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Long term effects of murine postnatal exposure to decabromodiphenyl ether (BDE-209) on learning and memory are dependent upon APOE polymorphism and age

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are a group of chemicals widely used as flame retardants; the lower brominated forms (1–5 bromine atoms) are highly neurotoxic and are presently not in commercial use. The highest brominated, the decabromodiphenyl ether (BDE-209) remains in use and its adverse and persistent effects are subject to debate. Of special concern are developmental exposures that can disrupt later-in-life adult health or aging. In this study, we investigated the effects of postnatal exposure to BDE-209 in combination with apolipoprotein E (apoE) genotype, a genetic factor that is associated with varied vulnerability for the development of neurodegenerative diseases. On postnatal day 10, transgenic mice of both sexes carrying apoE2, apoE3 and apoE4 were orally exposed to 0, 10 or 30 mg/kg of BDE-209. Spatial reference memory was assessed in a Morris Water Maze (MWM) task at 4 and 12 months of age. The levels of the brain-derived neurotrophic factor (BDNF) were determined in hippocampus and frontal cortex of mice at 5 months of age. Mice carrying different apoE polymorphisms showed differences in the acquisition and retention of the spatial navigation task both at 4 and 12 months of age. Postnatal exposure to BDE-209 induced long term effects in spatial learning, which were dependent upon age, sex and apoE genotype; these effects were more evident in apoE3 mice. BDNF levels were lower in the frontal cortex of apoE4 mice and higher in the hippocampus of exposed mice, independent of the genotype. The results of the present study provide evidence of long-lasting effects in spatial learning and memory after early exposure to BDE-209. Developmental exposure to this neurotoxicant may contribute to cognitive decline and abnormal aging.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs) used to reduce the flammability of combustible materials; they are divided into 10 congener groups (mono- to decabromodiphenyl ethers, based on the amount of the bromine atoms). PBDEs are of notable toxicological interest, because they may affect the endocrine and central nervous system (CNS) (Costa and Giordano, 2007). These compounds transfer from consumer goods to the environment by volatilization or waste incineration. Human exposures occur mainly through the diet and dust ingestion (Domingo, 2012). Of special concern is the developmental exposure to PBDEs since these compounds readily cross the placenta and accumulate at considerable levels in human breast milk (Talsness et al., 2008). Although in 2004, PBDE congeners containing five and eight bromine atoms were banned in the European Union, the decabrominated congener (BDE-209) is still produced worldwide. A European breast-feeding infant may ingest daily between 1 and 20 ng/kg body weight (bw) of BDE-209 (EFSA, 2011). Developmental neurotoxic effects vary depending on the congener, the dose and the age of exposure, with the low brominated compounds (1–5 bromine atoms) showing the highest adverse effects. In rodents, perinatal exposure to lower brominated congeners retarded neuromotor development and altered activity levels in adults (Branchi et al., 2002; Eriksson et al., 2002; Gee and Moser, 2008; Viberg et al., 2002). Postnatal exposure to hexa-BDE led to deterioration in spatial learning in mice 6 months after exposure (Viberg et al., 2003a). Similarly, the pentaBDE congener induced deficits in sustained attention and in inhibitory control in rats when administered chronically from birth to adulthood (Dufault et al., 2005). Learning deficits have been also reported in adult rats exposed to pentaBDE from postnatal days (PNDs) 6 to 12 (Driscoll et al., 2009, 2012). With the exception of Viberg et al. (2003b), where no effects on locomotor activity were noted in mice exposed to BDE-209 on PND 10, no other studies have assessed the behavioral effects of

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BDEs at this time point. The same authors demonstrated that BDE-209 readily crosses the blood–brain barrier and accumulates in the brain when administered on PND 10 (Viberg et al., 2003b). Developmental exposures to BDE-209, administered on PND 3 or from PND 2 to PND 15 led to delayed palpebral reflex acquisition and induced locomotor alterations in adult rodents (Johansson et al., 2008; Rice et al., 2007; Viberg et al., 2007, 2003b). Nevertheless, there is controversy about the neurotoxic effects of this compound (Hardy et al., 2009). In humans, PBDE levels in cord blood have been associated with lower cognitive and motor skills in young children (Gascon et al., 2011), while the concentration of PBDE in breast milk has been correlated with increased activity and impulsivity in toddlers (Hoffman et al., 2012). Moreover, in people aged 55–74, a link between serum concentrations of low brominated forms of PBDE and decreased verbal memory and learning was recently reported (Fitzgerald et al., 2012). However, no longitudinal studies on cognitive capacity after an early exposure to PBDEs are available in humans. A follow-up study concerning learning and memory after postnatal exposure of mice to BDE-209 showed impulsivity and perseverative responses in an operant task only in aged mice, suggesting that this compound may induce delayed toxicity (Rice et al., 2009). Several mechanisms have been proposed to explain PBDEs' neurotoxicity, including impairments in the cholinergic system (Viberg et al., 2003a), alterations in BDNF levels (Viberg et al., 2008), synaptophysin and tau expression (Viberg, 2009), most of which are also associated with neurodegenerative processes. The causes underlying neurodegenerative disorders involve complex interactions between genes and environment. Therefore, identifying genotypes that are associated with protective or vulnerable phenotypes to disease and toxic effects is of special relevance.

The ε4 allele of the apolipoprotein E gene is a well-established risk factor for Alzheimer's disease (AD). ApoE is the major transporter of brain cholesterol, showing three polymorphic forms in humans. ApoE is involved in the formation of synapses, plasticity and repair. Functionally, apoE4 has also been associated with higher vulnerability to neurocognitive impairment (Raber et al., 2004), as well as with worsening in spatial learning (Alexander et al., 2007). Transgenic mice carrying apoE4 showed impairments in spatial learning and memory tasks even at early stages of life (Grootendorst et al., 2005; Hartman et al., 2001; Pfankuch et al., 2005; Reverte et al., 2012). Moreover, transgenic apoE2 mice also showed impaired learning and memory (Reverte et al., 2012).

The present study was aimed at assessing spatial learning and memory in transgenic mice carrying different polymorphisms of apoE (ε2, ε3, ε4) at 4 and 12 months of age, upon a single postnatal oral exposure to BDE-209. Our main goal was to detect long-term effects of BDE-209, and to dissect interactions between toxic exposure, genetic factors, sex and age.

2. Materials and methods

2.1. Animals

The human apoE targeted replacement mice were used for this study. These mice have a C57BL/6NTac background and express functional human apoE isoforms at physiological levels, without disturbing any known endogenous regulatory sequences (Sullivan et al., 1997). Adult male and female mice homozygous for each of the three apoE human alleles (apoE2, apoE3, and apoE4) were obtained from Taconic (Taconic Europe, Lille Skensved, Denmark). After a quarantine period of 7 days, female mice were mated with males of the same genotype. Only litters sized 6–10 pups and with pups of both sexes were used (N = 51). The day of delivery was designated as postnatal day 0 (PND0). BDE-209 was administered on PND10. On PND30, the offspring were separated from the dam and housed in plastic cages containing 2–4 animals of the same sex. The animal room was maintained at a temperature of 22 ± 2 °C, a relative humidity of 50 ± 10%, and a 12-h light/dark automatic light cycle (light: 0800–2000 h). All animals were allowed free access to food (Panlab rodent chow, Barcelona, Spain) and tap water. A maximum of 2–3 animals from each litter and sex were assigned to one of the different experimental stages at 4 months, 12 months, or BDNF determination (Tables 1, 2). Spatial learning and memory were assessed at young adulthood (4 months) and middle-age/old (12 months). BDNF was determined in young adult mice (5–6 months), which were not behaviorally tested. The use of animals and the experimental protocols were approved by the Animal Care and Use Committee of the Universitat Rovira i Virgili (Tarragona, Spain).

2.2. Chemical and treatment

The DE-USC 209 or decabromodiphenyl ether (BDE-209) (technical) (IUPAC nomenclature: 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether) was purchased from LGC Promochem (Barcelona, Spain). The compound was dissolved in corn oil and adjusted to a concentration of 10 or 30 mg/kg in 1 μl/g of bw. The doses were similar to those used by Rice et al. (2007), but mice were exposed only on one day. The doses chosen are below those reported by the EFSA (2011) as the $BMDL_{10}$ for the most sensitive effects of the various BDE congeners. On PND10, animals were administered a single oral dose of 0, 10 and 30 mg/kg of BDE-209 with a micro-pipette of 2–20 μl. The time at the administration (PND10) was chosen because it coincides with the peak of brain growth and development in rodents (Eriksson et al., 2002). Body weight was measured on PND10 (day of treatment) and on PND21 (end of weaning) (Table 3).

2.3. Behavioral assessment at 4 months of age

A total number of 192 animals of both sexes were used for the experiment at 4 months of age (Table 1). Prior to behavioral testing, body weights were recorded. To assess spatial learning and memory, mice were trained in a Morris Water Maze (MWM) reference memory task.

The apparatus consisted of a circular pool (diameter: 1 m; height: 60 cm), virtually divided in four quadrants. An escape platform (10 cm diameter) was located 1 cm below the water surface. To acquire the task (localize the hidden platform), animals performed 2 trials per day for 10 days (60 min inter-trial interval). During each trial, mice were allowed to swim for a maximum of 90 s to find the hidden platform. If the animal failed to find the platform it was directly placed on it by the experimenter. Mice were maintained on the platform for 30 s. The starting position was changed for each trial (four different positions available; none of them was placed in the target quadrant). To avoid

Table 1
Number of animals evaluated in the MWM.

MWM	Males (4 months) Pups (litter)			Females (4 months) Pups (litter)			Females (12 months) Pups (litter)		
	apoE2	apoE3	apoE4	apoE2	apoE3	apoE4	apoE2	apoE3	apoE4
Vehicle	8 (4)	10 (4)	10 (5)	12 (6)	12 (5)	12 (6)	9 (6)	11 (5)	15 (5)
10 mg/kg	12 (6)	9 (4)	12 (5)	9 (4)	10 (4)	11 (5)	9 (7)	12 (5)	11 (5)
30 mg/kg	11 (5)	10 (4)	12 (5)	11 (5)	10 (5)	11 (5)	9 (5)	14 (6)	12 (6)

Data show the number of mice and litters evaluated in the Morris Water Maze at 4 and 12 months of age