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Primate Performance Decrements Following Acute Soman Exposure: Failure of Chemical Countermeasures

DENNIS W. BLICK,*[1] MICHAEL R. MURPHY,† G. CARROLL BROWN* AND STANLEY L. HARTGRAVES*

Systems Research Laboratories Division of Arvin/Calspan and †Radiofrequency Radiation Division, Occupational and Environmental Health Directorate, USAF Armstrong Laboratory, Brooks AFB, Texas 78235-5000

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BLICK, D. W., M. R. MURPHY, G. C. BROWN AND S. L. HARTGRAVES. *Primate performance decrements following acute soman exposure: Failure of chemical countermeasures.* PHARMACOL BIOCHEM BEHAV **49**(3) 503–510, 1994.—Three experiments are reported: 1) a feasibility study on using laboratory primates repeatedly in behavioral toxicity studies of organophosphate (OP) agents or of chemical countermeasures against OPs; 2) a study of the efficacy of pyridostigmine pretreatment and 2-PAM therapy; and 3) a study to determine the effects of these treatments on soman-induced cholinesterase (ChE) inhibition and its recovery. In rhesus monkeys, three repeated acute low-dose (2.1 to 2.8 μg/kg) soman exposures, separated by intervals > 5 weeks, did not change baseline compensatory tracking performance or the soman ED_{50}. Atropine therapy (97 μg/kg) alone had no effect on soman ED_{50}. Addition of pyridostigmine pretreatment (150 μg/kg) and 2-PAM therapy (17 mg/kg) to atropine therapy increased the soman ED_{50} for a performance decrement from 2.27 μg/kg to 2.58 μg/kg, an insignificant protective effect. At the soman ED_{50} for behavioral decrements, pyridostigmine pretreatment increased the inhibition of serum ChE observed immediately after soman exposure, but reduced the extent of permanent inhibition. The 2-PAM therapy reduced serum ChE inhibition from about 80% to less than 70%. These effects on the time course of ChE inhibition following soman exposure appear to combine additively. These chemical countermeasures do not prevent soman-induced performance decrements, even though they are effective in protecting lives after much higher doses. The soman doses used produce only small, transient performance decrements; animals so exposed can, thus, be used repeatedly in such studies.

Nerve agent Soman Behavioral toxicity Protective drugs Pyridostigmine Rhesus macaque Organophosphate Cholinesterase

SOMAN (pinacolylmethylphosphonofluoridate), an organophosphate (OP) nerve agent, is among the most toxic substances known. Soman and other OP agents are extremely toxic because they inhibit (i.e., inactivate) acetylcholinesterase (AChE), an enzyme necessary for the normal functioning of acetylcholine (ACh) as a transmitter substance at synapses in the nervous system and at neuromuscular junctions. Agent-induced reductions in AChE activity result in an excess of ACh, which can overstimulate and eventually block both neural and neuromuscular transmission. "After a large amount of nerve agent . . ., the clinical effects are precipitate in onset and catastrophic in magnitude" [(33), p. 172]. Immediate prostration and loss of consciousness are quickly followed by seizure activity and (minutes later) by cessation of respiration. Lower doses produce a variety of cholinergic signs and symptoms, including miosis, muscle fasciculations and tremor, excessive secretions (from sweat and salivary glands, nasal mucosa, gastrointestinal, and upper respiratory tracts), abdominal cramps, vomiting, diarrhea, bronchoconstriction, etc. The OP nerve agents readily cross the blood–brain barrier, so sublethal doses can easily disturb brain function and produce behavioral deficits (28,31). Because of its extreme toxicity, research on the effects of soman has addressed primarily lethality and its prevention; relatively few studies have investigated behavioral toxicity.

[1] Requests for reprints should be addressed to Dennis W. Blick, Systems Research Laboratories, P.O. Box 35313, Brooks AFB, TX 78235.

Most countries have fielded protective drugs as countermeasures in case of nerve agent warfare: a) carbamates such as pyridostigmine (PYR), which, if taken prior to exposure, can prevent soman from irreversibly inhibiting AChE; b) oximes such as 2-PAM Chloride or HI-6, to reactivate acetylcholinesterase (AChE); c) anticholinergics such as atropine, to block postsynaptic acetylcholine (ACh) receptors; and d) anticonvulsant drugs such as diazepam. Adding an anticonvulsant to the standard therapy following debilitating exposures reduces seizures (25) and brain lesions (27), and hastens return of function in exposed animals (7,27). The carbamate component of chemical defense packages is thought to protect acetylcholinesterase (AChE) by binding reversibly with AChE, thus sequestering a portion of the AChE from irreversible inhibition by the OP agent (22), which circulates in the body only briefly after exposure. After detoxification of the OP, AChE is released from the carbamylated form in sufficient quantities to sustain vital functions. While combinations of pretreatments and therapies have proven to be effective in preventing death in primates after high-dose soman exposure (12,22,34), preliminary studies have indicated a failure to prevent performance decrements (10,11). Promising new approaches to protection include pretreatment with exogenous cholinesterases, which act as circulating soman scavengers (16,35).

The PEP task used in these studies has proven to be a reliable measure of performance and its disruption by drugs, as demonstrated by previous studies with peripherally and centrally active anticholinergic and anticholinesterase drugs (2,4,6,8-11,18), as well as anticonvulsants (3,7,27), and ethanol (5). Hartgraves and Murphy (21) have recently reviewed its use in low-dose soman studies in our laboratories. The experiments reported here were intended to initiate a series of studies on the effects of nerve agents and of countermeasures against these agents on performance in laboratory primates. A practical concern was whether the scarce and expensive primate subjects could be reused in these and other behavioral studies after acute, low-dose exposure to nerve agents. The first objective of the experiments was to test whether such exposures permanently changed the performance of the subjects or their sensitivity to further nerve agent exposures.

Another objective of these experiments was to determine whether PYR pretreatment, in conjunction with standard therapeutic measures, could reduce the likelihood that low-dose soman exposure would cause decrements in the performance of the PEP task. Atropine sulfate has long been a standard treatment for OP poisoning. Autoinjectors containing atropine have been issued to military personnel for use in the event that symptoms of OP poisoning develop after a suspected chemical attack. Therefore, for the purpose of the current model, we assumed that soman exposure would generally be followed by atropine therapy. For this reason, as well as to minimize potential health risks to our subjects, we included a standard atropine therapy after every soman injection in these experiments. The atropine dosage selected was our best estimate of the monkey equivalent to the dosage received by a 70-kg human from two atropine autoinjectors. The atropine content of two Combo-Pens®, 4 mg, would produce a dosage of 57 μg/kg in a 70-kg person. However, there is evidence that rhesus monkeys are less sensitive to atropine than are humans. Mattsson et al. (26) developed a set of functions for specifying atropine dosages approximately equivalent in their effects on performance in the two species. Their human-to-monkey translation function: $X_m = 4.77(X_h)^{1.36}$, where X_h is the human dose in mg/kg, yielded an atropine dosage of 97 μg/kg as the approximate monkey equivalent to a dosage of 57 μg/kg in man. This dosage was injected immediately after each soman injection.

In the studies reported here, we determined the ED_{50} (the dose that produces a decrement in half the animals tested) of soman required to produce a small but reliably detectable decrement in the performance of the PEP task. ED_{50} determinations were performed under two treatment conditions: a control (C) condition in which standard atropine therapy was given immediately after soman exposure, and an experimental (E) condition in which both PYR pretreatment (30 min before soman) and oxime therapy (2-PAM chloride immediately after soman) were added to the standard atropine therapy. The fielded PYR pretreatment is intended to produce inhibition of circulating cholinesterases (ChEs) in the range of 30–40%. We, therefore, selected a PYR dose (150 μg/kg) that produced ChE inhibition of 40% 30 min after it was injected; we timed our soman injections to coincide with this peak of ChE inhibition. The 2-PAM injection (17 mg/kg) given immediately after soman exposure corresponds to the dosage a 70 kg person would receive from two Combo-Pen® autoinjectors. For the soman dosages used in these experiments, the pretreatment and therapy conditions tested were an idealized model of the best pretreatment and therapy that military personnel exposed in battle could expect to receive. Thus, we were modeling the best outcome (in terms of behavioral toxicity) that could be expected under conditions of minimal OP exposure with optimal pretreatment and therapy using the fielded countermeasures for OP nerve agents. The ratio of ED_{50E} to ED_{50C} is a measure of the protection against performance decrements afforded by the addition of PYR pretreatment and 2-PAM therapy to standard atropine therapy. ED_{50C} determinations were performed repeatedly, with sufficient time (>5 weeks) between soman exposures to allow complete recovery of serum ChE. After we determined that PYR pretreatment and 2-PAM therapy afforded significant protection (see the Results section) and produced complex changes in the inhibition and recovery of serum cholinesterase (ChE) following soman exposure, we performed a further experiment to determine the separate effects of PYR and 2-PAM on ChE changes after soman exposure, and how those effects combine.

METHOD

Subjects

A total of 18 adult male rhesus monkeys (*Macaca mulatta*), ranging in weight from 7 to 11 kg, were used in these studies. All were well trained in performance of the PEP task prior to exposure to any drugs. Routine care of the subjects was provided by the Veterinary Sciences division of the USAF Armstrong Laboratory (AL/OEVR). Fourteen of the subjects (Ss) were used in both the repeated ED_{50} determinations and (randomly divided into two groups of seven) in the PYR/2-PAM efficacy study. Twelve of these Ss plus 4 additional PEP-trained but soman-naive Ss were used in the ChE follow-up study.

Measurement of PEP Performance

The PEP task has been described previously (8,10,18). It is a continuous compensatory tracking task that measures the ability of a monkey to compensate for unpredictable perturbations in pitch induced by a filtered random noise signal. The experimental S sits in a chair that rotates about the pitch axis. Pitch angle of the chair is measured by a linear potentiometer coupled to the rotating shaft. The potentiometer and associ-

ated A-to-D converter are calibrated by reading in potentiometer voltages at 5° intervals over a 40° range. The computer then fits a line to the sampled values using a least squares procedure to determine a factor for converting input voltage to chair position in degrees. Repeated calibrations produce values that vary from one another by less than 1%. Platform position (angle in degrees) is measured 10 times per second, and the standard deviation (σ) of all the scores for each 5-min epoch, i.e., 3000 data points, is the metric for PEP performance. In the absence of joystick input, the random external input produces a large variation in platform position (σ of ~12°, with the largest excursions near the platform's limits of motion: ±40°). Well-trained Ss reduce this variation to a σ of ~2.5-4.0°. Performance is motivated by electric shocks (0.10 s at 1-Hz repetition rate, 5 mA maximum current) delivered to the tail whenever the chair platform deviates from the horizontal by more than 15°. For each S, tail-shock intensity is adjusted to the minimum level required to maintain motivated performance in baseline tests (well-trained Ss receive very few tail shocks, <1 shock/h on average). Ss perform this task for 120 min, with drug injections occurring after the first 30 min of testing.

Drugs and Injections

Soman at ≥98% purity was obtained from the US Army Medical Research and Development Command, Aberdeen Proving Ground, MD, at a concentration of 2 mg/ml. This solution was then diluted to a concentration of 20 μg/ml with normal saline and stored in single-dose vials in a Forma freezer at −70°C. Individual vials were removed from the freezer and thawed immediately before injection. Pyridostigmine bromide (Mestinon® Injection) was obtained from the Roche Laboratories division of Hoffmann-LaRoche Inc., Nutley, NJ. Subjects in the experimental group received PYR (150 μg/kg IM) injected 30 min prior to soman so as to produce approximately 40% inhibition of serum ChE at the time of soman injection, a condition determined by others (12,20,23) to produce nearly optimal protection from soman-induced toxicity without producing undesirable side effects in the absence of soman exposure. Atropine sulfate and 2-PAM chloride in the formulations used in the Combo-Pen® Autoinjector issued to US military personnel under threat of chemical attack were donated by Survival Technology, Inc., Bethesda, MD. Atropine (at a dosage equivalent to the contents of two Combo-Pens injected in a 70 kg human) was injected intramuscularly immediately after soman in all subjects. The subjects pretreated with PYR also received 2-PAM Chloride at the same dosage per kg (17 mg/kg) as a 70 kg person would receive from two Combo-Pens, injected intramuscularly immediately after soman.

Criterion for Drug-Induced Performance Decrements

Baseline tests were used to determine the range of normal performance for each S by the method of simultaneous tolerance limits described by Lieberman and Miller (24). Briefly summarized, this method consists of fitting a line to the baseline performance by the method of least squares. Simultaneous tolerance limits about this line ($p = 0.95$, $\alpha = 0.05$) are based on the residual variation about the fitted line. Our criterion for a performance decrement was met whenever at least two of the data points following drug injection exceeded the upper tolerance limits derived from the preceding baseline run for the same S. Examples of a baseline run, the calculated upper tolerance limits, and a soman test run in which a performance decrement occurred are shown in Fig. 1.

Determination of Median Effective Dose

To minimize the number of exposures required to estimate the ED_{50}, we adopted the up-and-down method described by Dixon and Massey (15). This method concentrates measurements in the dosage range of interest by using the response of each S to determine the dosage for the next S. Prior to the experiment, an initial dosage (2.78 μg/kg) and a logarithmic dosage step size (0.05 $\log_{10}$ units) were selected. After each soman test run, the S's performance was compared to the criterion for a soman-induced performance decrement. If the criterion was met, the next S received a dosage one step lower; if not, the dosage for the next S was one step higher. If the initial choices of dosage and step size were appropriate, i.e., if the initial dosage was within a few steps of the ED_{50}, and the step size approximated the standard deviation of the underlying distribution of effects, this up-and-down method could yield an adequate estimate of ED_{50} with as few as 6-10 tests (15). Testing the appropriateness of the selected initial dosage and step size was one of the objectives of this study. For the second and third ED_{50} determinations, adjustments in these parameters were made (see the Results section).

We hoped to base each of the three repeated ED_{50} determinations on performance results from at least 10 monkeys. Because of uncertainties regarding continued availability of all Ss for testing over an extended period, we began with 14 Ss. Each of the 14 monkeys received a soman dose within the dose range determined by the up-and-down method during each of the three ED_{50} testing series.

We have reported (8) that the recovery of serum ChE following soman exposures in the dose range used in these experiments is extremely rapid during the first week after exposure, followed by a slower phase that is completed within 5 weeks. For the current experiments, we chose a minimum time between soman exposures of 6 weeks. In some cases, 7 or 8 weeks elapsed between doses. The mean time between exposures for the second ED_{50} determination was 42.8 days. The mean time between the second and third exposures was 52.4

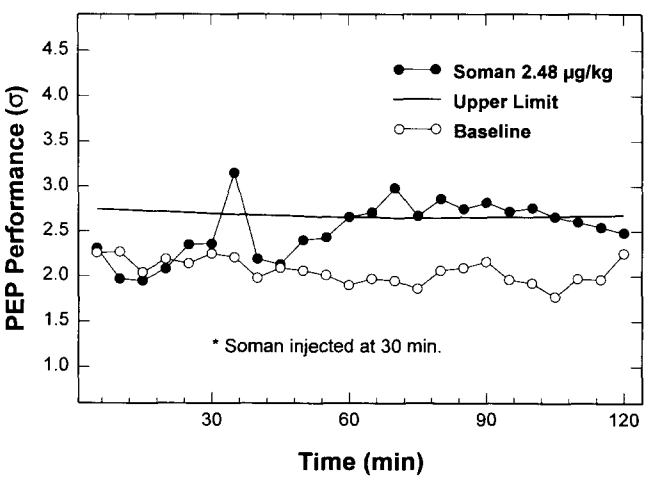

FIG. 1. Example of PEP performance measurement showing baseline performance, upper tolerance limits for normal performance, and a test in which soman (2.48 μg/kg) induced a performance decrement at about 60 min, when two measured points exceeded the upper limit.

days. After these three exposures showed that no carry-over effects were apparent, and that as few as 7 Ss could provide a stable ED_{50} estimate, the 14 subjects were divided randomly into two groups for the efficacy test of PYR pretreatment and 2-PAM therapy. When the ChE results from the efficacy study indicated the need for a factorially designed study of these effects, 12 of the original Ss were randomly assigned to the four groups, and four additional (new) Ss were added (one in each group) to bring the number per group up to four.

Blood Sampling and ChE Assay

Venous blood samples (about 1 ml) were drawn from a convenient leg vein and placed in chilled sample tubes in an ice-water bath. Within 10 min after the blood samples were drawn, they were centrifuged for 5 min at 5000 rpm in a refrigerated (4°C) centrifuge. Serum samples (20 μl) were then assayed for ChE activity, using a modification of the colorimetric method of Ellman et al. (17). Percent inhibition of ChE activity relative to baseline was calculated for each S and dose based on the mean of six assays of each sample. In the efficacy study, blood samples were drawn at −0.6, 1.5, 24, and 168 h after soman exposure. In the factorial study of PYR and 2-PAM effects on ChE recovery following a fixed dose of soman (2.58 μg/kg), samples were drawn at the following times relative to soman injection: −0.6 (baseline), −0.1 (pre), 0.75, 1.5, 3.0, 6.0, 24, and 168 h. The postsoman percent inhibition scores were analyzed by analysis of variance (ANOVA) in a 2 × 2 × 6 repeated measures design.

RESULTS

Repeated Soman Exposure

Figure 2 shows the up-and-down test sequence results for three consecutive ED_{50} determinations conducted at 6–8 week intervals. The first series, using a dosage step size of 0.05 $\log_{10}$ units, produced an ED_{50} estimate of 2.28 μg/kg, with a 95% confidence interval (CI) of 2.10 to 2.58 μg/kg. This ED_{50} estimate did not differ significantly from a previous study in a group of animals that did not receive atropine after soman [ED_{50} of 2.50 μg/kg with a 95% CI of 2.36 to 2.64 (8)], indicating that atropine treatment did not significantly reduce soman-induced PEP performance decrements. The combined results of these two up-and-down test sequences (with and without atropine therapy) indicated that our initial choice of dosage step size (0.05 $\log_{10}$ units) might be too large. The up-and-down method is maximally efficient when the step size approximates the standard deviation of the underlying distribution (assumed normal) of effect probability as a function of the logarithm of dose. With a dosage step size of 0.05 $\log_{10}$ units, only four doses appeared to be necessary to span the distribution:

Dosage (μg/kg):	1.97	2.21	2.48	2.78
f(PD*)/n:	0/1	2/5	4/7	5/5

* PD is the occurrence of a test meeting the performance decrement criterion.

With a step size equal to the σ, one would expect to take five to six steps to span a normal distribution, so we reduced the step size to 0.0333 $\log_{10}$ units for the second and third up-and-down test series. The second series produced an ED_{50} of 2.12 μg/kg with a 95% CI of 2.04 to 2.22. The third series produced an ED_{50} of 2.11 μg/kg with a 95% CI of 1.98 to 2.24. As shown in Fig. 2, the change in dosage step size reduced the extent of the 95% CI, thus increasing the sensitivity of the up-and-down procedure for detecting changes in the ED_{50}. Because the dosage step size enters into the estimation of both the σ of the underlying distribution of dosage thresholds and the standard error of the ED_{50} estimate, selecting too small a step would tend to bias conclusions based on up-and-down test series. Dixon (13) has stated that a step size too large tends to produce long series of alternating outcomes, while a step size too small tends to produce long runs of similar outcomes. With the right step size, there are usually two or three dosages at which some subjects respond and some do not. Because the smaller step size gave every indication of being in the right range [if anything, perhaps still too large, since estimates of σ calculated from the second and third test series by the methods of Dixon (13,14) were 0.0232 and 0.0286, respectively], it is an improvement in the method to use the smaller step size. The 95% CI ($ED_{50} \pm 1.96$ times the standard error of estimate) for the control condition then provides the simplest and most direct test for whether an ED_{50} from a treatment condition differs (with $\alpha < 0.05$) from the control ED_{50}.

Comparing the three repeated ED_{50} estimates in Fig. 2, although the first series produced the largest value, the 95% CI associated with this estimate includes the ED_{50}s derived from the second and third series, so the three values do not differ at the 0.05 level of significance. The second and third series produced very similar ED_{50} estimates. Thus, we found no evidence of a change in the soman dose required to produce a PEP performance decrement with repeated acute soman exposures separated by at least 6 weeks.

We also compared the baseline performances measured prior to any soman exposure to baseline performances before the second and third doses and 6 weeks after the third dose. Both the level and the variability of baseline performance remained constant. Low-level acute soman exposure, repeated every 6–8 weeks, did not significantly affect later baseline (nondrugged) performance. This comparison is shown in the last panel of Fig. 2.

Effects of Protective Drugs

Figure 3 shows the results of the two up-and-down testing series used in this experiment. The estimated soman ED_{50} to produce minimal but reliably detectable PEP performance decrements in the control Ss was 2.27 μg/kg (95% CI = 2.19–2.35 μg/kg). The addition of pretreatment with PYR and 2-PAM therapy to the control atropine therapy resulted in a soman ED_{50} of 2.58 μg/kg (95% CI = 2.26–2.95 μg/kg). Because the ED_{50} for the experimental group falls outside the 95% CI of the control group, this difference is statistically significant ($p < 0.05$). The ratio of the two soman ED_{50}s is 1.14. This protection ratio indicates that the addition of PYR pretreatment and 2-PAM therapy to atropine therapy permits Ss to be exposed to a soman dose 14% greater before they meet the same criterion for a performance decrement as Ss given atropine therapy alone. This degree of performance protection is small relative to the protection against the lethal effects of soman (LD_{50} protection ratio = 28) reported by others (12). The extent of protection also appears to vary substantially from subject to subject (see the Discussion section).

Figure 4 shows the average inhibition of serum ChE activity for the two groups of Ss 90 min, 1 day, and 1 week after

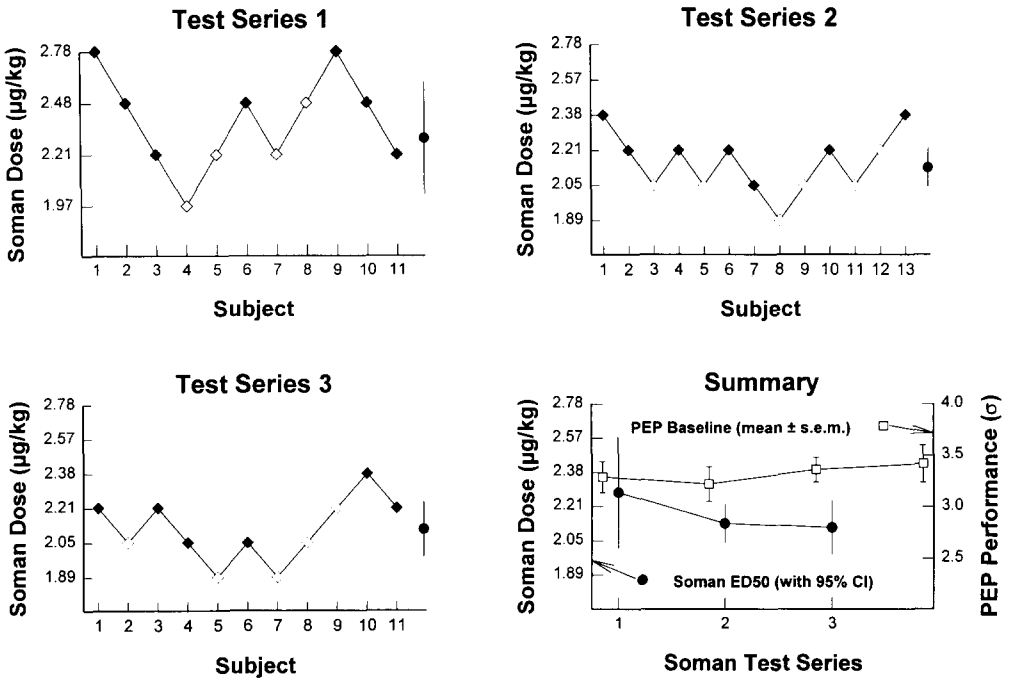

FIG. 2. Repeated measurements of soman ED_{50} for the production of minimal performance decrements in well-trained rhesus monkeys performing the Primate Equilibrium Platform (PEP) task. In each of the three test series, filled symbols indicate a test that met the criterion for a performance decrement; open symbols indicate that the criterion was not met. The size of the dosage step was changed between series 1 and the two succeeding series (see text). ED_{50} and its 95% confidence interval (CI = ED_{50} ± 1.96 times standard error) is indicated at the right end of each series, and in the summary graph (lower right). The summary graph also indicates the mean level and variability of baseline performance sampled during the week preceding each soman exposure and 6 weeks after the third exposure. Neither soman ED_{50} nor baseline performance changed significantly over the course of three low-dose soman exposures in the same subjects.

soman exposure. The inhibition levels observed were very similar at 90 min, but the experimental group showed significantly lower levels of inhibition (greater recovery of ChE activity) both 1 day and 1 week later. Although the up-and-down method assured that the average soman dose for the two groups was equated in terms of performance effects, it should be noted that the treated group that showed less ChE inhibition was actually exposed to a higher average dose (geometric mean = 2.437 µg/kg for the experimental group and 2.233 µg/kg for the control group). Because the effects of PYR pretreatment and 2-PAM therapy were completely confounded in these observations, an additional study was needed to measure the effects of each individual drug, as well as the combination. This was accomplished in a 2 × 2 factorial de-

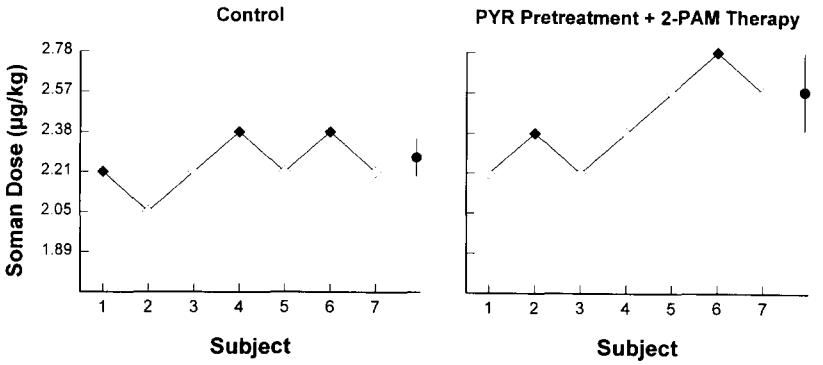

FIG. 3. Soman ED_{50} test series under control conditions (left) and with PYR pretreatment and 2-PAM therapy (right). Control ED_{50} was 2.27 µg/kg (CI: 2.19–2.35); protected ED_{50} was 2.58 µg/kg (CI: 2.26–2.95). $ED_{50E} \div ED_{50C}$ = 1.14, a statistically significant but practically insignificant protection factor.

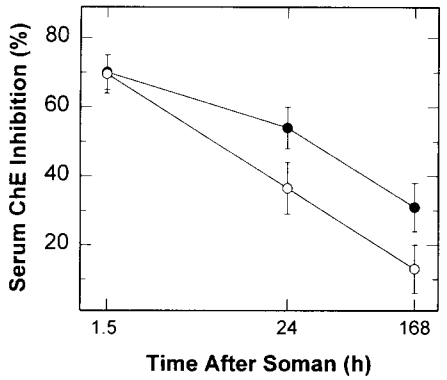

FIG. 4. Serum ChE inhibition and recovery following the soman exposures of the groups shown in Fig. 3. Filled symbols are for control Ss (n = 7), open symbols for PYR + 2-PAM treated Ss. Error bars indicate ± 1 SEM.

sign, with four subjects per group. The conditions of the previous experiment were replicated as nearly as possible, except that all Ss received the same soman dose (2.58 μg/kg, the ED_{50} for performance decrements in the protected animals), and performance testing was interrupted briefly to draw an additional blood sample 45 min after soman injection.

Serum ChE Effects of PYR, 2-PAM, and the PYR/2-PAM Combination

Figure 5 shows the time course of serum inhibition. For the effects of PYR pretreatment (Fig. 5A), the ANOVA showed that the PYR × time interaction was significant ($p < 0.0001$), indicating that PYR changes the time course of ChE recovery after soman exposure. Because PYR pretreatment produced higher levels of inhibition early (≥ 6 h) and lower levels later, the ANOVA result for the PYR main effect (averaged over time) was nonsignificant ($p > 0.5$).

The effects of 2-PAM therapy on serum ChE after soman exposure (Fig. 5B) differed in form from those of PYR pretreatment. The 2-PAM produced a significant ($p < 0.05$) reduction in the level of ChE inhibition. The mean reduction in serum ChE inhibition produced by 2-PAM therapy was 11%. This reduction did not vary significantly with time after soman exposure; the 2-PAM × time interaction in the ANOVA was nonsignificant ($p > 0.80$). This result indicates that essentially all of the 2-PAM effect of reversing ChE inhibition occurs within the first 45 min following nearly simultaneous soman exposure and 2-PAM therapy. Figure 4C shows the time course of serum ChE activity for the four treatment groups. The two treatments appear to act independently on serum ChE activity after soman exposure. The ANOVA showed that the PYR × 2-PAM interaction was nonsignificant ($p > 0.95$), as was the PYR × 2-PAM × time interaction depicted in Fig. 5C ($p > 0.99$). These results are consistent with the hypothesis that the effects of PYR and 2-PAM combine in a strictly additive fashion.

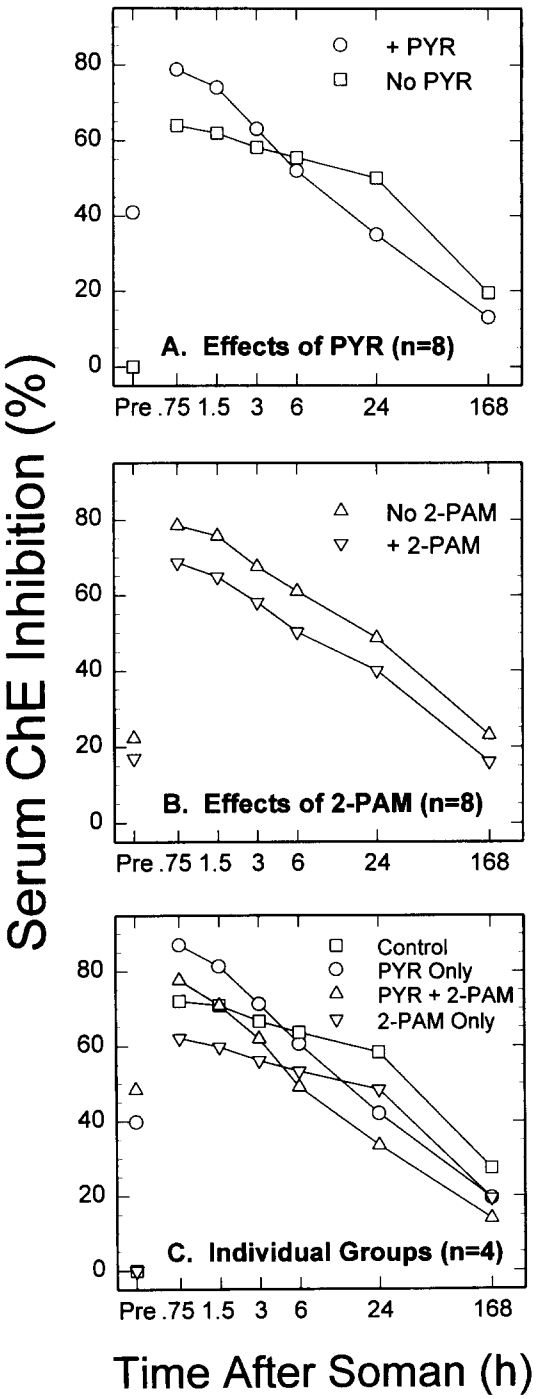

FIG. 5. Effects of PYR, 2-PAM, and both in combination on serum ChE recovery following an exposure to 2.58 μg/kg of soman. (A) PYR changes the time course of recovery, as indicated by the significant PYR × time interaction ($p < 0.0001$) illustrated here. (B) 2-PAM reduces inhibition by a constant amount throughout recovery, as indicated by a significant ($p < 0.05$) 2-PAM main effect and the nonsignificant ($p > 0.80$) 2-PAM × time interaction shown. (C) The nonsignificant ($p > 0.99$) PYR × 2-PAM × time interaction shown in this graph indicates that the main effects shown above combine in an additive fashion. This is also indicated by the nonsignificant ($p > 0.95$) PYR × 2-PAM interaction (not shown).

DISCUSSION

The primate equilibrium platform has proven to be a sensitive task for detecting low-dose soman-induced performance decrements (21). PEP performance is extremely stable over

long periods of time. Many investigators have reported extremely steep dose–response curves for behavioral, physiological, and lethal effects of soman. In agreement with prior work in rodents (29), the soman dose–effect function was extremely steep, indicating that very small changes in dose produce large changes in performance effects. However, in contrast to earlier findings in other species, including baboons (19), we were able to detect reliable changes in performance at doses much less than the estimated LD_{50} (approx. 35% of the LD_{50}), in the absence of any gross signs or symptoms of toxicity. Other investigators have generally found that doses large enough to produce reliable performance effects also produce toxic signs in some of their Ss, and that the ED_{50} for performance effects is closer to (i.e., greater than one-half of) the LD_{50} (19,22, 29,30). Our findings suggest either that the PEP performance task and associated criteria for performance decrements are more sensitive to soman effects than the tasks and criteria used by others, or that there are substantial species differences in the susceptibility of performance relative to the development of life-threatening symptoms. Furthermore, these results demonstrate that future efficacy studies like those reported here can be conducted using relatively small numbers (7–10 per condition) of well-trained primates, and that the use of these scarce and valuable animals can be minimized by their repeated use in such studies.

The dosage spacing used in these experiments (0.033 and 0.05 $\log_{10}$ units) were much smaller than those commonly used in toxicity studies. The sort of dosage step size more typically used in toxicology experiments (e.g., 0.30 $\log_{10}$ units) could easily lead to the erroneous conclusion that soman's performance effects follow an all-or-none law, because performance effects change from undetectable to complete debilitation with a change of less than 0.30 $\log_{10}$ units on the dosage scale. Unusually steep dose–response functions require unusually small dosage increments (e.g., the 0.033 $\log_{10}$ unit steps we used in the efficacy study). Although our results from several up-and-down determinations of the soman ED_{50} in untreated animals and in animals given atropine therapy after exposure are consistent with very low variability in the distribution of thresholds for soman-induced performance decrements ($\sigma \leq$ 0.033 $\log_{10}$ units), the results from this and other studies (10) suggest that the presence of PYR or of PYR-inhibited ChE may increase this variability. The up-and-down series for the PYR pretreated subjects in the current study, as well as in subjects chronically infused with PYR prior to and during daily repeated low-dose soman exposures (10) yield estimates of σ 2–3 times larger than the comparable controls not treated with PYR. The up-and-down data from both acute and chronic studies of PYR-soman interactions are consistent with the interpretation that some subjects receive little or no benefit from PYR pretreatment, while other subjects have their soman thresholds for performance decrements increased by approximately 0.06 to 0.15 $\log_{10}$ units (i.e., about 15 to 40%). We do not know if similar increases in variability occur when PYR pretreatment is used to increase the threshold for lethality following high-dose OP exposure.

Carbamate pretreatment and oxime/anticholinergic therapies provide significant protection against the lethal effects of soman in animals (12,20,22,23,32,34). However, our results suggest that even optimal use of the pretreatment and therapeutic drugs recommended for treatment of OP poisoning can do little to prevent transient behavioral decrements after OP exposure.

The minute extent to which fielded chemical countermeasures afford protection against performance decrements has implications for the mechanisms underlying these soman effects. Soman enters the central nervous system (CNS) readily, while PYR and 2-PAM do not. Atropine enters the CNS, but at a much slower rate of entry compared to soman. The performance decrements that we observe at low soman doses are likely to arise from CNS effects, because they occur at doses that do not evoke overt peripheral signs (miosis, salivation, muscle fasciculations) indicative of intoxication severe enough to interfere with the strictly motor or sensory aspects of the task. Conversely, the fact that chemical countermeasures produce much greater protection against soman-induced death seems to imply that some peripheral mechanisms are involved in producing the fatalities associated with soman exposure at these higher doses. In the blood, the combination of pretreatment with PYR and immediate 2-PAM therapy following soman exposure significantly reduces the fraction of serum ChE that is irreversibly inhibited by sublethal soman doses; this is especially noticeable at 24 h after soman exposure. These effects on ChE outside the CNS are presumably related to the protection against soman-induced mortality reported by others (12,20,22,23,32,35).

The studies reported here suggest that protection against OP performance disruption would require either administration of CNS-active countermeasures (e.g., physostigmine) before exposure, or the use of countermeasures to destroy or detoxify the OP in the blood before it enters into the CNS. A third possibility, larger postexposure doses of therapeutic anticholinergics (or alternatively, more potent anticholinergics) is less promising, because previously absorbed OP agents act so rapidly. Preliminary studies in our laboratory showed that higher postexposure atropine doses produced delayed atropined-induced performance decrements without affecting the earlier, OP-induced decrements. The use of centally active pretreatments is problematic, because antidotes to OP poisoning may be addictive, or cause behavioral deficits by themselves in the absence of soman exposure. The use of circulating OP scavengers holds more promise. In a recent study using fetal bovine serum AChE or horse serum butyrylcholinesterase, monkeys were able to tolerate 4 to 5 times the LD_{50} dose of soman (up to 32 μg/kg administered over a 2 h period) and still perform the PEP task (35). This level of protection represents a protection ratio of at least 14, compared to the 1.14 reported here. Although this approach is encouraging, potential problems with the immune system in humans must be considered.

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