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New perspectives for multi-level regulations of neuronal acetylcholinesterase by dioxins

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ABSTRACT

Acetylcholinesterase (AChE; EC 3.1.1.7) is a vital functional enzyme in cholinergic neurotransmission which can rapidly hydrolyze neurotransmitter, acetylcholine, in the central and peripheral nervous systems. Emerging evidence showed that in addition to classical environmental AChE inhibitors, e.g. organophosphate and carbamate pesticides, dioxins are a new type of xenobiotic causing impairment of AChE. Dioxin can transcriptionally or post-transcriptionally suppress AChE expression in human neuroblastoma cells or mouse immune cells via the aryl hydrocarbon receptor (AhR) pathway, respectively. Dioxins can affect gene expression through other mechanisms, such as cross-talk with other signaling cascades and epigenetic modulations. Therefore, in this review, by summarizing the known mechanisms of AChE regulation and dioxin-induced gene alteration, potential signaling cascades and epigenetic mechanisms are proposed for dioxin-mediated AChE regulation. Mitogen activated protein (MAP) kinase, 3′, 5′-cyclic adenosine monophosphate (cAMP) and calcium-related singaling pathways, as well as potential epigenetic mechanisms, such as DNA methylation, and post-transcriptional regulation via microRNAs, including hsa-miR-132, hsa-miR-212 and hsa-miR-25-3p are discussed here. These proposed mechanisms may be invaluable not only to promote comprehensive understanding of the action mechanisms for dioxin, but to illustrate the molecular basis of dioxin-induced health impacts.

1. Introduction

Acetylcholinesterase (AChE; EC 3.1.1.7) plays a key role in cholinergic synapses in central and peripheral nervous systems by rapid hydrolyzing the neurotransmitter, acetylcholine [1]. It has been known for a long time that organophosphate and carbamate pesticides are able to specifically inhibit the enzymatic activity of AChE in different species. In particular, high level of exposure to the pesticides leads to acute neurotoxicity in humans [2]. In recent years, various environmental chemicals are found to impair AChE function, such as persistent organic pollutants which are drawing particular attention recently. These chemicals are persistent in the environment, accumulate in the biota through the food chain, and exert ecological toxicities and health impacts even after being eliminated from the environment [3]. Dioxins are representative of such chemicals whose toxicity and action mechanisms have been extensively investigated [4]. Dioxins, including polychlorinated dibenzodioxins and dibenzofurans, as well as polybrominated dibenzo-p-dioxins and dibenzofurans, are unintentionally produced by industrial processes and incinerations [4]. They exhibit a wide range of toxic effects (e.g., liver toxicity, immune system impairment, developmental deficit in the nervous system etc.) [5]. Gestational or adult exposure of dioxin was associated with a loss of higher-order brain functions (e.g., emotion, cognition and psychological disorders) in animals and humans [6]. It is generally accepted that dioxins exert their biological and toxicological effects

Abbreviations: AChE, acetylcholinesterase; AhR, aryl hydrocarbon receptor; MAP, mitogen activated protein; cAMP, 3′, 5′-cyclic adenosine monophosphate; miRs, microRNAs.

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by transcriptional gene regulation via the aryl hydrocarbon receptor (AhR) pathway [6]. In addition, the cross-talk of AhR pathway with various intracellular signaling cascades was proposed to be involved in the toxicity triggered by dioxins [7].

Interference of dioxin and dioxin-like compounds with AChE and other cholinergic molecules were first reported as indirect effects due to hypothyroidism-induced developmental deficits in the nervous system [8]. Upon maternal administration of dioxin, the brain tissue of the offspring exhibited lower enzymatic activity of AChE compared to control rats [8]. Dioxin-induced hypothyroidism was proposed as the mechanism for the developmental defect of AChE [8]. This indirect effect was consistent with that of Aroclor 1254 (a mixture of dioxin-like and non-dioxin like polychlorinated biphenyl (PCB)), in which choline acetyltransferase (ChAT) activity was reduced [9]. Studies by us and others have demonstrated that dioxins suppress AChE expression directly in neuroblastoma cells and immune cells upon dioxin exposure, in which transcriptional regulation via AhR and post-translational regulation via microRNA (miR) were involved, respectively [10–12]. Since the dioxin-induced gene alterations involve a complex multi-level regulatory network, present knowledge on how dioxins affect AChE is not sufficient. Therefore, we have summarized intersectional mechanisms for AChE and dioxin-related gene regulation at the transcriptional and post-transcriptional levels, as to provide hints to uncover a full picture of the underlining mechanisms for dioxin effects.

2. Potential signaling cascades beyond AhR-dependent pathways

Our previous work demonstrated that dioxin decreased AChE activity by transcriptional suppression of the $AChE_T$ subunit, a major variant of AChE transcript in the nervous system, in SK-N-SH human neuroblastoma cells: this suppression was mediated via AhR-dependent pathway [10]. This transcriptional alteration of AChE led to a decrease in the protein expression, which consequently caused the decrease in AChE enzymatic activity[10]. There was no effect on the $AChE_R$ subunit, a rare splicing variant in the brain, nor on proline-rich membrane anchor (PRiMA), a structural subunit of neuronal AChE [10]. Consensus sequences of dioxin responsive element were found in the regulatory region upstream of the human *ACHE* gene, which might mediate the above transcriptional regulation [10]. Whether dioxin could influence AChE expression via acting on known AChE-related signaling pathways in muscle and neurons needs further investigation.

Mitogen activated protein (MAP) kinase has been demonstrated to be involved in the transcriptional up-regulation of $AChE_T$ and its structural subunit, PRiMA, during neuronal differentiation [13,14]. A recent study on neuro-beneficial effects of phyto-chemicals also suggested that the MAP kinase pathway could mediate xenobiotic-induced alterations of AChE in PC-12 cells [15,16]. Interestingly, the MAP kinase pathway also participated in the toxicological effects of dioxin. In cultured cerebellar granule cells, the MAP kinase was involved in dioxin-induced reactive oxygen species production [17]. Moreover, this pathway also had cross-talk with AhR and participated in dioxin effects in cancer and dendritic cells, as well as during osteoblast differentiation [18–20]. These pieces of evidence suggest a possible involvement of the MAP kinase pathway in AChE regulation triggered by dioxin.

As one of the secondary messengers, the 3′, 5′-cyclic adenosine monophosphate (cAMP) signaling was proposed to be involved in the tight control of AChE and collagen Q (ColQ) expression, during myogenesis and muscle innervation [21–25]. Accordingly, enhancement of cAMP-signaling by administration of db-cAMP or foskolin was found to interfere with dioxin-dependent induction of *CYP1A1* in mouse hepatoma cells [26]. On the other hand, the inhibitory effect of dioxin on cAMP-induced elevation of a glial differentiation marker, *GFAP*, was documented in cultured C6 glial cells [27]. Although there is no solid evidence showing a direct interaction of cAMP signaling and AhR signaling in neurons, the evidence of having cross-talk between these two signalings raises a possible involvement of cAMP in dioxin effects on neuronal AChE expression.

Apart from these two signaling cascades, calcium is another possible signaling molecule that is involved in dioxin-mediated AChE modulation. Dioxin was found to induce apoptosis by disruption of intracellular calcium homeostasis in human neuronal cell line SHSY5Y [28]. On the other hand, a signaling cascade triggered by calcium mobilization was involved in P2Y2 receptor-induced AChE expression in rat cortical neurons [14]. Thus, calcium-related signaling cascade(s) might be a candidate responsible for dioxin-induced transcriptional alterations of AChE.

3. Epigenetic regulatory mechanisms

3.1. Potential roles of DNA methylation and histone modification

Transcriptional activity of a gene can be regulated by epigenetic processes, involving DNA methylation and histone modification and microRNAs(miRs) expression [29]. Lau et al. demonstrated that after administration of a DNA methyltransferase inhibitor, the expression profile of AChE changed dramatically during the myogenesis process in C2C12 cells [30]. One SP1 site located 1826 bp upstream of the mouse *ACHE* gene was revealed to be methylated in this study [30]. Sailaja et al. investigated the epigenetic mechanism of the stress-induced long-lasting decrease of two AChE splicing variants distinct at the 5′ exons in hippocampus. Alteration of the splicing variants was due to reduced acetylation and elevated trimethylation of H3K9 at the corresponding promoter, in which histone deacetylase 4 served as a mediator [31].

Accordingly, emerging evidence has shown involvement of dioxins in epigenetic processes. Dioxin exposure has been associated with changes in DNA methylation status, that might in turn affect the dioxin-induced gene expression in splenocytes [32]. In addition to experimental data, high serum levels of dioxin OCDD and one dioxin-like PCB, PCB126, were associated with global DNA hypermethylation in an elderly population [33].

Thus the interference of dioxins with global DNA methylation might result in general alterations of a series of genes, perhaps including AChE. It is possible that altered DNA methylation levels induced by dioxin or dioxin-like compounds might particularly affect transcriptional properties of AChE in brain and/or in muscle.

3.2. Potential post-transcriptional modulations via dioxin-related miRs

MicroRNAs (miRs) are defined as short noncoding RNA molecules, 18–25 nucleotides in length, and their main function is to post-transcriptionally down-regulate expression of their target genes by inhibiting the translation or inducing the mRNA degradation. In the nervous system, miRs have been definitively linked to development, synaptic plasticity and neurodegenerative diseases [34]. Neuronal AChE is also regulated by miRs. In murine cell lines, miR-132 has been documented to target $AChE_T$ mRNA and consequently affect the AChE expression during stress-induced cognitive impairment [35]. In addition, primate-specific miR-608 was recently found to target AChE in human-derived cell lines [36]. By using PicTar, miRanda, miRbase, and microCosm algorithms, 47 miRs were predicted to target the human $AChE_T$ variant, and 81 miRs to target human $AChE_R$ [37]. By integrating the predicted data