Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 3, pp. 643–649, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0009-7

Behavioral Toxicity of Anticholinesterases in Primates: Effects of Daily Repeated Soman Exposure

DENNIS W. BLICK,*[1] FRANK R. WEATHERSBY, JR.,*
G. CARROLL BROWN* AND MICHAEL R. MURPHY†

*Systems Research Laboratories Division of Arvin/Calspan, Dayton, OH 45440
†Directed Energy Division, Occupational and Environmental Health Directorate,
USAF Armstrong Laboratory, Brooks AFB, Texas 78235-5000

Received 14 June 1993

BLICK, D. W., F. R. WEATHERSBY, JR., G. C. BROWN AND M. R. MURPHY. *Behavioral toxicity of anticholinesterases in primates: Effects of daily repeated soman exposure.* PHARMACOL BIOCHEM BEHAV **48**(3) 643–649, 1994.—The effects of repeated daily exposure to soman, an organophosphate nerve agent, on the performance of a well-learned, compensatory tracking task were tested in rhesus monkeys. The ED_{50} daily exposure required to produce a performance decrement on or before the fifth daily exposure (0.97 μg/kg) was about 40% of the acute dose required to produce a similar performance decrement. After repeated, low-dose exposures, performance decrements appeared when serum cholinesterase (ChE) activity was inhibited 85–90%. Acute exposures that produced similar performance effects were associated with lower levels of ChE inhibition (65–70%), suggesting that repeated daily exposure may lead to the development of a tolerance (physiological or behavioral) to low levels of ChE activity.

Organophosphates Chronic exposure Macaque Tracking performance

SINCE their development in Germany during World War II, large quantities of organophosphate (OP) agents have been produced, both for use as pesticides (e.g., malathion) and as chemical warfare (CW) nerve agents (e.g., soman, sarin, and tabun). OP agents are highly toxic because they irreversibly inhibit the enzyme acetylcholinesterase (AChE) and thus produce an excess of the transmitter acetylcholine (ACh) at central and peripheral cholinergic synapses in the nervous system and at neuromuscular junctions. Responses to toxic OP agents include muscle fasciculations and tremor, excessive salivation and other cholinergic autonomic signs, prostration, convulsions, respiratory failure, and death.

While the threat of mass casualties on the battlefield has fueled research into the lethal effects of organophosphate (OP) nerve agents such as soman (pinacolylmethylphosphonofluoridate), relatively little work has been directed toward behavioral effects of these OP agents or of anticholinesterases in general. The effects on behavior of relatively low-level exposures are relevant to concerns about environmental exposures to pesticides, many of which have similar modes of action but less toxicity than soman.

The present experiments used an animal model (the rhesus monkey) performing a compensatory tracking task to estimate the possible behavioral effects of repeated low-dose exposure that might be experienced by military personnel under combat conditions (e.g., medical personnel dealing with CW casualties, ordnance handlers generating repeated sorties from contaminated bases, or naval personnel conducting combat operations from contaminated ships). However, the results are relevant to many other possible repeated, low-dose exposure scenarios. The observed differences between single, acute exposures and repeated, low-dose exposures regarding toxic thresholds, variability of effects, the effects of exposure after detectable behavioral toxicity develops, and relations between ChE inhibition and performance effects have implications extending well beyond the particular animal model, behavioral task, exposure scenario, and OP agent tested here.

We previously examined the effects on primate perfor-

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

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. 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.

mance of acute exposure to soman, both with and without pretreatment and/or therapy (6,7,9,14,26,32). Our experimental approach was to determine threshold doses that produce minimal but reliably detectable decrements in the performance of a complex sensorimotor tracking task: the Primate Equilibrium Platform (PEP) task. Specifically, we determined the median effective dose (ED_{50}), a dose that will, under the conditions of our experiments, produce a reliably detectable (but minimal) performance decrement in 50% of the subjects exposed to that dose. Our results also provide an indication of the variability in response to doses near the ED_{50} among the test subjects (adult male rhesus monkeys). A dose that could be considered "safe" (i.e., one that would produce performance decrements in only a very small minority of subjects) can be estimated from the ED_{50} and the response variability.

The present experiment extended these observations to the performance effects of low-dose soman exposure repeated daily for up to five days. We measured the daily dose of soman that produced a decrement in PEP performance on or before the fifth day of exposure. This was the first study of the performance effects of daily exposure to a nerve agent in primates. It was followed by studies of interactions between repeated daily nerve agent exposure and chronic infusion of pyridostigmine bromide (3) or physostigmine salicylate (5).

METHODS AND PROCEDURES

Subjects

The subjects were seven adult male rhesus monkeys (*Macaca mulatta*) ranging in weight from 6 to 11 kg. All subjects had been performing the PEP task on a regular basis (at least weekly) for a minimum of two years. All subjects had a history of testing after exposure to cholinergic drugs (e.g., atropine sulfate, pyridostigmine bromide) and to low doses of soman (1.9–2.8 μg/kg). None of the animals had been exposed to any of these compounds for at least six weeks before this experiment. Previous experiments with repeated low-dose soman exposure (6) revealed no carryover effects with exposures separated by at least six weeks, a period sufficient for serum ChE activity to return to baseline levels. With as many as three acute doses, there were no changes in the ED_{50}, variability in performance effects of soman, baseline performance level or variability, or soman-induced changes in serum ChE (6). Since all of the subjects used in the current experiment had similar prior experience with acute, low-dose soman exposure, such prior exposure was not expected to affect the results. Although our paradigm for determining ED_{50} required soman exposure and behavioral testing for five days or until a performance decrement was detected (whichever came first), four animals were exposed for more than five days due to the exploratory nature of this study. The results of these extended exposures will be presented separately from those of the main (five-day) experiment, in which the data from these four subjects are included. Three of the subjects who had not met the criterion for a performance decrement by the fifth exposure day were exposed for seven days, at which time they did develop minimal performance decrements. One monkey that met the criterion for a performance decrement on the fifth exposure day was exposed for three additional days.

Behavioral Testing

The PEP task (16) is a continuous compensatory tracking task. The monkey is seated in a chair that rotates about his center of mass. A joystick control mounted directly in front of the monkey on a platform attached to the chair controls the chair's pitch. An externally applied filtered random noise signal also drives rotation of the chair about the pitch axis. The monkey's task is to use the joystick control to compensate for the external signal and thereby keep the platform as level as possible. The platform position (angle in degrees) is measured by a computer 10 times/s and the standard deviation (σ) of these measurements for each 5-min epoch (i.e., 3000 points) is the metric for PEP performance. Whenever the platform deviates from level by 15° or more, the monkey receives tail shocks (100-ms duration, 1-Hz repetition rate, 5-mA maximum current) until the platform returns to within the 15° limits. Subjects performed the PEP task for 2 h on each testing day. Soman or control injections (saline only, on baseline days) were administered by intramuscular injection at the end of the first 30 min of testing. If treatments produce deterioration in performance to the extent that the subject is unable to escape shock for 30–60 s, data collection is discontinued and the chair is pinned in the level position, so that shock is terminated. Testing is resumed after a 3–5-min rest period. If the monkey is still unable to perform, the test session is terminated. For any 5-min test epoch for which testing was interrupted due to such performance incapacitation, a σ score of 12° (i.e., the value obtained when no animal is in the system) is assigned.

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 (23) method of simultaneous tolerance limits. This method consists of fitting a line to the baseline performance data (2 h of testing divided into 24 5-min epochs) by the method of least squares. Simultaneous tolerance limits ($P = .95$, $\alpha = .05$) around this line are based on the residual variation about the fitted line. Whenever at least two of the data points collected following drug injection exceeded the upper tolerance limits derived from baseline runs for the same subject, the criterion for a performance decrement was met. Examples of a baseline run, the tolerance limit, and a test response that meets the criterion for a drug-induced performance decrement are shown in Fig. 1. Five baseline tests were performed on consecutive days dur-

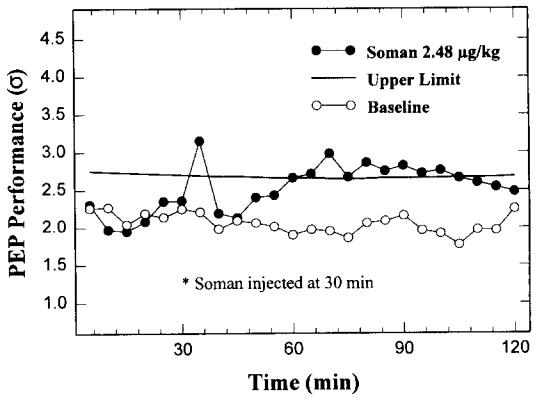

FIG. 1. Example of Primate Equilibrium Platform (PEP) performance measurement showing baseline performance, upper tolerance limits for "normal" performance, and a test in which soman induced a performance decrement.

ing the week preceding soman exposure. All five were used to set the criterion for a drug-induced performance decrement. By the nature of the up-and-down procedure for determining ED_{50}, some of the subjects were expected not to show performance decrements on or before the fifth daily soman exposure. In order further to explore the effects of daily exposure, observations were made on the effects of daily exposure continued past the fifth day in three cases in which no performance decrement was observed by the fifth day of exposure. In terms of the up-and-down procedure, these animals were considered "misses" (i.e., failures to meet the performance decrement criterion). In one animal that did meet the criterion, exposure was continued for three more days so we could observe the progressive development of more severe behavioral toxicity.

Normalized Performance Measure: PEP Impairment Score

To provide a performance score for each subject and dose as a basis for comparisons among different subjects, drugs, tasks, and even laboratories, normalized scores were computed for each drug test, based on the regression model used to compute the tolerance limits. For each 5-min performance sample following drug administration, the normalized PEP impairment score is

$$PI = (X_o - X_p)/\sigma_p,$$

where X_o = observed score, X_p = predicted score from the linear fit to the preceding baseline scores, and σ_p = standard error of prediction.

Calculation of Median Effective Dose (ED_{50})

The up-and-down method (13) 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 subject to determine the dosage for the next subject. An initial dosage and a logarithmic dosage step size were selected before the experiment. After each five-day soman test the subject's performance was compared to the performance-decrement criterion. If the subject's performance met the criterion, the next subject received a dosage one step lower; if not, the dosage for the next subject was one step higher. With appropriate choices of dosage and step size, this method can yield an adequate estimate of ED_{50} with as few as 6–10 tests (13). The initial dose in this experiment was 0.91 μg/kg, about 45% of the acute ED_{50}. Dosage step size was 0.033 $\log_{10}$ units, which had proven to be effective in acute experiments.

Drugs

Soman at ≥98% purity was obtained from the U.S. Army Medical Research and Development Command, Aberdeen Proving Ground, Maryland, 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.

Cholinesterase Assays

A venous blood sample (about 2 ml) was drawn from a convenient leg vein before the beginning of each daily test. Another sample was taken at the end of performance testing, 90–95 min after soman injection. The colorimetric method of Ellman et al. (15), using acetylthiocholine as a substrate, was used to measure the activity of all serum ChEs.

RESULTS

Performance Effects of Chronic Daily Soman Exposure

A comparison between the up-and-down test sequences for acute soman exposure and for the repeated daily soman exposure is shown in Fig. 2. The results for acute exposure from another experiment (6) showed that a dose of 2.25 μg/kg would produce a detectable performance decrement in 50% of exposed subjects (95% confidence limits: 2.02–2.51 μg/kg). Using the same dosage step size, the results for daily exposure for five days or until a performance decrement was observed (whichever came first) were somewhat more variable. The estimated daily dose (ED_{50}) required to produce a performance decrement on or before the fifth exposure day was 0.97 μg/kg/day (95% confidence limits: 0.80–1.18 μg/kg/day). The larger variability in response to daily exposure is reflected in the relative sizes of the two confidence intervals (CIs). In logarithmic terms, the confidence interval for the ED_{50} (0.169 $\log_{10}$ units) is several times as large as the confidence interval (0.048 $\log_{10}$ units) for acute exposure.

Several separate determinations of the acute ED_{50} in our laboratory (3–5) have yielded values ranging from 2.1 to 2.5

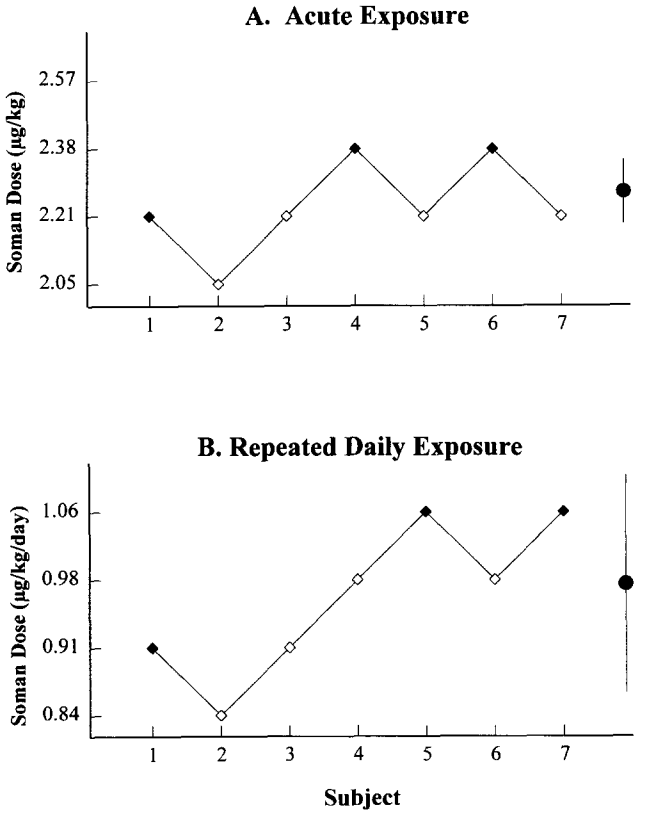

FIG. 2. Up-and-down test sequences to determine the ED_{50} soman dose for performance decrements following acute and repeated daily soman exposure. The ED_{50} and 95% confidence intervals are indicated at the right of each series. Filled symbols indicate tests in which performance decrements were produced.

μg/kg. The repeated daily dose required to produce similar performance decrements is 39–46% of the acute dose. Thus, the cumulative soman dose at the time the performance decrement was observed was 1.9 to 2.3 times the acute ED_{50}.

Changes in Cholinesterase Activity and Performance With Repeated Exposure

The mean PEP impairment scores and cumulative inhibition of ChE activity for four animals that did not show performance decrements (i.e., did not meet the preset criterion) within five exposure days and the same measures for the three animals that did show performance decrements are shown separately in Fig. 3. The results for impaired subjects on day 5 represent only two monkeys, since one monkey did not receive a fifth dose after showing a decrement on day 4. While the cumulative ChE inhibition changed dramatically for the first four to five days, the change in ChE activity produced by each dose was consistently 40–50%. The unimpaired subjects had an average daily change in ChE activity of 44.7 ± 4.4%, and cumulative inhibition of ChE activity on day 5 (86%) was still increasing from day 4 to day 5. The animals that showed performance decrements had average daily changes in ChE activity of 49.4 ± 6.0%, and cumulative inhibition reached an asymptotic level of 87% on day 4.

The consistent daily change in ChE activity and the asymptotic nature of cumulative inhibition after four to five days was confirmed in three animals that received daily soman doses for seven days (Fig. 4). Following chronic exposure, serum ChE activity was always depressed at least 85% from baseline level before performance decrements were observed. In previous acute experiments (8,9), performance decrements were associated with inhibition of ChE activity in the 65–70% range.

Neurotoxic Effects of Repeated Exposure

While the cumulative inhibition of serum ChE activity measured after each daily dose stopped changing after four to five doses, behavioral and (presumably) neurotoxic effects

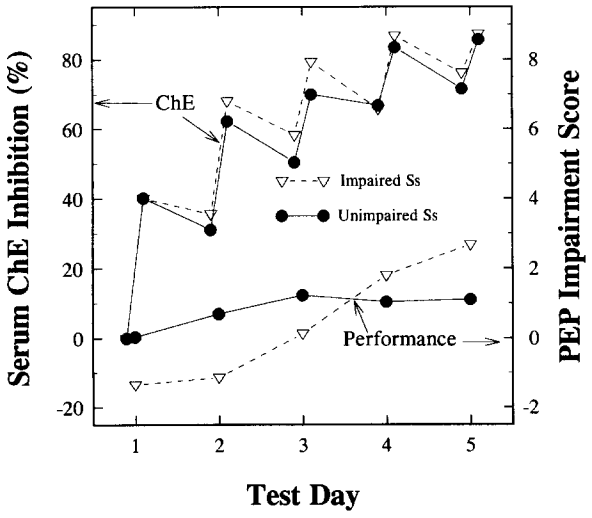

FIG. 3. Changes in serum cholinesterase (ChE) activity (left ordinate scales) and in Primate Equilibrium Platform (PEP) performance in subjects that did not show performance decrements and in subjects that met the criterion for performance decrements on or before the fifth daily exposure.

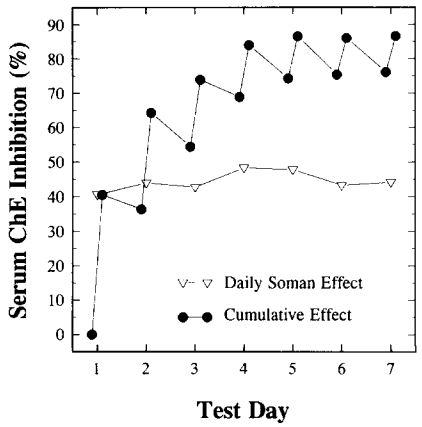

FIG. 4. Daily and cumulative changes in serum ChE activity in three animals exposed daily for seven days to soman doses near the threshold for inducing performance decrements.

continued to develop with continued exposure. The ChE and performance data from a single animal exposed for eight days are shown in Fig. 5. This example was the only case in which exposure was continued after a performance decrement occurred (on exposure day 5). This example was also the only case in which the performance failed to return to the normal range within a day or two after the last soman exposure. The magnitude of the performance decrement (Fig. 5) grew from exposure day 5 to day 8, the last exposure day. While there was a highly significant decrement in performance on exposure day 8, the subject remained able to perform the task and never completely lost control of the platform. In contrast, the subject was completely incapacitated on the day following the last exposure, displaying neurological symptoms including gross incoordination, weakness, and intention tremor. The weakness and incoordination appeared to be greatest for the proximal musculature. These symptoms persisted for several days, during which the animal remained unable to perform the PEP task. The subject's symptoms had abated seven days after the last soman dose, and PEP performance returned to nearly normal.

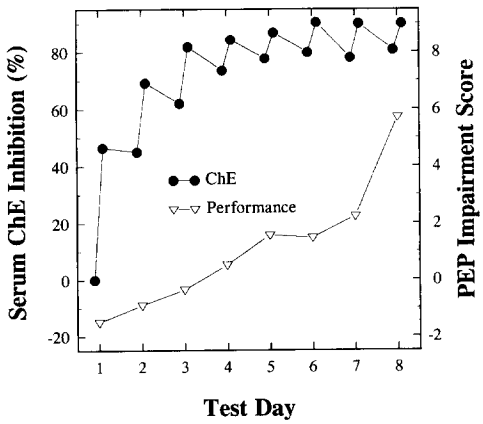

FIG. 5. Effects on PEP performance (right ordinate scale) and on ChE activity (left ordinate scale) in one subject exposed to a daily soman dose of 0.91 μg/kg/day for eight days.

DISCUSSION

Repeated Versus Acute Exposure Effects

The effects on performance of repeated daily exposure to soman differ from those of acute exposure in several important respects:

- The subject-to-subject variation in response to repeated exposure is greater than the variability of response to acute exposure.
- The maximum tolerable level of repeated daily dosage is, at most, 40–50% of the tolerable acute dose. Thus, with daily exposure, the tolerable cumulative exposure is only about two times the tolerable acute exposure.
- In a chronic exposure situation, performance degradation may provide a sensitive warning preceding the development of neurotoxic effects.
- With chronic exposure, performance decrements are exhibited at higher levels of ChE inhibition (about 90%) than with acute exposure (about 70%).

These differences will be discussed in turn.

Variability of Performance Effects

In our experience with more than 50 observations of the effects of acute soman exposure on PEP performance (6,9) dosages below 2.05 μg/kg have never produced significant performance decrements. In the absence of effective pretreatment/therapy combinations, dosages greater than 2.57 μg/kg have always produced significant performance decrements. Within this range, the frequency of performance effects increases monotonically with dosage. The PEP performance effects of acute soman are thus characterized by a very low level of variability; the dose that has always produced decrements differs from the one that never has by only about 0.1 $\log_{10}$ units (<26%). The dosage step we have used for acute studies (0.033 $\log_{10}$ units) appears to be a reasonable estimate of the standard deviation of the underlying distribution of performance effects.

Responses to the repeated exposure schedule are substantially more variable than those to acute exposure. Due to our much more limited experience with repeated daily exposure (seven observations in this experiment), we cannot yet specify the dosages corresponding to the extremes of the distribution of performance effects. Our results suggest that they differ by more than 0.15 $\log_{10}$ units (>41%), indicating substantially greater variability for the effects of repeated daily exposure than for acute exposure effects. Thus, the dose–effect function for repeated exposure may not be as steep as in the case of acute exposure. An attempt to estimate a "safe" exposure level must take this difference into account. This also implies that a larger dosage step size is more appropriate for repeated-exposure studies.

The greater variability in responses to repeated exposure may arise, at least in part, from mechanisms involved in the development of tolerance. Tolerance development to both the behavioral (2,21) and physiological (12,28) effects of repeated exposure to OP agents has been well documented. Research on the development of such tolerance effects has suggested several possible underlying mechanisms: downregulation in the number and/or sensitivity of ACh receptors (10,21,31), reductions in the production or release of ACh by presynaptic terminals (29,30,33), and compensatory changes in neural systems involving transmitter substances other than ACh (19,31). To the extent that these mechanisms contribute to the performance effects of repeated doses, they would also contribute to the variability of these effects. It is possible that the prior experience of our monkeys with soman (four acute exposures to doses from 1.9 to 2.8 μg/kg, separated by at least six weeks, at least six weeks prior to the beginning of this experiment) may have contributed to the observed variability in their response to daily low-dose exposure. Since all subjects had extremely similar prior experience, we think that this is unlikely. Other work (6) found no evidence of carryover effects of such exposures. Each of four acute ED_{50} determinations yielded similar values, and there was no change in either the mean level or the variability of baseline PEP performance tested before and six weeks after each exposure. Six additional ED_{50} determinations for five-day repeated exposures have been conducted, three in soman-naive monkeys (3) and three later in the same subjects (5). When these subjects were naive to soman, two of the three ED_{50} determinations had larger 95% CIs than reported in the present experiment; one was smaller. All three of the later ED_{50} determinations performed on these same subjects had 95% CIs similar to the one reported here. However, the possibility that prior soman exposure may contribute to variability in response to daily repeated exposure, even though it does not contribute to variability in response to acute exposure, can be completely eliminated only by further testing.

Tolerable Doses Under Varying Exposure Conditions

The PEP performance decrements after repeated daily soman exposure for up to five days occur at daily doses that are about 45% of the acute ED_{50}. Because of the observed small variability in responses to acute exposure, a dose only 0.1 $\log_{10}$ units (26%) less than the ED_{50} might be considered "safe" with regard to performance of the PEP task; such a dose would be expected to produce performance decrements in less than 5% of subjects like ours. The greater variability in response to repeated exposure implies a different relationship between the ED_{50} and the "safe" dose. To be considered "safe," a daily exposure would have to be a smaller fraction of the daily ED_{50} than the "safe" acute dose would be of the acute ED_{50}. Thus, although the cumulative total exposure associated with performance decrements at the repeated dosing ED_{50} is more than twice the acute ED_{50}, the higher variability of repeated dose effects suggests that the "safe" total exposure level under repeated exposure conditions would have to be somewhat less than twice the "safe" acute exposure level.

Performance Effects: Early Warning of Neurotoxicity?

Reliably detectable decrements in PEP performance occur at soman doses that generally do not produce extensive clinical symptoms (e.g., miosis, salivation, muscle fasciculations). In a previous experiment (6) we exposed animals repeatedly to doses near the acute ED_{50} for performance decrements (2.1–2.5 μg/kg), allowing sufficient time (six to eight weeks) between exposures for blood ChE activity to return to normal levels. Under those conditions there were no detectable carryover effects from one exposure to another. Performance generally recovered within 24 h; in many cases it returned to normal levels within 1 h.

In the repeated-exposure experiments reported here, animals received much larger total doses (4.5–10.8 μg/kg) spread over 4–12 days. When exposure was discontinued after the first detectable performance decrement, performance quickly returned to normal, and no symptoms of neurotoxicity were observed. In the single case in which an animal was exposed for 3 additional days after the first detectable performance

decrement, clear neurological symptoms developed. The symptoms were quite severe and the animal was incapable of performing the task for several days. The symptoms then abated gradually, disappearing in two to three weeks. While these results are insufficient to permit any firm conclusions about low-dose neurotoxic effects, they suggest an interesting hypothesis for further study: Subtle neurotoxic effects that occur at doses sufficient only to induce small performance decrements may be largely reversible, provided that no further exposure occurs before recovery is essentially complete. However, if the CNS function is already sufficiently compromised by soman exposure to reveal performance decrements, further exposure may produce more severe debilitations and possibly irreversible CNS damage. If this hypothesis were supported by further data, it would imply that long-term health risks may be associated with any soman exposure occurring within a few days to a few weeks after a soman exposure that produced a performance decrement. The subject exposed after showing performance decrements did not display neurological symptoms and inability to perform the PEP testing immediately after the last soman dose. The symptoms became obvious the following day, during what was to have been a test of performance recovery following cessation of exposure. A delayed neurotoxic effect may thus manifest itself after soman has been detoxified, while ChE activity levels are not changing rapidly. Gause et al. (18) reported that baboons exposed weekly to doses in the 2–5-μg/kg range sometimes developed seizure episodes that increased in severity after exposure was discontinued and blood ChE activity had substantially recovered. Although our gross examination of the CNS of the symptomatic monkey failed to reveal any obvious neuropathology, other workers have reported varying degrees of neural degeneration following sublethal soman exposure (24, 25,27). Future work should include a more sensitive neurohistological examination of affected subjects to more effectively examine a possible relationship between behavioral/neurological abnormalities and neural degeneration following repeated soman exposure.

The Relationship of ChE Activity to Performance Decrements

Since the primary physiological action of OP "nerve agents" such as soman is the inhibition of ChEs, including AChE, it would seem reasonable to expect that measures of ChE activity in body tissues could be readily related to the toxic effects of these agents. Acetylcholinesterase activity is essential to the normal function of ACh as a transmitter substance at the neuromuscular junction, in the CNS, and in the autonomic nervous system (1). The effects of certain OP agents on ACh-mediated neural and neuroeffector transmission have led to the common use of the term "nerve agent" to refer to these compounds. Investigations of these effects commonly include measures of tissue ChE activity, in the hope that such measurements can be related to observed toxic effects (e.g., fatality rates or the frequency or severity of neural lesions), performance decrements, or other observable symptoms of intoxication.

Lethality following OP nerve agent exposure has been attributed to respiratory failure produced by a buildup of excessive ACh at central or peripheral sites due to a reduction in ChE activity. The excessive ACh at a variety of sites can simultaneously produce several effects that interfere with normal respiration: increased airway resistance, weakness and reduced compliance of the respiratory musculature, and decreased driving signals from brain stem respiratory control centers (1). Effects of anticholinesterases can depend not only on the extent of ChE inhibition at multiple sites, but also on rates of change of ChE activity and on the prior history of exposure. Thus even the most readily measured outcome of soman intoxication, death by respiratory failure, appears to be difficult to predict from a measurement of ChE activity at any single site.

In spite of such difficulties, there is a long history of research on the relationship of tissue ChE activity to other measures of the effects of OP agents [e.g., (10,12,17,20,22)]. To summarize, studies of acute exposure indicate that there is a strong relationship between tissue ChE activity and symptoms during the initial, acute phase of toxicity that tends to weaken or disappear soon thereafter. The strength of the relationship also depends on the OP compound, the toxic signs observed, and the tissue from which ChE activity is assayed. The species of the subjects is also an important variable.

Relationships between ChE activity and signs of toxicity are even more complex following chronic OP exposure (11,20,33). Different repeated doses produce asymptotic levels of ChE inhibition that do not differ significantly, although the rate of approach to asymptote varies in the expected way with dose. The development of behavioral signs or symptoms of toxicity, as well as the development of tolerance, usually occur after the measured level of ChE activity has stopped changing. Thus, especially with chronic OP exposure, the dosage and dosage history generally provide much more accurate predictions of symptom development than do measurements of ChE activity.

Our results have also shown that performance decrements are not well predicted from a simple measure of ChE activity. With acute exposure, average serum ChE activity was inhibited about 70% when performance decrements occurred. With daily repeated exposure, we frequently observed ChE activity inhibited more than 70% 24 h after exposure in animals that showed no decrement at that time. The level of serum ChE inhibition measured after each daily dose had typically reached an asymptotic level of 85–90%, one to three days before any performance decrement could be observed. Not only was this inhibition level significantly different from that observed in the acute case, but similar levels of inhibition occurred in both the presence and absence of performance effects. Dosage and dosage history are thus much better predictors of performance effects than ChE measurements. Cholinesterase measurements can have limited usefulness if baseline ChE activity is known but exposure history is not known. Our experience with laboratory primates suggests that an exposure that leaves serum ChE activity more than 45–50% inhibited cannot safely be repeated within 24 h. However, if an exposure produces less than about 30% inhibition of serum ChE activity, then a similar exposure would be unlikely to produce a performance decrement or any other symptom if it were repeated at intervals of 24 h or more.

CONCLUSIONS

Primate Equilibrium Platform task performance provides a sensitive and reliable measure of soman's behavioral toxicity in laboratory primates. Performance decrements can be reliably detected in the absence of any overt signs of toxicity. However, if chronic exposure continues after the threshold for performance decrements has been crossed, adverse neurological symptoms may develop.

In terms of ED_{50}, the repeated daily dose required to pro-

duce detectable performance decrements within five days is 39–46% of the acute dose required to produce a similar decrement. However, since the effects of repeated exposure exhibit greater variability than the effects of acute exposure, a "safe" level of repeated daily exposure would necessarily be less than 39–46% of a "safe" level of acute exposure.

Measurements of ChE activity in readily accessible tissues like blood have very limited usefulness in predicting the occurrence of performance decrements or other toxic signs following soman exposure.

ACKNOWLEDGEMENTS

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

REFERENCES

1. Barrett, E. F.; Magleby, K. L. Physiology of cholinergic transmission. In: Goldberg, A. M.; Hanin, I., eds. Biology of cholinergic transmission. New York: Raven Press; 1976:29–99.
2. Bienhold, H.; Flohr, H. Role of cholinergic synapses in vestibular compensation. Brain Res. 195:476–478; 1980.
3. Blick, D. W.; Kerenyi, S. Z.; Miller, S. A.; 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.
4. Blick, D. W.; Miller, S. A.; Brown, G. C.; Murphy, M. R. Animal-to-human extrapolation: I. Ethanol effects on compensatory tracking performance in rhesus monkeys. Soc. Neurosci. Abstr. 18:107; 1992.
5. Blick, D. W.; Miller, S. A.; Brown, G. C.; Murphy, M. R. Behavioral toxicity of anticholinesterases in primates: Chronic physostigmine and soman interactions. Pharmacol. Biochem. Behav. 45:677–683; 1993.
6. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Hartgraves, S. L. Primate performance decrements following acute soman exposure: Failure of chemical countermeasures. Pharmacol. Biochem. Behav.; in press.
7. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Yochmowitz, M. G.; Fanton, J. W.; Hartgraves, S. L. Acute behavioral toxicity of pyridostigmine or soman in primates. Toxicol. Appl. Pharmacol.; in press.
8. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Yochmowitz, M. G.; Farrer, D. N. Effects of carbamate pretreatment and oxime therapy on soman-induced performance decrements and blood cholinesterase activity in primates. SAM-TR-87-23. Brooks AFB, TX: USAF School of Aerospace Medicine; 1987.
9. Blick, D. W.; Murphy, M. R.; Brown, G. C.; Yochmowitz, M. G.; Hartgraves, S. L. Effects of soman or pyridostigmine on primate equilibrium performance and blood cholinesterase. Soc. Neurosci. Abstr. 12:1203; 1986.
10. Bourdois, P. S.; Mitchell, J. F.; Somogzi, G. T.; Szerb, J. L. The output per stimulus of acetylcholine from cerebral cortical slices in the presence or absence of cholinesterase inhibition. Br. J. Pharmacol. 52:509–517; 1974.
11. Chippendale, T. J. G.; Zawolkow, G. A.; Russell, R. W.; Overstreet, D. H. Tolerance to low acetylcholinesterase levels: Modification of behavior without acute behavioral change. Psychopharmacology 26:127–139; 1985.
12. Costa, L. G.; Schwab, B. W.; Murphy, S. D. Tolerance to anticholinesterase compounds in mammals. Toxicology 25:99–111; 1982.
13. Dixon, W. J.; Massey, F. J., Jr. Introduction to statistical analysis, 4th ed. New York: McGraw-Hill; 1983.
14. Doctor, B. P.; Blick, D. W.; Gentry, M. K.; Maxwell, D. M.; Miller, S. A.; Murphy, M. R.; Wolfe, A. D. Acetylcholinesterase: A pretreatment drug for organophosphate toxicity. In: Shafferman, A.; Velan, B., eds. Multidisciplinary approaches to cholinesterase functions. New York: Plenum Press; 1992:277–284.
15. 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.
16. Farrer, D. N.; Yochmowitz, M. G.; Mattsson, J. L.; Lof, N. E.; Bennett, C. T. Effects of benactyzine on an equilibrium and multiple response task in rhesus monkeys. Pharmacol. Biochem. Behav. 16:605–609; 1982.
17. Freedman, A. M.; Willis, A.; Himwich, H. E. Correlation between the signs of toxicity and cholinesterase level of brain and blood during recovery from di-isopropyl fluorophosphate (DFP) poisoning. DFP Cholinesterase 157:80–87; 1949.
18. Gause, E. M.; Hartmann, R. J.; Leal, B. Z.; Geller I. Neurobehavioral effects of repeated sublethal soman in primates. Pharmacol. Biochem. Behav. 23:1003–1012; 1985.
19. Ho, I. K.; Fernando, J. C. R.; Sivam, S. P.; Hoskins, B. Roles of dopamine and GABA in neurotoxicity of organophosphorus cholinesterase inhibitors. Proc. West. Pharmacol. Soc. 27:177–180; 1984.
20. Hoskins, B.; Fernando, J. C. R.; Dulaney, M. D.; Lim, D. K.; Liu, D. D.; Watanabe, H. K.; Ho I. K. Relationship between the neurotoxicities of soman, sarin and tabun, and acetylcholinesterase inhibition. Toxicol. Lett. 30:121–129; 1986.
21. Hymowitz, N.; Brezenoff, H. E.; McGee, J.; Campbell, K.; Knight, T. Effect of repeated intraperitoneal injections of soman on schedule-controlled behavior in the rat. Psychopharmacology 86:404–408; 1985.
22. Jovic, R. C. Correlation between signs of toxicity and some biochemical changes in rats poisoned by soman. Eur. J. Pharmacol. 25:159–164; 1974.
23. Lieberman, G. J.; Miller, R. G. Simultaneous tolerance intervals in regression. Biometrika 50:155–168; 1963.
24. McDonough, J. H., Jr.; Jaax, N. K.; Crowley, R. A.; Mays, M. Z.; Modrow, H. E. Atropine and/or diazepam therapy protects agains soman-induced neural and cardiac pathology. Fundam. Appl. Toxicol. 13:256–276; 1989.
25. McLeod, C. G. Pathology of nerve agents: Perspectives on medical management. Fundam. Appl. Toxicol. 5:S10–S16; 1985.
26. Murphy, M. R.; Blick, D. W.; Dunn, M.; Fanton, J. W.; Hartgraves, S. L. Diazepam as a treatment for nerve agent poisoning. Aerosp. Med. 64:110–115; 1993.
27. Petras, J. M. Soman neurotoxicity. Fundam. Appl. Toxicol. 1: 242; 1981.
28. Russell, R. W.; Booth, R. A.; Jenden, D. J.; Roch, M.; Rice, K. M. Changes in presynaptic release of acetylcholine during development of tolerance to the anticholinesterase, DFP. J. Neurochem. 45:293–299; 1985.
29. Saelens, J. K.; Simpke, J. P.; Schuman, J; Allens, M. P. Agents which influence acetylcholine metabolism in mouse brain. Arch. Int. Pharmacodyn. Ther. 209:250–258; 1974.
30. Taylor, P. Anticholinesterase agents. In: Gilman, A. G.; Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics, 6th ed. New York: Macmillan; 1980:100–119.
31. Valdes, J. J.; Shih, T.-M.; Whalley, C. Soman intoxication alters regional brain muscarinic and GABA receptors. Pesticide Biochem. Physiol. 24:355–361; 1985.
32. 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.
33. Yamada, S.; Isogai, M.; Okudaira, H.; Hayashi, E. Correlation between cholinesterase inhibition and reduction in muscarinic receptors and choline uptake by repeated diisopropylfluorophosphate administration: Antagonism by physostigmine and atropine. J. Pharmacol. Exp. Ther. 266:519–525; 1983.