Polybrominated diphenyl ethers (PBDEs) as Ah-receptor agonists and antagonists

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Introduction

Polybrominated diphenyl ethers (PBDEs) are presently being used in large quantities as additive flame retardants in electronic equipment, plastics and building materials[1]. The chemical structure and properties of PBDEs are similar to the persistent polychlorinated biphenyls and dioxins. PBDEs have already been detected in human adipose tissue[2] and plasma[3], although at considerably lower levels than e.g. PCBs. Little is known about the toxicity of PBDEs. Studies have been performed almost exclusively on commercial PBDEs, which can be considered as more or less unspecified mixtures. A commercial PBDE-mixture (Bromkal 70-5DE, containing about equal amounts of tetra- and penta-congeners)[1] is shown to induce cytochrome P4501A1 and 1A2 both *in vitro*[4] and *in vivo*[5] , but it is not known which of the congeners is responsible for the observed effects. In this study we report on the potency of some pure PBDE-congeners (di-brominated to hepta-brominated) as Ah-receptor (ant)agonists.

Material and Methods

Pure PBDE-congeners were synthesized as described before[6,7]. PBDEs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were dissolved in dimethylsulfoxide (DMSO 99.9%; Janssen Chimica, Geel, Belgium). The Ah-receptor agonist and antagonist activities of the PBDEs were determined in a recombinant H4IIE rat hepatoma cell line showing Ah receptor mediated expression of a luciferase reporter gene[8]. This assay is known as the CALUX-assay and is described elsewhere[9]. Cells were exposed to single PBDE-congeners (concentrations varied from 0.01 to 25 μM) or combinations of a BDE with 15 pM TCDD during 24 hours, with a maximum DMSO concentration of 0.5%.

Results and Discussion

A series of 17 polybrominated diphenyl ethers were tested in the CALUX-assay. The results

in table I summarize the EC_{50}-values and maximum luciferase induction of the PBDEs alone and the IC_{50}-values for the inhibitory response observed in cells co-treated with 15 pM TCDD plus different concentrations of PBDEs.

Table I. EC_{50}-values, maximum luciferase induction and inhibition of TCDD-induced luciferase production by PBDEs in H4IIE CALUX cells[#].

BDE	Bromine substitution	EC_{50} (μM)	Luciferase induction by 25 μM BDE	Inhibition of TCDD induced luciferase (IC_{50}, μM)
15	4,4'	--	7.5 ± 0.6	8.8
28	2,4,4'	--	8.4 ± 0.3	n.r.
30	2,4,6	--	9.6 ± 0.5	--
32	2,4',6	n.r.	20.7 ± 0.6	--
47	2,2',4,4'	--	3.3 ± 0.2	3.6
51	2,2', 4,6'	--	10.1 ± 0.4	--
71	2,3',4',6	--	6.3 ± 0.7	--
75	2,4,4',6	--	13.2 ± 0.2	--
77	3,3',4,4'	--	12.8 ± 1.9	1.7
85	**2,2',3,4,4'**	**n.r.**	**28.3 ± 1.5**	**n.d.**
99	2,2',4,4',5	n.r.	24.1 ± 1.0	n.r.
100	2,2',4,4',6	--	8.0 ± 0.9	n.r.
119	2,3',4,4',6	n.r.	26.9 ± 0.6	n.r.
138	2,2',3,4,4',5'	--	9.7 ± 0.2	0.3
153	2,2',4,4',5,5'	n.r.	34.2 ± 1.6	--
166	2,3,4,4',5,6	1.4	65.8 ± 5.0	Induction
190	2,3,3',4,4',5,6	0.8	50.3 ± 4.0	--

[#] Results are expressed as mean ± standard deviation (n = 3). The EC_{50}-and IC_{50}-values were determined by fitting (1-site ligand, function $y = a_0*x/(a_1+x)$) the curves of the percent of maximal induced responses observed in cells cotreated with 15 pM TCDD plus different concentrations of the brominated diphenyl ethers. Maximal luciferase induction (100%) was obtained with 100 pM TCDD. N.r. = not reached, -- = no induction or inhibition at all. N.d. = not determined.

At PBDE-concentrations from 0.01 to 25 μM, induction of luciferase activity varied from 0 to 65.8% of the response observed for 100 pM TCDD (table I, figure 1). No cytotoxicity was noticed by microscopic observation. Seven of the 17 tested PBDEs induced luciferase expression, indicating that they are able to activate the Ah-receptor. EC_{50}-values could only be determined for BDE-166 (figure 1A) and BDE-190 and are in the same range as of the mono-ortho substituted polychlorinated biphenyls PCB-105 and PCB-118 (EC_{50}-values < 4 μM in H4IIE.luc cells)[10]. In cells co-treated with 15 pM TCDD plus different PBDE-concentrations, there was a concentration dependent decrease in TCDD-induced luciferase expression by 9 of the tested PBDEs as illustrated in figure 1B for BDE-138.

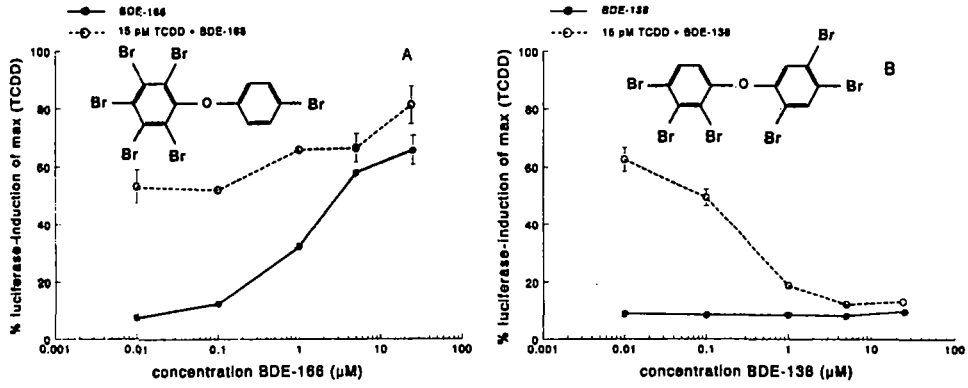
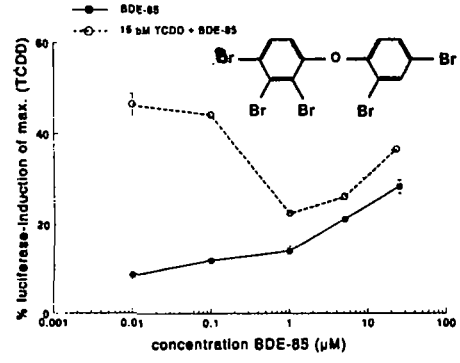


Figure 1. Response of BDE-166 (A) and BDE-138 (B) alone (straight line) or in combination with 15 pM TCDD (dotted line) in H4IIE.luc cells. Results are given as % luciferase induction compared to the maximum induction by TCDD as observed at 100 pM. Data present mean ± standard deviation (n = 3). If no error bar is visible, it is smaller than the marker.



BDE-85 was found to exhibit partial Ah-receptor agonist and antagonist activities. Concentrations from 0.01 to 1 μM of BDE-85 inhibit luciferase expression induced by 15 pM TCDD (= EC_{50}) to 22%. On the other hand, concentrations from 1 to 25 μM induce luciferase expression to a maximum of 37% (fig.2). Partial agonist and antagonist activities were also found for BDE-99 and BDE-119, but less pronounced.

Figure 2. Response of BDE-85 in H4IIE.luc cells.

These results show that some pure PBDE-congeners are able to act via the Ah-receptor signal transduction pathway as agonists, but mainly as antagonists. The potency of the agonists is comparable to the potencies of some mono-ortho PCBs[10]. The antagonist activities of BDE-47, BDE-77 and BDE-138 are similar as was observed for PCB-52 in recombinant Hepa1c1c7 cells[8]. Further studies will be performed to determine the Ah-receptor binding activities of the pure PBDE-congeners.

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