CHANGES IN HIPPOCAMPAL SPINE DENSITY AND PROTEIN KINASE C ISOFORMS FOLLOWING DEVELOPMENTAL EXPOSURE TO A MIXTURE OF PERSISTENT CHEMICALS

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Introduction

Polychlorinated biphenyls (PCBs) offer a unique model to understand the major issues related to complex environmental mixtures of persistent chemicals. These pollutants are ubiquitous, persistent, bioaccumulate in human body through the food chain, and exist as mixtures of several congeners in the environment. Human exposures to PCBs are associated with a variety of adverse health effects including neurobehavioral changes and neuroendocrine disruption[1]. It is of a particular concern that exposure to relatively low levels during development may be associated with neurological deficits such as 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 neurotoxic 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, 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 learning and memory, and the roles of PKC are subspecies specific. Also, PKC has been shown to modulate dendritic differentiation and growth[4]. In neurons, dendritic branching and spines parameters represent the anatomical substrate for the input of information to neurons. Indeed, dendritic parameters comprise over 95% of the surface area of the typical neuron. As such, change in dendritic morphology may result in a significant effect on the transfer of information in neural circuits and would inevitably influence neurobehavioral performance.

The purpose of the present study was to assess the effects of developmental exposure to a commercial mixture of PCBs (Aroclor 1254) on PKC isoforms and neuronal dendritic morphology of hippocampal CA1 pyramidal neurons.

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 ^{o}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 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 dams were weighed and dosed daily between 8:00 and 10:00 am.

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, only litters with more than 10 pups were culled to 10 pups/litter, five males and five females.

Immunoblotting for PKC isoforms

Hippocampi were dissected from male pups on PND4, 7, 14, 21, and 60. Cell fractionation and subsequent immunoblotting were performed as described previously[5]. Proteins (10 mg) from cytosolic and particulate fractions were separated by 7.5 % SDS-PAGE and transferred to nitrocellulose membrane by Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA). The nitrocellulose sheet was blocked with 5 % non-fat dry milk in Tris buffered saline. PKC isoforms were detected with isoform-specific monoclonal antibodies for a, g and e 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).

Dendritic morphology

Cerebral hemispheres were obtained from male rats on PND22 and 60. The hemispheres were formalin-fixed for Rapid Golgi staining using the Golgi-Cox method. Coded slides of hippocampus were prepared. For branching and spine analysis of hippocampal neurons, 6 CA1 pyramids were randomly selected from each brain. Camera lucida drawings of the basilar dendritic tree were analyzed using the Sholl method of concentric circles[6]. This provides a profile of the extent and distribution of dendritic material in the dendritic arbor. For dendritic spine analysis, counts were made along internal and terminal tip segments of 6-7 neurons from each brain. Statistical analysis of the Sholl data was evaluated using the Wilcoxon rank-sign test. Analysis of the dendritic spines was evaluated using a one-way ANOVA and the Tukey post-hoc test.

Statistics

The PKC 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).

Protein kinase C isoforms

PKC has been implicated as an important factor in learning and memory processes and the etiology

of some neurological diseases[7]. 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 (a & g)and one Ca^{++}-independent forms (e)[8-10].

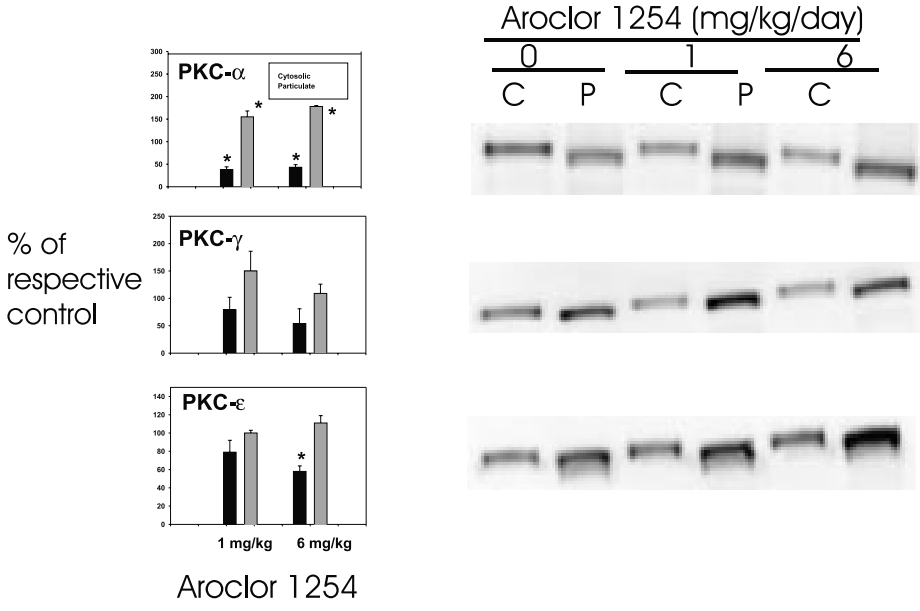

Figure 1. PKC isoforms in Hippocampus following developmental exposure to a mixture of PCBs. C = cytosolic fraction; P = particulate fraction.

Since cognitive deficits are considered as a significant developmental neurotoxic effect of PCBs, hippocampus was chosen as a target region. Although the study was performed at different age groups starting from PND4 through 60, significant observations made on PND14 are presented here due to the limited space (Fig. 1). Immunoblot analysis of PKC-a from hippocampus revealed that developmental exposure to Aroclor 1254 caused significant changes in PKC-a in cytosolic and particulate fractions. Analysis of PKC-g showed no significant changes either in cytosolic or particulate fractions. But, the ratio between the fractions showed a marginal increase (p=0.06). Analysis of PKC-e showed a significant decrease in cytosolic PKC-e 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 developmental PCB exposure were PKC isoform- and developmental stage-specific.

Dendritic branching and spine density in CA1 hippocampal pyramids

Developmental exposure to PCBs affected neuronal maturation. In 22 day-old rats, PCB exposure impaired normal dendritic development of CA1 pyramids. In neurons of the PCB-exposed rats, Sholl analysis revealed that there is significantly less branch material in the distal 2/3rds of the basilar dendritic arbor compared to the controls (Fig. 2). In 60 day-old rats, there is continued neurostructural disruption of the CA1 dendritic arbor following PCB exposure. Note however, that PCB exposure has now resulted in significantly more dendritic branching in the outer 2/3rds of their dendritic domains (Fig. 2). This hyperplastic “overshoot” in dendritic branching represents the structural basis for long-

lasting neurobehavioral effects of PCBs. In 22 day-old PCB-exposed rats, there is a significantly smaller spine density. By 60 days, the spine density returned to control levels (Fig. 2). This dysmorphic cytoarchitecture could be the structural basis for long-lasting neurocognitive deficits in PCB-exposed rats.

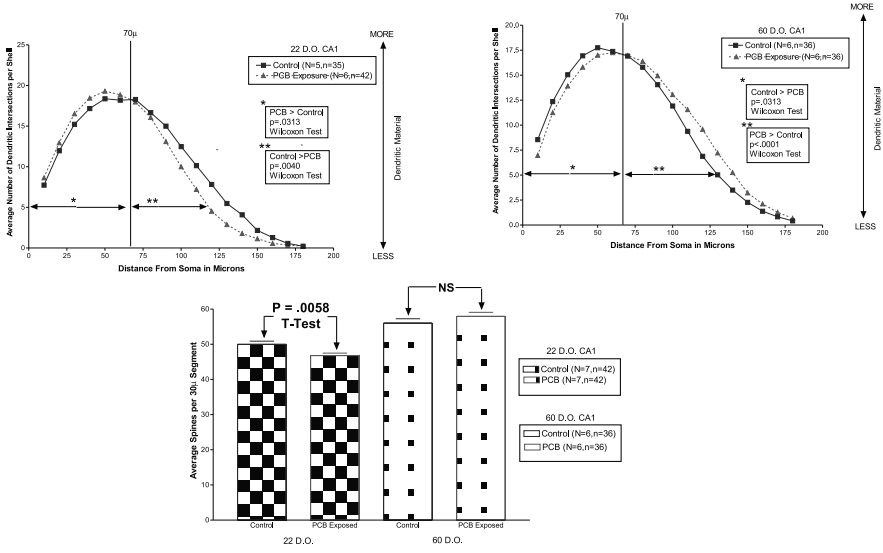

Figure 2. Dendritic branching and spine density in hippocampal CA1 pyramid neurons at PND22 and PND60 following developmental exposure to PCBs.

Results from this study indicate that developmental exposure to a PCB mixture resulted in altered cellular distribution of PKC isoforms which can subsequently disrupt the normal maintenance of signal transduction in developing neurons. The perturbations in intracellular signaling events could lead to structural changes in hippocampus. Our findings suggest that altered subcellular distribution of PKC isoforms may be a possible mode of action for PCB-induced cognitive dysfunction.

Acknowledgments

This abstract does not necessarily reflect USEPA policy.

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