Biochemical Pharmacology, Vol. 31, No. 24, pp. 4085-4088, 1982.
Printed in Great Britain.

0006-2952/82/244085-04$03.00/0
Pergamon Press Ltd.

PLASMA ALIESTERASE — A POSSIBLE DEPOT FOR SOMAN (PINACOLYL METHYLPHOSPHONOFLUORIDATE) IN THE MOUSE*

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(Received 29 July 1982; accepted 28 September 1982)

Poisoning by soman (pinacolyl methylphosphonofluoridate), an extremely potent organophosphate anticholinesterase, is resistant to treatment using atropine and conventional oximes (e.g. PAM, TMB-4, toxogonin). New bispyridinium oximes such as HI-6, HGG-12 and HGG-42, when combined with atropine, are an effective antidote of soman poisoning (1).

It was previously assumed that free soman was detoxified very rapidly *in vivo*; however, Wolthuis and associates (2, 3) found that 75 min after the initial soman injection there was still free soman in the blood stream which was capable of inhibiting exogenously administered purified acetylcholinesterase circulating in the blood. They suggested that soman was stored in some sort of depot and was gradually released. Sterri *et al.* (4), in an investigation into the development of tolerance to soman poisoning, suggested that "the tolerance to soman may be linked to the storage of the lipid soluble compound in adipose tissue or binding of soman to plasma and liver proteins". In further investigations, Sterri *et al.* (5) suggested that the storage depot(s) for soman may be plasma cholinesterase and more likely plasma aliesterase. Polak and Cohen (6, 7) reported that ^{32}P-sarin bound to aliesterase *in vivo*. The purpose of this study was to examine the possibility that plasma aliesterase may be a soman depot.

METHODS

Male CD-1® mice (25 – 30 g) used in this study were obtained from Canadian Breeding Farms and Laboratories Ltd., St. Constant, Quebec, Canada. Mice were injected intraperitoneally (i.p.) with atropine (17.4 mg/kg) and HI-6 (50 mg/kg), in the same solution, 5 min before receiving soman (287 μg/kg; subcutaneously (s.c.)). Control mice received saline injections only. Mice were decapitated and exsanguinated 1 hr later and whole blood was collected in a beaker containing a drop of heparin. Heparinized whole blood was centrifuged and the plasma drawn off. Plasma aliesterase and acetylcholinesterase activity was determined ("0" time); then the samples were incubated at 37°C in small (2 ml) capped centrifuge tubes. At various times, aliquots were removed and enzyme activities were determined.

* Suffield Report No. 353

Plasma aliesterase activity was assessed by the pH-stat technique using tributyrin suspended in 0.9% saline as the substrate. To a reaction vessel (23°C), tributyrin (10 ml of 0.2% solution) was added and the solution titrated to pH 7.9 using 0.01 N NaOH by a Radiometer titragraph. To start the reaction 50 μl of plasma was added. Rate of addition of 0.01 N NaOH from the first to the fourth min was used in determining enzyme activity. The reaction was linear over this time period. All solutions were made CO_2-free by bubbling pure nitrogen through them for at least 5 min and the reaction vessel was purged continuously with nitrogen while the reaction was in progress. Aliesterase activity was expressed as nmoles tributyrin (TBT) hydrolysed/ml plasma/min.

Plasma acetylcholinesterase activity was determined according to the procedure of Siakotos *et al.* (8). Ten μl of plasma was incubated with ^{14}C-acetylcholine iodide for 5 min. Acetylcholinesterase activity in plasma was expressed as nmoles acetylcholine (ACh) hydrolysed/ml plasma/min.

RESULTS AND DISCUSSION

The results in Table 1 demonstrate that soman inhibition of aliesterase activity decreases over the 24 hr incubation period concomitant with an increase in the inhibition of plasma acetylcholinesterase activity (Table 1). The further decrease in acetylcholinesterase activity in plasma from soman-treated mice over the 24 hr incubation period was not due to a destruction of enzyme as there was no significant difference in control plasma acetylcholinesterase activity between 4 and 24 hr. Between 4 and 24 hr it appeared that free soman was available which then further inhibited the plasma acetylcholinesterase. The results suggest that the free soman came from plasma aliesterase.

TABLE 1

Effect of *in vitro* Incubation of Plasma from Control and Soman-Treated Mice on Plasma Aliesterase and Acetylcholinesterase Activity

Time (hr)	Aliesterase Activity (nmoles TBT/ml/min)			Acetylcholinesterase Activity (nmoles ACh/ml/min)		
	Control	Soman	% Control	Control	Soman	% Control
0	1980 ± 114[a] (4)	367 ± 18 (5)	19	1419 ± 68 (3)	58.4 ± 8 (3)	4
2	1993 ± 44 (4)	413 ± 30 (5)	21	1255 ± 40 (3)	72.5 ± 5 (3)	6
4	1953 ± 34 (4)	400 ± 27 (5)	20	1119 ± 61 (3)	29.1 ± 12 (3)	3
24	1807 ± 58 (3)	807 ± 128 (5)	45	1138 ± 85 (3)	1.7 ± 1.7 (3)	0.15

[a] mean ± S.E. (N)

In mice, aliesterase plays a very important role in the detoxification of soman as indicated by the potentiation of soman toxicity 15 – 19 fold by CBDP (2-(o-cresyl)-4H-1:3:2-benzodioxaphosphorin-2-oxide), a metabolite of tri-o-cresyl phosphate, which is a potent, irreversible inhibitor of aliesterase (9; Clement, J.G., unpublished observations†).

The recovery of activity of soman-inhibited aliesterase *in vitro* was similar to that reported *in vivo*, i.e. 24 hr after soman poisoning, aliesterase was completely recovered (5). Similar results were found following sarin (isopropyl methylphosphonofluoridate) poisoning in rats. At 24 hr, aliesterase activity had recovered whereas cholinesterase activity was still inhibited (6). In contrast, the inhibition of aliesterase produced by CBDP was essentially irreversible. One hour after CBDP (50 mg/kg, s.c.) serum aliesterase was inhibited 98%, while at 24 hr aliesterase was still 89% inhibited. Control aliesterase activity returned within 6 days (Clement, J.G., unpublished observations). The results presented above suggest that soman does not irreversibly inhibit aliesterase as it does acetylcholinesterase.

In vivo the rapid return to control value of soman-inhibited plasma aliesterase is perhaps due to a combination of regeneration of free enzyme by the release of soman and new enzyme synthesis. The ability of aliesterase to hydrolyse soman is not known; however, since aliesterase hydrolyses uncharged carboxylic esters, it is doubtful that it would enzymatically hydrolyse soman. *In vivo* the slow release of soman from aliesterase would allow other detoxification mechanisms such as the A-esterase, somanase, to hydrolyse soman thus preventing soman from inhibiting vital tissue acetylcholinesterase.

Maliwal and Guthrie reported that the binding of various organophosphate insecticides to high and low density lipoproteins (10) and albumin (11) may play a significant role in the transport of insecticides *in vivo*. However, Polak and Cohen (6) found that ^{32}P-sarin was associated primarily with the α-globulin fraction which contained aliesterase activity. Thus aliesterase and, perhaps to a limited extent, lipoproteins and/or albumin comprise a soman depot alluded to by previous investigators (2, 3, 4, 5) in cases of poisoning by **high** soman concentrations. Sterri *et al.* (12) recently concluded that there may be "very little, if any, storage of soman" following poisoning by **sub-lethal** doses of soman.

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† The s.c. LD_{50} value for soman was reduced from 130 μg/kg to 8.6 μg/kg by pretreating mice with CBDP (50 mg/kg, s.c.) one hour prior to receiving soman.

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