ALTERATIONS IN BRAIN PROTEIN KINASE C ISOFORMS FOLLOWING DEVELOPMENTAL EXPOSURE TO A PCB MIXTURE

Jae-Ho Yang[1,2], Ethel C. Derr-Yellin[2], and Prasada Rao. S. Kodavanti[2]

[1]National Research Council, Washington, D.C. and [2]Neurotoxicology Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

Introduction

Polychlorinated biphenyls (PCBs) offer a unique model to understand the major issues related to complex environmental mixtures. These environmental pollutants are ubiquitous, persistent, bioaccumulate in human body through the food chain, and exist as mixtures of several congeners in the environment. Human exposure to PCBs are associated with a variety of adverse health effects such as skin disorders, cancer, immune dysfunction, neurobehavioral changes and neuroendocrine disruption[1]. It is of a particular concern that exposure to relatively low level during development may be associated with neurological deficits such as motor dysfunction and impairments in learning and memory[2]. During the past decades there has been an attempt to understand the cellular and molecular basis of PCB-induced behavioral and neurological effects in animal models. Recent *in vivo* studies from our laboratory indicated that developmental exposure to a commercial PCB mixture, Aroclor 1254[R], caused perturbations of calcium homeostasis and changes in protein kinase C (PKC) activities in rat brain[3]. While PCBs are known to disturb several neurochemical endpoints and are implicated in the etiology of some neurological diseases, it is not known which molecular substances are targets for PCB-induced developmental neurotoxicity. The PKC signaling pathway has been implicated in the modulation of motor behavior as well as learning and memory, and the roles of PKC are subspecies specific. In this context, the objective of the present study is to analyze PKC isoforms in selected brain regions following developmental exposure to a PCB mixture.

Methods

Animals. Long-Evans rats were obtained from Charles River Laboratory (Portage, MI) on gestational day (GD) 3 arrival (the day of insemination was GD 0) and housed in AAALAC approved animal facilities. The animals were housed individually in standard plastic hanging cages with sterilized pine shavings as bedding. Food (Purina lab chow) and water were provided *ad libitum*. Temperature was maintained at 21 ± 2°C and relative humidity was maintained at 50 ± 10% with a 12 h light/dark cycle (6:00-18:00 h). All the experiments were approved in advance by the National Health and Environmental Effects Research Laboratory animal care committee of the USEPA.

Dosing of animals. A commercial PCB mixture, Aroclor 1254 (Lot # 124-191; purity >99%) was purchased from AccuStandard, Inc (New Haven, CT). The dosing solutions were prepared by dissolving this PCB mixture in corn oil. At least 10 dams per dosage were given Aroclor 1254 (0, 1 or 6 mg/kg) in corn oil (2 ml/kg) by oral gavage starting from GD 6 through postnatal day (PND) 21, except on PND1 when the dams were left undisturbed. The rats were dosed daily between 8:00 and 10:00 am. The dams were weighed every day before dosing.

Beginning on GD22, rats were checked twice daily (AM and PM) for births, and the date that birth was first discovered was assigned PND 0. All dams (>90% success of pregnancy) gave birth within few hours apart and the litter size varied between 4-17 pups. On PND4, litters were culled to 10 pups/litter, five males and five females.

Circulating thyroid hormones. Blood samples were collected on PND 4, 7, 14, 21, and 60 from at least one pup/litter/treatment. After collection of blood samples, they were placed on ice for an hour to allow for blood clotting. The samples were centrifuged at 1000xg for 15 min to separate serum. These serum samples were stored at -80°C until radioimmunoassay. Total T4 and T3 were determined using radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA) based on the competitive protein binding technique.

Immunoblotting. Cerebellum and hippocampus were dissected from male pups. Cell fractionation and subsequent immunoblotting were performed as described previously[5]. Proteins (10 μg) from cytosolic and particulate fractions were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membrane by Semi-Dry Tranbsfer Cell (Bio-Rad, Hercules, CA). The nitrocellulose sheet was blocked with 5% non-fat dry milk inTris buffered saline. PKC isoforms were detected with isoform-specific monoclonal antibodies for α, γ and ε isoforms (Transduction Lab, Lexington, KY). The blots were reacted with a peroxidase-conjugated anti-mouse IgG and detected by the Super Signal (Pierce, Rockford, IL)

Statistics. The data were analyzed by Two-Way Analysis of Variance (ANOVA) with age as one factor and treatment as the other followed by Dunnett's post-hoc test. All analyses were performed with PROC GLM in SAS (SAS Institute Inc., 1989).

Results and Discussion

General Health and Development. Arolcor 1254 treatment did not alter the maternal body weights during gestation or lactation. The pregnancy rate was 91%, 90%, and 100% for dams in the control, 1 mg/kg and 6 mg/kg Aroclor 1254 treatment groups, respectively. The litter size ranged from 4 to 17 pups with a mean of 12.6 pups per litter. The % pup mortality in control was not significantly different from either 1 or 6 mg/kg/day treatment groups. Developmental exposure to Aroclor 1254 caused a small, but transient decrease in body weight gain of offspring in high dose group (data not shown).

Circulating Thyroid Hormones. Developmental exposure to Aroclor 1254 caused hypothyroxinemia in the dams and early postnatal offsprings. Maternal T4 on PND22 was depressed by 29% in 1 mg/kg and 59% in 6 mg/kg dose group. Circulating total T4 levels in the offspring were also lower following perinatal exposure to this mixture. The effect was much greater on PND 14 and the T4 levels recovered to control levels on PND60 (data not shown). There was no significant effect of Aroclor 1254 on maternal or offspring T3 levels. These results are in agreement with previously published information and indicates that the selected dosages cause significanthypothyroxinemia in the absence of overt maternal toxicity.

Protein kinase C isoforms. PKC has been implicated as an important factor in learning and memory process and etiology of some neurological diseases[5]. While roles of all the individual subspecies are

considered crucial, we selected to focus on three of the most frequently studied isoforms implicated in the neurological diseases; two Ca^{++}-dependent (α & γ)and one Ca^{++}-independent forms (ε)[6-8]. Since cognitive deficit and motor dysfunction are considered as significant developmental neurotoxicities of PCBs, hipppocampus and cerebellum were chosen as target regions. Although the study was performed at 5 different age groups starting from postnatal day (PND)4 through 60, significant observations made on PND 14 will be presented here due to the limited space (Fig. 1). Immunoblot analysis of PKC-α from cerebellum revealed that developmental exposure toAroclor 1254 caused a significant decrease in cytosolic fraction and an increase in particulate fraction and the ratio (particulate/cytosol) was also significantly increased in a dose-dependent manner. Developmental exposure to Aroclor 1254 also caused significant changes in PKC-αin cytosolic and particulate fractions of hippocampus. There was no significant difference between these regions on the level of fractional changes. Analysis of PKC-γ in cerebellum showed a decrease ofcytosolic fraction at the low dose and increase of particulate fraction at the high dose groups relative to controls (Fig. 1).The ratio between the two fractions was increased in the high dose group. In the hippocampus, there were no significant changes of PKCγ either in cytosolic or particulate fractions. But, the ratio between the fractions showed a marginal increase ($p=0.06$). Analysis of PKC-ε in cerebellum did not show any significant changes either in cytosolic or particulate fractions following developmental exposure to Aroclor 1254. But in hippocampus, there was a significant decrease in cytosolic PKC-ε and an increase of ratio in the high dose group. The results from this study indicate that the patterns of subcellular distributions of PKC isoforms following a developmental PCB exposure were brain region-, PKC isoform- and developmental stage-specific. It appears that developmental exposure to a PCB mixture was, in part, responsible for the altered cellular distributions of PKC isoforms which may be related to the subsequent disruption of the normal maintenance of signal transduction in developing neurons. Our findings suggest that alteredsubcellular distribution of PKC isoforms may be a possible mechanism of PCB-induced neurologicaltoxicities and that PKC-α, γ and ε may be among the target molecules implicated with PCB-induced neurological impairments during developmental exposure. In addition, it is speculated that altered distribution of theseisoforms may be associated with the lowered levels of circulating thyroid hormones, since thyroid hormones are known to regulate PKC proteins. It is believed that this is the first report successfully identifying PKC isoforms responding to the developmental exposure to a PCB mixture.

Acknowledgments

Jae-Ho Yang is a recipient of National Research Council Senior Research Associateship Award. We thank Michael Vernon and David Mason for their excellent help in this study. This abstract does not necessarily reflect USEPA policy.

References

1. Swanson GM, Ratcliffe HE and Fischer LJ (1995). Regul. Toxicol. Pharmacol. 21, 136.
2. Jacobson JL and Jacobson SW (1996). N. Engl. J. Med. 335, 783.
3. Kodavanti PRS, Derr-Yellin E, Mundy WR, and Tilson HA (2000)The toxicologist. 361, 76
4. Yang JH, Vogel C and Abel J (1999). Carcinogenesis 20, 13.
5. Matsushima H, Shimohama S and Kimura J (1996). J. Neurochem. 67, 317
6. Leahy JC, Luo Y and Vallano ML (1993). Neuroscience, 52, 563
7. Chen C, Kano M,, and Tonegawa S (1995). Cell 83, 1233
8. Guizetti M, Wei M and Costa LG (1998). Eur. J. Pharmacol. 359, 223.

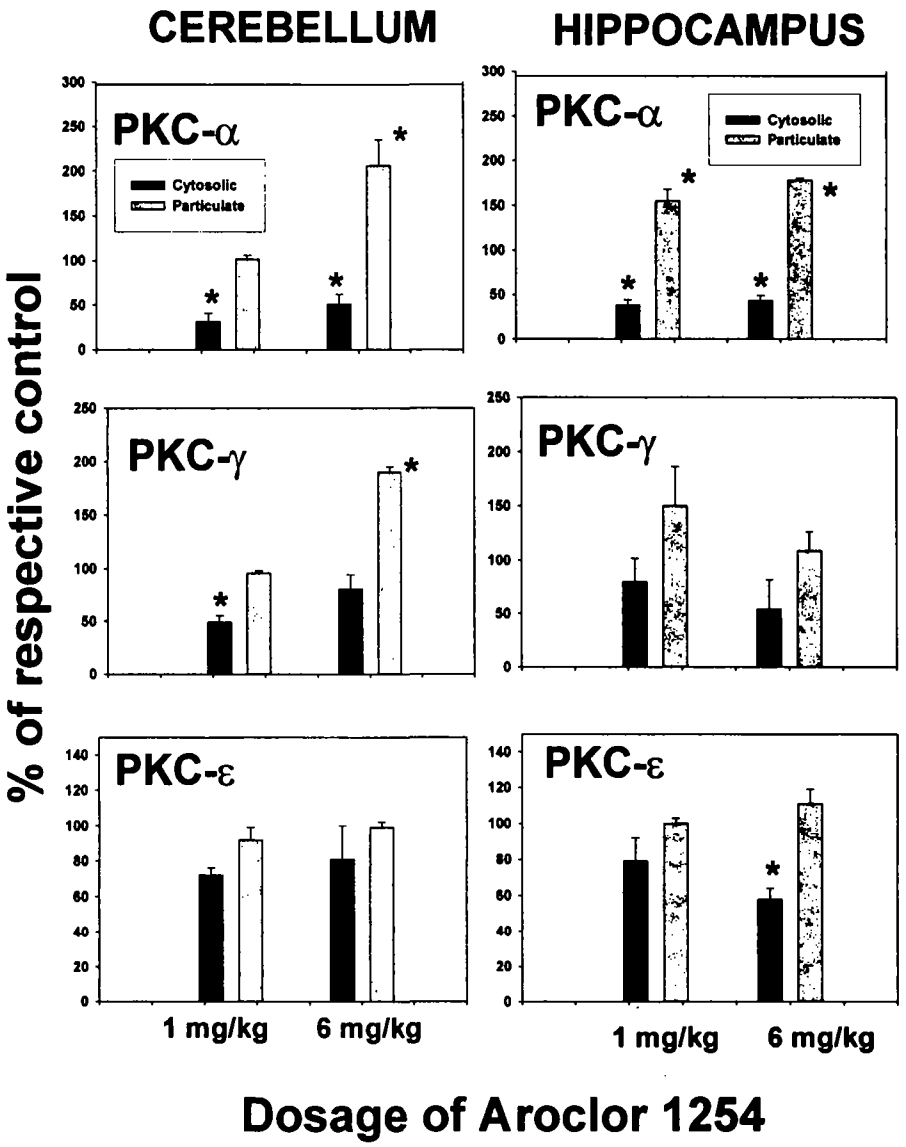

Figure 1. Changes in subcellular distribution of brian PKC isoforms at PND14 following developmental exposure to Aroclor 1254. Values are mean ± SD of 3 separate experiments. *Significantly different from control at $p < 0.05$. The variability in the controls was always < 10%.