AN ALTERATION IN ENZYMATIC ACTIVITY OF NEURONAL ACETYLCHOLINESTERASE IN RESPONSE TO DIOXIN

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Introduction

Dioxins (polychlorinated dibenzo dioxins and polychlorinated dibenzo furans) or dioxin-like compounds (DLCs), cause multiple toxic effects, including increasing the risk of cancer, interference with the function and development of nervous, immune and reproductive systems[1]. More attention has been given to the toxicological effect of dioxins on advanced brain functions, as deficit in cognitive function was found in victims exposed to dioxins[2,3], as well as in experimental animals[4,5]. The influence of dioxins on neurotransmission associated with cognitive functions has been considered as one of the major mechanisms directly mediating the adverse effect of the dioxins. Evidence showed that glutamatergic, dopaminergic and 5-HT neurotransmission systems could be involved in dioxin- or DLC-induced adverse effect on cognition, such as in memory and learning functions[6]. However, due to the extreme complexity of multiple neurotransmission systems involved in advanced brain functions, the understanding of the mechanisms on dioxin- or DLC -induced neurotoxicity is far from clear. While cholinergic neurotransmission and acetylcholinesterase (AChE), a vital functional enzyme in the nervous system, play important roles in memory, cognition and development of the nervous system, the effects of dioxins or DLCs on the cholinergic system have not been clarified[7,8]. Therefore, in the present study we investigated the effects of dioxins or DLCs on enzymatic activity of AChE in cultured neuronal cells, in order to find possible direct interference with cholinergic systems by dioxins or DLCs.

Materials and methods

Cell culture and exposure to dioxin SK-N-SH, human neuroblastoma cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated at 37 ^{o}C in a water-saturated 5% CO_2 incubator. All reagents for cell cultures were from Invitrogen (Carlsbad, CA). The cells were seeded in 6-well-plates at 500,000 cells per well 24 hours before exposure to dioxin . The most potent congener of dioxins, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was purchased from Wellington Laboratories Inc. (Ontario, Canada) and employed at low doses of 10^{-12} ~10^{-9} M. The concentration of the solvent dimethyl sulfoxide (DMSO) was present at 0.1% for all treatments. Treated cultures were compared with cultures exposed to 0.1% DMSO alone.

Determination of AChE enzymatic activity AChE enzymatic activity was determined according to the method of Ellman[9] , modified by the addition of 0.1 mM tetra- isopropylpyrophosphoramide (iso-OMPA), an inhibitor of butyrylcholinesterase (BChE). After 6 to 48-hour-exposure, cells were collected and total protein extraction was performed in 200 μL of low salt lysis buffer (80 mM Na_2HPO_4, pH 7.4) supplemented with 0.5% Triton X-100 and protease inhibitor . About 30 μL cell lysate was incubated with 0.1 mM iso-OMPA for 10 min to inhibit the BChE activity, followed by addition of acetylthiocholine iodide (ATCh) to 0.625 mM and 5, 5'-dithiobis(2-nitrobenzenoic acid) to 0.5 mM. Absorbance at 410 nm was recorded and the rate of its increase was determined as a function of the total protein amount. All reagents were obtained from Sigma (St. Louis, MO). Protein concentrations were measured routinely by Bradford's method with a kit from Bio-Rad Laboratories (Hercules, CA).

Results and discussion

To investigate the dioxin-induced influence on the enzymatic activity of AChE in neurons, quiescent SK-N-SH neuroblastoma cells derived from human were exposed to TCDD (10^{-12} ~10^{-9} M) for 48 hours to observe the cytotoxicity. No obvious cell death could be found according to the observed morphological changes and cell viability assays (data not shown) after the TCDD treatments. Therefore time and dose effects of the treatment of TCDD on the enzymatic activity of AChE were determined. As compared to DMSO alone, the enzymatic

activities of AChE were reduced by ~20% after the 24-hour-exposure to TCDD. The suppression of AChE was first observed at 12 hours and continued through 48 hours of treatment (Figure 1B).

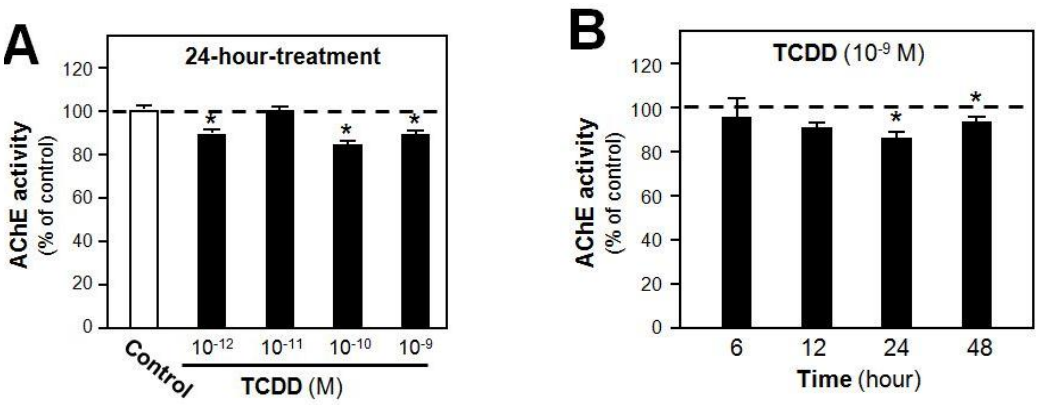

Figure 1 Dose and time effects of TCDD to the enzymatic activity of neuronal AChE. Cultured SH-N-SH cells were incubated with TCDD with doses from 10^{-12} to10^{-9} M or with solvent alone at 0.1% for 6 to 48 hours and AChE activity was determined as described in Materials and Methods. Dose (A) and time (B) effect of TCDD to AChE activity are shown. Values, calculated as % of solvent alone, were expressed as Mean±SEM, n=4, each with triplicate samples, * p<0.05, significant difference as compared to DMSO-treated controls, was determined by ANOVA.

The present finding is consistent with the decrease of AChE activity in the brains from the rats perinatally exposed to TCDD[10]. Furthermore, this study provides new evidence showing the influence of dioxin on the enzymatic activity of neuronal AChE, suggesting that dioxins may directly interfere with the cholinergic neurotransmission. This finding may pave the way for new research to understand the deleterious effects of dioxins on cognitive function. However, exploration of the transcriptional regulation of AChE by dioxin and the role of the aryl hydrocarbon receptor (AhR or dioxin) -dependent pathway, are worthy of further investigations. Considering the species specificity in dioxin-AhR interaction[11], whether the suppressive effect of dioxin on AChE is ubiquitous among various species needs to be addressed.

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