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Pharmacological dissociation of the motor and electrical aspects of convulsive status epilepticus induced by the cholinesterase inhibitor soman*

Steven Sparenborg^a, Lucas H. Brennecke^b and Everette T. Beers^a

^aNeurotoxicology Branch and ^bComparative Pathology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010, USA

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Key words: Organophosphorus agent; Convulsions; Seizures; Anticonvulsants; Pathology; Status epilepticus; Power spectral analysis

In an effort to validate methods to be used in a screen for drugs effective as anticonvulsants for soman-induced convulsions, scopolamine (0.2 mg/kg) or diazepam (1 mg/kg) were given (i.m.) to male guinea pigs as a pretreatment 30 min before a convulsant dose of soman. Pyridostigmine, atropine and pralidoxime chloride also were given to counteract the lethality of soman. All animals challenged with soman and which did not receive either diazepam or scopolamine exhibited convulsive status epilepticus (SE), identified by continuous electrographic seizure activity (EGSA) and continuous motor convulsions. Despite the presence of continuous motor convulsions in all animals pretreated with diazepam and challenged with soman, EGSA was not observed in five of the seven animals. Continuous motor convulsions developed in four of seven animals pretreated with scopolamine and challenged with soman, but EGSA was not observed in any scopolamine-pretreated guinea pig. Neuronal necrosis was observed in the hippocampus, thalamus, amygdala, and cerebral and pyriform cortices in each animal with EGSA, but no brain damage was found in subjects without EGSA. Thus, although convulsions, EGSA and brain damage normally occur together in animals exposed to soman, the convulsions can be pharmacologically dissociated from the EGSA and brain damage, demonstrating that the clinically manifested convulsions are not dependent on EGSA recorded from the cortex or on abnormal activity which leads to neuronal necrosis in the forebrain.

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* The experiment reported here was conducted according to the 'Guide for Care and Use of Laboratory Animals' (document 85-23; revised 1985) as prepared by the Committee on Care and Use of Laboratory Animals, National Research Council, United States of America. The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense, United States of America.

Correspondence to: S. Sparenborg, Division of Neuropharmacological Drug Products, HFD-120, FDA, 5600 Fishers Lane, Rockville, MD 20857, USA.

Introduction

Exposure to the organophosphorus (OP) anticholinesterase soman (pinacolyl methylphosphorofluoridate) results in peripheral signs of cholinergic poisoning, convulsions, central neuronal damage, respiratory arrest and death^{10,16,26}. These toxic effects are thought to be related to the excessive buildup of acetylcholine resulting from the inhibition of cholinesterases which become irreversibly bound by soman³³. Soman-induced convulsions in guinea pigs are characterized by a mild to robust tonic extension of all limbs. Tonic extension

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predominates, but bouts of rapid fore- and hind-limb clonus may occur. Tonic limb extension may endure for 2 days following an acute dose of soman, although the degree of tonicity is much reduced after a few hours³⁰. Electrographic seizure activity (EGSA) is observed in the EEG, consisting of spikes, sharp wave paroxysms, and, occasionally, a spike and wave pattern. Neuronal damage in some limbic and cortical regions is observed following soman poisoning and has been highly correlated with the occurrence of clinically observed convulsions^{22,26,31}.

Cholinolytics, including scopolamine, have often been considered as antidotes to soman poisoning for their ability to generally counteract any toxic effect of excessive ACh^{9,11,20,21}. Shortly after World War II, researchers from this institution reported that scopolamine could restore a convulsant EEG record, resulting from the cholinesterase inhibitor DFP, to normal low-voltage fast activity³⁶. The restorative effect of scopolamine was attributed to its antimuscarinic property, offsetting the excessive levels of ACh resulting from the action of DFP. Recent studies of scopolamine as an antidote to soman poisoning have focused on its potential as an anticonvulsant, demonstrating that it prevents convulsions⁹ or that it arrests both EGSA and convulsions when given 1 min after the onset of convulsions^{2,18}. Similarly, atropine has been used to arrest EGSA induced by DFP or sarin, another irreversible cholinesterase inhibitor³⁵. Although it has been known for many years that diazepam can block convulsions, EGSA, and brain damage caused by soman^{19,23,25,27}, a more effective treatment for soman neurotoxicity is sought.

An important step in screening potential anticonvulsants effective against soman poisoning is to verify that those candidates which stop motor convulsions also prevent EGSA and brain damage. In the development of a module to measure electrographic seizure activity in a screen for anticonvulsants we tested diazepam and scopolamine as exemplary test compounds.

Methods

Subjects and surgical preparation

Twenty-eight male albino C1L:(HA)BR VAF/Plus

guinea pigs (*Cavia porcellus*), weighing 300–350 g, were quarantined on arrival and screened for evidence of disease. They were individually housed in polycarbonate shoebox cages on contact corn cob bedding changed two times per week and provided with commercial certified guinea pig chow (Purina # 5026) and tap water ad libitum. Animal rooms were maintained at 20–22°C, relative humidity of 50 ± 10%, on a 12-h light:dark cycle with no twilight.

The guinea pigs were chronically instrumented for electrocorticogram (ECoG) recording under ketamine (30 mg/kg, i.m.) and xylazine (6 mg/kg, i.m.) anesthesia. Lidocaine (2%) was injected into the scalp prior to a midline incision. Small stainless steel screws were placed in holes drilled bilaterally in the skull 3 mm anterior to and 3 mm lateral to bregma for recording the ECoG. The tips of these screw electrodes rested just above the dura overlying sensorimotor cortex. Wires connected the electrodes to a plastic socket secured to the skull with dental cement. Antibiotic ointment was applied to all areas of the incision, which was then closed with wound clips.

Experimental design

One week after surgery, the guinea pigs were placed in individual, plexiglas cubicles (side length = 30 cm) for ECoG recording and experimental drug administration. The experiment began with a 30-min baseline period of behavioral observation and ECoG sampling, followed by a 30-min pretreatment period. At the beginning of the pretreatment period, the following combinations of substances were administered to groups of seven subjects each: scopolamine/atropine/pyridostigmine (SCO/ATR), diazepam/atropine/pyridostigmine (DIA/ATR), saline/atropine/pyridostigmine (SAL/ATR), saline/saline/pyridostigmine (SAL/SAL). At the beginning of the soman treatment period, each subject in all groups received 2 × LD₅₀ soman followed 30 s later by pralidoxime. All subjects were observed for 2 h immediately following the soman injection and rated for the severity of convulsive activity.

Drugs

The following forms and dosages of the drugs

were used: pyridostigmine bromide (0.22 mg/kg; Hoffman-La Roche), atropine sulfate (4 mg/kg; Sigma Chemical Co.), diazepam (1 mg/kg; Hoffman-La Roche), scopolamine HBr (0.2 mg/kg; Sigma Chemical Co.), pralidoxime chloride (25 mg/kg; Wyeth-Ayerst), and soman (56 μ g/kg). All drugs were dissolved in normal saline, except diazepam (vehicle = 40% polyethylene glycol, 10% ethanol, 1.5% benzyl alcohol, 48.5% water) and all were administered in 0.5 ml/kg volume. All injections were given intramuscularly except soman, which was given subcutaneously.

Military personnel may be issued pyridostigmine, atropine, pralidoxime and diazepam as antidotes to soman poisoning. The use of these substances as therapy for nerve agent exposure has been reviewed elsewhere¹⁷. Pyridostigmine was given as a pretreatment to prevent soman from irreversibly inhibiting virtually all peripheral cholinesterase molecules. Soman and pyridostigmine both inhibit cholinesterase by binding at the same site. After soman had either bound tightly to the remaining cholinesterase molecules or had been metabolized, some cholinesterase molecules became free to perform their enzymatic function as pyridostigmine dissociated from them. Pralidoxime is routinely given to victims of OP poisoning because it can separate the OP-cholinesterase complex, although its effectiveness against soman is limited. Atropine was used to protect muscarinic receptors from excessive stimulation.

The $2 \times LD_{50}$ dose of soman was used to produce unequivocal seizures in each subject which did not receive either diazepam or scopolamine. The doses of atropine, pyridostigmine, and pralidoxime used in this experiment were selected to promote survival, yet not interfere with seizures. Diazepam and scopolamine were added to this drug regimen as representative anticonvulsant test drugs.

Data collection and analysis

Motor activity was categorized in three levels: stage 1-preconvulsive, muscle tremors in the face, limbs, or body; stage 2-convulsive, running-bouncing clonus or tonic forelimb extension or clonic hindlimb activity; and stage 3-profoundly convulsive, rigid tonic fore- and hindlimb extension.

The severity of convulsive activity was compared between groups by submitting the most severe stage level attained for each subject to the Kruskal-Wallis test, a non-parametric, multiple-group method. When a significant outcome was obtained, post hoc paired comparisons of groups were made with a procedure which is the non-parametric analog of the Fisher least significant difference test¹³.

For ECoG recording, the difference voltage from the two electrodes was amplified and band-pass filtered (half amplitude cut-offs: 0.1 and 100 Hz). This signal was passed to an analog-to-digital converter, an FM tape recorder, an oscilloscope, and a paper chart recorder. The paper chart records were examined for electrographic seizure activity, characterized by spikes or sharp waves (2–5/s) with an amplitude twice that of the baseline ECoG signal.

The digitized signal was sampled 12 times per minute (sample epoch = 1 s) and a fast Fourier transform performed on each epoch. Separate power spectral estimates (PSE) for each subject were produced by combining spectral amplitudes for single frequencies into five bands, 1–4, 5–7, 8–13, 14–21, 22–32 Hz, and for the total (a composite of 1–32 Hz).

The magnitude of ECoG activity was standardized between subjects by converting the PSE to *z*-scores. To accomplish this, a 15-min sample of PSE from each of the baseline, pretreatment, and soman periods was extracted. The frequency band and total means of the baseline sample PSE were subtracted from the appropriate pretreatment and soman sample PSE means and then divided by the standard deviation. The baseline PSE sample began 10 min after the beginning of the baseline period. The pretreatment PSE sample began 10 min after drug injections during the pretreatment period. The soman PSE sample began with the onset of spike or sharp wave activity during the soman period. If a subject did not exhibit EGSA, the soman sample was considered to have begun 10 min after soman administration. Group differences in PSE were compared by analyzing *z*-scores with one-way ANOVAs. Paired comparisons of treatment group means were made with the Fisher least significant difference test following significant outcomes in the ANOVAs.

Histopathology

Those guinea pigs surviving at 48 h after the administration of soman were deeply anesthetized with pentobarbital sodium and transcardially perfused with saline followed by 10% formalin. The brains were removed and processed for hematoxylin and eosin staining and evaluated for pathology by a board certified (DAVCP) pathologist according to methods described elsewhere³².

Results

Lethality

All SAL SAL subjects died within 1 h after soman challenge. The addition of atropine to the antidotal regimen enabled three of the seven subjects from the SAL ATR group to survive for 48 h after soman. One subject from each of the DIA/ATR and SCO ATR groups died within 48 h after soman.

Electrocorticogram data

EGSA developed within approximately 10 min of soman injection in each subject from the SAL/SAL and SAL/ATR groups. EGSA was continuous in the SAL/ATR subjects throughout the 2-h observation period, and continued in each SAL/SAL subject until minutes before death. Bursts of rhythmic activity, which might signify an ictal event, were rarely observed in these animals. Pretreatment with diazepam completely prevented the appearance of EGSA, or any paroxysmal activity, in five guinea pigs. One of the two subjects from the DIA/ATR group that did develop EGSA was the one member of this group that died. No evidence of any seizure-like activity was found in the paper chart, oscilloscope, or digitized records of those subjects given scopolamine (see Fig. 1).

Diazepam and scopolamine significantly reduced power spectral estimates during the soman period ($P < 0.01$) relative to those of the SAL/ATR group. Although scopolamine pretreatment slightly elevated power in the lower frequencies in the pretreatment period, the combination of scopolamine and soman returned PSE to baseline levels in the soman period. The PSE of these two diazepam-pretreated subjects that exhibited seizure activity are included in the average for the DIA/

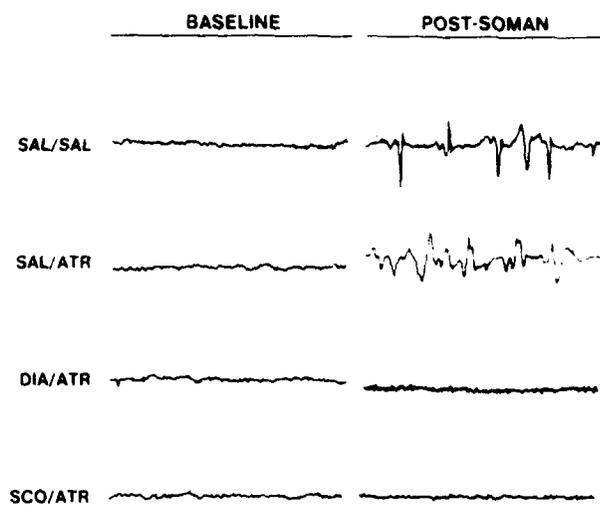


Fig. 1. Electrocorticogram (ECOG) recorded from dural surface of guinea pigs. Animals were implanted bilaterally with stainless steel screws that rested on the dural surface overlying sensorimotor cortex. Bioelectric potentials were amplified and continuously displayed on rectilinear chart paper during the course of the experiment. Left panel: baseline activity was recorded for 30 min prior to any drug treatment. Right panel: subjects pretreated with only pyridostigmine. SAL SAL, exhibited robust soman-induced electrographic seizure activity (EGSA) that continued from within minutes after soman administration until just prior to death (within 1 h post soman). Guinea pigs pretreated with pyridostigmine and atropine (4 mg/kg), SAL/ATR, exhibited robust seizures (right panel) that continued for hours. Five of seven animals pretreated with pyridostigmine, atropine and diazepam (1 mg/kg), DIA ATR, did not exhibit seizure activity. Pretreatment with scopolamine (0.2 mg/kg) plus pyridostigmine and atropine, SCO ATR, prevented EGSA in all subjects. Calibration bar is 0.5 s and 0.7 mV.

ATR group in Fig. 3. A separate plot of the PSE of these two subjects is found in Fig. 4, along with a plot of the remaining five subjects that did not have EGSA. A relatively high level of power was found in the 22–32 Hz frequency band of the non-seizing subjects.

Analyses of PSE revealed a variety of drug effects in the pretreatment period. The two drugs that protected against the seizure-inducing properties of soman also affected PSE prior to soman administration. Scopolamine significantly increased power in the lower frequencies and diazepam significantly increased power in the higher frequencies (see Fig. 2). Pyridostigmine and atropine did not affect PSEs. These statements are based on planned comparisons of PSE during the pretreatment

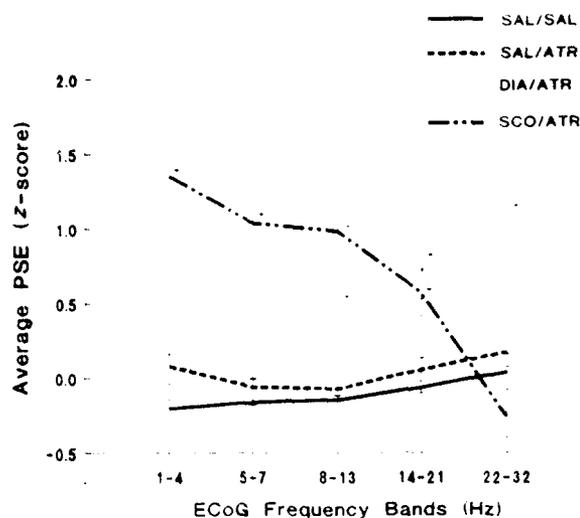


Fig. 2. Spectral power plot of ECoG activity, pretreatment period. Power spectral estimates (PSE) of the SAL/SAL and SAL/ATR groups were not affected by atropine and/or pyridostigmine pretreatment. Scopolamine and diazepam pretreatment, however, inversely affected spontaneous ECoG activity. Standardized PSE of ECoG activity are plotted in five frequency bands, each point representing PSE during a 15-min sample starting at 10 min after pretreatment drugs were given (mean \pm SEM). ECoG data from this period were converted to PSE and then standardized as z-scores based on the mean and standard deviation of data from the baseline period. Standardized baseline period activity for each group at each frequency band is represented by $z = 0$. Asterisks indicate differences from the SAL/ATR group, $P < 0.01$.

period between the SAL/ATR group and each of the other three groups in each frequency band ($P < 0.01$).

Motor convulsions

Convulsive activity was observed in all subjects except in three animals that were pretreated with scopolamine. In pairwise comparisons following a significant outcome in the Kruskal-Wallis test ($P < 0.05$), only the group that received scopolamine had significantly reduced convulsions relative to any other group. The average maximum convulsion stages for the SAL/SAL, SAL/ATR, DIA/ATR, and SCO/ATR groups were 3.0, 2.86, 2.57, and 1.57, respectively.

The severity of convulsions in each subject in the SAL/SAL group progressed from stage 1 through stage 3. None of the subjects in the group pretreated with scopolamine, however, reached stage 3 level convulsions. The two subjects from the

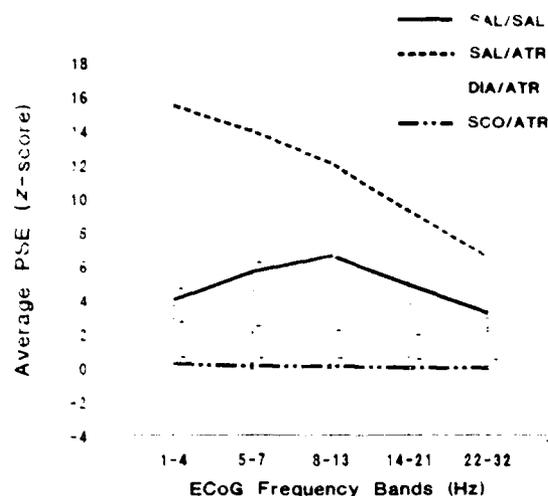


Fig. 3. Spectral power plot of ECoG activity, soman period. Scopolamine pretreatment prevented seizure activity and kept PSE of the SCO/ATR group at baseline levels. Seizures were also prevented in five of seven subjects pretreated with diazepam. Average PSE of the DIA/ATR group depicted here include the subjects with EGSA. The atropine pretreatment given to the SAL/ATR group enhanced power in the two lowest frequency bands compared to the control group given pyridostigmine as the only pretreatment drug. Asterisks indicate differences from the SAL/ATR group, $P < 0.01$. See Figs. 1 and 2 for additional explanations.

group pretreated with diazepam that went into status epilepticus also reached the stage 3 level of convulsions. However, five subjects from that group had convulsions (three at stage 3), but without EGSA or SE detectable from the electrodes at the surface of the brain. None of the scopolamine-pre-

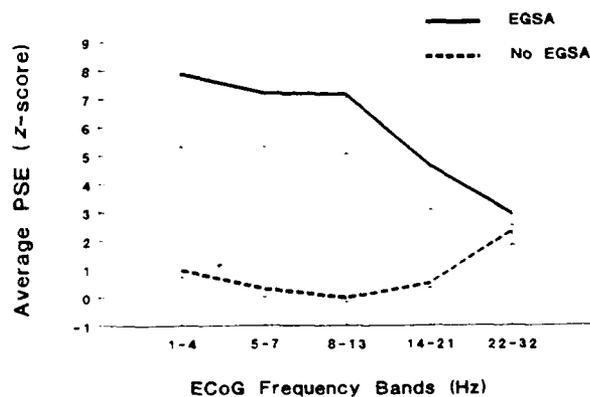


Fig. 4. Spectral power plot of ECoG activity, diazepam-pretreated subjects. Average power spectral estimates during the soman period for the two subjects with EGSA from the DIA/ATR group are plotted separately from the five subjects without EGSA. Data points are mean minus the SEM. See Fig. 2 for further explanations.

treated subjects, even the four subjects that convulsed at the stage 2 level, exhibited EGSA or SE.

Guinea pigs tested in this model, utilizing pyridostigmine atropine pretreatment, ordinarily express robust and continuous tonic extension and/or clonus for at least 2 h following soman exposure. The nature of the convulsions exhibited by animals in this experiment was consistent with the model in that they were robust and continuous. All subjects that reached stage 3 recovered to stage 2 within 5 min and remained in that stage for the remainder of the observation period. All subjects whose maximal convulsive stage was 2 remained in that stage except for one subject in both the SAL/ATR and SCO/ATR groups, which recovered to stage 1 by the end of the observation period.

The onset of convulsions occurred within ± 7 min of the development of EGSA, one not consistently preceding the other across subjects. No change in the rate of spiking was observed at the emergence of tonic hindlimb extension (stage 3). We observed no correlation of the timing of spikes recorded at the cortex with the occurrence of limb clonus.

Histopathology

Necrotic neurons were found in abundance in all five brain structures examined from each of the three surviving SAL/ATR subjects. Necrosis was relatively slight in the thalamus and moderate to extreme in the hippocampus, amygdala, and the cerebral and pyriform cortices. Only one DIA/ATR subject had necrotic neurons and the extent of the damage in that subject was somewhat less in the hippocampus and pyriform cortex than that observed in the SAL/ATR subjects. This subject was one of the two DIA/ATR subjects which experienced SE. Necrotic neurons were not found in the brains of those guinea pigs treated with scopolamine.

Discussion

We have described an absence of electrical seizure activity and brain damage in the forebrain of guinea pigs that exhibited convulsive status epilepticus for at least 2 h. The clinical nature of the

convulsions exhibited by subjects in the group pretreated with diazepam was indistinguishable from that of the control groups given soman without diazepam. The guinea pigs in these control groups exhibited robust motor convulsions, SE, and brain damage. The severity and pattern of brain damage matched that observed in other reports of seizure-related brain damage induced by soman^{22,23,27,33}. In guinea pigs given diazepam, however, EGSA and brain damage were prevented. No evidence of EGSA or brain damage was found in the animals pretreated with scopolamine, even those that did convulse. The absence of brain damage in these animals suggests that prolonged abnormal activity did not take place in their forebrain structures.

Another example of the weak relation between EGSA recorded from the forebrain and clinically observed motor convulsions was the disparity between the onset times of these two measures. In some subjects, the appearance of spikes occurred prior to the development of stage 1 activity while, in other subjects, spikes first occurred during stage 1 or 2 activity. It is evident from Fig. 1 that the spike activity recorded from the saline groups during the soman period was not rhythmical. These facts suggest that the EGSA recorded from these guinea pigs was inter-ictal epileptiform activity, which typically does not correlate well with tonic clonic activity.

We have reported a similar dissociation between ECoG recordings and convulsions in guinea pigs poisoned with soman when caramiphen was given as a pretreatment³⁰. Caramiphen is a drug with anticonvulsant, antitussive, and anticholinergic properties^{11,14,21,34}. Robust, soman-induced, motor convulsions were observed in each subject given a relatively low dose of caramiphen (10–30 mg/kg), but in some of these subjects, no EGSA or brain damage was observed. The presence of motor convulsions without EGSA or brain damage was more apparent in groups given the lower doses of caramiphen. All subjects given a higher dose (100 mg/kg) displayed significantly reduced motor convulsions and complete prevention of EGSA and brain damage. In other work using doses of diazepam higher than that used here³¹, EGSA and motor convulsions were arrested simultaneously, suggesting that the threshold dose for arrest of

EGSA may be lower than that for arrest of motor convulsions.

In the absence of a strong relationship between forebrain EGSA brain damage and motor convulsions in these experiments, alternative neural regions may be considered as the critical locus for driving the motor aspects of convulsions. Burnham and Browning have hypothesized that the tonic-clonic convulsions culminating in tonic hind-limb extension observed in many animal models are driven by epileptic activity in the non-specific 'core' of the brainstem and spinal cord⁸. Their hypothesis is based, in part, on the similarity of responses to anticonvulsant drugs, results of lesion experiments, and on the common aspects of motor patterns seen among various models. The pattern of convulsive activity seen in the present experiment is similar to that identified by Burnham and Browning as common to models such as the maximal electroshock and maximal pentylenetetrazol.

Browning and colleagues have demonstrated that tonic extension and certain forms of clonus similar to that expressed by guinea pigs in this experiment require an intact brain stem, but not a functional forebrain. Rats with precollicular transections displayed tonic extension/flexion and running-bouncing clonus in response to pentylenetetrazol or minimal/maximal electroshock, just as sham-operated rats did³. The tonic components of convulsions induced by these two methods were attenuated by lesions in the pontine reticular formation^{4,5}. Convulsions similar to those seen in soman-exposed guinea pigs have been produced by electrical stimulation of the brainstem, yet without accompanying forebrain EGSA⁷. The genetically epilepsy prone rat (GEPR) offers another example of tonic convulsive activity without EGSA at the cortical surface. These rats do not exhibit EGSA during acoustically induced seizures until the seizures have been repeated²⁸. After a few daily repetitions, polyspikes appeared at the cortical surface of GEPR-9 rats during tonic limb extension. Such polyspike activity is not unlike that exhibited in soman-exposed guinea pigs as seen in Fig. 1 of this report. Thus, diazepam and scopolamine may have acted preferentially on the forebrain to block EGSA and to a lesser extent on the brainstem, a

probable locus of control over tonic clonic activity in the guinea pig.

With respect to the power spectral analysis results, a mutual antagonism occurred between soman and the two test compounds. Although scopolamine, diazepam and soman individually produced changes in PSE, a combination of soman plus one of the other two drugs resulted in normal or near-normal power levels. Scopolamine produced an expected increase in low frequency ECoG power²⁴ during the pretreatment period. The subsequent administration of soman not only failed to induce EGSA, but returned PSE to normal levels. Scopolamine may have exactly counteracted the muscarinic agonist effect of excess acetylcholine resulting from acetylcholinesterase inhibition by soman. Diazepam increased power in the higher frequencies^{12,29} (8-32 Hz) before soman, and like scopolamine, returned PSE to nearly baseline-like levels during the soman period for five of the seven subjects tested. The continued elevation of power in the 22-32-Hz band after soman, however, is attributable to the effect of soman, and not of diazepam. A similar pattern of increased high frequency activity has been found in guinea pigs with soman-induced seizures that have been arrested with MK-801³² or in humans and monkeys that have been exposed to other organophosphorus cholinesterase inhibitors and were not treated with a benzodiazepine⁶.

In conclusion, the motor aspect of soman-induced convulsive status epilepticus was not driven by the synchronous activity of neurons whose electrical manifestations were detectable at the cortex. Furthermore, brain damage was not an unavoidable consequence of soman-induced motor convulsions. The weak relationship between forebrain EGSA and convulsions in soman-poisoned guinea pigs is similar to the relationship between these measures in humans with certain types of epilepsy. An absence of EGSA recorded from the cortex during clinically manifested convulsions in humans with somato-motor or generalized secondary epilepsies is occasionally observed^{1,15}. In such instances, however, no specific pharmacological manipulations were undertaken to produce the apparent dissociation between electrical recordings and clinical manifestations. In soman-induced, convul-

sive status epilepticus, a pharmacological interference is apparently required to eliminate forebrain EGSA and yet permit motor convulsions. An intervention such as the one used here demonstrates a difference in the thresholds for pharmacological protection against EGSA (along with brain damage) and motor convulsions.

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