U.S. Army Medical Research
Institute of Chemical Defense

USAMRICD-TR-10-06

Experimental Therapeutics against the Toxic and Lethal Effects Resulting from Acute Exposure to Nerve Agents without Carbamate Pretreatment in Guinea Pigs

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September 2010

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
September 2010	Technical Report	1 March 2009 to 30 June 2010

4. TITLE AND SUBTITLE

Experimental therapeutics against the toxic and lethal effects resulting from acute exposure to nerve agents without carbamate pretreatment in guinea pigs

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Chang, F-CT, DeBus, SJ, Spriggs, DL

5d. PROJECT NUMBER

5e. TASK NUMBER

6.2

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDR-I
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-10-06

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDZ-I
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

14. ABSTRACT

Our current countermeasure against nerve agent poisoning involves carbamate pretreatment followed by a post-exposure therapy (atropine and oxime). For military/civilian populations not enrolled in the carbamate pretreatment program, atropine and oxime may not offer adequate protection against the toxic/lethal effects of nerve agents. To reduce the reliance on carbamate pretreatment, we have developed a post-exposure therapy mix that can protect against the lethal effect of a 2xLD50 dose of tabun, sarin, soman, cyclo-sarin or VX. Guinea pigs chronically instrumented for concurrent recordings of EEG, cardiorespiratory activities, diaphragm and skeletal muscle EMG were used in this study. The post-exposure therapy mix consisted of scopolamine (0.5 mg/kg), methylatropine (2 mg/kg), physostigmine (0.015 mg/kg), MMB4 (26.1 mg/kg), and phenobarbital (25 mg/kg). Results showed that only mild and short-lasting acute cholinergic effects (salivation, dystonia, tremor) were seen after exposure/therapy. During convalescence, none of the animals exhibited seizures/convulsions, signs of anomalous cardiorespiratory activities, or any debilitating effects. The animals were asymptomatic within 30 min following therapy and survived the agent challenge 24 hr later. In conclusion, the therapy mix used in this study was effective not only in antagonizing nerve agent-induced lethality, but also in protecting the functional integrity of the CNS and cardiorespiratory system.

15. SUBJECT TERMS Chemical warfare agents, GA, GB, GD, GF, VX, organophosphates, acetylcholinesterase inhibitors, carbamate pretreatment, pyridostigmine, physostigmine, bispyridinium oximes, MMB4, scopolamine, phenobarbital, methylatropine, respiration, cardiovascular physiology, seizures, convulsions, guinea pigs

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Fat-Chun T. Chang
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	38	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-1343

ABSTRACT

Currently, the medical countermeasure adopted by the U.S. Armed Forces against nerve agent poisoning involves pyridostigmine pretreatment followed by a post-exposure therapy consisting of atropine, an oxime and, if needed, diazepam. For military/civilian populations not enrolled in the pyridostigmine pretreatment program, however, the post-exposure component of this modality may not offer adequate protection against the toxic and lethal effects of nerve agents. To reduce the reliance on carbamate pretreatment, we have developed a post-exposure therapy mix that can protect against the lethal effects produced by a lethal dose ($2xLD_{50}$) of tabun (GA), sarin (GB), soman (GD), cyclo-sarin (GF) or VX in unanesthetized guinea pigs. For a thorough efficacy evaluation, the animals were chronically instrumented to permit concurrent recordings of central nervous system activity (electrocorticogram), cardiorespiratory activity profiles, diaphragmatic and skeletal muscle electromyograms. The post-exposure therapy mix, given within a min after the nerve agent, consisted of scopolamine (0.5 mg/kg, im), atropine methylnitrate (2 mg/kg, im), physostigmine (0.015 mg/kg, im), MMB4 (a bispyridinium oxime, 26.1 mg/kg, im), and phenobarbital (25 mg/kg, ip). Results showed that only mild and short-lasting acute cholinergic effects (salivation, dystonia, tremor) and phenobarbital-induced sedative effects were seen during the first 20 min after exposure/therapy. Throughout the course of intoxication/therapy and convalescence, none of the animals exhibited seizures, convulsions, signs of anomalous cardiorespiratory activities, incapacitation, or other debilitating central effects typically associated with nerve agent-induced cholinergic crisis. The animals were generally asymptomatic within 30 min following post-exposure therapy. All animals in this study survived the agent challenge 24 hr later. Animals exposed to the agents (GA, GB, GD, GF and VX) mentioned without any pretreatment/therapy on the other hand, all exhibited generalized seizures, convulsions, cardiorespiratory depression, and died about 25-30 min following exposure. In conclusion, our findings indicated that the therapy mix used in this study was effective not only in antagonizing nerve agent-induced lethality, but also in protecting the functional integrity of the CNS and cardiorespiratory system.

INTRODUCTION

Tabun (GA), sarin (GB), soman (GD), cyclo-sarin (GF) and VX are extremely toxic organophosphorus (OP) chemical warfare agents (CWAs). Table 1 lists the chemical nomenclature and guinea pig LD_{50} values of these agents.

Table 1. CWA Nomenclature and LD_{50}s

Chemical Agent	Chemical Nomenclature	LD_{50}
Tabun (GA)	Ethyl N-dimethylphosphoramidocyanidate	119 μg/kg
Sarin (GB)	Isopropyl methylphosphonofluoridate	43 μg/kg
Soman (GD)	Pinacolyl methylphosphonofluoridate	28 μg/kg
Cyclo-Sarin (GF)	Cyclohexyl methylphosphonofluoridate	57 μg/kg
VX	S-(2-diisopropylaminoethyl) O-ethyl methyl phosphonothiolate	8 μg/kg

Acute exposure to CWAs can cause a myriad of cholinergic hyperfunctions, seizures, convulsions, cardiorespiratory depression, and death (Somani *et al*., 1992). Currently, the U.S. military medical management doctrine for nerve agent poisoning involves a three-tier strategy: i) carbamate pretreatment (pyridostigmine) that binds reversibly with AChE and creates a "reserved pool" of acetylcholinesterase (AChE; Koster, 1946; Koelle, 1981), ii) blockade of cholinergic receptor over-stimulation with atropine, and iii) reactivation of AChE with 2-PAM (pralidoxime, an AChE reactivator; Berry and Davis, 1970; Gordon *et al*., 1978; Hobbiger, 1957; Taylor, 1985). For the protection of central nervous system (CNS), an anticonvulsant (e.g., diazepam; see Lipp, 1972; 1973) can also be added to this regimen to block the development, or terminate the progression, of nerve agent-induced seizures, incapacitations and brain damage (Churchill *et al*., 1985; Gordon *et al*., 1978; Lallement *et al*., 1991; 1992; 1993; Lemercier *et al*., 1983; McLeod *et al*., 1984; McLeod, 1985; Petras, 1994).

The protective effect of carbamate pretreatment in the medical management of CWA poisoning is worthy of further note. Without carbamate pretreatment, the post-exposure component (equivalent to the Mark I Kit) of the existing pretreatment and therapy (P&T) regimen, which consists of atropine and 2-PAM, is only marginally effective against the toxic effects of sarin and VX, and completely ineffective against the toxic/lethal effects of other agents such as tabun, soman, and cyclo-sarin. For combat personnel and civilian support units not enrolled in the carbamate pretreatment program, therefore, the consequence could be rather dire in the event of a hostile or accidental chemical incident.

In recognition of such inadequacy in our existing medical preparedness, we have designed and evaluated a post-exposure therapy mix that can protect, without carbamate pretreatment, against the toxic and lethal effects of up to $5xLD_{50}$ (130 μg/kg, sc) of soman in guinea pigs

(DeBus *et al*, 2008; Spriggs *et al*, 2007). These findings were as significant as they were exciting because, of all the G and V nerve agents, the toxic and lethal effects resulting from soman intoxication are the most difficult to manage medically using our existing countermeasure doctrine. Moreover, in addition to a myriad of cholinergic effects that soman engenders, its toxic effects are also compounded by such phenomena as "aging" and "depot effect." Brief descriptions of these phenomena are provided in Appendix I.

In this report, we will offer a detailed description of the effectiveness of our post-exposure therapy mix (without carbamate pretreatment) against the toxic/lethal effects of not only GD, but also a wide range of other CWAs such as GA, GB, GF and VX.

Table 2. Description of Post-Exposure Therapy Mix

Pharmacological Component	**Route**	**Dosage**
Scopolamine (Cholinolytic)	*im*	*0.5 mg/kg*
Methylatropine (Cholinolytic)	*im*	*2 mg/kg*
Physostigmine salicylate (Carbamate)	*im*	*0.015 mg/kg*
MMB4 (AChE Reactivator)	*im*	*26.1 mg/kg*
Phenobarbital (Anticonvulsant)	*ip*	*25 mg/kg*

MMB4: Bispyridinium Oxime - *1,1'-methylene bis[4-(hydroxyiminomethyl)pyridnium] dimethylsulfinate)*

Table 2 is a description of the constituents, routes of administration, and dosage of the post-exposure therapy mix used in the present investigation. The rationale based on which each constituent was chosen in the design of a broad-spectrum efficacy post-exposure therapy mix, as well as a brief account of each constituent's pharmacological attributes, are provided in the narratives to follow.

1. **Scopolamine** (Action: Cholinolytic. Dose: 0.5 mg/kg, im). Scopolamine has long been known to possess antidotal action against nerve agent poisoning (Wescoe *et al*., 1948; Wills, 1963). The use of scopolamine in medical management of nerve agent toxicities, however, pales in comparison to atropine despite evidence suggesting that scopolamine may be a far more effective neuroprotectant than atropine in the treatment of nerve agent poisoning (Anderson *et al*., 1994; 1997; Bertram *et al*., 1977; Chang *et al*., 2003c; Harris *et al*., 1991; 1994; Jovic and Milosevic, 1970; Leadbeater *et al*., 1985; Lennox *et al*., 1992; Shih *et al*., 1991; Solana *et al*., 1991; Wescoe *et al*., 1948; Wills, 1963). In addition to its anti-muscarinic effects, scopolamine appeared to be a notably more powerful anticonvulsant than benzodiazepine derivatives (such as diazepam and midazolam) in animals intoxicated by soman (Anderson, 1994; 1997; Chang *et al*., 2003c; Harris *et al*., 1994). In consideration of scopolamine's profound anticonvulsant and anticholinergic properties, these investigators further suggested that it could replace atropine, or diazepam, or both, as a therapeutic compound against soman toxicities. Other evidence has shown that scopolamine can even be used as a pretreatment formulation against a variety of

organophosphorus nerve agent poisoning (von Bredow *et al*., 1991a; 1991b; Harris *et al*., 1991; Lennox *et al*., 1992; Philippens *et al.,* 2000).

The nature and extent of CNS protection by scopolamine and atropine following organophosphate (OP) intoxication has yet to be systematically investigated. Despite the widespread impression that scopolamine and atropine are virtually identical in their pharmacological actions, they do differ qualitatively and quantitatively in their anti-muscarinic activities (for review, see Brimblecombe, 1974). In clinical applications, atropine appears to have a significantly less CNS depressant effect in doses that are used for most anticholinergic/vagolytic purposes, and is therefore given in preference over scopolamine. In applications such as pre-anesthesia medication where CNS depression is clinically inconsequential or desired, scopolamine is often chosen over atropine. The rationale for these practices is in basic agreement with *in vitro* data which showed that, compared to atropine, scopolamine's antimuscarinic activity was about 16-fold more potent in the CNS, and about 3-fold more potent in the peripheral neural tissues (Freedman *et al*., 1988). Thus, to the extent that cholinergic mechanisms are known to play a functionally and pathophysiologically significant role in nerve agent-induced seizures (Shih *et al*., 1991) and dysfunctional central respiratory drive (Brimblecombe, 1977), a more centrally active antimuscarinic compound, such as scopolamine, would clearly have a distinct therapeutic advantage over a less centrally active compound such as atropine. Moreover, Capacio and co-workers (1992) showed that rats did not exhibit performance deficit in an accelerating rotorod test when given an anticonvulsant dose of scopolamine (0.43 mg/kg), which, incidentally, is comparable to the dose level (0.5 mg/kg) we used in the present study. Finally, findings from our "proof of concept" studies showed that substitution of scopolamine with either methylscopolamine (a quaternary amine; with doses up to 2 mg/kg) or atropine sulfate (dose range: equimolar, 8 and 16 mg/kg) consistently resulted in "less-than-optimal" therapeutic outcome in animals intoxicated by any one of the five CWA prototypes.

2. **Methylatropine** (Action: Ganglionic Blocker. Dose: 2 mg/kg, im). Methylatropine (aka, atropine methylnitrate) is a quaternary nitrogen compound derived from methylation of nitrogen in atropine. Methylatropine is a potent ganglionic blocker. As a quaternary compound, methylatropine's activity is confined primarily to the periphery. Pharmacologically, methylatropine is about 3-5 times more potent than other ganglionic blockers such as tetraethylammonium (TEA), mecamylamine, and atropine (Fink and Cervoni, 1953; Jansen and Dellinger, 1989; Lönnerholm and Widerlöv, 1975). As findings from our earlier research showed, methylatropine is ideally suited for reversing nerve agent-induced ganglionic perturbations (e.g., Chang *et al*., 2002; 2003a; 2003b; 2003c; Chang, 2006; Chang *et al.*, 2006). In our "proof of concept" investigations, methylatropine was also found to be exceptionally effective in suppressing CWA-induced airway blockage and acute autonomic effects such as mucoid-salivary hypersecretion, reduced airway patency, bradycardia, atrioventricular (AV) blockade, cardiac dysrhythmia, negative inotropic effects, etc.

3. **MMB4** (Oxime, Action: AChE Reactivator. Dose: 26.1 mg/kg, im). The CWAs used in the present study are potent inhibitors of AChE. The enzyme inhibition is mediated through a reaction known as phosphorylation whereby the nerve agent binds to the serine residue of AChE's esteratic site, inactivates AChE's enzymatic activity, results in accumulation of

acetylcholine at the endplate, and disrupts central and peripheral cholinergic neurotransmission. It has long been shown in the OP literature that the OP-AChE bond is resistant to spontaneous hydrolytic cleavage. In theory, dephosphylation of OP-inhibited AChE, if possible, could produce a reactivated cholinesterase (see Dawson, 1994 for review). That the dissociation of OP-AChE bond could indeed be achieved by a nucleophilic attack on the phosphorus atom using either choline or hydroxylamine was first demonstrated by Wilson (1951). Wilson's findings later formed the basis for the development of a number of much stronger nucleophilic agents such as pralidoxime (Wilson and Ginsburg, 1955), trimedoxime (Poziomek *et al*., 1958), obidoxime (Luttringhaus and Hagedorn, 1964) and HI-6 (Clement, 1982a; Oldiges and Schoene, 1970).

The relative effectiveness of various oximes has been the focus of considerable research over the decades (Dawson, 1994; Koplovitz and Stewart, 1994). To this date, reactivation of OP-inhibited AChE (oxime therapy) continues to be a critical component of current medical management doctrine. Several oxime variants are currently available for clinical and research use. These include i) mono-pyridinium oximes (e.g., 2-PAM), ii) obidoxime (such as Toxogonin), and iii) bispyridinium oximes such as H-series oximes (HI-6, HS-3, HS-6, HGG-12, HGG-42, HGG-52, HLö-7) and MMB4 (used in the present study).

MMB4 (1,1'-methylenebis[4-[(hydroxyimino)methyl]-pyridinium] dimethanesulfonate; aka MMB4.DMS) is being evaluated by the U.S. Armed Forces to replace the currently fielded 2-pralidoxime chloride (2-PAM) in the Antidote Treatment Nerve Agent, Auto-Injector (ATNAA). MMB4 appears to be quite promising and is presently being considered by FDA for Phase I clinical trial (see CBMS Information Package for Type B Pre-IND, 2006). On the basis of safety/toxicity/efficacy findings derived from our earlier research, and of findings derived from our "proof of concept" study mentioned above, we believe MMB4's protective effects against CWA poisoning either are comparable to, or surpass those of mono-pyridinium oximes (2-PAM), obidoxime, and H-series oximes such as HI-6 (Chang *et al*., 2005; Chang, 2006).

4. **Physostigmine** (Action: Inhibits AChE Reversibly. Dose: 0.015 mg/kg, im). As pretreatment drugs, both pyridostigmine and physostigmine are known to reversibly inhibit AChE and protect a critical portion of this enzyme from irreversible binding to nerve agents (Somani and Dube,1989). Pyridostigmine is a quaternary amine and it does not readily cross the blood-brain barrier (BBB) to mediate its protective effect in the CNS (Blick *et al.*, 1991; Kerenyi *et al*, 1990). Physostigmine, on the other hand, is a tertiary amine; as such, it readily crosses the BBB. For this reason, it has been argued that physostigmine may afford equal protection to cholinesterases in the CNS as well the periphery, and ultimately, deliver a more favorable therapeutic outcome (Blick *et al.*, 1991; von Bredow *et al*., 1991a; 1991b; Kerenyi *et al*, 1990; Harris *et al*, 1984; Leadbeater *et al*, 1985; Miller *et al*., 1993).

However, physostigmine's ability to cross the BBB has also raised the concern of possible untoward central (psychological, neurobehavioral and physiological) side effects (D'Mello and Sidell, 1991; Somani and Dube, 1989). Moreover, the half-life of physostigmine is somewhat rapid (≈1 hr; Somani *et al*., 1991) which may necessitate frequent repeated dosing so the protective effects of pretreatment could be continuously maintained over time. In the present study, this concern may be of little relevance because physostigmine was used as a post-exposure treatment drug in what were considered "life-or-death" situations. As such, the benefit of

surviving a chemical agent exposure should far outweigh any potential untoward central (psychological, neurobehavioral and physiological) effects. Similarly, repeated dosing in a post-exposure situation, if needed, would more likely be administered for the purpose of facilitating a favorable recovery, and NOT for producing, or maintaining, an optimal pretreatment effect.

As shown in our "proof of concept" study, the inclusion of physostigmine in the post-exposure mix did not appear to exacerbate the CWA toxicities as we first suspected. Quite to the contrary, physostigmine proved to be a critical component in our post-exposure therapy mix. Pilot data derived from CWA-intoxicated animals showed that substitution of physostigmine with pyridostigmine consistently resulted in varying degrees of sensorimotor anomalies, incapacitation, and higher incidents of lethality.

5. **Phenobarbital** (Action: Mediates an Inhibitory Modulatory Influence Over Uncontrolled CNS Excitability. Dose: 25 mg/kg, ip). Seizures and convulsions are among the major pathophysiological developments following G or V intoxication. In the present study, phenobarbital was used to provide a tonic level of inhibitory influence over the unstable CNS excitability following agent exposure.

Phenobarbital is the most widely used antiepileptic drug in the developing world (Baulac, 2002). Although phenobarbital's propensity to cause sedation and other cognitive and behavioral side effects has relegated it to second- or third-line use in many parts of the industrialized world, it remains a choice, though not as popular as diazepam, in many developed countries (Baldy-Moulinier *et al*., 1998). The reasons we have chosen phenobarbital over diazepam in the present study are as follows.

While both phenobarbital and diazepam are capable of suppressing the development and progression of CNS hyper-excitability, they do so via different allosteric binding sites on the postsynaptic Cl^- ionophore (Macdonald and Twyman, 1991; Rogawski and Porter, 1990). More specifically, phenobarbital affects the Cl^- ionophore in a dose-dependent fashion (Skolnick *et al.*, 1981; Study and Barker, 1981). At low (sub-anesthetic) doses, phenobarbital augments the affinity of the $GABA_A$ receptor for GABA and increases the mean channel opening time induced by GABA without affecting open frequency or conductance. The resultant increase in Cl^- flux hyperpolarizes the postsynaptic neuronal cell membrane and subsequently impedes further progression of synchronous CNS hyper-excitability. At moderate (sub-anesthetic) to high (anesthetic; ca. 150 mg/kg) concentrations, phenobarbital can directly increase Cl^- channel openings, even in the absence of GABA! Diazepam, on the other hand, has no direct effects on Cl^- channel opening; it only enhances the affinity of the receptor for GABA and causes an increase in the frequency of GABA-activated channel openings (Macdonald and Twyman, 1991; Study and Barker, 1981). The importance of this distinction is twofold. First, although diazepam can augment GABA's actions at low synaptic GABA concentrations, it will have little to no additional effect at saturating concentrations of synaptic GABA. In other words, diazepam only shifts the concentration–response curve for GABA *slightly* to the left (Zorumski and Isenberg, 1991). Phenobarbital, on the other hand, can continue to mediate an inhibitory modulatory influence over CNS excitability regardless of fluctuations in the synaptic GABA concentrations. Second, the magnitude of inhibitory modulatory influence resulting from

increases in Cl^- channel open dwell time (as in the case of phenobarbital) would be far greater than that from increases in open frequency (as in the case of diazepam).

Another reason for choosing phenobarbital over diazepam in the present investigation has to do with their relative efficacies in modulating CNS excitability. The $GABA_A$ receptors can be positively or negatively modulated along a "continuum" by compounds that range in activity from full agonists such as 3-aminopropane-sulfonic acid (3-APS) and gamma-hydrobutyric acid (GHB) to full inverse agonists such as imidazobenzodiazepine Ro15-1788 (flumazenil), a selective antagonist that binds with high affinity to $GABA_A$ receptors (Haefely, 1990). Along this continuum lie compounds with different degrees of intrinsic efficacy - that is, compounds with varying degrees of partial agonist actions (e.g., diazepam and a host of other benzodiazepine derivatives). Behaviorally, full agonists (such as pentobarbital and phenobarbital) have anesthetic/sedative properties, whereas full inverse agonists are pro-convulsants (Breier and Paul, 1990). Somewhere in the middle are partial agonists and a number of them are still in development by pharmaceutical companies as anxiolytics. From the standpoint of pharmaceutical industries, partial $GABA_A$ receptor agonists such as diazepam are ideally suited for the treatment of anxiety syndromes because they are devoid of the sedative effects generally associated with full agonists (Breier and Paul, 1990; Haefely, 1990; Hommer *et al.*, 1987) and can therefore be used clinically as an anxiolytic medication without significantly affecting the patients' ability to carry out their normal activities. But exactly how effective is diazepam against a lethal dose of CWA in animals that are not carbamate pretreated? As earlier research showed, diazepam appeared to be adequate in managing uncontrolled CNS excitability in carbamate-pretreated animals exposed to low-to-moderate levels of nerve agent challenge (e.g., Lipp, 1972; McDonough *et al.*, 1989). Even with that background, we were still somewhat skeptical about diazepam's effectiveness against the neurotoxic effects of a lethal or supra-lethal dose of nerve agent without the benefit of pretreatment. Indeed, as borne out by our "proof of concept" data, when phenobarbital (25 mg/kg) was replaced by diazepam (up to 5 mg/kg) in our post-exposure therapy mix, the therapeutic outcome (depending on the type of chemical agent in question) in animals given diazepam is either comparable to, or less favorable than, those receiving phenobarbital.

That Cl^- channels can be opened by phenobarbital without the involvement of synaptic GABA is a phenomenon worthy of further note. The use of benzodiazepine derivatives (such as diazepam) is widely recognized as an effective means of managing nerve agent-induced seizures and convulsions. But the mechanism through which benzodiazepines mediate their inhibitory/anticonvulsant effect depends critically on the presence of synaptic GABA. As such, in circumstances where GABA receptors are irreversibly blocked by such acylating ligands as tetrabutylbicycloorthobenzoate (TBOB), t-butyl bicyclophosphorothionate (TBPS), and *meta*- and *para*-isothiocyanato-t-butylbicycloorthobenzoate; see Hawkinson et al., 1991; Lewin *et al*., 1989; Rossi *et al.,* 2001), benzodiazepines would be completely ineffective. Parenthetically, TBPS and TBOB mentioned above can either be purchased from a variety of commercial sources or synthesized in a minimally equipped laboratory. The likelihood of simultaneously encountering these agents along with G/V agents in combat and/or civilian environments clearly exists and should therefore be taken into consideration in the design of medical countermeasure strategies.

Taken together, substituting benzodiazepine derivatives with phenobarbital (or other similar anticonvulsants) may not only greatly broaden the efficacy spectrum of our existing anticonvulsant/neuroprotection regimens for nerve agents, but also significantly expand our overall preparedness and capability in the management of uncontrolled CNS excitability.

In addition to the reasons mentioned above, phenobarbital has many other desirable features (see Baulac, 2002): i) broad spectrum efficacy against all seizure types other than absences, ii) wide clinically effective starting dose range, iii) greater effectiveness in seizure termination than those associated with modern drugs (e.g., diazepam), iv) a very low risk of life threatening adverse effects, v) linear pharmacokinetics, vi) long half life, vii) low propensity to be a target for drug interactions (except for the inhibition of its metabolism by valproate), viii) low potential for abuse, ix) availability in a wide variety of parenteral formulations, x) long shelf life, and xi) extremely low cost (1-2 U.S. dollars per pound, pharmaceutical grade).

MATERIALS AND METHODS

Animal Use and Care. Sixty (60) barrier raised male Hartley albino guinea pigs (*Cavia porcellus*; Charles River) weighing between 400-600 gm were used in this study. The animals were randomly assigned to ten (10) experimental groups (n=6/group). An experimental group description is provided in Table 3.

Table 3. Experimental Group Design

Group (n=6/Group)	Experimental Condition
1	GA (Intoxication Only with No Other Intervention)
2	GB (Intoxication Only with No Other Intervention)
3	GD (Intoxication Only with No Other Intervention)
4	GF (Intoxication Only with No Other Intervention)
5	VX (Intoxication Only with No Other Intervention)
6	GA (Post-Exposure Therapy / No Pretreatment)
7	GB (Post-Exposure Therapy / No Pretreatment)
8	GD (Post-Exposure Therapy / No Pretreatment)
9	GF (Post-Exposure Therapy / No Pretreatment)
10	VX (Post-Exposure Therapy / No Pretreatment)

All animals were surgically instrumented to allow for concurrent electrophysiological recordings of a variety of CNS and cardiorespiratory activities (Chang *et al*., 1998). Survival surgeries were performed under aseptic conditions. Surgical plane of anesthesia was induced

with sodium pentobarbital (38 mg/kg, ip; suppl. 5-10 mg/kg). Each animal was instrumented for the concurrent electrophysiological recordings of i) electrocorticogram (ECoG, to evaluate CWA-induced seizure activities); ii) diaphragmatic EMG (DEMG, to monitor changes in respiratory status and signs of compromise in diaphragmatic function; Chang and Harper, 1989); iii) integrated DEMG (IntDEMG, τ=50 msec; to more clearly identify changes in the temporal attributes of diaphragmatic contractions throughout the course of intoxication and therapy); iv) neck skeletal muscle electromyogram (NEMG, to assess the extent of anomalous neuromuscular activity changes such as fasciculation, tremors and convulsions); v) Lead II electrocardiogram (ECG_{II}, to document changes in the functional integrity of the myocardium); and vi) core temperature (to assess CWA-induced alterations in thermostasis) using an implanted thermistor (Part# 014-44004-NA-IP-6-ST; YSI, Dayton, Ohio). The animals were allowed at least two weeks to recover before being used for experimental purposes.

Methods of chronic surgical instrumentation procedures for electrophysiological recordings of diaphragmatic electromyogram (Chang and Harper, 1989), NEMG, electrocorticogram and ECG_{II} (Chang *et al*., 1998) have been described elsewhere. Readers are referred to the reports cited above for technical details.

Experimental Procedure.

1. Intoxicants. GA, GB, GD, GF and VX were obtained from the Chemical Surety Material Facility, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland. Working solutions using saline as diluent were prepared minutes prior to intoxication. Each animal was challenged with a $2xLD_{50}$ subcutaneous dose (dose volume = 0.5 ml/kg) of GA (238 µg/kg), GB (86 µg/kg), GD (56 µg/kg), GF (114 µg/kg), and VX (16 µg/kg).

2. Post-Exposure Therapy. The pharmacological constituents, routes of administration and dosage of the post-exposure therapy mix used in this investigation are described in Table 2 (*vide supra*).

Scopolamine, methylatropine, physostigmine and MMB4 (Chang, *et al*., 2005; Chang, 2006) were prepared as an intramuscular admixture (dose volume: 0.25 ml/kg). Phenobarbital was given intraperitoneally (dose volume, 1.0 ml/kg). The post-exposure therapy was administered 1 min following a $2xLD_{50}$ dose of each CWA mentioned above.

3. Palliative Care. To prevent the development of hypothermia, a servo-controlled thermal blanket or a heat lamp was used to maintain the animals' body temperature between 38.5-39.5 ^{o}C.

4. Data Collection. Each animal served as its own control. A 15-min period of control electrophysiological recording was made prior to intoxication. Immediately following the control recording, a single dose of agent was administered subcutaneously. Data collection continued until the animals either i) succumbed to the lethal effects of intoxication as indicated by a state of cardiorespiratory collapse (seen in Groups 1-5 animals that received no pretreatment or therapy), or ii) responded positively to the post-exposure treatment regimen and survived for at least 24 hr (typically seen in Groups 6-10 animals).

5. Electrophysiological Signal Processing. DEMG, NEMG and ECoG signals were amplified and band-pass filtered (DEMG, NEMG, 50-7.5k Hz; ECoG, 0.5-500 Hz) prior to conversion to digital signals. DEMG, NEMG, ECoG, integrated DEMG (IntDEMG, τ=50 msec), ECG_{II}, and body temperature were analog-to-digital converted prior to digital storage and off-line analysis.

Data Analysis. Data analyses were performed off-line and analyzed in accordance with the following scheme.

1. ECoG, DEMG and NEMG Activities. Power spectral analyses (Childers, 1978) were performed to evaluate the extent of changes in ECoG, DEMG and NEMG activities. ECoG data were sampled at a rate of 625 Hz for 32.768 sec (20 epochs; 1024 points/epoch). DEMG and NEMG data were sampled at a rate of 10 kHz for 32.768 sec (20 epochs; 8192 points/epoch). "Zero Mean" was applied to DEMG, NEMG and ECoG data. The running sums of DEMG and NEMG data were "cosine-tapered" before Fast Fourier Transforms (FFTs) were computed. All final ECoG and DEMG power spectra were smoothed with a 15-point polynomial filter.

2. Cardiorespiratory Data. "Repeated Measures ANOVA" was performed to evaluate the overall changes in respiratory rate and heart rate. Tukey post-hoc pair-wise comparisons were performed to more clearly identify the differences, if necessary.

RESULTS

Chemical Warfare Agent-Induced Pathophysiology (Groups 1-5). GA, GB, GD, GF and VX in this study all produced CNS and cardiorespiratory responses characteristic of organophosphorus poisoning. The initial signs of intoxication were restlessness, exaggerated alerting/startle responses to novel sensory (auditory, visual, tactile) stimuli, unusually frequent bouts of exploratory behavior and sniffing reflexes and orofacial movements indicative of mucoid/salivary hypersecretion and bronchoconstriction/laryngospasm. As the toxicity progressed, more severe symptoms began to emerge. The most prominent of these were arrhythmia, seizures, convulsions, bradycardia, dyspnea, declining respiratory frequency, diminished magnitude of diaphragmatic contractions (a sign of reduced central respiratory drive), cyanosis, a state of converging acidemia/hypercapnia/hypoxia, bradypnea, and death. An idioventricular rhythm (60-120 bpm), which signaled a failing myocardium, invariably appeared shortly after the development of a bradypneic profile.

GD- and VX-induced pathophysiological changes have been selected for illustration here in consideration of the similarity in CWA-induced responses across the five CWAs tested in this investigation. Figure 1 (1A and 1B) is a representative electrophysiographic depiction of CNS and cardiorespiratory activity profiles during the control period and throughout the course of intoxication by GD (Fig. 1A) and VX (Fig. 1B). The pathophysiological changes following intoxication by GD and VX are reduced, for the clarity and brevity of presentation, into four (4) experimental stages: Control stage, Arrhythmia stage, Bradypnea stage and Terminal (apnea) stage.

Figure 1 (A and B). Electrophysiographic depiction of pathophysiology induced by a 2-LD_{50} dose of GD (Fig. 1A) and VX (Fig. 1B). Note i) the development of ectopic cardiac rhythm during Arrhythmia stage; ii) sustained full-blown seizure activities in GD- and VX-intoxicated animal; iii) progressive respiratory rate depression as indicated by time-dependent increases in respiratory cycle duration; iv) idioventricular cardiac rhythm during Bradypnea and Terminal stages; and, v) robust diaphragmatic bursts even at the end of the Terminal stage. Signal trace description: **ECoG**, electrocorticogram; **DEMG**, diaphragmatic electromyogram; **NEMG**, electromyographic recording from the neck muscle (dorsal portion of cervical trapezius); and **ECG**, Lead II electrocardiogram. Voltage calibrations: ECoG, 121 μV; DEMG, 1.32 mV; NEMG, 1.01 mV.

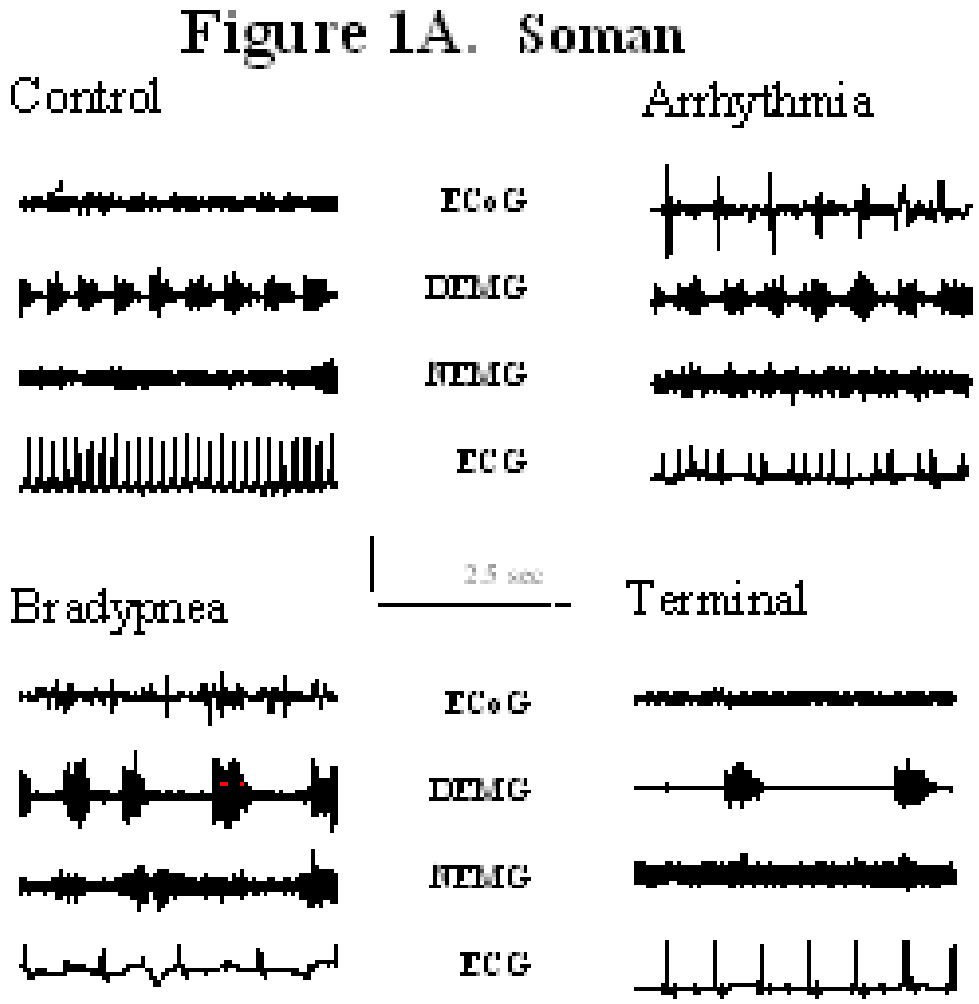
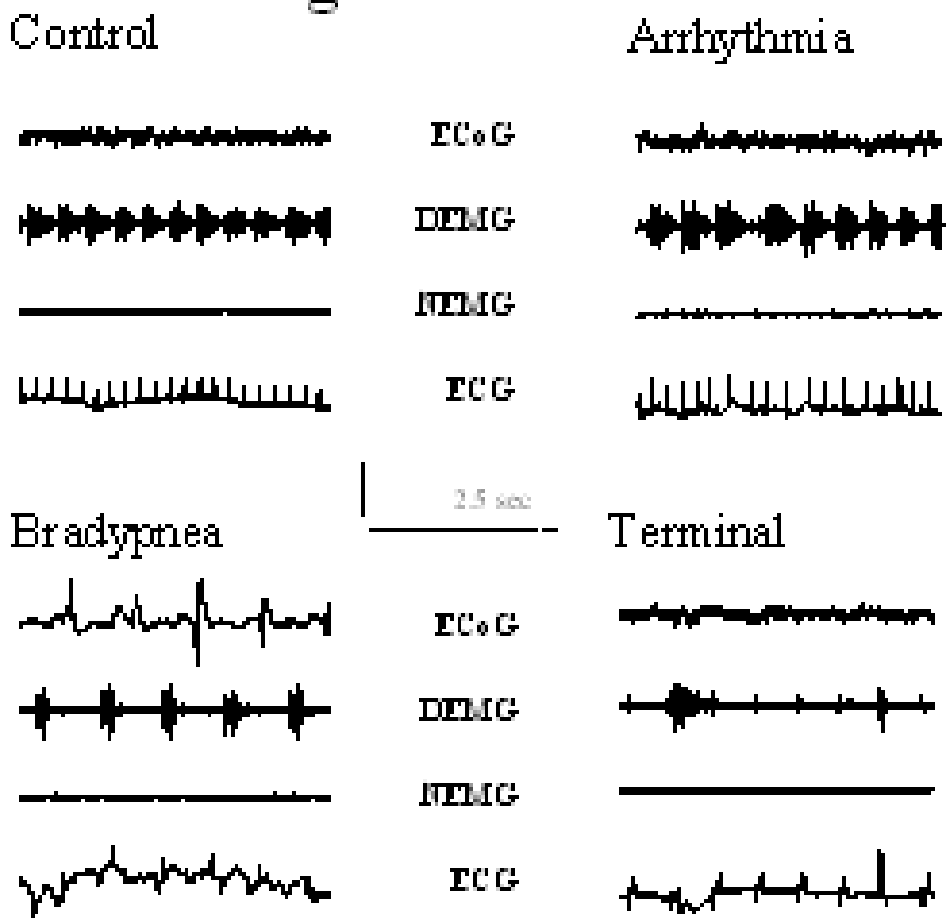


Figure 1B. VX



1. The Arrhythmia stage began approximately 10-15 min following CWA exposure. The development of an arrhythmia profile was typically preceded by a bradycardiac profile which progressed to cardiac arrhythmia characterized by premature atrial or ventricular contractions with discernible QT prolongation. The ectopic cardiac rhythm generally continued for about a minute before the myocardium regained a relatively stable rhythmicity albeit at a rate about 50-70% of control. Seizures and convulsions were generally seen during the later part (10-15 min post-CW exposure) of this stage.

2. The Bradypnea stage began approximately 20 min following CWA intoxication. The "Bradypnea" stage was generally preceded by episodes of ataxic or dyspneic breathing. Seizures and convulsions typically continued during this state. This stage was characterized by a marked reduction in respiratory frequency (≈30-40% of control). Other more severe myocardial dysfunctions such as J-Point elevation (downward convex varieties), T wave inversion, and second/third degree atrioventricular block were also typically seen during this stage. Signs of diaphragmatic muscle fatigue and an idioventricular rhythm (prolonged QRS complexes with a regular rhythm of 60-120 beats per minute) typically began to develop at the later part of this stage.

3. The Terminal stage was a period lasting approximately a minute prior to respiratory arrest (apnea). This stage began about 25 min after CWA intoxication. The most notable feature during this period was a profound reduction in respiratory frequency (5-10% of control). ECoG amplitudes were either significantly reduced or iso-electric at this time. Time-averaged spectral analyses of DEMG spectral profiles revealed that the overall distribution of DEMG frequency components was not altered considerably by any of the CWAs. Averaged DEMG amplitudes, on the other hand, did indicate a trend toward augmentation as the toxicity progressed. These results as well as observations that the diaphragm continued to respond to intermittent centrally mediated respiratory drive (gasping reflexes) during the "Terminal" stage suggest that the neuromuscular blockade of the diaphragm by the agents used in this study did not constitute an important factor in the induction of respiratory failure.

As for the cause of death, our data suggested that it was a state of combined hypercapnia, hypoxia and a progressively dysfunctional cardiovascular status, which ultimately converged and imposed such an anoxic burden on the central respiratory mechanism as to cause respiratory drive to cease altogether.

Efficacy Evaluation of Post-Exposure Therapy Mix (Groups 5-8). Figure 2 (2A-2E) is a series of electrophysiographic depictions of CNS and cardiorespiratory responses to the post-exposure therapy mix along the 24-hr course of intoxication and recovery in animals intoxicated by GA (Fig. 2A), GB (Fig. 2B), GD (Fig. 2C), GF (Fig.2D) and VX (Fig. 2E). In consideration of the enormity of data, and of the complexity of responses to the post-exposure regimen across CWAs, only representative periods of CNS and cardiorespiratory activity profiles during the 24-hr timeframe are shown in Figure 2. These periods are "Control", "20 Min" post-exposure, "2 Hours" post-exposure, "6 Hours" post-exposure and "24 Hours" post-exposure.

Figure 2 (A-E). Representative electrophysiographic depiction of CNS and cardiorespiratory responses to the post-exposure therapy mix in animals challenged with a 2-LD_{50} dose of GA (Fig. 2A), GB (Fig. 2B), GD (Fig. 2C), GF (Fig. 2D) and VX (Fig. 2E). These animals all recovered 24 hr following intoxication/therapy. Note i) the absence of any aberrant cortical activity throughout the course of intoxication/therapy and recovery, and ii) the CNS and cardiorespiratory activity profiles were all restored to a state comparable to that of control at 24 hr post-intoxication. Signal trace description: **ECoG**, electrocorticogram; **DEMG**, diaphragmatic electromyogram; IntDEMG, integrated diaphragmatic electromyogram (τ = 50 msec); **NEMG**, electromyographic recording from the neck muscle (dorsal portion of cervical trapezius); and **ECG_{II}**, Lead II electrocardiogram. Voltage calibrations: ECoG, 128 μV; DEMG, 1.22 mV; NEMG, 1.11 mV.

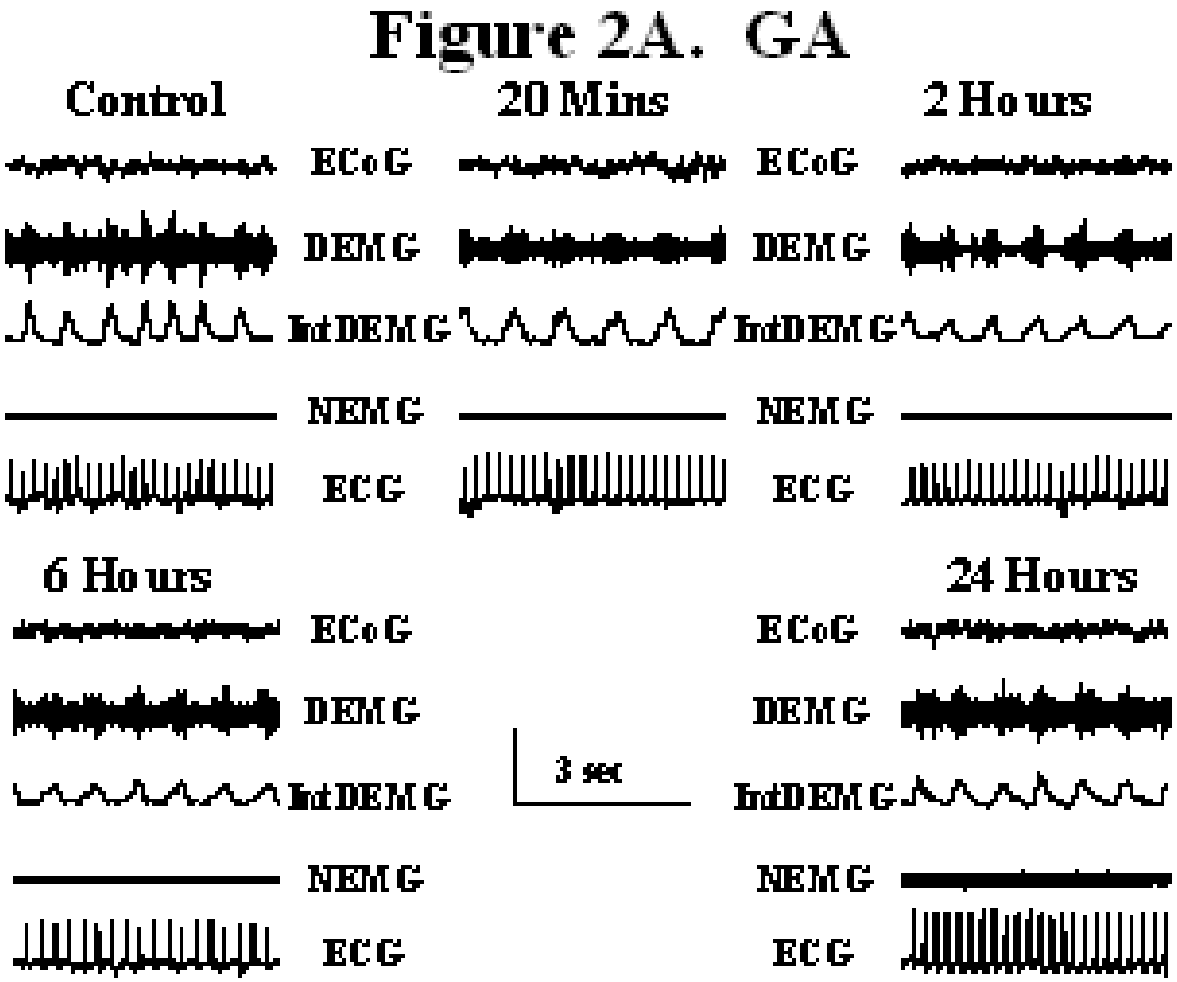


Figure 2B. GB

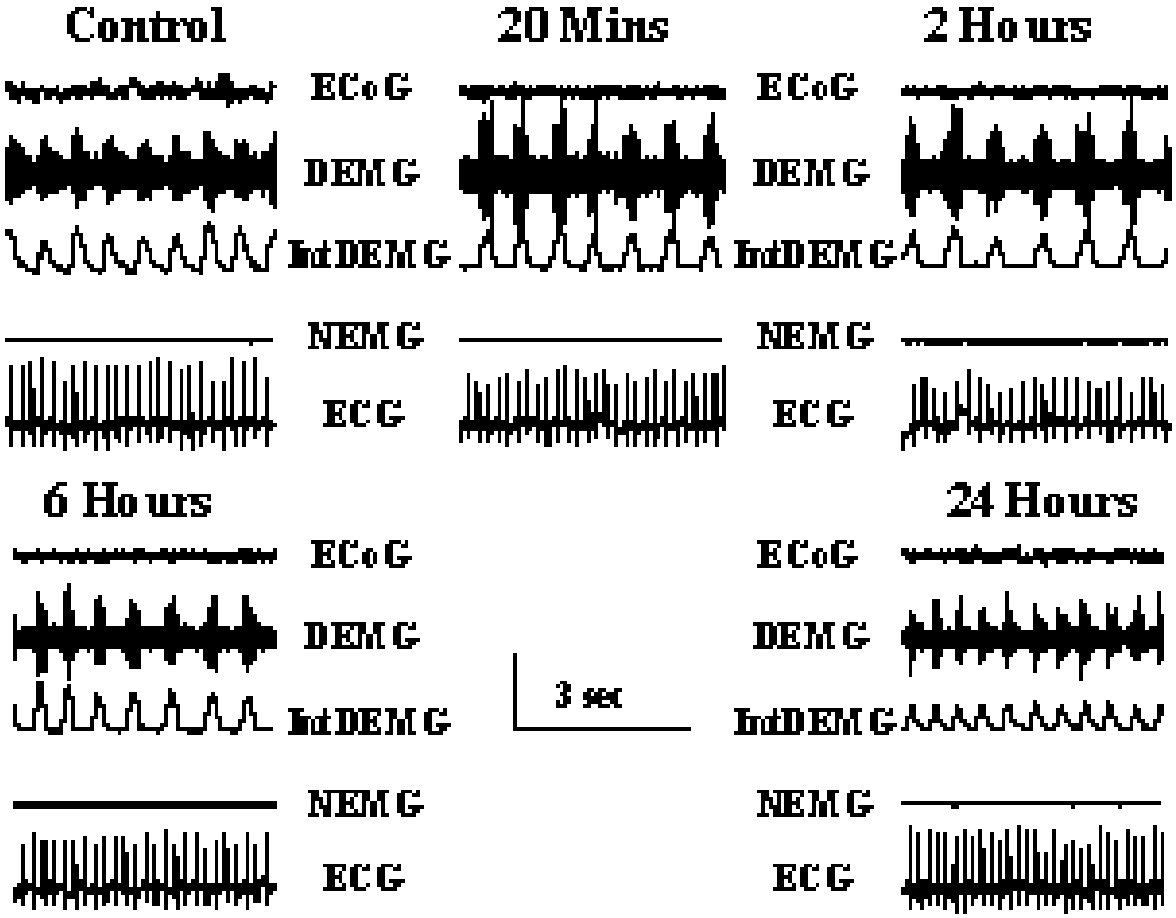

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Figure 2C. Soman

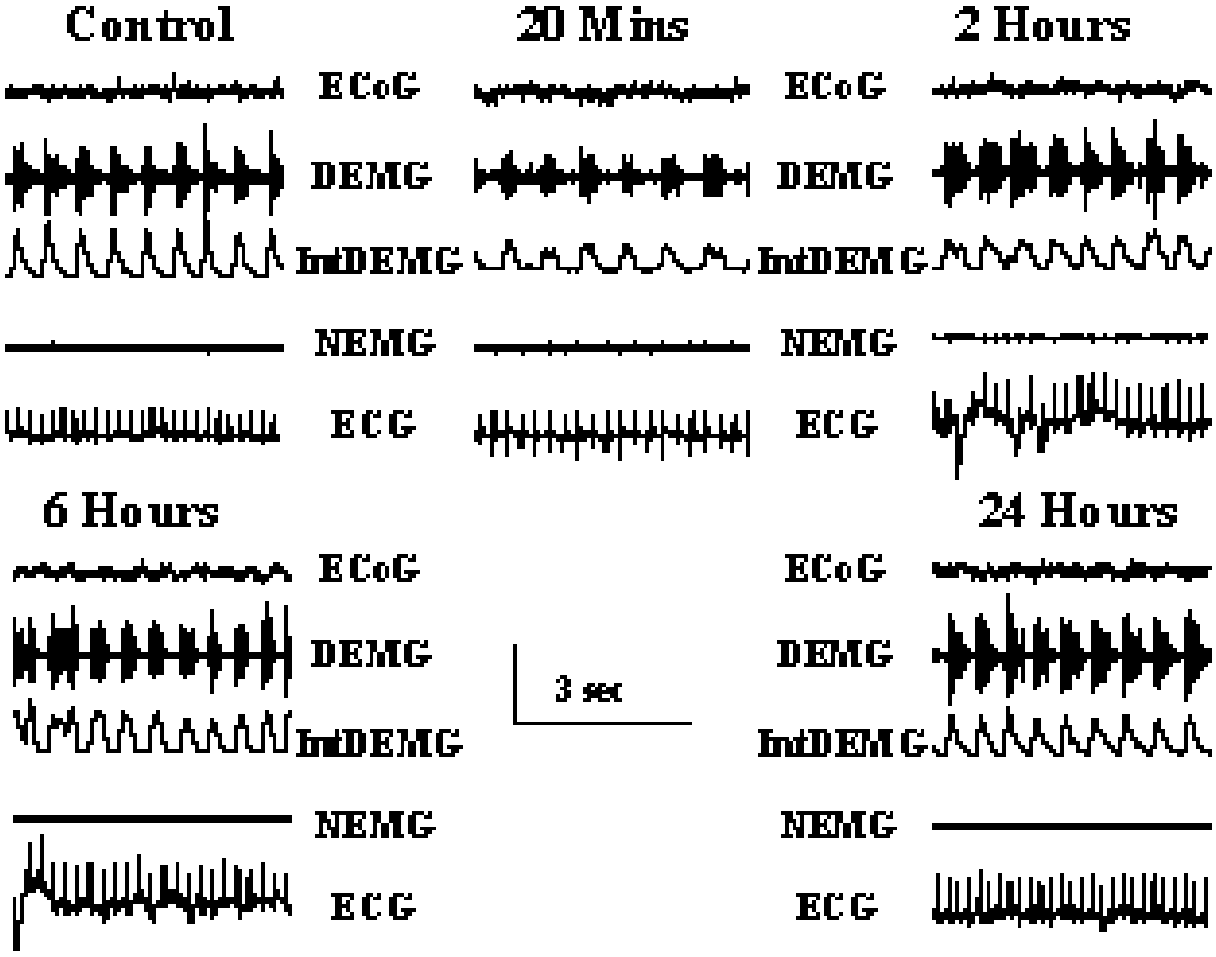
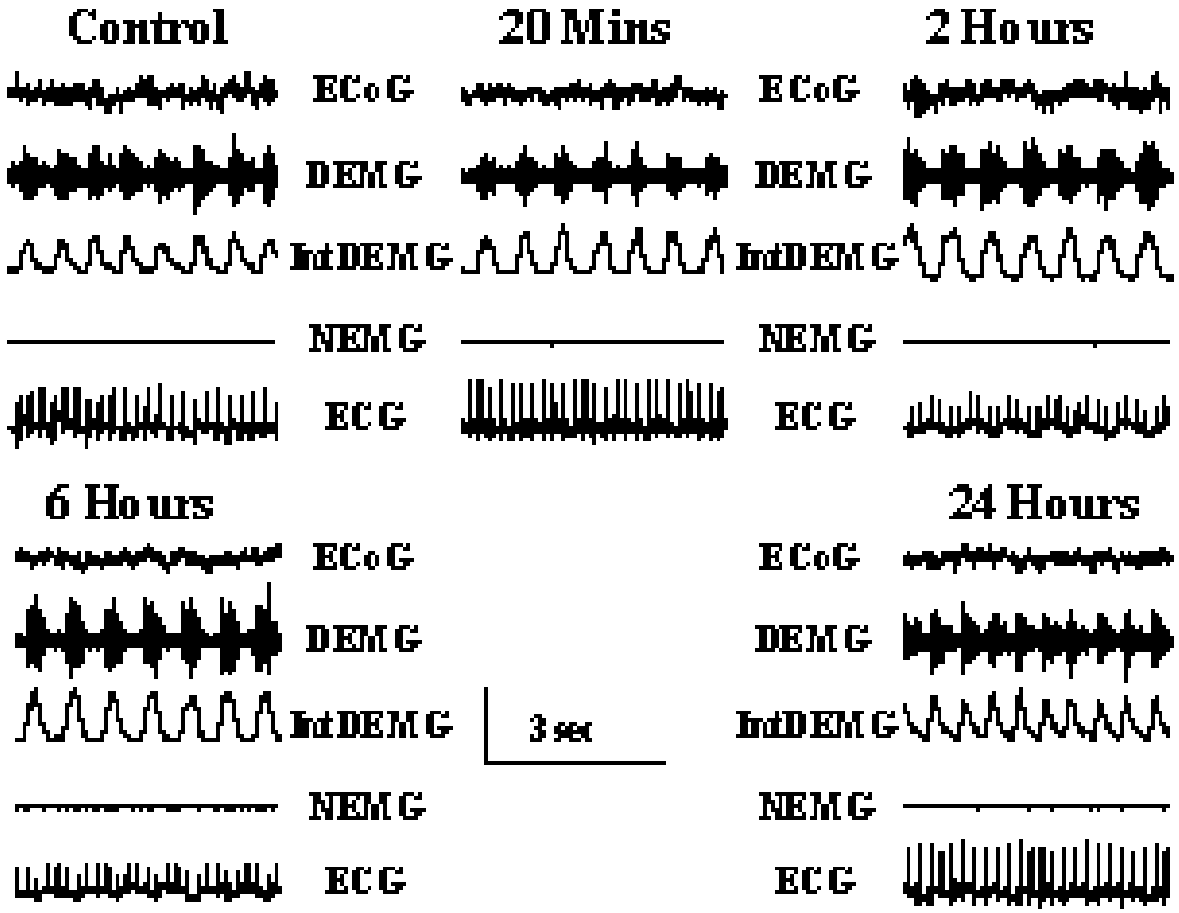


Figure 2D. GF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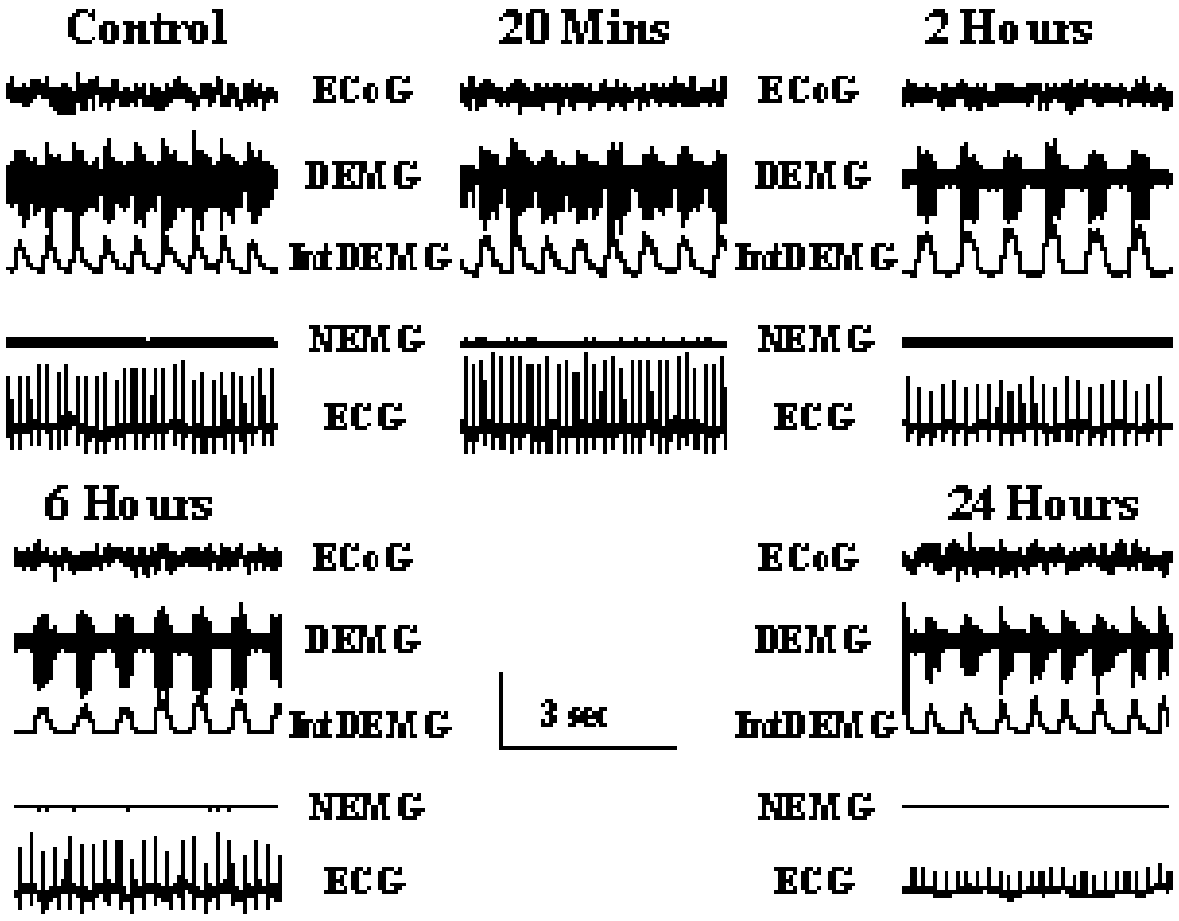
24 Hours
3 sec

Figure 2E. VX



All animals challenged with CWA (GA, GB, GD, GF or VX) responded favorably to the post-exposure treatment regimen and survived 24 hr after intoxication. Notable behavioral and pathophysiological changes attributable to intoxication and therapy were only seen during the first 30 min after acute agent exposure. These included oro-facial movements (indicative of immoderate secretion), mild degree of dystonia/ataxia, and a short period of phenobarbital-induced sedative effects. Animals were able to recover from these neurobehavioral deficits in about 5-10 min. Parenthetically, recovery was defined by the first incident of volitional and successful completion of postural modification (e.g., transition from walking/standing to normal crouching) or a series of discernible normal behavioral repertoire (e.g., grooming, exploratory behaviors, eating). Generally speaking, the animals were typically asymptomatic and able to engage in behavioral repertories comparable to those of the control condition ≈30 min after intoxication/therapy.

Throughout the course of intoxication/therapy and 24-hr convalescence, none of the animals exhibited seizures, convulsions or signs of aberrant cardiorespiratory activities or other debilitating neurobehavioral changes. These findings indicated that the post-exposure therapy mix was effective not only in antagonizing CWA-induced lethality, but also in protecting the functional integrity of the CNS and cardiorespiratory system.

Cardiorespiratory Responses Following Intoxication and Post-Exposure Therapy. Figure 3 is a summary of changes in heart rate and respiratory rate in response to the post-exposure treatment regimen across animals intoxicated by CWAs (GA, GB, GD, GF and VX).

Figure 3. Cardiorespiratory Response Profiles Throughout the Course of Intoxication and Post-Exposure Therapy

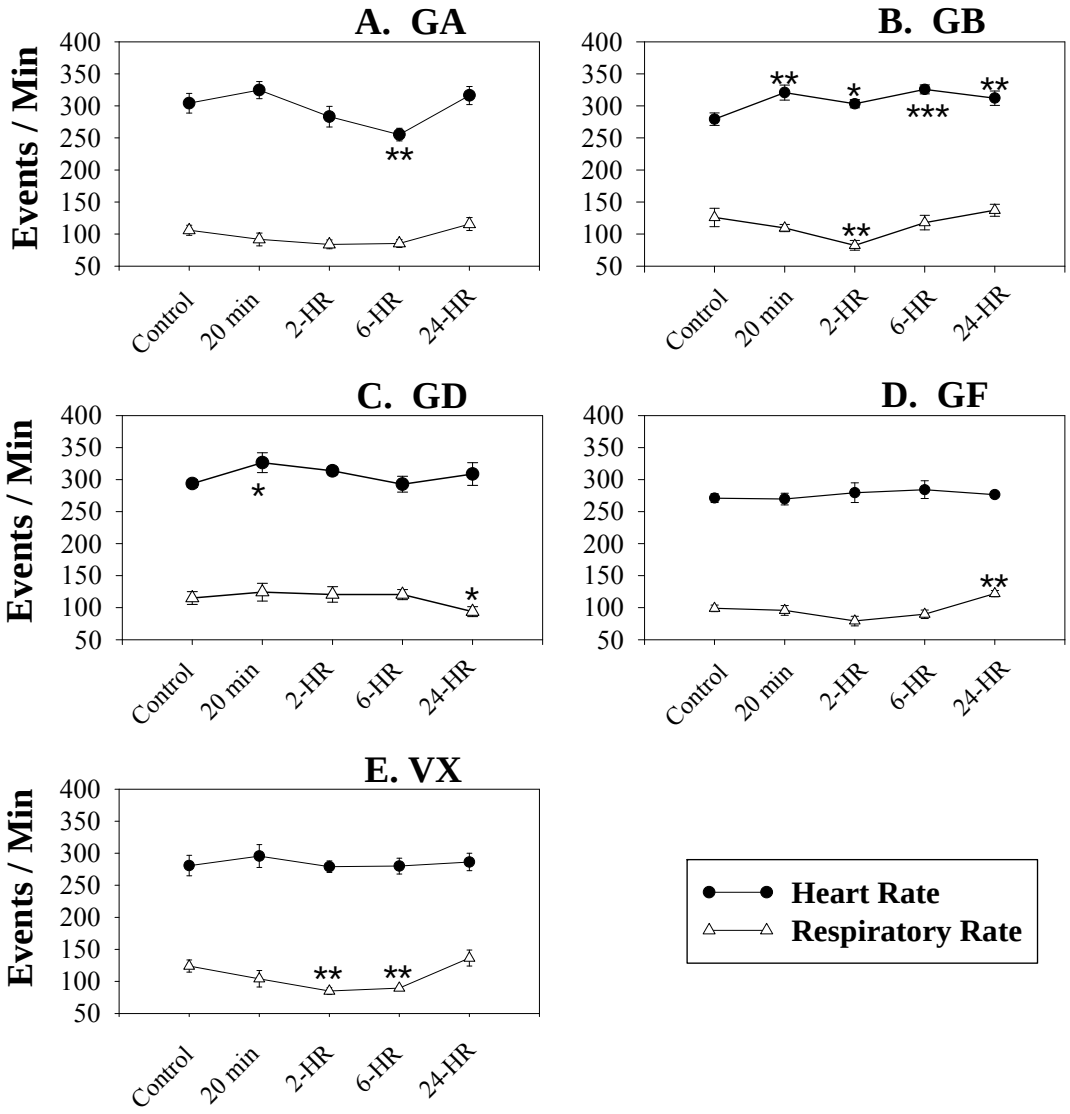


Figure 3 (A-E). A summary of changes in heart rate (filled circle) and respiratory rate (open triangle) in response to post-exposure therapy regimen in animals exposed to a 2-LD_{50} dose of GA (Fig. 3A), GB (Fig. 3B), GD (Fig. 3C), GF (Fig. 3D), and VX (Fig. 3E) across control and various post-exposure experimental stages. Asterisks denote significant differences between control and post-OP data: (***), $p<0.001$; (**), $p<0.005$; (*), $p<0.01$. Error bar = Standard Error of the Mean (SEM).

1. Heart rate and Electrocardiographic Attributes (Fig. 3; filled circle). With the exception of GF animals (Group 9; see Fig 3D), the myocardial activities in animals challenged with GA, GB, GD or VX all showed the development of a positive chronotropic ramp profile following intoxication and therapy. The positive chronotropic effects seen here can be attributed primarily to the vagolytic actions of methylatropine nitrate and scopolamine in the post-exposure therapy mix. The heart rate typically peaked within the first 5 min post-agent exposure and returned to a level comparable to that of control over a course of approximately 2 hr.

The myocardial protective effects of the cholinolytic adjunct mentioned above were unequivocal. Throughout the course of intoxication/therapy and convalescence, we saw no sign of myocardial perturbation or aberrant electrocardiographic attributes (such as bradycardia, dysrhythmia, QT-prolongation, T-inversion, J-Point elevation, atrio-ventricular block, etc) in any of the animals across Groups 6-10.

2. Respiratory Rate (Fig. 3; open triangle). Moderate degree of respiratory frequency depression was noted in GA-, GB-, GF- and VX-intoxicated animals ≈1-3 hr following intoxication/therapy. Although respiratory rate depression could be seen in GD-intoxicated animals, the magnitude of reduction was typically quite trivial compared to those seen in GA-, GB-, GF- and VX-intoxicated animals. At 24 hr post-CWA exposure, both the respiratory rate and breathing patterns across Groups 6-10 were comparable to those of control.

3. Diaphragmatic Effects. Time-averaged spectral analyses and breath-by-breath examination of power spectrograms showed that all animals (Groups 6-10) displayed signs of diaphragmatic perturbation to varying degrees of severity during the 20-30 minutes following intoxication/ therapy. Figure 4 (A-E) is a power spectrographic depiction of the extent of perturbation of diaphragmatic neurotransmission. Despite a profound reduction in diaphragmatic activity amplitudes and signs of fatigue (characterized by ramp reversals of integrated DEMG signals; see Figs. 2A-2E) at 20-min post-intoxication/therapy, the diaphragm still appeared to oscillate with sufficient vigor to sustain ventilation throughout the course of dyspnea mentioned earlier.

Figure 4. DEMG Power Spectrographs

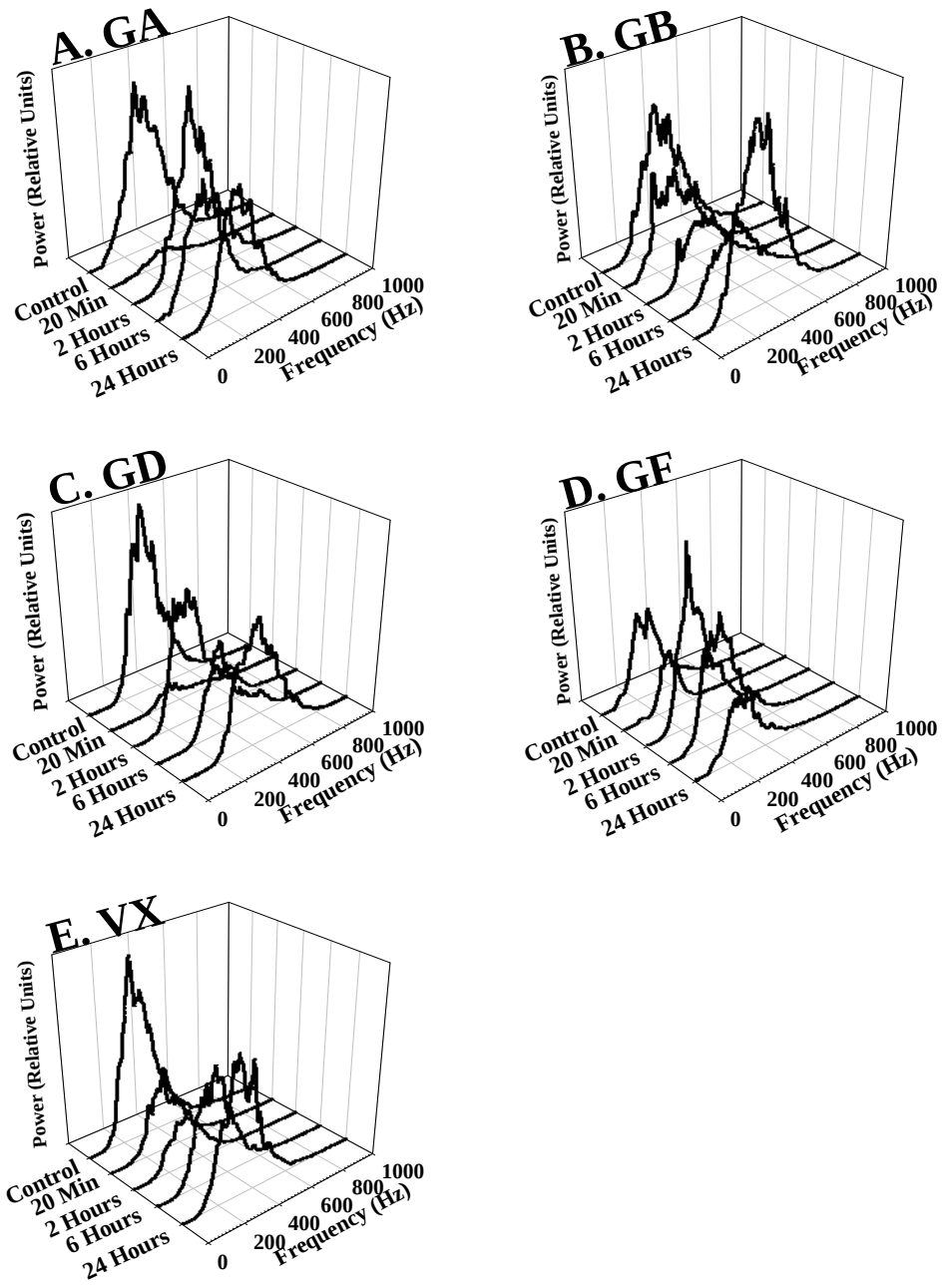


Figure 4. Power spectrographic depictions of changes in the functional integrity of the diaphragm in response to post-exposure therapy mix one minute following exposure to a 2-LD_{50} dose of GA (Fig. 4A), GB (Fig. 4B), GD (Fig. 4C), GF (Fig. 4D), and VX (Fig. 4E) across control and various post-exposure experimental stages. To permit direct visual comparison, the power spectrographic profiles in each agent category were normalized against the profile of the greatest spectral power. Note i) a sizeable reduction in DEMG spectral powers at 20-min post intoxication/therapy across CWA groups; and ii) a markedly diminished DEMG spectral power in GA- and GD-intoxicated animals at 20-min post-exposure/therapy. When the spectral profiles at the 20-min time point were examined at a magnified scale, fragmentation of DEMG power spectrum into distinct spectral complexes were also seen in GA- and GD-intoxicated animals (see text for more information).

In all CWA-intoxicated animals (Fig. 4, A-E), power spectral analyses indicated that the reductions in diaphragmatic spectral powers typically spanned the entire diaphragmatic frequency spectrum (1-1000 Hz), suggesting an overall reduction in central respiratory drive. The temporal course along which diaphragmatic spectral power was restored varied somewhat across CWAs. Signs of recovery generally began to emerge about 1 hr following intoxication and therapy.

Another notable observation (not indicated in Fig.4) was that in animals intoxicated by GA and GD, the pattern of declines in diaphragmatic spectral amplitudes was fragmented (i.e., the spectral powers were grouped into a variety of spectral components or spectral ranges). This phenomenon is characteristic of infirmities of peripheral respiratory mechanism (i.e., intermittent upper airway occlusion and/or diaphragmatic neuromuscular blockade). What can be inferred from Figures 4A-E is that, at 20-min post-intoxication/therapy, in addition to a developing state of respiratory rate depression (bradypnea), the functional integrity of the diaphragm in GA- and GD-intoxicated animals was also compromised to some extent.

DISCUSSION

The efficacy of a post-exposure therapy mix (see Table 2) against the toxic and lethal effects of GA, GB, GD, GF and VX was evaluated. Findings from this study indicated that the therapy mix was effective not only in antagonizing lethality and other life-threatening pathophysiological developments resulting from exposure to various CWAs, but also in protecting the functional integrity of the CNS and cardiorespiratory system. All animals receiving the post-exposure therapy survived the challenge 24 hr later. Although brief periods of acute cholinergic effects (mucoid-salivary secretion, dystonia, ataxia, tremors and fasciculations) were seen shortly after intoxication/therapy, these effects were generally quite mild. The only notable side effect of the post-exposure therapy mix was a transient period of sedation attributable to the central inhibitory effects mediated by phenobarbital - one of the constituents in the mix. No other side effects, benign or untoward, associated with the post-exposure therapy mix were observed.

The extent of CNS protection conferred by the post-exposure therapy mix was particularly notable. That is, throughout the course of intoxication/therapy and convalescence, none of the animals exhibited seizures, convulsions, signs of incapacitation, or other debilitating central effects typically seen in OP intoxication. With the exception of brief periods of compensatory changes in cardiorespiratory activity profiles during the first 30 min following intoxication/therapy, the functional integrity of the overall cardiorespiratory activity profile remained fundamentally intact. Animals typically began to show signs of recovery 30 min after intoxication/therapy; at which time, the animals were able to resume behavioral repertories comparable to those of the control condition.

Future Research and Efficacy Enhancement of Post-Exposure Therapy Mix.

Efforts are currently being made to further enhance the effectiveness of our post-exposure therapy mix by modifying and streamlining the constituents in the mix and their dose levels. For instance, the addition of mecamylamine (a ganglionic blocking agent) together with minor adjustments to the scopolamine dose level may obviate the need for methylatropine. Another possibility is to optimize the therapeutic outcome with co-administration of physostigmine (tertiary amine) and pyridostigmine (quaternary amine). Preliminary data indicated that the combined use of these carbamates can mediate a less than synergistic but greater than additive therapeutic effect even at dose levels considerably lower than when they are used individually. Moreover, the combined use of these carbamates may also expand the efficacy spectrum against the toxic effects of other CWAs (e.g., VR, Chinese VX, etc.) not examined in this investigation.

Thus far, we have only investigated the effectiveness of a bolus dose of our post-exposure therapy mix. The added benefits of supplemental dosing will also be systematically examined particularly in the case of GD intoxication (at doses greater than $2xLD_{50}$) in which the phenomena of "aging" and "depot effect" (see Appendix 1) may delay, complicate, or thwart the recovery process.

Alternative routes of administration of our post-exposure therapy mix are also under consideration. At this point, our post-exposure mix is delivered via a combination of intramuscular (MMB4, scopolamine, methylatropine and physostigmine) and

intraperitoneal (phenobarbital) routes. Whether the therapeutic outcome can be further optimized when the constituents are administered intravenously (iv, and perhaps at a greatly reduced dose levels) awaits future research. In the present study, phenobarbital was administered separately so that the osmolarity of the im admixture could be maintained at a reasonable level. Should the iv route be deemed more appropriate in our future investigations, the added osmolarity load resulting from inclusion of phenobarbital in the iv solution (a much greater dose volume than the im solution) would certainly not be too much of a concern.

The objective of this research effort is to develop a maximally effective and minimally debilitating post-exposure therapy regimen. In the process of accomplishing this goal, critical inquiries will be made at various phases of future development to systematically identify any untoward drug-to-drug interaction(s) amongst the constituents in the mix so that an optimally effective post-exposure therapy mix can be ultimately formulated.

Dual Military/Civilian (Duality) Medical Countermeasure .

The uses of GB (a binary nerve agent) by Aum Shinrikyo terrorist group in a residential area of Matsumoto in1994 and in a crowded Tokyo subway in 1995 are testimonies that nerve agent attacks can inflict massive casualties and ultimately present not only a formidable challenge to the emergency medical system (Kenichiro, 2005; Ogawa *et al*., 2000; Tu, 2000; Yanagisawa *et al.*, 2006), but also a serious environmental-toxicological problem (Nishiwaki *et al*., 2001). Moreover, in the wake of an elusive and ever-widening terrorist web, the increasing likelihood of a chemical attack in a civilian environment has not only heightened concerns for our safety/security awareness, but also our less than adequate medical readiness in emergency chemical casualty management.

For the protection of the civilian population, the Centers for Disease Control and Prevention (CDC) has implemented a CHEMPACK Program (Chempack Program Description, 2004; Young, 2004), a nerve agent antidote initiative, and made it available to public health systems on a voluntary participation basis nation-wide. Unfortunately, the current version of the CHEMPACK Program does not involve carbamate pretreatment. As mentioned in the "Introduction" section of this report, carbamate plays a critical role in nerve agent countermeasure. Therefore, the most we can hope to benefit from the CHEMPACK Program is its "*marginal*" effectiveness in antagonizing sarin- and VX-induced toxicities. Taken together, without the protective benefits of carbamate pretreatment, the effectiveness of Chempacks or Mark I Kits strictly as a post-exposure treatment is quite doubtful because such interventions offer virtually no protection against the toxic effects of other nerve agents, such as tabun, soman, and cyclo-sarin, that are known to be much more difficult to medically manage with just atropine and 2-PAM alone.

Thus, the development of a post-exposure therapy regimen like the one described in this report can benefit not only military personnel not enrolled in the carbamate pretreatment program, but also civilian populations worldwide.

Concluding Comments.

Even in its current rudimentary stage of development, our post-exposure therapy mix is already able to antagonize the toxic and lethal effects of not only soman (a chemical agent that is widely recognized as being most resistant to treatment by the existing DoD pretreatment and therapy regimen), but also a wide range of other CWAs such as GA, GB, GF and VX. On the basis of findings derived from this investigation, we are optimistic that our post-exposure therapy mix will provide a sound framework upon which further research may be conducted to extend its efficacy spectrum against the toxic and lethal effects of a wide range of chemical threat agents without requiring pretreatment. We are mindful that site/mechanism-specific scavengers, antibodies or other immunopharmacological products may become the therapeutic compounds of tomorrow. In their current state of R&D, however, the therapeutic use of these compounds in an actual chemical attack remains questionable. Other considerations such as immunogenicity, limited *in vivo* half-life, availability, thermo-stability, shelf-life, and the exorbitant cost of these compounds could also significantly discourage their use, particularly in extreme climatic conditions, as well as geographically isolated and economically disadvantaged areas. For the foreseeable future, we believe, pharmacological compounds will continue to play a critical role in the management of toxic/lethal effects of chemical threat agents.

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APPENDIX I

Aging. Reactivation of OP-inhibited AChE by oxime remains, to this date, one of the most critical therapeutic components against the toxic and lethal effects of nerve agents. Oxime reactivation of inhibited AChE is not always predictable, however. In the case of soman, dealkylation of the soman moiety occurs with the inhibited AChE, and the inhibited complex can then become refractory to reactivation by oxime over time (Benschop and Keijer, 1966; Berends *et al.*, 1959; Berry and Davis, 1966; Fleisher and Harris, 1965; Fleisher *et al.*, 1967). This phenomenon is called "aging." The rate of aging varies across nerve agents, and to some extent, animal species. For tabun, sarin and VX, aging occurs over a period ranging from hours to days. For soman, aging occurs in 2 minutes. The rapid aging of soman-inhibited AChE, which significantly limits the therapeutic effectiveness of oximes, constitutes one of the most insidious toxicity components of soman intoxication.

Depot Effect. Following exposure, soman can be sequestered and stored intact in a number of tissues, organs and even enzymes such as plasma aliesterase, and subsequently released back into the system to wreak havoc (Clement, 1982b; Kadar *et al*, 1985 Norgren *et al.*, 1985). The deposition of soman in the so-called "soman depots" represents another significant toxicity component that renders medical management of soman-induced toxicities more difficult than those of other nerve agents.