data). No significant statistical differences (Student's *t*-test) were found between these various inhibitions and those of PG-induced contractions. All these inhibitions were reversed by washing out chloroquine from the bath and, as previously demonstrated for electrical stimulations (Famaey & others, 1975) small amounts of PGE_1 were able to restore the contractions to control levels.

It appears from our data that (i) non-specific antagonism was observed between PGs and chloroquine in the guinea-pig ileum, at even higher concentrations than those used in the rat mesenteric vascular bed, (ii) chloroquine behaves like an overall spasmolytic agent on guinea-pig ileal smooth muscle, (iii) this chloroquine inhibition of contractions to acetylcholine, histamine, nicotine, 5-HT and electrical stimulations is reversed by small amounts of PGE_1 added to the bath. This could be due to an inhibition by chloroquine of the endogenous ileal synthesis of PGs which would be necessary for eliciting a normal smooth muscle contraction with all these agonists (including PGs themselves) or more probably, as suggested by us (Famaey & others, 1977b) and others (Chong & Downing, 1973; Bennett, Eley & Stockley, 1975; Schulz & Cartwright, 1976) to a non-specific smooth muscle sensitization induced by PGs to any kind of stimulation. The overall inhibition induced by chloroquine could, in this case, be related to its well known membrane stabilizing properties (Weissmann, 1965) which might affect the smooth muscle membrane reactivity.

Chloroquine (diphosphate) was a generous gift from Bayer, prostaglandins were from Upjohn and acetylcholine from Roche. We thank Mrs C. De Bruyne-Pastiels for her skilful technical assistance and the Belgian 'Fonds national de la recherche scientifique' for financial support.

June 24, 1977

REFERENCES

BENNETT, A., ELEY, K. G. & STOCKLEY, H. L. (1975). *Br. J. Pharmac.*, **54**, 197–204.

CHONG, E. K. & DOWNING, O. A. (1973). *J. Pharm. Pharmac.*, **25**, 170–171.

COLLIER, H. O. J. (1974). In: *Prostaglandin Synthetase Inhibitors*, pp. 121–133. Editors: Robinson, H. J. & Vane, J. R. New York: Raven Press.

FAMAEY, J. P., FONTAINE, J. & REUSE, J. (1975). *Agents and Actions*, **5**, 354–358.

FAMAEY, J. P., FONTAINE, J. & REUSE, J. (1977a). *Br. J. Pharmac.*, **60**, 165–171.

FAMAEY, J. P., FONTAINE, J. & REUSE, J. (1977b). *Prostaglandins*, **13**, 107–114.

FAMAEY, J. P., FONTAINE, J. & REUSE, J. (1977c). *Ibid.*, **14**, 119–124.

GREAVES, M. W. & MACDONALD-GIBSON, W. J. (1972). *Br. med. J.*, **3**, 527.

LOCKIE, L. M. (1972). In: *Arthritis and allied conditions*, 8th edn, pp. 483–494. Editors: Hollander, J. L. & McCarty, D. J. Philadelphia: Lea & Febiger.

MANKU, M. S. & HORROBIN, D. F. (1976a). *Prostaglandins*, **12**, 789–801.

MANKU, M. S. & HORROBIN, D. F. (1976b). *Lancet*, **2**, 1115–1117.

PATON, W. D. M. (1955). *J. Physiol. Lond.*, **127**, 40P–41P.

ROLLO, I. M. (1975). In: *The pharmacological basis of therapeutics*, 5th edn, pp. 1045–1068. Editors: Goodman, L. S. & Gilman, A. New York: MacMillan.

SCHULZ, R. & CARTWRIGHT, C. (1976). *Naunyn-Schmiedebergs Arch. Pharmac.*, **294**, 257–260.

THOMPSON, P. E. & WERBEL, L. M. (1972). *Antimalarial agents: chemistry and pharmacology*. New York: Raven Press.

WEISSMANN, G. (1965). *New Engl. J. Med.*, **273**, 1143–1149.

Antidotal action of the oxime HS6 at the soman poisoned neuromuscular junction of the rat and guinea-pig

ANDREW P. SMITH*, ALAN W. MUIR, *Chemical Defence Establishment, Porton Down, Salisbury, Wilts SP4 0JQ U.K.*

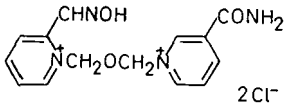
Treatment with atropine and oxime is ineffective in animals poisoned by the organophosphorus cholinesterase inhibitor soman (*O*-pinacolyl-methylphosphonylfluoride) (Loomis & Salafsky, 1963; Heilbronn & Tolagen, 1965). This is due to the soman-inhibited acetylcholinesterase rapidly 'ageing' to a form which is resistant to oxime reactivation (Fleisher & Harris, 1965). In addition, the small increase in protection obtained against soman poisoning in animals pretreated with the oximes Toxogonin and P_2S (Wolthuis & Cohen, 1967) suggests that reactivation of the inhibited enzyme is difficult even before 'ageing' has occurred.

The oxime HS6 (1-{2-hydroxyiminomethyl-pyri-

* Correspondence.

dinium}-1-{3-carboxamidopyridinium}-dimethyl ether) (Schoene, 1967) has structural similarities to Toxogonin, but provides some protection against soman poisoning in various species (Oldiges & Schoene, 1970; Schenk, Loffler & Weger, 1975) and delays the onset of respiratory paralysis for up to several hours in rats poisoned with 4 and 6 LD50's of soman (Wolthuis, Clason-Van der Wiel & Visser, 1976).

HS6



These results demonstrate the superiority of HS6 over other oximes in the treatment of soman poisoning. The following study was undertaken to relate the therapeutic efficacy of HS6 to its ability to restore neuromuscular function depressed by soman poisoning in the rat and guinea-pig.

Experiments were carried out in male albino Porton strain rats (300–400 g) and guinea-pigs (500–600 g) anaesthetized with a 2·5%/25% chloralose/urethane mixture. The gastrocnemius muscle and sciatic nerve were prepared according to the technique described for the cat by the Edinburgh Staff (1970). Isometric single twitch (0·1 Hz) and tetanic responses (50 Hz for 5 s) were recorded from the muscle using supramaximal pulses (0·2 ms pulse width, 2 V intensity) by stimulation of the sciatic nerve via bipolar platinum electrodes. Responses were recorded on a Devices M19 polygraph using a type 4150 force transducer. The animals were infused intravenously with doses of soman sufficient to produce 80–90% blockade of

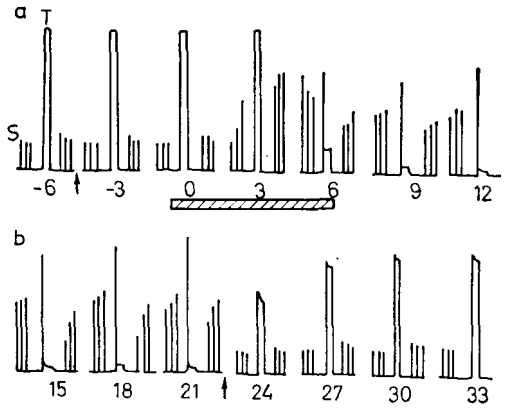


FIG. 1. Experimental (traced) record demonstrating the effect of HS6 in restoring tetanic tension blocked by intravenous infusion of soman in the atropinized guinea-pig/gastrocnemius muscle preparation. T, tetanus; S, single twitch. Tetanic tension is expressed as a percentage of the mean sustained height before infusion of soman. a—arrow indicates atropine sulphate (10 μmol kg^{-1}, i.v. at −5 min), hatch bar indicates soman infusion (0·36 μmol kg^{-1}, i.v.). b—arrow indicates HS6 (130 μmol kg^{-1}, i.v. at 22 min).

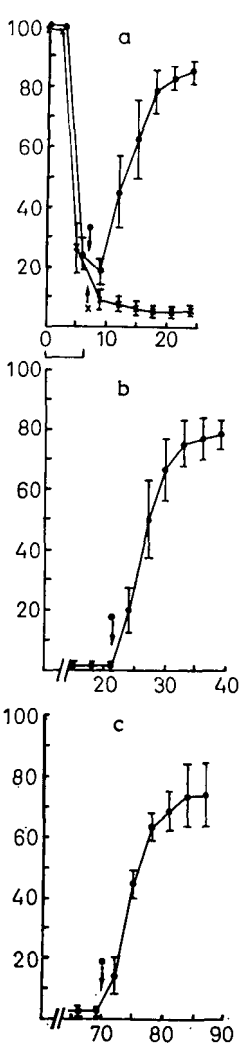


FIG. 2.

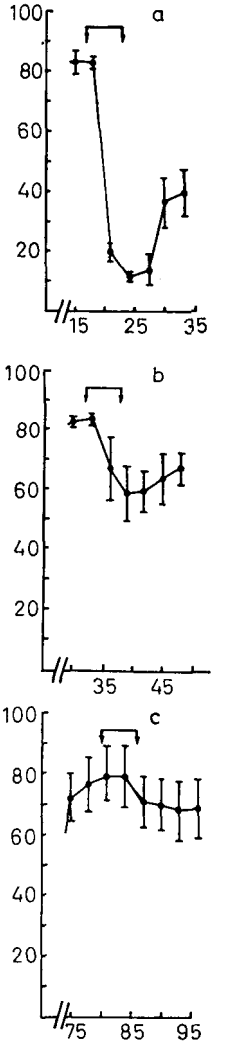


FIG. 3.

FIG. 2. Effects of 130 μmol kg^{-1} HS6 (●) and P_2S (×, lower curve in a) on tetanic tension blocked by 0·36 μmol kg^{-1} soman infusion (↑——↑) in the guinea-pig. Each point represents the mean of 3 experiments ± s.e. Ordinate—Tetanic height as % of maximum. Abscissa—Time (min). HS6 was given 1, 16 and 64 min (a, b, c) after the soman infusion.

FIG. 3. Effects of a second infusion of 0·36 μmol kg^{-1} soman on tetanic tension restored by HS6 given 1, 16 and 64 min (a, b and c, respectively) after a primary neuromuscular blocking dose (0·36 μmol kg^{-1}). The second soman infusions (↓——↓) were given 10 min after oxime therapy. Ordinate—Tetanic height as % of maximum. Abscissa—Time (min).

tetanic tension within 6 min. This was achieved with 0·36 μmol kg^{-1} (4 i.v. LD50's) and 0·47 μmol kg^{-1} (1 i.v. LD50) soman in the guinea-pig and rat, respectively. Animals were pretreated with atropine sulphate (10 μmol kg^{-1}) 5 min before soman infusion and respiration maintained with a Palmer miniature pump. One, 16 and 64 min after completion of nerve agent infusion 130 μmol kg^{-1} HS6 or P_2S was injected intravenously into both species.

In the guinea-pig HS6 restored 80–90% tetanic tension at each time interval (Figs 1 and 2) whereas P_2S therapy even when given 1 min after soman infusion had no effect. Similar results were obtained with HS6 and P_2S in the rat following neuromuscular blockade with 0·47 μmol kg^{-1} of soman. In experiments with higher doses of soman HS6 restored 40–70% tetanic tension in guinea-pigs given 4 and 16 times the 6 min blocking dose of nerve agent. In contrast, only partial and statistically non-significant reversal was obtained with HS6 in the rat following infusions of 2 and 4 times the 6 min blocking dose of soman respectively.

Blood samples were removed after oxime treatment in rats initially poisoned with 0·47 μmol kg^{-1} soman to determine whether there was any relation between recovery of neuromuscular function and regeneration of acetylcholinesterase as measured by the method of Ellman, Courtney & others (1961). In the rat 17% ($\pm$2·2 s.e.) cholinesterase activity was regenerated within 10 min by HS6 given 1 min soman infusion but no reactivation was detected when oxime administration was delayed for 64 min ($P > 0{\cdot}05$). In similar experiments in the guinea-pig no cholinesterase reactivation could be detected even when oxime therapy was given 1 min after soman infusion. While blood cholinesterase concentrations do not necessarily reflect functional acetylcholinesterase activity at the neuromuscular junction (Wills, 1972), the results obtained are difficult to explain in terms of reactivation of the soman inhibited enzyme being the sole therapeutic action of HS6.

To investigate further the relation between restoration of neuromuscular function and acetylcholinesterase reactivation the effects of a second soman infusion upon HS6-restored tetanic tension were studied in the guinea-pig. Blockade of tetanus produced by initial infusions of 0·36 μmol kg^{-1} soman was reversed with HS6 given after 1, 16 and 64 min. Second infusions of 0·36 μmol kg^{-1} soman were then given 10 min after oxime therapy and produced approximately 85, 30 and 5% falls in tetanic tension respectively (Fig. 3). Thus the decline of tetanic tension produced by secondary soman infusion decreased as delay to oxime therapy increased. If it can be assumed the decline of tetanic tension produced by the second soman infusion is solely due to inhibition of previously reactivated acetylcholinesterase, it is unlikely that enzyme reactivation is responsible for restoration of neuromuscular function when therapy is delayed. These results further suggest that at least one other action of HS6 is involved in the restoration of soman blocked neuromuscular function in the guinea-pig.

We thank Dr M. C. French and Mrs J. Wetherell for determination of blood cholinesterase levels.

September 20, 1977

REFERENCES

EDINBURGH STAFF. (1970). *Pharmacological Experiments on Intact Preparations*. Edinburgh: E. and S. Livingstone.
ELLMAN, G. L. COURTNEY, K. D., ANDRES, V. & FEATHERSTONE, R. M. (1961). *Biochem. Pharmac.*, **7**, 88–95.
FLEISHER, J. H. & HARRIS, L. W. (1965). *Biochem. Pharmac.*, **14**, 641–650.
HEILBRONN, E. & TOLAGEN, B. (1965). *Biochem. Pharmac.*, **14**, 73–77.
LOOMIS, T. A. & SALAFSKY, B. (1963). *Toxic. app. Pharmac.*, **5**, 685–701.
OLDIGES, H. & SCHOENE, K. (1970). *Arch. Toxic.*, **26**, 293–305.
SCHENK, J., LOFFLER, W. & WEGER, N. (1975). *Wehrmed. Mschr.*, **1**, 15–17.
SCHOENE, K. (1967). Thesis, Rota-Druck Buchbinderei, J. Krause, Freiburg.
WILLS, J. H. (1972). *Crit. Rev. Toxic.*, **1**, 153–202.
WOLTHUIS, O. L., CLASON-VAN DER WIEL, H. J. & VISSER, R. P. L. S. (1976). *Eur. J. Pharmac.*, **39**, 417–421.
WOLTHUIS, O. L. & COHEN, E. N. (1967). *Biochem. Pharmac.*, **16**, 361–367.