POSSIBLE MOLECULAR TARGETS FOR DIOXIN IN CEREBELLAR GRANULE CELLS

Jae-Ho Yang, Sun-Young Kim and Ji-Hye Lee

Department of Pharmacology/Toxicology. Catholic University of Daegu, School of Medicine. Daegu, Republic of Korea

Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to show direct effects on neuronal and glial cell development and disruption of neurotransmitters and endocrine systems. Both human and animal studies addressed neurotoxic effects of TCDD such as cognitive impairment and motor dysfunctions as neurodevelopmental outcomes [1,2]. While TCDD may lead to neurodevelopmental and neurobehavioral deficit[3], it is not known which molecular substances are targets for TCDD-induced developmental neurotoxicity. Since TCDD accumulates in brain and the brain contains the Ah receptor, it is possible that TCDD may act at the target site such as cerebellum. Evidence suggests that cerebellum is a storage site for the memory traces for discrete motor learning and classical conditioning of eyeblink response[4].

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[5]. Alteration of PKC in cerebellum is suggested to be associated with impaired motor dysfunction[6]. Since PKC isoforms are differentially distributed in the brain cells and their roles are isoform-specific and species-specific[7], it is important to identify the individual isoforms involved in the neurotoxic effects to understand the mechanism of action. A recent study using cerebellar granule cells demonstrated a translocation of PKC-alpha and epsilon following the PCB exposure[12]. However, it remains unknown which subspecies are target molecules for TCDD.

To identify the target molecules for TCDD in the developing brain, the present study attempted to analyze the PKC isoforms in the cerebellar granule cells

Materials and Methods

Cerebellar granule cell culture

Cerebellar granule cell cultures were prepared from the cerebella of 7-day old Sprague-Dawley rat pups as described previously[8]. Cells were plated at 3 x 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 0, 1, and 10 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (>99 % purity; KOR, Boston) for 15 min. 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. For the inhibition study, cells were treated with a-naphthoflavone (10 μM) for 1 hr prior to the exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin .

Immunoblotting

Immunoblot analysis was performed as described previously[9]. 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). 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 α,γ,δ,ε,λ, and Ι 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). The density of respective bands was analyzed by the Fluor-S (Bio-Rad, Hercules, CA). The data was represented as % controls.

Results and Discussion

TCDD is known to be sensitive to the developing brain and to affect the central nerve system[10,11]. The TCDD-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 [5]. Since functional roles and subcelluar distributions of individual PKC isoforms are isoform-specific and species-specific, identification of specific isoforms targeted for TCDD is required to understand the mechanism of the TCDD-induced neurotoxicity.

Immunoblot analysis revealed that translocations of PKC-α from cytosol to memebrane fractions were increased in a dose-dependent manner (Fig. 1). A translocation of PKC-α in this study provides an evidence on the involvement of this particular isoform in the TCDD-altered signal transduction pathway. Since PKC-α is Ca^{2+}-dependent isoform, the translocation may be mediated by the increased free calcium from the exposure of TCDD. As the study identified a specific PKC isoform that responds to TCDD, it is suggested that this isoform may be a target molecule. While the functional roles of PKC-α 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 cognitive deficits.

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 and present mainly in the presynaptic component[12]. 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)[13]. Thus, PKC-ε has been suggested to be a candidate isoform associated with this presynaptic mechanism of LTP. In the present study, the translocational effects of PKC-ε were most outstanding at the high dose (Fig. 2). It is suggested that alteration of this particular isoform may perturb the normal signaling pathway and induce the dysregulation of neuronal cell proliferation, which may result in the neurological diseases. 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 isoform may play important roles in the TCDD-induced neurotoxicity. Moreover, a translocation of PKC-ε in this study suggests that the TCDD-induced neurochemical changes may be, at least in part, mediated via calcium-independent pathway.

Translocation of PKC-α &-ε with exposure to TCDD was inhibited by the Ah receptor blocker, á -naphthoflavone (Fig. 1 and 2), indicating that TCDD-induced PKC translocation in the developing cerebellar cells is the Ah receptor-mediated action. In the previous study[14], only non-dioxin-like PCBs, which do not bind with the Ah receptor, revealed the significant translocation of PKC-α in the cerebellar granule cells, while dioxin-like PCBs did not. The present findings suggest that the structure-activity relation existing between PCB congeners does not apply for TCDD, the strongest Ah

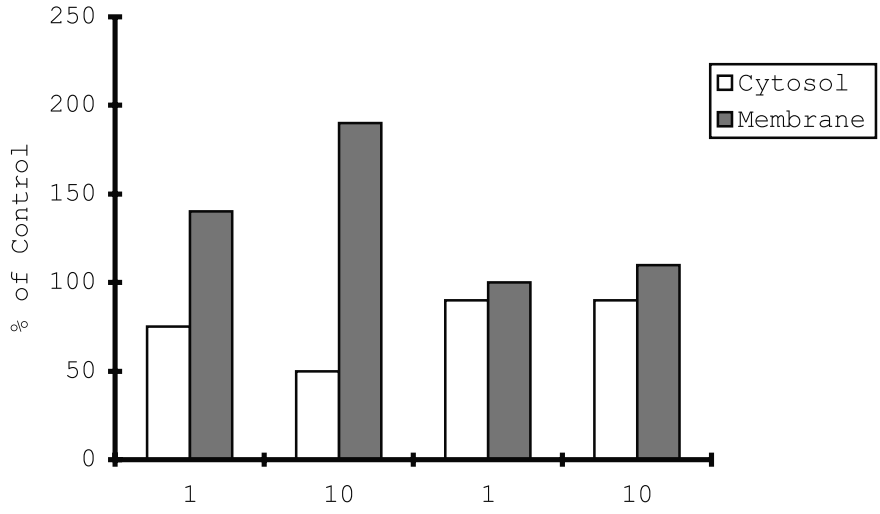

Figure 1. Effects of TCDD on subcellular distribution of PKC-α in SD rat cerebellar granule cells in presence or absence of α-naphthoflavone(α-NF).

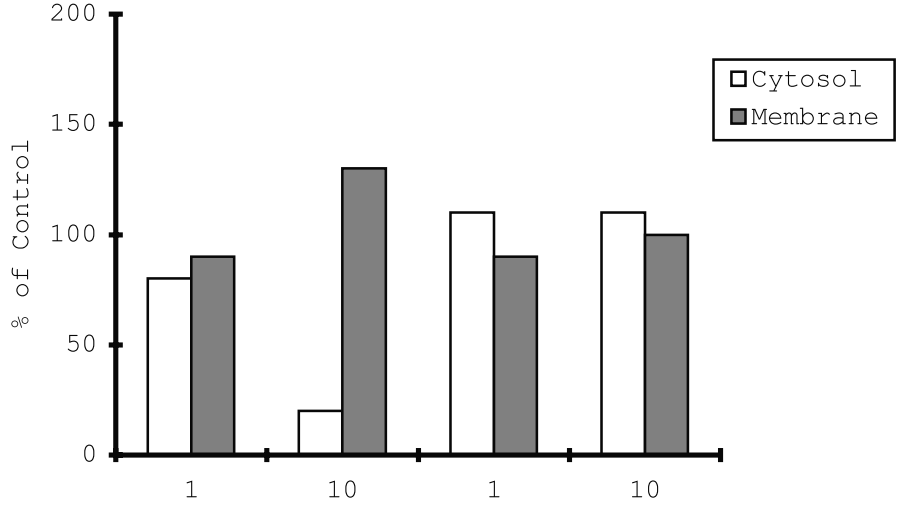

Figure 2. Effects of TCDD on subcellular distribution of PKC-ε in SD rat cerebellar granule cells in presence or absence of α-naphthoflavone(α -NF).

receptor agonist. It is speculated that the responses to TCDD via the Ah receptor may have a unique pathway different from other Ah receptor agonists.

The study provided the evidence that TCDD altered selective PKC isoforms in the developing neurons via the Ah receptor. It is believed that this study is a first report identifying specific PKC isoforms involved in the TCDD-induced PKC translocation. Identification of target molecules for

TCDD and confirmation of the Ah receptor-mediated pathway may contribute to further understanding of TCDD-induced neurotoxic mechanism of action in neuronal cells, thereby improving the health risk assessment in humans.

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