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Efficacy of biperiden and atropine as anticonvulsant treatment for organophosphorus nerve agent intoxication

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Abstract The ability of the nerve agents tabun, sarin, soman, GF, VR, and VX to produce brain seizures and the effectiveness of the anticholinergics biperiden HCl or atropine SO_4 as an anticonvulsant treatment were studied in a guinea-pig model. All animals were implanted a week prior to the experiment with cortical electrodes for electroencephalogram (EEG) recordings. On the day of exposure, the animals were pretreated with pyridostigmine (0.026 mg/kg, i.m.) 30 min prior to challenge with a $2 \times LD_{50}$ dose (s.c.) of a given agent. In separate experiments, animals were challenged with $5 \times LD_{50}$ (sc) of soman. One minute after agent challenge, the animals were treated intramuscularly (i.m.) with 2 mg/kg atropine SO_4 admixed with 25 mg/kg 2-PAM Cl and then observed for the onset of seizure activity. Five minutes after the start of nerve agent-induced EEG seizures, animals were treated i.m. with different doses of biperiden HCl or atropine SO_4 and observed for seizure termination. The anticonvulsant ED_{50} of biperiden HCl and atropine SO_4 for termination of seizures induced by each nerve agent was calculated and compared. With equally toxic doses ($2 \times LD_{50}$) of these agents, continuous EEG seizures (status epilepticus) developed in all animals challenged with soman, tabun, or VR, and in more than 90% of the animals challenged with GF or sarin. In contrast, only 50% of the animals developed seizures when challenged with VX. The times to onset of seizures for soman, tabun, GF, and sarin were very similar (5–8 min) while for VR, it was about 10 min. In the case of VX, not only was the time to seizure development longer (20.7 min), but the seizure activity in 19% of the animals terminated spontaneously within 5 min after onset and did not return. Under these conditions, the anticonvulsant ED_{50}s of biperiden HCl for soman, GF, VR, tabun, sarin, and VX were 0.57, 0.51, 0.41, 0.2, 0.1, and 0.09 mg/kg, respectively, while those of atropine SO_4 for soman, VR, tabun, GF, sarin, and VX were 12.2, 11.9, 10.4, 10.3, 5.1, and 4.1 mg/kg, respectively. In separate experiments, the anticonvulsant ED_{50} doses of biperiden for animals challenged with 2 or $5 \times LD_{50}$ of soman were 0.48 (95% confidence limits 0.25–0.73) or 0.57 (95% CI 0.38–0.84) mg/kg, respectively, while the anticonvulsant ED_{50}s for atropine (12.2 mg/kg, i.m.) were identical under these same two challenge conditions. The present study demonstrates that all nerve agents can produce status epilepticus and that the therapeutic effectiveness of atropine and biperiden roughly paralleled the seizurogenic potential of these agents.

Key words Organophosphorus compounds · Cholinesterase inhibitors · Soman · Sarin · Tabun · GF · VX · Convulsions · Seizures · EEG activity · Anticonvulsants · Atropine · Biperiden · Anticholinergic compounds

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Introduction

The potential for exposure to chemical warfare nerve agents, such as sarin, VX, tabun, and soman, exists on the battlefield (e.g., Iran-Iraq war, Desert Storm), in the civilian sector as a threat by a terrorist group (e.g., Tokyo subway incident), or as an accident as part of current demilitarization efforts. These nerve agents are organophosphorus (OP) cholinesterase (ChE) inhibitors. Exposure causes a progression of toxic signs, including hypersecretions, fasciculations, tremor, convulsions, coma, respiratory distress, and death (Taylor 1985). These toxic effects are due to hyperactivity of the cholinergic system as a result of inhibition of ChE, in particular, acetylcholinesterase (AChE), and the subsequent increase in the concentration of the neurotransmitter acetylcholine (ACh) at central and peripheral sites

(Taylor 1985). A combined regimen of prophylaxis and therapy is now generally considered the most effective medical approach for dealing with the threat of nerve agent poisoning of military personnel (Dunn and Sidell 1989; Moore et al. 1995). Pretreatment with carbamate ChE inhibitors, such as pyridostigmine, shields a fraction of ChE in the periphery from irreversible inhibition by the nerve agents. In the event of poisoning, immediate therapeutic treatment with an anticholinergic drug, such as atropine sulfate, antagonizes the effects of excess ACh at muscarinic receptor sites, and an oxime, such as pyridine-2-aldoxime methylchloride (2-PAM), is used to reactivate any unaged inhibited enzyme. The use of carbamate pretreatment in conjunction with atropine and oxime therapy has been shown to significantly increase the survival rate of experimental animals exposed to multiple LD_{50} doses of some OP nerve agents (Berry and Davis 1970; Dirnhuber et al. 1979; Kluwe et al. 1987; Lennox et al. 1985).

This combined prophylaxis and therapy regimen, however, does not appear to ameliorate nerve agent-induced, centrally mediated seizure activity and concomitant motor convulsions (Dirnhuber et al. 1979; Kluwe et al. 1987; Shih et al. 1996). Seizure activity in OP intoxication creates a problem for the medical management of exposed subjects. Additionally, the seizure activity rapidly progresses to status epilepticus (Glenn et al. 1987; Koplovitz and Skvorak 1998; Lipp 1968; McDonough and Shih 1993) and contributes to the profound brain damage and cardiac pathology that develop as a consequence of exposure to these highly toxic compounds (Thornton and Fukuyama 1961; Thornton and Brigden 1962; Petras 1981, 1994; Lemercier et al. 1983; McLeod et al. 1984; McDonough et al. 1989, 1995; Kadar et al. 1995). Effective control of nerve agent-induced seizures continues to be a major goal of military medical research (Dunn and Sidell 1989). Therefore, concomitant administration of an adjunct compound selected for its anticonvulsant activity is required to improve the currently utilized regimen of carbamate pretreatment plus atropine and oxime therapy (Dunn and Sidell 1989; Moore et al. 1995).

Diazepam, a commercially available benzodiazepine anticonvulsant, was provided to US military forces in the early 1990s in an autoinjector that delivers a dose of 10 mg, i.m. Other countries have similar devices containing diazepam or similar benzodiazepines (e.g., avizafone) (Clement and Broxup 1993). The decision to use a benzodiazepine drug was based on animal research data from many laboratories (Hayward et al. 1990; Boskovic 1981; Johnson and Wilcox 1975; Lipp 1972, 1973; Rump et al. 1973). When viewed as a whole, these studies showed that diazepam or other benzodiazepines could block/terminate nerve agent-induced seizures/convulsions and enhance survival, especially when given in conjunction with carbamate pretreatment and atropine and oxime therapy. However, diazepam has been shown in experimental studies to provide less than total protection against the neuropathological consequences of nerve agent exposure (Hayward et al. 1990; McDonough et al. 1995). Although the neuropathology was significantly reduced compared with animals that did not receive diazepam, the incidence and degree of protection afforded were never complete (Hayward et al. 1990). This may relate to the findings that soman-induced seizures can recur after diazepam treatment (McDonough and Shih 1993). Additionally, if anticholinergic drugs are not given prior to or concurrently with diazepam, the lethal effects of the agent exposure are enhanced (McDonough and Shih 1993; Shih et al. 1991a). These observations suggest that there is a continuing need to find better drugs to treat seizures elicited by OP nerve agents. Recently, it has been shown that anticholinergic drugs have significant anticonvulsant activity against soman-induced seizures and potentially could provide greater or more enhanced protection than is now provided by diazepam (McDonough et al. 1989; Capacio and Shih 1991; McDonough and Shih 1993; Shih et al. 1996).

In the past, nerve agent anticonvulsant studies have focused almost exclusively on seizures elicited by the nerve agent soman (Shih et al. 1991a, 1996; McDonough and Shih 1993, 1997). The development of improved anticonvulsant treatments should focus on efficacy against all nerve agents. The ability of different nerve agents to produce seizures has only very recently been systematically explored (Shih and McDonough 1996). As part of a total effort to develop an advanced anticonvulsant, the drug of choice should possess the ability to terminate seizure activity elicited not only by soman, but also by other OP nerve agents. Furthermore, the anticonvulsant efficacy of a treatment should also extend across a wide range of exposure levels of nerve agents.

This study compared the development of electroencephalographic (EEG) seizures produced by six potential chemical warfare nerve agents, namely, tabun (ethyl *N,N*-dimethyl phosphoramidocyanidate), sarin (isopropyl methylphosphonofluoridate), soman (pinacolyl methylphosphonofluoridate), GF (cyclohexyl methylphosphonofluoridate), VR (*O*-isobutyl *S*-(2-(diethylamino)ethyl)methylphosphonothioate) and VX (*O*-ethyl *S*-(2-(diisopropylamino)ethyl)methylphosphonothioate). In addition, the anticonvulsant efficacy of atropine sulfate and biperiden HCl against seizures produced by these agents and by different challenging doses of soman was assessed. These experiments were conducted in a guinea-pig model that closely simulates the US Army fielded pretreatment/therapy regimen (i.e., pyridostigmine pretreatment and atropine sulfate plus 2-PAM therapy) (Keeler et al. 1991; Moore et al. 1995).

Materials and methods

Male Hartley guinea-pigs (Crl: (HA) BR COBS; Charles River Labs, Wilmington, Mass., USA), of 250–300 g body weight at the start of the study, served as subjects. Animals were housed individually in temperature (21° ± 2 °C) and humidity (50% ± 10%)

controlled animal quarters and maintained on a 12-h light-dark full spectrum lighting cycle with lights on at 0600 h. Laboratory chow and tap water were freely available except during the experimental period. The animal care program of the US Army Medical Research Institute of Chemical Defense is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

The animals used in this study were approved by the Institute's Animal Care and Use Committee and handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, proposed by the Committee to Revise the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, and published by National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

Saline (0.9% NaCl) injection, USP, was purchased from Cutter Labs (Berkeley, Calif.). Atropine sulfate was purchased from Sigma Chemical (St. Louis, Mo.). Pyridostigmine bromide was obtained from Hoffmann-La Roche (Nutley, NJ), 2-PAM from Ayerst Labs (New York, NY), and pentobarbital sodium injection from A.J. Buck & Son (Cockeysville, Md.). Tabun, sarin, soman, GF, VR, and VX were obtained from the US Army Edgewood Research, Development and Engineering Center (Aberdeen Proving Ground, Md.). The nerve agents were diluted in ice-cold saline prior to injection. Atropine sulfate and 2-PAM were prepared in saline solution. Biperiden HCl and pentobarbital were prepared and diluted in a vehicle containing 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol, and 48.5% distilled water. The injection volume was 0.5 ml/kg for 2-PAM, atropine sulfate, biperiden HCl, pyridostigmine, and all nerve agents. All drug solutions were prepared and injected separately, with the exception of the atropine sulfate (2 mg/kg) and 2-PAM therapy, which were admixed.

Surgery

All animals were prepared approximately 1 week before experimentation with cortical stainless steel screw electrodes using previously described procedures (McDonough and Shih 1993; Shih et al. 1996).

EEG recordings were made using QND software and amplifiers supplied by Neurodata (Pasadena, Calif.) (low frequency filter = 0.3 Hz; high frequency filter = 40 Hz; sampling rate = 128 Hz) and displayed on a computer monitor. During EEG recordings, all animals were housed in individual plastic recording chambers that allowed free movement with the exception of the recording leads attached to the electrode connector on the top of the head.

On the day of the experiment, the guinea-pigs were continuously monitored for EEG activity. After a 15-min recording of baseline EEG measures, the animals received pyridostigmine (0.026 mg/kg, im) to produce ~30% whole blood ChE inhibition (Lennox et al. 1985). Thirty minutes later, the animals were challenged with $2 \times LD_{50}$ subcutaneous (s.c.) dose of tabun (240 μg/kg), sarin (84 μg/kg), soman (56 μg/kg), GF (114 μg/kg), VR (22 μg/kg), or VX (16 μg/kg). In a separate series of experiments, animals were challenged with either 1 or $5 \times LD_{50}$ (28 or 140 μg/kg, s.c.) of soman. In the case of $1 \times LD_{50}$ challenge, one set of animals received pretreatment with pyridostigmine while a separate set did not (i.e., saline pretreatment). One minute after nerve agent challenge, all animals were treated with atropine sulfate (2 mg/kg, i.m.) plus 2-PAM (25 mg/kg, i.m.). This dose of 2-PAM closely approximates the total dose of 2-PAM in 3 autoinjectors given to a 70–75 kg human. The 2 mg/kg dose of atropine sulfate was chosen to be sufficient to prevent rapid lethal effects yet not to interfere significantly with seizure development (Shih et al. 1996; McDonough and Shih 1997). This dose (2 mg/kg, i.m.) was not included in the final calculation of the anticonvulsant ED_{50} for the study of atropine sulfate. Five minutes after the onset of EEG seizure activity, atropine sulfate or biperiden HCl was given intramuscularly. Animals were observed continuously for the 1st h following exposure and treatment and periodically thereafter for at least 6 h. EEGs were monitored continuously throughout this time and for 30 min at 24 h. Seizure onset was operationally defined as the appearance of ≥10 s of rhythmic high amplitude spikes or sharp wave activity in the EEG. Each animal was rated as having the seizure terminated or not terminated based on the overall appearance of the EEG record at the end of the experimental day. Animals still alive 24 h after nerve agent exposure were euthanized with an overdose of sodium pentobarbital (85 mg/kg, i.p.).

Data analysis

Dose-effect curves and the median effective dose (ED_{50}) for anticonvulsant activity of atropine sulfate or biperiden HCl administered 5 min after nerve agent-induced seizures were determined by probit analysis (Bliss 1952) using 4–7 doses with 5–6 animals per group. Anticonvulsant ED_{50}s for atropine sulfate or biperiden HCl were then compared among different nerve agents and for different challenging doses of soman.

Results

In this model, guinea-pigs were given a dose of pyridostigmine to produce ~30% inhibition of blood ChE and then challenged with a multiple lethal dose of a nerve agent. A combined therapy of atropine and 2-PAM was given 1 min after the nerve agent. Thus, it simulates the fielded pyridostigmine pretreatment and combined atropine and 2-PAM therapy that was proposed for military personnel in anticipation of a chemical attack (Keeler et al. 1991; Moore et al. 1995). All six OP nerve agents were capable of inducing brain seizure activity in this model. Either atropine sulfate or biperiden HCl, when given 5 min after onset of seizures, was capable of terminating on-going seizures induced by these nerve agents. When atropine sulfate or biperiden HCl treatment failed to stop the seizure, epileptiform activity was evident continuously throughout the 6-h experimental period and could still be observed in some animals 24 h after nerve agent exposure.

Table 1 summarizes and compares the seizure-producing effects of the 6 different nerve agents in this guinea-pig model. The data show that under the conditions of this model, soman (100%), VR (100%), tabun (98%), and GF (96%) produced a greater incidence of seizures than sarin (86%) and VX (53%). With VX challenge, the seizure activity spontaneously stopped in 19% of the animals that developed seizures. In these cases, epileptiform EEG activity would begin, wax, and wane for a short time (30 s to 4 min), then spontaneously terminate before anticonvulsant treatment was given. These animals would remain free of further epileptiform EEG activity for the rest of the 6-h recording period and the next day. Such spontaneous termination of epileptiform seizure activity was not observed with any other nerve agent. A comparison of the initial EEG seizure patterns between VX and the other 5 nerve agents is shown in Fig. 1. The onset of EEG seizures was rapid (~7 min) after soman, tabun, GF, or sarin, and slightly slower after VR (~10 min), while the onset of

Table 1 Seizure-producing effects of soman, tabun, GF, sarin, VX, and VR in the guinea-pig model [guinea-pigs received pyridostigmine (26 μg/kg, i.m.) 30 min before exposure to nerve agents ($2.0 \times LD_{50}$), followed 1 min later by atropine sulfate (2 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.). Anticonvulsant treatment was given intramuscularly 5 min after seizure onset]

	Soman	Tabun	GF	Sarin	VX	VR
Group size (*n*)	60	47	44	57	137	51
Seizure onset time (min)	8.32 ± 0.26 (60)	5.94 ± 0.24 (46)	7.09 ± 0.51 (42)	7.14 ± 0.38 (49)	20.73 ± 0.77 (73)	10.25 ± 0.34 (51)
Seizure occurrence	60/60 (100%)	46/47 (97.9%)	42/44 (95.5%)	49/57 (86%)	73/137 (53.3%)	51/51 (100%)
No evidence of EEG seizure	0/60 (0%)	1/47 (2.1%)	2/44 (4.5%)	8/57 (14%)	64/137 (46.7%)	0/51 (0%)
Seizure stopped before treatment	0/60 (0%)	0/46 (0%)	0/42 (0%)	0/49 (0%)	14/73 (19.2%)	0/51 (0%)
Died before EEG seizure	0/60 (0%)	0/47 (0%)	2/44 (4.5%)	0/57 (0%)	2/137 (1.5%)	0/51 (0%)
Died before treatment	0/60 (0%)	0/46 (0%)	0/42 (0%)	0/49 (0%)	0/73 (0%)	0/51 (0%)
Died after treatment	15/60 (25%)	11/46 (23.9%)	22/42 (52.4%)	8/49 (16.3%)	15/73 (20.5%)	11/51 (21.6%)
24-h cumulative mortality	15/60 (25%)	11/47 (23.4%)	24/44 (54.6%)	8/57 (14%)	17/137 (12.4%)	17/51 (33.3%)

seizures after VX was significantly slower (~21 min). In the case of GF (2 out of 44 animals) and VX (2 out of 137 animals) exposure, the possibility existed that a small percentage of animals would die before any evidence of EEG activation. One other notable aspect is the substantially higher 24-h accumulated mortality rate for GF (55%) than for any of the other five nerve agents (12%–33%).

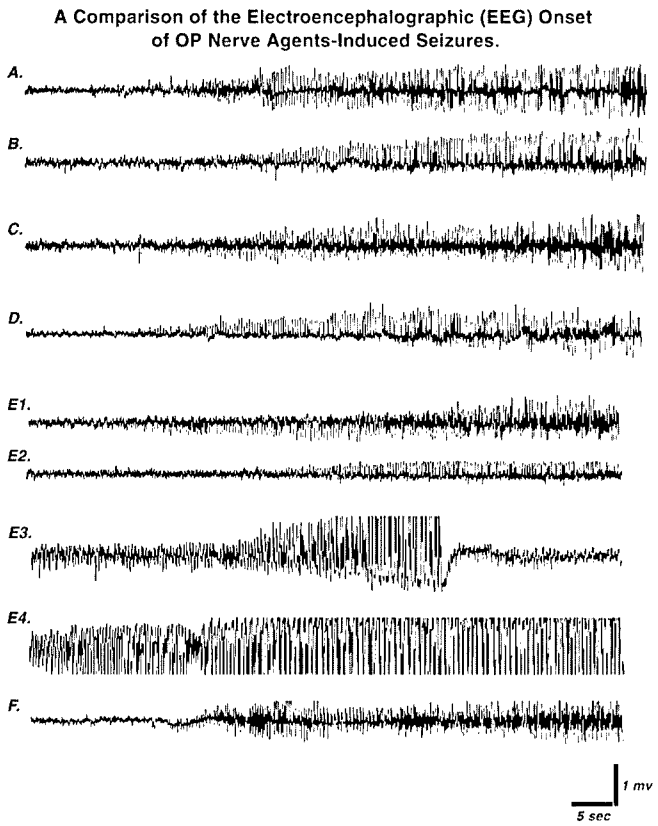

Fig. 1 A comparison of the electroencephalographic (EEG) onset of organophosphorus (OP) nerve agent-induced seizures. Tabun (*A*), sarin (*B*), soman (*C*), GF (*D*), and VR (*F*) have similar patterns of seizure onset and progression. The average time to onset for tabun = 6.30 min, sarin = 7.67 min, soman = 7.57 min, GF = 7.53 min, and VR = 10.53 min. VX (*E*) showed a different pattern of EEG activity. The seizures would begin as with the other agents (*E1*), then the spike amplitude would wax and wane (*E2*, *E3*, and *E4*) for a short time (30 s to 4 min), and then the amplitude would increase and continue on without further modulation in spike amplitude or frequency. In some cases, the EEG seizures spontaneously terminated by themselves and remained that way for the rest of the recording period and for 24 h

Table 2 summarizes the anticonvulsant efficacy (ED_{50}) of atropine sulfate and biperiden HCl for different nerve agents in this guinea-pig model. The efficacy of atropine sulfate as an anticonvulsant when given 5 min after seizure onset was similar for soman-, VR-, tabun-, and GF-induced seizures, with ED_{50} doses of 12.2, 11.9, 10.4, and 10.3 mg/kg i.m., respectively, while the anticonvulsant ED_{50} doses for sarin and VX were relatively lower, 5.1 and 4.1 mg/kg i.m., respectively. However, the 95% confidence limits of the atropine ED_{50} for sarin overlapped with those obtained for soman, tabun, GF, and VX, and the 95% confidence limits of the atropine ED_{50} for VX overlapped with those obtained for GF and sarin. The anticonvulsant ED_{50}s of biperiden HCl for soman, GF, VR, tabun, sarin, and VX were 0.57, 0.51, 0.41, 0.2, 0.1, and 0.09 mg/kg, respectively. The 95% confidence limits of the biperiden ED_{50} for tabun overlapped with those obtained for all other nerve agents, while the biperiden ED_{50}s for sarin and VX were completely different from those obtained for soman, GF, and VR. The doses required for biperiden HCl to terminate on-going seizures are consistently and remarkably lower than those required for atropine sulfate across all six nerve agents. When the doses of either anticholinergic drug were sufficient to terminate on-going seizures, the EEG pattern changes from high-amplitude and high-frequency spikes to a normal baseline activity were sudden and rapid, usually within minutes. A typical example is shown in Fig. 2, where the EEG epileptiform seizure activity terminated in 50 s around 16 min after a dose of biperiden.

Table 3 summarizes and compares the seizure-producing effects of different challenge doses of soman in this guinea-pig model. When challenged with a lower dose of soman ($1 \times LD_{50}$) after pyridostigmine pretreatment, the incidence of seizure was lower (57% vs 100%), and the latency to seizure production was longer (17.6 min vs 8.32 min) than when challenged with a $2 \times LD_{50}$ dose of soman. The results (the incidence of

Table 2 Anticonvulsant efficacy of atropine sulfate and biperiden hydrochloride against nerve agent-induced seizures in the guinea-pig model [guinea-pigs received pyridostigmine (26 μg/kg, i.m.) 30 min before receiving nerve agents (2.0 × LD_{50}), followed 1 min later by atropine sulfate (2 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.). Atropine sulfate or biperiden HCl as anticonvulsant therapy was administered intramuscularly 5 min after seizure onset. ED_{50}s calculated based on blocking of cortical EEG seizure activity with 95% confidence limits in parentheses]

ED_{50}s (mg/kg, i.m.)	Soman	Tabun	GF	Sarin	VX	VR
Atropine sulfate	12.2 (8.5–16.7)	10.4 (7.2–14.2)	10.3 (6.2–16.1)	5.1 (2.9–8.4)	4.1 (2.7–6.4)	11.9 (8.5–15.3)
Biperiden HCl	0.57 (0.38–0.84)	0.20 (0.09–0.47)	0.51 (0.32–1.93)	0.10 (0.06–0.17)	0.09 (0.08–0.12)	0.41 (0.24–0.64)

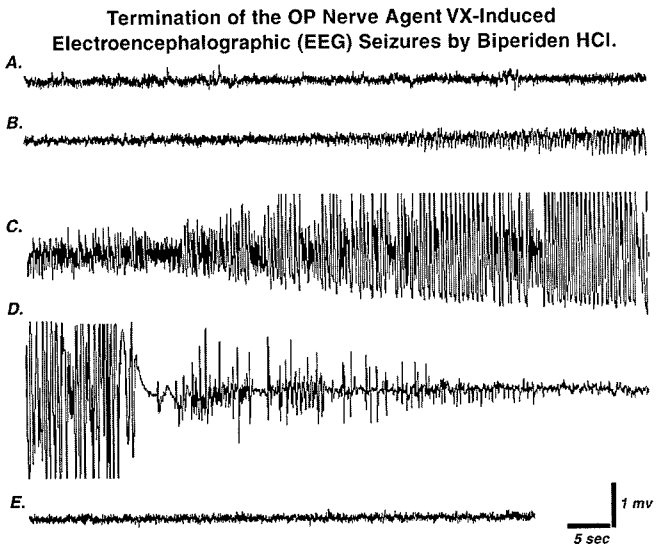

Fig. 2 Termination of the OP nerve agent VX-induced EEG seizures by biperiden HCl. **A** Baseline EEG activity. **B** Seizure started at 20 min 15 s after VX administration. **C** EEG seizure activity increased in spike amplitude. **D** Sudden and rapid (within 50 s) termination of seizure activity 16 min 30 s after treatment with biperiden HCl (0.25 mg/kg, i.m.). **E** EEG tracing returned to baseline activity after biperiden treatment and remained that way for the rest of the recording period and for 24 h

seizure and the latency to seizure) with a 1 × LD_{50} challenge of soman were virtually the same when pyridostigmine pretreatment was omitted. However, without pyridostigmine pretreatment, the 24-h lethality increased 3-fold, from 5% to 15%. When animals were challenged with 2 or 5 × LD_{50} doses of soman, there were remarkable differences in the response. Animals challenged with a 2 × LD_{50} dose of soman never lost their righting reflex during the initial development of toxic signs and seizures, and EEG epileptiform activity was continuously evident. In contrast, when challenged with the 5 × LD_{50} dose of soman, the animals would rapidly lose their righting reflex, and some animals then entered a comatose state in which respiratory efforts became erratic and of a diaphragmatic nature, the EEG recording became isoelectric, and the animal was totally unresponsive. This comatose state would last 1–4 min, after which the EEG activity slowly returned as respiratory efforts became more frequent and regular; epileptiform seizure activity emerged as the EEG returned toward a normal activity pattern (see Fig. 3). Three of the 31 animals challenged under these conditions died without any expression of EEG seizure activity during this comatose state, and two of the seizing animals died before anticonvulsant treatment could be administered. The latency to seizure onset was more rapid than after 2 × LD_{50} dose of soman.

Table 4 summarizes the anticonvulsant effects of atropine sulfate and biperiden HCl following different challenging doses (1, 2, or 5 × LD_{50}, s.c.) of soman. When challenged with a lower dose of soman (1 × LD_{50}) after pyridostigmine pretreatment, the anticonvulsant ED_{50} dose for atropine sulfate was lower (4.4 vs 12.2 mg/kg) than with 2 × LD_{50} doses of soman. The anticonvulsant ED_{50} for atropine sulfate with a 1 × LD_{50} challenge of soman was virtually the same when pyridostigmine pretreatment was omitted. When

Table 3 Seizure-producing effects of soman with or without pyridostigmine pretreatment in guinea-pigs [guinea-pigs received either pyridostigmine (26 μg/kg, i.m.) or no pyridostigmine 30 min before soman challenge. All animals received atropine sulfate (2 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.) 1 min after soman administration. Anticonvulsant treatment was given intramuscularly 5 min after seizure onset]

	No pyridostigmine 1 × LD_{50}	With pyridostigmine pretreatment (−30 min)		
		1 × LD_{50}	2 × LD_{50}	5 × LD_{50}
Group size (*n*)	39	42	60	54
Seizure onset time (min)	13.23 ± 1.08 (21)	17.64 ± 1.19 (24)	8.32 ± 0.26 (60)	5.10 ± 0.26 (51)
Seizure occurrence	21/39 (53.8%)	24/42 (57.1%)	60/60 (100%)	51/54 (94.4%)
Seizure stopped before treatment	1/21 (4.8%)	0/24 (0%)	0/60 (0%)	2/51 (3.9%)
No evidence of EEG seizure	18/39 (46.2%)	18/42 (42.9%)	0/60 (0%)	3/54 (5.6%)
Died before EEG seizure	0/39 (0%)	0/42 (0%)	0/60 (0%)	3/54 (5.6%)
Died before treatment	0/39 (0%)	0/42 (0%)	0/60 (0%)	2/51 (3.9%)
Died after treatment	6/20 (30%)	2/24 (8.3%)	15/60 (25%)	14/51 (27.5%)
24-h cumulative mortality	6/39 (15.4%)	2/42 (4.8%)	15/60 (25%)	26/54 (48.2%)

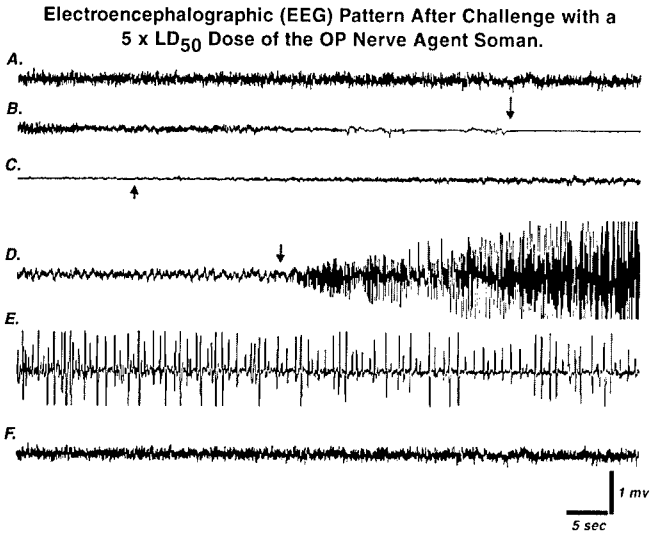

Fig. 3 EEG pattern after challenge with a $5 \times LD_{50}$ dose of the OP nerve agent soman. **A** Baseline activity. **B** Four minutes after soman administration, the animal entered a comatose state marked by an isoelectric EEG tracing that could last 1–4 min. Animals lost righting reflex and were totally unresponsive. **C** EEG activity gradually resumed 2 min 15 s after coma. **D** Seizure activity started 6 min 50 s after soman administration. **E** Seizure activity reduced in frequency 14 min 55 s after treatment with atropine sulfate (16 mg/kg, i.m.). **F** EEG returned to baseline activity 21 min 45 s after atropine sulfate treatment

animals were challenged with $5 \times LD_{50}$ dose of soman, the anticonvulsant ED_{50} dose (12.2 mg/kg, i.m.) for atropine was virtually the same as that for $2 \times LD_{50}$ dose of soman. This is also the case when biperiden HCl was used as anticonvulsant treatment.

Discussion

The present findings show that all six OP nerve agents (tabun, sarin, soman, GF, VR, and VX) when given at toxic doses, $2 \times LD_{50}$ in guinea-pigs, can induce EEG epileptiform activity. In most cases, the seizure activity rapidly develops into status epilepticus and persists for many hours if not successfully treated. The results show that in the guinea-pig model used in the present study the anticholinergic drugs atropine sulfate and biperiden HCl are capable of stopping these epileptiform seizures induced by any of the nerve agents tested. Furthermore, the effective anticonvulsant dose for either atropine or biperiden is, with the exception of VX, relatively the same across all nerve agents. The dose of biperiden HCl required to terminate seizure activity appear to be considerably (20- to 50-fold) lower than that of atropine sulfate across all nerve agents.

Soman has been the focus of nerve agent mechanistic and antidote research during the past several decades because of its resistance to standard therapy employing atropine sulfate and the oxime clinically available in the USA, 2-PAM (Shih et al. 1991b). Soman-inhibited ChE is only minimally reactivated by 2-PAM, TMB-4, or toxogonin (an oxime clinically available in Europe), but is susceptible to reactivation with the experimental oxime HI-6 (Dawson 1994). In addition, following its reaction with ChE, soman rapidly undergoes a chemical change ('aging' process) that makes reactivation of ChE activity by any oxime no longer possible (Fleisher and Harris 1965). However, animal studies have shown that significant survival after soman poisoning can be achieved by pretreatment with a carbamate (pyridostigmine) in conjunction with subsequent atropine sulfate and oxime (2-PAM) therapy (Berry and Davis 1970; Dirnhuber et al. 1979; Lennox et al. 1985; Kluwe et al. 1987). More recently, the focus of research has shifted to determine appropriate anticonvulsant drugs to control nerve agent seizures and prevent subsequent brain pathology (Shih et al. 1991a; McDonough et al. 1995; McDonough and Shih 1997). Previous studies of a variety of compounds with anticonvulsant potential, primarily benzodiazepines, anticholinergics, and *N*-methyl-D-aspartate antagonists, have evaluated seizure activity induced only by the nerve agent soman. Given that other nerve agents pose real military (sarin, tabun, GF during the Gulf War) and terrorist (sarin and VX used in different incidents in Japan) threats, the present series of experiments investigated the ability of all potential nerve agents to produce generalized epileptic seizures and the efficacy of the anticholinergic drugs atropine sulfate and biperiden HCl to control the brain epileptic activity elicited by these nerve agents.

In earlier studies, we have shown that the development of neuropathology following intoxication with soman (Petras 1981, 1994; Lemercier et al. 1983; McLeod et al. 1984; Hayward et al. 1990) is a result of the prolonged seizure activity (McDonough et al. 1995). Although EEG studies have not yet been done with

Table 4 Anticonvulsant efficacy of atropine sulfate and biperiden HCl following different challenging doses of soman in guinea-pigs [guinea-pigs received either pyridostigmine (26 μg/kg, i.m.) or no pyridostigmine 30 min before soman challenge. All animals received atropine sulfate (2 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.) 1 min after soman administration. Atropine sulfate or biperiden HCl was administered intramuscularly 5 min after seizure onset. ED_{50}s calculated based on blocking of cortical EEG seizure activity with 95% confidence limits in parentheses] (*NA* not studied)

ED_{50}s (mg/kg, i.m.)	No pyridostigmine $1 \times LD_{50}$	With pyridostigmine pretreatment (−30 min)		
		$1 \times LD_{50}$	$2 \times LD_{50}$	$5 \times LD_{50}$
Atropine sulfate	4.1 (2.5–6.9)	4.4 (2.6–7.9)	12.2 (8.5–16.7)	12.2 (7.5 18.1)
Biperiden HCl	NA	NA	0.57 (0.38 0.84)	0.48 (0.25 0.73)

other OP nerve agents prior to the present study, it has also been reported that nerve agents such as sarin (Kadar et al. 1995) and VX (Thornton and Fukuyama 1961; Thornton and Brigden 1962) produce neuropathology in animals. Therefore, it has been speculated that OP nerve agents other than soman may induce prolonged brain seizure as well. Indeed, the present study shows that all six nerve agents tested can induce prolonged brain seizures in our guinea-pig model. However, there are differences among these nerve agents. Soman, VR, tabun, and GF induce brain seizures fairly rapidly (6–10 min) and with a high incidence (>90%), while VX induces seizure with a long latency (21 min) and low incidence of seizure development (50%) under these conditions. With VX, the epileptiform EEG activity spontaneously stopped in one-fifth of the animals that developed seizures. Such spontaneous termination of seizure activity was not observed with any other nerve agents, although a low incidence of seizure development (73%) was observed after sarin intoxication. Based on the present findings and the neuropathology observed after prolonged soman-induced seizures (McDonough et al. 1995), neurological damage is a predictable outcome for all nerve agents, if an effective anticonvulsant is not given rapidly to terminate the seizure activity.

Atropine sulfate in combination with an oxime has traditionally been utilized as the mainstay of therapy against the lethal effects of OP anti-ChE compounds, including commercial pesticides as well as nerve agents (Taylor 1985). Recently, the anticonvulsant effects of atropine sulfate in larger doses as well as other anticholinergic compounds, such as biperiden, scopolamine, and trihexyphenidyl, in much smaller doses, have been recognized and documented against seizures elicited by the OP nerve agent soman (McDonough et al. 1989; Capacio and Shih 1991; Shih et al. 1991a, 1996). Unfortunately, the anticonvulsant effect of atropine sulfate and biperiden HCl against nerve agents other than soman has not been reported. The present study shows that either atropine sulfate or biperiden HCl can terminate seizure activity induced by all six nerve agents (tabun, sarin, soman, GF, VR, and VX) if given shortly (5 min) after seizure onset. However, the doses required for biperiden HCl to terminate on-going seizures are consistently and remarkably lower than those required for atropine sulfate, not only for soman as previously reported (Capacio and Shih 1991; Shih et al. 1991a, 1996), but for all the nerve agents. As shown in the present study, soman, VR, tabun, and GF induce a higher incidence of seizure development (100%, 100%, 95%, and 92%, respectively) than that induced by sarin and VX (73% and 50%, respectively) for exposure to a $2 \times LD_{50}$ dose. The reason for this difference is not known, but may reflect the lower ChE reactivating ability of 2-PAM against the former four agents (Shih et al. 1991b; Dawson 1994; Maxwell et al. 1997). Furthermore, higher doses of atropine sulfate or biperiden HCl were required to control soman-, VR-, tabun-, or GF-induced seizures than were required for sarin- and VX-induced seizures. Thus, it appears that the anticonvulsant ED_{50} of atropine sulfate for a nerve agent was highly correlated with the incidence of seizures.

The overall data show that a higher dose of atropine sulfate or biperiden HCl was required to terminate on-going seizure activity induced by soman than by other nerve agents. This suggests that a drug capable of stopping soman-induced seizures would be equally effective for the treatment of seizures elicited by other OP nerve agents as well. It reaffirms our earlier selection of soman as the nerve agent to use in animal models to evaluate potential anticonvulsant drugs (Shih 1990; Shih et al. 1991a, 1996; McDonough and Shih 1993).

This study also shows that, with or without pyridostigmine pretreatment, the incidence of seizure development is about 55% in both groups challenged with a $1 \times LD_{50}$ dose of soman, and the anti-seizure ED_{50} of atropine sulfate was similar in these two groups (4.4 or 4.1 mg/kg). However, as expected, a higher mortality was observed for the group that did not receive prior pyridostigmine treatment. When the challenge dose of soman was increased from $1 \times LD_{50}$ to $2 \times LD_{50}$, the incidence of seizure development increased to 100%, and the ED_{50} of atropine to terminate seizure increased to 12.2 μg/kg. This result may reflect the similarity in low incidence (~55%) of seizure development in these two $1 \times LD_{50}$ groups as compared with the $2 \times LD_{50}$ group (Table 3). However, there was no similar increase in the anticonvulsant ED_{50} of atropine sulfate or biperiden HCl between the 2 and $5 \times LD_{50}$ challenge dose of soman. Thus, it may be reasonable to speculate that once the functional threshold of cholinergic receptors has been activated by the increased ACh that follows ChE inhibition by a nerve agent to trigger epileptiform seizure activity, the same amount of atropine sulfate or biperiden HCl is required to compete with and block these functional receptors.

In conclusion, the present study demonstrates that prolonged EEG seizure activity (status epilepticus) can be induced by all potential chemical warfare nerve agents (tabun, sarin, soman, GF, VR, and VX). Both atropine sulfate and biperiden HCl can stop on-going seizure activity generated by these OP nerve agents if given shortly after seizure onset, although the doses required to terminate seizure activity appear to be markedly higher for atropine sulfate than for biperiden HCl. Among the agents studied, VX had the lowest incidence of seizure and was much easier to control by atropine sulfate or biperiden HCl. Regardless of the challenge dose (2 or $5 \times LD_{50}$) of soman used to initiate seizures, the dose of atropine sulfate or biperiden HCl needed to terminate the seizures remained the same. This study reaffirms the selection of soman as the nerve agent to use in animal models to evaluate potential anticonvulsant drugs (Shih 1990; Shih et al. 1991a, 1996; McDonough and Shih 1993), since all effective doses of atropine sulfate or biperiden HCl for other OP nerve agents were equal to or lower than those for soman.

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