EFFECTS OF DIOXIN AND DIOXIN-LIKE COMPOUNDS ON NEURONAL ACETYLCHOLINESERASE ACTIVITY VIA Ah RECEPTOR

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Introduction

Exposure to the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs) and related dioxin-like compounds (DLCs) increases the risk of cancer and interfere with the function and development of the nervous, immune and reproductive systems[1-3]. Ah Receptor (AhR) is a member of the bHLH-PAS family of DNA-binding proteins, which activates gene expression in a ligand-dependent manner[4]. Upon activation by dioxin and DLCs, AhR translocates to the nucleus where it forms a heterodimer with ARNT, binds to xenobiotic responsive elements (XRE) in the promoter of its target genes, and initiates transcription [5].

As an important functional enzyme in cholinergic neurotransmission, acetylcholinesterase (AChE) plays vital roles in advanced brain functions[6]. It was reported that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can interfere with the development of the central cholinergic system[7], so that dioxins may act as a neuroendocrine disruptor. Moreover, the effects of TCDD on brain AChE are related to the alterations in thyroid development[7]. Recent research showed that AChE is not only a target of organophosphorus pesticides, but also of many other kinds of chemicals[8]. Generally, activity of AChE can be affected either by direct inhibition of enzymatic activity or by suppression of gene transcription[9]. Our previous study showed that TCDD can reduce AChE activity via transcriptional down regulations, mediated by AhR in SK-N-SH neuroblastoma cells[10]. Given that a large number of natural and synthetic AhR ligands (agonist and antagonists) have been identified and characterized[11], whether they could affect AChE expressions through the common mechanism, by activation of AhR and AhR-dependent signaling pathway, remains to be investigated.

Materials and methods

Cell culture. SK-N-SH, a cell line derived from human neuroblastoma cells, was purchased from the cell resource center of the Chinese Academy of Medical Sciences. These cells express both AChE and muscarinic acetylcholine receptor[12]. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated at 37 °C in a water-saturated 5% CO_2 incubator.

Chemical treatment. The cells were seeded in 6-well-plates at 500,000 cells per well 24 hours before exposure to dioxin or other drug treatment for AChE activity determination. The most potent congener of dioxins, TCDD was employed at low concentrations of 10^{-9} M. CH223191, an inhibitor of the AhR-dependent pathway[13], was employed at 10^{-6} M. 1,2,3,7,8-PCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, and 2,3,7,8-TBDD were employed respectively at concentrations of 10^{-9} M, 10^{-8} M, 3×10^{-9} M and 10^{-9} M. 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 or other indicated groups.

Luciferase assay. Cells were transfected with human AChE promoter-reporter construct (pAChE-Luc) together with cDNA encoding the β-galactosidase gene at 10:1 weight ratio. Twenty-four hours later, cells were treated with chemicals as described in the preceding Chemical treatment section. For luciferase measurement, sample wells were washed twice with phosphate-buffered saline, followed by the addition of cell lysis buffer (Promega) and shaking of the plates for 10 min at room temperature to allow cell lysis. Insoluble material was removed by centrifugation, and the resulting lysates were transfered to white 96-well microplates for measurement of luciferase activity using a TECAN Infinite F200 Pro luminometer with automatic injection of Promega stabilized luciferase reagent.

Determination of AChE enzymatic activity. AChE enzymatic activity was determined according to the method of Ellman[14], 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 BChE activity, followed by the 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.

Results and discussion

Compared to DMSO treatment, TCDD treatment significantly decreased the activity of AChE. In contrast, pretreatment with CH223191 reversed the suppressive effect of TCDD, indicating that AhR was involved in the dioxin-induced effect. Consistent with AChE activity, a significant decrease in human ACHE promoter activity was found upon TCDD (10^{-9} M) exposure as compared to DMSO treatment. Similar to the effects on AChE activity, pre-treatment with CH223191 (10^{-6} M) significantly reversed the suppressive effect of TCDD (10^{-9} M) on the promoter activity of human ACHE. The transcriptional regulation of AChE by dioxin was further confirmed by real time PCR analyses to determine expression levels of AChET mRNA. Results showed a 25% decrease in AChET mRNA level in response to TCDD exposure at 10^{-9} M. We concluded that exposure to dioxin leads to a decrease in the mRNA expression of AChE catalytic subunit resulting in decreased expression of the active form of AChE (Figure 1A, B).

In order to study the toxic effects of dioxin-like compounds on neurons, other AhR ligands, such as 1,2,3,7,8-pentachlorooxanthrene (1,2,3,7,8-PCDD), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PCDF), 2,3,7,8-tetrabromodibenzo-p-dioxin (2,3,7,8-TCDF) and 2,3,7,8-tetrabromodibenzo-p-dioxin (2,3,7,8-TBDD) were also tested to examine their effects on AChE activities in SK-N-SH cells. Our results showed that AChE activity were decreased by exposure to all tested AhR agonists suggesting that these chemicals may interfere with cholinergic functions in neurons. Compared with the 2,3,7,8-TCDD treatment, 2,3,7,8-TCDF and 2,3,4,7,8-PCDF treatments exerted more obvious effects on the AChE activities decrease. 1,2,3,7,8-PCDD, with one more chlorine atoms at position 1 can also strengthen the effects; 2,3,7,8-TBDD, with bromine atoms instead of chlorine atoms, did not show significant difference in the suppression of AChE. These results further support the role of AhR-dependent pathway in manipulating the expression of neuronal AChE (Figure 1D).

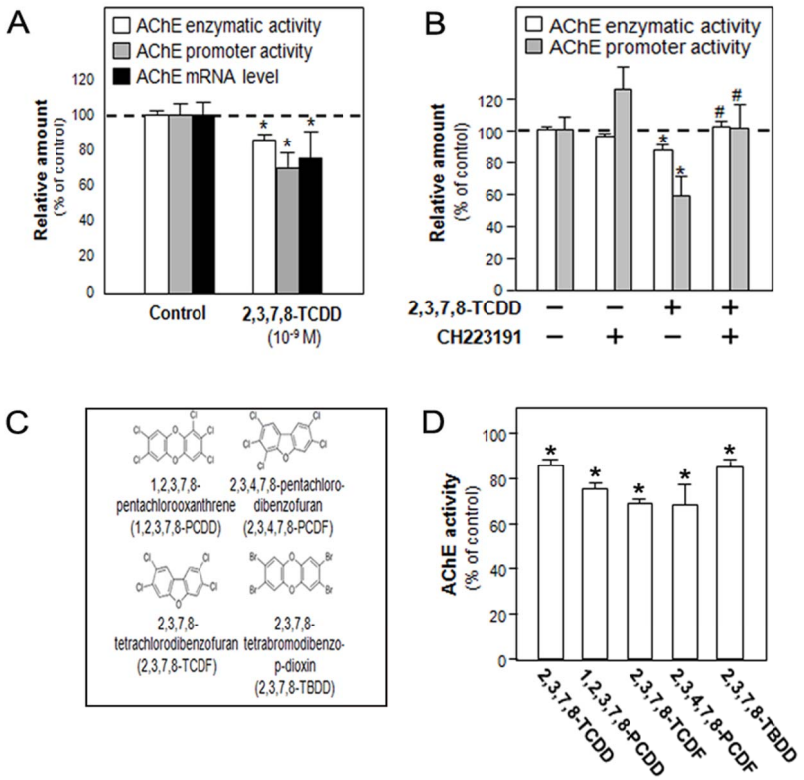

Figure 1. Effects of dioxin and dioxin-like compounds on the neuronal AchE activity, promoter activity and mRNA levels; Values, calculated as % of solvent alone, were expressed as Mean±SEM, n=4, each with triplicate samples, * p<0.05, significant difference as compared to DMSO-treated controls, was determined by ANOVA.

The finding may pave the way for new research to understand the deleterious effects of dioxins on advanced brain functions. All tested AhR agonists (dioxin and dioxin-like) could suppress neuronal AchE activity. The results support the part of AhR-dependent pathway in manipulating the expression of AchE activity. Explorations on the transcriptional regulation of AChE by DLCs or other AhR ligands are worthy of further investigations. Inhibition of AChE activity has been used as an indicator of organophosphorus insecticide (OP) exposure, because OPs irreversibly inhibit the activity of AChE by binding to its catalytic residue[9]. Our study, for the first time, disclosed that dioxin and DLCs could affect cholinergic neurotransmission system through a common and novel mechanism-AhR mediated transcriptional down-regulation of the neuronal AchE activity. These findings can establish a foundation for the application of AchE activity as an indicator of dioxin and DLCs exposure.

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References
1. Marinkovic N, Pasalic D, Ferencak G, Grskovic B, Stavljenic Rukavina A. (2010) Arh Hig Rada Toksikol. 61(4): p. 445-53.
2. Boucher O, G. Muckle, and C.H. Bastien. (2009) Environ Health Perspect. 117(1): p. 7-16.
3. White S.S. and L.S. Birnbaum. (2009) J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 27(4): p. 197-211.
4. Denison M.S. and S. Heath-Pagliuso. (1998) Bull Environ Contam Toxicol. 61(5): p. 557-68.
5. Beischlag T. V, Luis Morales J, Hollingshead B. D, Perdew G. H. (2008) Crit Rev Eukaryot Gene Expr. 18(3): p. 207-50.
6. Woolf N.J. and L.L. Butcher. (2011) Behav Brain Res. 221(2): p. 488-98.
7. Ahmed, R.G. (2011). Food Chem Toxicol. 49(6): p. 1276-84.
8. Xie W, Stribley J. A, Chatonnet A, Wilder P. J, Rizzino A, McComb R. D, Taylor P, Hinrichs S. H, Lockridge O. (2000) J Pharmacol Exp Ther. 293(3): p. 896-902.
9. Farahat F. M, Ellison C. A, Bonner M. R, McGarrigle B. P, Crane A. L, Fenske R. A, Lasarev M. R, Rohlman D. S, Anger W. K, Lein, P. J, Olson J. R. (2011) Environ Health Perspect. 119(6): p. 801-6.
10. Xie H. Q, Xu, H. M, Fu, H. L, Hu Q, Tian W. J, Pei X. H, Zhao B. (2013) Environ Health Perspect. 121(5): p. 613-8.
11. Denison M.S. and S.R. Nagy. (2003) Annu Rev Pharmacol Toxicol. 43: p. 309-34.
12. Ezoulin M. J, Ombetta J. E, Dutertre-Catella H, Warnet J. M, Massicot F. (2008) Neurotoxicology. 29(2): p. 270-7.
13. Zhao B, Degroot D. E, Hayashi A, He G, Denison M. S. (2010) Toxicol Sci. 117(2): p. 393-403.
14. Ellman G. L, Courtney K. D, Andres V, Jr.Feather-Stone R. M. (1961) Biochem Pharmacol. 7: p. 88-95.