Cod: 1.1014

EFFECTS OF DIOXIN ON NEURONAL ACETYLCHOLINESERASE ACTIVITY DURING NGF-INDUCED NEURONAL DIFFERENTIATION

Y. Chen[1], L. Xu[1], T. Xu[1], H.Q. Xie[1], H. Fu[1], B. Zhao[1]

[1] *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences,chinese academy of sciences, Beijing, China*

Introduction

Dioxin and Dioxin-like compounds could induce adverse effect on advanced brain functions, such as deficit in cognitive function1,2. Cholinergic neurotransmission system, especially acetylcholinesterase (AChE) plays an important role in advanced brain functions3. It was reported that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) could reduce AChE activity via transcriptional down regulation, mediated by aryl hydrocarbon receptor (AhR) in SK-N-SH neuroblastoma cells4,5. Thus AChE could be considered as a target of the neuronal toxicity of dioxin.

Neuronal differentiation is an essential basis of neuronal development and advanced brain functions. Generally, the expression level and enzymatic activity of AChE would be increased during neuronal differentiation6,7. In this study, we used PC12 cells as the study model, investigated the change of AChE activities upon the treatment of NGF and the effect of TCDD on AChE during different stages of NGF induced PC12 differentiation.

Material

Cell culture and drug treatment

Pheochromocytoma PC12 cells, a cell line derived from rat adrenal medulla, were obtained from American Type Culture Collection (ATCC, Manassas, VA), and which were maintained in Dulbecco's modified Eagle's medium supplemented with 6% fetal calf serum, 6% horse serum, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified CO2 (7.5%) incubator at 37 °C. Fresh medium was supplied every other day. All culture reagents were purchased from Life Technologies (Carlsbad, CA). During the treatment with NGF and TCDD, cultured PC12 cells were serum starved for 3 hours in Dulbecco's modified Eagle's medium supplemented with 1% fetal calf serum, 1% horse serum and penicillin-streptomycin, and then which were treated with the drug for certain time.

Determination of AChE enzymatic activity

AChE activities were determined by Ellman assay8. After certain treatments, cultured PC12 cells were collected in PBS (3 wells of cells from 6-well-plate were combined in one tube) and washed for 3 times and then resuspended in 1mL of low salt lysis buffer (pH=7.5) without Triton X-100 and all of the samples were diluted to equal cell density. Each sample was then equally divided into 3 parts: (1) anchored on the cell surface (cells were suspended in low salt lysis buffer without Triton X-100, AChE activity of the cell suspension was determined), (2) the background reading (cells were centrifuged and AChE activity in the supernatant was determined) (3) the total AChE activity from both intracellular and surface (Triton X-100 was added to the final concentration of 0.5%, AChE activity of total cell lysate was determined). Readings of sample (1) and (3) minus readings of sample (2) obtained the absolute AChE activity on the cell surface and from total cell, respectively.

Results and discussion

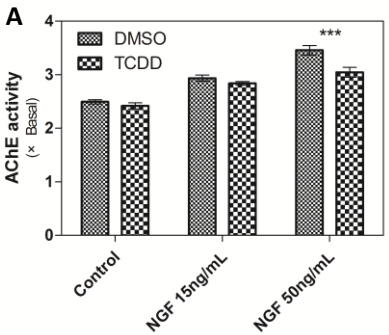
TCDD at the concentration of 1×10-9 M was applied to the cultured PC12 cells to investigate its influence on AChE activity during NGF induced neuronal differentiation. Results show that at the earlier stage of differentiation (NGF treated for 12 hours), TCDD cannot affect AChE activity; however, at later stage of differentiation (NGF treated for 72 hours), TCDD could slightly decrease AChE activity (Figure1A). The ratio of surface AChE activity to total AChE activity was also detected. Result shows that TCDD slightly increased the percentage of cell surface AChE activity (Figure1B).

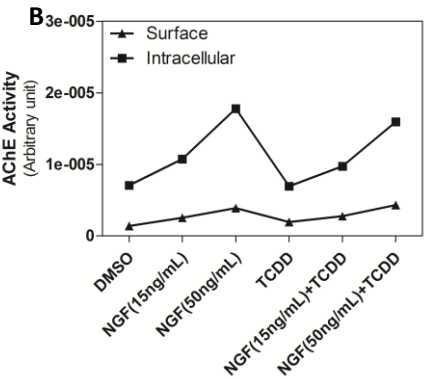
The TCDD concentration we used in this experiment is close to environmental levels. Previous work in our laboratory declared that dioxin could decrease AChE activity by about 15% in human-derived neuronal cells SK-N-SH5. In the present study, we found that total AChE activity of PC12 cells only could be decreased when the cells were differentiated and showed neuronal properties, while un-differentiated cells did not response to the treatment of TCDD.

Figure 1: TCDD affects the AChE activity of NGF induced differentiated PC 12 cells.
(A) To investigate the effect of TCDD on total AChE activity of NGF induced differentiated PC12 cells. PC12 cells were treated with TCDD with NGF, and equal volume of DMSO and NGF for 12 hours (left panel) and 72 hours (right panel). Total protein was extracted from each sample to perform the Ellman assay, and results were normalized by the protein concentrations. Values are expressed as the fold of change (X Basal) against the control (the AChE activity of PC12 cells treated by DMSO for 12 hours; set as 1), and in Mean ± SEM, n = 3, each with triplicate samples. ***where $p<0.01$ compared to the value of NGF 50ng/mL treatment without TCDD.
(B) To quantify the effect of TCDD on cell surface AChE activity of NGF induced differentiated PC12 cells. PC12 cells were treated with TCDD with NGF, and equal volume of DMSO and NGF for 72 hours. AChE activity on cell surface and total AchE activity was determined respectively. Values are expressed as the arbitrary unit, and in Mean ± SEM, each with triplicate samples.

Acknowledgements
This work was supported by grants from Natural Science Foundation of China (21407171, 21377160, 21525730), the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB14030400).

References
1. Barrett, D.H., et al., Serum dioxin and cognitive functioning among veterans of Operation Ranch Hand. Neurotoxicology, 2001. 22(4): p. 491-502.
2. Bouchard, M.F., et al., Polychlorinated biphenyl exposures and cognition in older U.S. adults: NHANES (1999-2002). Environ Health Perspect, 2014. 122(1): p. 73-8.
3. Soreq, H. and S. Seidman, Acetylcholinesterase - new roles for an old actor. Nature Reviews Neuroscience, 2001. 2(4): p. 294-302.
4. Xie, H.Q., et al., AhR-mediated effects of dioxin on neuronal acetylcholinesterase expression in vitro. Environ Health Perspect, 2013. 121(5): p. 613-8.
5. Xu, H.M., et al., Dioxin and dioxin-like compounds suppress acetylcholinesterase activity via transcriptional downregulations in vitro. J Mol Neurosci, 2014. 53(3): p. 417-23.
6. Choi, R.C., et al., Regulation of PRiMA-linked G(4) AChE by a cAMP-dependent signaling pathway in cultured rat pheochromocyoma PC12 cells. Chem Biol Interact, 2008. 175(1-3): p. 76-8.
7. Bi, C.W., et al., Quantification of the transcripts encoding different forms of AChE in various cell types: real-time PCR coupled with standards in revealing the copy number. J Mol Neurosci, 2014. 53(3): p. 461-8.
8. Ellman, G.L., et al., A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol, 1961. 7: p. 88-95.


A
DMSO
TCDD
***
AChE activity
(× Basal)
4
3
2
1
0
Control
NGF 15ng/mL
NGF 50ng/mL


B
Surface
Intracellular
AChE Activity
(Arbitrary unit)
3e-005
2e-005
1e-005
0
DMSO
NGF(15ng/mL)
NGF(50ng/mL)
TCDD
NGF(15ng/mL)+TCDD
NGF(50ng/mL)+TCDD