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Reactivation of organophosphate inhibited acetylcholinesterase activity by α,ω-bis-(4-hydroxyiminomethylpyridinium)alkanes *in vitro*

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Summary
In this article, we have followed up the relationship between the ability to reactivate acetylcholinesterase inhibited by organophosphorus compounds and the length of linking chain between two 4-hydroxyiminomethylpyridinium rings of acetylcholinesterase reactivators. α,ω-bis(4-hydroxyiminomethylpyridinium) alkanes have been used as the tested acetylcholinesterase reactivators. These oximes differ in the number of methylene groups on the connecting chain. A three or four membered linking chain seems to be the ideal length for the satisfactory reactivation potency with the exception of reactivators of cyclosarin-inhibited acetylcholinesterase. In this case, the most efficacious acetylcholinesterase reactivator has one methylene group on the connecting chain.

Keywords: acetylcholinesterase – organophosporus compounds – tabun – sarin – cyclosarin – VX

INTRODUCTION

Poisonings with organophosphorus compounds (OPC) are frequent because OPC are widely used as insecticides. According to World Health Organization (WHO), more than one serious accidental and 2 million suicidal poisonings with insecticides occur worldwide every year, and of these approximately 200,000 die, mostly in developing countries (Jayaratnam 1990). Also among lethal chemical weapon (CW) agents, the organophosphorus nerve agents have had an entirely dominant role since World War II (Schrader 1963). All nerve agents belong to the group of highly toxic organophosphorus compounds and represent a serious risk of military or terrorist misuse [for example Iraq-Iran war in 1983 and 1984 (Balali-Mood et al. 1998) or the sarin terrorristic attack in Tokyo subway (Maekawa 1995)].

The mechanism of toxic effect of organophosphates is based on acetylcholinesterase (AChE) inhibition in the nerve system. OPC block the active site of the serine by covalently binding to the serine oxygen (Mars 1993). The complex of AChE and organophosphate can be reversed by introducing an oxime into the system. These compounds, with an ionized oxime group, will break the bond between AChE and organophosphate and restore enzyme activity as illustrated in Figure 1 (Kassa 2002).

MATERIAL AND METHODS

*Enzyme preparation***.** As a source of the enzyme, a crude homogenate of the whole rat brain (male rats of Wistar strain) of individuals weighing 180–220 g was used. The animals were killed under ether narcosis by cutting the carotids and the brains were excised, rinsed in physiological saline and homogenized in an Ultra-Turrax homogenizer in saline. Resulting homogenate was filtered across

mule, divided into test-tubes and treasured up in a freezing box at –18 °C until use.

Reagents. All oxime reactivators were synthesized in our laboratory earlier by Dr Bielavsky (Poziomek et al. 1958). Their structural formulae and melting points (determined on a Boetius apparatus and uncorrected) are summarized in Table 1.

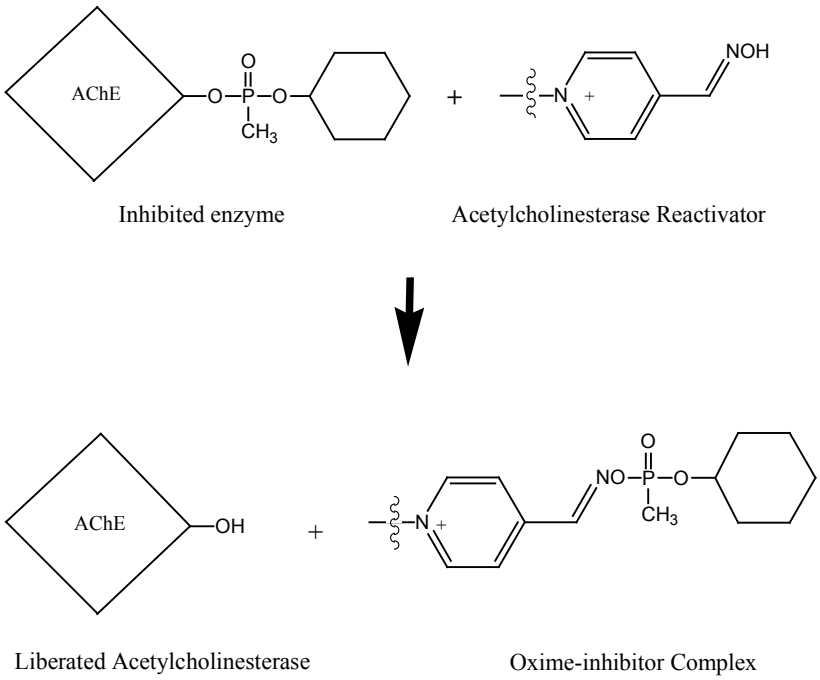
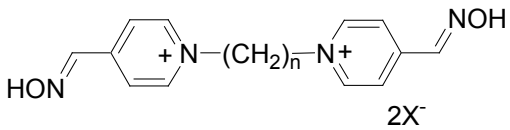


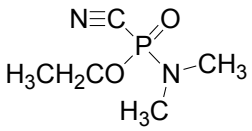
Fig. 1. **Reactivation of acetylcholinesterase inhibited by cyclosarin**

Table 1. **Melting points of the tested acetylcholinesterase reactivators**

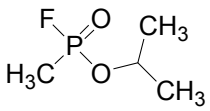


Number of methylene groups *n*	Melting points of the tested oximes [C °]*	Anionts of the tested oximes X^-
1	235.0	Br
2	298.0	Br
3	241.0	Br
4	242.0 – 243.0	Br
5	210.0 – 212.0	Br
6	216.0	Br
8	228.0 – 230.0	Br
10	223.0 – 225.0	Br

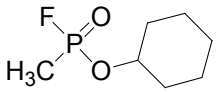
* Melting points were measured with the accuracy 0.1 °C



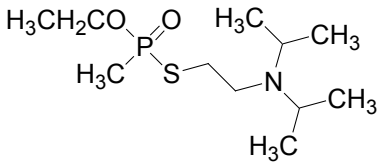
Tabun



Sarin



Cyclosarin



VX

Fig. 2. **Structures of the tested OPC**

RESULTS AND DISCUSSION

The regeneration of phosphorylated AChE by oximes is strongly dependent not only upon the chemical structure of the reactivators (Petrova et al. 1992, Kuča et al. 2002) but also on the OPC type (Cabal 1992, Cabal 1996) and enzyme source (Worek et al. 2002). Rat brain AChE as a source of enzyme was used, because this homogenate is considered to be a suitable source of the enzyme (Patočka et al. 1970, Kassa and Cabal 1999a, Kuča et al. 2003a). The phosphorylation of the enzyme by tabun, sarin, cyclosarin, and VX was performed by 30 min incubation of the enzyme with a particular OPC at the convenient concentration (10^{-5} M for tabun, 10^{-5} M for sarin, 10^{-6} M for cyclosarin, 10^{-6} M for VX) which resulted in 98% inhibition of the enzyme. Thereafter, oximes in a final concentration of 1 mmol/litre were added to the reaction mixture and the activity of AChE was measured after 10 min incubation at 25 °C and pH 8.0. The percentage of the reactivated enzyme was estimated from the following equation:

% reactivation $= 100 - 100(a_o - a_r) / (a_0 - a_i)$

where a_o is the activity of the intact enzyme, a_i is the activity of the OPC inhibited enzyme, and a_r is the activity of the OPC inhibited enzyme after incubation with the oxime reactivator.

In a series of measurements, we followed the dependence of the percentage of reactivation on the number of methylene groups in the oxime molecule. The results for all OPC used are summarized in Table 2.

As can be seen from Figure 3, the percentage of reactivation increased with the increasing number of methylene groups in the oxime molecule up to n=3 or 4 and then decreased at higher homologes

Table 2. **Reactivation potency of the tested reactivators of OPC inhibited acetylcholinesterase**

n*	Tabun [%]	Sarin [%]	Cyclosarin [%]	VX [%]
1	1 ± 1	21 ± 4	37 ± 6	45 ± 7
2	0 ± 1	44 ± 6	0 ± 1	70 ± 7
3	41 ± 3	76 ± 6	0 ± 3	85 ± 10
4	46 ± 6	54± 5	0 ± 1	72 ± 6
5	8 ± 2	34 ± 6	0 ± 1	41 ± 6
6	2 ± 1	22 ± 3	0 ± 2	20 ± 4
8	0 ± 1	4 ± 2	0 ± 1	2 ± 1
10	0 ± 2	0 ± 1	0 ± 1	0 ± 1

*n…number of methylene groups

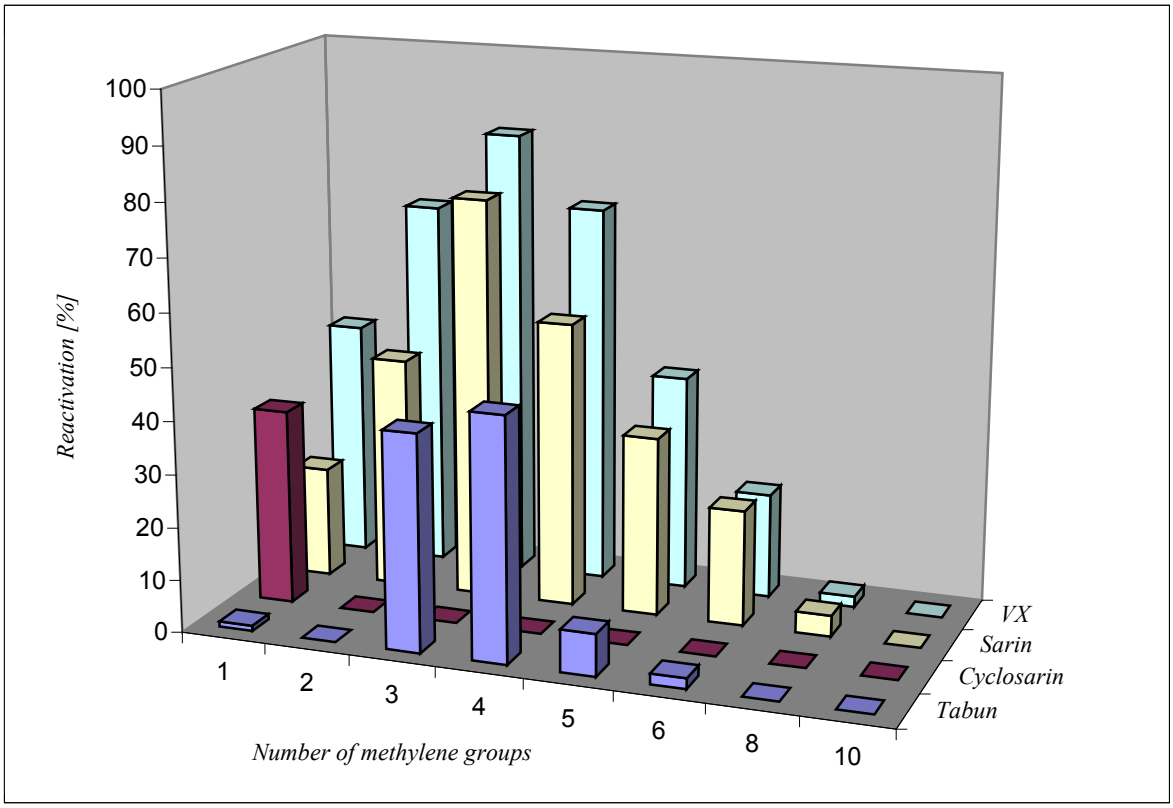


Fig. 3. **Dependence of the reactivation potency on the number of methylene groups and on the type of the OPC inhibitors**

Very similar results were obtained for tabun, sarin and VX as shown in figure 3. In the case of cyclosarin, only the first member of the set of oximes (n=1) was expressed as an effective reactivator.

The group of oximes studied in this paper represents a homologous series of compounds that differ only in the length of the connecting polymethylene chain between the two quaternary nitrogens of the reactivator. With an increasing number of carbon atoms, the lipophilicity of the compound increases and the dissociation ability of the two oxime groups increases as well as (Patočka et al. 1972).

The differences of the values of reactivation potency depend on the type of difficulties with nucleophilic attack (Wilson et al. 1957).

The low values for tabun-inhibited AChE, demonstrated in this paper, were expected, because of difficulties with nucleophilic attack (Kuča et al. 2003b).

The values of reactivation potency for VX and sarin-inhibited AChE are similar for all tested concentrations of the reactivators. The described results were expected because of the same electron effect of the phosphorylated enzyme, which differs only in one methyl group (EtO- by VX, iPrO- by sarin) (Cabal et al. 1996).

In the case of cyclosarin inhibited AChE, the cyclohexyl moiety of the cyclosarin protects the inhibited enzyme against the nucleophilic attack of the reactivators thanks to its large steric extension. This is the reason why only small molecules of the reactivators can reactivate cyclosarin inhibited AChE (Kassa and Cabal, 1999b).

Our results confirm the former results, that the most promising reactivators of OPC-inhibited acetylcholinesterase are bisquaternary pyridinium oximes with three or four methylene membered chains (Petrova et al. 2001). Synthetic approaches in the future should keep this rule in mind.

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