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Possible Molecular Targets of Halogenated Aromatic Hydrocarbons in Neuronal Cells

Jae-Ho Yang
Catholic University of Daegu, School of Medicine

Halogenated Aromatic Hydrocarbons (HAH) is a class of widely dispersed, environmentally persistent compounds. Intensive industrial use and improper disposal of these chemicals resulted in a global contamination. One of the major class of HAH is PCBs with different structural characteristics of congeners. PCBs are stable, lipophilic and bioaccumulate in wildlife and foodstuffs. Human exposure to PCBs has been associated with several abnormalities. 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. Animal studies also demonstrated the neurotoxic potentials of PCBs including psychomotor dysfunction and cognitive deficits. While neurotoxic mechanism of PCBs still remains to be elucidated, structure-activity relationship has been described for some PCBs on neuronal tissues. Studies from our laboratory found that the ortho-, non-coplanar PCB altered intracellular Ca2+ homeostasis by inhibiting Ca2+ 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. Perturbation of Ca2+ 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. While translocation of PKCis one of the key effects of ortho-PCBs, it remains unknown which subspecies are target molecules. Evidence suggests that cerebellumis a storage site for the memory traces for discrete motor learning and classical conditioning of eyeblink response. Alteration of PKC in cerebellum is suggested to be associated with impaired motor dysfunction. The present study attempted to assess structure-activity relationship on PCB-induced subcellular changes in PKC isoforms and to identify target molecules sensitive to the PCB exposure in cerebellar granule cells in culture.

Cells were exposed to 2,2'-dichlorobiphenyl (ortho PCB; 2,2'-DCB), or 4,4'-dichlorobiphenyl (non-ortho PCB; 4,4'-DCB) for 15 min, respectively, and subsequently fractionated and immunoblotted against the selected PKC monoclonal antibodies (a,r,d,e,l, and I). While 2,2'-DCB induced a translocation of PKC-a [cytosol (% control): 54+12 at 25 uM and 66+10 at 50 uM; membrane (% control): 186+37 at 25 uM and 200+48 at 50 uM] and –e [cytosol (% control): 92+12 at 25 uM and 97+15 at 50 uM; membrane (% control): 143+23 at 25 uM and 192+24 at 50 uM] from cytosol to membrane fraction in a concentration-dependent manner, 4,4'-DCB had no effects. 2,2'-DCB induced translocation of PKC-a was blocked by the treatment of sphingosine, suggesting a possible role of sphingolipid pathway. Although reports on implication of PKC-r with learning and memory are relatively extensive, the expression of this particular isoform in the primary cerebellar granule cells was below the detectable level. PKC-d,-l and I were present in these cells, but were not altered by PCB exposure. These results suggest that the effects of 2,2'-DCB on PKC is subspecies-dependent and PKC-a as well as PKC-e may be target molecules for ortho-PCBs in neuronal cells.

PKC-a is specifically involved in PCB-induced activation of PLA2 in neutrophils and is selectively associated with lithium-induced memory impairments. Translocation of PKC-a has been associated with long-term potentiation in a hippocampus region. A significant translocation of PKC-a 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. While the functional roles of PKC-a in the cerebellar granule cells are not clear, altered subcellular distribution of this particular isoform may cause the disruption of normal signal pathways in the developing brain, which ultimately may lead to motor dysfunction and learning deficits.

Although the physiological roles of Ca2+-independent forms have not been fully clarified, it is known that PKC-e is most abundant in the brain and present mainly in the presynaptic component. PKC-e has been suggested to be a candidate isoform associated with this presynaptic mechanism of LTP. In addition, the altered activation of PKC-e may induce the dysregulation of neuronal cell proliferation, which may result in the neurological diseases. PKC-e is known to regulate the glial cell cycle and its overexpression is associated with astro-glial tumor. Since PKC-e has been associated with a variety of pivotal biological events in neuronal cells, it is feasible that altered subcellular distribution of this particular isoform may play important roles in the ortho-substituted PCB-induced neurotoxicity. Moreover, a significant translocation of PKC-e

in this study suggests that the DCBP-induced neurochemical changes may be, at least in part, mediated via calcium-independent pathway. Since neurochemical changes observed following DCB exposure have been observed only in the presence of extracellular calcium and most of neurochemical events have been considered Ca2+-dependent, altered subcellular distribution of the Ca2+-independent isoform in this study may shed a new light in the mechanistic studies of PCB-induced neurotoxicity.
Since the PKC translocation was considered Ca2+-dependent event, several potential routes of calcium entry into cerebellar granule cells were previously examined. Kodavanti et al. tried a variety of receptor-activated calcium channel blockers but none of theses blockers inhibited DCBP-induced translocation of PKC except sphingosine, a physiological and naturally occurring PKC inhibitor (unpublished data). Consistent with previous [3H]PDBu binding data, in the present study, preincubation of sphingosine for 2hr blocked the DCBP-induced translocation of PKC-a in the cerebellar granule cells. The result indicates that sphingosine is an effective blocker for the activation of PKC-a and suggests that the role of shingolipid signaling pathway may be associated with PCB effects. While roles of sphingosine in PKC inhibitory action are not known, it is speculated that change of fluidity on lipid bilayer membrane may be associated with inhibitory mechanism. However, roles of sphingosine on inhibitory effects of PKC translocation warrant further studies in the future.

The present study supports the previous findings that an ortho-substituted PCB congener is active on the PKC translocation, while a non-ortho-substituted 'dioxin-like' PCB is not, providing the additional evidence that the ortho-substituted PCB is neuroactive. It is believed that this study is a first report identifying specific PKC isoforms involved in a ortho-substituted PCB-induced PKC translocation. Identification of target molecules specifically responding to this class of environmental pollutants may contribute to understanding their mechanism of action, thereby improving the quality of the health risk assessment.