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Effect of reversible ligands on oxime-induced reactivation of sarin- and cyclosarin-inhibited human acetylcholinesterase

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HIGHLIGHTS

- We investigated the inhibition type and potency of human AChE by ligands *in vitro*.
- We investigated the effect of AChE ligands on oxime reactivation of OP-inhibited human AChE.
- AChE ligands did not improve reactivation of human AChE by obidoxime and HI-6.

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ABSTRACT

Poisoning by organophosphorus compounds (OP) used as pesticides and nerve agents is due to irreversible inhibition of the enzyme acetylcholinesterase (AChE). Oximes have been widely recognized for their potency to reactivate the inhibited enzyme. The limited efficacy of currently available oximes against a broad spectrum of OP-compounds initiated novel research efforts to improve oxime-based treatment. Hereby, oxime-induced reactivation of OP-inhibited non-human AChE was reported to be accelerated by different AChE-ligands. To investigate this concept with AChE from human source, the inhibitory potency, binding properties and the potential enhancement of oxime-induced reactivation of OP-inhibited AChE by structurally different AChE-ligands was assessed. Several ligands competed with the oxime for the AChE binding-site impairing reactivation of OP-inhibited AChE whereas a markedly accelerated reactivation of sarin-inhibited enzyme by obidoxime was recorded in the presence of edrophonium, galanthamine and donepezil. Enhancement of oxime-induced reactivation with ligands was presumably subject to prevention of re-inhibition by the reaction product phosphonyloxime (POX). In the end, the results of the present study did not confirm that AChE-ligands directly accelerate the reactivation of OP-inhibited AChE by oximes, but indirectly by prevention of re-inhibition by the reaction product POX. This may be due to different experimental conditions and species differences between human and non-human AChE of previous experiments with non-human AChE.

1. Introduction

Acetylcholinesterase (AChE) (EC 3.1.1.7), a serine hydrolase present at the synapses of the cholinergic nervous system, terminates cholinergic synaptic transmission by hydrolysis of the neurotransmitter acetylcholine (Quinn, 1987; Taylor et al., 1995). Irreversible inhibition of AChE is the primary mechanism of action of many organophosphorus (OP) esters, including pesticides and highly toxic nerve agents. These compounds exert their acute toxicity through phosphylation (denotes phosphorylation and phosphonylation) of the γ-oxygen of the AChE active site serine (Holmstedt, 1959; Taylor et al., 1995). Thus, impaired hydrolysis of acetylcholine leads to accumulation of the neurotransmitter at muscarinic and nicotinic receptors. The following overstimulation of peripheral and central cholinergic receptors causes disruption of vital body functions, respiratory arrest and finally death (Grob and Harvey, 1953; Holmstedt, 1959; Wright, 1954).

Since the early 1950s, numerous nucleophilic oxime compounds, including monopyridinium and bis pyridinium compounds as pralidoxime, obidoxime and HI-6, were synthesized and their properties to reactivate the OP-inhibited enzyme shown (Eyer, 2003; Eyer and Worek, 2007; Worek et al., 2007). However, efficacy of oxime-induced reactivation of OP-inhibited AChE is limited in poisoning by different nerve agents, such as soman,

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tabun and cyclosarin as reported by several *in vivo* and *in vitro* studies (Dawson, 1994; Eyer and Worek, 2007; Marrs et al., 2006; Worek and Thiermann, 2013). One reason for impaired reactivation by oximes is the limited accessibility of the attacking nucleophilic oxime group to the phosphylated binding site as recorded by site-specific mutagenesis studies (Ashani et al., 1995; Taylor et al., 1995). In other cases, reactivation of the OP-enzyme-conjugate is affected by a post-inhibitory process known as aging comprising a dealkylation reaction, which makes the phosphylated AChE, particularly soman-inhibited AChE, resistant to reactivation by oximes in living species (Segall et al., 1993). Another mechanism suggested to explain the dramatically reduced reactivation rates of oximes is the inevitable formation of phosphylated oxime (POX) during reactivation which is able to induce re-inhibition of the regenerated enzyme *in vitro* (de Jong and Ceulen, 1978; Harvey et al., 1986; Schoene, 1973). However, only highly stable POX, as *e.g.*, derived from obidoxime and other pyridinium-4-aldoximes conjugated with specific OP-residues, such as sarin, exert anticholinesterase activity (Ashani et al., 2003; Hackley and Owens, 1959; Kiderlen et al., 2000, 2005; Worek et al., 2000; Worek and Thiermann, 2013).

These drawbacks of oxime efficacy reinforced the search for more effective oximes and also initiated new therapeutic strategies to provide an improved oxime-based treatment of OP poisoning (Luo et al., 1998; Musilek et al., 2011; Sit et al., 2011). Previous studies proposed that oxime-induced reactivation of AChE–OP-conjugate may be accelerated in the presence of different non-oxime AChE-ligands, such as edrophonium and decamethonium, using AChE from diverse non-human sources, including fetal bovine serum and mouse AChE (Luo et al., 1998, 1999a,b). In order to verify the potential acceleration by reversible AChE-compounds using human erythrocyte AChE and to shed light into its underlying mechanism of action, the inhibitory potency and mode of inhibition by eight reversible, structurally different AChE-ligands (Fig. 1) as well as their effect in oxime-induced reactivation of OP-inhibited AChE was investigated in the present study.

2. Materials and methods

Acetylthiocholine iodide (ATCh) and 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) were purchased from Sigma–Aldrich (Taufkirchen, Germany) and obidoxime (1,1′-[oxybis(methylene)] bis[4-(hydroxyimino) methyl] pyridinium dichloride) from Merck (Darmstadt, Germany), HI-6 (1-[[[4-(aminocarbonyl) pyridinio] methoxy]methyl]-2-[(hydroxyimino)methyl]pyridinium dichloride monohydrate) was provided by Dr. Clement (Defense Research Establishment Suffield, Ralston, Alberta, Canada). Imipramine hydrochloride, donepezil hydrochloride, pancuronium bromide and propidium iodide were obtained from Sigma–Aldrich. Edrophonium chloride, itopride hydrochloride and desoxypeganine hydrochloride were from Santa Cruz Biotechnology (Santa Cruz, Dallas, Texas, USA) and galanthamine hydrobromide from Tocris Bioscience (Wiesbaden, Germany).

All other chemicals were purchased from Merck Eurolab GmbH (Darmstadt, Germany) at the purest grade available.

Sarin (isopropyl methylphosphonofluoridate; >98% by GC–MS, ^{1}H NMR and ^{31}P NMR) and cyclosarin (cyclohexyl methylphosphonofluoridate; >95% by GC–MS, ^{1}H NMR and ^{31}P NMR) were made available by the German Ministry of Defense. Stock solutions of sarin and cyclosarin (0.1% v/v) were prepared in acetonitrile, stored at 20 °C and appropriately diluted in distilled water just before use.

Oxime and ligand stock solutions were prepared in distilled water and stored at −80 °C. Working solutions were diluted as required in 0.1 M phosphate buffer. All solutions were kept on ice until the experiment.

2.1. Blood samples

Hemoglobin-free human erythrocyte membranes ('ghosts') were prepared from human whole blood and served as source of human erythrocyte AChE (Worek et al., 2002). Aliquots of erythrocyte ghosts were adjusted to an AChE activity physiologically found in whole blood (~9000 U/l) and stored at −80 °C. Prior

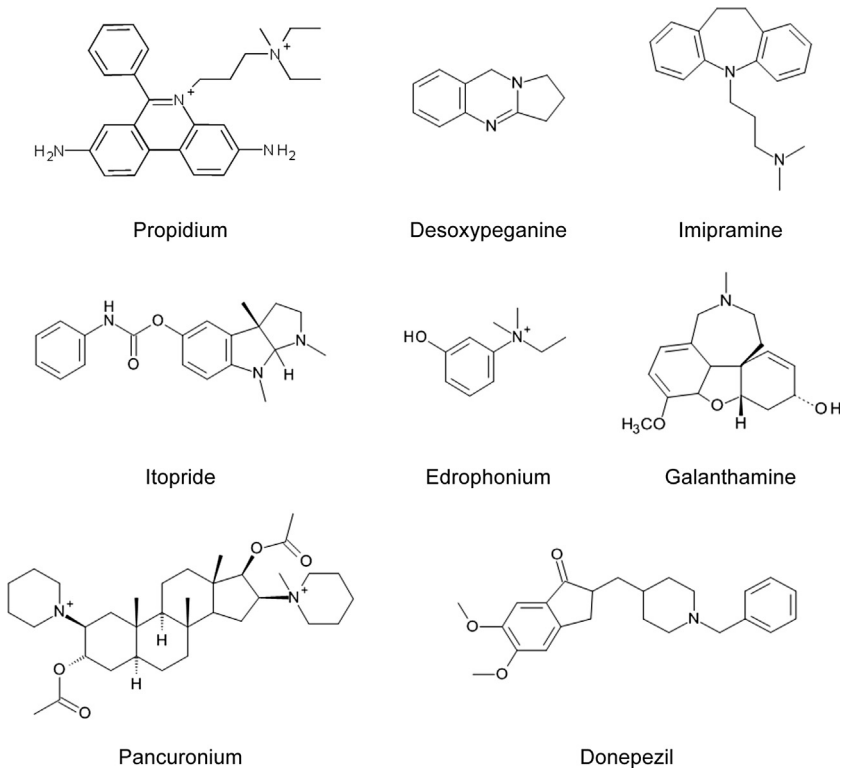

Fig. 1. Structures of AChE-ligands used in this study.