Correlation Between Accumulation of Polybrominated Diphenyl Ether (PBDE) Congeners in Rat Neuronal Cultures and Their Effects on PKC Translocation

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Introduction

Polybrominated diphenyl ethers (PBDEs) are commercially produced with three degrees of bromination, i.e., pentaBDE, octaBDE, and decaBDE, indicating the average bromine content. Since direct bromination of aromatic compounds is non-selective, mixtures of homologues and isomers are formed with 209 possible congeners having low vapor pressure at room temperature and a high lipophilicity; log Kow ranges between 4.28 and 9.9[1]. Commercially produced PBDE mixtures contain a limited number of PBDE congeners and are less complex than the corresponding technical polychlorinated biphenyl (PCB) mixtures because of steric hindrance due to the large bromine atom. PBDEs are extensively used as flame-retardants in the electronic, textile, and computer industry in circuit boards, computer housing, television, capacitors, furniture, and automobile cushions. Since PBDEs are used as additive flame-retardants and do not bind chemically to the polymers, they can leach from the surface of the product and easily reach the environment[2,3].

PBDEs are structurally similar to dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs). PBDEs are now ubiquitous; they can be found in air, water, fish, birds, marine mammals, and humans, and in many cases, their concentrations are increasing over time[4]. In spite of their widespread occurrence in the environment, only limited information is available on the toxicology of PBDEs[5]. Recent studies showed that PBDE exposure caused aberrations in spontaneous behavior and reduced learning and memory in mice[6,7]; these effects are similar to those seen after exposure to DDT or PCBs[8]. However, the mode of action for this group of chemicals remains unclear. The underlying molecular mechanisms of the adverse health effects of PCBs have been associated with perturbations in intracellular signaling mechanisms including Ca^{2+} homeostasis and translocation of protein kinase C (PKC)[9]. These two intracellular signaling events are critical for the normal function of the nervous system and development[10,11]. In support of this hypothesis, our studies indicated that commercial PBDE mixtures such as DE-71 affected intracellular signaling events in a similar way to those of PCBs[12,13]. The objective of the present study is to evaluate: (a) the effects of environmentally relevant PBDE congeners on PKC translocation; (b) the accumulation of PBDE congeners by rat cerebellar neurons; and (c) to correlate PBDE accumulation with their effects on PKC translocation in neurons.

Materials and Methods

Chemicals. Radiolabeled [^{3}H]phorbol 12,13-dibutyrate (20 Ci/mmol) was purchased from Dupont NEN Corporation (Boston, MA). Radiolabeled [^{14}C]-PBDE congeners (96-99% pure) were custom synthesized by NEN Life Sciences Products (Boston, MA). PBDE congener, 2,2',4,4'-tetrabromodiphenyl ether [PBDE 47]) was a gift from Great Lakes Chemical Corporation (West Lafayette, IN). PBDEs 99 (2,2',4,4',5-pentabromodiphenyl ether) and 153 (2,2',4,4',5,5'-hexabromodiphenyl ether) were purchased from AccuStandard, Inc (New Haven, CT). All PBDEs were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the assay buffer (0.2 % v/v) did not significantly affect ^{3}H-phorbol ester binding.

Cerebellar granule cell culture. Primary cultures of rat cerebellar granule neurons (CGCs) were prepared from 6-8 day old Long Evans rat pups as outlined by Gallo *et al*.[14] with modifications[15]. Cultures were grown in DMEM with 10% FBS and 30 mM KCl in 12-well plates (Corning Costar), with a plating density of 1.0×10^6 cells/ml. Cytosine arabinoside was added 48 hours after plating to prevent the proliferation of non-neuronal cells. Cultures were assayed at 7 days *in vitro* when they were fully developed.

PKC translocation in cerebellar neurons. The effects of PBDE congeners (0 to 30 μM) were studied on the translocation of PKC in cerebellar granule neurons. PKC translocation from cytosol to the membrane is one of the key events in intracellular signaling, and was determined by measuring [^{3}H]-phorbol ester (PDBu) binding according to the method of Vaccarino et al.[16].

^{14}C-PBDE accumulation: Cerebellar granule neurons at 7 days in culture were incubated with 0.05 μCi of ^{14}C-PBDE congeners (0.67 μM) along with different concentrations of cold PBDEs (0 to 30 μM) for 15 min. After incubation,

cells were washed twice with cold Locke's buffer and then dissolved in 1 ml NaOH. The ^{14}C-accumulation was represented as nanomoles of PBDE accumulation in 15 min.

Statistics. The data (n = 4-8 experiments, assayed in triplicates) were analyzed by a two-way analysis of variance (ANOVA) with chemical as one factor and concentration as the other (SigmaStat, version 3.0, SPSS Inc., Chicago, IL). In the case of significant interaction, step-down ANOVAs were used to test for main effects of PBDEs. Pair-wise comparisons between groups were made using Fisher's LSD test. The accepted level of significance was $p < 0.05$.

Results and Discussion

^{14}C-PBDE accumulation by cerebellar granule neurons:

Cerebellar granule neurons accumulated all three PBDE congeners (PBDEs 47, 99, and 153). The accumulation of PBDEs increased with time of exposure (data not shown). At the lowest concentration, about 13-18% of the total dose was accumulated by these neurons. There were distinct differences in the pattern of accumulation between PBDE congeners. The accumulation of PBDE 47 was linear with dose, but that was not the case with PBDEs 99 and 153 (Figure 1A).

PBDE congener effects on [^{3}H]-phorbol ester binding:

PBDE congeners 47, 99, and 153 increased [^{3}H]PDBu binding and a significant effect was seen at 10 μM (Figure 1B). Among the three PBDE congeners, PBDE 47 increased [^{3}H]PDBu binding to a greater extent and in a concentration-dependent manner. The effect seen with PBDE 47 was much greater than that of DE-71, which is a pentabrominated diphenyl ether mixture[13].

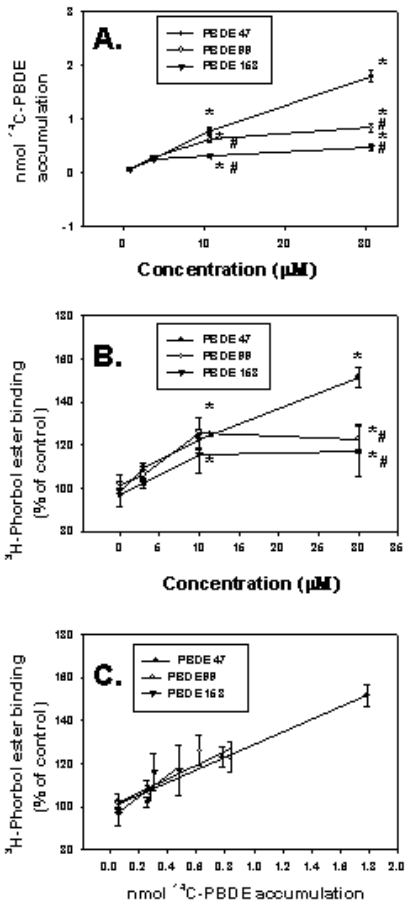

Figure 1. Accumulation of ^{14}C-PBDE congeners (Figure 1A) and their effects on PKC translocation (Figure 1B) in cerebellar granule neurons. The correlations between the PBDE accumulation and their effects on PKC translocation are shown in Figure 1C. Values are mean ± SE of 4-8 experiments, assayed in triplicates. *Significantly different from control. $^{\#}$Significantly different from PBDE 47;

Correlations between accumulation of PBDEs and their effects on PKC translocation in cerebellar granule neurons:

The pattern of PBDE accumulation correlates well with PKC translocation. Of the three PBDEs tested, PKC translocation was stimulated to the greatest extent with PBDE 47 and this congener was also most readily

accumulated by the cerebellar granule neurons. When PKC translocation is plotted against nmol accumulation, a strong correlation (r^2 = 0.991) was found for PBDE 47 (Figure 1C). The effects of PBDE 99 and 153 were different when compared to PBDE 47. The dose-response curves for both the stimulation of PKC translocation and accumulation for both PBDEs 99 and 153 reached plateau at 10 μM. Although stimulation of PKC translocation reached a maximum at 10 μM, while the accumulation of these congeners continued to rise at a much slower rate between 10 and 30 μM. These results suggest that PKC translocation is a critical neuronal effect for some of the PBDE congeners, as observed for PCBs.

We have demonstrated previously that PCBs, which are known to cause developmental neurotoxicity, perturbed intracellular signaling processes[9], critical for nervous system development and associated with learning and memory processes. In agreement with the effects seen with commercial PBDE mixtures, PCBs, and other organohalogens, the environmentally relevant PBDE congeners in this study also altered PKC translocation. In addition, PBDE mixtures showed similar potency and efficacy on a molar basis compared to those of PCB mixtures[13]. PBDEs are as ubiquitous and persistent as PCBs in human blood and breast milk samples[17], and the levels of PBDEs are rapidly rising in North Americans[18]. Considering the structural similarity of PBDEs with PCBs and the known health effects of PCBs, these two groups of chemicals could conceivably work through the same mode of action, to cause developmental neurotoxicity.

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