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Long-term Effects of the Anticholinesterases Sarin and Soman on Latencies of Muscle Action Potentials in Mouse Diaphragm Muscle

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Abstract—In-vivo administration of the irreversible antichlolinesterases sarin and soman has been shown to produce long-term effects on latency and variability of latency of muscle action potentials in in-vitro mouse diaphragm muscle preparations. The maximum observed effects occurred three days post-soman administration and seven days post-sarin administration, and were no longer detectable 28 days later. With both anticholinesterases the increase in latency, and variability of latency, was reduced by pyridostigmine pretreatment. Therapeutic administration of pralidoxime mesylate effectively prevented the sarin-induced effects when given after a delay of 24 h. In contrast, the effectiveness of pralidoxime mesylate declined rapidly when its administration was delayed following soman. These findings are consistent with this action of soman and sarin being a product of acetylcholinesterase inhibition. The results obtained with sarin suggest that a period of acetylcholinesterase inhibition in excess of 24 h is required to trigger the events leading to the production of this long-term effect.

In addition to the acute effects of anticholinesterases, which result in blockade of cholinergic junctions following prolonged exposure to high transmitter levels (Grob 1963; Bowman & Rand 1980), long-term effects, which result in necrosis of muscle fibres, have also been described (Ariens et al 1969; Kibler 1973; Wecker et al 1978; Dettbarn 1984; Gebbers et al 1986). In rodents, muscle necrosis is associated with doses of anticholinesterase producing tremor, fasciculation and muscular weakness (Kibler 1973; Laskowski et al 1975). Generally, the damage first appears after 6–12 h, peaks at 3–8 days, and is no longer detectable 14–28 days after poisoning. It is produced by a wide range of anticholinesterases, and can be prevented by carbamate pretreatment (Wecker et al 1978) and oxime therapy (Ariens et al 1969; Wecker et al 1978) suggesting it to be a product of cholinesterase inhibition.

Muscle necrosis, however, is only produced in a small proportion (5–7%) of muscle fibres (Kibler 1973; Laskowski et al 1975; Wecker & Dettbarn 1976) and the question arises as to whether fibres without structural damage are more subtly affected. Kelly et al (1990) employed a modification of the single fibre electromyography (SFEMG) recording technique of Stalberg & Trontelj (1979) to investigate the long-term effects of anticholinesterases further. SFEMG is a clinical method, extremely sensitive to neuromuscular disorders, in which variability of latency (jitter) between pairs of muscle action potentials from a single motor unit is measured. Using this technique, adapted to record from stimulated mouse diaphragm in-vitro, Kelly et al (1990) demonstrated that ecothiophate, DFP (diisopropyl phosphorofluoridate) and BOS (pinacolyl *S*-(2-trimethylaminoethyl) methylphosphonothioate) markedly increased jitter in diaphragms removed from animals given necrotizing doses of the anticholinesterases. Since necrotic fibres will be electrically silent, this finding, together with the fact that DFP and BOS increased jitter when administered at subnecrotizing doses, suggests that the long-term effect of anticholinesterases is more extensive than is suggested by necrosis of muscle fibres.

The objectives of this study were to determine whether increase in jitter is a general property of organophosphate acetylcholinesterase inhibitors by investigating the long-term actions of soman (pinacolyl methylphosphonofluoridate) and sarin (isopropyl methylphosphonofluoridate), to investigate the relationship between the jitter increase and acetylcholinesterase inhibition, and to confirm that the jitter increase is a product of acetylcholinesterase inhibition by measuring the efficacy of pyridostigmine pretreatment and pralidoxime mesylate therapy, established antidotes against acute poisoning by organophosphate anticholinesterases, against induced increase in jitter.

Materials and Methods

Methods

Experiments were carried out using male Balb C mice (Charles River), 23–30 g.

Pyridostigmine bromide, pralidoxime mesylate and the anticholinesterases, sarin and soman (both of purity > 95%), were synthesized at the Chemical and Biological Defence Establishment, Porton Down, UK. Sarin and soman were stored at 4°C in solution in isopropanol at concentrations of approximately 5 mg mL^{-1}. Atropine sulphate was purchased from Sigma, UK, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and *S*-acetylthiocholine iodide were purchased from BDH, UK. All drugs and chemicals were diluted in saline (0·9% NaCl, w/v) on the day of the experiment to the final concentrations used. Compounds injected were administered subcutaneously into the scruff at a dose volume of 10 mL kg^{-1}.

Mice were killed by cervical dislocation and the left hemidiaphragms were dissected and prepared for the intracellular recording of muscle action potentials according to the technique described by Kelly et al (1990). A strip of intact

muscle fibres extending 2·5 mm either side of the point of entry of the phrenic nerve was pinned to Sylgard 184 (Dow Corning) in a perspex bath and perfused with physiological saline solution of the following composition (mM): NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 1, KCl 5, $MgCl_2$ 1, glucose 25 and $CaCl_2$ 2. The saline was maintained at 37°C and gassed with 95%O_2–5%CO_2. The phrenic nerve, cut 1–2 mm away from the muscle, was stimulated via a suction electrode connected to a Grass S44 stimulator adjusted to produce supramaximal pulses of 0·02 ms duration.

Muscle action potentials (MAPs) were recorded intracellularly at the tendinous end of the preparation via glass microelectrodes, filled with 4 M KCl, of 20–40 Mohm resistance. MAPs were amplified using a WPI M701 microprobe system, displayed on a Tektronix D13 dual beam storage oscilloscope and recorded on an FM tape-recorder running at 30 in s^{-1}.

Signals were replayed through a Biodata Microlink AD Converter fitted with a transient capture unit and analysed using an Apricot PC computer to measure the amplitude and latency of individual MAPs. The sampling rate of recordings was 1 MHz. Using a stimulator (Grass S44) to simulate MAPs at 10 and 30 Hz, the recording and analysis possessed an intrinsic variation in latency of ± 2 μs. Trains of 16 MAPs were recorded at 30 Hz from fibres in each preparation, and, if recording conditions were stable, 10 Hz in the same fibre. Recordings were made only from fibres with a resting membrane potential greater than −60 mV, and were accepted for analysis only if the peak amplitudes of the muscle action potentials did not vary by more than ±10% during the period of stimulation. The delay between the stimulus and a point on the rising phase of the MAP at 30% of the mean peak amplitude was measured for each action potential in the train. Changes in the delay to impulse 6 (D6), and the mean consecutive difference (MCD) of impulses 6 to 16, were then calculated.

MCD is a measure of variability in latency of MAPs in which effects of long-term drifts in latency are minimized (Stalberg & Trontelj 1979). The formula used to calculate the MCD in the present study was:

$$[MCD = (L6 - L7) + (L7 - L8) + (L8 - L9) + \ldots . (L15 - L16)/10]$$

where $Ln - L[n+1]$ represented the difference in latency between each pair of MAPs from 6 to 16.

D6 was measured to monitor effects on latency of MAPs at the start of a train, the section where progressive increases in latency are most apparent (Kelly et al 1990).

Results obtained at the two frequencies of stimulation tested in this study were similar and only data for 30 Hz are presented.

Cholinesterase assays

Erythrocyte acetylcholinesterase and diaphragm acetylthiocholine hydrolase activity were measured using the spectrophotometric method of Ellman et al (1961). Erythrocyte samples were washed twice and remade to the original volume in saline, and then diluted 1 in 200 in phosphate buffer (0·1 M, pH 8) before assay. The total acetylcholinesterase activity was expressed as μmol acetylthiocholine hydrolysed min^{-1} (mL blood)$^{-1}$.

Diaphragms were homogenized in phosphate buffer (0·1 M, pH 8) and stored at −20°C until assayed. The protein content of the diaphragms was determined by the method of Lowry et al (1951). The diaphragm total acetylthiocholine hydrolase activity was expressed as μmol acetylthiocholine hydrolysed min^{-1} (g protein)$^{-1}$.

Statistical analysis

All results are expressed as mean ± s.e.m. of values from at least three animals unless otherwise stated. Following doses of anticholinesterases, frequency distributions of D6 and MCD values were markedly skewed to the right as shown for MCD in Figs 2, 3. To take into account the skewed distribution of the data, control and test groups were compared using the Kolmogorov-Smirnov non-parametric test (Siegel 1956), and results taken to be significantly different if $P < 0.05$ (2-tailed test).

Experimental approach

Sarin and soman were administered at doses producing 80–90% inhibition of acetylcholinesterase, the level previously being found to be associated with jitter increases following administration of the anticholinesterase ecothiophate (Kelly et al 1990). Atropine sulphate (0·7 μmol kg^{-1}) was administered immediately following the organophosphate to control the effects of excessive muscarinic stimulation. Measurements of D6 and MCD were made at increasing intervals after anticholinesterase administration until values were no longer significantly different from controls. Further animals were tested at the observed maximum response times following lower doses of sarin and soman to investigate the relationship between cholinesterase inhibition and D6 and MCD increase. The effects of single dose pralidoxime mesylate therapy, and pyridostigmine pretreatment, were tested on the D6 and MCD increases produced by the highest doses of sarin and soman used in this study. Pralidoxime mesylate was administered at various times post-poisoning and pyridostigmine pretreatment administered 30 min before challenge with anticholinesterase agent.

Results

In diaphragms from untreated mice stimulated at 30 Hz, the latency of successive MAPs increased progressively and reached a steady-state by the 6th potential of the train. The mean delay to impulse 6 (D6) of the control muscle fibre population was 28·5 ± 3·7 μs, and mean consecutive difference in latency of the subsequent 10 impulses (MCD) 9·9 ± 0·7 μs. Individual fibre values of D6 and MCD were distributed unimodally in these animals.

Effects of anticholinesterases

Subcutaneous (s.c.) injection of sarin (1·6 μmol kg^{-1}) or soman (0·82 μmol kg^{-1}) produced signs of acetylcholinesterase inhibition within 10–15 min of their administration. These included periodic general mild fasciculation and tremor which persisted for up to 45 min after which the animals appeared slightly subdued for 24–36 h before returning to normal. These doses caused significant increases in MCD and D6 to the order of 250–350% (Fig. 1). The maximum observed increases in D6 and MCD coincided for each anticholinesterase, 7 days post-sarin and 3 days post-

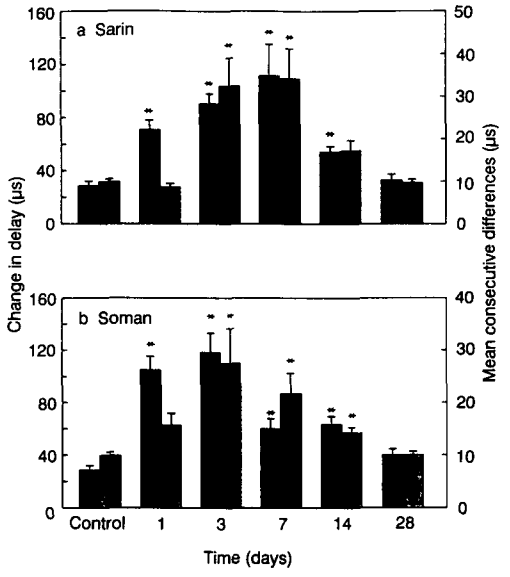

FIG. 1. Effects of (a) sarin 1·6 μmol kg^{-1} and (b) soman 0·82 μmol kg^{-1} on D6 (■) and MCD (■) in mice. * $P < 0.05$ compared within control.

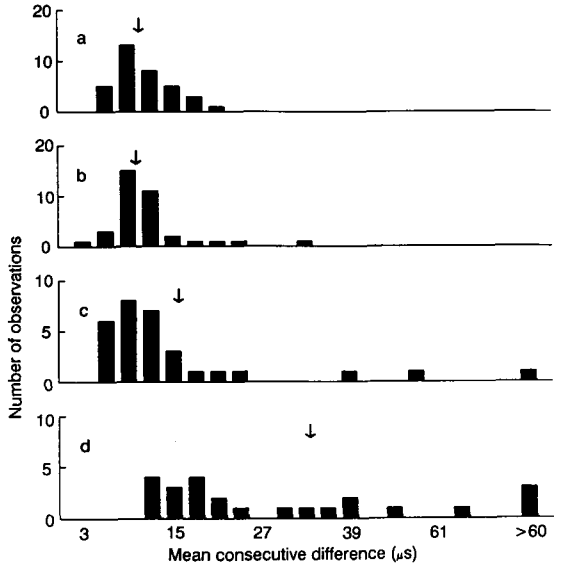

FIG. 2. Distribution of mean consecutive difference values of action potentials in (a) untreated muscle and muscle removed 7 days after sarin given at doses of (b) 1·24, (c) 1·42 and (d) 1·6 μmol kg^{-1}. The arrows indicate the mean value of the population.

soman administration, and returned to control levels after 28 days. With both anticholinesterases, the effects developed in the same sequence, an increase in D6 appearing 24 h post-poisoning followed by an increase in MCD at 3 days. In animals given atropine alone, the D6 and MCD values were not significantly different from control values either 3 or 7 days post-drug administration, although a transient significant 69% increase in D6 was observed at 24 h.

At the times of maximum observed increase following anticholinesterase poisoning, some fibres tested could not be analysed because the trains lacked at least one MAP. Approximately 13% of the fibres tested following sarin and soman poisoning were misfiring, a similar proportion being observed with each organophosphate. Of the remaining fibres, 4% of which exhibited multiple action potentials in response to single stimuli, the populations of D6 and MCD were no longer unimodal, and many values were greater than those observed in control preparations (Figs 2d, 3e). There was no correlation between D6 and MCD increase, fibres with a large increase in MCD not necessarily showing a large increase in delay.

Acetylthiocholine hydrolase activity in diaphragms removed from animals 24 h after organophosphate administration was 56% inhibited in sarin-dosed mice, and 73% inhibited in soman-dosed mice. Table 1 shows that erythrocyte acetylcholinesterase inhibition in these animals was 93 and 85%, respectively.

Whilst a formal histopathological investigation of the tissues for muscle damage was not undertaken in this study, trans-illuminated diaphragms, pinned out for electrophysiological recording, were studied for signs of damage using the low power (×20) binocular microscope normally used to position intracellular glass microelectrodes. With both anticholinesterases, areas of opacity in the endplate zone of the diaphragms were clearly evident at the respective maximum observed jitter times.

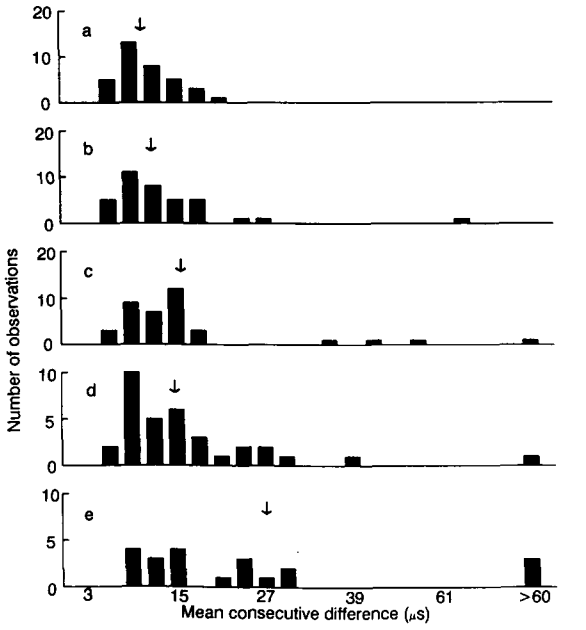

FIG. 3. Distribution of mean consecutive difference values of action potentials in (a) untreated muscle and muscle removed 3 days after soman given at doses of (b) 0·42, (c) 0·53, (d) 0·66, and (e) 0·82 μmol kg^{-1}. The arrows indicate the mean value of the population.

Effects of lower sarin and soman doses

The effects of lower doses of sarin and soman on D6 and MCD were measured at the respective observed maximum response times for each anticholinesterase. With both doses of sarin, signs of poisoning were observed following its administration. These were less marked than those observed following 1·6 μmol kg^{-1} sarin, but included transient local fasciculation at the site of injection. The effects of the organophosphates on diaphragm acetylthiocholine hydro-

Table 1. Acetylcholinesterase activity in washed red blood cells (RBC) and total acetylthiocholine hydrolase activity in diaphragm muscle 24 h after administration of sarin or soman.

Dose (μmol kg^{-1})	Washed RBCs (μmol min^{-1} (mL blood)$^{-1}$)	n	Inhibition (%)	Diaphragm (μmol min^{-1} (g protein)$^{-1}$)	n	Inhibition (%)
Untreated	0·26±0·02	8	0	18·14±0·89	15	0
Sarin						
1·24	0·15 (0·052–0·24)	2	42	11·32 (10·34–12·30)	2	38
1·42	0·038±0·002	3	85	9·91±0·84	3	45
1·60	0·018±0·003	3	93	7·99±0·56	3	56
Soman						
0·42	0·17±0·02	3	35	13·26±0·78	3	27
0·53	0·17±0·05	3	35	9·68±1·23	3	47
0·66	0·12±0·04	3	54	9·16±2·38	3	50
0·82	0·04±0·01	3	85	4·99±1·00	3	73

Values are mean ± s.e.m. of n observations except for sarin 1·24 μmol kg^{-1} where limits of variation are shown.

lase levels measured 24 h post-poisoning are shown in Table 1.

Table 2 shows that sarin, given at doses of 1·24 and 1·42 μmol kg^{-1}, caused no significant increase in D6 or MCD. The highest sarin dose tested which produced no significant change in D6 or MCD, 1·42 μmol kg^{-1}, inhibited diaphragm acetylthiocholine hydrolase by 45% and erythrocyte acetylcholinesterase by 85% (Table 1).

Table 2 shows that 0·53 and 0·66 μmol kg^{-1} soman produced significant increases in D6. MCD increases produced by these doses of soman were not significant at the $P<0·05$ value. The highest dose of soman tested, which failed to produce statistically significant increases in either D6 or MCD, was 0·42 μmol kg^{-1}. This dose inhibited diaphragm acetylthiocholine hydrolase by 27% and erythrocyte acetylcholinesterase by 35%. As with sarin, signs of poisoning were produced by the lower doses of soman which were similar to, but less severe than those associated with 0·82 μmol kg^{-1}. Fasciculations observed following the lower soman doses appeared to be confined to the site of injection. Using low-power light microscopy, no muscle damage was evident in diaphragms removed from mice dosed with sarin at either 1·24 or 1·42 μmol kg^{-1}. In diaphragms from soman-poisoned mice, minor muscle damage was evident in one animal following 0·53 μmol kg^{-1} and one animal following 0·66 μmol kg^{-1} soman. No damage was seen in diaphragms removed from mice treated with 0·42 μmol kg^{-1} soman.

Figs 2, 3 show that the lower doses of both anticholinesterases caused an increase in the MCD in a small number of fibres. This was, however, insufficient to produce a significant change in the frequency distribution as assessed by the Kolmogorov-Smirnov two-sample test.

Table 2. Effects of various doses of sarin and soman on D6 and MCD. Values were measured 7 days post-sarin and 3 days post-soman.

Dose (μmol kg^{-1})	n	D6 (μs)	MCD (μs)
Untreated	35	28·5±3·7	9·9±0·7
Sarin			
1·24	36	35·0±3·6	10·0±0·9
1·42	30	34·1±4·9	14·2±2·7
1·60	24	111·6±23·6*	34·2±7·0*
Soman			
0·42	37	17·8±3·3	12·0±1·3
0·53	38	76·0±8·2*	16·0±4·3
0·66	36	70·3±5·7*	15·1±2·1
0·82	21	118·2±14·8*	27·4±6·7*

Values are mean ± s.e.m. of n observations. * Significantly different from untreated mice ($P<0·05$).

Table 3. Effects of pralidoxime mesylate (130 μmol kg^{-1}) on maximum observed increases in D6 and MCD produced by 1·6 μmol kg^{-1} sarin and 0·82 μmol kg^{-1} soman.

Pralidoxime mesylate	n	D6 (μs)	MCD (μs)
a Sarin			
None	24	111·6±23·6	34·2±7·0
+30 s	30	35·2±4·8*	12·7±1·8*
+24 h	29	35·8±5·7*	10·6±1·2*
+48 h	38	73·2±7·2	23·4±2·4
b Soman			
None	21	118·2±14·8	27·4±6·7
+30 s	35	53·6±4·9*	10·6±1·0*
+15 min	34	84·7±8·3*	19·0±5·4*
+60 min	21	140·3±14·4	28·8±5·7

Values are mean ± s.e.m. of n observations. * Significantly different from those in mice not given pralidoxime mesylate ($P<0·05$).

Effects of pralidoxime mesylate therapy on anticholinesterase-induced increases in latency and MCD

Pralidoxime mesylate (130 μmol kg^{-1}, s.c.) was given at increasing intervals after administration of the highest doses of sarin and soman until the therapeutic effectiveness of the oxime was lost. The oxime significantly reduced increases in D6 and MCD induced by 1·60 μmol kg^{-1} sarin when it was given immediately post-poisoning or after a delay of 24 h (Table 3a). The effectiveness of pralidoxime mesylate was lost only when administration was delayed for 48 h. Administration of pralidoxime mesylate 30 s after sarin, prevented the anticholinesterase-induced fasciculation and tremor, the animals appearing slightly subdued. Pralidoxime mesylate did not produce any improvement in the condition of mice when administered 24 or 48 h after sarin.

Table 4. Effects of pyridostigmine bromide pretreatment on maximum observed increases in D6 and MCD produced by 1·6 μmol kg^{-1} sarin and 0·82 μmol kg^{-1} soman.

Pretreatment	n	D6 (μs)	MCD (μs)
a Sarin			
None	24	111·6 ± 23·6	34·2 ± 7·0
Pyridostigmine	37	50·6 ± 5·4*	13·4 ± 1·0*
b Soman			
None	21	118·2 ± 14·8	27·4 ± 6·7
Pyridostigmine	36	38·9 ± 4·9*	12·5 ± 0·9*

Values are mean ± s.e.m. of n observations. * Significantly different from values in mice not given pyridostigmine pretreatment ($P < 0·05$). Drug doses used were as follows: pyridostigmine bromide 0·38 μmol kg^{-1}, s.c., at −30 min; sarin 1·60 μmol kg^{-1}, s.c., at 0 min; soman 0·82 μmol kg^{-1}, s.c., at 0 min; atropine sulphate 0·70 μmol kg^{-1}, s.c., at +0·5 min.

In contrast, Table 3b shows that the effectiveness of pralidoxime mesylate against soman-induced D6 and MCD increases declined more rapidly, and the oxime was ineffective when given 60 min after soman. The administration of pralidoxime mesylate, at all of the times tested, did not produce any improvement in the condition of soman-poisoned mice.

Pralidoxime mesylate, given 30 s after sarin, effectively prevented any induced increases in D6 or MCD, the values not differing significantly from those obtained in unpoisoned mice. In contrast, pralidoxime mesylate administered 30 s after soman effectively prevented an increase in MCD, but not D6 value, which remained significantly higher than that obtained in unpoisoned mice.

Effects of pyridostigmine on anticholinesterase-induced increases in latency and MCD

Pyridostigmine bromide (0·38 μmol kg^{-1}, s.c.), given 30 min before the higher doses of sarin or soman, significantly reduced the organophosphate-induced increases in D6 and MCD, both at the time of the maximum observed increase post-poisoning, and at recording times either side of this time point. Table 4 shows the maximum observed values for D6 and MCD following sarin and soman poisoning for pyridostigmine-pretreated animals, and animals receiving no pretreatment.

Pyridostigmine effectively prevented D6 and MCD increases following sarin, both values not differing significantly from control values in unpoisoned mice. Following soman poisoning, however, although pyridostigmine significantly reduced the increase in D6 and MCD, the values remained significantly higher than those in unpoisoned mice. Pyridostigmine bromide by itself did not produce a significant change in either D6 or MCD. The carbamate dose tested (0·38 μmol kg^{-1}) produced 29% inhibition of erythrocyte acetylcholinesterase activity 30 min after its administration in these mice.

Discussion

The increase in D6 and MCD recorded in unpoisoned diaphragm strips is consistent with that described by Kelly et al (1990) to be a feature of normal neuromuscular transmission. In the present study, in-vivo administration of sarin and soman was shown to produce marked increases in both D6 (latency) and MCD (Fig. 1) of a magnitude and time profile similar to that produced by ecothiophate, BOS and DFP (Kelly et al 1990). This finding, together with the fact that the doses of sarin and soman producing these effects also caused muscle fibre damage in the diaphragm, supports the suggestion of Kelly et al (1990) that necrotic doses of anticholinesterases affect a larger proportion of muscle fibres than that measured solely in terms of the physical damage produced. The finding that there was no relationship between the D6 and MCD increase in individual fibres, and the difference in time course for the increase in these parameters (Fig. 1), supports the suggestion of Kelly et al (1990) that the increases in latency and MCD are the product of separate mechanisms.

Correlation of the jitter-increasing properties of organophosphates with their cholinesterase-inhibiting properties has not been well established. Kelly et al (1990) reported that jitter increases were generally associated with doses of organophosphates producing 87–91% peak inhibition of diaphragm cholinesterase. They observed inconsistencies, however, in that the doses of different organophosphates producing a similar level of cholinesterase inhibition caused different degrees of jitter increase. In the present study, sarin and soman produced increases in jitter at dose levels inhibiting diaphragm acetylthiocholine hydrolase activity by 56–73%. The increases in jitter appeared to be maximal at different times, however; 7 days post-sarin poisoning and 3 days post-soman poisoning. The apparent difference in time course of sarin- and soman-induced jitter increase cannot yet be explained, but might account for some of the inconsistencies described by Kelly et al (1990) who compared organophosphate-induced jitter increases at a single time point, 5 days post-poisoning, when effects were not necessarily maximal.

In the present study, the lowest sarin and soman doses tested, 1·24 and 0·42 μmol kg^{-1}, respectively, failed to produce statistically significant increases in mean values of D6 or MCD (Table 2). Analysis of the frequency distributions of MCD values, however, showed the presence of a small number of fibres with increased MCD in these experiments (Figs 2a, 3a). Since no muscle damage was observed in the diaphragms of these animals, the increase in MCD supports the suggestion of Kelly et al (1990) that increases in jitter need not necessarily be associated with muscle necrosis.

The oxime pralidoxime mesylate is an effective therapeutic agent in the treatment of anticholinesterase poisoning due to its ability to reactivate inhibited cholinesterase (Hobbiger 1963). The finding in the present study that pralidoxime mesylate therapy, given 30 s post-poisoning, effectively prevented sarin-, and partially reduced soman-induced increases in jitter (Table 2) suggests, therefore, that the jitter increase is related to organophosphate-induced cholinesterase inhibition. Pralidoxime mesylate reduced both sarin- and soman-induced jitter increases when given immediately post-poisoning, but rapidly declined in effectiveness against soman as the delay to administration was increased. This is consistent with the ageing of inhibited enzyme to a form resistant to oxime reactivation occurring more quickly following soman than sarin posoning (Fleisher & Harris

1965; Hobbiger 1976). Pralidoxime mesylate prevented sarin-induced jitter increase when given 24 h after poisoning (Table 3a), indicating that sarin-inhibited acetylcholinesterase was not completely aged after 24 h, and suggesting that a long period of cholinesterase inhibition is required to trigger the process leading to increase in jitter.

Pyridostigmine-pretreatment prevented sarin-induced jitter increase and partially reduced soman-induced jitter increase in this study. Carbamate-pretreatment exerts its protective action against organophosphate poisoning by reversibly inhibiting a portion of acetylcholinesterase, hence shielding it from irreversible inhibition by organophosphates. Spontaneous reactivation of the carbamoylated enzyme then regenerates sufficient acetylcholinesterase to restore normal neuromuscular function (Dirnhuber & Green 1978; Green & Smith 1983). The effectiveness of pyridostigmine in protecting against sarin- and soman-induced increases in jitter supports the suggestion that the jitter increases are due to the cholinesterase-inhibiting properties of organophosphates.

The findings from the present study that organophosphate-induced jitter increase is triggered by a period of prolonged cholinesterase inhibition, and is sensitive to carbamate-pretreatment and oxime therapy, is consistent with similar findings for organophosphate-induced muscle necrosis (Ariens et al 1969; Wecker et al 1978). These results suggest that jitter increase and muscle necrosis may be the end products of a common mechanism progressing to differing degrees. Fenichel et al (1972) and Leonard & Saltpeter (1979) reported calcium accumulation in muscle to be associated with myopathy produced by the organophosphates. They suggested that a calcium activated protease may be triggered to produce muscle damage if prolonged acetylcholine-receptor interactions elevate intracellular calcium levels sufficiently. The recent finding that calcium accumulation precedes myopathic damage following sarin in mice (Bright et al 1991) supports this suggestion. The link between organophosphate-induced jitter increase and calcium accumulation, however, remains to be established.

References

Ariens, A. T., Meeter, E., Wolthuis, O. L., Van Benthem, R. M. J. (1969) Reversible necrosis at the end-plate region in striated muscles of the rat poisoned with cholinesterase inhibitors. Experientia 25: 57–59

Bowman, W. C., Rand, M. J. (1980) Textbook of Pharmacology. 2nd edn, Blackwell Scientific Publications

Bright, J. E., Inns, R. H., Tuckwell, N. J., Griffiths, G. D., Marrs, T. C. (1991) A histochemical study of changes observed in the mouse diaphragm after organophosphate poisoning. Human Exp. Toxicol. 10: 9–14

Dettbarn, W. D. (1984) Pesticide induced muscle necrosis: mechanisms and prevention. Fund. Appl. Toxicol. 4: S18–S26

Dirnhuber, P., Green, D. M. (1978) Effectiveness of pyridostigmine in reversing neuromuscular blockade produced by soman. J. Pharm. Pharmacol. 30: 419–425

Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88–95

Fenichel, G. M., Kibler, W. B., Olsen, W. H., Dettbarn, W. D. (1972) Chronic inhibition of cholinesterase as a cause of myopathy. Neurology 22: 1026–1033

Fleisher, J. H., Harris, L. W. (1965) Dealkylation as a mechanism for ageing of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate. Biochem. Pharmacol. 14: 641–650

Gebbers, G. O., Lotscher, M., Kobel, W., Portmann, R., Laissue, J. A. (1986) Acute toxicity of pyridostigmine in rats: histological findings. Arch. Toxicol. 58: 271–275

Green, D. M., Smith, A. P. (1983) The reversal by pyridostigmine of soman induced neuromuscular blockade in primate respiratory muscle. Br. J. Pharmacol. 79: 252P

Grob, D. (1963) Anticholinesterase intoxication in man and its treatment. In: Koelle, G. B. (ed.) Cholinesterase and Anticholinesterase Agents. Hanbuch der Experimentellen Pharmacokologie. Vol. 15, Springer-Verlag, Berlin, Gottingen, Heidelberg, pp 989–1027

Hobbiger, F. (1963) Reactivation of phosphorylated acetylcholinesterase. In: Koelle G. B. (ed.) Cholinesterase and Anticholinesterase Agents. Hanbuch der Experimentellen Pharmakologie. Vol. 15, Springer-Verlag, Berlin, Gottingen, Heidelberg, pp 921–988

Hobbiger, F. (1976) Pharmacology of anticholinesterase drugs. In: Zaimis, E. (ed.) Neuromuscular Junction. Handbuch der Experimentellen Pharmakologie. Vol. 42, Springer Verlag, Heidelberg, New York, pp 487–581

Kelly, S. S., Ferry, C. B., Bamforth, J. P. (1990) The effects of anticholinesterases on the latencies of action potentials in mouse skeletal muscles. Br. J. Pharmacol. 99: 721–726

Kibler, W. B. (1973) Skeletal muscle necrosis secondary to parathion. Toxicol. Appl. Pharmacol. 25: 117–122

Laskowski, M. B., Olson, W. H., Dettbarn, W. D. (1975) Ultrastructural changes at the motor end-plate produced by an irreversible cholesterase inhibitor. Exp. Neurol. 47: 290–306

Leonard, J. P., Saltpeter, M. M. (1979) Agonist induced myopathy at the neuromuscular junction is mediated by calcium. J. Cell Biol. 82: 811–819

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Siegel, S. (1956) Non parametric Statistics for the Behavioural Sciences. McGraw-Hill Book Co, New York, Toronto, London

Stalberg, E., Trontelj, J. (1979) Single Fibre Electromyography. The Mirvalle Press Ltd, Old Woking, Surrey, UK

Wecker, L., Dettbarn, W. D. (1976) Paraoxon-induced myopathy: muscle specificity and acetylcholine involvement. Exp. Neurol. 51: 281–291

Wecker, L., Kiauta, T., Dettbarn, W. D. (1978) Relationship between acetylcholinesterase inhibition and the development of a myopathy. J. Pharmacol. Exp. Ther. 206: 97–104