Pharmacology Biochemistry and Behavior, Vol. 45, pp. 677-683, 1993

0091-3057/93 $6.00 + .00

Behavioral Toxicity of Anticholinesterases in Primates: Chronic Physostigmine and Soman Interactions

DENNIS W. BLICK,*[1] STEPHANIE A. MILLER,† G. CARROLL BROWN* AND MICHAEL R. MURPHY†

Systems Research Laboratories Division of Arvin/Calspan and †Directed Energy Division, USAF Armstrong Laboratory, P.O. Box 35313, Brooks AFB, TX 78235-5000

Received 29 July 1992

BLICK, D. W., S. A. MILLER, G. C. BROWN AND M. R. MURPHY. *Behavioral toxicity of anticholinesterases in primates: Chronic physostigmine and soman interactions.* PHARMACOL BIOCHEM BEHAV **45**(3) 677-683, 1993.—Dose rates for continuous infusion of physostigmine salicylate required to inhibit 30 and 60% of normal serum cholinesterase activity in rhesus monkeys were determined. The effects of continuous physostigmine infusion at these dose rates on the behavioral toxicity of daily repeated low-dose soman were determined not to be deleterious; in fact, they were slightly (and variably) protective.

Organophosphates Carbamates Anticholinesterases Macaque Tracking performance

PHYSOSTIGMINE (PHY), the first known anticholinesterase (anti-ChE) (32), is a tertiary carbamate that has been considered for inclusion in a pretreatment regimen that could greatly reduce the toxicity of exposure to organophosphate (OP) nerve agents such as soman (pinacolyl methylphosphonofluoridate). Koster (25) first observed that PHY could protect against the toxic effects of OPs in cats in 1946. Because PHY readily crosses the blood-brain barrier, it might be much more efficacious against OP-induced performance decrements than pyridostigmine Br, a quaternary carbamate that has already been fielded as part of the military chemical defense package. While the fielded chemical defense package has substantial efficacy against the lethal effects of OP agents, its efficacy against OP-induced performance decrements is so small as to be considered operationally insignificant both when the soman exposure is acute (7) and when it occurs in low dosages repeated daily for up to 5 days (6).

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 (26,29,32), 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. Because it penetrates the CNS, PHY might protect central as well as peripheral AChE and thus prevent or reduce the OP-induced performance decrements that have been observed in pyridostigmine-protected animals.

Numerous studies in mice (4), rats (4,10,19), rabbits (4), and guinea pigs (26,27) demonstrated that PHY is more efficacious than pyridostigmine in protecting against OP-induced lethality. Studies in rats (17,18), guinea pigs (26), and monkeys (33) have also shown that PHY is more efficacious than pyridostigmine in reducing the severity and/or duration of behavioral incapacitation that follows near-lethal acute OP exposures.

For studies of the effects of sublethal acute or chronic exposure on complex behavior, primates, including marmosets and macaques, clearly provide better models than rodents. With regard to the kinetics of PHY in blood, the rhesus macaque (*Macaca mulatta*) appears to be a much better model of the human than either the marmoset or the guinea pig (34). The latter two species have long been considered better models of OP effects in primates than rats, rabbits, or mice (26).

Sustained military operations in environments heavily contaminated with nerve agents entail a risk of repeated, low-dose exposure of personnel, for example, during donning/doffing of protective apparel or through small leaks in protective suits or masks. We previously showed that repeated daily soman exposure (for up to 5 days) produced performance decrements in monkeys only after the cumulative dose exceeded twice the acute dosage that produced similar decrements in performance

[1] To whom requests for reprints should be addressed.

(9). These results were obtained in the absence of any pretreatment or therapy. With chronic infusion of pyridostigmine Br (0.52 or 1.35 mg/kg/day, sufficient to inhibit 30 or 60% of serum ChE, respectively), the daily soman dose required to produce a criterion deficit in performance of the Primate Equilibrium Performance (PEP) task in 50% of subjects increased by 41 or 24%, respectively. While these levels of protection were statistically reliable, they were too small to be considered operationally significant.

The present experiment tested whether PHY, which readily penetrates to the CNS, might provide significantly greater protection than that previously shown for pyridostigmine Br against the behavioral toxicity of repeated, low-dose soman exposure. Thus, we replicated the previous experiment (6) using chronic PHY in the place of chronic pyridostigmine.

GENERAL METHOD

BEHAVIORAL TESTING

The PEP task (6,14) is a continuous compensatory tracking task. The monkey is seated in a chair that rotates about his center of mass. Rotation about the pitch axis is driven by a low-pass-filtered random noise signal. The monkey's task is to manipulate a joystick control to compensate for the unpredictable perturbations in pitch induced by the noise signal. Performance is motivated by a mild electric shock delivered to the subject's tail whenever the chair platform position deviates from the horizontal by more than 15°. The variability of platform position indicates the quality of the performance. The SD of platform position is the performance measure. The random noise input, in the absence of joystick input, produces large variations in platform position (SD of 12–15°). A well-trained subject typically reduces this variation to about 2–4° by the joystick manipulations, thus receiving almost no tail-shocks. Platform position is sampled by computer at a rate of 10 Hz; SD is computed and stored every 5 min. Subjects (Ss) performed the PEP task for 2 h on each testing day.

SUBJECTS

Ss were adult, male rhesus monkeys (*M. mulatta*), ranging in weight from 4.5–8 kg. All Ss had been performing the PEP task on a regular basis (at least weekly) for a minimum of 1 year. None of the Ss had a prior history of exposure to PHY. All Ss had participated in the previous repeated low-dose soman study of the efficacy of chronic pyridostigmine 4–6 mo earlier. Routine care of animals was provided by the Armstrong Laboratory Veterinary Resources Branch (AL/OEVR).

CRITERION FOR PERFORMANCE DECREMENT

To provide a criterion for a soman-induced performance decrement, baseline runs were used to define the range of "normal" performance by the Lieberman and Miller method of simultaneous tolerance limits (28). This method consists of fitting a line to five previous baseline performances by the method of least squares. Simultaneous tolerance limits (p = 0.99, α = 0.01) around this line are based upon the residual variation about the fitted line. Within a soman test session, our criterion for a performance decrement was met whenever at least two of the data points collected after drug injection exceeded the upper tolerance limits derived from baseline runs for the same S. The five baseline tests were performed during the week preceding soman exposure.

DETERMINATION OF MEDIAN EFFECTIVE DOSE OF SOMAN

The up-and-down method (11) was used to minimize the number of soman exposures required for a reliable estimate of the median effective dose (ED_{50}). 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. An initial dosage and a logarithmic dosage step size are selected before the experiment. After each soman test session, the S's performance is compared to the criterion for a soman-induced performance decrement. If the S's performance meets the criterion, the next S receives a dosage one step lower; if not, the dosage for the next S is one step higher. If the initial choices of dosage and step size are appropriate (i.e., if the initial dosage is within a few steps of the ED_{50} and the step size approximates the SD of the underlying distribution of effects), this up-and-down method would yield an adequate estimate of ED_{50} with as few as 6–10 tests. The initial dose in this experiment was 1.26 μg/kg/day, the dosage from the prior pyridostigmine–soman interaction study nearest to the collective ED_{50} for all Ss in that study (6). Dosage step size was 0.05 $\log_{10}$ units.

CHRONIC PHY INFUSION

Alzet osmotic pumps (Alza Corp., Palo Alto, CA, Model 2ML1, 10 μl/h, 7 days) were implanted SC to provide constant-rate infusion of PHY in both experiments. After a surgical level of anesthesia was achieved with a short-acting barbiturate, a small (6–8 mm) skin incision was made under aseptic conditions near the dorsal midline, between the scapulae. Blunt dissection was used to open an SC pocket to accommodate the pump. The pumps were soaked in sterile saline at 37°C for 5 min prior to insertion to prevent a thermally induced bolus release of PHY (23). After pump insertion, the incision was closed with interrupted sutures.

CHOLINESTERASE ASSAYS

Venous blood samples (about 2 ml) were drawn from a convenient leg vein. A modification of the colorimetric method of Ellman et al. (13) was used to measure serum ChE activity.

EXPERIMENT 1: EFFECTS OF CONSTANT-RATE PHY INFUSION ON SERUM CHE ACTIVITY

EXPERIMENTAL DESIGN

Eighteen PEP-trained male rhesus monkeys (4.5–8 kg) were assigned randomly to three groups of six. Ss in these three groups were implanted with osmotic pumps that delivered PHY at rates of 75, 150, or 300 μg/kg/day. We expected that this range of delivery rates would produce chronic inhibition of serum ChE activity ranging from about 20 to about 60%.

PROCEDURES

Six animals (two from each dose rate group) were implanted on each of three successive Fridays. Several baseline venous blood samples were drawn during the week preceding implantation. Additional blood samples were taken daily on days 3–7 after implantation. PEP performance was tested on days 3, 5, and 7 after implantation.

RESULTS

None of the Ss in Experiment I met our criterion for a PEP performance decrement during chronic PHY infusion;

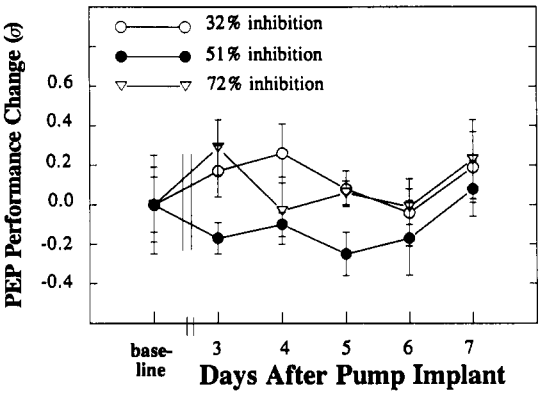

FIG. 1. Day-to-day variation in mean Primate Equilibrium Performance (PEP) performance during continuous physostigmine (PHY) infusion at three dose rates: 0.075 (32% inhibition), 0.150 (51% inhibition), and 0.300 μg/kg/day. No dose-related changes were found.

Fig. 1 shows that changes in performance from days 3-7 following pump implantation were small and variable. The changes were not systematically related either to PHY dose rate or to time after pump implantation.

Postimplantation ChE activity measures for serum were converted to % inhibition (%I) scores for each S. A repeated-measures analysis of variance (ANOVA) was performed, with dose rate (between Ss), postimplantation time (within Ss), and Ss (nested within dose rates) as factors.

The three dose rates produced highly significant ($p < 0.0001$) variation in serum ChE inhibition, as shown in Fig. 2. The relationship between %I and log(dose rate) was linear ($r > 0.99$), so the regression line for this relationship was used to determine dose rates of 71 and 200 μg/kg/day for use in Experiment 2 to produce serum ChE inhibitions of 30 and 60%, respectively.

EXPERIMENT 2: EFFECTS OF CHRONIC PHY-INDUCED CHE INHIBITION ON DAILY REPEATED SOMAN ED_{50} FOR PEP PERFORMANCE DECREMENTS

EXPERIMENTAL DESIGN

The up-and-down method (11) was used to estimate the soman ED_{50} for PEP performance decrements induced on or before the fifth day of exposure to a fixed daily dose of soman under each of three PHY infusion conditions: a) control, implanted with pumps containing only vehicle [70% glacial acetic acid (1 : 2,000) in water, 20% propylene glycol, and 10% absolute ethanol]; b) PHY30, implanted with pumps containing sufficient PHY (71 μg/kg/day) to inhibit 30% of serum ChE activity; or c) PHY60, implanted with pumps containing sufficient PHY (200 μg/kg/day) to inhibit 60% of serum ChE activity.

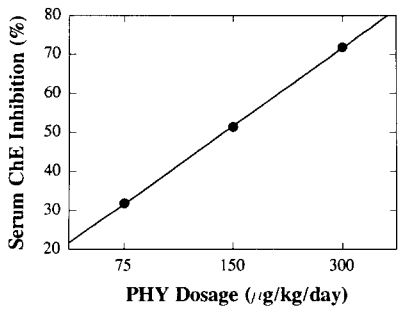

FIG. 2. Inhibition of serum cholinesterase (ChE) activity produced by continuous physostigmine (PHY) infusion at varying dose rates. Each point is the mean of 30 observations (6 animals × 5 days).

Each of the three groups included six Ss from Experiment 1 (two from each group) plus two additional randomly selected PEP-trained animals. Within each group of eight animals, order of testing was randomly determined. To avoid possible carryover effects of chronic ChE inhibition, pumps were not implanted in Experiment 2 until at least 6 weeks after pumps from Experiment 1 were removed. We previously demonstrated that no carryover effects occur between acute, low-dose soman injections spaced at 6-week intervals (8). Because these soman injections produced larger and longer-lasting depressions of ChE activity than the PHY exposures in Experiment 1, carryover effects in Experiment 2 were unlikely to occur. However, as an additional precaution the groups in Experiment 2 were balanced with respect to the PHY dose rates received in Experiment 1.

Pumps were implanted in one member of each PHY group on Fridays. Daily soman exposures began the following Monday and continued through Friday. Venous blood samples (2 ml) were taken 30 min before and 90 min after each soman dose. The first S in each group received a soman dosage of 1.26 μg/kg/day, the dosage from the prior pyridostigmine–soman interaction study nearest to the collective ED_{50} for all Ss in that study (6). The ratio step between soman dosages was 0.05 $\log_{10}$ units, or 12.20%. According to the up-and-down method, the dosage for each S after the first in a group was one step below or above the dosage received by the preceding animal, depending upon whether the preceding animal did or did not meet the criterion for a performance decrement.

RESULTS

The outcomes of the three up-and-down testing sequences are shown in Fig. 3. When the pump contained only vehicle (PHY0), the daily soman dose estimated to produce a performance decrement by the fifth daily exposure in 50% of subjects (ED_{50}) was 1.22 μg/kg/day [95% confidence interval (CI): $1.076 \leq ED_{50} \leq 1.383$ μg/kg/day]. With continuous PHY infusion at a rate of 71 μg/kg/day (PHY30), the daily soman ED_{50} was 1.317 μg/kg/day (95% CI: $1.162 \leq ED_{50} \leq 1.493$ μg/kg/day). The PHY30 condition did not produce a significant change in ED_{50}. With continuous PHY infusion at a rate of 200 μg/kg/day (PHY60), the daily soman ED_{50} was 1.610 μg/kg/day (95% CI: $1.453 \leq ED_{50} \leq 1.784$ μg/kg/day). The ED_{50} for the PHY60 condition was greater than the upper bound of the confidence interval for the control condition; thus, the difference between this condition and control is significant ($p < 0.05$). By the same criterion, the ED_{50} for the PHY60 condition was also significantly ($p < 0.05$) greater than that for the PHY30 condition. Therefore, chronic PHY infusion at this dose rate confers statistically significant ($p < 0.05$) protection against the deleterious effects on PEP performance of daily exposure to low-dose soman. The extent of this protection is, however, small and variable. The protective ratios, that is, the ratios of treated ED_{50} to untreated ED_{50}, were only 1.08 for PHY30 (n.s.) and 1.24 for PHY60 ($p < 0.05$).

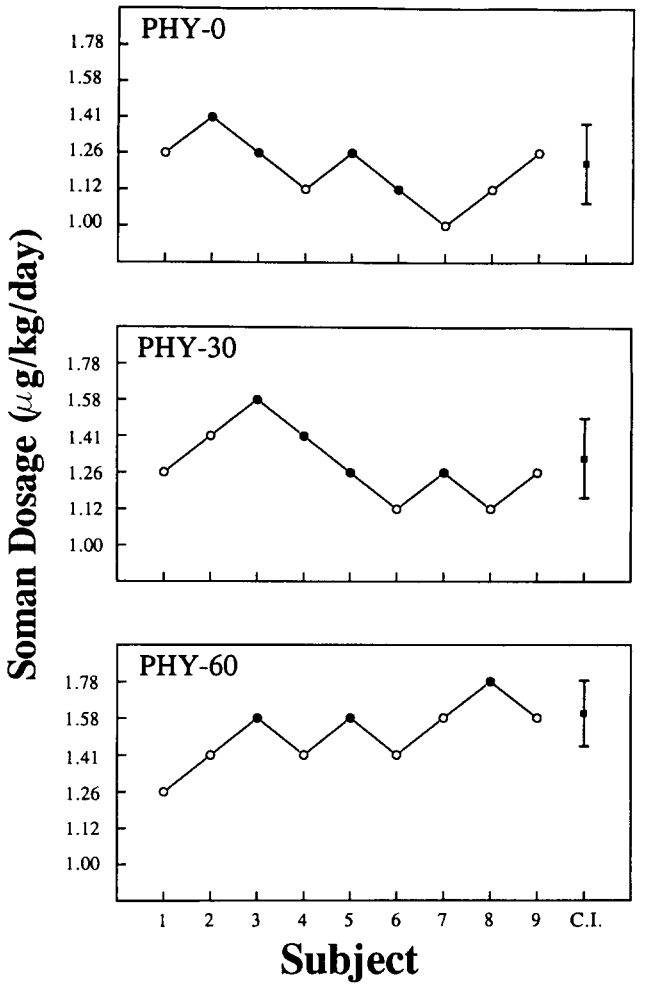

FIG. 3. Up-and-down soman-effects tests for the three groups implanted with osmotic pumps. Subjects that met the criterion for a soman-induced performance decrement on or before the fifth exposure day are indicated by filled symbols. ED_{50} and the associated 95% confidence interval is indicated at the end of each series.

Mean levels of serum ChE inhibition before and after each daily soman exposure for the PHY0, PHY30, and PHY60 Ss are shown in Fig. 4. Although the average soman dosages for the three groups differ (geometric means = 1.196, 1.292, and 1.506 μg/kg, respectively), the up-and-down method approximately equates the groups in terms of the performance effects of the soman on the fifth day of exposure. The mean %I values before the first soman dose and 90 min after the final soman dose are shown in Table 1.

The presoman values show that the pump dose rates selected produced inhibition near the target values. The cumulative effects of five daily soman exposures on serum ChE nearly overwhelm the effects of chronic PHY so that final %I values are essentially identical.

GENERAL DISCUSSION

These experiments have shown that chronic PHY, while not detrimental when continued in conjunction with repeated, low-dose soman exposure, does not provide substantial protection against the behavioral toxicity of such soman exposure. The protection afforded was in the same range as we found previously (6) for chronic pyridostigmine Br. There were several reasons to expect that PHY would provide substantially more protection than pyridostigmine:

1. PHY readily crosses the blood-brain barrier, while pyridostigmine does not, so PHY should provide additional protection against CNS soman toxicity.
2. Similar experiments in rats (30) found that PHY was significantly more effective than pyridostigmine in protecting against both lethality and severe weight loss produced by repeated soman exposure;
3. In numerous studies of the behavioral toxicity of acute, single soman exposure, PHY pretreatment (in conjunction with various therapies) was significantly more protective than pyridostigmine pretreatment (4,10,17,18,19,27).

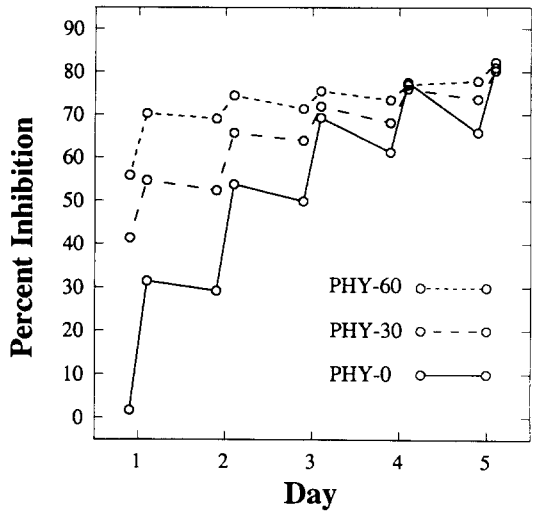

FIG. 4. Inhibition of serum cholinesterase (ChE) activity induced by five daily soman exposures plus continuous physostigmine (PHY) infusion.

There are two major differences between the present study and previous studies that demonstrated greater efficacy for PHY than pyridostigmine:

1. No other studies used PEP performance as an end-point; and
2. None of the other studies used soman dosages as small as those used in this experiment. Only one study in rats used repeated daily low-dose soman exposure with continuous PHY infusion.

TABLE 1

MEAN %I VALUES BEFORE THE FIRST SOMAN DOSE AND 90 min AFTER THE FINAL SOMAN DOSE

Group	Before 1st Soman Dose	After 5th Soman Dose
PHY-0	1.8	81.0
PHY-30	41.3	80.2
PHY-60	55.8	82.2

The possibility that these differences may account for our unexpected result will be discussed in turn.

The failure of PEP performance to reveal a difference between PHY (which has both central and peripheral actions) and pyridostigmine (which has only peripheral actions) might be explained by an insensitivity of the PEP task to all cholinergic effects or by a relative insensitivity of the task to central effects. However, the results of a large number of previous PEP studies of cholinergic compounds (3,5–9,12,35) do not support either of these possibilities.

The sensitivity of the PEP task to central cholinergic effects has been well documented (21). For acute soman, which penetrates the CNS rapidly, PEP performance decrements have a rapid onset (0–10 min) and an extremely steep dose–response function; statistically reliable performance decrements are detectable at relatively low dosages ($0.25–0.50 \times LD_{50}$). In well-trained groups of monkeys, $ED_{90}/ED_{10} \leq 0.26$, that is, a dosage change of only 0.10 $\log_{10}$ units produces a change in performance from a nearly undetectable decrement to nearly complete incapacitation (5,7,8). At the ED_{50} for PEP performance decrements (2.0–2.5 μg/kg), there are no detectable long-term effects and acute signs of toxicity are mild to nonexistent. By contrast, a study using a more cognitive discrimination task (matching-to-sample) in baboons failed to demonstrate significant performance decrements until the soman dosage closely approximated the estimated LD_{50} for that species and resulted in both acute and persistent signs of toxicity (15).

Acute PHY produces PEP performance effects similar to those of soman, with a rapid onset of decrements (0–10 min) and a steep dose–response function (Bennett et al., unpublished). Atropine, which penetrates the CNS less rapidly, produces PEP performance decrements with delayed onset (40–120 min) and a shallower dose–response function ($ED_{90}/ED_{10} \approx 0.8$; a dosage change of ≈ 0.25 $\log_{10}$ units produces a response change from nearly undetectable decrements change to near incapacitation). This atropine effect (3) is centrally mediated, as it can be canceled by an appropriately timed injection of PHY. Dosages of PHY and atropine that produce substantial performance decrements when given alone can produce a complete cancellation of effects when given together, if injections are timed so that the peak effects of each drug coincide. For complete cancellation of effects, atropine must be injected 40–80 min before PHY. The finding of a cancellation of effects suggests that both atropine and PHY produce their performance decrements by actions at populations of central sites with extensive overlap. Although ED_{50}s for atropine and for PHY were not estimated in these studies, the results indicate that the acute PHY ED_{50} for PEP performance decrements would be less than 60 μg/kg while the atropine ED_{50} would be less than 160 μg/kg.

Pyridostigmine, which does not enter the CNS in significant quantities, presents a different pattern of effects after acute injections (5). While soman, PHY, and atropine can produce substantial performance decrements with relatively minor cholinergic signs (3–5,8,9), pyridostigmine dosages large enough to produce substantial deficits are always accompanied by clear peripheral signs (muscle fasciculations, salivation, occasional vomiting, diarrhea, etc.). We have not explored the high end of the dosage range, but the dose–response curve appears to be much less steep than for the centrally active cholinergic compounds discussed above. The ED_{50} for PEP performance decrements, 660 μg/kg, was associated with inhibition of serum ChE of 77%, while the soman ED_{50} produced only 67% inhibition of serum ChE (5). If serum ChE indicates the peripheral effects of these compounds, it is clear that the soman effect on performance is not primarily a peripheral effect because soman dosages that produce 77% inhibition of serum ChE cause severe to incapacitating performance decrements in all exposed subjects. Pyridostigmine-induced serum ChE inhibition of the same magnitude is associated with relatively mild performance decrements in about half the subjects. The soman ED_{50} is associated with mild to nonexistent peripheral cholinergic signs, while the pyridostigmine ED_{50} produces clear cholinergic signs in almost all subjects (5).

The results of the many PEP studies of the acute performance effects of cholinergic compounds suggest that this behavioral end-point compares favorably with other behavioral tasks in sensitivity to both central and peripheral effects and that this task is relatively sensitive to central cholinergic effects. Thus, the different behavioral end-point used in this study cannot explain the failure to find that PHY is more efficacious than pyridostigmine against the behavioral effects of repeated, low-dose soman exposure.

The daily repeated low-dose soman exposures used in this study may have contributed to the unexpected lack of superior efficacy for PHY over pyridostigmine. In this paradigm, the fifth small daily dose of soman must be sufficient to reach and modify the central target sites despite the detoxifying effect of peripheral cholinesterases that tend to bind the circulating soman before it reaches the target sites. Figure 4 shows that the final level of serum ChE at the soman ED_{50} was identical in both the PHY-infused groups and the vehicle-only group despite large differences in both average soman dosage (up to 26%) and PHY-induced ChE inhibition (up to 56%). We have no direct measures of changes in brain AChE, but a similar dosage regimen in rats (daily exposure to a soman dose of 25–30% of the acute LD_{50}) produced stair step-like increases in AChE inhibition (22,31), reaching about 70% inhibition after five daily doses in the most sensitive brain regions (the hippocampus and frontal and piriform cortex). The daily dosage in the current study was somewhat smaller (about 15–25% of the estimated acute LD_{50}), probably because our end-point was more sensitive than those used in the rat studies. The rat study (30) that parallels the present monkey study found a protective effect for chronic PHY infusion of the same magnitude as the protective effect demonstrated here. This was larger than the protective effect of pyridostigmine measured in an earlier study in rats (24) only because that study found no protective effect, in contrast to the earlier monkey study (6), which demonstrated a small but reliable protective effect. There may be a species difference in the protective effect of pyridostigmine infusion against the behavioral toxicity of repeated, low-dose soman exposure, but the results for PHY infusion are consistent across the two species.

Peripheral ChEs, especially circulating ChEs, can be viewed as a sink for toxic OP agents, binding these agents as they circulate and reducing their toxic effects (35). Reversible binding of peripheral ChEs with carbamates does not appear to reduce the size of this sink significantly because OP toxicity does not increase in the presence of carbamate pretreatment, even when no therapy is administered after OP exposure (4,16,24,30). This implies that reversibly carbamylated ChEs become rapidly available to bind OP agents as these agents begin to reduce the available unbound circulating ChEs. Without postexposure therapy, reversible inhibition of ChE with carbamates produces little or no change in the lethality of OP agents (16). Supportive therapy apparently slows the lethal processes sufficiently that AChE sequestered by carbamyla-

tion can be released in sufficient quantities to maintain life. A major advantage of PHY over pyridostigmine is that it reduces the duration and severity of the behavioral incapacitation that invariably accompanies such high-dose OP exposures despite carbamate pretreatment and standard (cholinolytic + oxime) therapy. Where PHY has been shown to be efficacious in preventing behavioral effects of OP exposure (26,33), it has in general been given as a pretreatment in conjunction with a cholinolytic (e.g., scopolamine), which would serve as a therapeutic agent if given after OP exposure. Thus, it appears that PHY demonstrates its efficacy against soman-induced lethality or behavioral toxicity only in the presence of cholinolytic therapy drugs. Because no such drugs were used in this study, it is not surprising that chronic PHY infusion had little effect on the behavioral toxicity of repeated daily low-dose soman exposure.

In the present experiment, PHY was chronically infused at a rate sufficient to reversibly carbamylate significant fractions (30–60%) of circulating ChEs but not sufficient to produce behavioral toxicity by itself. The effect of this chronic infusion appears to have been to slightly increase the size of the soman sink so that slightly higher daily soman dosages were required to produce the same eventual behavioral toxicity and (perhaps coincidentally) the same final level of serum ChE inhibition. Apparently, the reversibly carbamylated circulating ChEs serve as part of the soman sink, and some process involved in the development of tolerance to chronically low levels of circulating ChE contributes to an effective increase in the size and/or efficacy of this soman-detoxifying sink.

Most of the studies showing PHY to be more effective than pyridostigmine in protecting against various toxic effects of acute exposure to soman and other OP agents used acute dosages of the order of magnitude of the acute LD_{50} (e.g., 0.5–5.0 times the acute LD_{50}). In acute studies, we have shown that pyridostigmine has a slight and variable protective effect (protective ratio about 1.4) against soman-induced PEP performance decrements, using acute soman dosages less than 0.33 times the acute LD_{50} (7). However, we have not performed the comparable experiment using PHY as the pretreatment drug. Thus, we cannot say whether the PEP performance task would yield results like those reported by others using higher soman dosages.

While neither chronic pyridostigmine Br nor chronic physostigmine salicylate is particularly effective against the toxic effects of repeated, low-dose soman exposure, both have been shown to provide substantial protection against the lethal effects of high-dose, acute exposure. Our experiments in both rats (24,30) and monkeys [(6) and this article] suggest that pretreatment with either drug can and should be continued, even in subjects who receive repeated, low-dose exposure, as long as there is a realistic threat of more severe, acute soman exposure.

ACKNOWLEDGEMENT

This work was supported by U.S. Air Force Armstrong Laboratory (Human Systems Division, Air Force Materiel Command) under Contract F33615-87-C-0625, with funding from the U.S. Naval Medical Research and Development Command. The views expressed herein are those of the authors and are not to be construed as official policy of the U.S. Air Force or of the U.S. Department of Defense. Soman, at least 97% pure, was supplied by the U.S. Army Chemical Research, Development and Engineering Center. Animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

REFERENCES

1. Albuquerque, E. X.; Deshpande, S. S.; Kawabuchi, M.; Aracava, Y.; Idriss, M.; Rickett, D. L.; Boyne, A. F. Multiple actions of anticholinesterase agents on chemosensitive synapses: Molecular basis for prophylaxis and treatment of organophosphate poisoning. Fund. Appl. Toxicol. 5:S182–S203; 1985.
2. Anderson, D. R.; Harris, L. W.; Lennox, W. J.; Solana, R. P. Effects of subacute pretreatment with carbamate together with acute adjunct pretreatment against nerve agent exposure. Drug Chem. Toxicol. 14:1–19; 1991.
3. Bennett, C. T.; Lof, N. E.; Farrer, D. N.; Mattsson, J. L. Equilibrium performance changes produced by atropine in *M. mulatta* and *M. fascicularis*. SAM-TR-81-29. USAF School of Aerospace Medicine, 1981.
4. Berry, W. K.; Davies, D. R. The use of carbamates and atropine in the protection of animals against poisoning by 1,2,3-trimethylpropyl methylphosphonofluoridate. Biochem. Pharmacol. 19:927–934; 1970.
5. Blick, D. W.; Brown, G. C.; Murphy, M. R.; Yochmowitz, M. G.; Hartgraves, S. L.; Fanton, J. W. Effects of soman or pyridostigmine Br on primate equilibrium performance and serum cholinesterase activity. SAM-TR-87-20. USAF School of Aerospace Medicine, 1987.
6. Blick, D. W.; Kerenyi, S. Z.; Miller, S.; Murphy, M. R.; Brown, G. C.; Hartgraves, S. L. Behavioral toxicity of anticholinesterases in primates: Chronic pyridostigmine and soman interactions. Pharmacol. Biochem. Behav. 38:527–532; 1991.
7. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Hartgraves, S. L.; Yochmowitz, M. G. Effects of carbamate pretreatment and oxime therapy on soman-induced performance decrements and blood cholinesterase activity in primates. Soc. Neurosci. Abstr. 13:1716; 1987.
8. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Yochmowitz, M. G. Primate equilibrium performance following soman exposure: Effects of repeated acute exposure with atropine therapy. SAM-TR-87-43. USAF School of Aerospace Medicine, 1987.
9. Blick, D. W.; Murphy, M. R.; Weathersby, F. R.; Brown, G. C.; Yochmowitz, M. G.; Fanton, J. W.; Harris, R. K. Primate equilibrium performance following soman exposure: Effects of repeated daily exposures to low soman doses. SAM-TR-87-19. USAF School of Aerospace Medicine, 1987.
10. Deshpande, S. S.; Viana, G. B.; Kauffman, F. C.; Rickett, D. L.; Albuquerque, E. X. Effectiveness of physostigmine as a pretreatment drug for protection of rats from organophosphate poisoning. Fund. Appl. Toxicol. 6:566–577; 1986.
11. Dixon, W. J.; Massey, F. J., Jr. Introduction to statistical analysis. 4th ed. New York: McGraw-Hill; 1983.
12. Ellin, R. I.; Kaminskis, A. Carbamoylated enzyme reversal as a means of predicting pyridostigmine protection against soman. J. Pharm. Pharmacol. 41:633–636; 1989.
13. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88–95; 1961.
14. Farrer, D. N.; Yochmowitz, M. G.; Mattsson, J. L.; Lof, N. E.; Bennett, C. F. Effects of benactyzine on an equilibrium and multiple response task in rhesus monkeys. Pharmacol. Biochem. Behav. 16:605–609; 1982.
15. Geller, I.; Hartmann, R. J., Jr.; Moran, E.; Leal, B. Z.; Haines, R. J.; Gause, E. M. Acute soman effects in the juvenile baboon: Effects on a match-to-sample discrimination task and on total blood acetylcholinesterase. Pharmacol. Biochem. Behav. 22:961–966; 1985.
16. Gordon, J. J.; Leadbeater, L.; Maidment, M. P. The protection

of animals against organophosphate poisoning by pretreatment with a carbamate. Toxicol. Appl. Pharmacol. 43:207–216; 1978.
17. Harris, L. W.; Lennox, W. J.; Stitcher, D. L.; McDonough, J. H.; Talbot, B. G.; Barton, J. A. Effects of chemical pretreatment on soman-induced lethality and physical incapacitation. Pharmacologist 23:224; 1981.
18. Harris, L. W.; Lennox, W. J.; Talbot, B. G.; Anderson, D. R.; Swanson, D. R. Toxicity of anticholinesterases: Interactions of pyridostygmine and physostigmine with soman. Drug Chem. Toxicol. 7:507–526; 1984.
19. Harris, L. W.; Stitcher, D. L. Protection against diisopropylfluorophosphate intoxication by pyridostigmine and physostigmine in combination with atropine and macemylamine. Arch. Pharmacol. 327:64–69; 1984.
20. Harris, L. W.; Talbot, B. G.; Lennox, W. J.; Anderson, D. R.; Solana, R. P. Physostigmine (alone and together with adjunct) pretreatment against soman, sarin, tabun, and VX intoxication. Drug Chem. Toxicol. 14:265–281; 1991.
21. Hartgraves, S. L.; Murphy, M. R. Behavioral effects of low-dose nerve agents. In: Somani, S. M., ed. Chemical warfare agents. San Diego, CA: Academic Press; 1992:125–154.
22. Howerton, T. C.; Murphy, M. R.; Miller, S. A.; Hartgraves, S. L. Differential sensitivity of CNS regions to acetylcholinesterase inhibition following chronic low-dose soman treatment in the rat. Psychopharmacology (Berl.) 105:400–406; 1991.
23. Kerenyi, S. Z.; Hartgraves, S. L. Premature excess release from the Alzet osmotic pump. Pharmacol. Biochem. Behav. 27:199–201; 1987.
24. Kerenyi, S. Z.; Murphy, M. R.; Hartgraves, S. L. Toxic interaction between repeated soman and chronic pyridostigmine in rodents. Pharmacol. Biochem. Behav. 37:261–271; 1990.
25. Koster, R. Synergism and antagonisms between physostigmine and diisopropyl fluorophosphate in cats. J. Pharmacol. Exp. Ther. 88:39–46; 1946.
26. Leadbeater, L.; Inns, R. H.; Rylands, J. M. Treatment of poisoning by soman. Fund. Appl. Toxicol. 5:S225–S231; 1985.
27. Lennox, W. J.; Harris, L. W.; Talbot, B. G.; Anderson, D. R. Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. Life Sci. 37:793–798; 1985.
28. Lieberman, G. J.; Miller, R. G. Simultaneous tolerance intervals in regression. Biometrika 50:155–168; 1963.
29. Maxwell, D. M.; Brecht, K. M.; Lenz, D. E.; O'Neill, B. L. Effect of carboxylesterase inhibition on carbamate protection against soman toxicity. J. Pharmacol. Exp. Ther. 246:986–991; 1988.
30. Miller, S. A.;, Blick, D. W.; Kerenyi, S. Z.; Murphy, M. R. The efficacy of physostigmine as a pretreatment for organophosphate poisoning. Pharmacol. Biochem. Behav. (in press).
31. Murphy, M. R.; Miller, S. A.; Kerenyi, S. Z.; Switzer, R. C., III; Blick, D. W.; Hartgraves, S. L. Soman toxic syndrome: Effects of soman on biochemistry, brain, and behavior in rats. SAM-TR-90-15. USAF School of Aerospace Medicine, 1990.
32. Somani, S. M.; Dube, S. N. Physostigmine—an overview as pretreatment drug for organophosphate intoxication. Int. J. Clin. Pharmacol. Ther. Toxicol. 27:367–387; 1989.
33. von Bredow, J.; Corcoran, K.; Maitland, B.; Kaminskis, A.; Adams, N.; Wade, J. Efficacy evaluation of physostigmine and anticholinergic adjuncts as a pretreatment for nerve agent intoxication. Fund. Appl. Toxicol. 17:782–789; 1991.
34. Wetherell, J. R.; French, M. C. A comparison of the decarbamoylation rates of physostigmine-inhibited plasma and red cell cholinesterases of man with other species. Biochem. Pharmacol. 42:514–520; 1991.
35. Wolfe, A. D.; Blick, D. W.; Murphy, M. R.; Miller S. A.; Gentry, M. K.; Hartgraves, S. L.; Doctor, B. P. Use of cholinesterases as pretreatment drugs for the protection of rhesus monkeys against soman toxicity. Toxicol. Appl. Pharmacol. 117:189–193; 1992.