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Organophosphorus Nerve Agents-Induced Seizures and Efficacy of Atropine Sulfate as Anticonvulsant Treatment

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SHIH, T.-M. AND J. H. McDONOUGH, JR. *Organophosphorus nerve agents-induced seizures and efficacy of atropine sulfate as anticonvulsant treatment.* PHARMACOL BIOCHEM BEHAV **64**(1) 147–153, 1999.—The ability of five organophosphorus nerve agents (tabun, sarin, soman, GF, and VX) to produce brain seizures and the effectiveness of atropine as an anticonvulsant treatment against these nerve agents were studied in two different animal models—the rat and guinea pig. All animals were implanted with cortical electrodes for EEG recordings. Five minutes after the start of nerve agent-induced EEG seizures, animals were treated intramuscularly (IM) with different doses of atropine sulfate and observed for seizure termination. The anticonvulsant ED_{50} of atropine sulfate for termination of seizures induced by each nerve agent was calculated and compared. In the rat model, selected oximes were administered either before, concurrent with, or following challenge with a 1.6 × LD_{50} dose of a given nerve agent to maximize seizure development with certain agent/oxime combinations. The choice and the timing of oxime administration significantly effected the incidence of seizure development by different nerve agents. When oxime administration did not effect seizure development (tabun, soman) the anticonvulsant ED_{50} for atropine sulfate was the same, regardless of the nerve agent used to elicit the seizure. When oxime administration reduced the incidence of seizure occurrence (sarin, GF, VX), the anticonvulsant ED_{50} dose of atropine sulfate for a nerve agent was lower. In the guinea pig model, animals were pretreated with pyridostigmine prior to challenge with 2 × LD_{50} of a given agent, and treated 1 min later with atropine sulfate (2 mg/kg) and 2-PAM (25 mg/kg). Under these conditions, the incidence, latency of seizure development, and anticonvulsant ED_{50}s of atropine for soman-, tabun-, and GF-elicited seizures were virtually identical. With sarin, although the latency of seizure development was the same as with soman, tabun, and GF, seizures occurred with a lower incidence, and the anticonvulsant ED_{50} of atropine was lower. With VX, the latency of seizure development was notably longer, while the incidence of seizure development and anticonvulsant ED_{50} of atropine were significantly lower than with soman, tabun, or GF. In both models, a lower incidence of seizure development predicted a lower anticonvulsant dose of atropine. In the rat, the incidence of seizure development and the anticonvulsant effectiveness of atropine was highly dependent on the oxime used. In the guinea pig, higher doses of atropine sulfate were required to control soman-, tabun-, or GF-induced seizures, perhaps reflecting the lower cholinesterase reactivating ability of 2-PAM against these agents. © 1999 Elsevier Science Inc.

Organophosphorus compounds Cholinesterase inhibitors Nerve agents Soman Sarin Tabun GF VX Convulsions Seizures EEG activity Anticonvulsants Atropine sulfate Anticholinergic compounds

POTENTIAL for exposure to chemical warfare nerve agents, such as sarin, 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 incidence), or as an accident as part of current demilitarization efforts. These nerve agents are organophosphorus (OP) cholinesterase (ChE) inhibitors, and exposure causes a progression of toxic signs, including hypersecretions, fasciculations, tremor, convulsions, coma, respiratory distress, and death (35). These toxic effects are due to hyperactivity of the cholinergic system

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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 (35). A combined regimen of prophylaxis and therapy is now generally agreed upon as the most effective medical countermeasure for dealing with the threat of nerve agent poisoning to military personnel (8,26). 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.

This combined prophylaxis and therapy regimen, however, does not ameliorate nerve agent-induced centrally mediated seizure activity and concomitant motor convulsions (7,14,31). Seizure activity in OP intoxication creates a problem for casualty management of exposed subjects and can progresses to status epilepticus (10,15,18,23), which contributes to the profound brain damage and cardiac pathology that can develop as a consequence of exposure to these highly toxic compounds (13,16,21,22,25,27,28,36,37). Although diazepam or other similar benzodiazepines are currently utilized to control OP-induced seizures (3,5,11,12,19,20,29), there are significant drawbacks to the use of benzodiazepines as the sole treatment of nerve agent-induced seizures (24,33). Recently, several animal studies have shown that anticholinergic drugs can prevent or stop seizures induced by the nerve agent soman (4,22, 23,31). Potentially, these drugs could provide greater or more enhanced protection from nerve agent-induced seizures than is now provided solely by diazepam.

In the past, nerve agent anticonvulsant studies have focused almost exclusively on seizures elicited by the nerve agent soman (23,24,31,32). Ideally, an improved anticonvulsant for the treatment of nerve agent-induced seizures should possess the ability to terminate seizure activity elicited 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. To address these questions, the present study assessed the development of electroencephalographic (EEG) seizures produced by five chemical warfare nerve agents, namely, tabun (ethyl *N,N*-dimethyl phosphoramidocyanidate), sarin (isopropyl methylphosphonofluoridate), soman (pinacolyl methylphosphonofluoridate), GF (cyclohexyl methylphosphonofluoridate), and VX (0-ethyl s-2-diisopropylaminoethyl methylphosphonothiolate). In addition, the anticonvulsant efficacy of a currently used OP nerve agent antidote, atropine sulfate, was assessed against seizures produced by these agents and by different challenge doses of soman. These experiments were conducted initially in a rat model (30,32) in which the animals were pretreated with oximes such as HI-6 (*N,N′*-(oxydimethylene)(pyridine-4-carboxyamide)(pyridine-2-aldoxime) dichloride), 2-PAM or TMB-4 (*N,N′*-trimethylene bis(pyridine-4-aldoxime) bromide monohydrate), and subsequently, in a guinea pig model that simulates the fielded pyridostigmine pretreatment and atropine sulfate plus 2-PAM therapy (26).

METHOD

Subjects

Male Crl:CDBR Vaf/Plus Sprague–Dawley rats (250–300 g) and male Hartley guinea pigs (250–300 g), obtained from Charles River Labs (Wilmington, MA), served as subjects. Animals were housed individually in temperature (21 ± 2°C) and humidity (50 ± 10%)-controlled animal quarters and maintained on a 12 L:12 D full spectrum lighting cycle with lights on at 0600 h. Laboratory chow and tap water were freely available.

Surgery

All animals were prepared approximately 1 week before experimentation with cortical stainless screw electrodes while under isoflorane anesthesia. The screws were placed approximately equidistant between bregma and lamda, and bilaterally ±3.0 mm from the midline. The screws were connected by wires to minature plugs; the screws, wires, and plugs were then anchored to the skull and insulated with dental acrylic. Animals that survived 24 h after nerve agent exposure were euthanized with an overdose of sodium pentobarbital (85 mg/kg, IP).

Apparatus

EEG recordings were made using QND software and amplifiers supplied by Neurodata Inc. (Pasadena, CA) (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.

Materials

Saline (0.9% NaCl) injection, USP, was purchased from Cutter Labs. Inc. (Berkeley, CA); atropine sulfate, atropine methylnitrate and TMB-4 were obtained from Sigma Chemical Co. (St. Louis, MO); pyridostigmine bromide was obtained from Hoffmann–LaRoche Inc. (Nutley, NJ); 2-PAM was obtained from from Ayerst Labs, Inc. (New York City, NY). HI-6 was a gift from the Defence Research Establishment Suffield (Canada). Tabun, sarin, soman, GF, and VX were obtained from the U.S. Army Edgewood Research, Development and Engineering Center (Aberdeen Proving Ground, MD). Agents were diluted in ice-cold normal saline and maintained on ice prior to injection. All compounds were prepared in normal saline solution; injection volume was 0.5 ml/kg for all drugs and all nerve agents. All drug solutions were prepared and injected separately, with the exception of the atropine sulfate and 2-PAM therapy used in the guinea pig study, which was admixed.

Procedure: Rat Models

On the day of the experiment, rats were continuously monitored for EEG activity. After a 15-min recording of baseline EEG, animals received HI-6 (125 mg/kg, IP) and 30 min later were challenged with 1.6 × LD_{50} (SC) of tabun (448 μg/kg), soman (176 μg/kg), sarin (200 μg/kg), GF (336 μg/kg), or VX (26 μg/kg) based on the test model described earlier (30). At 5 min after seizure onset atropine sulfate was injected IM at varying doses to establish its anticonvulsant efficacy against these nerve agents. With this HI-6 pretreatment model, however, one-third of the animals challenged with sarin and none of the animals challenged with VX developed seizures. Therefore, another oxime, 2-PAM (25 mg/kg, IM), was given either 30 min before or immediately after sarin or VX challenge. When 2-PAM was given as a pretreatment or immediately after VX challenge, only about 33% of all rats challenged with

VX developed seizures. Thus, in an additional series of rats, 2-PAM treatment was delayed and given at the onset of VX-induced seizures. In another experiment, TMB-4 (20 mg/kg, IM) was given 30 min before the tabun challenge. Regardless of the timing of oxime treatments, all animals received 2 mg/kg, IM, atropine methylnitrate, immediately after nerve agent challenge, to minimize peripheral mucus secretions and salivation, which interfered with respiration. The doses of oxime were chosen based on earlier findings that 125 mg/kg HI-6 provided the best overall lethality protection against this soman challenge (30,34), whereas the doses of 2-PAM and TMB-4 were based on literature values commonly used against these agents (respectively sarin, VX, and tabun) (6). Animals were observed continuously for the first hour following exposure and treatment and periodically thereafter for at least 6 h. EEGs were monitored continuously throughout this time and at 24 h. Seizure onset was operationally defined as the appearance of $\geqslant$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.

Procedure: Guinea Pig Model

On the day of the experiment, guinea pigs were continuously monitored for EEG activity. After a 15-min recording of baseline EEG measures, animals received pyridostigmine (0.026 mg/kg, IM), to produce ~30% whole blood ChE inhibition (17). Thirty minutes later, animals were challenged with 2 × LD_{50} (SC) of tabun (240 μg/kg), sarin (84 μg/kg), soman (56 μg/kg), GF (114 μg/kg), or VX (16 μg/kg). In a separate experiment animals were challenged with 1 (28 μg/kg) or 5 (140 μg/kg) × LD_{50} (SC) of soman. One minute after nerve agent challenge, all animals were treated with atropine sulfate (2 mg/kg, IM) plus 2-PAM (25 mg/kg, IM). This dose of 2-PAM closely approximates the total dose of 2-PAM in three 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 significantly interfere with seizure development (24,31). Five minutes after the onset of EEG seizure activity atropine sulfate was given intramuscularly. Observation of the animal's behavioral response to the drug, EEG monitoring after drug treatment, and assessment of seizure response to the treatment were identical to the criteria outlined in the rat model.

Data Analysis

Dose–effect curves and the median effective dose (ED_{50}) for anticonvulsant activity of atropine sulfate when administered 5 min after nerve agent-induced seizures were determined by probit analysis (2) using four to seven doses with five to six animals per group. Anticonvulsant ED_{50}s for atropine sulfate were then compared among the different nerve agents and among the different challenge doses of soman.

RESULTS

Rat Model

All five OP nerve agents were capable of producing brain seizure activity in rats. However, the development of seizures was highly dependent on the challenge agent, the oxime used, and, in some cases, the timing of oxime administration. When atropine sulfate 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 even 24 h later (15,23).

With HI-6 pretreatment (Table 1), both soman and tabun produced seizures in 100% of the subjects, whereas GF and sarin produced seizures in 77 and 68% of the animals tested, respectively. In contrast, none of the eight rats challenged with VX developed seizure activity, and all of them survived 24 h without any additional treatment. The time to onset of seizures was rapid and essentially the same (2.7–4.2 min) for soman, tabun, GF, and sarin. The 5-min anticonvulsant ED_{50}s of atropine sulfate for seizures elicited by soman and tabun were 59.8 and 48.5 mg/kg, respectively, which was significantly higher than those for GF and sarin, which were 20.5 and 11.0 mg/kg, respectively. With GF and sarin challenge, one and two animals, respectively, died rapidly after exposure without any trace of developing EEG spike activity.

The low incidence (sarin) or absence (VX) of seizure activity when the oxime HI-6 was used as a pretreatment prompted the use of a different oxime, 2-PAM, to further examine the seizure-producing effects of these two agents and the anticonvulsant efficacy of atropine sulfate. When 2-PAM was used as the pretreatment oxime instead of HI-6, a different pattern of results developed (Table 2). Virtually all animals challenged with soman, tabun, GF, and sarin developed seizures, again with short latencies. However, challenge with soman, tabun, or GF was so rapidly lethal that it prevented an

TABLE 1

SEIZURE PRODUCING EFFECTS OF SOMAN, TABUN, GF, SARIN, AND VX IN RATS: HI-6 PRETREATMENT MODEL

	Soman	Tabun	GF	Sarin	VX
Seizure onset time (min)	3.69 + 0.28 (28)	4.22 + 0.56 (37)	2.73 + 0.13 (23)	3.58 + 0.31 (27)	—
Seizure occurrence	28/28 (100%)	37/37 (100%)	23/30 (76.7%)	27/40 (67.5%)	0/8 (0%)
No EEG seizure	0/28 (0%)	0/37 (0%)	7/30 (23.3%)	13/40 (32.5%)	8/8 (100%)
Died before EEG seizure	0/28 (0%)	0/37 (0%)	1/7 (14.3%)	2/13 (15.4%)	0/8 (0%)
Died before treatment	1/28 (3.6%)	8/37 (21.6%)	3/23 (13.0%)	5/27 (18.5%)	—
Died after treatment	6/28 (21.4%)	2/37 (5.4%)	1/23 (4.3%)	3/27 (11.1%)	—
24-h mortality	7/28 (25%)	10/37 (27%)	5/30 (16.7%)	10/40 (25%)	0/8 (0%)
ED_{50}s (mg/kg, IM) for atropine sulfate	59.8 (50.9 – 71.6)	48.5 (39.0 – 59.4)	20.5 (14.7 – 25.3)	11.0 (7.7 – 15.3)	—

Rats were treated with atropine sulfate (IM) 5 min after the onset of nerve agent-induced seizures. All animals received HI-6 (125 mg/kg, IP) 30 min prior to 1.6 × LD_{50} dose of nerve agent administration. ED_{50}s calculated based on blocking of cortical EEG seizure activity with 95% confidence limits in parentheses.

accurate assessment of any anticonvulsant potential of atropine sulfate. With VX, seizures developed at a substantially lower incidence (33.3%) and a significantly longer latency (19.05 min) than with the other four agents. The 5-min anticonvulsant ED_{50} of atropine sulfate for sarin was 51.8 mg/kg under these conditions, whereas for VX it was 19.4 mg/kg.

In a further effort to enhance the incidence of seizure development in VX-challenged animals, 2-PAM was administered immediately after sarin or VX challenge, rather than as pretreatment. This procedural manipulation did not appreciably change the results; seizures developed with the same incidence (sarin = 93%; VX = 28%), latencies (sarin = 5.64 ± 1.13 min; VX = 22.31 ± 3.96 min), and responsiveness to atropine sulfate treatment (atropine ED_{50}: sarin = 59.8 mg/kg, 47.9–71.9 = 95% confidence limits; VX = 27.5 mg/kg, 2.5–39.5 = 95% confidence limits) as when 2-PAM was given as a pretreatment. Therefore, an additional experiment was performed in which 2-PAM treatment was given only after the onset of seizures induced by VX challenge. Under these conditions, the 1.6 X LD_{50} challenge of VX elicited seizures in 62% of the animals tested; the other 38% of the animals died before a seizure developed. In the animals that did seize, the 5-min anticonvulsant ED_{50} of atropine sulfate was calculated to be 57.6 mg/kg (42.1–117.8 mg/kg = 95% confidence limits). In another experiment, the oxime TMB-4 (20 mg/kg, IM) was given 30 min before animals were challenged with tabun. Under these conditions, all animals developed seizures with short onset latencies ($\overline{X}$ (or mean) = 3.46 ± 0.20 min) and an anticonvulsant ED_{50} of atropine = 53.1 mg/kg (46.8–60.4 mg/kg = 95% confidence limits). These results were virtually identical to those obtained when HI-6 pretreatment was used (Table 1).

Guinea Pig Model

Similar to what was observed in rats, all five OP nerve agents were capable of inducing brain seizure activity in guinea pigs under the treatment conditions used. When atropine sulfate 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 even 24 h after nerve agent exposure (31).

Table 3 summarizes the findings comparing the seizure-producing effects of the different agents and the anticonvulsant efficacy of atropine sulfate in this guinea pig model. The data show that under the conditions of this model, soman (100%), tabun (95%), and GF (92%) produced a greater incidence of seizures than sarin (73%) and VX (50%). With the VX challenge, the seizure activity spontaneously stopped in 18% of the animals that developed seizures. In these cases, the seizures would begin, wax, and wane for a short time (30 s to 4 min), then spontaneously terminate before atropine treatment was given. These animals would remain free of further epileptiform activity for the rest of the 6-h recording period and the next day. Such spontaneous termination of seizure activity was not observed with any other nerve agent. The onset of EEG seizures was rapid (~7 min) after soman, tabun, GF or sarin, while the onset of seizures after VX was significantly slower (~21 min). The efficacy of atropine sulfate as an anticonvulsant when given 5 min after seizure onset was similar for soman-, tabun-, and GF-induced seizures, with ED_{50} doses of 12.2, 10.4, and 10.3 mg/kg, IM, respectively, while the anticonvulsant ED_{50} doses of atropine sulfate for sarin and VX were relatively lower—5.1 and 4.1 mg/kg, IM, 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. One other notable aspect of this data is the substantially higher 24-h mortality rate for GF than any of the other four nerve agents.

Table 4 summarizes the results comparing the anticonvulsant effects of atropine sulfate following different challenging doses (1, 2, or 5 × LD_{50}, SC) of soman. When challenged with the low dose of soman (1 × LD_{50}), the incidence of seizure was lower (57%), the latency to produce seizure was longer (17.6 min), and the anticonvulsant ED_{50} dose for atropine sulfate was lower (4.4 mg/kg, IM) than with either of the higher challenge doses of soman. The results 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 by threefold, from 5 to 15%. When animals were challenged with 2- or 5 × LD_{50} doses of soman, the latency to seizure onset (~6–7 min) and the anticonvulsant ED_{50} doses (12.2 mg/kg, IM) for atropine were virtually the same for either condition. This was despite remarkable differences in the response to these challenge doses. 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 activity was always evident. In contrast, when challenged with the 5 × LD_{50} dose of

TABLE 2

SEIZURE PRODUCING EFFECTS OF SOMAN, TABUN, GF, SARIN, AND VX IN RATS: 2-PAM PRETREATMENT MODEL

	Soman	Tabun	GF	Sarin	VX
Seizure onset time (min)	3.90 ± 0.72 (6)	3.17 ± 0.35 (8)	2.58 ± 0.24 (6)	4.67 ± 0.64 (29)	19.05 ± 2.93 (18)
Seizure occurrence	6/6 (100%)	8/8 (100%)	6/6 (100%)	29/30 (96.7%)	18/54 (33.3%)
No EEG seizure	0/6 (0%)	0/8 (0%)	0/6 (0%)	1/30 (3.3%)	36/54 (66.7%)
Seizure stopped before treatment	0/6 (0%)	0/8 (0%)	0/6 (0%)	0/29 (0%)	3/18 (37.5%)
Died before treatment	2/6 (33.3%)	7/8 (87.5%)	6/6 (100%)	8/29 (27.6%)	0/15 (0%)
Died after treatment	4/6 (66.7%)	1/8 (12.5%)	—	7/29 (24.1%)	0/15 (0%)
24-h mortality	6/6 (100%)	8/8 (100%)	6/6 (100%)	16/30 (53.3%)	0/54 (0%)
ED_{50}s (mg/kg, IM) for atropine sulfate	—	—	—	51.8 (41.1 – 61.9)	19.4 (0.80 – 49.71)

Rats were treated with atropine sulfate (IM) 5 min after the onset of nerve agent-induced seizures. All animals received 2-PAM (25 mg/kg, IM) 30 min prior to 1.6 × LD_{50} dose of nerve agent administration. ED_{50}s calculated based on blocking of cortical EEG seizure activity with 95% confidence limits in parentheses.

TABLE 3

SEIZURE PRODUCING EFFECTS OF SOMAN, TABUN, GF, SARIN, AND VX: GUINEA PIG MODEL

	Soman	Tabun	GF	Sarin	VX
Seizure onset time (min)	7.57 ± 0.34 (30)	6.30 ± 0.44 (19)	7.53 ± 0.79 (22)	7.67 ± 0.42 (22)	21.26 ± 1.04 (38)
Seizure occurrence	30/30 (100%)	19/20 (95%)	22/24 (91.7%)	22/30 (73.3%)	38/76 (50%)
No EEG seizure	0/30 (0%)	1/20 (5%)	2/24 (8.3%)	8/30 (26.7%)	38/76 (50%)
Seizure stopped before treatment	0/30 (0%)	0/19 (0%)	0/22 (0%)	0/22 (0%)	7/38 (18.4%)
Died before EEG seizure	0/30 (0%)	0/20 (0%)	2/24 (8.3%)	0/30 (0%)	2/76 (2.6%)
Died before treatment	0/30 (0%)	0/19 (0%)	0/22 (0%)	0/22 (0%)	0/31 (0%)
Died after treatment	10/30 (33.3%)	7/19 (36.8%)	13/22 (59.1%)	4/22 (18.2%)	12/31 (38.7%)
24-h mortality	10/30 (33.3%)	7/20 (35%)	15/24 (62.5%)	4/30 (13.3%)	14/76 (18.4%)
	12.2	10.4	10.3	5.1	4.1
ED_{50}s (mg/kg, IM) for atropine sulfate	(8.5 – 16.7)	(7.2 – 14.2)	(6.2 – 16.1)	(2.9 – 8.9)	(2.7 – 6.4)

Guinea pigs received pyridostigmine (26 μg/kg, IM) 30 min before receiving nerve agents ($2.0 \times LD_{50}$), followed 1 min later by atropine sulfate (2 mg/kg, IM) and 2-PAM (25 mg/kg, IM). Atropine sulfate 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.

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, and then EEG activity slowly returned as respiratory efforts became more frequent and regular; seizure activity emerged as the EEG returned toward a normal activity pattern. 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 atropine sulfate treatment could be administered.

DISCUSSION

The present findings show that all five OP nerve agents when given at toxic doses, $1.6 \times LD_{50}$ in rats and $2 \times LD_{50}$ in guinea pigs, respectively, can induce brain seizure activity. In most cases, the seizure activity develops into status epilepticus, and persists for many hours if not successfully treated. The results also show that in both animal models the anticholingergic drug atropine sulfate is capable of stopping seizures induced by any of the nerve agents tested, and that the effective dose is, with the exception of VX, relatively the same across all agents within a given model. The doses of atropine sulfate required to terminate seizure activity to be considerably higher in rats than in guinea pigs.

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 only oxime clinically available in the United States, 2-PAM (34). Soman-inhibited ChE is only minimally reactivated by clinically available oximes (6), and, soman-inhibited ChE very rapidly undergoes a chemical change ("aging" process) that makes reactivation of ChE activity by any oxime no longer possible (9). However, animal studies have shown that significant survival from soman poisoning can be achieved by pretreatment with a carbamate (pyridostigmine) in conjunction with subsequent atropine sulfate and oxime (2-PAM) therapy (1,7,14,17). More recently, the focus of research has shifted to determine appropriate anticonvulsant drugs to control nerve agent seizures and prevent subsequent brain pathology (21,24,32). 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 so-

TABLE 4

ANTISEIZURE EFFECTS OF ATROPINE SULFATE FOLLOWING DIFFERENT CHALLENGING DOSES OF SOMAN IN GUINEA PIGS

	No Pyridostigmine	With Pyridostigmine Pretreatment (−30 min)		
	$1 \times LD_{50}$	$1 \times LD_{50}$	$2 \times LD_{50}$	$5 \times LD_{50}$
Seizure onset time (min)	13.23 ± 1.08 (21)	17.64 ± 1.19 (24)	7.57 ± 0.34 (30)	5.80 ± 0.37 (28)
Seizure occurrence	21/39 (53.8%)	24/42 (57.1%)	30/30 (100%)	28/31 (90.3%)
Seizure stopped before treatment	1/21 (4.8%)	0/24 (0%)	0/30 (0%)	0/28 (0%)
No EEG seizure	18/39 (46.2%)	18/42 (42.9%)	0/30 (0%)	3/31 (9.7%)
Died before EEG seizure	0/39 (0%)	0/42 (0%)	0/30 (0%)	3/31 (9.7%)
Died before treatment	0/39 (0%)	0/42 (0%)	0/30 (0%)	2/28 (7.1%)
Died after treatment	6/20 (30%)	2/24 (8.3%)	10/30 (33.3%)	12/26 (46.2%)
24-h mortality	6/39 (15.4%)	2/42 (4.8%)	10/30 (33.3%)	17/31 (54.8%)
	4.1	4.4	12.2	12.2
ED_{50}s (mg/kg, IM) for atropine sulfate	(2.5 – 6.9)	(2.6 – 7.9)	(8.5 – 16.7)	(7.5 – 18.1)

Guinea pigs received either pyridostigmine (26 μg/kg, IM) or no pyridostigmine 30 min before soman challenge. All animals received atropine sulfate (2 mg/kg, IM) and 2-PAM (25 mg/kg, IM) 1 min after soman administration. Atropine sulfate 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.

man (24,32,33). 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 seizures and the efficacy of the anticholinergic atropine sulfate to control seizures elicited by these nerve agents.

The HI-6 pretreatment model was originally developed to allow the study of potential anticonvulsant compounds in soman-intoxicated rats (30,32,34). The purpose was to be able to reliably (100%) elicit convulsions/seizures with a fixed challenge dose of soman ($1.6 \times LD_{50}$) yet prevent the rapid acute lethal effects of such a toxic dose that may obscure the potential anticonvulsant actions of test drugs. However, the present results showed that the choice of and timing of oxime administration had a marked effect on the results, depending upon which nerve agent was being tested. In the rat model, HI-6 pretreatment attenuated the development of seizures with sarin and GF, and totally prevented them in the case of VX, while pretreatment or treatment immediately following agent challenge with 2-PAM influenced the incidence of VX-induced seizures in both rat and guinea pig models. The choice of oxime was more critical in studies with rats. This species is relatively resistant to the ability of carbamate prophylaxis to enhance the protective effects of atropine and oxime therapy against rapidly aging agents (soman) or those resistant to reactivation by 2-PAM (soman, tabun, GF) (6). In the 2-PAM pretreatment condition, the lethal effects of soman, tabun, and GF occurred so quickly that it was impossible to determine the anticonvulsant potential of atropine sulfate. Thus, in the case of tabun poisoning, pretreatment with the oxime TMB-4, which has good reactivating properties against tabun-inhibited ChE, prevented the acute lethal effects of agent challenge and allowed an assessment of the anticonvulsant properties of atropine sulfate. In the case of VX exposure, the time to develop seizures in both the rat and guinea pig is longer (three- to fivefold) than other nerve agents. This additional time before onset of agent effects, along with the good reactivating activity of 2-PAM against VX-inhibited ChE, possibly explains the significantly lower incidence of seizures with this agent in both models. Taken as a whole, these data indicate that with an appropriate amount of an effective circulating oxime in conjunction with a slower acting agent, the amount of agent may not reach sufficient levels in the brain to initiate seizure activity. Further study is required to test this hypothesis. In terms of seizure and neuropathology protection, VX exposure seems to be an easy intoxication to treat with 2-PAM, the only clinically available oxime in the United States.

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 (35). Recently, the anticonvulsant effects of atropine sulfate in larger doses as well as other anticholinergic compounds (4,22,31,32) have been recognized and documented against seizures elicited by the OP nerve agent soman. Unfortunately, the anticonvulsant effect of atropine sulfate against other nerve agents has not been reported. The present study shows that atropine sulfate can terminate seizure activity induced by all five nerve agents (tabun, sarin, soman, GF, and VX) if it was given shortly (5 min) after seizure onset. In the rat, the dose of atropine sulfate required for an anticonvulsant effect with any particular nerve agent was highly dependent on the oxime used. Additionally, in the case of VX poisoning, the timing of oxime administration significantly affected the incidence of seizure development. When oxime administration had minimal impact on incidence of seizure development, the anticonvulsant ED_{50} for atropine sulfate was the same (48.5–59.8 mg/kg, IM) regardless of the nerve agent used. In situations where oxime administration reduced the incidence of seizures, the anticonvulsant ED_{50} of atropine was lower. The mechanism of this effect is unclear. It does indicate that the choice of oxime can greatly affect assessments of the anticonvulsant efficacy of test drugs against a specific nerve agent in the rat. In the guinea pig, soman, tabun, and GF induce a higher incidence of seizure development (100, 95, and 92%, respectively) than that induced by sarin and VX (73 and 50%, respectively), when exposed to a $2 \times LD_{50}$ dose of these agents. Higher doses of atropine sulfate were required to control soman-, tabun-, or GF-induced seizures than were required for sarin- and VX-induced seizures. The reason for this difference is not known, but perhaps reflects the lower ChE reactivating ability of 2-PAM against the former three agents. Thus, in both rat and guinea pig models, the anticonvulsant ED_{50} of atropine sulfate for a nerve agent was highly correlated with the incidence of seizures.

In both the rat HI-6 pretreatment model (Table 1) and the guinea pig model (Table 3), a higher dose of atropine sulfate was required to terminate ongoing 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 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 (30–32).

This study also shows that in the guinea pig, with or without pyridostigmine pretreatment, the antiseizure ED_{50} of atropine sulfate against a $1 \times LD_{50}$ challenge dose of soman was the same, although, as expected, higher mortality was observed for the group that received no prior pyridostigmine treatment. Again, this result may reflect the similarity in low incidence ($\sim$55%) of seizure development in these two groups (Table 4). 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 also. However, there was no similar increase in the anticonvulsant ED_{50} of atropine from the 2 to $5 \times LD_{50}$ 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 seizure activity, the same amount of atropine sulfate is required to compete with and block these functional receptors to terminate that seizure activity.

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