NEUROTOXICOLOGY OF PCBs: A NOVEL STRUCTURE/ACTIVITY RELATIONSHIP

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ABSTRACT

We used *in-vitro* procedures to determine the relationship between congener structure and neurotoxicity and found that ortho-substituted, but not planar dioxin-like congeners reduced cellular dopamine. When non-human primates were exposed to Aroclor 1016 we noted decreased dopamine levels and detected only three congeners (2,4,4', 2,4,2',4' and 2,5,2',5) in brain.

INTRODUCTION

Although PCBs have been long recognized as putative carcinogens (1) and hepato- and immunotoxicants (2,3), evidence has only slowly accumulated suggesting that PCBs may also be significant environmental neurotoxicants. Both epidemiological (4,5) and laboratory-derived data (6) indicate that perinatal exposure to PCBs is associated with decreased cognitive or intellectual function--behaviors controlled, in part, by brain dopamine (DA) (7).

In this manuscript we describe *in-vitro* and *in-vivo* experiments that provide important insights to the mechanisms by which PCBs alter central nervous system function (CNS). In the *in-vitro* experiments we exposed pheochromocytoma (PC12) cells to individual congeners. These cells are a continuous cell line derived from a rat adrenal gland tumor that synthesize, store, release and metabolize DA in a manner similar to that observed in the mammalian CNS (8). In the *in-vivo* experiments we determine the effects of sub-chronic exposure of *Macaca nemestrina* to Aroclor 1016 on CNS DA and measure the accumulation of individual congeners in brain.

EXPERIMENTAL

***In-Vitro* Experiments.** PC12 cells were maintained in 100 mm plastic culture dishes (Costar, Cambridge, MA) at 37°C in a 95%O_2/5%CO_2 humidified incubator and fed a complete growth medium consisting of RPMI 1640 medium supplemented with 5% fetal bovine serum and 10% selected horse serum. For all experiments cells, at a density of 10^5 cells/well, were transferred to 24-well culture trays and allowed to grow for 6-7 days before use. Growth medium was changed every two days. PC12 cells were exposed to individual congeners dissolved in DMSO and diluted in growth medium. The final DMSO concentration depended on which congener was being tested and varied between 0.1 and 0.5% (v/v). PC12 cells were exposed to either control vehicle or the PCB for 6 hr and the results for each congener are expressed as a percent of the appropriate concentration DMSO value.

***In-Vivo* Experiments.** Non-human primates, *Macaca nemestrina*, (pig-tailed macaque) were orally-exposed to Aroclor 1016 at dose levels of 0.8, 1.6 or 3.2 mg (kg/day). At the end of twenty weeks the animals were sacrificed. Regional brain concentrations of DA and other neurotransmitters were determined by high-performance liquid chromatography with electrochemical detection (9) and individual PCB congeners were determined by gas-chromatography with electron capture detection (10).

RESULTS AND DISCUSSION

***In-Vitro* Experiments.** Exposure of PC12 cells to individual congeners has allowed us to demonstrate a novel relationship between the structure of a congener and its ability to alter cellular DA concentrations. Results are presented in Table 1 where congeners are grouped according to the IC_{50} values for decreases in cellular DA content.

Table 1. IC_{50} values for selected PCB congeners determined *in vitro*.

Range					
<100 μM		251-500 μM		>1000 μM	
Congener	IC_{50}	Congener	IC_{50}	Congener	IC_{50}
2---,2----	64	--4--,-----	335	--4--,--4--	>1000
2-4-6,2----	71	23456,2-4--	370	2---6,2---6	>1000
2-4-6,-3---	90	-3---,-----	300	-34--,-34--	>1000
2-5-,2----	83	-3-5-,--4--	310	-34--,-345-	>1000
		-3---,--4--	410		

These studies have allowed us to reach the following conclusions. (1) Ortho-substituted congeners which do not assume a planar configuration are the most potent congeners tested to date with 2,2' and 2,4,6,2' having IC_{50} of 64 and 71 μM respectively. Archetypical dioxin-like congeners such as 3,4,3',4' and 3,4,5,3',4' are without effect. (2) Chlorination in the para position of at least one ring increased congener potency. (3) Chlorination in a meta position decreased activity. (4) Increasing congener chlorination did not correlate with decreased potency, though saturation of a ring appeared to reduce potency.

One possible reason for the relative potencies of the different PCB congeners is their ability to accumulate in cells. Several congeners with different IC_{50} values were used to describe dose-dependent changes in both cellular DA and PCB content. There was no correlation between potency and accumulation

indicating that PCB partitioning into cells is not directly related to potency.

***In-Vivo* Experiments.** All PCB-exposed animals gained weight at the same rate as control animals and did not show any observable signs of chlorobiphenyl poisoning. Exposure to Aroclor 1016 for twenty weeks significantly decreased brain concentrations of DA in the caudate, putamen, substantia nigra and hypothalamus (Table 2). These results were confirmed by linear regression analysis. Concentrations of DA in other brain regions and concentrations of norepinephrine and serotonin were unaffected.

Table 2. Linear regression analysis of brain dopamine concentrations following exposure of the non-human primate, *Macaca nemestrina*, to Aroclor 1016.

Brain Region	Intercept	Slope	r	*p*
Caudate Nucleus	19.1	-1.1	0.57	0.05
Putamen	20.2	-1.1	0.65	0.02
Substantia Nigra	3.52	-0.76	0.74	0.01
Hypothalamus	0.80	-0.16	0.79	0.002

Gas chromatographic analysis of brain tissue demonstrated that only three congeners (2,4,4'; 2,4,2',4' and 2,5,2',5') accumulated to detectable level (0.5 ppb) and made up more than 95% of the total PCB residue in brain. These latter results suggest that these congeners may be responsible for the observed decrease in brain DA concentrations.

In order to directly determine whether the above congeners were responsible for the *in-vivo* decreases in DA we exposed PC12 cells to the three congeners, either separately or as admixtures that reflected the ratios of the congeners as found in brain. Both the individual congeners and mixtures significantly decreased PC12 cellular DA concentrations (Figure 1) with the mixtures more effective than the individual congeners.

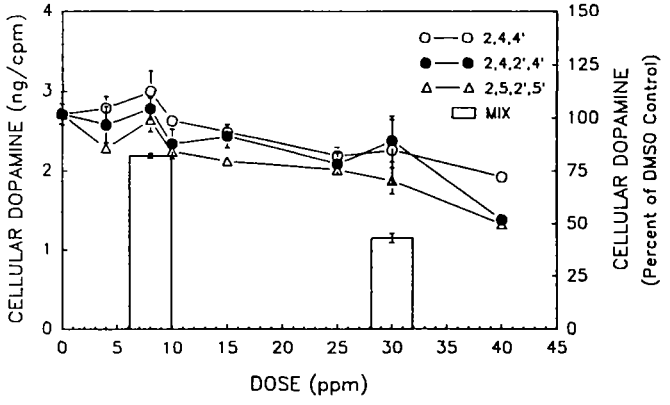

Figure 1. Dose-dependent changes in dopamine concentrations in pheochromocytoma (PC12) cells.

These results, using highly disparate test systems, indicate that PCB neurotoxicity involves the interaction of ortho-substituted non-planar congeners with specific sites that regulate dopaminergic function in both *in-vivo* and *in-vitro* preparations. As such, these findings bring insights to the neuronal mechanisms responsible for decreases in cognitive functioning in perinatally-exposed humans.

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