Polybrominated Diphenyl Ether (PBDE) Effects in Rat Neuronal Cultures: 14C-PBDE Accumulation, Biological Effect, and Structure-Activity relationships

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame-retardants in many types of consumer products such as electrical equipment, plastics, and building materials. 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, they are increasing over time[1]. In spite of their widespread occurrence in the environment, only limited information is available on the toxicology of PBDEs[2]. Recent studies showed that PBDE exposure caused aberrations in spontaneous behavior and reduced learning and memory in mice[3,4]; these effects are similar to those seen after exposure to DDT or PCBs[5]. However, the mode of action for this group of chemicals remains unclear.

Previously, we demonstrated that PCBs, which are known to cause neurotoxic effects, affected intracellular signaling pathways including [^{3}H]arachidonic acid ([^{3}H]AA) release, calcium homeostasis, and translocation of protein kinase C (PKC)[6]. Regarding PBDEs, we have reported that PBDEs altered [^{3}H]AA release in neuronal cultures like PCBs[7]. These signaling pathways have been associated with learning and memory, and the development of the nervous system[8]. The objectives of the present study were to test: (a) whether biologically relevant

PBDE congeners affected PKC translocation in neuronal cultures in a similar way to those of other organohalogens; (b) compare the potency and efficacy of PBDE congeners with their ^{14}C-accumulation; and (c) understand the structure-activity relationships among PBDE congeners.

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). PBDE 77 (3,3',4,4'-tetrabromodiphenyl ether) was commercially available from the Biochemical Institute for Environmental Carcinogens, Lurup 4, D-22927 Grosshansdorf, Germany. PBDEs 99 (2,2',4,4',5-pentabromodiphenyl ether), 100 (2,2',4,4'6- 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*.[9] with modifications[10]. 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 x 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. The effects of PBDE congeners (0 to 50 μM) were studied on the translocation of PKC in cerebellar granule neurons. PKC translocation is one of the key events in intracellular signaling. The [^{3}H]-phorbol ester (PDBu) binding, which indicates PKC translocation from cytosol to the membrane, was determined according to the method of Vaccarino et al.[11].

^{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 to 1 hr. After incubation,

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

Statistics. The data (n = 5-8 experiments, assayed in triplicates) were analyzed by a two-way analysis of variance (ANOVA) with chemical as one factor and concentration as the other using SigmaStat software, 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

PBDE congener effects on [^{3}H]-phorbol ester binding:
All the tested PBDE congeners increased [^{3}H]PDBu binding and a significant effect was seen as low as 10 μM (Figure 1). Among the congeners tested, 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[12]. In agreement with previous studies, non-coplanarity of PBDE seems to play a major role in the biological effect. PBDE 77, which is a non-ortho-substituted non-coplanar congener is active while PCB 77, which is a non-ortho-substituted coplanar congener, was inactive[13].

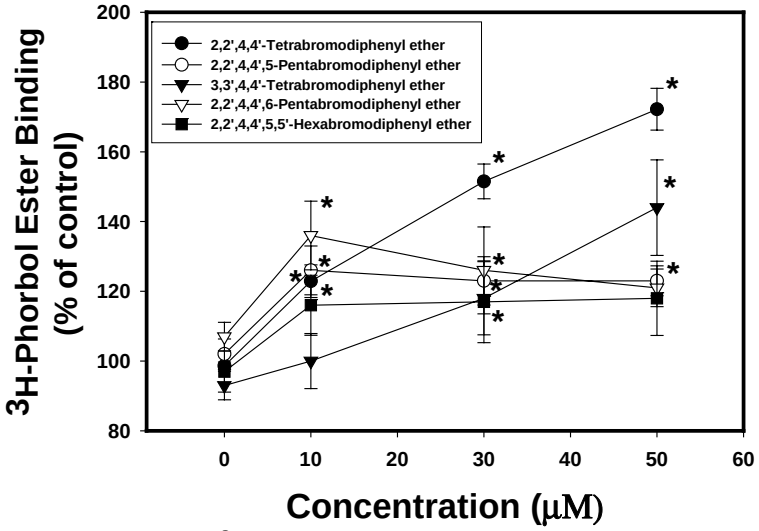

Figure 1. Increased ^{3}H-phorbol ester binding following exposure to PBDE congeners in cerebellar granule neurons. Values are mean ± SE of 6-8 experiments, assayed in triplicates. *Significantly different from control.

<u>^{14}C-PBDE accumulation by cerebellar granule neurons:</u>

Cerebellar granule neurons accumulated all three PBDE congeners tested (PBDEs 47, 99, and 153). The accumulation of PBDEs either as percentage or nmoles, increased with time of exposure (Figure 2). 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 percentage of accumulation was much lower for the 30 μM PBDE99 and 10-30 μM PBDE153 than at the lower concentrations, which may be due to solubility of these congeners. The accumulation pattern with PBDE47 did not vary with concentration. On a nanomole accumulation basis, PBDE 47, 99, and 153 accumulation is linear with time, however, they are not linear with concentration (Figure 2). The pattern of PBDE accumulation seems to correlate with the biological effect, which is PKC translocation.

We demonstrated previously that PCBs, which are known to cause developmental neurotoxicity, perturbed intracellular signaling processes[6,10] 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 selected biologically relevant PBDE congeners in this study

also altered PKC translocation. Some of the PBDE congeners showed similar potency and efficacy on a molar basis compared to those of PCB congeners[12]. PBDEs are as ubiquitous and persistent as PCBs in human blood and breast milk samples[14], and the levels of PBDEs are rapidly rising in North Americans[15]. 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 mechanism(s), to cause developmental neurotoxicity.

Acknowledgments

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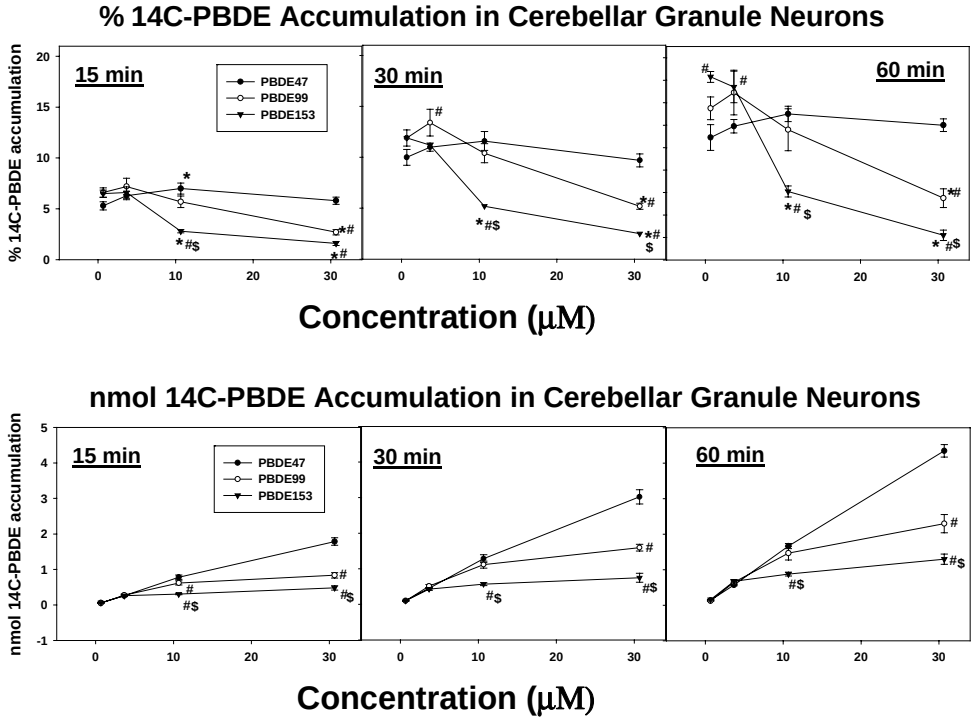

Figure 2. Accumulation (represented as % and nmol) of ^{14}C-PBDE congeners in cerebellar granule neurons. Values are mean ± SE of 4 experiments, assayed in triplicates. *Significantly different from 0.67 μM. $^{\#}$Significantly different from PBDE 47; $^{\$}$Significantly different from PBDE 99;

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