done this does not rule out its metabolic formation as suggested by Beckett & others (1971). Because of our efforts to minimize the possible oxidation of methadone to methadone *N*-oxide during the extraction and assay and because the percentage of methadone *N*-oxide did not increase while the tissue extracts were held at 4°, we have concluded that the methadone *N*-oxide detected in this study is a metabolic product of methadone in the Rhesus monkey.

May 4, 1977

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Differences between some biological properties of enantiomers of alkyl *S*-alkyl methylphosphonothioates

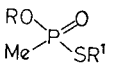
C. RICHARD HALL, THOMAS D. INCH*, ROBERT H. INNS, ALAN W. MUIR, DAVID J. SELLERS, ANDREW P. SMITH,
Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 0JQ, U.K.

During recent attempts to clarify some aspects of the therapeutic properties of oximes and anti-acetylcholine drugs against poisoning by organophosphorus anticholinesterases, it was observed that whereas a mixture of atropine and pyridine-2-aldoxime methylmethanesulphonate (P2S) provided considerable protection against poisoning by *S*(−)ethyl-*S*-propyl methylphosphonothioate [I(−)], the same mixture provided insignificant protection against the enantiomer, (+)ethyl-*S*-propyl-methylphosphonothioate [I(+)] (see below). This observation prompted a more detailed examination of the series of alkyl-*S*-alkyl methylphosphonothioates listed in Table 1. The following aspects were investigated: (i) inhibition of acetylcholinesterase (EC 3.1.1.7); (ii) reactivation of inhibited acetylcholinesterase by P2S; (iii) blockade of tetanic response of the *in vitro* rat phrenic nerve/diaphragm preparation and its reversal by P2S; (iv) restoration by P2S of neuromuscular function *in vivo* in the gastrocnemius muscle of the rat, previously blocked by administration of 2 LD50's of an anticholinesterase; (v) LD50 values and the protection given by atropine and P2S. The results of these studies are summarized or appropriate examples are given in Table 2 and Figs 1 and 2.

The rank order of the results obtained from the *in vitro* experiments involving measurements of the second order rate constants of inhibition of acetylcholinesterase and of concentrations giving equal degrees

* Correspondence.

Table 1. *Alkyl S-alkyl methylphosphonothioates.* The racemic alkyl-*S*-alkyl methylphosphonothioates were prepared from the appropriate thioacids and alkyl bromides (Gazzard, Sainsbury & others, 1974). The enantiomers were prepared similarly but using optically active thioacids (Boter & Platenburg, 1967). The optical purity of the thioates was checked by the nmr method using a chiral shift reagent (Hall, Inch & others, 1975) or by stereospecific synthesis (Cooper, Hall & Inch, 1975).



Compound No.	R	R¹	Configuration	$[\alpha]_D^0$ (in $CHCl_3$, *c* 2)
I(±)	Et	nPr	Rac	
I(+)	Et	nPr	*R*	+51
I(−)	Et	nPr	*S*	−52
II(±)	Et	iPr	Rac	
II(+)	Et	iPr	*R*	+40
II(−)	Et	iPr	*S*	−40
III(±)	Et	nBu	Rac	
III(+)	Et	nBu	*R*	+51
III(−)	Et	nBu	*S*	−52
IV(±)	Et	nPentyl	Rac	
IV(+)	Et	nPentyl	*R*	+47
IV(−)	Et	nPentyl	*S*	−47
V(±)	cy.Pentyl	Me	Rac	
V(+)	cy.Pentyl	Me	*R*	+70
V(−)	cy.Pentyl	Me	*S*	−68
VI(±)	cy.Pentyl	nPr	Rac	
VI(+)	cy.Pentyl	nPr	*R*	+44
VI(−)	cy.Pentyl	nPr	*S*	−45
VII(±)	Et	$CH_2CH_2NiPr_2$	Rac	
VII(+)	Et	$CH_2CH_2NiPr_2$	*R*	−12
VII(−)	Et	$CH_2CH_2NiPr_2$	*S*	

Table 2. *Acetylcholinesterase inhibition rate constants, toxicity and blockade of tetanic response on the rat phrenic nerve/diaphragm of some alkyl S-alkyl methylphosphonothioates.*

Compound No.	Enzyme inhibition*[a] M^{-1} s^{-1}	Toxicity†[b] μmol kg^{-1}	Equieffective dose‡[c] μmol $litre^{-1}$
I(±)	8·0	2·97 (2·91–3·24)	65·9
I(+)	1·0	2·47 (2·31–2·69)	659·3
I(−)	15·0	12·31 (11·54–12·91)	32·9
II(±)	—	—	—
II(+)	0·8	15·5 (13·41–17·97)	—
II(−)	4·0	12·80 (10·56–13·42)	—
III(±)	19·0	8·88 (8·21–10·29)	19·1
III(+)	2·9	12·24 (11·84–12·70)	1275
III(−)	44·0	11·68 (10·56–13·42)	9·5
IV(±)	44	9·33 (8·52–10·29)	14·2
IV(+)	3·2	78·6 (70·80–84·8)	238
IV(−)	79·0	11·24 (8·33–15·90)	7·1
V(±)	—	—	—
V(+)	10·7	38·2 (32·80–44·2)	—
V(−)	$6·8 \times 10^2$	2·73 (2·52–2·89)	—
VI(±)	$1·8 \times 10^2$	10·59 (10·09–11·26)	—
VI(+)	41	15·14 (14·28–15·77)	—
VI(−)	$3·2 \times 10^2$	7·79 (7·43–7·97)	—
VII(±)	$8·1 \times 10^5$	0·049 (0·042–0·061)	0·011
VII(+)	$4·7 \times 10^4$	0·21 (0·19–0·24)	0·21
VII(−)	$7·5 \times 10^5$	0·033 (0·028–0·038)	0·0076

* Apparent second order rate constant. † LD50/95% limits.
‡ On rat diaphragm.

[a] The experiments were carried out with acetylcholinesterase (EC 3.1.1.7) as described previously (Gazzard & others, 1974), except that acetylcholine was $2·5 \times 10^{-4}$M. Reactivation experiments were carried out as follows. A solution of AChE (10 units ml^{-1}) and inhibitor in phosphate buffer (pH 7·4, 5×10^{-5}M phosphate, 0·1M NaCl) was stirred at 4° for 30 min. Excess of inhibitor was removed by ultrafiltration through a XM-50 membrane in an Amicon ultrafiltration cell using pressures of 25–30 lb in^{-2}. Reactivations were carried out in a solution (2·5 ml) containing 20 units of inhibited AChE, in 5×10^{-2}M phosphate, pH 7·4, 0·1M NaCl at P2S concentrations of 5×10^{-5}M at 37°. Aliquots (0·15 ml) were removed, diluted, and assayed on a Radiometer pH stat using $4·1 \times 10^{-4}$M acetylcholine iodide.

[b] Subcutaneous LD50's were determined using Porton strain male Wistar albino rats (180–230 g). Compounds were dissolved in saline (except IV which was dissolved in PEG300) and administered in a volume of 1 ml kg^{-1}. Five groups of animals were used (six animals per group) for each compound and the LD50 calculated using the method of probit analysis.

[c] The preparation was as described by Bülbring (1946). The tissue was suspended in Krebs solution at 32 ± 0·2° and gassed with 5% CO_2 in oxygen. Single twitches (0·1 Hz) and tetanic contractions (50 Hz for 5 s) were recorded using a Devices force transducer, type 4151, amplified and displayed on a Devices MX212 recorder. Concentrations of compounds required to produce a 90–95% block of tetanic tension (i.e. a 90–95% reduction of the maximum tension observed during stimulation) were determined.

of blockade of the tetanic response of the rat phrenic nerve/diaphragm preparation are in good agreement (Table 2). For all the compounds investigated the (−)-enantiomers were generally much more active than the corresponding (+)-enantiomers and approximately twice as active as the corresponding racemates. The rank order of potency of all the compounds was similar in the two tests. Furthermore, the addition of P2S to acetylcholinesterase inhibited with any of the ethyl-methylphosphonothioates, caused reactivation of >50% of the enzyme in 60 min and on the rat phrenic nerve/diaphragm preparation the neuromuscular blocking activity of all the ethyl-methylphosphonothioates was also reversed by P2S (e.g. Fig. 1). As expected, because acetylcholinesterase inhibited with cyclopentyl derivatives 'ages' rapidly (Coult, Marsh & Read, 1966), the effects of the cyclopentyl derivatives were not reversed by P2S in either test.

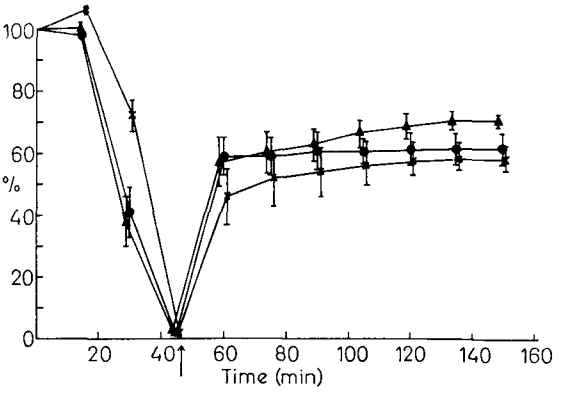


FIG. 1. Changes in the tetanic tension (% of maximum) of the isolated rat phrenic nerve/diaphragm produced by I(−) [▲ at 32·9 μmol], I(±) [● at 65·9 μmol] and I(+) [X at 659 μmol] and the effects of P2S (0·5 mmol) in reversing the changes. The P2S was added (↑) shortly after a 90–95% block of tetanic tension was observed.

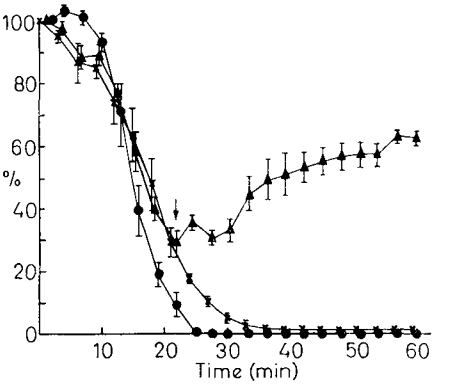


FIG. 2. The effect of P2S (130 μmol kg^{-1}, i.v.) administered during poisoning as indicated by ↓ on the tetanic tension (% of maximum) of the rat gastrocnemius muscle preparation *in vivo*, dosing (i.v.) with II(−) [▲ 24·6 μmol kg^{-1}], II(±) [● 5·94 μmol kg^{-1}] and II(+) [× 4·94 μmol kg^{-1}]. Experiments were carried out on male Wistar rats (300–400 g) anaesthetized with 2·5% chloralose—25% urethane (3·5 ml kg^{-1}, s.c.). Single twitches (0·1 Hz) and tetanic contractions (50 Hz for 5 s) were recorded from the gastrocnemius muscle using supramaximal pulses by stimulation of the sciatic nerve via bipolar platinum electrodes. Responses were amplified and recorded on a Devices M19 Polygraph using a type 4150 force transducer. Adequate ventilation of the rats was maintained with a Palmer miniature respiration pump. All the compounds exerted a typical anticholinesterase effect by producing potentiation of single twitch tension and a progressive decline in tetanic tension.

The effects of both the (+)- and (−)-enantiomers of VII were reversed by P2S in both tests in the same manner as the effects of any of the ethyl *S*-alkyl-methylphosphonothioates. It is noteworthy that although the (+)- and (−)-isomers of VII inhibit acetylcholinesterase at different rates and substantially faster than the (+)- and (−)-isomers of the *S*-alkyl methylphosphonothioates (e.g. I(+) and I(−)), the reactivation profiles of all four compounds were indistinguishable.

The results of the *in vivo* experiments showed marked differences from those obtained *in vitro*. The rank order of the LD50 values did not correspond with the rank order of potency of the compounds in the *in vitro* tests (Table 2). Moreover there was sometimes no significant difference between the LD50 values of an enantiomeric pair even though the (−)-isomer was *in vitro* clearly a more potent anti-acetylcholinesterase than the (+)-isomer. In the case of one compound, ethyl *S*-propyl methylphosphonothioate (I), the (+)-isomer was significantly more toxic than the (−)-isomer in contrast to the results of the *in vitro* results.

In the gastrocnemius muscle, as is shown in Fig. 2, the administration of P2S at a dose of 130 μmol kg^{-1}, during poisoning by the (−)-enantiomer of I, re-established neuromuscular function whereas similar P2S treatment of poisoning by the (+)-enantiomer and the racemate failed to restore neuromuscular function. Compounds II and III showed similar behaviour. In marked contrast, the effects of both the (+)- and (−)-enantiomers of VII on the gastrocnemius muscle preparation were reversed by administration of P2S.

The difference in response to P2S of the (+)- and (−)-enantiomers of the ethyl-methylphosphonothioates I, II and III was consistent with the preliminary observation that rats poisoned by (+)ethyl-*S*-propyl methylphosphonothioate (I+) failed to respond to P2S—atropine treatment. Thus whereas for I(−), treatment by P2S—atropine under standard conditions (Green, Muir & others, 1977) gave a protection ratio of 8·5 (protection ratio = LD50 of Sarin in treated animals/LD50 of Sarin in untreated animals) the protection ratios of I(+) and I(±) were 2·2 and 1·2 respectively. In marked contrast, but in agreement with the results on the gastrocnemius preparation, P2S—atropine treatment of poisoning by VII(±), VII(+) and VII(−) gave protection ratios of 24, 70 and 55 respectively.

To our knowledge there is no recorded precedent for the difference of the (+)- and (−)-enantiomers of the ethyl-methylphosphonothioates observed *in vivo*. Previous comparisons (Fukuto, 1971) of the enantiomers of alkylphosphonothioate anticholinesterases have been concerned mainly with the insecticidal activity and stereoselectivity of cholinesterases from different sources and so no indications of possible mechanistic *in vivo* differences in animals were obtained. That metabolic activation of the (+)-isomers occurs, is an attractive proposition.

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The effects of storage upon *in vitro* and *in vivo* characteristics of soft gelatin capsules containing digoxin

B. F. JOHNSON*†, P. V. MCAULEY, P. M. SMITH, J. A. G. FRENCH, **Clinical Research Division, Wellcome Research Laboratories, Beckenham and Wellcome Development Laboratories, Dartford, Kent, U.K.*

Encapsulation of a solution of digoxin in soft gelatin is associated with greater bioavailability than from an aqueous solution (Johnson, Bye & others, 1976) or from the solution used to fill the capsules (Mallis, Schmidt & Lindenbaum, 1975). Whereas intestinal absorption of digoxin from tablets is incomplete and variable between individuals (Johnson & Bye, 1975), absorption from soft gelatin capsules is virtually complete and shows less between-subject variation (Johnson & others, 1976). The mechanism of the enhanced absorption from such capsules is unclear, but seems likely to be related to either the integrity or chemical composition of the capsule wall. The possibility that altered characteristics of the capsule wall might occur under differing conditions of storage, and

† Correspondence and present address: Medical Division, Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, N.C. 27709, U.S.A.