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Ageing and reactivation of acetylcholinesterase inhibited with Soman and its thiocholine-like analogue

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THE ANTIDOTAL effect of oximes in combination with atropine is negligible in *O*-pinacolyl methylphosphonofluoridate (Soman) poisoning.[1, 2] According to Loomis and Johnson[3] this is due to rapid ageing of the Soman inhibited acetylcholinesterase (AChE). Experiments *in vitro* seem to support this assumption.[3, 4] However, studies of ageing in the brain of the rat *in vivo* (measured by the rate of dealkylation) have shown that maximum dealkylation was reached only 30 min after poisoning with ^{32}P Soman.[5]

It follows from this, and from other results *in vivo*,[6, 7] that in spite of ageing, there might be enough time for an oxime to reactivate effectively Soman inhibited AChE and restore function at cholinergic synapses. Since treatment fails even if the oxime is given before Soman,[1, 2] some other factors besides ageing have been considered. One of them was that bulky alkoxy side chain of Soman hinders the binding of oxime to the anionic site of the enzyme (Heilbronn and Tolagen[2]). Alternatively the toxicity of TMB-4 could limit the dose of oxime required for an effective concentration *in vivo* (Berry *et al.*[8]).

To check the hypothesis that the alkoxy side chain of soman limits the effectiveness of oximes, we studied the reactivation of AChE, ageing and protective effect of 1,3-trimethylenebis(4-hydroxyiminomethylpyridinium chloride) (TMB-4) in poisoning by Soman and its thiocholine-like analogue (compound I). Compound I differed from Soman in containing a -S-(2-diethylaminoethyl) methylsulphomethylate group instead of fluorine.

EXPERIMENTAL AND RESULTS

One volume of washed human haemolysed erythrocytes was mixed at 0° with 1 vol. of a cold aqueous solution of the organophosphorus compounds. The final concentration of the organophosphates was 10^{-7} M and inhibition of the enzyme amounted to about 90 per cent; 15 min later 0·08 ml of the mixture was transferred to a vessel kept in a water bath at the 37° and containing either acetylcholine chloride (ACh) or ACh plus TMB-4 and the activity of AChE was determined by a modification of Michel's electrometric method.[9] The percentage reactivation of AChE (Fig. 1) was calculated as suggested by Hobbiger.[10]

To study ageing *in vivo*, 5 groups of 5 rabbits were used. Soman (0·015 mg/kg) was injected into the ear vein of all rabbits, 10 min after an i.p. injection of atropine sulphate (20 mg/kg) had been given. TMB-4 (20 mg/kg, i.v.) was then given to individual groups of animals at the time stated in Table 1, i.e. 5, 10, 20, 40 or 80 min after Soman. Blood samples were taken before Soman (control

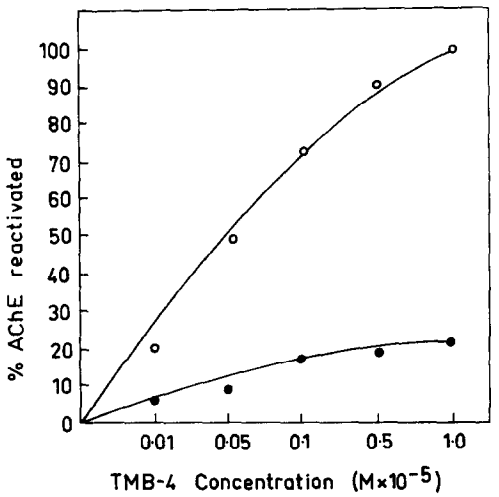

FIG. 1. Reactivation by TMB-4 of human erythrocytes cholinesterase inhibited with Soman (●) and compound I (○) in 30 min at 37° *in vitro*.

activity), before TMB-4 (inhibited enzyme) and 5 min after TMB-4 (reactivated enzyme) and AChE activity was determined with acetyl-β-methylcholine chloride as substrate. The difference between the enzyme activities before and after the injection of TMB-4 was used for assessment of the rate of ageing.[12]

TABLE 1. AGEING OF PHOSPHORYLATED AChE IN ERYTHROCYTES IN RABBITS POISONED BY SOMAN (mean values of 5 animals; 95 per cent confidence limits)

TMB-4 (20 mg/kg, i.v.) injected after Soman (min)	Acetylcholinesterase activity (% of normal)		Difference
	Before TMB-4	5 min after TMB-4	
5	28·7 ± 6·2	55·1 ± 5·2	26·4
10	27·7 ± 10·4	47·3 ± 9·7	19·6
20	35·1 ± 7·2	51·5 ± 5·8	16·4
40	32·6 ± 2·0	39·2 ± 2·4	6·6
80	33·4 ± 5·2	30·6 ± 4·8	−2·8

t 1/2 for ageing: 16 min.

The group of 5 rabbits (Table 2) was injected with compound I (0·1 mg/kg, s.c.). Blood samples were taken at intervals and the AChE activity was determined with acetyl-β-methylcholine chloride as substrate. To get information on ageing, the *in vitro* effect of 10^{-5} M TMB-4 on enzyme activity was determined and the difference between the enzyme activities in the absence and presence of *in vitro* added TMB-4 was used for assessment of the rate of ageing.[12] TMB-4 (20 mg/kg, i.v.) also readily reactivated the inhibited erythrocyte AChE *in vivo*, raising the activity of the enzyme from 27·0 ± 5·4 to 84·0 ± 9·2 per cent (difference 57·0 per cent).

The results of these experiments (Tables 1 and 2) show that the ageing of the phosphorylated AChE found by Soman is much faster than that of phosphorylated AChE by compound I.

Furthermore, the antidotal effect of TMB-4 and atropine was much greater in mice poisoned with Compound I than in mice poisoned with Soman (Table 3).

TABLE 2. AGEING OF PHOSPHORYLATED AChE IN ERYTHROCYTES IN RABBITS POISONED BY COMPOUND I

(mean values of 5 animals; 95 per cent confidence limits)

Time after injection of compound I (hr)	Acetylcholinesterase activity (% of normal)		Difference
	a	b	
1	7·9 ± 2·4	91·0 ± 5·0	83·1
24	37·6 ± 9·0	80·6 ± 3·8	43·0
48	44·7 ± 4·0	68·6 ± 7·2	23·9
72	80·0 ± 9·7	89·0 ± 9·2	9·0
96	98·7 ± 2·2	98·7 ± 2·2	0·0

t 1/2 for ageing: 1500 min.

Activity in absence (a) and presence (b) of 10^{-5} M TMB-4 added *in vitro*.

TABLE 3. EFFECT OF TMB-4 (18 mg/kg) IN COMBINATION WITH ATROPINE (10 mg/kg) ON THE TOXICITY OF ANTICHOLINESTERASES TO MICE

Anticholinesterases	LD_{50}, s.c. (mg/kg)		Protection factor*
	Untreated animals (a)	TMB-4 and atropine† (b)	
Soman	0·16 (0·14–0·19)	0·23 (0·19–0·29)	1·4
Compound I	0·32 (0·24–0·4)	10·5 (8·1–14·5)	32·8

* LD_{50} in group (a)/LD_{50} in group (b).

† TMB-4 and atropine were given together i.p., 10 min before the anticholinesterase. LD_{50} values were calculated from 24 hr mortality figures, by method of moving averages (Thompson[11]).

DISCUSSION

According to Tammelin[13] the reaction between phosphorylthiocholines and cholinesterase leads to the liberation of thiocholine and a simultaneous phosphorylation of the esteratic site of the enzyme. We, therefore propose that the marked difference between the *in vivo* and *in vitro* reactivatability of AChE inhibited by Soman and Compound I might be explained as follows (Fig. 2).

Compound I aligns with both the esteratic and anionic site of the enzyme and after elimination of thiocholine-like residue, the alkoxy side chain is in a different position relative to the anionic site of the enzyme than the corresponding alkoxy group of Soman. Since the anionic site of AChE inhibited by compound I remains free a quaternary oxime can be bound to it and become effectively orientated to the phosphoryl group of the esteratic site. On the other hand, the reaction between Soman and AChE leads to the elimination of F and the alkoxy group of inhibitor prevents access of quaternary oxime to the anionic site (Heilbronn and Tolagen[2]). Thus reactivation is relatively easy when the phosphorylating agent is compound I but difficult when phosphorylating agent is Soman.

Soman inhibited AChE of rabbit erythrocytes ages extremely rapidly *in vivo* by comparison with AChE inhibited by compound I (Tables 1 and 2); the corresponding *t* 1/2 values being 16 and 1500 min, respectively. This means that the structure of the alkyl ester group[4] as well as its orientation at the enzyme surface play an essential part in ageing.

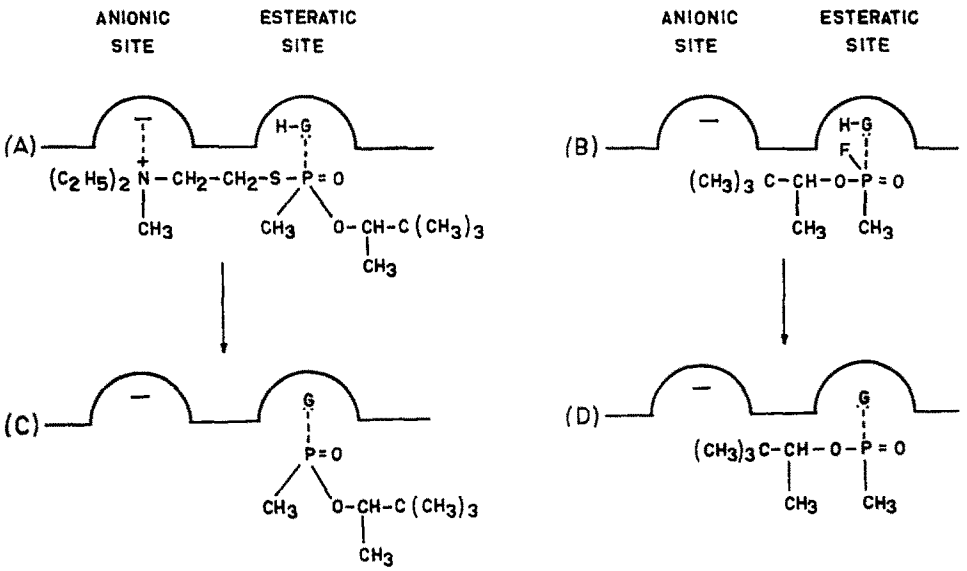

FIG. 2. Proposed interaction of compound I (A) and Soman (B) with AChE.

We do not at the present dismiss the importance of ageing, but feel that steric disturbances are at least as responsible as ageing in the failure of oximes to reactivate effectively AChE inhibited by Soman.

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