Interactive Effects of Environmentally Relevant PCBs and Dioxins on [^{3}H]Phorbol Ester Binding in Neuronal Cell Cultures

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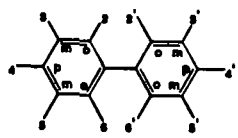
Abstract

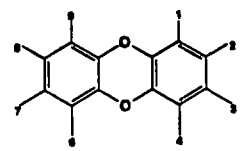
Present data suggest that the biological effects of some PCB congeners are dose-additive and coplanar congeners do not alter the activity of non-coplanar congeners indicating a common mechanism for all non-coplanar congeners that is distinct from that of coplanar congeners. Some interactions showed inhibition of activity of very active congener by the moderately active congener indicating that these congeners act at the same site. The "dose-additive" model, which implies a single site of action, seems to be more appropriate for predicting the results. Since samples from human and environmental mixtures of PCB congeners were biologically active, careful attention must be paid for the role of non-coplanar congeners in PCB risk assessment process.

Introduction

Studies from our laboratory indicated that *ortho*-substituted PCB congener, 2,2'-dichlorobiphenyl (-DCB; IUPAC # 4) altered intracellular Ca^{2+}-homeostasis by inhibiting the Ca^{2+}-buffering systems, increased phosphoinositide hydrolysis, and caused protein kinase C (PKC) translocation at concentrations where no cytotoxicity was observed, whereas the non-*ortho* substituted PCB congener, 3,3',4,4',5-pentachlorobiphenyl (-PeCB; IUPAC # 126), was not effective in inhibiting Ca^{2+}-buffering systems, did not alter Ca^{2+}-homeostasis to a great extent, and was not cytotoxic [1,2]. Studies on structure-activity relationships (SAR) indicate that that the activity of most PCB congeners was associated with chlorination patterns that favored non-coplanarity[3-5]. Further *in vivo* studies generally support these *in vitro* observations and suggest that intracellular signal transduction processes including Ca^{2+}-homeostasis, inositol phosphates, and PKC translocation play a crucial role in the effects of non-coplanar PCBs on nervous system[6].



Polychlorinated Biphenyls



Polychlorinated Dibenzo-*p*-dioxins

FIG. 1. Chemical structures of polychlorinated biphenyls and dioxins. The letters (o), (m) and (p) indicate *ortho*, *meta*, and *para* positions, respectively. The numbers indicate chlorine substitutions.

Human exposure to PCBs is mainly through an oral intake of contaminated food products such as dairy products, meat and fatty fish, which contain a mixture of non-coplanar and coplanar congeners[7,8]. Although few reports on the interactive effects of mixtures exist either on toxicokinetics, immunotoxic and teratogenicity end points or on fish early stage mortality[9-11], most of the laboratory research on these compounds has been carried out with the commercial mixtures or with single

congeners. Hence, the present study was undertaken to study the interactive effects of different PCB congeners and dioxins on PKC translocation measured as [^{3}H]phorbol ester binding ([^{3}H]PDBu) in neuronal preparations. In the neuron, PKC activation/translocation could be due to increased intracellular free Ca^{2+}, formation of diacylglycerol, and increased levels of free fatty acids such as arachidonic acid and lysophospholipids[12] and reported to be involved in the pathogenesis of neuronal injury[13]. The present study examined binary combinations of PCBs, hydroxy-PCB or dioxin to determine; 1) if the active congeners are "dose additive" indicating that they act through a common site or "effect additive" indicating that, although the endpoint ([^{3}H]PDBu binding) is common, the congeners act through independent sites[14]; 2) if the non-active congeners enter the active site and are ineffective at inducing [^{3}H]PDBu binding or if these congeners simply are unable to enter the active site; 3) if dioxin or hydroxy-PCB alters the response of PCB congeners. In addition, mixtures were generated to match the ratio of congeners found in environmental and biological samples and studied their effects on [^{3}H]PDBu binding in neuronal cultures.

Experimental Methods

Different PCB congeners, hydroxy-PCB, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (purity >99%) were purchased either from Ultra Scientific (North Kingstown, RI) or from Accu-Standard (New Haven, Conn.). Stock solutions of PCB congeners were prepared by dissolving them in dimethyl sulfoxide (DMSO). A 2 µl aliquot of stock solution (different concentrations) was added to the buffer to yield the desired final concentrations. DMSO (2 µl/ml) had no significant effect on [^{3}H]-PDBu binding. Granule cells from rat (postnatal day 6-9) cerebella were isolated by the enzymatic disruption[15] with modifications[1] and cultured on 12 well plastic trays (Costar), which were tested at 7 days in culture for [^{3}H]PDBu binding[16]. The data were analyzed by a two-way analysis of variance (ANOVA) followed by Dunnett's *post-hoc* test. The accepted level of significance was $p < 0.05$.

Results and Discussion

Interactive effects between coplanar and non-coplanar PCBs: The combined effects of an active non-coplanar (2,2',5,5'-tetrachlorobiphenyl [-TeCB]) and an inactive coplanar (3,3',4,4'-TeCB) PCB congener indicated no significant interaction (Table 1). 3,3',4,4'-TeCB (10-100 µM) had no significant effect on [^{3}H]-PDBu binding, nor did it affect the response of 30 µM 2,2',5,5'-TeCB. 2,2',5,5'-TeCB significantly increased [^{3}H]-PDBu binding from 10-100 µM, an effect that was not modified by the presence of 50 µM 3,3',4,4'-TeCB. A similar result was obtained with the combination of an active non-coplanar (2,2',4,6-TeCB) and an inactive non-coplanar (2,2',6,6'-TeCB) congener (Table 1). Table 1 also shows the lack of any combined effects of the inactive non-coplanar (2,2',6,6'-TeCB) congener with an inactive coplanar (3,3',4,4'-TeCB) congener. There is also lack of any interaction between two inactive coplanar congeners (3,3',4,4'-TeCB and 4,4'-DCB). The inactivity of 2,2',6,6'- and 3,3',4,4'-TeCBs could be either they do not enter the active site or they enter the active site but are not efficacious in eliciting the response. The non-active congener failed to block the effects of the active congeners, suggesting that these non-active congeners do not bind to the response-inducing site.

The combined effects of an active non-coplanar (2,2'-DCB) and a moderately active coplanar (3,5-DCB) congener indicated additivity between these two congeners. 2,2'-DCB (10-100 µM) increased [^{3}H]-PDBu binding. Fifty µM 3,5-DCB increased [^{3}H]-PDBu binding by 40% and did not interact significantly with 2,2'-DCB. 3,5-DCB also produced a concentration-dependent increase in [^{3}H]-PDBu binding. There was no significant interaction with the presence of 50 µM 2,2'-DCB. The results from two active non-coplanar (2,2',4,4'-TeCB and 2,2'-DCB) congeners also showed no interaction and the effects were additive. 2,2'-DCB increased [^{3}H]-PDBu binding. In the presence of 50 µM 2,2',4,4'-TeCB, ANOVA indicated no significant interaction ($p = 0.235$). 2,2',4,4'-TeCB also increased [^{3}H]-PDBu binding in a concentration-dependent manner and this response was not affected by the presence of 10 or 30 µM 2,2'-DCB. The effects between another non-coplanar congener (2,2',3,5',6-PeCB) and a moderately active congener (2,2',4,4',5-PeCB) indicated a very strong interaction ($F_{4,30} = 5.01$, $p < 0.0033$). *Post hoc* test shows that 2,2',3,5',6-PeCB increased [^{3}H]-PDBu

binding in a concentration-dependent manner with maximal effect over 250% of control at 100 μM and this effect was reduced significantly in the presence of 50 μM 2,2',4,4',5-PeCB indicating that the less active congener interacted at the same site but being less efficacious lowered the total activity.

Table 1. Results of the interactive effects of PCBs, hydroxy-PCB or dioxin on [^{3}H]-PDBu binding in cerebellar granule cells.

Binary combination	Selected chemicals	Type of interaction
Active non-coplanar and inactive coplanar	2,2',5,5'- and 3,3',4,4'-	No interaction
Active non-coplanar and inactive non-coplanar	2,2',4,6- and 2,2',6,6'-	No interaction
Active non-coplanar and active coplanar	2,2'- and 3,5-	Additivity
Two active non-coplanar	2,2'- and 2,2',4,4' 2,2',3,5',6- and 2,2',4,4',5-	Additivity Significant interaction
Two inactive coplanar	3,3',4,4'- and 4,4'	No interaction
Inactive non-coplanar and inactive coplanar	2,2',6,6'- and 3,3',4,4'-	No interaction
TCDD and non-coplanar	TCDD and 2,2',5,5'-	No interaction
TCDD and coplanar	TCDD and 3,3',4,4'-	No interaction
Non-coplanar PCB and non-coplanar hydroxy-PCB	2,4,4',5- and 2,2',5'-ol	Additivity

Interactive effects between PCBs (coplanar and non-coplanar) and TCDD: The results of the present study confirm and extend the hypothesis that the PCB congeners in neuronal system do not act through a dioxin-like mechanism. For instance, TCDD at concentrations up to 100 nM was ineffective in this system. In addition, TCDD (100 nM) did not interact with an active congener (2,2',5,5'-TeCB) or a non-active congener (3,3',4,4'-TeCB). These results indicate that PKC translocation in neuronal cultures is independent of the Ah receptor mode of action and any TEF approach would be inappropriate in comparing these effects. Therefore, application of the TEF approach for risk assessment of PCBs must be used with caution.

Interactive effects between a parent PCB and a hydroxy metabolite: There is growing concern about the contribution of metabolites to the biological effects of PCBs, since hydroxy-PCBs have been detected in mammlian tissue and blood samples[17]. 2,4,4',5-TeCB (10-100 μM) increased [^{3}H]-PDBu binding and this did not interact significantly with the hydroxy metabolite. 2,2',5'-Trichloro-4-biphenylol increased [^{3}H]-PDBu binding by approximately 20% at 50 μM. These data indicate that the increase in [^{3}H]PDBu binding induced by certain PCB congeners and their hydroxy metabolites results from interaction at a specific site and not a generalized non-specific interaction.

Interactive effects of environmental mixtures: Complex mixtures were generated to more nearly mimic human exposure. These mixtures are representative on the ratios of the main constituents of the PCB load (Table 2). Mixtures 1 & 2 were generated to represent the ratios of PCB congeners found in human milk[7, 18] and would be a measure of infant exposure. Mixture 3 represents the PCB congener ratio found in Wisconsin fish[19] which represents adult and infant exposures. The mixture 4 is the congener's mix from human blood representing maternal exposure[20]. Mixture 5 represents the ratio of PCB congeners detected in the brains of rats at postnatal day 21 which had been exposed to PCBs from conception to weaning via feeding dams with adulterated chow containing commercial mixture, Aroclor 1254 [21].

TABLE 2

The ratios of selected PCB congeners in each of the environmental mixtures. The selected PCB congeners represent 46-99% composition of these mixtures.

Mixture # / Source	IUPAC # of PCB congeners															
	28	52	74	82	85	95	99	101	105	110	118	128	138	153	179	180
Mixture 1 (Human milk)[a]													35%	43%		21%
Mixture 2 (Human milk)[b]	8%		11%								7%		10%	12%		
Mixture 3 (Wisconsin fish)[c]	8%					7%		6%		7%			8%	11%		
Mixture 4 (Human blood)[d]	4%	3%		4%								5%	17%	9%		4%
Mixture 5 (Rat brain)[e]					12%		10%		13%						14%	

Source:
[a]Schecter *et al.* [18]
[b]Safe *et al.* [7]
[c]Maack and Sonzogni [19]
[d]Bush et al. [20]
[e]Shain *et al.* [21]

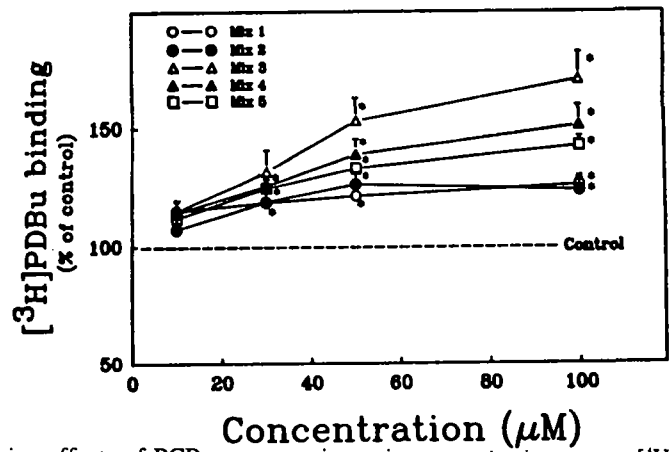


FIG. 2. Interactive effects of PCB congeners in environmental mixtures on [^{3}H]PDBu binding in cerebellar granule cells. The binding was represented as percentage of control (361 ± 12 fmol/mg protein/15 min; N = 29). Values are mean ± SEM of 5-6 preparations, assayed in triplicate.

All the mixtures increased [^{3}H]PDBu binding in a concentration-dependent manner (Fig. 2). *Post-hoc* analysis indicated that mixtures 1, 4, and 5 were significantly different from control starting at 30 μM while mixtures 2 and 3 were significantly different from control starting at 50 μM (Fig. 2). Of all the selected mixtures, mix 3 was the most active. In order to understand the interactions between PCB congeners in these mixtures, the data was fit into a previously established "logistic second order response surface model" which is based on dose-addition assumption[22]. The observed and predicted E50 values (concentration that increases the control activity by 50%) were presented in table 3. The observed E50 values for the mixtures are very close to the predicted E50 values from the model that is based on dose-addition assumption suggesting that PCB congeners in the selected mixtures exhibit dose-additivity.

Table 3. The observed and predicted E50 values for the interactive effects of selected environmental mixtures on [^{3}H]PDBu binding in rat cerebellar granule cells

Mixture # / Source	Observed E50 values (μM)[a]	Predicted E50 values (μM)[b]
Mixture 1	200	222
Mixture 2	188	192
Mixture 3	61	77
Mixture 4	88	194
Mixture 5	107	121

[a]Calculated E50 values were obtained from the regression line fit to the linear portion of the curve and represent the effective concentration that increases the control activity by 50%.
[b]Predicted E50 values were obtained from the logistic second order response surface model based on dose-addition assumption described in Svendsgaard *et al*[17].

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