The link between adult behavioral effects of PBDEs and alterations in protein levels in the neonatal brain.

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Introduction

Polybrominated diphenyl ethers (PBDEs), a group of chemical substances used to inhibit combustion, are additive flame retardants, meaning they have a tendency to leak from products into the environment [1]. PBDEs have subsequently been found in human matrices measured, including breast milk and indoor dust and particulate matter has been shown to contain high concentrations of PBDE. The two main routes of exposure to highly brominated PBDEs in infants are suckling and inhalation and/or ingestion of dust or particulate matter. It has been seen that newborns and toddlers are exposed to higher levels of PBDEs than the average adult individual, especially regarding the highly brominated PBDEs [2, 3]. Several PBDEs can act as neurotoxicants when exposure coincides with the neonatal brain developmental period [4-10], when several important developmental processes ooccur, for example proliferation, axonal and dendritic outgrowth, synaptogenesis, myelinezation and synaptic pruning [11].

Materials and methods

Mouse pups were exposed to different brominated flame-retardants, PBDEs (99, 203, 206 and 209) and TBBPA (tetrabromo-bisphenol A), during the brain growth spurt, on postnatal day 10, except for PBDE 209, where exposure occurred on postnatal day 3. For comparative reasons additional mouse pups were exposed to the fluorinated compounds PFOS and PFOA, or the artificial sweetener and organoclorine compound, sucralose, on postnatal day 10. Animals were either sacrificed 24 hours after the neonatal exposure or left to mature to adult age.

In the animals sacrificed 24 hours after exposure the brains were dissected and different brain regions (cerebral cortex and hippocampus) were homogenized and analyzed for the different proteins CaMKII (calcium calmodulin-dependent kinase II), GAP-43 (growth-associated protein 43), synaptophysin and tau, with Slot-blot technique.

In adult animals, exposed neonatally to different compounds, spontaneous behavior was measured for a period of 60 minutes, split into 3 consecutive 20-minute periods. Locomotion (horizontal movement) and rearing (vertical movement) was measured

Results and discussion

In the spontaneous behavior test the control groups showed a high activity during the first 20-minute period in both locomotion and rearing. This activity gradually decreased during the 60-minute period, to a low level of activity during the last 20-minute period, showing a normal and significant habituation.

Animals exposed to PBDE 99, 203, 206, 209, PFOS or PFOA during the postnatal period all showed significantly altered spontaneous behavior compared to the control groups. For both locomotion and rearing the activity during the first 20-minute period was significantly lower than in control animals and during the last 20-minute period the activity was significantly higher than in the control group, showing a lack of habituation during the 60-minute test period.

In neonatal brains of animals exposed to PBDE 99, 203, 206, 209, PFOS or PFOA, significant differences in the levels of one or more proteins were seen, compared to the control animals. CaMKII was the protein most often affected followed by synaptophysin, GAP-43 and tau, in decreasing order. Protein levels in both hippocampus and cortex were changed depending on the exposure, but most often changes were seen in hippocampus, leading to the assumption that hippocampus is the more sensitive brain region of the two.

In the spontaneous behavior test, animals exposed neonatally to TBBPA or sucralose, showed no differences in activity, during any of the three 20-minute periods, compared to the control animals, meaning that normal habituation was seen.

When analyzing the levels of the four different proteins in the neonatal brains of animals exposed to TBBPA or sucralose, no significant differences could be seen in any of the different brain regions for any of the four proteins, compared to the control animals.

These studies indicate a very interesting finding, in that all exposures where adult behavioral disturbances are seen, the levels of CaMKII are also increased in hippocampus. In contrast, in the two exposures, TBBPA and sucralose, where no adult behavioral disturbances are seen, no changes in protein levels could be seen for CaMKII, GAP-43, synaptophysin or tau.

CaMKII, GAP-43, synaptophysin and tau all show distinct ontogeny patterns during the neonatal period, with CaMKII and synaptophysin increasing drastically during the first four weeks of life, while GAP-43 and tau increases during the first week of life, to reach a peak during the second week and then decrease, showing a bell-shaped ontogeny curve. All four proteins are involved in important developmental processes, such as neurite outgrowth, dendritic arborization, synaptogenesis and pruning with its subsequent apoptosis. Since all these four proteins are involved in the normal development of the brain, changes in the levels of these proteins during this period of rapid brain growth and development, could interfere with normal development, giving rise to cognitive and behavioral alterations in the adult individual.

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