Available online at www.sciencedirect.com
ScienceDirect
Toxicology 233 (2007) 97–107
TOXICOLOGY
www.elsevier.com/locate/toxicol

Adaptive changes in acetylcholinesterase gene expression as mediators of recovery from chemical and biological insults

Tama Evron [a], David Greenberg [a], Tsafrir S. Mor [b], Hermona Soreq [a,*]

[a] *Department of Biological Chemistry, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*
[b] *School of Life Sciences and The Biodesign Institute, P.O. Box 874501, Arizona State University, Tempe, AZ 85287-4501, USA*

Received 27 April 2006; received in revised form 10 August 2006; accepted 11 August 2006
Available online 22 August 2006

Abstract

Both organophosphate (OP) exposure and bacterial infection notably induce short- and long-term cholinergic responses. These span the central and peripheral nervous system, neuromuscular pathway and hematopoietic cells and involve over-expression of the "readthrough" variant of acetylcholinesterase, AChE-R, and its naturally cleavable C-terminal peptide ARP. However, the causal involvement of these changes with post-exposure recovery as opposed to apoptotic events remained to be demonstrated. Here, we report the establishment of stably transfected cell lines expressing catalytically active human "synaptic" AChE-S or AChE-R which are fully viable and non-apoptotic. In addition, intraperitoneally injected synthetic mouse ARP (mARP) elevated serum AChE levels post-paraoxon exposure. Moreover, mARP treatment ameliorated post-exposure increases in corticosterone and decreases in AChE gene expression and facilitated earlier retrieval of motor activity following both paraoxon and lipopolysaccharide (LPS) exposures. Our findings suggest a potential physiological role for overproduction of AChE-R and the ARP peptide following exposure to both chemical warfare agents and bacterial LPS.

Keywords: Acetylcholinesterase; Body temperature; Gene expression; Motor activity; Paraoxon

1. Introduction

Exposure to chemical warfare agents, as well as sub-lethal exposure to pesticides, and exposure to bacterial endotoxin, all induce short- and long-term responses that involve cholinergic elements (Taylor, 1996). A solidly grounded understanding of the cholinergic events that follow such exposures will allow a more effective treatment of the symptoms and the underlying pathology. These events involve the corresponding genes, transcriptional and post-transcriptional processes and signal transduction events that depend on protein–protein interactions of the resultant products and which initiate signaling pathways. The biological systems that respond to organophosphate (OP) and other anti-cholinesterase (anti-ChE) agents include the central and peripheral nervous system, the neuromuscular pathway and hematopoietic cells (Soreq and Seidman, 2001). Due to the common response elements, these systems are involved in responding to both chemical and biological warfare (CBW) agents. The result of acetylcholinesterase (AChE) inhibition is the accumulation of acetylcholine (ACh) at synapses of the central and peripheral nervous systems, leading to short-term severe symptoms (e.g. drop in blood pressure, convulsions, respiratory failure). Longer-term effects

* Corresponding author. Tel.: +972 2 6585109; fax: +972 2 6520258.
E-mail address: soreq@cc.huji.ac.il (H. Soreq).

0300-483X/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2006.08.018

of sub-lethal exposure are cognitive decline including memory impairments and muscle fatigue (Rosenstock et al., 1991).

Chemical agents initiate immune response as well. The nerve agent soman induces an increase in the pro-inflammatory cytokine interleukin 1β (IL-1β) in rats (Svensson et al., 2001). OP exposure was shown to inhibit human lymphocyte and natural killer (NK) cell activity (Dyer et al., 2001). The long-term effects of OP exposure are reminiscent of those of stress, and profound effects of stress have been demonstrated on the immune system: it increases production of pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, IL-6 and IFN-γ, and the negative immunoregulatory cytokines, IL-10 and IL-4 (Maes et al., 1998), and it depresses serum cortisol levels and increases receptor numbers, resulting in a greater sensitivity to exogenous corticoids and wide diurnal variation (Golier and Yehuda, 1998).

The "cholinergic anti-inflammatory pathway" involves efferent vagus nerve cholinergic signaling in modulation of the inflammatory response (Bernik et al., 2002; Borovikova et al., 2000; Metz and Tracey, 2005; Tracey et al., 2001; Wang et al., 2003). Acetylcholine, the principal vagal neurotransmitter, significantly attenuates the release of the cytokines, TNF, IL-1β, IL-6 and IL-18, but not the anti-inflammatory cytokine IL-10, in lipopolysaccharide (LPS)-stimulated human macrophage cultures. AChE inhibitors, which lead to increased levels of ACh, reduce IL-1β levels in the circulation as well as within the brain (Pollak et al., 2005). Moreover, immune stimulation by LPS influences AChE activity in human serum (Cohen et al., 2003) and in pro-megakaryocytes (Pick et al., 2006).

Human and mouse AChE pre-mRNA harbors alternative splicing sites at both 3′ (Li et al., 1991; Soreq and Seidman, 2001) and 5′ ends (Luo et al., 1998; Meshorer et al., 2004), the latter with corresponding promoters (Atanasova et al., 1999; Meshorer and Soreq, 2006). Alternative splicing at the 3′ ends yields three AChE variants with different carboxy terminal sequences which determine the protein product multimerization capacities and its cellular localization (Meshorer and Soreq, 2006; Soreq and Seidman, 2001). Synaptic AChE-S, the main transcript expressed in the nervous system and neuromuscular junctions (NMJs) contains a C-terminal cysteine, allowing dimerization by disulphide bonds. This dimer can form tetramers by the attachment of additional monomers (Bon et al., 1997) and can adhere to the membrane through the ColQ and PRiMA structural subunits (Ohno et al., 1999; Perrier et al., 2002). The erythrocytic, AChE-E transcript includes a glycyl bond in its C-terminus which allows the attachment of a glycophosphatidylinositol group to the protein, followed by its anchoring to erythrocyte membrane as a dimer. The normally rare, stress-induced "readthrough", AChE-R variant remains a soluble monomer (Soreq and Seidman, 2001). In the mouse gene the expression of four alternative exons at the 5′ end, named mE1a-d was confirmed experimentally (Meshorer et al., 2004). In the human gene the expression of three exons, named hE1a, hE1b and hE1e was confirmed. hE1e was shown to extend the known open reading frame (ORF) of ACHE and to yield a novel membrane-attached protein, N-AChE, detected in several brain regions and hematopoietic cells (Meshorer et al., 2004). The combination of 3′ splicing with the novel N-terminus may attach monomeric AChE-R and AChE-S to the membrane, leading to new, yet unidentified functions.

Recent reports that AChE overexpression leads to apoptosis (Park et al., 2004; Zhang et al., 2002) raised the question whether the post-exposure changes in AChE gene expression reflect exposure-induced damages or, inversely, adaptive reactions with protective value. To distinguish between these two possibilities, we performed experiments in cultured CHO cells and in live mice. First, we established cell lines stably over-expressing either AChE-S or AChE-R of human origin, which demonstrated threshold values of AChE levels that are fully compatible with cell viability. Second, we exposed live mice to sublethal levels of either the organophosphate poison paraoxon or bacterial lypopolysaccharide (LPS) with or without co-administration of mouse (m)ARP and followed their post-exposure phenotype and AChE production. Our findings are compatible with the hypothesis of adaptive AChE overproduction and suggest the post-exposure use of synthetic ARP for facilitating rapid recovery.

2. Materials and methods

2.1. Establishment of stable cell lines over-expressing specific AChE variants

CHO cells were grown as described (Perry et al., 2004). Transfection involved the AChE-S and AChE-R encoding cDNAs (Soreq and Seidman, 2001) as well as cDNA encoding green fluorescent protein (GFP, Perry et al., 2002) and followed standard selection procedures.

2.2. In vivo experiments

A 7–8 weeks CD-1 male mice were housed in a specific pathogen free (SPF) environment (maximum six animals per cage). Animals were provided ad libitum, a commercial rodent diet (Harlan Teklad TRM Rat/Mouse Diet, Rehovot,