Pharmacology Biochemistry & Behavior, Vol. 38, pp. 527–532. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Behavioral Toxicity of Anticholinesterases in Primates: Chronic Pyridostigmine and Soman Interactions[1,2,3]

DENNIS W. BLICK,* SANDRA Z. KERENYI,† STEPHANIE MILLER,†
MICHAEL R. MURPHY,* G. CARROLL BROWN* AND STANLEY L. HARTGRAVES†

**Systems Research Laboratories Division of Arvin/Calspan and †Radiation Sciences Division*
USAF School of Aerospace Medicine, Brooks AFB, TX 78235-5301

Received 20 August 1990

BLICK, D. W., S. Z. KERENYI, S. MILLER, M. R. MURPHY, G. C. BROWN AND S. L. HARTGRAVES. *Behavioral toxicity of anticholinesterases in primates: Chronic pyridostigmine and soman interactions.* PHARMACOL BIOCHEM BEHAV **38**(3) 527–532, 1991.—Dose rates for continuous infusion of pyridostigmine bromide required to inhibit 30% and 60% of normal serum cholinesterase activity in rhesus monkeys were determined. The effects of continuous pyridostigmine infusion at these dose-rates on the behavioral toxicity of 5 daily repeated low-dose exposures to a toxic organophosphate (soman) were determined not to be deleterious; in fact, they were slightly (and variably) protective. Relative to controls (5-day soman ED_{50} = 0.89 μg/kg/day), pyridostigmine infusions producing 30% and 60% inhibition produced 5-day ED_{50}s of 1.25 and 1.11 μg/kg/day, respectively. Variability in response to the pyridostigmine-soman combinations appeared to be greater than in response to daily soman exposure without pyridostigmine infusion.

Behavioral toxicology Behavioral pharmacology Organophosphate Carbamate Cholinergic systems Rhesus monkeys Compensatory tracking

PRETREATMENT with the quaternary carbamate pyridostigmine bromide (PYR) has been shown to provide protection against the deadly effects of the nerve agent soman (pinacolyl methylphosphonofluoridate) in a number of species (1, 17, 18, 23, 24, 30), including primates (8,21). Military personnel under the threat of chemical attack are expected to undertake a pretreatment regimen consisting of 90 mg/day of PYR divided into 3 equal oral doses. PYR tablets appropriate for such pretreatment have been fielded in Europe for use by NATO troops. The effect of this pretreatment is to reversibly bind (carbamylate) 30–40% of the cholinesterase (ChE), protecting it from permanent inactivation by the nerve agent. ChE so protected during agent exposure later becomes available to restore near-normal neuromuscular and autonomic function (13,16).

Numerous experiments in humans and animals have shown that the prophylactic use of PYR is safe, since it produces only relatively minor side-effects (e.g., transient gas, intestinal cramps, or diarrhea) in only a small fraction of treated subjects. In human subjects, no deleterious effects on cardiopulmonary functions (22,29) or on the performance of simulated aircrew tasks (14,29) resulted from the recommended pretreatment regimen. In animals, testing over a much larger range of doses showed that performance decrements began to develop only at 6–10 times the recommended pretreatment dosage, when serum ChE inhibition approaches 90% (5,27).

Previous research has shown that PYR (with atropine therapy) is safe and efficacious in preventing death following exposure to high soman doses (8,21). A question that remained was whether the effects of chronic PYR would interact positively or negatively with the effects of repeated, low-dose exposure to soman.

Sustained military operations in environments heavily contaminated with nerve agents entail a risk of repeated, low-dose exposure of personnel, e.g., during donning/doffing of protective apparel or through small leaks in protective suits or masks. We

[1]This work was supported by U.S. Air Force School of Aerospace Medicine (Human Systems Division, Air Force Systems Command) Contract Number F33615-87-C-0625, with funding from the U.S. Naval Medical Research and Development Command. The views expressed 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.

[2]Soman, at least 97% pure, was supplied by the U.S. Army Chemical Research, Development and Engineering Center.

[3]The 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.

have previously shown that repeated daily soman exposure (for up to 5 days) produces performance decrements in monkeys only after the cumulative dose exceeds twice the acute dosage that would produce similar decrements (3,7). These results were obtained in the absence of chronic PYR treatment. The experiments reported here extend the previous studies by determining whether chronic PYR modifies the effects of repeated daily low-dose soman exposure on primate performance.

Preliminary studies in rodents have shown that the chronic ChE inhibition induced by continuous infusion of PYR has little effect on performance capabilities or on the lethality of repeated soman exposure (20). However, since the rodent model is complicated by the presence of a high level of blood carboxylesterase that drastically reduces (relative to primates) its sensitivity to organophosphates (26), there was no assurance that similar results would be obtained in primates.

In order to provide a better model of the PYR-soman interaction effects that might occur in humans, these interactions were studied in a laboratory primate, the rhesus macaque. The effects of chronic PYR on the inhibition of serum ChE were investigated in Experiment I. Interactions between chronic PYR exposure and daily repeated soman exposure in their effects on the performance of the Primate Equilibrium Platform (PEP) task by well trained rhesus monkeys were examined in Experiment II. Based on the results of Experiment I, we selected two PYR dose-rates for use in Experiment II that were estimated to produce 30% or 60% inhibition of serum ChE activity. ChE inhibition of 30% was chosen because this level is near the lower end of the target range for prophylactic efficacy. If chronic ChE inhibition at this level were found to increase significantly the risk of performance decrements due to long-term low-dose (LTLD) soman exposure, it would pose a problem for military planners. On the other hand, if no significant detrimental effects were found at chronic inhibition levels of 60%, planners could be confident that such effects in humans are unlikely.

The PEP task was chosen for these studies for two reasons: first, it has been shown to be quite sensitive to the effects of nerve agents and other cholinergic agents; and, second, it models aspects of sensorimotor tasks of interest to military planners, e.g., maintaining the stability of a weapons platform in spite of unpredictable external perturbations, or tracking a moving target with a gun-sight. Previous work (2, 4–7) has shown that reliable and graded performance effects of soman can be demonstrated at doses so low (e.g., 0.3–0.4·ED_{50}) that no overt signs of toxicity are apparent. Using other behavioral tasks, other studies have generally found that the ED_{50} for performance effects is closer to the LD_{50} (15, 17, 23, 27, 30). For example, baboons performing a more cognitive task (match-to-sample) did not display reliable performance decrements until the soman dose closely approached the estimated LD_{50} for that species and produced clear symptoms of toxicity (15). For the purposes of the present experiments, a task that provides information about behavioral toxicity with little or no risk to the subjects was considered preferable.

GENERAL METHOD

Behavioral Testing

The PEP task (12) 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 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 standard deviation (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 performed the PEP task for 2 h on each testing day.

Subjects

The subjects (*S*s) were 24 adult male rhesus monkeys (*Macaca mulatta*), ranging in weight from 4.5–8 kg. All *S*s had been performing the PEP task on a regular basis (at least weekly) for a minimum of 6 months. None of the *S*s had a prior history of exposure to soman or PYR. Routine care of the animals was provided by the Veterinary Sciences Division, USAF School of Aerospace Medicine (USAFSAM).

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 (25). This method consists of fitting a line to the baseline performances (5 sets of 24 SDs for 5-min sampling periods) by the method of least squares. Simultaneous tolerance limits ($p = 0.99$, $\alpha = 0.01$) around this line are based on the residual variation about the fitted line. Within a soman test session, our criterion for a performance decrement was met whenever at least 2 of the data points collected after drug injection exceeded the upper tolerance limits derived from baseline runs for the same *S*. This is a conservative criterion, since the occurrence of one data point in excess of the tolerance limit would meet the usual statistical criterion for null hypothesis rejection. In studies involving so few subjects, however, an occasional 'false alarm' could add unacceptably to the variability of the results. The use of a more conservative criterion provides more confidence that the performance decrement observed in a single subject after a single exposure is 'real' and unequivocal. While this may lead to a slight overestimation of the ED_{50}, it does not affect the comparisons of ED_{50}s measured for different treatment conditions. Five baseline tests performed during the week preceding soman exposure were used to derive the tolerance limits for each *S*.

Determination of Median Effective Dose of Soman

The up-and-down method (10) was used to minimize the number of soman exposures required for a reliable estimate of 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 standard deviation of the underlying distribution of effects) this up-and-down method yields an adequate estimate of ED_{50} with as few as 6–10 tests. The significance of differences between ED_{50} estimates can be tested using *t*-tests (10). The ini-

tial dose in this experiment was 1.00 μg/kg/day, approximately the ED_{50} for 5-day repeated soman determined in a previous experiment (7). Dosage step size was 0.05 $\log_{10}$ units.

Chronic PYR Infusion

Alzet® osmotic pumps (Alza Corp., Palo Alto, CA, Model 2ML1, 10 μl/h, 7 day) were implanted subcutaneously to provide constant-rate infusion of PYR 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 a subcutaneous pocket to accommodate the pump, which was inserted, delivery orifice first, at body temperature (19). The incision was then 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. (11) was used to measure serum ChE activity.

EXPERIMENT I. EFFECTS OF CONSTANT RATE PYR INFUSION ON SERUM ChE ACTIVITY

Experimental Design

Eighteen PEP-trained male rhesus monkeys (4.5–8 kg) were assigned randomly to 3 groups of 6. The *S*s in these 3 groups were implanted with osmotic pumps that delivered PYR at rates of 0.25, 0.50, or 1.0 mg/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 (2 from each dose-rate group) were implanted on each of 3 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 *S*s in Experiment I met our criterion for a PEP performance decrement during chronic PYR infusion, so the performance data were not analyzed further.

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 *S*s), postimplantation time (within *S*s), and *S*s (nested within dose-rates) as factors (28).

The 3 dose-rates produced significant ($p<0.0001$) variation in serum ChE inhibition, as shown in Fig. 1. Linear interpolation and extrapolation, using the line connecting the means for the two highest dose-rates, yielded dose-rates of 0.52 and 1.35 mg/kg/day for use in Experiment II to produce serum ChE inhibitions of 30 and 60%, respectively.

The ANOVA also indicated significant ($p<0.05$) variation in mean ChE inhibition over days. A nonsignificant dose-rate × days interaction ($p>0.35$) indicated that the day effect was independent of dose-rate. A post hoc test [Bonferroni (28)] on the differences among days showed that the highest observed mean inhibition (on day 4) differed from the lowest mean inhibition (on day 6). No other differences among days were significant. An examination of the raw data showed that unusually low inhibition values for day 6 occurred only in the first group of 6 animals implanted. Of these 6 animals, 4 had their lowest inhibition values on day 6. The third group of implanted animals showed unusually high inhibition values on day 4, with 5 of the 6 animals showing their highest values on that day. The unusual results on day 6 of week 1 and day 4 of week 3 may have resulted from artifacts in the assay procedures. However, since the aberrant results were independent of dose-rate and in opposite directions, they would introduce, at most, a very small bias in the dose-rate means used to determine the PYR dose-rates for Experiment II.

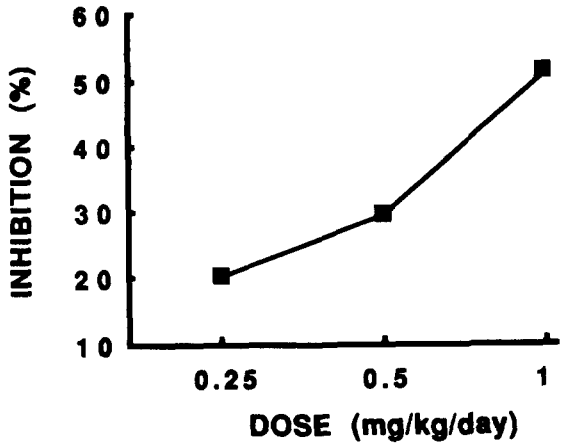

FIG. 1. Inhibition of serum ChE activity produced by continuous PYR infusion at varying dose-rates. Each point is the mean of 30 observations (6 animals × 5 days).

EXPERIMENT II. EFFECTS OF CHRONIC PYR-INDUCED ChE INHIBITION ON DAILY REPEATED SOMAN ED_{50} FOR PEP PERFORMANCE DECREMENTS

Experimental Design

The up-and-down method was used to estimate the soman ED_{50} for PEP performance decrements induced on or before the 5th day of exposure to a constant daily dose of soman under each of 3 PYR infusion conditions: 1) Control—implanted with pumps containing only vehicle (1 M acetic acid buffered to a pH of 5.0 with sodium hydroxide); 2) PYR30—implanted with pumps containing sufficient PYR (0.52 mg/kg/day) to inhibit 30% of serum ChE activity; or 3) PYR60—implanted with pumps containing sufficient PYR (1.35 mg/kg/day) to inhibit 60% of serum ChE activity.

Each of the 3 groups included 6 *S*s from Experiment I (2 from each group) plus 2 additional randomly selected PEP-trained animals. Within each group of 8 animals, order of testing was randomly determined. To avoid possible carryover effects of chronic ChE inhibition, pumps were not implanted in Experiment II until at least 6 weeks after pumps from Experiment I were removed. We had previously demonstrated that no carryover effects occur between acute soman injections spaced at 6-week intervals (4). Since these soman injections produced larger and longer-lasting depressions of ChE activity than the PYR exposures in Experiment I, carryover effects in Experiment II were unlikely to occur. However, as an additional precaution, the groups in Experiment II were balanced with respect to the PYR dose-rates received in Experiment I.

Pumps were implanted in one member of each PYR group on

Fridays. Daily soman exposures began the following Monday, and continued through the following Friday. Soman, diluted to 10 μg/ml with distilled water, was intramuscularly injected into the large muscles of the upper leg. 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 1.00 μg/kg/day, approximately the ED_{50} for performance decrements on or before the 5th daily exposure determined in a previous experiment (3). 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 each group was one step below or above the dosage received by the preceding animal, depending on whether the preceding animal did or did not meet the criterion for a performance decrement.

RESULTS

The outcomes of the 3 up-and-down testing sequences are shown in Fig. 2. When the pump contained only vehicle (PYR-0), the daily soman dose estimated to produce a performance decrement by the 5th daily exposure in 50% of subjects (ED_{50}) was 0.891 μg/kg/day [95% confidence interval (CI): $0.834 \leq ED_{50} \leq 0.952$]. With continuous pyridostigmine infusion at a rate of 0.52 mg/kg/day (PYR-30), the daily soman ED_{50} increased, $t_{\infty} = 4.28$, $p < 0.001$, to 1.254 μg/kg/day (CI: $0.931 \leq ED_{50} \leq 1.689$). With continuous pyridostigmine infusion at a rate of 1.35 mg/kg/day (PYR-60), the daily soman ED_{50} increased, $t_{\infty} = 2.74$, $p < 0.01$, relative to PYR-0, to 1.11 μg/kg/day (CI: $0.809 \leq ED_{50} \leq 1.521$). Both rates of pyridostigmine infusion increased the soman ED_{50} relative to the control condition; however, the ED_{50} values for the 2 infusion rates did not differ significantly, $t_{\infty} = 1.54$, $p > 0.10$. Therefore, chronic pyridostigmine infusion in this range of dose-rates confers statistically significant ($p < 0.01$) 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, i.e., the ratios of treated ED_{50} to untreated ED_{50} were only 1.41 for PYR-30 and 1.24 for PYR-60. The variability of this protective effect is indicated by the larger confidence intervals (Fig. 2) associated with the PYR-infusion conditions, and by the broad range of soman dosages in which PYR-treated animals may or may not show soman-induced performance decrements. In the 2 up-and-down tests with PYR-treated animals, performance decrements resulted from soman dosages ranging from 1.00 to 1.41 μg/kg/day, while soman dosages ranging from 0.89 to 1.41 μg/kg/day failed to produce performance decrements in some subjects. By contrast, in 2 up-and-down tests without pyridostigmine infusion [one of which was performed earlier (3) with results very similar to the PYR-0 series], soman-induced performance decrements appeared at soman dosages ranging only from 0.91 to 1.06 μg/kg/day. The range of soman dosages that failed to produce decrements in these tests was only from 0.79 to 0.98 μg/kg/day. Pyridostigmine infusion thus appears not only to increase (on average) the tolerable level of daily soman exposure; it also appears to increase the variability of response to such exposures. Based on these data, the variability increase induced by the PYR-30 treatment is estimated to be about 3-fold. The increase in variability associated with the PYR-60 treatment is even larger, 4- to 5-fold (9). No statistical tests for the significance of such changes in variability have been developed (9), so these observations must be taken as suggestive. Despite the fact that the protective effects of chronic pyridostigmine are small and variable, it is clear that chronic pyridostigmine, at the dose-rates tested, does not exacerbate the effects on performance of LTLD soman exposure.

Mean levels of serum ChE inhibition before and after each daily soman exposure for the PYR-0, PYR-30 and PYR-60 *S*s are shown in Fig. 3. Although the average soman dosages for the 3 groups differ (geometric means = 0.917, 1.122, and 1.059, respectively), the up-and-down method approximately equates the groups in terms of the performance effects of soman. The mean %I values before the first soman dose and 90 min after the final soman dose were:

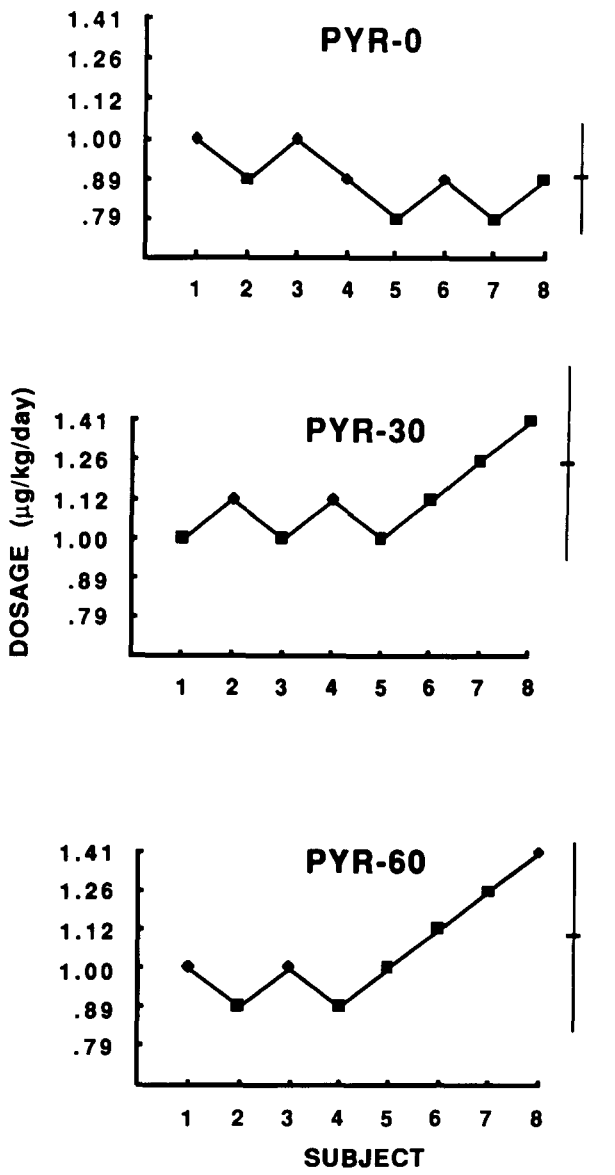

FIG. 2. Up-and-down soman-effects tests for the 3 groups implanted with osmotic pumps. Subjects that met the criterion for a soman-induced performance decrement on or before the 5th exposure day indicated by ◆. ED_{50} and the associated 95% confidence interval is indicated at the end of each series.

Group	Before 1st Soman Dose	After 5th Soman Dose
PYR-0	6.2	74.9
PYR-30	26.6	77.7
PYR-60	59.1	81.3

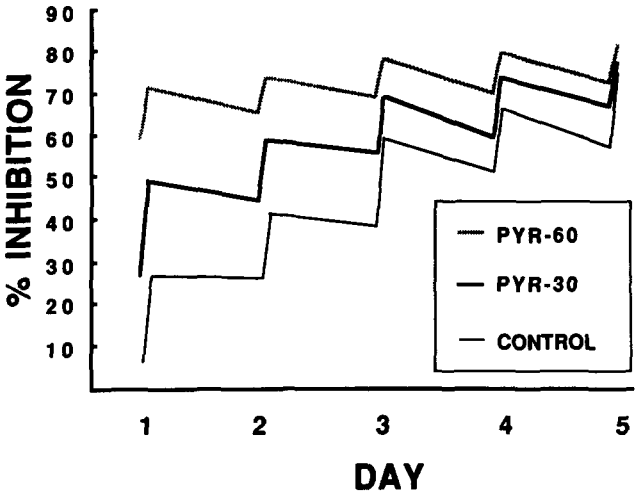

FIG. 3. Inhibition of serum ChE activity induced by 5 daily soman exposures plus continuous PYR infusion.

The presoman values show that, within normal variability, the pump dose-rates selected produced inhibition at the target values. The cumulative effects of 5 daily soman exposures on serum ChE nearly overwhelm the effects of chronic PYR, so that final %I values are quite similar.

DISCUSSION

These experiments addressed the question of whether PYR pretreatment should be continued during actual or threatened LTLD soman exposure. If chronic ChE inhibition due to PYR increased the toxicity of such soman exposure, a recommendation to discontinue PYR in personnel working in soman-contaminated environments might be appropriate. On the other hand, if PYR either did not change or reduced the toxicity of LTLD soman exposure, then PYR should be continued, given its proven efficacy against the lethal effects of high-dose exposure. Based on the PEP performance results in monkeys, chronic PYR effects may range from little or no protection against LTLD soman-induced performance decrements to an increase of 40% in tolerable daily exposure. Therefore, PYR pretreatment should be continued during threatened or actual LTLD soman exposure.

Studies of the interaction between chronic PYR and LTLD soman effects on lethality in rats have also supported this conclusion (20). In agreement with studies in humans (22,29), we found that chronic PYR exposure alone does not produce dose-related changes in performance, even at doses higher than those recommended for pretreatment under the threat of nerve gas exposure. At most, chronic PYR exposure at recommended doses produces mild and tolerable symptoms of peripheral ChE inhibition.

We have previously demonstrated (2) that PYR pretreatment has a reliable but operationally insignificant protective effect against PEP performance decrements induced by single, acute soman exposures. Since PYR poorly penetrates the blood-brain barrier, while soman-induced performance decrements are probably due to changes in brain function, the limited protection provided by PYR against performance decrements is not surprising. To protect performance against low-dose soman exposure, pretreatment drugs that penetrate the blood-brain barrier may be required.

REFERENCES

1. 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.
2. 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.
3. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Kerenyi, S. Z.; Hartgraves, S. L. Effect of daily repeated soman exposure on tracking performance and blood cholinesterase in rhesus monkeys. Soc. Neurosci. Abstr. 14:1136; 1988.
4. 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. USAF School of Aerospace Medicine, SAM-TR-87-43; 1987.
5. Blick, D. W.; Brown, G. C.; Murphy, M. R.; Yochmowitz, M. G.; Hartgraves, S. L.; Fanton, J. W. Effects of soman or pyridostigmine bromide on primate equilibrium performance and serum cholinesterase activity. USAF School of Medicine, SAM-TR-87-20; 1987.
6. Blick, D. W.; Murphy, M. R.; Dawson, D. P.; Brown, G. C. The effect of oral pyridostigmine on serum cholinesterase activity in *Macaca mulatta*. USAF School of Aerospace Medicine, SAM-TR-86-34; 1986.
7. 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. USAF School of Aerospace Medicine, SAM-TR-87-19; 1987.
8. Dirnhuber, P.; French, M. C.; Green, D. M.; Leadbeater, L.; Stratton, J. A. The protection of primates against soman poisoning by pretreatment with pyridostigmine. J. Pharm. Pharmacol. 31:295–299; 1979.
9. Dixon, W. J. Personal communication, Feb 9, 1990.
10. Dixon, W. J.; Massey, F. J., Jr. Introduction to Statistical analysis. 4th ed. New York: McGraw-Hill; 1983.
11. 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.
12. 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.
13. French, M. C.; Wetherell, J. R.; White, P. D. The reversal by pyridostigmine of neuromuscular block produced by soman. J. Pharm. Pharmacol. 31:290–294; 1979.
14. Gawron, V. J.; Schiflett, S. G.; Miller, J. C.; Slater, T.; Ball, J. F. Effects of pyridostigmine bromide on inflight aircrew performance. Human Factors 32:79–94; 1990.
15. Geller, I.; Hartmann, R. J.; 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.; Maidmont, 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.; Stitcher, D. L.; Heyl, W. C. The effects of pretreatments with carbamates, atropine and mecamylamine on survival and on soman-induced alterations in rat and rabbit brain acetylcholine. Life Sci. 26:1885–1891; 1980.
19. Kerenyi, S. Z.; Hartgraves, S. L. Premature excess release from the Alzet osmotic pump. Pharmacol. Biochem. Behav. 27:199–201; 1987.

20. 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.
21. Kluwe, W. M.; Chinn, J. C.; Feder, P.; Olson, C.; Joiner, R. Efficacy of pyridostigmine pretreatment against acute soman intoxication in a primate model. Proc. Sixth Med. Chem. Def. Biosci. Rev. 227–234; 1987.
22. Krutz, R. W., Jr.; Burton, R. R.; Schiflett, S. G.; Holden, R.; Fischer, J. Interaction of pyridostigmine bromide with mild hypoxia and rapid decompression. Proc. Sixth Med. Chem. Def. Biosci. Rev. 601–604; 1987.
23. Leadbeater, L.; Inns, R. H.; Rylands, J. M. Treatment of poisoning by soman. Fundam. Appl. Toxicol. 5:S225–S231; 1985.
24. 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.
25. Lieberman, G. J.; Miller, R. G. Simultaneous tolerance intervals in regression. Biometrika 50:155–168; 1963.
26. Maxwell, D. M.; Brecht, K. M.; O'Neill, B. L. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol. Lett. 39:35–42; 1987.
27. Murphy, M. R.; Blick, D. W.; Brown, G. C.; Romano, J. A.; Goddard, G. A. Physiologic and performance effects of pyridostigmine bromide in rats and monkeys. Fifth Ann. Chem. Def. Biosci. Rev. Abstr., U.S. Army Medical Research Institute of Chemical Defense, P12; 1985.
28. SAS Institute Inc. SAS® user's guide: Statistics, 5th ed. Cary, NC: SAS Institute Inc.; 1985.
29. Schiflett, S. G.; Stranges, S. F.; Slater, T.; Jackson, M. K. Interactive effects of pyridostigmine and altitude on performance. Proc. Sixth Med. Chem. Def. Biosci. Rev. 605–608; 1987.
30. Xia, D.; Wang, L.; Pei, S. The inhibition and protection of cholinesterase by physostigmine and pyridostigmine against soman poisoning in vivo. Fundam. Appl. Toxicol. 1:217–221; 1981.