THE EFFECT OF BROMINATED FLAME RETARDANTS ON CELL DEATH AND FREE RADICAL FORMATION IN CEREBELLAR GRANULE CELLS

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Introduction

Some brominated flame retardants (BFRs) such as polybrominated diphenylethers (PBDE), tetrabromobisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD) have attracted considerable concern due to their occurrence and persistence in the environment and humane exposure for the last two decades. Their environmental distribution seems to be similar to those of PCB's and DDT. There is an increase in BFRs in human milk in Sweden (1). In contrast to the meanwhile elaborated knowledge about PCB's mechanisms of action there is almost no exact information about the toxicity of BFRs, especially as neurotoxicity is concerned. A few toxicological studies have been carried out and some BFRs can elicit serious health effects such as thyroidogenic, estrogenic and dioxin-like activity (2,3). There is also reported a rapid oscillation of calcium in neuron cell populations after exposure to PBDEs (4).

In the present paper we show that exposure to three different BFRs lead to death of cultured cerebellar granule cells. In some cases the NMDA-receptor antagonist, MK-801, and the antioxidant vitamin E, reduce this effect. The productions of free radicals after exposure of these cells by BFRs have also been investigated.

Material and Methods

Chemicals

Pentabromodiphenyl ether (DE-71, Great Lakes), octabromodiphenyl ether (techn), decabromodiphenyl ether (techn), hexabromobiphenyl (techn), tetrabromobisphenol-A (BA-598, Great Lakes), and hexabromocyclododecane (GD-788, Great Lakes) were all obtained from Promochem (Sweden). (+)-MK-801 (hydrogen maleat), Vitamin E and 2,7-dichlorofluorescin (DCF-DA) were purchased from Sigma Chemical (USA).

Preparation of cerebellar granule cells

Primary cultured neurons from rat cerebellum were isolated mainly as described by Schousboe *et al*. (5). The cerebella from 6- to 8-day-old pups were dissected under sterile conditions and the brain tissue was then mechanically dissected from the meninges, chopped in buffered solution and grown for 6-8 days in basal Eagle's medium (containing fetal calf serum, penicillin/streptomycin, KCl and glutamine) before exposure. The cells were exposed for the BFRs in culture media without fetal bovine serum. Cell death was measured by using the tryphan blue exclusion assay. In some cases the cells were exposed simultaneously to BFRs and compounds expected to reduce their response.

Free radical measurements

Formation of ROS was measured by using the fluorescent probe DCF-DA as previously described

(6). The cells were preincubated with DCF-DA, which is permeable across cell membranes, and inside the cell the acetate moiety is cleaved by cellular esterases. DCFH readily react with ROS such as peroxynitrite and lipid peroxides to the fluorescent DCF. The formation of the fluorescent oxidized derivate of DCFH, namely DCF, was measured with a luminescence spectrometer at 37º C for 180 minutes.

Results and Discussion

In this study we investigated the effect of different BFRs on cerebellar granule cells. We found that relatively low concentrations of these compounds induced cell death as demonstrated by their failure to exclude trypan blue. Some of the BFRs were even more toxic to neuronal cell cultures than PCB (7). HBCD was the most toxic BFR in this study, and the LC_{50} was about 3µM after 24 hours of exposure. TBBP-A and PBDE showed LC_{50} values about 7µM. There was a vide span in toxicity in the diphenylether series with pentabrom being fairly toxic compared to octabrom and decabrom, which did

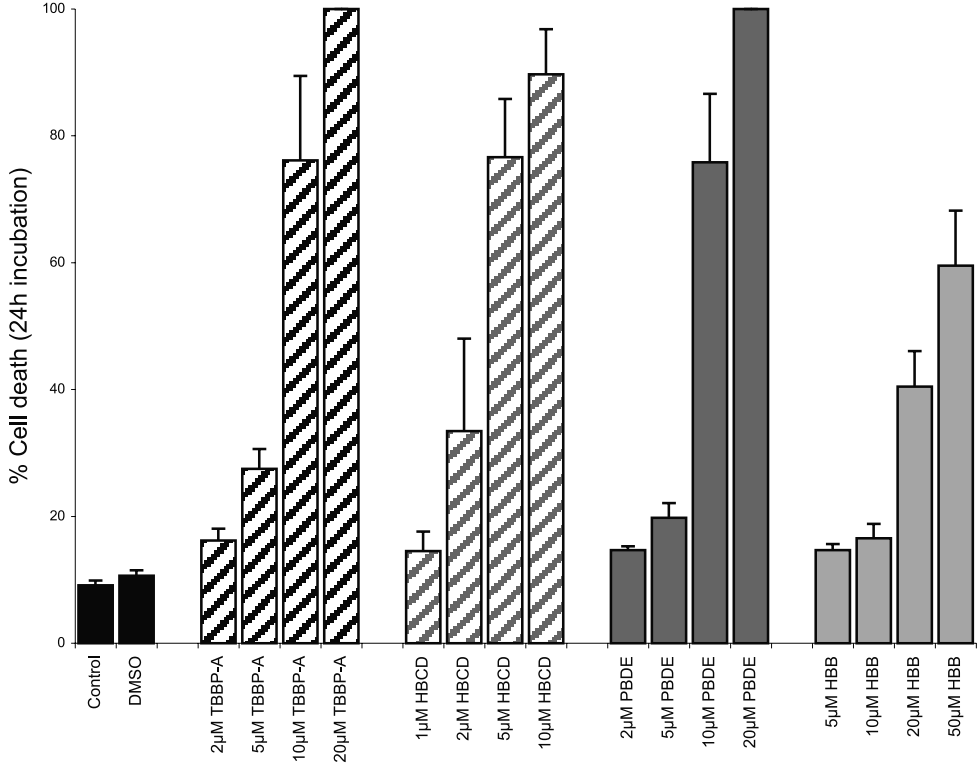

Figure 1. Death of cultured cerebellar granule cells after exposure to increasing concentrations of TBBP-A, HBCD, PBDE and HBB for 24 hour. The LC_{50} values were estimated to approximately 7 µM, 3 µM, 7 µM and 30-40 µM for TBBP-A, HBCD, PBDE and HBB, respectively. Values are presented as percent death (mean ± SEM).

not show any effect. HBB showed a LC_{50} about 30-50 µM, and the other BFRs tested was much less active (Fig. 1). The effect of TBBP-A, HBCD and PBDE on cell death were all reduced by the NMDA-receptor antagonist, MK-801, indicating calcium passage through the NMDA-receptor. In agreement, Wiegand *et al*. (4) showed dysfunction of calcium homeostasis in a series of different cell preparations, including neurons, after exposure for PBDE's. Activation of the NMDA receptor may trigger several downstream pathways leading to ROS formation and cellular damage. An involvement of ROS was indicated because high concentrations of vitamin E had a protective effect in our experiment. One should therefore believe that BFRs in agreement with previous studies on non-planar PCBs lead to formation of free radicals or reactive oxygen species. ROS formation was measured using the fluorescent probe DCF-DA as previously described (7,8) Interestingly, TBBP-A, was the only BFR that induced production of ROS in this study (Table 1), this is probably due to its strong activation of the NMDA-receptor. DCF do not measure ROS in cell membranes. Therefore the effect of vitamin E may be due to its protective properties in biological membranes as discussed by Mariussen et al (7).

The findings that several BFRs induce cell death of nerve cells at concentrations even lower than observed for the PCBs make it important to be aware of the BFRs as potential hazardous environmental toxins.

Table 1. Relative fluorescence as a measure for formation of ROS in rat cerebellar granule cells with increasing concentration of TBBP-A, HBCD and PBDE.

Compound	Relative fluorescence % of control	Compound	Relative fluorescence % of control
DMSO	96 ± 2		
TBBP-A (1µM)	190 ± 20	HBCD (12,5µM)	109 ± 9
TBBP-A (3µM)	319 ± 42	HBCD (25µM)	119 ± 15
TBBP-A (6µM)	412 ± 42	PBDE (12,5µM)	115 ± 8
TBBP-A (12,5µM)	647 ± 63	PBDE (25µM)	114 ± 9

Values are presented as percent of control (percent ± SEM)

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