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Behavioral effects and oxidative status in brain regions of adult rats exposed to BDE-99

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants. Although developmental neurotoxicity of PBDEs has been already investigated, little is still known about their potential neurotoxic effects in adulthood. In this study, we assessed the oxidative damage in brain sections and the possible behavioral effects induced by exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99). Adult male rats (10/group) received BDE-99 by gavage at single doses of 0, 0.6 or 1.2 mg/kg/body weight. Forty-five days after exposure, the following behavioral tests were conducted: open-field activity, passive avoidance and Morris water maze. Moreover, cortex, hippocampus and cerebellum were processed to examine the following oxidative stress (OS) markers: reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and thiobarbituric acid reactive substances (TBARS). In cerebellum, BDE-99 significantly decreased SOD, CAT and GR activities at the highest BDE-99 dose. A decrease in CAT and SOD activities was also observed in cortex and hippocampus, respectively. In the behavioral tests, no BDE-99 effects were observed, while histopathological examination of the brain regions was normal. The current results show that the brain antioxidant capacity is affected by BDE-99 exposure, mainly in cerebellum. Oxidative damage could be a mechanism for BDE-99 neurotoxicity in adult rats.

1. Introduction

Brominated flame retardants are a diverse group of industrial compounds used to retard, suppress or inhibit combustion processes to reduce fire risks (IPCS, 1994). Polybrominated diphenyl ethers (PBDEs), used as additive flame retardants, have the chemical formula $C_{12}H_{(0-9)}Br_{(1-10)}O$, being 209 the theoretical number of possible congeners is 209. Products that contain PBDEs are textiles, building materials, as well as a wide variety of electrical and electronic appliances, including cases for television sets and computers (Branchi et al., 2003; Birnbaum and Staskal, 2004).

The chemical characteristics and some toxicological properties of PBDEs are comparatively similar to those of polychlorinated biphenyls (PCBs) (Kodavanti and Ward, 2005; Coburn et al., 2007). Like PCBs, they are persistent organic pollutants and bioaccumulate in the environment (Schecter et al., 2005; Law et al., 2006). A number of studies have indicated the presence of PBDEs in sediments, soil, outdoor and indoor air, house dust, foods, birds, fish and marine and terrestrial animals (Darneurd et al., 2001; De Wit, 2002; Bocio et al., 2003; Domingo, 2004). Furthermore, human levels of PBDEs have been detected in adipose tissue, serum and breast milk (Costa and Giordano, 2007). In contrast to PCBs, dioxins and furans, whose concentrations in human tissues and breast milk have been declining in the past 20 years, levels of PBDEs have been increasing (Meneses et al., 1999; Schecter et al., 2005; Schuhmacher et al., 2007, 2009). One of the PBDE congeners most frequently found in the environment is the 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) (McDonald, 2002; Viberg et al., 2006; Lundgren et al., 2009). In particular, this congener is the most commonly found in human milk and cord blood (Norén and Meironyté, 2000; Schuhmacher et al., 2009).

During development, mammals may be exposed to toxicants either as fetuses via maternal intake, during the newborn period via intake of breast milk, or by direct ingestion or contact with toxicants (Viberg et al., 2006). A number of experimental studies in mice and rats indicate that PBDEs can cause developmental neurotoxic effects (Costa and Giordano, 2007; Fischer et al., 2008; Cheng et al., 2009). Kuriyama et al. (2005) showed that

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prenatal exposure to BDE-99 in rats induces hyperactivity in the offspring and impaired spermatogenesis. In turn, Branchi et al. (2005) demonstrated that prolonged developmental exposure of mice to BDE-99, from gestational day (GD) 6 to postnatal day (PND) 21, induced hyperactivity, manifested mainly during adolescence. Furthermore, neurotoxic effects were also observed when BDE-99 was administered during a critical period of neonatal brain development. These effects are manifested as disrupted spontaneous behavior, reduced habituation, and impaired learning/memory abilities (Eriksson et al., 2002; Branchi et al., 2003; Fischer et al., 2008).

Little information exists on possible cellular mechanisms underlying the neurotoxic effects of PBDEs. It has been reported that PBDEs exert a disrupting effect on thyroid function, reducing circulating thyroxine (T_4), when administered during the perinatal period (Zhou et al., 2002). Some studies have also demonstrated that PBDEs can affect signal transduction pathways, particularly the homeostasis of calcium and protein kinase C (PKC) (Madia et al., 2004; Kodavanti and Ward, 2005).

Recently, some studies have been focused on assessing the role of oxidative stress (OS) on PBDE-induced neurotoxicity (He et al., 2008; Giordano et al., 2008; Tagliaferri et al., 2010). The inherent biochemical and physiological characteristics of the brain, including a high lipid content and energy requirements, make it particularly susceptible to free radicals mediated insult. Reactive oxygen species (ROS) are generated continuously in nervous during normal metabolism and neuronal activity. Moreover, the natural antioxidant system consists of a series of antioxidant enzymes that are able of reacting with and inactivating ROS. When ROS production exceeds the antioxidant defense capacity of the cell, OS ensues, leading to damage to DNA, proteins and membrane lipids (Giordano et al., 2008).

In vitro studies in neuronal and astroglial cells have shown that PBDEs may produce neurotoxic effects by a mechanism that involves OS. In primary cultured rat hippocampal neurons, He et al. (2008) showed that 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) caused oxidative damage. Furthermore, Giordano et al. (2008) examined the neurotoxicity of a pentapolybrominated diphenyl ether mixture (DE-71) in mouse neurons and astrocytes. DE-71 induced a decrease in the levels of reduced glutathione (GSH), as well as an increase in ROS levels and in lipid peroxidation (LPO). Recently, Cheng et al. (2009) demonstrate that developmental BDE-99 exposure caused OS in the hippocampus of rat offspring by altering the activity of SOD and GPx and producing LPO. In addition, BDE-99 affected motor activity and learning and memory functions. The authors suggested that disturbance of the hippocampus, a tissue with a crucial role during brain development, might cause serious neurobehavioral effects.

Although developmental neurotoxicity of PBDEs has been already studied by a number of researchers, information on the potential neurotoxic effects when exposure occurs during adulthood is scarce. In this study, we assessed in adult rats exposed to BDE-99 the neurobehavioral effects and the oxidative status in cerebellum, cortex and hippocampus. BDE-99 is among the BDE congeners found more often and at highest levels in sentinel species and human tissues. Moreover, by means of histopathological examination, the possible effects of redox response in these brain areas were also investigated.

2. Materials and methods

2.1. Chemical

BDE-99 (CIL-BDE-99-CS) was obtained from LGC Standards S.L.U. (Barcelona, Spain). It was dissolved in corn oil as vehicle and administered orally (gavage) by intragastric tubing.

2.2. Animals and treatment

Sexually mature male Sprague–Dawley rats (220–240 g) were obtained from Criffa (Barcelona, Spain). Animals were housed in plastic cages in a climate-controlled facility with a constant day–night cycle (light: 08.00–20.00 h) at a temperature of 22 ± 2 °C, and a relative humidity of 50 ± 10%. Food (Panlab rodent chow, Barcelona, Spain) and tap water were available *ad libitum*. The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the "Rovira i Virgili" University (Tarragona, Spain).

After a quarantine period of 2 weeks, rats were randomly divided into two groups (10 animals per group). Each group of rats received by gavage single doses of 0.6 or 1.2 mg BDE-99/kg body weight. The selection of the BDE-99 doses used in this study was based on a previous report describing neurobehavioral effects of this congener in rat offspring (Kuriyama et al., 2007). A third group of 10 rats (control group) received only the vehicle, corn oil. After 45 days of exposure to BDE-99 and during 9 days, rats were evaluated for several skills by performing different behavioral tests. The time interval between the tests was 1 day. When behavioral tests were completed, rats were euthanized with ketamine–xylazine. Animals were decapitated and brain was removed. Brains were immediately dissected. Cortex, hippocampus and cerebellum were obtained from the right hemisphere. Samples were washed in 0.9% saline and processed to examine the oxidative stress markers, while samples from the left hemisphere were used to histopathological examination.

2.3. Functional observation battery (FOB)

The FOB protocol (Moser, 2000) was used at 3, 21 and 44 days after administration of BDE-99. Following a brief assessment of the rat in the home cage, each animal was removed and placed in an experimental white box (60 cm × 90 cm) to evaluate autonomic, neuromuscular and sensorimotor functions (Fuentes et al., 2007).

2.4. Behavioral tests

At the end of the experimental period, all rats were weighed and the following behavioral tests were carried out.

2.4.1. Open-field activity

At day 46 after exposure to BDE-99, general motor activity was measured in an open-field apparatus consisting of a wood square (80 cm × 80 cm) surrounded by a 47 cm high dark wall. During the test, rats were allowed to move freely around the open field and to explore the environment for 15 min. The path and movements of the animals were recorded by a video camera (Sony CCD-IRIS model) that was placed above the square and was connected to a VHS videocassette recorder (Panasonic AG-5700 model). The video tracking program Etho-Vision® from Noldus Information Technologies (Wageningen, The Netherlands) was used to measure the distance traveled and the time spent as a measure of horizontal activity. The number of rearings was also determined as a measure of vertical activity (Bellés et al., 2005).

2.4.2. Passive avoidance test

After 48 days of exposure to BDE-99, the recent memory of animals was tested in a passive avoidance test. The apparatus consisted of a shuttle box separated into two compartments by a wall and a sash door (Ugo Basile, Comerio, Italy). One compartment was illuminated, while the second one was dark. Animals were placed in the illuminated compartment, and after a period of 30 s, the door was pulled up. After the rats spontaneously entered the dark compartment (recorded as T1), the door was shut and the animals received an electric shock of 1 mA for 3 s. Twenty-four hours later, the same procedure was repeated with a delay period of 10 s before opening the door. The time elapsed before entering the dark compartment (maximum 5 min) was recorded as T2 (Bellés et al., 2005; Colomina et al., 2005).

2.4.3. Morris water maze test

At day 51 after exposure to BDE-99, spatial learning and retention were tested in a water maze according to a test modified from the procedure of Morris (1984). The water maze consisted of a circular tank (diameter, 160 cm; height, 60 cm) divided into four equal-sized quadrants. During testing, the tank was filled with water at 23 ± 2 °C. A transparent platform (diameter, 12 cm; height, 20 cm) was set inside the tank being the top submerged 2 cm below the water surface, in the center of one of the four quadrants of the maze. Extra-maze clues were located around the pool to provide a spatial configuration of the task. The path of the animals was recorded by a video camera placed above the maze and data were analyzed by the video tracking program Etho-Vision®.

Animals were subjected to five trials per day for tree consecutive days (training sessions). Each trial started from one of four points assigned on different arbitrary quadrants of the circular tank. The maximum duration of each trial was 60 s, being each trial separated by a 60 s intertrial interval. At the beginning of each trial, the rat was placed into the pool with the nose pointing towards the wall from one of five starting positions. If the rat did not locate the platform within 60 s, the animal was then placed on the platform for 30 s. Twenty-four hours after the last training session; retention of the task was assessed by a probe trial which consisted of a 60 s free swim without the escape platform. The swim-path length and the latency to find the escape platform during the training sessions, as well as the total time and