THE EFFECT OF PENTABROMODIPHENYL ETHER, HEXABROMOCYCLODODECANE AND TETRABROMOBISPHENOL-A ON DOPAMINE UPTAKE INTO RAT BRAIN SYNAPTOSOMES

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Introduction

Findings of brominated flame retardants (BFRs) in marine, freshwater and terrestrial environment have indicated a worldwide distribution of these compounds (1-3). The environmental levels of the BFRs are still generally lower than for PCB, approximately 10-100 times lower. However, the environmental levels are increasing, which is a matter of concern. Currently the most used BFRs are Tetrabromobisphenol-A (TBBPA), followed by the brominated diphenyl ethers (BDE) and hexabromocyclododecane (HBCD). Most focus regarding BFRs have been on the brominated diphenyl ethers. However, recent studies have shown that considerable levels of the more recently developed BFRs such as TBBP-A and HBCD are found in biota (4-6). Several of the BFRs have chemical and physical characteristics, which resembles the chlorinated organic compounds, such as PCBs, making them potential harmful environmental toxins. Very little information is available regarding toxicity of the BFRs. Studies by Eriksson et al (7) have shown that neonatal exposure to some of the diphenyl ethers produces persistent aberrations in spontaneous behavior and affects learning and memory function in the adult mice as found for the PCBs. Although the BFRs are regarded as relatively non-toxic and have a low acute toxicity these findings suggest that the BFRs have a neurotoxic potential. Several studies have shown that environmental contaminants, such as PCBs and methylmercury, affect the neurotransmitter system in CNS, especially the dopaminergic system (8-11). These findings have led to the suggestion that chronic exposure to environmental contaminants may lead to increased incidents of Parkinsons' disease (12,13). Since the environmental levels are increasing it is important to be aware of their potential as environmental toxins. In this study we have therefor studied the effect of three different mixtures of BFRs on the membrane properties of the neurons. The synaptosomes are the detached nerve terminal particle, which maintain a membrane potential and many of the transport properties of the neuron, such as the uptake of dopamine.

Material and Methods

Chemicals

Pentabromodiphenyl ether (DE-71, Great Lakes), hexabromocyclododecane (CD-75P-Great Lakes), tetrabromobisphenol A (BA-59P-Great Lakes) were purchased from Promochem (Sweden). [2,5,6-^{3}H]-dopamine (^{3}H-dopamine, 6-14 Ci/mmol) and tetra[^{3}H]phenylphosphonium bromide (^{3}H-TPP$^+$, 27Ci/mmol) were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden).

Preparation of synaptosomes

Rat brain synaptosomes were prepared as described previously (11). Male Wistar rats (150-200g) were killed by decapitation and the brains were quickly removed and kept on ice. The brains were

homogenized (~5 % wt/vol) in 0.32 M sucrose and the homogenate was centrifuged for 10 min (1000 g, Sorvall SS-600 rotor). The supernatant was then mixed with 1.3 M sucrose to obtain a 0.8 M suspension to remove myelin on further centrifugation. The supernatant was centrifuged for 30 min (20 000 g) to get a crude synaptosomal pellet (P2). The synaptosomal pellet was gently resuspended in 0.32 M sucrose and used in the experiment on the same day.

Assay for uptake of dopamine into synaptosomes

High affinity uptake of dopamine was determined as described previously (11). Synaptosomes (32µg protein/ml) were preincubated at 25 °C for 15 minutes in absence or presence of BFRs in Tris-Krebs buffer. The uptake was started by adding substrate containing approximately 100 nM 3H-dopamine (0.5 µCi). The mixtures (500 µl final volume) were incubated for 9 minutes and the reaction was terminated by filtration with a solution of 0.15 M NaCl and 0.05 % (w/v) bovine serum albumin in a cell harvester onto Skatron filtermats. The filters were dissolved in 10 ml of Filter Count (Packard) and counted for retained radioactivity in a liquid scintillation spectrophotometer (Packard Tri-Carb 300).

Assay for uptake of ^{3}H-TPP^{+} into synaptosomes

Accumulation of ^{3}H-TPP^{+} into synaptosomes to measure the effect on the membrane potential was in principle performed as described previously (14). Synaptosomes (0.2 mg/ml) were washed once in Tris-Krebs buffer and preincubated at 30 °C for 15 minutes in absence or presence of BFRs in Tris-Krebs buffer. The uptake was started by adding substrate containing 10 nM ^{3}H-TPP^{+} (0.1 µCi). The mixtures (250 µl final volume) were incubated for 10 minutes and stopped by adding ice cold 0.9 % NaCl followed by filtration (Whatman GF/B filters). The filters were counted for retained radioactivity in a liquid scintillation spectrophotometer

Results and Discussion

In this study we show for the first time that two of the most frequently used flame retardants have a neurotoxic potential. We found that the technical mixtures of HBCD and TBBPA inhibited the high affinity uptake of dopamine at a similar concentration level as previously shown for the PCBs (11) with an EC50 concentration of 5µM and 10µM respectively (Fig 1). The PBDE mixture had only a small effect on the dopamine uptake at relatively high concentrations. Kinetic study did not indicate that HBCD or TBBPA inhibited dopamine uptake competitively. Since uptake of dopamine into nerve terminals is dependent on a high transmembrane sodium gradient, we further hypothesized that the BFRs influenced the ion-fluxes of the synaptosomal membrane. TPP^{+} is a probe which uptake is a function of the membrane potential (15). TBBPA inhibited uptake of TPP^{+} in a concentration range similar to the inhibition of dopamine uptake (Fig 2). HBCD also inhibited TPP^{+} uptake, but to a smaller degree than TBBPA (Fig 2). This finding indicates that TBBPA disturb the synaptosomal membrane potential in a way that reduces the uptake mechanism of dopamine. Since inhibition of TPP^{+} by HBCD was not directly in accordance to inhibition of dopamine uptake we suggest a more selective, although not yet identified, mechanism of action of this compound. The PBDE had only minor effect on TPP^{+} uptake.

The finding that HBCD have a neurotoxic potential is of major concern. HBCD is used as an additive BFR and has to certain degree replaced the use of PBDE. Partly because of analytical challenges and the lack of analytical standards there are very few data on environmental levels of this compound, however some recent studies have shown that its environmental levels may be even higher than for the PBDEs (5). HBCD has a low acute toxicity, probably, as for the DecaBDE, because fairly little of the compound is absorbed and taken up in the body when eaten. However, it is suggested that

HBCD has the potential to bioaccumulate, which may, over time, induce damage related to chronic exposure. Since TBBPA mainly is used as a reactive BFR one should not expect high environmental levels of this compound, however recent studies have observed this compound in human blood with the highest levels found in breastfed children (6). It is also shown that residual unreacted TBBPA may leak from TBBPA treated products (4). Bearing in mind that the BFRs have a bioaccumulative potential these findings should merit further investigation to improve the analytical methods available for monitoring the levels of these compounds and to elucidate possible toxic effects.

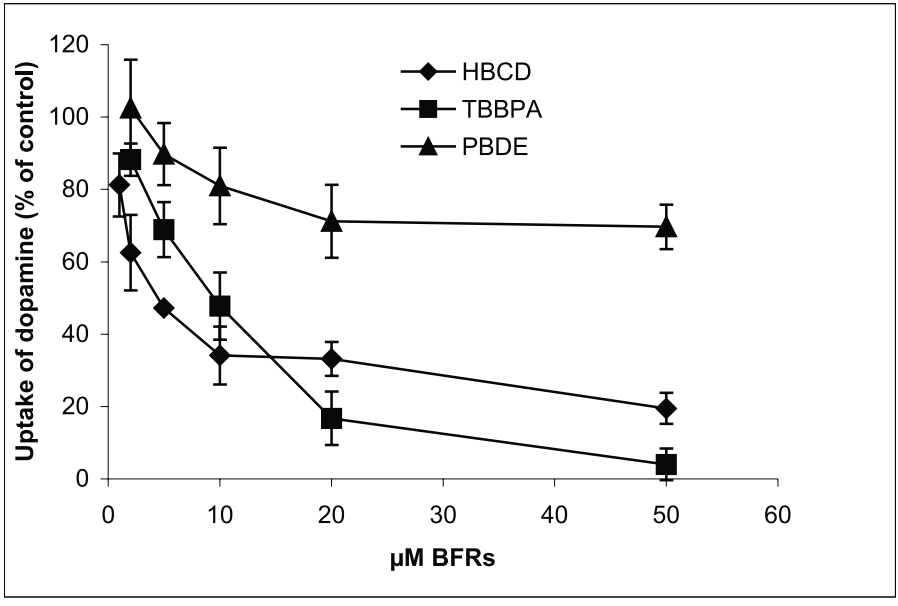

Figure 1. The effect of BFRs on the uptake of the neurotransmitter dopamine into detached nerve terminals. The results are expressed as mean values (± SD) of four separate experiments assayed in duplicate.

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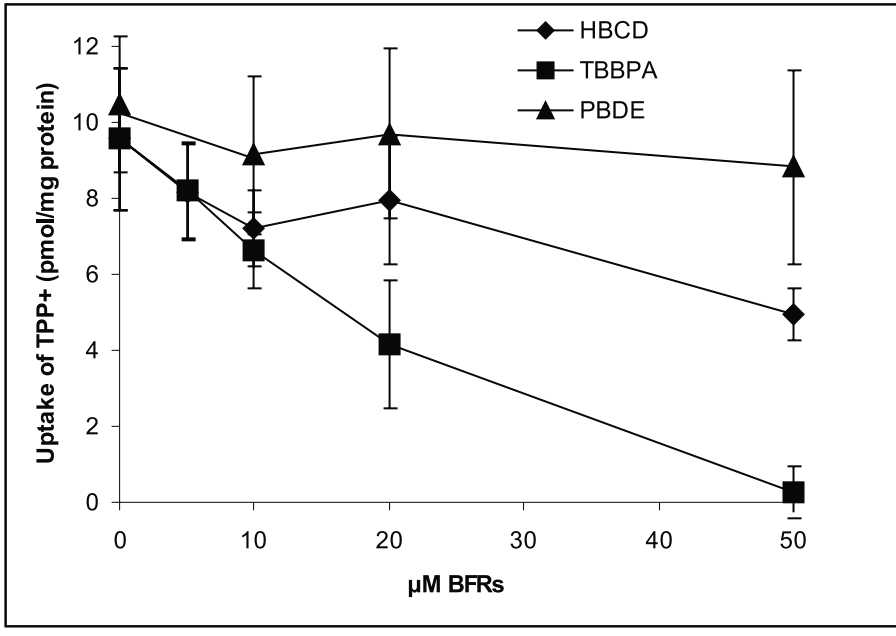

Figure 2. The effect of BFRs on the uptake of the membrane potential marker TPP^+ into detached nerve terminals. The results are expressed as mean values (± SD) of four separate experiments assayed in duplicate.

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