Pharmacology Biochemistry & Behavior, Vol. 22, pp. 961–966, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Acute Soman Effects in the Juvenile Baboon: Effects on a Match-to-Sample Discrimination Task and on Total Blood Acetylcholinesterase

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Received 8 November 1984

GELLER, I., R. J. HARTMANN, E. MORAN, B. Z. LEAL, R. J. HAINES AND E. M. GAUSE. *Acute soman effects in the juvenile baboon: Effects on a match-to-sample discrimination task and on total blood acetylcholinesterase.* PHARMACOL BIOCHEM BEHAV 22(6) 961–966, 1985.—Male juvenile baboons, trained on a match-to-sample operant discrimination task, were given acute intramuscular injections of soman (methyl pinacolyl phosphonofluoridate) at 1.0, 2.0, 3.0, 4.0, and 5.0 μg/kg. The different doses were given in a mixed order just before a behavioral test session. Just prior to administration of each soman dose and immediately following the 2-hr behavioral test session, a sample of blood (0.5 ml) was drawn from the baboon and analyzed for inhibition of acetylcholinesterase activity. Thereafter, blood sampling was accomplished at weekly intervals and soman was administered again only when whole blood acetylcholinesterase reached at least 80% of pre-soman control level. Behavioral effects of soman included a slowing of response times, a decrease in extra inconsequential responses, a decrease in responsiveness to the visual stimuli and an increase in errors. These effects were observed when acetylcholinesterase (AChE) levels fell to 25 μmoles/hr/ml blood or less. The threshold dose for behavioral effects was very close to the dose of soman which induced seizures.

Soman Baboon Delayed match-to-sample discrimination Irreversible acetylcholinesterase inhibition

SOMAN (0-1,2,2-trimethylpropylmethylphosphonofluoridate) is a potent, rapidly-acting, irreversible inhibitor of acetylcholinesterase, an enzyme which is critically important to both central and peripheral nervous systems. There is evidence that persistent psychological and neurobehavioral disorders can develop after sublethal exposure to organophosphates.

Studies of humans, accidently exposed to other acetylcholinesterase inhibitors, indicate EEG changes that persist as long as a year post exposure [2]. A single or limited period of low level exposure to organophosphates produced persistent disorders of affect, emotion and memory [10], psychological dysfunctions such as disturbed memory, impairment of alertness or attention focussing [1,5] and slowing of intellectual and motor responses, agitation and tenseness [1].

The present study has addressed the question of whether sublethal exposure to soman might significantly impair an individual's ability to perform a complex task requiring integration of CNS functions. An appropriate animal behavioral model for the study of anticholinesterase compounds should include an animal whose body systems approximate those of the human and a behavioral task that measures cognitive ability, alertness, memory and focussing of attention. The juvenile baboon trained on a match-to-sample (MTS) discrimination task satisfies these requirements, and it was employed in this study.

METHOD

The subjects were six male juvenile baboons (*Papio cynocephalus*) ranging in age from 1 year to 20 months when obtained from the breeding colony of Southwest Foundation for Biomedical Research. They were housed in behavioral test chambers with an intelligence panel on one wall. The panel contained a row of three translucent discs (levers) on which visual stimuli could be projected. Under the appropriate experimental conditions, pressing either side disc activated a feeder which delivered a banana pellet reward. Experimental sessions of 2-hr duration were conducted on Monday through Friday of each week. The procedure for training the baboons on the MTS task was as follows. When a session timer was activated a variable-interval (VI) programming tape was set in motion. The tape programmed the occurrence of a stimulus on the center lever on the average of once every 3 minutes. The VI tape was inoperative during each trial, which began with the illumination of one of the

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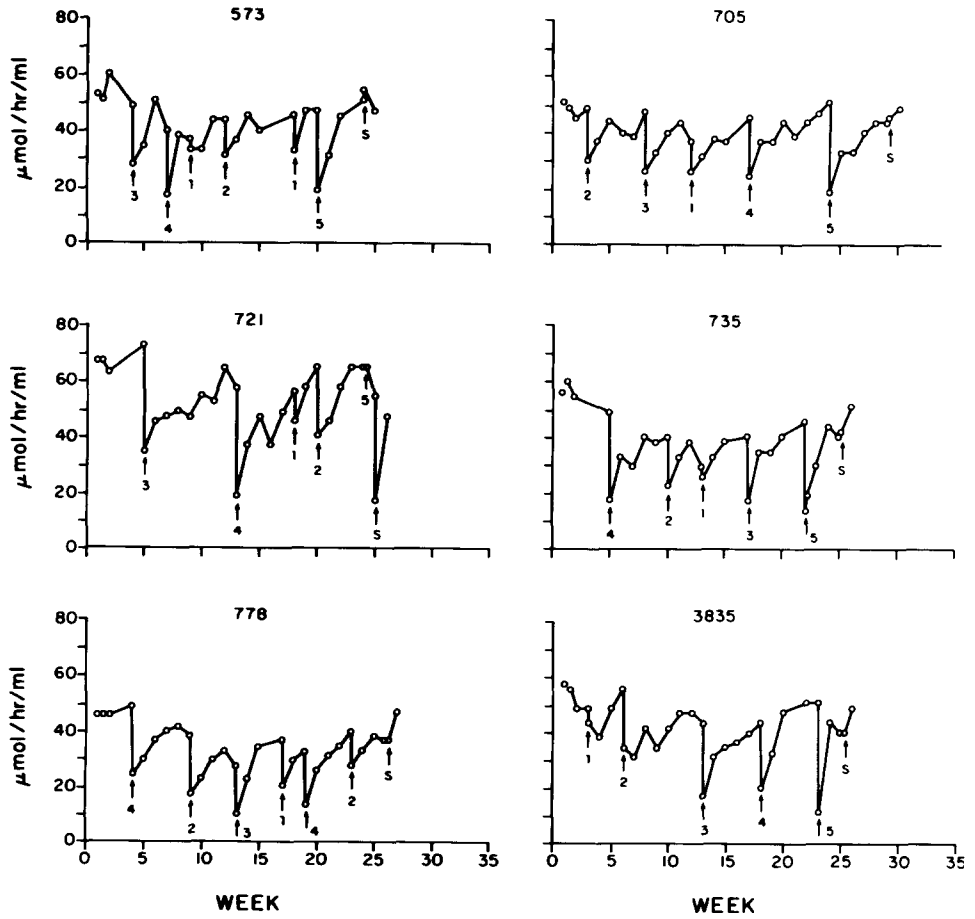

FIG. 1. Effects of soman on blood AChE of six baboons. The numbers and arrows indicate the dose of soman in µg/kg and the days of soman administration respectively. S represents saline administration. AChE activity is expressed as µmoles/hr/ml.

stimuli on the center lever or probe stimulus. This stimulus was terminated at the end of a 30-sec period or by a response on the lever. Termination of the stimulus activated a timer for 2 min, called the delay interval. At the end of the delay interval, stimuli appeared on both levers adjacent to the center lever. The correct matching stimulus was varied between these two levers in a mixed order. A response on the correct lever, when the stimulus matched the center lever stimulus, terminated the stimuli, activated the feeder and produced a banana pellet reward. Responses on the incorrect lever simply terminated the stimuli and again set the VI tape in motion.

A record was kept of the number of probe stimuli presented during each 15-min segment of a 2-hr session, the number of incorrect matching responses on the right and left levers and the number of extra responses that occurred on any of the three levers in the absence of discriminative stimuli or during the delay interval. Also measured were the percent of trials worked by the baboon and the time it took the baboon to respond, once a stimulus had been activated (response time). A 20-sec time limit was imposed so that if an animal did not respond in 20 seconds, the side stimuli were terminated.

Soman was kept at −80 degrees C until ready for use. It was prepared in saline at the appropriate dilutions so that the different doses might be administered in equivalent volumes. The baboons were given intramuscular (gastrocnemius muscle) injections of soman in doses of 1.0, 2.0, 3.0, 4.0 or 5.0 µg/kg. One baboon did not receive the 5.0 µg/kg dose since the effects observed under 4.0 µg/kg suggested that 5 µg/kg might prove to be too toxic for this animal. The drug was given on Wednesday or Thursday immediately prior to the behavioral session. The order of dosing was mixed so that it differed for all animals. Time between dosings was determined by monitoring blood acetylcholinesterase activity. Blood samples were taken from the baboons 10 min before soman and 130 min later, 10 min after termination of the behavioral session. Total blood acetylcholinesterase activity was assayed immediately by the spectrophotometric coupled assay [3]. Post-soman acetylcholinesterase activity was expressed as a percent of the pre-soman control activity obtained 2 hr earlier. Thereafter, blood samples were obtained at weekly intervals to monitor recovery of total acetylcholinesterase activity. When the value reached at least 80% of control, the animal was given the next soman injection.

RESULTS

Whole blood AChE activity was monitored throughout the period of the acute soman administration experiments

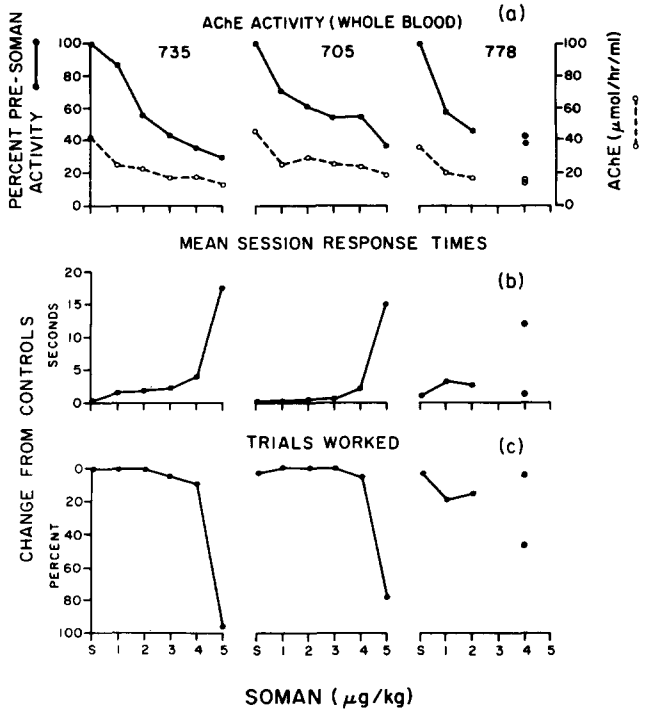

FIG. 2. Effects of soman on whole blood AChE activity, response times and percent trials worked on a match-to-sample discrimination task. AChE activity is expressed as μmole/hr/ml and as percent pre-soman activity; response times in seconds are shown as change from pre-drug day controls; percent trials worked are shown as change from pre-drug day controls.

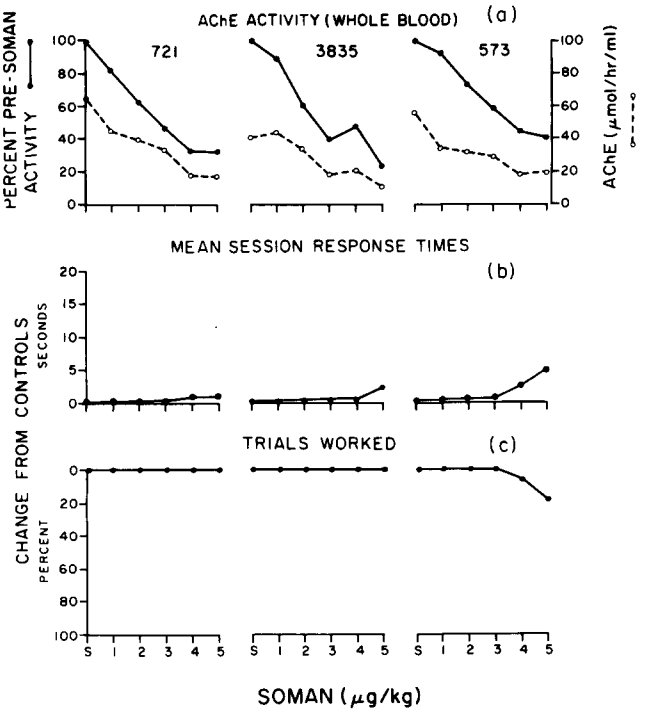

FIG. 3. Effects of soman on whole blood AChE activity, response times and percent trials worked on a match-to-sample discrimination task. AChE activity is expressed as μmole/hr/ml and as percent pre-soman activity; mean session response times in seconds are shown as change from pre-drug day controls; percent trials worked are shown as change from pre-drug day controls.

and is shown in Fig. 1 for each baboon. Acetylcholinesterase activities are expressed as μmoles/hr/ml blood; the numbers and the arrows indicate, respectively, the doses and days on which soman was given. The times required for blood AChE levels to return to at least 80% of control level varied between baboons; recovery times were generally 1–2 weeks for baboon 573, 2–4 weeks for baboon 735, 3–4 weeks for baboon 778, 3–6 weeks for baboons 705 and 3835 and 4–7 weeks for baboon 721.

Figures 2 and 3 contain data of six juvenile baboons showing various behavioral parameters as a function of soman dose: (a) changes in blood AChE expressed as μmoles/hr/ml and as a percent of pre-soman AChE activity, (b) mean session response time in seconds, expressed as difference between pre-soman day and exposure day; and (c) number of trials worked during the session, expressed as percent change from control day.

There was a marked dose-effect of this level of soman exposure upon whole blood AChE activities. For the 5 baboons which received the 5 μg/kg dose, blood AChE activity levels were reduced to 24–40% of pre-soman activity levels, corresponding to 60–76% enzyme inhibition (Figs. 2(a) and 3(a)). The sixth baboon (778) was not given 5 μg/kg; however, because of computer problems resulting in loss of behavioral data, this animal received 4 μg/kg on three separate occasions and blood AChE activities were reduced to 50, 40, and 41 percent of control levels, respectively, by this dose.

At the highest soman doses, mean session response times increased for all animals. The largest increases were observed for the three animals shown in Fig. 2(b); for these animals, the mean response times increased from 12 to 17 sec compared to the prior (baseline) day.

The percentage of trials worked during the session was reduced for four of the six baboons by soman (Figs. 2(c) and 3(c)); 3 of these animals were the 3 which exhibited maximal increases in mean response times (Fig. 2(b)), and the other was Baboon 573 (Fig. 3(b)).

Of the four baboons which exhibited increases in mean response times and decreases in percent trials worked after soman (Figs. 2 and 3 (b)–(c)), only two of these animals made errors—i.e., incorrect responses—during the sessions immediately following 4–5 μg/kg soman administration. The occurrence of errors for two animals are shown in Fig. 4; on two occasions, 778 received 4 μg/kg (on a third occasion, all behavioral data was lost) and made errors both times (Figs. 4(a) and (b)) while 573 made errors after 5 μg/kg (Fig. 4(c)). The trial-by-trial response times during the pre-soman control day and soman day sessions are plotted and the trials on which errors occurred are indicated by "E." After completion of the session shown in Fig. 4(c), post-session blood was drawn as usual; 573 subsequently assumed a crouching position and held his midsection, remaining in this position for approximately 1 hr.

The onset of behavioral effects appeared to correlate better with the depression of blood AChE activity to actual levels of 24 μmole/hr/ml or less, than with the percentage AChE inhibition produced. This absolute level of activity was produced by the 4–5 μg/kg doses, primarily, and represented 43–77 percent inhibition of the pre-soman AChE activity. The one exception was baboon 721, in which effects

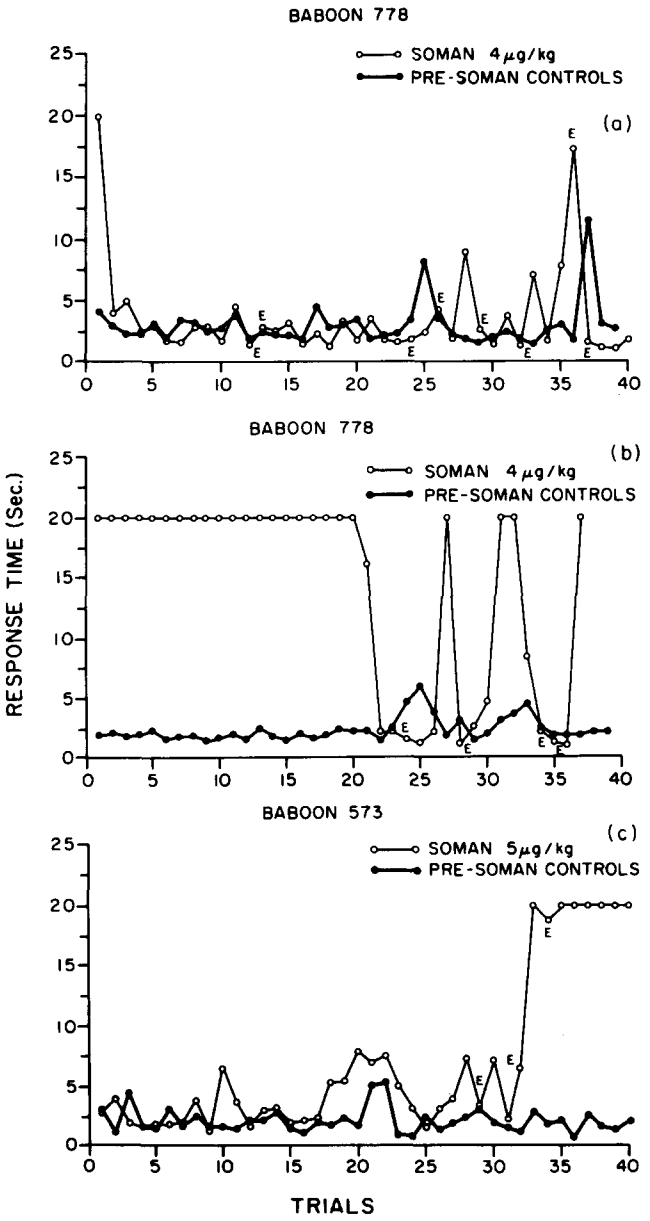

FIG. 4. Effects of soman on response times. Data obtained under 4 μg/kg for baboon 778 are shown in the top two panels. Data obtained under 5 μg/kg for baboon 573 are shown in the bottom panel. Closed circles represent pre-drug day soman controls. Open circles represent soman data. Errors under soman are represented by E's.

upon behavior were observed with a blood AChE level of 35 μmol/hr/ml (equivalent to 52% inhibition) after 3 μg/kg soman.

Administration of soman produced a reduction in the number of extra responses during the soman day session in most instances. However, during the weeks following soman administration, the number of extra responses generally increased above pre-soman baseline levels for all animals. This effect is shown in Fig. 5 as the maximum number of extra responses per session observed each week for all animals. For five of the six baboons, the incidence of extra responses appeared to have returned to the pre-soman baseline range within 5–10 weeks after the last soman injection; the level of extra responses by baboon 721 was still elevated 7 weeks after the last soman injection (5 μg/kg), but had returned to control range within 16 weeks after soman (Fig. 5).

DISCUSSION

Administration of 1–5 μg/kg soman to juvenile baboons resulted in a dose-dependent inhibition of blood AChE. Behavioral parameters were affected in almost all instances at the highest dose levels. One animal (735) experienced several convulsions at the 5 μg/kg dose. Another baboon (573) began to exhibit behavioral effects at the 5 μg/kg dose and after the behavioral test session was observed in a crouched position holding its midsection. This behavior lasted for 1 hr.

These observations are of interest in light of previous clinical data in which EA-1701, an organophosphate anticholinesterase compound similar in properties to soman, was applied topically to male volunteers [1]. These investigators reported that while the most severely affected subjects exhibited both psychological and gastrointestinal symptoms which were frequently separated in time by several hr, less severely affected subjects usually exhibited either one kind of symptom or the other. The psychological syndrome was usually seen prior to gastrointestinal symptomatology when both were encountered in the same subject; the onset of symptoms was observed from 3.5 to 18 hr after administration of EA-1701. These investigators concluded that there was no close correlation between the psychological and gastrointestinal manifestations. Psychological effects reported were that any activity requiring retention of information was impaired. The subjects were unable to carry out tasks of simple arithmetic and had minor problems with orientation. However, there was little effect on these psychological variables unless the blood acetylcholinesterase fell to at least 40% of control level [1]. Similarly, when blood acetylcholinesterase was reduced to 40% of control for the baboon administered 5 μg/kg soman, his ability to perform the MTS task was impaired within 1 hr, and he appeared to be experiencing gastrointestinal problems within 2.5–3 hr.

Following soman administration, blood AChE recovery time to at least 80% of control varied between baboons from 1–3 weeks to as much as 4–7 weeks. Recovery times of approximately 3 weeks would be expected for regeneration of erythrocytes, and this mechanism is probably reflected in the observed 3–4 week recovery times. Recovery periods of 4–7 weeks might involve other renewal processes, or alternatively might possibly reflect a scarcity of bone marrow erythrocyte precursor cells or some other individually determined characteristic.

As illustrated in Figs. 3 and 4, the onset of soman-induced behavioral effects appears to be associated with the actual level of peripheral AChE activity produced. Behavioral effects were always observed when AChE activity fell to 25 μmole/hr or less. Behavioral effects were not observed at higher blood AChE levels except for baboon 721 who exhibited behavioral effects at 3 μg/kg soman with a blood AChE activity of 35 μmol/hr. These data suggest that when total blood AChE activity falls below 25 μmol/hr, the capacity for "scavenging" of soman by blood enzymes becomes marginal, and the remaining peripheral scavenger sites become unable to compete for soman, with the result that additional inhibitor becomes available to CNS sites.

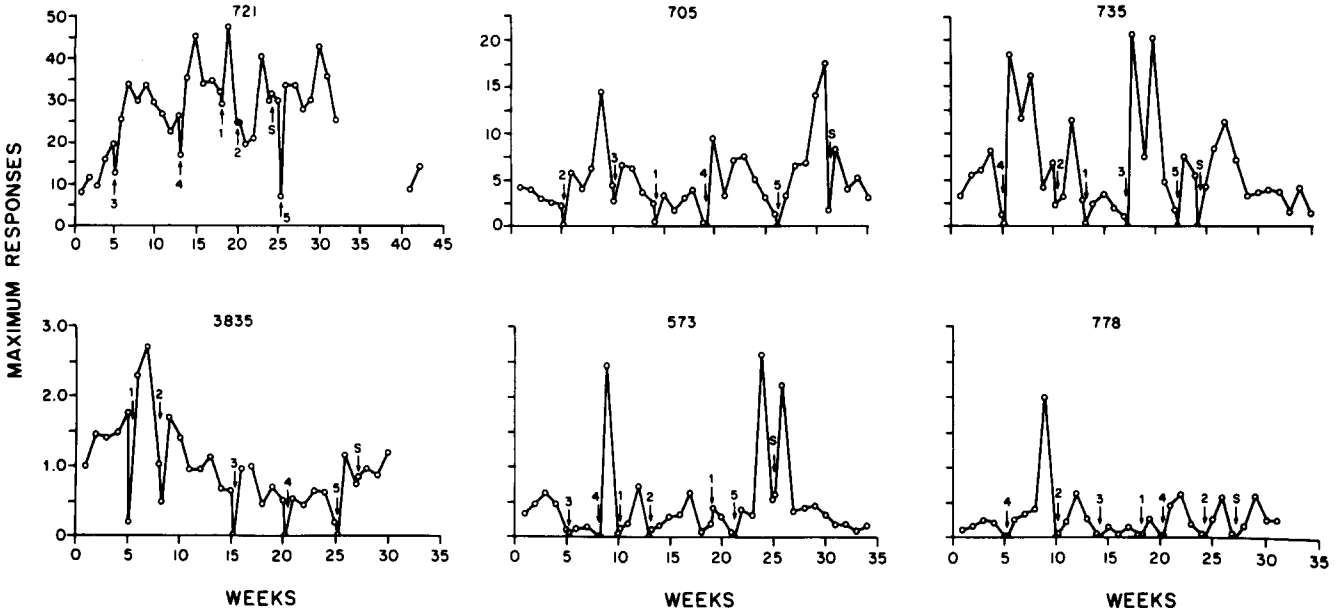

FIG. 5. Data for six individual baboons showing the maximum daily extra responses occurring during each week following soman or saline administration. The arrows and numbers indicate day of injection and μg/kg soman administered.

Baboon 778 appeared to be the most susceptible of all subjects to the behavioral effects of soman; indications of this susceptibility can be seen in Figs. 2 and 4. This animal also exhibited the lowest level of blood AChE activity prior to initial exposure to soman which would suggest the existence of fewer peripheral binding sites to scavenge AChE inhibitors, thereby resulting in increased CNS sensitivity to soman. This speculation derives support from a previous report [6] proposing that peripheral AChE protects the CNS by acting as a scavenger for AChE inhibitors.

The findings of these experiments suggest that the dose-effect of soman upon discrimination behavior is exponential. For the juvenile male baboons employed here, behavioral effects were minimal below 4 μg/kg, but increased sharply in the range of 4–5 μg/kg soman. The initial effects appear to be a slowing of mean session response times and a reduction in the number of trials worked during a 2-hr period of MTS task performance (Figs. 2–4). Errors in the performance of the task were observed for two of six animals in these experiments (Fig. 4). However, the monitoring of errors was complicated by the lack of responding to a number of the trials during the sessions. Similar dose-effects upon behavior were reported for the OP, parathion; 0.5 mg/kg had no effects on performance of a visual discrimination task by monkeys, while 1.0 mg/kg abolished all behavior, but neither dose produced incorrect responses [7].

An interesting observation was that extra (inconsequential) responses, which decreased on the day of soman administration, consistently increased significantly in the weeks following exposure (Fig. 5). The next dose of soman again produced a decrease in the number of extra responses, followed in turn by another increase in subsequent weeks. The maximal numbers of extra responses appeared to occur 2–4 weeks post soman. After the last soman injection, the extra responses had returned to pre-soman baseline levels for five of the six baboons, within 1 to 10 weeks; however, one animal (721) did not recover within 10 weeks, but had recovered by 16 weeks post-soman.

These post-soman increases in numbers of extra responses were unexpected and were not systematically investigated in the present study. This data must therefore be regarded as an incomplete characterization of this response. Similar alterations in behavior following inhibition of AChE have been reported [8]. A slowing of avoidance extinction and an increase in responding was associated with a decrease in brain ChE below 60% of normal. It was suggested that a decrease in brain ChE activity might be related to behavioral hyperactivity. Other investigators have reported a resistance to extinction of fixed ratio behavior in rats treated with diisopropyl fluorophosphate (DFP) and speculated that resistance to extinction might be related to peripheral cholinesterase inhibition [4]. Since a further overshoot in responses above control levels was made on the first extinction day, the authors attributed this to *de novo* synthesis of brain ChE allowing brain levels to increase above 40% of normal levels.

Russell *et al.* [9] measured food intake, water intake and laps of rats during tolerance development to and withdrawal from DFP. They observed suprabaseline drinking performance during withdrawal from DFP.

The possibility that the increases in operant and non-operant behaviors in the above-cited studies may be related through a common underlying mechanism to the observed increases in extra responses in the present study is speculative at best, since a different species was used and different behaviors were evaluated.

ACKNOWLEDGEMENT

This research was supported by Department of the Army Contract No. DAMD17-82-C-2161 and was presented in part at the 1984 meetings of the Western Pharmacology Society, Reno, NV.

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