ACETYLCHOLINESTERASE IS A TARGET GENE OF DIOXIN AND DIOXIN LIKE COMPOUNDS IN NEURONS

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Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) plays important roles in cholinergic neurotransmission in central and peripheral nervous system. Recently, AChE has drawn more and more attention in research area of environmental sciences. Apart from being used as a biomarker of organophosphorus pesticide (OP) exposure, emerging evidence has suggested that other classes of environmental pollutants, such as heavy metals, nanoparticles, as well as dioxin are able to interfere with the enzymatic activity of AChE[1, 2, 3]. Aryl hydrocarbon receptor (AhR) mediated transcriptional down regulation has been proposed as mechanism for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) to exert its adverse effect to AChE[3].

Dioxin and dioxin like compounds (DLCs) has long been one of the major focuses in environmental toxicology studies, including halogenated dibenzo-p-dioxins, dibenzofurans and coplanar polychlorinated biphenyls (PCBs)[4]. Long-term exposure to dioxins increases the risk of several types of cancer and interferes with the development and physiological function of the immune, nervous, endocrine, and reproductive systems. Dioxin and DLCs exert their biological and toxicological effects primarily via AhR-mediated pathway, in which dioxin and DLCs bind to AhR followed by nuclear translocation and binding to dioxin-responsive element (DRE) in target gene promoters[5]. Given the coexistence of dioxin and DLCs in environment, it is significant to address if AChE is a target gene consistently for other dioxins.

Therefore, in this study, the promoter activity, transcriptional expression as well as enzymatic activity of AChE were examined in SK-N-SH neuroblastoma cells, after exposure to several other classes of dioxins and DLCs, including 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PCDD), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PCDF).

Materials and methods

Cell culture

SK-N-SH cells were purchased from the cell resource center of the Chinese Academy of Medical Sciences (Beijing, China). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, and 1% penicillin-streptomycin, and was incubated at 37°C in a water saturated 5% CO2 incubator.

Exposure experiments

2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were purchased from Wellington Laboratories Inc. (Ontario, Canada). 1,2,3,7,8-PCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PCDF were dissolved in nonane (10% toluene). 2,3,7,8-TCDD was used as a positive control and was employed at concentration of 10^{-9} M. PCDD/Fs exposure concentrations were calculated based on World Health Organization (WHO) toxic equivalency factors (TEFs)[4]. Thus, 1,2,3,7,8-PCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-PCDF were employed at 10^{-9} M, 10^{-8} M and 3 x 10^{-9} M, respectively, which were equivalent to 2,3,7,8-TCDD at 10^{-9} M. The solvent was present in all treatments at 0.1%. The cells were seeded in 24-well-plates at 50,000 cells/well 24 h before exposure to chemicals for 24 h.

Reporter gene construction, transfection and luciferase activity determination

Cells were seeded in 96-well-plates at 10,000 cells/well 24 h before transient transfection using PolyJet™ reagent following the manufacturer's instructions (SignaGen Laboratories, Rockville, MD). The Renilla luciferase encoding plasmid pRL-SV40 (Promega) was used as a normalization control, which was co-transfected with pAChE -Luc into the cells. The mass ratio of target plasmid and control plasmid was 50:1. Twenty four hours thereafter, the cells were exposed to the chemicals for 24 h, and then washed with PBS and

underwent luciferase activity determination using a spectrometer with automatic injection of luciferase substrate (TECAN Infinite F200 Pro).

Real-time quantitative PCR

The total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was prepared using total RNA and Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen). Real-time PCR was performed on equal amounts of cDNA using SYBR Green Master mix and Rox reference dye (Promega). For human $AChE_T$ transcript (NM_000665), the primers were 5´-GGG GTT CCC CAG GTA AGT GAC CT-3´ (forward) and 5´-TGA GCA GCG ATC CTG CTT GCT GTA G-3´ (reverse). For human 18S rRNA (NR_003286), the primers were 5´-GAA CGA GGA ATT CCC AGT AAG -3´ (forward) and 5´- GAT AGT CAA GTT CGA CCG TC-3´ (reverse). The SYBR and Rox signal was measured by the MX3005P quantitative PCR system (Stratagene, La Jolla, CA). ΔΔCT method was used to quantify the relative mRNA expression level[6].

AChE enzymatic activity determination

AChE enzyme activity was determined by a modified method of Ellman et al[7]. First, cell lysates were prepared in low salt lysis buffer (80 mM disodium hydrogen phosphate, pH 7.4) supplemented with 0.5% Triton X-100 and 2.5 mM benzamidine (a protease inhibitor), then incubated with 0.1 mM tetraisopropyl pyrophosphoramide (iso-OMPA), an inhibitor of butyrylcholinesterase (BChE), and 0.5 mM 5,5'-dithiobis-(2-nitrobenzoic acid) for 30 min at room temperature for inhibiting BChE activity and allowing saturation of unspecific reactions. Acetylthiocholine iodide (0.625 mM) was subsequently added to initiate the AChE-specific reaction. Optical density (OD) at 410 nm was recorded with a microplate spectrometer (TECAN Infinite F200 Pro, Männedorf, Switzerland).

Results and discussion

The effects of DLCs on the transcriptional expression of AChE were investigated by promoter activity and real time PCR analysis. Results showed that human *ACHE* promoter activity, determined by co-transfection of pAChE-Luc together with control plasmid pRL-SV40, was significantly decreased after exposure to the DLCs (Figure 1). We further confirmed the transcriptional expression of AChE. $AChE_T$ mRNA expression levels were significantly decreased after 24-hour-exposure to 2,3,7,8-PCDD, 2,3,7,8-TCDF and 2,3,4,7,8-PCDF by approximately 25% (Figure 1). These results indicate that AChE is probably a common target gene of dioxin and DLCs, which consistently suppress the expression of AChE at the transciptional level in neurons. Dioxin and DLCs exert their biological and toxicological effects via AhR-dependent pathway. After passing the cell membrane, dioxin and DLCs bind to AhR in the cytoplasm, then AhR is transformed and translocated into the nucleus, in order to bind to ARNT to form a heterodimer. The heterdimer subsequently binds to DRE located upstream of human *ACHE* gene, whose function has been studied by mutagenesis previously[3]. However, the involvement of post-transcriptional and epigenetic regulations in dioxin and DLCs-induced suppression needs further investigations.

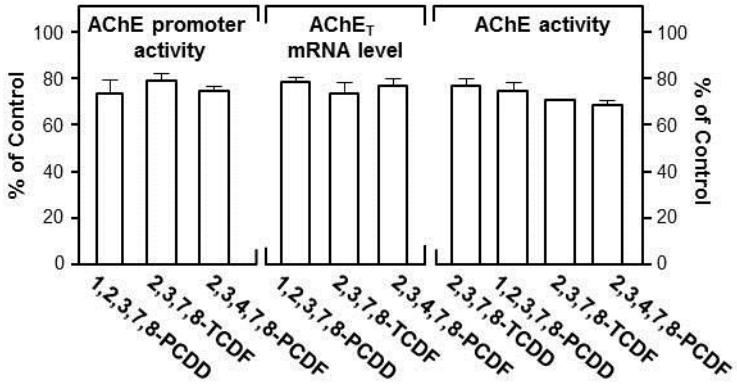

Figure 1 Effects of dioxin and DLCs to AChE expression in neurons

In line to the transcriptional down regulation, the enzymatic activity of AChE was decreased in SK-N-SH cells after exposure to dioxin and DLCs. Compared with the solvent control, AChE activity was reduced by approximately 20-30% after exposure to the chemicals (Figure 1). The results further support the notion that AChE is a functional molecule sensitive to dioxin and DLCs in neurons. However, whether DLCs could directly interact with the enzyme still needs to be examined, and comprehensive study on the oligmerisation and membrane localization of AChE need to be carried out in order to reveal the inteference of dioxins with AChE function.

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