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Neurotoxicity of Halogenated Aromatic Hydrocarbons; Structure-Activity Relationship

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Halogenated aromatic hydrocarbons (HAHs) including TCDD and PCBs are known to cause neurotoxic effects in both man and animals such as cognitive impairment and motor dysfunctions. While theses chemicals may lead to neurodevelopmental and neurobehavioral deficit, structural activity relationship and molecular targets for these chemicals are not elucidated. Since TCDD and certain congeners of PCBs accumulates in brain and the brain contains the Ah receptor, it is possible that these chemicals may act at the target site such as cerebellum, which is responsible for cognitive abilities and motor function.

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. Alteration of PKC in cerebellum is suggested to be associated with impaired motor dysfunction. Since PKC isozymes are differentially distributed in the brain cells and their roles are isozyme-specific and species-specific, it is important to identify the individual isoforms involved in the neurotoxic effects to understand the mechanism of action. A recent *in vitro* studies using cerebellar granule cells demonstrated a translocation of PKC-α and ε following the TCDD or PCB exposure. RACK (receptor for activated C-kinase) proteins play a key role in PKC activation and in membrane anchoring. Activation of certain isoforms (PKC-β, δ, and ε) is preferentially associated with RACK-1 which plays a significant role in PKC signaling pathway . However, it is not known whether RACK is a possible intracellular target for HAH-mediated signaling pathway.

To identify the intracellular target for HAHs and understand a signaling pathway in the developing brain, the present study attempted to analyze the PKC isoforms, RACK and

neurodevelopmental factors in the cerebellar granule cells following exposures to TCDD, coplanar PCB, non-coplanar PCB and PCB mixture (Aroclor 1254).

Certain types of HAHs are known to be sensitive to the developing brain and to affect the central nerve system. The HAH-induced neurodevelopmental deficits include the cognitive disability and motor dysfunction. PKC is implicated in learning and memory as well as in LTP. 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. Since functional roles and subcelluar distributions of individual PKC isoforms are isoform-specific and species-specific, identification of specific isoforms targeted for HAH is required to understand the mechanism of the HAH-induced neurotoxicity.

Ca^{2+}-independent PKCs have different substrate specificity or phospholipid dependency as compared to Ca^{2+}-dependent isoforms. Ca^{2+}-independent forms are suggested to be involved in the different cellular functions than Ca^{2+}-dependent forms. Although the physiological roles of Ca^{2+}-independent forms have not been fully clarified, it is known that PKC-ε, one of the Ca^{2+}-independent forms, is most abundant in the brain.

In the present study, the translocational effects of PKC-ε and δ were observed after a high dose exposure of TCDD for 60 min. Translocation of PKC-a and -ε was also observed after a high dose exposure of non-coplanar PCBS but not with coplanar PCBS. These PKC effects clearly demonstrate structure-activity relationship among PCB congeners.

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, it is speculated that PKC-ε may play a role in expression of GAP-43 in neuronal cells. In addition, PKC-ε has been suggested to be a candidate isoform associated with a mechanism of LTP. Thus, it is suggested that alteration of this particular isoform may perturb the normal maintenance of LTP and lead to the impairment of learning and memory. A subsequent abnormal expression of GAP-43 by the altered PKC-ε may disturb structural formation of neuronal cells. Regulation of neurotransmitter release may be interfered via altered

cytoskeleton networks. mRNA expression of GAP-43 and RC-3 was elevated with exposure to non-coplanar PCBs but the level was decreased following TCDD or coplanar PCBs, suggesting that these factors may be useful indicators for identifying the moieties of PCB struictures.

PKC-δ is known to play an important role in regulating cell cycle of cerebellar granule cells. Inhibition of PKC-δ led to the apoptosis of cerebellar granule cells derived from 8-day old rat cerebellum. Thus, the altered activation of PKC-δ may induce the dysregulation of neuronal cell proliferation, which may result in the neurological diseases. Since PKC-ε and δ have been associated with a variety of pivotal biological events in neuronal cells, it is feasible that altered subcellular distribution of these isoforms may play important roles in the HAH-induced neurotoxicity.

RACKs are 30- and 36-kDa proteins located in cytoskeletal compartment. RACK is one of proteins that anchor the activated PKC at the site of translocation. RACKs bind PKC only in the presence of PKC activators. Since different PKC isoforms translocate to distinct subcellular sites on activation, it is suggested that isoform-specific RACK may be present. Whereas isoform specificity of RACK is not known, PKC-β, δ, and ε tend to bind RACK more preferentially than other isoforms. Immunoblot analysis of RACK in the present study showed that level of RACK protein was increased with exposure to TCDD, suggesting that RACK may be an intracellular target molecule for TCDD. Dequalinium is known to PKC activity by blocking RACK-1 binding domain[22]. Pretreatment of dequalinium for 1 hr blocked the increase of RACK protein induced by TCDD. This result further suggests that TCDD be involved in the alteration of PKC signaling pathway in this neuronal cell system. Since RACK is a homolog of the G-protein beta subunit, altered level of RACK with TCDD exposure may disrupt normal signal pathway in brain, which ultimately may lead to neurobehavioral and cognitive deficit. Altered expression of RACK was not observed with exposure to non-coplanar/coplanar PCBs or PCB mixture, Aroclor 1254

Translocational effects of PKC-δ and ε with exposure to TCDD was slightly dampened by dequalinium chloride, indicating that TCDD targets PKC signaling system by altering a binding potential between RACK and activated PKCs. The results suggest that certain

PKC isoforms and their anchoring protein are possible intracellular targets of TCDD and alteration of these proteins may be associated with a mechanism of TCDD-induced neurotoxicity.

While RACKs are known to bind activated PKC in the presence of phosphatidylserine(PS), diacylglycerol(DAG) and calcium, PKC isoforms associated with these protein seems not to be limited to classical PKC isoforms, which require both calcium and DAG. The present study showed that RACK might be, at least, in part, involved in activation of novel PKC class, which does not require calcium for its activation.

The study provided the evidence that HAH has a clear structure-activity relationship with respect to neurodevelopmental factor, PKC activation and intracellular target molecules. Identification of target molecules and understanding of SAR as shown in the present study may contribute to understanding HAH-induced neurotoxic mechanism of action in neuronal cells, thereby improving the health risk assessment in humans.

Acknowledgments

This work was supported by an endocrine disrupter research project 2003 from Korea Food and Drug Administration.