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Effects of PCB Congeners in Rodent Neuronal Cells in Culture

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We attempted to analyze the mechanism of polychlorinated biphenyl (PCB)-induced neurotoxicity and identify the target molecules in the neuronal cells for PCBs.Since the developing neuron is particularly sensitive to PCB-induced neurotoxicity, we isolated cerebellar granule cells derived from 7-day old Sprague Dawley (SD) rats and grew cells in culture for additional 7 days to mimic PND-14 conditions. Only non-coplanar PCBs at a high dose showed a significant increase of total protein kinase C (PKC) activity at phobol 12,13-dibutyrate ([^{3}H]PDBu) binding assay, indicating that non-coplanar PCBs are more neuroactive than coplanar PCBs in neuronal cells. PKC isozymes were immunoblotted with the selected monoclonal antibodies. PKC-α, δ, and ε were activated with non-coplanar PCB exposure. Receptor for activated C kinase-1 (RACK-1), anchoring protein for activated PKC, was more induced with exposure to coplanar PCBs than non-coplanar PCBs. Reverse transcription PCR (RT-PCR) analysis showed induction of neurogranin (RC-3) and growth associated protein-43 (GAP-43) mRNA with non-coplanar PCBs. The results indicate that these factors may be useful biomarkers for differentiating non-coplanar PCBs from coplanar PCBs. The present study demonstrated that non-coplanar PCBs are more neuroactive congeners than coplanar PCBs.

Key Words: PCB, Neurotoxicity, Structure-activity relation, Cerebellum, Culture, PKC

INTRODUCTION

Polychlorinated biphenyls (PCBs) are stable compounds which have been identified as environmental contaminants in almost every component of the global ecosystem. Although PCBs are no longer manufactured in the industrialized countries, these compounds continue to be found as environmental pollutants due to continued release from the hazardous waste sites, accidental breakdown of electrical transformers and slow degradation. PCBs consist of 209 distinct congeners with different structural characteristics. PCBs are stable, lipophilic and bioaccumulative in wildlife and foodstuffs. Human exposure to PCBs has been associated with several abnormalities (Swanson et al, 1995). It is of a particular concern that exposure to the relatively low concentrations of PCBs may be associated with subtle behavioral and neurological deficits if exposure occurs during development (Jacobson et al, 1996). Animal studies also demonstrated the neurotoxic potentials of PCBs including psychomotor dysfunction and cognitive deficits (Schantz et al, 1995). While neurotoxic mechanism of PCBs still remains to be elucidated, structure-activity relationship has been described for some PCBs on neuronal tissues. Recent studies found that the ortho-, non-coplanar PCB altered intracellular Ca^{2+} homeostasis by inhibiting Ca^{2+} buffering system and caused protein kinase C (PKC) translocation at low micromolar concentrations, while non-ortho, coplanar PCB did not have any effects on these second messenger systems (Kodavanti et al, 1993; Kodavanti et al, 1994; Kodavanti et al, 1996). Perturbation of Ca^{2+} homeostasis leading to the sustained elevation of calcium ions can trigger many second messenger systems, which may result in altered function and development of neurons. One of the most pivotal second messenger molecules involved in neuronal function and development is PKC. PKC signaling pathways have been implicated as an important factor in learning and memory processes (Matsushima et al, 1996). While translocation of PKC is one of the key effects of ortho-PCBs, it remains unknown which subspecies of PKC are target molecules. Evidence suggests that cerebellum is a storage site for the memory traces for discrete motor learning and classical conditioning of eyeblink response (Molchan et al, 1994). Alteration of PKC in cerebellum is suggested to be associated with impaired motor dysfunction (Chen et al, 1995). The present study attempted to assess structure-activity relationship on PCB-induced subcellular changes in PKC isozymes and to identify target molecules sensitive to the PCB exposure in cerebellar granule cells in culture.

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METHODS

Cerebellar granule cell culture

Cerebellar granule cell cultures were prepared from the cerebella of 7-day old Sprague-Dawley (SD) rat pups as

ABBREVIATIONS: PCBs, polychlorinated biphenyls; PKC, protein kinase C; RACK-1, receptor for activated C kinase-1; PND, post natal days; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

described previously (Kodavanti et al, 1993). Cells were plated at 3×10^6 cells/well in 6-well plates. After plating, cells were incubated at 37°C in a humidified incubator with 5% CO_2 atmosphere. Cytosine arabinoside (5 μM) was added after 24 hr to prevent growth of non-neuronal cells. Cells were used for the experiments after 7 days in culture. Cultures typically contained >95% neurons.

Exposure

Cerebellar granule cells grown on 6-well culture plates were exposed to 50 μM of coplanar PCBs {2,2'-dichlorobiphenyl (DCB), 2,3,3',4,4',5-hexachlorobiphenyl (HCB) and 2,3',4,4',5-pentachlorobiphenyl (PCB)} or non-coplanar PCBs {Aroclor 1254 and 4,4'-dichlorobiphenyl (DCB)} (>99% purity; Accu-Standard, New Haven CT) for 15 min. For phobol 12,13-dibutyrate ([^{3}H]PBDu) assay, the concentrations exposed were 0.1, 1, 25 and 50 μM. In order to get enough protein for immunoblots, 4 culture plates were used for each concentration. After the exposure, cultures were washed twice with Lock's buffer and the cells were harvested in a final volume of 2 ml buffer A.

[^{3}H]PDBu binding binding assay

Cerebellar granule cells grown on 12-well plastic trays (Costar) were tested at 7 days in culture. [^{3}H]phorbol ester binding method was adopted from Vaccarino et al (1991). Briefly, the monolayers were washed with Locke's buffer (154 mM NaCl; 5.6 mM KCl; 3.6 mM $NaHCO_3$; 2.3 mM $CaCl_2$; 5.6 mM d-glucose; 5 mM Hepes, pH 7.4) containing 0.1% fatty acid-free BSA. Following washing, the cells were incubated in Locke's buffer containing 1 nM 4-β-[^{3}H] phorbol 12,13-dibutyrate ([^{3}H]PDBu; 0.1 μCi/ml) for 15 min at room temperature with the test chemicals (0.1~50 μM). An equal amount of DMSO was added to controls. After incubation, the medium was aspirated, and cells were washed three times with Locke's buffer and suspended with 1 ml of 0.1 M NaOH. An aliquot of this sample (0.7 ml) was added to 9 ml Ultima Gold (Packard Inst. Co., Meriden, CT) and the radioactivity was determined using scintillation spectroscopy (Beckman LS 6000LL). A small aliquot was used for protein determination (Bradford, 1976). Nonspecific binding was determined in the presence of 1.6 μM phorbol myristate acetate, which was always <20%, and subtracted from all the values. The unit of [^{3}H]PDBu binding was fmol/mg protein/15 min.

Cell fractionation

Cells were scraped off into buffer A (20 mM Tris-HCl. pH7.5, Containing 0.25 M sucrose, 2 mM EDTA, 2 mM EDTA and cocktail of protease inhibitors including 0.5 mM phenylmethylsulfonylfluoride (PMSF), 10 μg/ml leupeptin, and 10 μg/ml pepstatin). The cells were briefly sonicated and centrifuged at 100,000 g for 1 h. The supernatants were designated as cytosolic fraction. The membrane proteins in the pellets were extracted with buffer B (20 mM Tris-HCl, pH 7.5, containing 1% Nonidet P-40, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA and protease inhibitors) on ice for 30 min followed by centrifugation at 15,000 g, and the supernatants were saved as detergent-soluble-membrane fraction.

Immunoblotting

Immunoblot analysis was performed as described previously (Yang et al, 1999). Proteins (10 μg) from cytosolic and membrane fractions were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membrane by Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA). For the detection of receptor for activated C kinase-1 (RACK-1), 7 μg of whole cell lysate was analyzed by 10% SDS-PAGE. The nitrocellulose sheet was blocked with 5% non-fat dry milk in Tris buffered saline. PKC isozymes were detected with isozyme-specific monoclonal antibodies for α, γ, δ and ε isozymes (Transduction Lab, Lexington, KY). The blots were reacted with a peroxidase-conjugated anti-mouse IgG and detected by the Super Signal (Pierce, Rockford, IL). The density of respective bands was analyzed by the Fluor-S (Bio-Rad, Hercules, CA). The data was represented as % controls.

Reactive oxygen species (ROS) detection

Formation of ROS was elucidated by use of the fluorescent probe 2,7-dichlorofluoresicin diacetate (DCFH-DA). The detection method was adopted from Mariussen et al (2002). Briefly, the cells were preincubated with DCFH-DA (5 μM), dissolved in methanol, at 37°C in 5% CO_2 atmosphere for 20 min. DCFH-DA is permeable across cell membranes and inside the cell, the acetate moieties are cleaved by cellular esterases. DCFH readily reacts with ROS such as peroxynitrite (ONOO-) and lipid peroxides to the fluorescent DCF. The medium with the DCFH-DA was then removed, and the cells were added to 1.5 ml prewarmed Hepes-buffered (20 mM) HBSS (pH 7.4) with 5 mM glucose containing PCB congeners. The dishes were incubated for 15 min at room temperature. Thereafter, the cells were gently removed from the dishes and transferred in triplicate to 250-μl wells (microtiter plate, 96 wells) for ROS measurement. The formation of the fluorescent oxidized derivative of DCFH, namely DCF (fluorescent at 530 nm), was monitored with a luminescence spectrometer (LS50B, PerkinElmer) at 37°C for 180 min.

RT-PCR analysis

Total RNAs were prepared with a RNA isolation kit (Gibco-BRL, Gaithersburg, MD), according to manufacturer's instruction followed by digestion with RNase-free DNase I. RT-PCR was performed as described previously (Yang, 1999). Briefly, for cDNA synthesis 1 μg total RNA was heated in a final volume of 10 μg with 2 μg oligo (dT)15 primer for 5 min at 60°C, chilled on ice, and reverse transcribed in a final volume of 40 μl containing 1 mM of each dNTP 8 μl 5x M-MLV buffer, 60 units RNase inhibitor, 10 mM DTT, and 400 units M-MLV reverse transcriptase. Samples were incubated at 37°C for 1h and subsequently denatured for 10 min at 70°C. PCRs were carried out in a final volume of 50 μl containing 2.5 μl RT samples, 5 μl 10x Taq DNA polymerase, the presence of 0.2 μM of each primer, 2.5 units Taq DNA polymerase, and 1 μCi [α-^{32}P] dCTP. Three separate experiments were repeated for each gene. Analysis of respective bands was performed by the image analyzer (Bio-Rad, Hercules, CA).

Statistics

The data were analyzed by one way analysis of variance followed by Tukey's multiple comparison test. The significance was set at $p<0.05$.

RESULTS

Effects of PCBs on PKC activities

To determine total PKC activity following PCB exposure, [^{3}H]PDBu binding assay was performed. Compared to the coplanar PCBs, non-coplanar PCBs showed higher values of PKC translocation (Table 1). Increase of PKC translocation was dose-dependent with exposure to non-coplanar PCBs. However, coplanar PCBs did not show such an effect.

Table 1. [^{3}H] PDBu binding following the exposure of substances (μM) (% control)

Compounds	Conc.			
2,2'-dichlorobiphenyl (μM)	0.1	1	25	50
	103±8	105±11	128±18	195±14
2,3,3',4,4',5-hexachloro-	0.1	1	25	50
biphenyl (μM)	108±12	112±25	115±16	131±18
2,3,4,4',5-pentachloro-	0.1	1	25	50
biphenyl (μM)	95±18	102±5	132±25	128±16
Aroclor 1254 (μM)	0.1	1	25	50
	100±8	108±12	135±15	181±19
4,4'-dichlorobiphenyl (μM)	0.1	1	25	50
	106±12	98±8	115±15	111±12

Structure-activity relationship on subcellular changes in PKC isozymes

To examine effects of PCBs on PKC isozymes, immunoblot analysis was performed. The cells were fractionated and subsequently immunoblotted against the selected monoclonal antibodies. When 50 μM of the test compounds were exposed, translocation of PKC-α, δ, ε and isozymes was

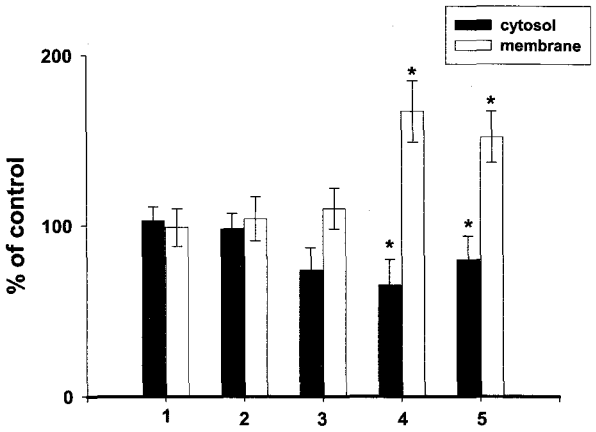

Fig. 2. Translocation of PKC-δ following PCB (50 μM) exposure. Immunoblot analysis was performed against PKC-δ monoclonal antibody after cells were treated with PCBs for 15 min; 4,4'-DCB (1), 2,3,3',4,4',5-HCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4) or 2,2'-DCB (5). Proteins (10 μg) from cytosolic and membrane fractions were separated by 7.5 % SDS-PAGE. The blots were reacted with a peroxidase-conjugated anti-mouse IgG. The values are means of triplicate separate experiments. The data was represented as % of controls. Student t-test was used to determine the significance (*$p<0.05$).

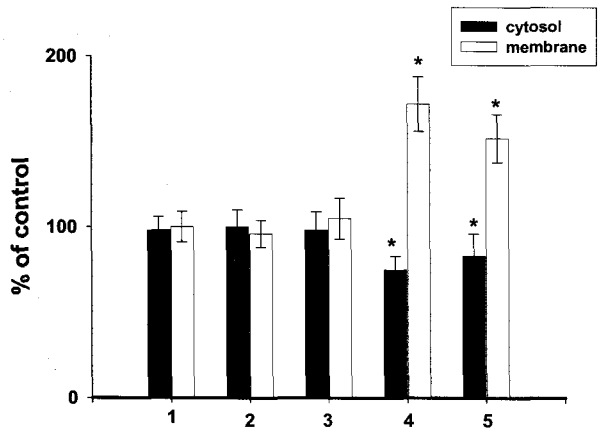

Fig. 1. Translocation of PKC- following PCB(50 μM) exposure. Immunoblot analysis was performed against PKC-α monoclonal antibody after cells were treated with PCBs for 15min; 4,4'-DCB (1), 2,3,3',4,4',5-HCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4) or 2,2'-DCB (5). Proteins (10 μg) from cytosolic and membrane fractions were separated by 7.5 % SDS-PAGE. The blots were reacted with a peroxidase-conjugated anti-mouse IgG. The values are means of triplicate separate experiments. The data was represented as % of controls. Student t-test was used to determine the significance (*$p<0.05$).

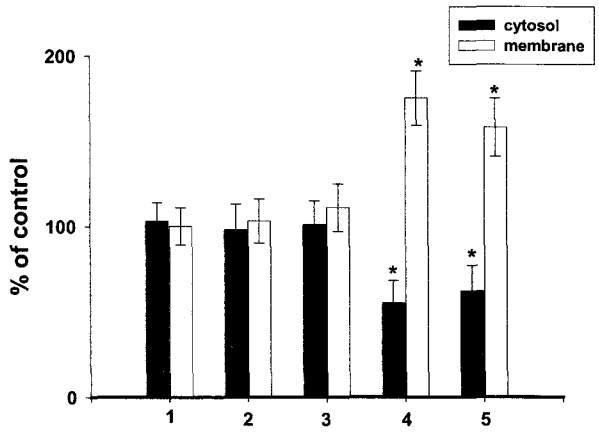

Fig. 3. Translocation of PKC-ε following PCB (50 μM) exposure. Immunoblot analysis was performed against PKC-ε monoclonal antibody after cells were treated with PCBs for 15 min; 4,4'-DCB (1), 2,3,3',4,4',5-HCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4) or 2,2'-DCB (5). Proteins (10 μg) from cytosolic and membrane fractions were separated by 7.5 % SDS-PAGE. The blots were reacted with a peroxidase-conjugated anti-mouse IgG. The values are means of triplicate separate experiments. The data was represented as % of controls. Student t-test was used to determine the significance (*$p<0.05$).

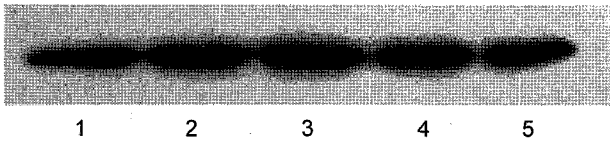

Fig. 4. Induction of RACK-1 with PCB (50 μM) exposure. Levels of RACK-1 were measured by the immunoblot analysis after cells were treated with PCBs for 30 min; 0.1% DMSO (1), 4,4'-DCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4) or 2,2'-DCB (5). RACK-1 monoclonal antibody (Transduction Lab) was used. 7 μg of whole cell lysate was separated by 10% SDS-PAGE. A typical result of three separate experiments is shown.

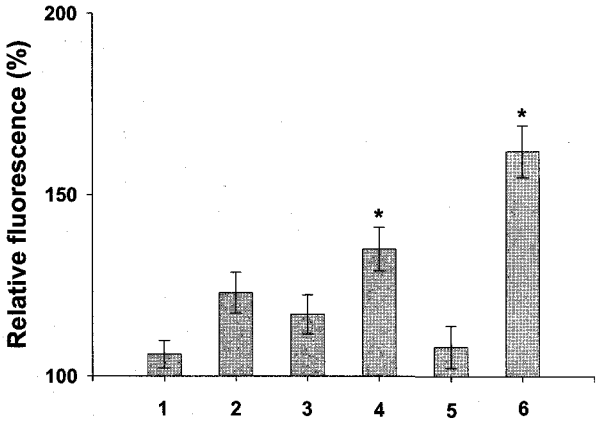

Fig. 5. Relative fluorescence for formation of ROS in cerebellar granule cells treated with PCBs (50 μM) and TCDD (10 nM) exposure; 4,4'-DCB (1), 2,3,3',4,4',5-HCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4), 2,2'-DCB (5) or TCDD (6). Formation of ROS was measured as described in Method. All values are relative to the response of control cerebellar granule cells (0.1% DMSO only, 100%). Values are means of three separate experiments. Student t-test was performed to determine the significance (*$p<0.05$).

observed with exposure to non-coplanar PCBs (Fig. 1~3). Aroclor 1254 induced decreases of cytosol (% of control; 82±12 at PKC-α, 78±10 at PKC-δ and 66±15 at PKC-ε) and increases of membrane fractions (% of control; 167±19 at PKC-α, 158±15 at PKC-δ and 188±11 at PKC-ε). 2,2'-DCB induced decreases of cytosol (% of control; 88±15 at PKC-α, 92±18 at PKC-δ and 75±13 at PKC-ε) and increase of membrane fractions (% of control; 153±11 at PKC-α, 148±12 at PKC-δ and 168±18 at PKC-ε). In contrast, coplanar PCBs did not show such changes.

Effects of PCBs on RACK-1 induction

RACK-1 was analyzed by immunoblot analysis. While increase of RACK-1 was observed with exposure to 4,4'-DCB (135±18%) or 2,3,4,4',5-PCB (142±18%), Aroclor 1254 and 2,2'-DCB did not show such an increase. The results indicate that RACK-1 induction requires coplanar moiety of the PCB structure (Fig. 4).

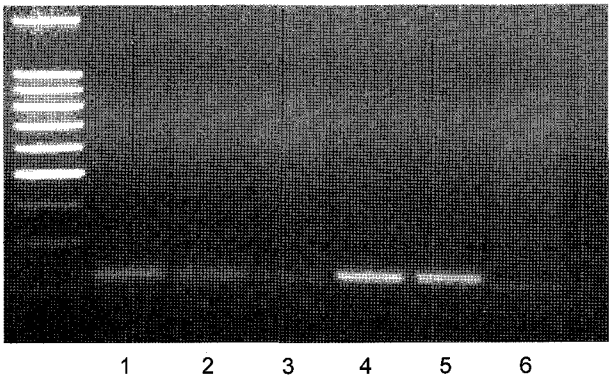

Fig. 6. RT-PCR analysis of RC-3 with PCBs (50 μM) and TCDD (10 nM) exposure; 0.1% DMSO (1), 4,4'-DCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4), 2,2'-DCB (5), TCDD (6). After cells were treated with TCDD or PCB congeners for 12 hrs, total RNA was extracted as described in Methods. GAPDH was used as a loading control. A typical result of three separate experiments is shown.

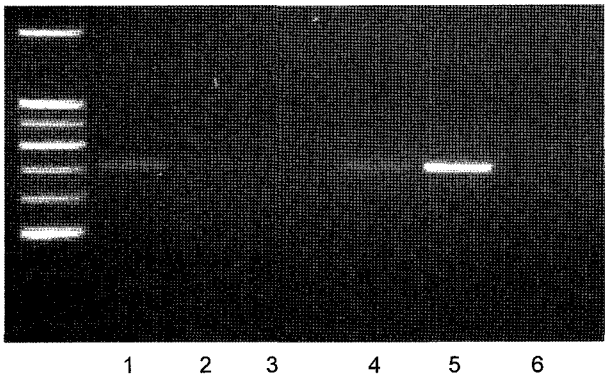

Fig. 7. RT-PCR analysis of GAP-43 with PCBs (50 μM) and TCDD (10 nM) exposure; 0.1% DMSO (1), 4,4'-DCB (2), 2,3,4,4',5-PCB (3), Aroclor 1254 (4), 2,2'-DCB (5), TCDD (6). After cells were treated with TCDD or PCB congeners for 12 hrs, total RNA was extracted as described in Methods. GAPDH was used as a loading control. A typical result of three separate experiments is shown.

Effects of PCBs on ROS production

PCB exposure increased ROS production. However, the production was not structurally related. Order of ROS production among the test substances was as followed; TCDD > Aroclor1254 > 2,3,3',4,4',5-HCB > 2,3,4,4',5-PCB > 2,2'DCB > 4,4'-DCB (Fig. 5). There was no structure-activity relationship observed between the test substances.

Extracellular matrix associated with neuronal cell growth

RT-PCR analysis revealed that mRNA levels of RC-3 were increased with non-coplanar PCBs only. TCDD and other coplanar PCB structures did not show such an effect (Fig. 6). mRNA level of GAP-43 was most increased with exposure to 2,2'-DCB and followed by Aroclor1254. TCDD and other coplanar PCBs did not show such an effect (Fig. 7).

DISCUSSION

Previous report revealed that the ortho-substituted, non-coplanar PCB caused the perturbation of calcium homeostasis and PKC translocation in cerebellr granule cells, while the non-ortho-substituted, coplanar PCBs did not show such effects (Kodavanti et al, 1993; Kodavanti et al, 1994; Kodavanti et al, 1996). Since PKC translocation as measured by the [^{3}H]PDBu binding was observed only in the presence of the extracellular calcium (Kodavanti et al, 1994), it is assumed that the classical PKCs, Ca^{2+}-dependent isozymes, may be involved in this translocation process. However, it has not been identified which isozyme is associated with the non-coplanar PCB-induced PKC activation. Since PKC subspecies are located in different subcellular compartment and have their unique activation profile (Nishizuka et al, 1988), it is important to analyze the individual PKC isozymes to understand their biological significance in the cellular system. PKCs are abundant in neuronal tissue and are involved in neuronal survival and functions of neuronal trophic factors, suggesting a crucial role for PKC in the signal transduction between neurons and the etiology of the neuronal diseases (Hama et al, 1986; Matsushima et al, 1996). In an attempt to analyze effects of PCB on PKC signaling pathways and identify their possible intracellular molecular targets in the cerebellar granule cells, we performed immunoblot analysis of the selected PKC isozymes and RACK-1, an anchoring protein for PKC. In addition, the extracellular matrix associated with neuronal growth were analyzed with RT-PCR. Since PCBs have many distinct congeners with their unique structural properties, the study examined the structure-activity relationship of biological responses among PCB congeners to achieve the better assessment of biological and ecological impacts.

Among PKC isozymes analyzed in the present study, PKC-α, -δ and -ε, were detected in both cytosol and membrane fractions, but the γ-form was below the detectable level. PKC-γ is known to be abundant in the brain and the most studied PKC isozyme associated with the learning and memory (Riedel, 1997). Lack of detection observed in this study may be due to the use of antibodies from different sources, since different antibodies detect the different epitopes of antigen. However, cerebellar tissue isolated from the same animal at PND 7 revealed the good expression of PKC-γ with the same antibody (Yang et al, 2003). The monoclonal antibody used in this study does not have any cross-reactivity with other PKC isozymes. Thus, this result demonstrated a good example of the different outcomes between in vivo and in vitro experiments.

PKC-α is specifically involved in PCB-induced activation of PLA_2 in neutrophils (Olivero et al, 2000) and is selectively associated with lithium-induced memory impairments (Manji et al, 1993). Translocation of PKC-α has been associated with long-term potentiation in a hippocampus region (Son et al, 1996). A significant translocation of PKC-α from cytosol to membrane fraction in this study provides an evidence for the involvement of this particular isoform in the PCB-altered signal transduction pathway. As the study identified a specific PKC isozyme that responds to the non-coplanar PCBs, it is suggested that this isozyme may be a target molecule in the neuroactive process of the non-coplanar structures.

Ca^{2+}-independent PKCs have different substrate specificity or phospholipid dependency as compared to Ca^{2+}-dependent isozymes. Ca^{2+}-independent forms are suggested to be involved in the different cellular functions than Ca^{2+}-dependent forms (Konno et al, 1989). Although the physiological roles of Ca^{2+}-independent forms have not been fully clarified, it is known that PKC-ε is most abundant in the brain and present mainly in the presynaptic component (Saito et al, 1993). Presynaptic activation by arachidonic acid and by diacylglycerol generated after metatrophic glutamate receptor activation plays a pivotal role in the maintenance of long-term potentiation (LTP) (Herreo et al, 1992). Thus, PKC-ε has been suggested to be a candidate isozyme associated with this presynaptic mechanism of LTP. In addition, PKC-ε is known to regulate the glial cell cycle and its overexpression is associated with astroglial tumor (Guizzetti et al, 1998; Sharif et al, 1999). Since PKC-ε has been associated with a variety of pivotal biological events in neuronal cells, it is feasible that altered subcellular distribution of this particular isozyme may play important roles in the non-coplanar PCB-induced neurotoxicity.

Biological importance of PKC-δ in the cellular system has been recently highlighted. PKC-δ plays a key role in the glioblastoma thru the transactivation of EGFR, a protein tyrosine kinase (Amos et al, 2004). Caspase-3- dependent proteolytic activation of PKC-δ is associated with manganese-induced apoptosis of dopaminergic neuronal cells (Latchoumycandane et al, 2004). The study suggests that alteration of this isozyme may be associated with changes in cross talk between the kinases and cell death signals.

A significant translocation of PKC-ε and -δ in this study suggests that the non-coplanar PCB-induced neurochemical changes may be, at least in part, mediated via calcium- independent pathway. Since neurochemical changes observed following the non-coplanar PCB exposures have been observed only in the presence of extracellular calcium and most of neurochemical events have been considered Ca^{2+}-dependent, altered subcellular distribution of the Ca^{2+}-independent isozymes in this study may shed a new light in the mechanistic studies of PCB-induced neurotoxicity.

While the functional roles of PKC-α, δ and in the cerebellar granule cells are not clear, altered subcellular distribution of these isozymes may cause the disruption of normal signal pathways in the developing brain, which ultimately may lead to motor dysfunction and cognitive deficits.

Neurogranin (RC-3) and growth-associated protein-43 (GAP-43) play pivotal roles in neuronal cell growth and developments (Pasinelli et al, 1995; Casoli et al, 2003). Alteration of extracellualr matrix such as GAP-43 is associated with the various biological responses in neurons. Because regulation of PKC-ε mRNA showed a similar time course to GAP-43 mRNA and both PKC-ε and GAP-43 are located in the presynaptic terminals (Saito et al, 1993; Bendotti et al, 1994), it is speculated that PKC-ε may play a role in expression of GAP-43 in neuronal cells. GAP-43 is the neuron-specific phosphoprotein associated with axonal development and regeneration (Meiri et al, 1988). Thus, abnormal expression of GAP-43 by the altered PKC-ε may disturb structural formation of neuronal cells. The results suggest that both RC-3 and GAP-43 may be sensitive molecules altered by non-coplanar PCBs. In particular, since GAP-43 was sensitive to the most non-coplanar moiety, it is suggested that this protein can be utilized as a biomarker to differentiate the non-coplanar structures

from the coplanar ones. Because the environmental samples contain both non-coplanar and coplanar PCBs as a mixture, identification of biomarker such as GAP-43 will contribute to improving the risk assessment of the PCB-induced neurotoxicities in humans.

RACKs are essential components in PKC signaling pathways (Schechtman et al, 2001). Although RACKs are not active substrates for PKC, their presence substantially increases substrate phosphorylation by the corresponding PKC isozyme (Csukai et al, 1999). RACK-1 is known as the selective adaptor protein for PKC-βII (Ron et al, 1999), but it also binds other signaling enzymes. Phospholipase Cγ (PLCγ) binds RACK-1 through the C2 domain, which is the RACK-1 binding site for PKC (Disatnik et al, 1994). RACK-1 localizes PKC-βII next to activated PLCγ for the efficient PKC-mediated signaling. Modulation of RACK-1 by coplanar PCBs and TCDD may affect a cross talk between tyrosine kinase pathway and serine/threonine kinase pathway. Therefore, it is suggested that increased expression of RACK-1 in the cerebellar granule cells by TCDD and coplanar PCBs, as demonstrated in this study, causes the disrupted regulation of signaling cascade in neuronal cells, which may lead to the neurological disorders observed in PCB-exposed subjects.

The study provided the evidence that the non-coplanar moiety of PCBs favors neuroactive impacts in the developing neuronal cells. It suggests that non-coplanar PCBs, which have been neglected in the risk assessment processes, should be incorporated in the future to improve the quality and accuracy of risk assessment on the neuroendocrinal adverse effects of PCB exposures. Identification of target molecules specifically responding to this class of environmental pollutants may contribute to understanding their mechanism of action, thereby improving the health risk assessment in humans.

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