NEUROTOXICITY OF DIFFERENT POLYBROMINATED DIPHENYL ETHERS, INCLUDING PBDE 209

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Introduction

The polybrominated diphenyl ethers, widely used as flame retardants in electrical appliances and textiles [1], have recently been shown to be present in the global environment, such as sediment, fish, etc [2, 3] and that levels of PBDEs are increasing in the Swedish environment [4, 5]. A recent report has shown the presence of PBDEs in Swedish human milk and also that the PBDEs have increased exponentially since 1972, whereas PCBs are steadily decreasing [6, 7]. The sum of PBDEs found in Swedish human milk increased from 0.07 ng/g lipids, 1972, to 4.02 ng/g lipids, 1997. The most abundant PBDE congeners in Swedish human milk, 1997, were 2,2',4,4'-tetraBDE (PBDE 47), 2,2',4,4',5-pentaBDE (PBDE 99), 2,2',4,4',5,5'-hexaBDE (PBDE 153), and 2,2',4,4',6-pentaBDE (PBDE 100) [6]. In human blood plasma the total PBDE concentration was 2.1 ng/g lipids [8], with the same pattern of congeners. Occupational exposure to PBDEs has been demonstrated and in blood serum the total concentration was 37 pmol/g lipids with the same pattern of congeners [9]. Furthermore computer technicians show levels of PBDE 209 in their blood [10]. This indicates that humans are exposed to PBDEs both as infants and as adults.

In several studies we have reported that low-dose exposure to environmental agents, such as DDT, PCBs and organophosphates, during the period of rapid brain growth, known as the "brain growth spurt", in neonatal mice can lead to disruption of the adult brain function, and also to an increased susceptibility to toxic agents at adult ages [11, 12]. In recent studies we have shown that neonatal exposure to PBDE congeners 2,2',4,4',-tetraBDE (PBDE 47), 2,2',4,4',5-pentaBDE (PBDE 99) and 2,2',4,4',5,5'-hexaBDE (PBDE 153) [13-16]can induce persistent dysfunction in adult mice, manifested as deranged spontaneous behaviour, e.g. hyperactivity, and altered behavioural response to the cholinergic agent nicotine [16]. We have also shown that the effects are inducible during a restricted period of neonatal life and that the behavioural effect gets worse with age [13-16].

Above mentioned studies call for further investigations. Can the decabrominated diphenyl ether (PBDE 209) induce similar effects after neonatal exposure in mice? Furthermore, are the neurotoxic effects of PBDE 99 restricted to male mice only and are these effects inducible only in a certain strain of mice (NMRI)?

Materials and methods

The polybrominated diphenyl ethers 2,2',3,3',4,4',5,5',6,6'-decaBDE (PBDE 209), ^{14}C-labelled 2,2',3,3',4,4',5,5',6,6'-decaBDE ([U-^{14}C]PBDE 209) and 2,2',4,4',5-pentaBDE (PBDE 99) were synthesized at the Wallenberg Laboratory, Stockholm University, Sweden, and kindly donated by the research group of Åke Bergman.

[U-^{14}C]PBDE 209: NMRI male mice were given 1.5 MBq [U-^{14}C]PBDE/kg body weight (40.5 µCi/kg body weight), as a single oral dose, via a metal gastric-tube at the age of 3, 10 or 19 days. 24 hours or 7 days after administration, the male mice were sacrificed and their brains were solubiliszed individually and radioactivity counted in a scintillation analyser.

PBDE 209: NMRI male mice were given 1.34 – 20.1 mg PBDE 209/kg body weight (1.4 - 21 µmol/kg body weight) as a single oral dose, via a metal gastric-tube at the age of 3, 10 or 19 days. Mice serving as control animals received the 20% fat emulsion vehicle. At the age of 2, 4 and 6 months the male mice were observed for spontaneous behaviour. The test measures locomotion (horizontal movement), rearing (vertical movement) and total activity (all types of vibration in the test cage, including movement, shaking (tremors) and grooming.

PBDE 99: C57 Black/J male and female mice were given 0.4 – 16 mg PBDE 99/kg body weight (0.7 – 28 µmol/kg body weight) as a single oral dose, via a metal gastric-tube at the age of 10 days. Mice serving as control animals received the 20% fat emulsion vehicle. At the age of 2, 5 and 8 months both male and female mice were observed for spontaneous behaviour in the same manner as described above.

Results and discussion

The retention study of [U-^{14}C]PBDE 209 showed that PBDE 209 is taken up in neonatal mice. Mice administered PBDE 209 on postnatal day 3 or 10 contained 4.8 or 4.0‰ of the total amount radioactivity administered, in the brain 24 hours after administration , while mice administered PBDE 209 on postnatal day 19 only contained 0.6 ‰ of the total amount radioactivity administered, in the brain 24 hours after administration. After 7 days the amount of radioactivity had increased to 7.4 or 10.5 ‰ of the total administered amount, in mice administered on postnatal day 3 or 10, while mice administered PBDE 209 on day 19 showed the same amount of radioactivity 7 days after administration. Compared to certain PCBs and PBDE 99 this is a different pattern of retention, because PCBs and PBDE 99 have been seen to be the same or decrease in the brain of neonatal mice over the 7-day-period [14, 17].

The spontaneous motor behaviour data showed that PBDE 209 induced a significant disruption of habituation in 2, 4, and 6 months old male NMRI mice, exposed to 20.1 mg PBDE 209/kg body weight, on postnatal day 3. Habituation here defined as a decrease in locomotion, rearing and total activity variables in response to the diminishing novelty of the test chamber, over the 60 min test period. For mice exposed to 2.22 mg PBDE 209/kg body weight on postnatal day 3 there are significant differences in the spontaneous behaviour, but not in all three variables measured. Mice exposed to PBDE 209 on postnatal day 10 or 19 did not show any significant disruption of habituation. The spontaneous behaviour tests also showed that the disruption of habituation, in mice exposed to PBDE 209 on postnatal day 3, is irreversible and actually worsen with increasing age. In recent studies we have shown that PBDE 47, 99 and 153 induce their neurotoxic effects when exposure occurs on postnatal day 10, during the peak of the "brain growth spurt" [13-16]. This and the fact that the pattern of retention differs with this congener of PBDE, indicate that the effect might be due to the presence of metabolites of PBDE 209, during the critical period of rapid brain development, because the amount of radioactivity in the brain of mice administered PBDE 209 on postnatal day 10 should be enough to cause a similar effect.

The spontaneous motor behaviour tests showed that exposure to PBDE 99, on postnatal day 10, induced a disruption of habituation in adult C57 Black/J mice. A clear dose-response relation can be seen for this kind of neurotoxic effect. The same kind of neurotoxicity has earlier been seen in adult NMRI male mice, neonatally exposed to PBDE 99. The doses that here is seen to induce neurobehavioural effects in adult C57 Black/J mice are the same as earlier seen in studies of NMRI mice [14-16]. This indicates that the neurotoxic effects of PBDE 99 are not strain specific. PBDE 47, 153, and 209 have also been shown to induce neurobehavioural effects after neonatal

exposure on day 10. These three congeners induce their effects at about the same doses, on a molar level, as PBDE 99. The effects seen in the present study persist over the eight months period and can be considered irreversible. The effects not only persist, but also get worse with increasing age for the highest dose. This has also been seen in adult NMRI mice, after exposure to PBDE 47, 153 and 209, on postnatal day 10 [13-16]. Furthermore, the neurotoxic effect induced by PBDE 99, manifested as deranged spontaneous behaviour and disrupted habituation, effects that get worse with increasing age, is also seen in adult female C57 Black/J mice. The same clear dose-response relation is seen in the female mice and the effect is irreversible and get worse with increasing age, which was also seen in male C57 Black/J mice. We conclude that the neurotoxic effect induced by PBDE 99 is not gender specific, but can in fact be induced in both sexes.

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