaction between gender and the exposure group was observed. Data for BL, BW and BMI were adjusted for sex and litter size. Means of exposed groups for BW, BL and body-mass index were compared with those of the control group by the Dunnett test. All data for IGF-1 and blood glucose were treated separately for male and female pups and adjusted for litter size. Least square means of exposed groups were compared with that of control group by the Dunnett test. All results are expressed as adjusted means (least square means) and standard error of the means (SE). A linear model was applied to test individual IGF-1 and weigh relationships and effect of the exposure group on individual IGF-1.

3. Results

3.1. Developmental landmarks

No significant relations of litter size and dose of exposure were found, the number of pups varying from 10 to 17 per litter. The means ± SD for litter sizes were as follows: 13.0 ± 2.0, 12.9 ± 1.7, and 13.3 ± 2.2 for the control group and the groups exposed to 0.02 and 0.2 mg/kg BW BDE-47 respectively. Fur development, incisor eruption, eye opening, ear unfolding, vaginal opening, testis descent and preputial separation were not altered by exposure to BDE-47.

3.2. Weight and body length

No weight differences were observed between the control and exposed dams throughout the experiment.

BW of pups from the most exposed group (0.2 mg/kg BW) was significantly ($p < 0.05$) higher on PND 1, 5, 10, 15, 20, 25, 30, and 35 (Fig. 1A and B). BL was also higher in this group on PND 1, 10, 15, 25, and 30 (Fig. 1C and D). BW and BL were not different between pups exposed to low doses of PBDE (0.002 mg/kg) and controls during development except BL on PND 1 which was greater in the exposed group ($p = 0.01$) then in the control. To verify the impact of increased longitudinal growth and transversal increase in tissue volume (presumably mainly due to fat accumulation) on the resulting increase in weight in exposed pups, we analysed the body-mass index (Fig. 1E). It was significantly ($p < 0.05$) smaller in the most exposed group on PND 1 and 15 and in the least exposed group on