Neurochemical Effects of PCBs are Structure and Recipient Age-Dependent

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1. Introduction

Until recently, only *meta*-, *para*-substituted, dioxin-like (*i.e.*, coplanar) polychlorinated biphenyl (PCB) congeners were thought to be toxic. The reasons for this assumption were based on the fact that dioxins, furans and coplanar PCB congeners interact with the aryl hydrocarbon (Ah) receptor to induce cytochrome P-4501A activities and hepato- and immunotoxicity [1,2] while *ortho*-substituted congeners do not interact with the Ah receptor. However, using pheochromocytoma (PC12) cells in culture Seegal [3] and Shain *et al.* [4] have demonstrated a novel structure-activity relationship (SAR) in which *ortho*-substituted, non-coplanar PCB congeners significantly reduce cellular content of dopamine (DA), an important neurotransmitter involved in the control of learning and memory [5-7] and motor function [8-10]. The reductions in cellular DA content are similar to the reductions in brain DA concentrations observed following exposure of the both adult non-human primate and rodent to Aroclor mixtures [11-13] and individual PCB congeners [14]. In order to determine if: (i) the SAR observed using *in-vitro* procedures is also seen *in-vivo*; and (ii) the developing rat responds to PCBs in a neurochemically similar manner to the adult, we have examined the neurochemical effects of developmental exposure of the rat to individual *ortho*-substituted and coplanar PCB congeners. The objectives of this study are to compare and contrast the neurochemical effects of *in-vitro* and *in-vivo* developmental exposure to coplanar and *ortho*-substituted PCB congeners.

2. Methods

a. *In-vitro* studies

Experimental procedures are described in detail in Seegal *et al.* [3]. Briefly, PC12 cells were grown in RPMI growth media supplemented with 10% horse serum and 5% fetal calf serum at 37°C in 95% O_2/5% CO_2; seeded in 24 well culture plates and exposed to individual congeners in growth media containing 0.1-0.3% dimethylsulfoxide (DMSO) for 1-6 h. Control cells were exposed to the same concentration of DMSO. Cells were harvested and cell and media concentrations of DA and its metabolites (3,4-dihydroxyphenylacetic acid [DOPAC] and 3-methoxy-4-hydroxyphenylacetic acid [HVA]) were determined by high-performance liquid-chromatography with electrochemical detection (HPLC) [15].

b. *In-vivo* studies

Pregnant rat dams were exposed from gestational day (GD) six through weaning to either the coplanar PCB congener #77 (3,4,3',4') at doses of 0.1 or 1.0 mg/(kg·day), or congener #47 (2,4,2',4') at doses of 1.0, 10 or 20 mg/(kg·day). Male and female offspring

were sacrificed on postnatal days (PND) 35, 60 or 90 and concentrations of DA and its metabolites were measured in the frontal cortex and substantia nigra by HPLC.

3. Results

a. *In-vitro* studies

We exposed PC12 cells to more than fifty individual PCB congeners [4] and have classified them in terms of their ability to reduce cellular DA content, based on their EC_{50} values--that is the dose, expressed on a molar basis, required to reduce cellular DA content by 50%. In some cases, it was not possible to directly determine an EC_{50} and results are based on extrapolating a predicted EC_{50} from the existing dose response data (*i.e.*, those cases in which EC_{50} values were greater than 200 μM). Representative congeners from each of the major subdivisions are presented below in Table I.

TABLE I. Representative EC_{50} Values for PCB Congener-Mediated Decreases in Cellular Dopamine Content Determined *In vitro* using Pheochromocytoma Cells (PC-12 Cells) in Culture

Di- and Tri-*Ortho* Congeners	EC_{50}	Mono-*Ortho* Congeners	EC_{50}	Mono-*Para* Congeners	EC_{50}	Di-*Para* Congeners	EC_{50}
2,2'	64	2	182	3,5,4'	310	4,4'	n.e.
2,4,6,2'	71	2,4,4'	196	4	335	3,4,3',4'	n.e.
2,5,2',5'	86			3,4'	410	3,4,3',4',5'	n.e.
2,5,2'	88						
2,4,2',4'	115						
2,4,6	150						
Mean	96	Mean	189	Mean	352		

n.e.=no effect observed

b. *In-vivo* Results

For the sake of clarity, we present the neurochemical results combined across both gender and age of the animal at sacrifice. Perinatal exposure of rats to the di-*ortho*-substituted congener 2,4,2',4' resulted in significant reductions in DA concentrations in the frontal cortex and substantia nigra--results similar to those observed in the adult. In contrast, perinatal exposure to the coplanar congener, 3,4,3',4', resulted in small, but significant, elevations in DA concentrations in the frontal cortex and substantia nigra. Although the data are not presented here these alterations in brain DA concentrations were evident in animals sacrificed on PND90. These data are presented in presented in Figures 1A and B.

Brain PCB residues (data not shown) for 3,4,3',4' were undetectable (*i.e.*, <0.05 ng/g brain) demonstrating that the persistent elevations in DA concentrations were due to PCB-induced alterations in brain biochemistry and development during gestation and lactation and were not due to the continued presence of the congener in the brain at the time the neurochemical measurements were collected.

Figure 1A

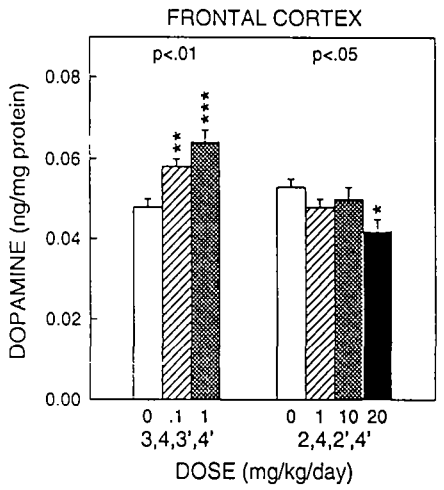

Figure 1B

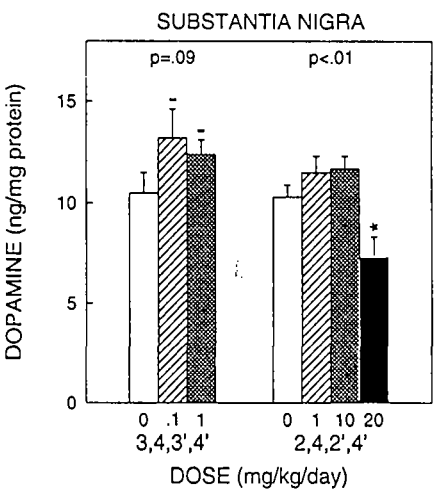

Figure 1. Results of perinatal exposure of rats to 3,4,3',4' and 2,4,2',4' at the above doses on dopamine in the frontal cortex **(A)** and substantia nigra **(B)** analyzed using one-way analysis of variance. Bonferroni-corrected t-tests comparing each exposure group with the respective control group (-$p \leq 0.1$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; N=30-78 animals per group).

4. Discussion

Data obtained using the *in-vitro* PC12 system has allowed us to determine the relationship between the structure of individual PCB congeners and reductions in cellular DA content [4]. These results clearly demonstrate that *ortho*-substituted, non-coplanar congeners reduce cellular DA content and that the most active congener is 2,2'. The position of the chlorines on the biphenyl moiety, rather than the number of chlorines is responsible for the neurochemical activity. This conclusion is based on the following observations. First, other congeners with the same number of chlorines, including 3,3'(EC_{50}=195 μM) and 4,4' (no effect observed), are either considerably less active than 2,2' or are totally inactive. Secondly, the coplanar congeners 3,4,3',4' and 3,4,5,3',4' are totally inactive while congeners that have two or more *ortho*-substitutions with the same number of chlorines are active (*e.g.*, 2,4,2',4' [EC_{50}=114 μM]; 2,4,6,2',4' [EC_{50}=158 μM]). Thirdly, the total number of chlorines is a poor predictor of neurochemical activity since 2,3,4,6,2',3',4' has an EC_{50} of 134 μM while 2,3,4,5,6,2',4' has an EC_{50} greater than 200 μM. We suggest that the mechanism most likely responsible for the decreases in cellular DA content involves inhibition of synthesis of DA at the level of the rate limiting enzyme, tyrosine hydroxylase (TH) [16]. This statement is based on the following lines of evidence. First, the lack of alterations in media concentrations of DA or its metabolites suggest that PCBs inhibit DA synthesis [3]. Secondly, using N1E-115 cells that do not express the enzyme aromatic amino acid decarboxylase (AADC), we have shown that 2,2'-DCB significantly depresses media concentrations of L-DOPA reinforcing the

hypothesis that PCBs inhibit the synthesis of DA at the level of TH.

Additional studies carried out in adult rats exposed to Aroclor mixtures and NSD-1015, a compound that inhibits the conversion of the intermediate product L-DOPA to DA, confirms that the reductions in brain DA, induced by exposure to PCBs, are due to inhibition of DA synthesis at the level of TH. In the absence of conflicting data we suggest that the reductions in brain DA seen in the developing rat following exposure to 2,4,2',4' are also due to inhibition of newly synthesized DA at the level of TH.

The elevations in brain DA following perinatal exposure to 3,4,3',4' was unexpected in light of the PCB-induced decreases in DA concentrations seen in cells in culture and in the adult animal. The inability of coplanar congeners to alter neurochemical function in these two preparations indicates that these congeners are neuroteratogens, compounds that are inactive when administered to the adult but are active during development. We suggest that the developmental neurochemical activity of this class of congeners is due to their ability to alter key hormonal systems during development that indirectly influence the neurochemical development of the brain. This statement is based on: (i) the well established role of coplanar congeners to alter both steroidal and thyroid hormones during development [17-19] and (ii) the fact that alterations in these hormones have been shown to influence levels and activity of biogenic amines, including DA [20-22]. If the major effect of these congeners is to alter hormonal systems during development, the lack of activity of these congeners when administered to the adult can be explained by the fact that alterations in hormonal systems in the adult are limited to activation while alterations during development influence the neuronal organization of the brain [23-25] and hence would induce long-lasting changes in neurochemical function. We present below a table summarizing the effects of *ortho*-substituted and coplanar congeners on neurochemical and endocrine function. These data provide important insights to the potential mechanisms by which the two major classes of PCB congeners may influence both neurochemical and neurobehavioral function in both the mature and developing central nervous system.

PCB EXPOSURE: NON-COPLANAR VS. COPLANAR

CONGENER STRUCTURE	BRAIN DOPAMINE CONCENTRATIONS		ESTROGENIC ACTIVITY	THYROID FUNCTION
	Perinatal	Adult		
Non-coplanar	↓[a]	↓[c]	↑[e]	↓[g]
Coplanar	↑[a] ↓[b]	→[d]	↓[f]	↓[g]

[a]Seegal *et al.*, unpublished data.
[b]Agrawal *et al.*, Toxicol. Lett. 7:417-424 (1981).
[c]Seegal *et al.*, Toxicology 66:145-163 (1991).
Chishti and Seegal, Toxicologist 12:320 (1992).
[d]Tilson *et al.*, Environ. Res. 18:466-474 (1979).
Chishti and Seegal, Toxicologist 12:320 (1992).
[e]Korach *et al.*, Mol. Pharmacol. 33:120-126 (1988).
[f]Gierthy *et al.*, Biochem. Biophys. Res. Commun. 157:515-520 (1988).
Safe *et al.*, Pharmacol. Toxicol. 69:400-409 (1991).
[g]van den Berg *et al.*, Toxicol. Lett. 41:77-86 (1988).

In summary, *ortho*-substituted PCB congeners reduce cell and brain DA concentrations, most likely due to their ability to inhibit the formation of newly synthesized DA. Although the mechanisms by which coplanar congeners elevate brain DA concentrations have not been firmly established, the available data suggest that the effects of these congeners on neurochemical function are most likely due to their ability to alter key hormones during development.

5. References

1) Safe, S. (1988): Polychlorinated biphenyls - Human health effects, in *Hazards, Decontamination, and Replacement of PCB - A Comprehensive Guide*. J.P. Crine ed., pp. 51-69, New York and London, Plenum Press
2) Silkworth, J.B., L. Antrim, and L.S. Kaminsky (1984): Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus and liver microsomal enzyme induction in C57BL/6J and DBA/2 mice. *Toxicol. Appl. Pharmacol.* 75, 156-165
3) Seegal, R.F., K. Brosch, B. Bush, M. Ritz, and W. Shain (1989): Effects of Aroclor 1254 on dopamine and norepinephrine concentrations in pheochromocytoma (PC-12) cells. *Neurotoxicology* 10, 757-764
4) Shain, W., B. Bush, and R.F. Seegal (1991): Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. *Toxicol. Appl. Pharmacol.* 111, 33-42
5) Brozoski, T.J., R.M. Brown, H.E. Rosvold, and P.S. Goldman (1979): Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* 205, 929-931
6) Brandt, I. (1986): Metabolism-related tissue-binding of halogenated hydrocarbons. *Upsala J. Med. Sci.* 91, 289-294
7) Archer, T., W. Danysz, A. Fredriksson, G. Jonsson, J. Luthman, E. Sundström, and A. Teiling (1988): Neonatal 6-hydroxydopamine-induced dopamine depletions: Motor activity and performance in maze learning. *Pharmacol. Biochem. Behav.* 31, 357-364
8) Iversen, S.D. (1977): Brain Dopamine Systems and Behavior, in *Handbook of Psychopharmacology*. L.L. Iversen, S.D. Iversen, and S.H. Snyder eds., pp. 333-384, New York, Plenum Press
9) Sokolowski, J.D. and J.D. Salamone (1994): Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. *Brain Res.* 642, 20-28
10) Cooper, J.A., H.J. Sagar, S.M. Doherty, N. Jordan, P. Tidswell, and E.V. Sullivan (1992): Different effects of dopaminergic and anticholinergic therapies on cognitive and motor function in Parkinson's disease. *Brain* 115, 1701-1725
11) Seegal, R.F., B. Bush, and W. Shain (1990): Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol. Appl. Pharmacol.* 106, 136-144
12) Seegal, R.F., B. Bush, and K.O. Brosch (1991): Comparison of effects of Aroclors 1016 and 1260 on nonhuman primate catecholamine function. *Toxicology* 66, 145-163
13) Seegal, R.F., B. Bush, and K.O. Brosch (1991): Sub-chronic exposure of the adult rat to Aroclor 1254 yields regionally-specific changes in central dopaminergic function. *Neurotoxicology* 12, 55-66
14) Geremia, E., D. Baratta, S. Zafarana, R. Giordano, M.R. Pinizzotto, M.G. La Rosa, and A. Garozzo (1990): Anti-oxidant enzymatic systems in neuronal and glial cell-enriched fractions of rat brain during aging. *Neurochem. Res.* 15, 719-723
15) Seegal, R.F., K.O. Brosch, and B. Bush (1986): High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. *J. Chromatogr.* 377, 131-144

16) Seegal, R.F. and W. Shain (1992): Neurotoxicity of polychlorinated biphenyls: The role of ortho-substituted congeners in altering neurochemical function, in *The Vulnerable Brain and Environmental Risks. Volume 2. Toxins in Food.* R.L. Isaacson and K.F. Jensen eds., pp. 169-191, New York, Plenum Press
17) Gierthy, J.F., D.W. Lincoln, S.J. Kampcik, H.W. Dickerman, H.L. Bradlow, T. Niwa, and G.E. Swaneck (1988): Enhancement of 2- and 16α-estradiol hydroxylation MCF-7 human breast cancer cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biochem. Biophys. Res. Commun.* 157, 515-520
18) Safe, S., B. Astroff, M. Harris, T. Zacharewski, R. Dickerson, M. Romkes, and L. Biegel (1991): 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds as antiestrogens: Characterization and mechanism of action. *Pharmacol. Toxicol.* 69, 400-409
19) Morse, D.C., D. Groen, M. Veerman, C.J. van Amerongen, H.B. Koeter, A.E. Smits van Prooije, T.J. Visser, J.H. Koeman, and A. Brouwer (1993): Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. *Toxicol. Appl. Pharmacol.* 122, 27-33
20) Euvrard, C., C. Oberlander, and J.R. Boissier (1980): Antidopaminergic effect of estrogens at the striatal level. *J. Pharmacol. Exp. Ther.* 214, 179-185
21) Di Paolo, T., C. Rouillard, and P. Bedard (1985): 17β-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. *Eur. J. Pharmacol.* 117, 197-203
22) Van Hartesveldt, C. and J.N. Joyce (1986): Effects of estrogen on the basal ganglia. *Neurosci. Biobehav. Rev.* 10, 1-14
23) Puymirat, J. (1985): Effects of dysthyroidism on central catecholaminergic neurons. *Neurochem. Int.* 7, 969-977
24) Kaylor, W.M., Jr., C.H. Song, S.J. Copeland, F.P. Zuspan, and M.H. Kim (1984): The effect of estrogen on monoamine systems in the fetal rat brain. *J. Reprod. Med.* 29, 489-492
25) Slotkin, T.A. and R.J. Slepetis (1984): Obligatory role of thyroid hormones in development of peripheral sympathetic and central nervous system catecholaminergic neurons: Effects of propylthiouracil-induced hypothyroidism on tranmitter levels, turnover and release. *J. Pharmacol. Exp. Ther.* 230, 53-61