Computational Biology and Chemistry 75 (2018) 74–81

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

Mechanisms of acetylcholinesterase protection against sarin and soman by adenosine A_1 receptor agonist N^6-cyclopentyladenosine

Ariana Beste[a,*], DeCarlos E. Taylor[a], Tsung-Ming Shih[b], Thaddeus P. Thomas[a,b]

[a] US Army Research Laboratory, Deer Creek Loop, Aberdeen Proving Ground, MD 21005-5069, USA
[b] US Army Medical Research Institute of Chemical Defense, 2900 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA

ARTICLE INFO

Article history:
Received 31 January 2018
Received in revised form 25 April 2018
Accepted 29 April 2018
Available online xxx

Keywords:
Nerve agent
Antidote
Enzyme inhibition
Reactivation
Thermodynamic analysis
Density functional theory

ABSTRACT

Organophosphorus nerve agents (NAs) irreversibly inhibit acetylcholinesterase (AChE), the enzyme responsible for breaking down the neurotransmitter acetylcholine (ACh). The over accumulation of ACh after NA exposure leads to cholinergic toxicity, seizure, and death. Current medical countermeasures effectively mitigate peripheral symptoms, however; the brain is often unprotected. Alternative acute treatment with the adenosine A_1 receptor agonist N^6-cyclopentyladensosine (CPA) has previously been demonstrated to prevent AChE inhibition as well as to suppress neuronal activity. The mechanism of AChE protection is unknown. To elucidate the feasibility of potential CPA-AChE interaction mechanisms, we applied a truncated molecular model approach and density functional theory. The candidate mechanisms studied are reversible enzyme inhibition, enzyme reactivation, and NA blocking prior to enzyme conjugation. Our thermodynamic data suggest that CPA can compete with the NAs sarin and soman for the active site of AChE, but may, in contrast to NAs, undergo back-reaction. We found a strong interaction between CPA and NA conjugated AChE, making enzyme reactivation unlikely but possibly allowing for CPA protection through the prevention of NA aging. The data also indicates that there is an affinity between CPA and unbound NAs. The results from this study support the hypothesis that CPA counters NA toxicity *via* multiple mechanisms and is a promising therapeutic strategy that warrants further development.

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1. Introduction

Acetylcholinesterase (AChE) is an enzyme in the nervous system that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh), thereby, terminating neurotransmission at cholinergic synapses. The active site is a catalytic triad consisting of a nucleophile (serine), a base (histidine), and an acid (glutamic acid) (Sussman et al., 1991). The nucleophilic serine covalently binds ACh involving hydrogen transfer from serine to the histidine base (Quinn, 1987). Release of choline leads to the formation of an acyl enzyme that is, subsequently, hydrolyzed to yield acetic acid and the regenerated enzyme (Colovic et al., 2013). Exposure of AChE to organophosphorus nerve agents (NAs), such as soman, sarin, or tabun, results in inhibition of the active site through covalent binding of the agents at the catalytic serine (phosphorylation) similar to binding of the ACh substrate (Colovic et al., 2013). Whereas the substrate is, afterwards, released from the active site *via* deacylation, the organophosphorus NAs undergo dealkylation (also called “aging”) which causes irreversible inhibition of AChE and loss of biological activity (Colovic et al., 2013). During the aging reaction, the imidazolium of the catalytic histidine stabilizes the developing negative charge on the phosphoryl oxygen, allowing a water molecule to attack the adjacent carbocationic center, which leads to carbon-oxygen bond breaking. The aging product is stabilized by the formation of a salt bridge (Carletti et al., 2008; Millard et al., 1999). Without sufficient AChE activity, there is an over accumulation of ACh at cholinergic receptor sites and consequential hyper-stimulation of the nervous system and, eventually, death (Weinbroum, 2005).

The traditional treatment for NA poisoning is a combination of atropine and a pyridinium oxime. While atropine counteracts the effects of excess concentration of ACh at the cholinergic synapses, oximes predominantly function as a reactivator of phosphorylated AChE (Maxwell et al., 2013; Chambers et al., 2015; Worek et al., 2016). Oximes may also bind reversibly to AChE (Simeon-Rudolf et al., 1999), in which case the therapeutic effect is due to a competition between the NA and the oxime for the active site. Available oximes such as 2-PAM, HI-6, TMB-4, LüH-6, and MMB-4

* Corresponding author.
E-mail address: ariana.beste.ctr@mail.mil (A. Beste).

https://doi.org/10.1016/j.compbiolchem.2018.04.017
1476-9271/Published by Elsevier Ltd.

(all containing pyridinium rings) carry the oxime functional group at different ring positions (Jokanovic, 2012). The effectiveness of NA removal by the oxime depends on structural properties of the NA as well as oxime type. Currently, there is no universal antidote available that protects against all organophosphorus NAs. Moreover, current medical countermeasures are often ineffective at protecting the brain from seizure and neuropathology. Mono- and bipyridinium oximes have a limited ability to cross the blood-brain barrier (Joosen et al., 2011), and seizures become refractory to inhibitory pharmacologics after a period of time. Limited efficacy of delayed oxime treatment is due to the onset of the "aging" reaction that prevents reactivation (Carletti et al., 2008; Hornberg et al., 2007).

The adenosine A1 receptor (A1AR) is a promising therapeutic target due to its efficacy in suppressing pathological neuronal activity and consequential minimization of brain damage (Jacobson and Gao, 2006; Cunha, 2016). Toward investigating its neuroprotective potential for counteracting NAs, the A1AR agonist N^6-cyclopentyladensosine (CPA) has been tested in small animal NA-induced seizure models. It has been shown that treatment with CPA effectively prevents soman and VX seizure from occurring when given acutely, and even more remarkably, CPA terminates NA-induced sustained seizure activity (Acon-Chen et al., 2016; Thomas and Shih, 2014). Moreover, adenosine's suppression of excitotoxicity translated to neuroprotection and prevention of brain damage as assessed by neurohistopathology (Thomas et al., 2017). The results from those studies strongly suggest CPA's neuroprotective effect is a consequence of direct inhibition on the central nervous system *via* pre- and postsynaptic neuronal mechanisms. Data from further studies (Thomas and Shih, 2016) aimed to elucidate CPA's neuroprotective mechanism by measuring possible effects on AChE activity. To do so, AChE activity was measured in rats that received a seizure inducing dose of soman, and then treated with saline or CPA at one or fifteen minutes later (Thomas and Shih, 2016). Surprisingly, treatment with CPA at 1 min after exposure protected the brain's AChE from NA inhibition, *i.e.*, 83% (relative to baseline) of central AChE remained active. In contrast, central AChE activity in animals not receiving CPA was drastically reduced to 6.2% of baseline activity (Thomas and Shih, 2016). Unlike the acute treatment group, delayed CPA treatment (15 min after soman) did not protect the animal's AChE; their activity levels were comparable to untreated rats. However, even though AChE was not protected, delayed CPA effectively terminated cholinergic symptoms (*e.g.*, convulsions, hypersecretions). No changes in AChE activity were observed in rats that were not exposed to soman but treated with CPA (Thomas and Shih, 2016). The protection of AChE with acute CPA treatment was unexpected; *in vitro* studies conducted at The Netherlands Organization for Applied Scientific Research (TNO) indicated that CPA does not interact with AChE (Bueters et al., 2003).

The mechanism of AChE activity preservation with acute CPA treatment is unclear. Fig. 1 shows postulated pathways of enzyme protection that are, in part, motivated by previous experimental findings (Thomas and Shih, 2016). On one hand, the observation that CPA is ineffective if treatment is delayed may indicate that CPA has to be present at the active site before soman poisoning. This suggests a mechanism involving reversible enzyme inhibition by CPA in competition with the NA, Fig. 1A. On the other hand, oximes are unable to reactivate AChE after the conjugated form undergoes aging. Since the aging half-time for soman inhibited AChE is only a few minutes (Worek et al., 2004), the ineffectiveness of the delayed treatment might, therefore, be due to the progression of the aging reaction, if CPA protects through enzyme reactivation, Fig. 1B. In addition, CPA may bind to the NA prior to enzyme conjugation, thereby blocking the agent from reaching the active site, Fig. 1C. Here, we investigate these candidate mechanisms using computational modeling. We are not aware of prior computational work on interactions of CPA with NA, AChE, or NA inhibited AChE.

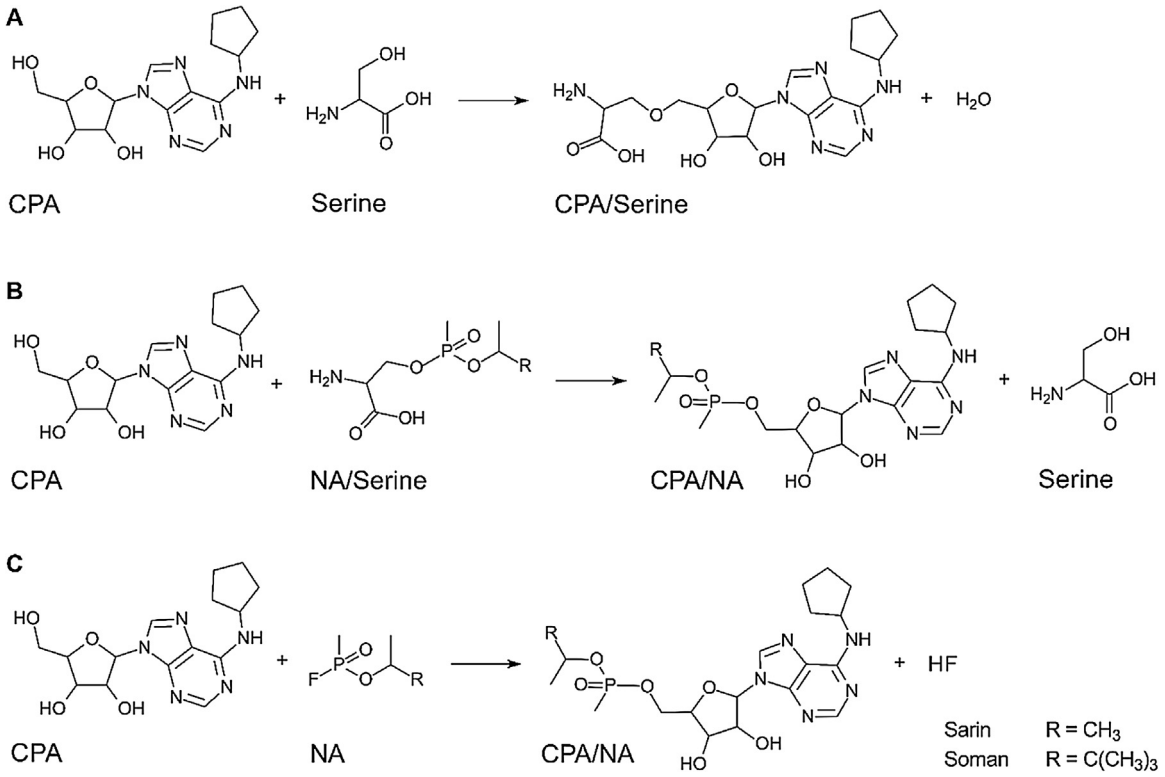

Fig. 1. Potential mechanisms of CPA protection against NAs soman and sarin, AChE is modelled as serine, **A** reversible enzyme inhibition by CPA, **B** enzyme reactivation through serine substitution by CPA, **C** NA blocking by CPA/agent binding prior to conjugation.