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Behavioral and Immunological Effects of Exogenous Butyrylcholinesterase in Rhesus Monkeys

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MATZKE, S. M., J. L. OUBRE, G. R. CARANTO, M. K. GENTRY AND G. GALBICKA. *Behavioral and immunological effects of exogenous butyrylcholinesterase in rhesus monkeys.* PHARMACOL BIOCHEM BEHAV **62**(3) 523–530, 1999.—Although conventional therapies prevent organophosphate (OP) lethality, laboratory animals exposed to such treatments typically display behavioral incapacitation. Pretreatment with purified exogenous human or equine serum butyrylcholinesterase (Eq-BuChE), conversely, has effectively prevented OP lethality in rats and rhesus monkeys, without producing the adverse side effects associated with conventional treatments. In monkeys, however, using a commercial preparation of Eq-BuChE has been reported to incapacitate responding. In the present study, repeated administration of commercially prepared Eq-BuChE had no systematic effect on behavior in rhesus monkeys as measured by a six-item serial probe recognition task, despite 7- to 18-fold increases in baseline BuChE levels in blood. Antibody production induced by the enzyme was slight after the first injection and more pronounced following the second injection. The lack of behavioral effects, the relatively long in vivo half-life, and the previously demonstrated efficacy of BuChE as a biological scavenger for highly toxic OPs make BuChE potentially more effective than current treatment regimens for OP toxicity. © 1999 Elsevier Science Inc.

Butyrylcholinesterase Serial probe recognition (SPR) Rhesus monkeys Immunology Operant behavior Accuracy Latency Memory

ORGANOPHOSPHATES (OPs) pose potential neurotoxic threats to both military and civilian populations, as evidenced in recent terrorist attacks, as well as occupational hazards to individuals exposed to certain insecticides. OP toxicity results from the irreversible binding to and inactivation of acetylcholinesterase (AChE), the enzyme that normally catalyzes the hydrolysis of acetylcholine (ACh), at neuromuscular junctions and other cholinergic synapses. Accumulation of ACh in the synapse causes repetitive neuronal firing, resulting ultimately in convulsions, respiratory failure, and/or death (17). Fatal respiratory failure can be prevented by timely intervention after OP exposure. However, if not sequestered in the periphery, lipophilic OPs such as soman and sarin can readily cross the blood–brain barrier and lead to neuronal death (2).

Conventional treatments for OP toxicity include administration of carbamates, cholinergic antagonists, and oxime reactivators. Carbamates, such as pyridostigmine, reversibly bind to cholinesterase (ChE), and thus protect against the irreversible binding of OP compounds (12). Atropine and other cholinergic antagonists block ACh receptors, preventing repetitive neuronal firing due to excess ACh in the synapse. Oxime reactivators, such as HI-6 [1-(2-(hydroxyimino)methyl)pyridinium-2-(4-(aminocarbonyl)pyridinium) dimethylether], work by reactivating inhibited AChE (14). Although these interventions used alone, or in combination, have effectively prevented OP lethality, their use results in various untoward side effects. For example, pyridostigmine pretreatment, and atropine posttreatment, while preventing the fatal effects of nerve agent exposure, generated substantial behavioral incapacitation in subjects so treated (14). Adding diazepam to the pyridostigimine/atropine treatment decreased convulsions and reduced the behavioral impairments after nerve agent ex-

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posure in rhesus monkeys as measured by performance on a recognition memory task (6). Even with this treatment regimen, however, performance on the task still took 6 days to return to baseline (6). These and other side effects associated with the above treatment regimens prompt a continued search for alternatives.

One recently explored option has been the administration of exogenous ChEs such as butyrylcholinesterase (BuChE) and AChE, to sequester the OP compound before it reaches its physiological targets. The efficacy of ChEs as biological scavengers to protect against OP poisoning has been demonstrated in mice (1,7,14,18,20,23), rats (2,9,16), and rhesus monkeys (3,5,15,19,22). In almost all cases, doses of ChEs sufficient to prevent death due to OP exposure have been devoid of behavioral side effects. Rats pretreated with purified equine BuChE (Eq-BuChE) then exposed either to the OP, 7-methylethoxy-phosphinyloxy-1-methylquinolinium iodide (MEPQ), or its vehicle, did not display a decrement in responding under a variable-interval schedule of food reinforcement (9). In the same study, rats performing a passive avoidance task showed no deficits in acquisition or retention of the task after Eq-BuChE, whereas atropine disrupted retention but not acquisition of the passive avoidance task (9). Human serum BuChE also has no apparent effect on performance of certain spatial tasks [e.g., a Morris water-maze task (2)].

Although behavioral effects of exogenous Eq-BuChE have generally been absent in rats, behavioral deficits due to the enzyme have been reported in rhesus monkeys. For example, monkeys trained on a recognition memory task and pretreated with commercial grade Eq-BuChE failed to respond to the task at 30 min postenzyme exposure (3). In that study, subsequent nerve agent exposure may have exacerbated the behavioral incapacitation; however, the enzyme given alone clearly disrupted responding 30 min postinjection. In a subsequent study, rhesus monkeys treated with commercial grade Eq-BuChE alone and then tested on the same task did not respond until 8 h postenzyme injection (5). Although these studies seem to indicate that Eq-BuChE produces behavioral deficits in rhesus monkeys, other studies suggest the opposite—exposure to purified Eq-BuChE did not disrupt performance of rhesus monkeys on a Primate Equilibrium Platform (PEP) task (22), or a spatial discrimination task (19). None of these studies examined the effects of a second dose of BuChE.

The present study sought to characterize the effects of repeated administration of BuChE alone on behavioral and immunological responses of rhesus monkeys. The behavioral task was a replication of the six-item serial probe recognition (SPR) task used in the studies listed above (3,5). The SPR task is a multiple item memory task adapted from a task used to evaluate memory in humans (21), and has already been established as a neurobehavioral model that reliably evaluates changes in cognitive function of nonhuman primates [e.g., (4,13,24)]. The task involves the successive presentation of six items followed by a test item. The monkeys must choose whether the test item was in the previous list or not. In the present study, rhesus monkeys were given a similar preparation of Eq-BuChE as described in previous studies (3,5) and evaluated on the SPR task. Regular samples of blood were withdrawn and assayed for BuChE activity and antibody levels. Once SPR performance, BuChE activity, and antibody levels returned to baseline, a second injection, twice the original amount of Eq-BuChE, was given to the same monkeys. Data from the present study should help clarify the role of BuChE in disrupting complex cognitive performance in monkeys. In addition, the present immunological data provide the first set of such measures from rhesus monkeys treated in this fashion.

METHOD

Subjects

Four experimentally naive male rhesus monkeys (*Macaca mulatta*), weighing between 8.9–9.4 kg at the time of the study, served as subjects. Three of the monkeys were 7 years of age at the beginning of the study and were obtained from Charles River (Wilmington, MA). One monkey was obtained from the Uniformed Services University of the Health Sciences (Bethesda, MD) and was 16 years old at the beginning of the study. The monkeys were individually housed in stainless steel cages (61 cm W $\times$ 71 cm D $\times$ 86 cm H). They were provided with tap water ad lib, and with sufficient commercial certified primate rations (Purina Mills, Inc., St. Louis, MO) to maintain healthy body weights. The monkeys' diet was supplemented with fresh fruit daily. Animal rooms were maintained at 20–22°C, relative humidity of 50% ($\pm$20%), using at least 10 complete air changes per hour of 100% conditioned fresh air. The colony was maintained on a 12-h light/dark cycle with no twilight (lights on at 0600 h).

Apparatus

The subjects were tested unrestrained in their home cages. A 14″ Mitsubishi Diamond Scan touchscreen monitor (Model MITS 381, Microtouch Systems, Inc., Methuen, MA) was attached to the front wall of each cage. Food pellets (300 mg banana flavored pellets, Bioserve Inc., Frenchtown, NJ) were delivered by a pellet dispenser (BRS/LVE Model QNB-400 1) into a food well positioned in the front of the test chamber, centered directly under the touchscreen and 2 cm from the chamber floor. A PC, running a custom-written Pascal routine, was interfaced to the touchscreen and controlled all experimental events and collected all data. The stimuli presented to each monkey and a trial-by-trial performance report could be observed outside the testing chamber on a color and a monochrome monitor, respectively.

Behavioral Procedures

The SPR task involves presentation of a successive list of six items followed by a test item, with subjects classifying the test item as matching or not matching an item in the list. For monkeys to acquire this performance, training was conducted under three successive conditions. Using standard shaping procedures, the monkeys were first trained to press the touchscreen with their hands until they eventually touched only the area where the stimuli were being displayed. Stimuli presented on the monitor were either a white rectangle (3.5 $\times$ 4.5 cm), or a compound stimulus comprised of two superimposed ASCII characters. The two characters were of different sizes, were each randomly selected from the ASCII set, and each colored randomly with the constraint that they be different. This combination of characters and colors generated several thousand different stimuli each day. The characters ranged from 2.5 to 5.5 cm in length and varied in width up to 3 cm. Stimuli could be displayed alone or simultaneously, either on the top center, bottom left, or bottom right of the touchscreen monitor. The white box only appeared in the lower positions on the touchscreen. The center of the top stimulus was displayed 5 cm from the top of the touchscreen and 14.5 cm from the left edge of the touchscreen. The center of the bottom left stimulus was displayed 7.5 cm from the left edge of the touch-

screen and 4.5 cm above the lower edge of the touchscreen. The center for the bottom right stimulus was displayed 5.5 cm from the right edge of the touchscreen and 4.5 cm above the lower edge of the touchscreen.

Same–different discrimination training began when the monkey consistently approached and pressed the touchscreen stimuli. On all trials, the target stimulus (list) item was a compound stimulus presented in the top center position, and the test stimulus (probe) item was a compound stimulus presented in either the bottom right or left of the screen. A white rectangle always appeared in the opposite position. On same trials, the list and probe stimuli were identical, and touching the probe stimulus generated a food pellet. On different trials, they were two nonmatching stimuli, and touching the white rectangle produced a pellet. Touching the incorrect stimulus in either same or different trials produced no food. Same and different trials were presented in a pseudorandom sequence and occurred with equal frequency throughout each session.

The delayed same–different discrimination training began when the monkey responded correctly on 80% of the probe trials on the same–different discrimination. During this condition, a list item was presented in the top center position of the screen for a fixed duration, and then was removed. After a fixed delay of 1.5 s, the probe stimulus and the rectangle were presented as before. Reinforcement contingencies for the delayed same–different responses were the same as those used in the previous condition.

Once responding reached 80% correct for three successive sessions during the delayed same–different discrimination condition, multiple-item serial probe recognition training began, by incrementally introducing an additional list item in the top center position of the screen, until a total of six items were presented. Monkeys were advanced to the next higher list number after achieving an 80% correct performance criterion for 3 consecutive days on all probe trials. During SPR training, each list item was displayed for a maximum of 4.0 s with a 1.5-s delay between successive items (interstimulus interval). Touching the stimulus at any time terminated it and initiated the interstimulus interval. The probe item was displayed 1.5 s after the presentation of the last list item and remained on the screen until a response was made or 10 s had elapsed. Correct responses were followed immediately by a short tone (4000 Hz, 0.25-s duration), a food pellet, and a 5-s intertrial interval. Incorrect responses were followed by a 5-s intertrial interval. If a monkey failed to make a response within 10 s of probe stimulus presentation, the trial terminated and a 5-s intertrial interval ensued. As before, half of all probe items matched a list item, and half did not. Probe items matched list items at each serial position with equal frequency on same trials. On different trials, probes were stimuli that were not contained in any list for that session. Each monkey received 240 trials per day, 5 days per week.

Enzyme

Horse serum BuChE was purchased from Sigma Chemical Co. (St. Louis, MO; Lot No. 125H7035) and used as supplied. The enzyme preparation and dose was similar to that used in studies described previously (3,5) and without any further purification. Several batches of commercial Eq-BuChE were routinely tested for their catalytic activity. Based upon their enzymatic activity, essentially all batches were 55–60% pure BuChE. The experiments described in this report were performed with the same batch of enzyme. All calculations used to make up the enzyme solution, however, were based upon information provided by Sigma Chemical Co. For each injection, enzyme solution was made up fresh and used within 1–2 h. Injections and blood withdrawals took place in a standard primate restraint chair to which the animals had been adapted. Eq-BuChE was dissolved in sterile normal saline and administered intravenously (saphenous vein). Blood samples were taken from a catheter in the saphenous vein. Eq-BuChE activity in blood samples was determined by the method of Ellman et al. (8). Heparinized monkey blood was diluted 10-fold in distilled water, and assays were conducted at pH 8.0 and 25°C.

Following acquisition of the six-item SPR task, monkeys received an injection of 27,000 IU of Eq-BuChE in 5 ml normal saline. Performance on the SPR task was assessed 1 h after Eq-BuChE administration, and daily testing continued for the duration of the study. Blood samples were taken immediately before injection and 1, 4, 8, 24, 48, and 72 h, and 1, 2, 4, and 8 weeks postinjection. Twenty-one weeks after the first injection a second injection of 54,000 IU of Eq-BuChE in normal saline was administered to the same monkeys. The interval between the first and second injection was determined to allow sufficient time for the enzyme to clear from circulation. SPR testing and blood withdrawals were conducted in a manner similar to that following the first injection. Additional blood samples were taken at 12, 16, and 20 weeks after the injection of 54,000 IU Eq-BuChE.

Determination of Anti-BuChE

The development of antibodies to the injections of Eq-BuChE was determined by ELISA. Microtiter plates were coated with 0.2 U/well of highly purified horse serum BuChE; whole blood lysates were assayed from 1/10 to 1/30,000 (volume/volume) in log and half-log dilutions. Antibody binding to BuChE was detected with peroxidase-labeled rabbit anti-monkey IgG (Sigma Chemical Co., St. Louis, MO) using 2.2′-azino-di[3-ethylbenzthiazoline sulfonate] (ABTS) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Concentration of antibody was determined by interpolation from standard curves (GraphPad Prism, San Diego, CA) using purified rhesus monkey IgG.

Statistical Analysis

Overall daily performance scores (accuracy and latency) were analyzed using a one-way repeated measures analysis of variance (ANOVA, GraphPad Prism, San Diego, CA). Any significant ($p \leqslant 0.05$) main effects were further examined using a post hoc comparison (Dunnet's multiple comparison test) with the control day as the reference value. Performance scores on control days and the day of treatment as a function of list position were analyzed using a multifactor ANOVA (Minitab 11, Minitab, Inc.) with treatment, subject, and list position as factors.

BuChE activity levels were derived by subtracting the individual baseline measurements from postinjection determinations. Those levels were considered significantly different from baseline when they exceeded the upper limit of a 95% confidence interval for the group mean of normal enzyme activity. To detect changes in antibody levels, the amount of antibody detected in the baseline sample was subtracted from the amount in each subsequent sample. The median difference for each measurement was then calculated and compared to zero using a Wilcoxon signed rank test of the medians (Minitab 11, Minitab, Inc.). Median difference values

significantly ($p \leq 0.05$) greater than zero were considered to be an increase from baseline. The peak antibody levels, in terms of amount and time of peak, were analyzed in a similar manner, using peak values after the first injection and subtracting the peak values after the second injection to obtain difference values.

RESULTS

Behavioral Performance

Unless otherwise indicated, the enzyme injection did not cause any significant effects on behavioral measures (all ps $>$ 0.05). Administration of 27,000 IU Eq-BuChE caused no observable effect on the monkeys' SPR performance overall or as a function of serial list position. Overall performance remained above 80% correct both before and after injection, as seen in Fig. 1A. A one-way repeated measures ANOVA did not reveal a significant main effect of days, $F(4, 12) = 1.241$. Figure 1B illustrates the average performance as a function of serial list position on control (mean of 4 days before injection) and Eq-BuChE (1 h after injection) trials. A three-way ANOVA showed that neither a treatment $\times$ subject interaction, $F(3, 36) = 1.80$, nor the main effect of treatment, $F(1, 36) = 0.01$, were significant. A treatment $\times$ list position interaction, $F(6, 36) = 2.00$, was not significant, indicating that performance remained relatively the same pre- and postinjection at all list positions. Response latencies are presented in Fig. 1C and D. Daily latencies overall, analyzed with a repeated measures ANOVA, did not significantly differ before or after enzyme exposure, $F(4, 12) = 0.8304$. A treatment $\times$ list position interaction was also not significant, $F(6, 36) = 1.33$, demonstrated in a three-way ANOVA, indicating no change in response latencies due to the enzyme.

The injection of 54,000 IU Eq-BuChE, twice the amount of the first injection, likewise caused no observable effect overall or as a function of serial list position after enzyme injection. A repeated measures one-way ANOVA showed that the effect of days was not significant, $F(4, 12) = 1.308$. Figure 2A illustrates that overall performance remained consistent 1 day before and 4 days postinjection of 54,000 IU. As depicted in Fig. 2B, the serial position curves show no difference in the monkeys' performance before (average of 4 days) or after Eq-BuChE exposure (the day of injection). A treatment $\times$ subject interaction, $F(3, 36) = 1.39$, and a treatment $\times$ list position interaction, $F(6, 36) = 0.76$, shown in a three-way ANOVA, were not significant. The main effect of treatment, $F(1, 36) = 0.619$, was also not significant. A repeated-measures one-way ANOVA revealed a difference in overall response latencies shown in Fig. 2C, $F(4, 12) = 3.828$, $p < 0.05$. However, a post hoc comparison (Dunnet's multiple comparison test) of means to the control day (day 1) showed that response latencies on the day of enzyme injection did not significantly differ from the control day. Only day 3 differed significantly (mean difference $= -0.1427$ s, $p < 0.05$) from the control day, revealing that the average response latency on that day was slightly higher than the control day. Figure 2D shows response latencies of the mean of 4 days before (circles) and the day of (triangles) injection as a function of serial list position. A three-way ANOVA demonstrated no significant treatment $\times$ subject or treatment $\times$ list position interaction, $F(3, 36) = 0.25$, and $F(6, 36) = 0.58$, respectively, for response latencies.

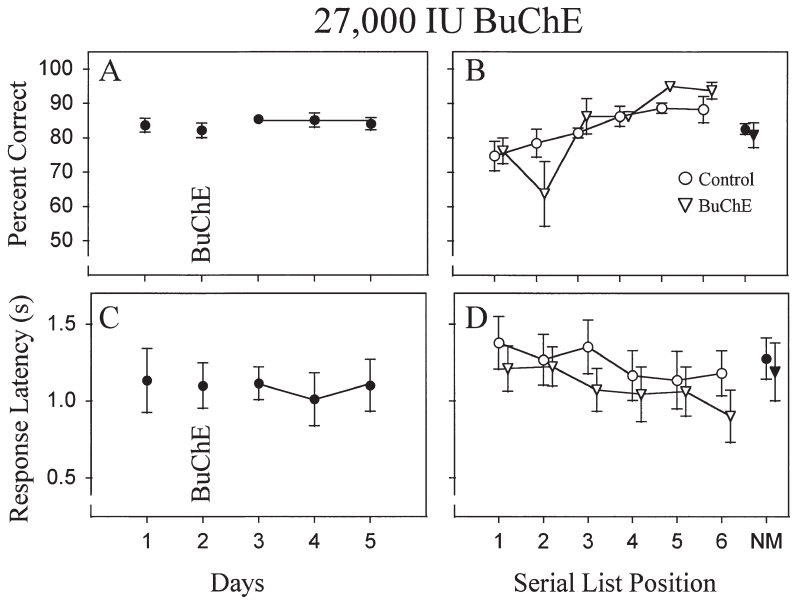

FIG. 1. Behavioral effects of IV injection of 27,000 IU Eq-BuChE (mean of four monkeys ± SEM). (A) The mean percent correct overall on the SPR task 1 day before, the day of, and 3 days after injection. Testing was conducted at approximately the same time every day and 1 h after injection on the day marked "BuChE." (B) Mean percent correct as a function of serial list position. Control represents the average of the 4 days before, and BuChE represents the session the day of Eq-BuChE injection. Open symbols represent performance when probe trials were matching and closed symbols represent performance when probe trials were not matching (NM). (C,D) The average response latency to the probe trials on the SPR task seen in the figures directly above. (A–D) No significant effects of the enzyme were found (ps > 0.05).

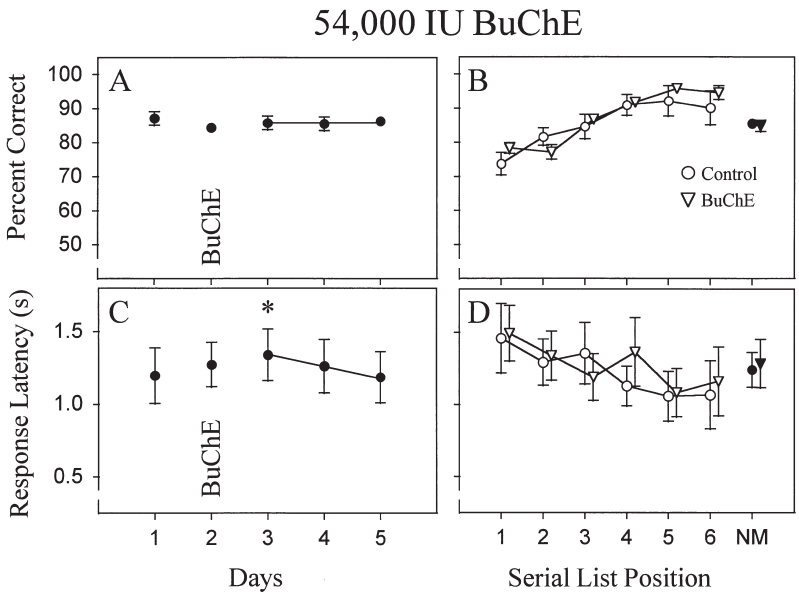

FIG. 2. Behavioral effects of the second IV injection, 54,000 IU Eq-BuChE, 21 weeks following the first injection (mean of four monkeys ± SEM). Plotting characteristics are identical to those in Fig. 1.*Denotes $p < 0.05$, Dunnet's multiple comparison test.

BuChE Activity Levels and Immunological Response

As illustrated in Fig. 3A, administration of 27,000 IU Eq-BuChE increased the amount of BuChE activity in blood between 25 and 32 U/ml above baseline levels within 1 h postinjection. All subjects' BuChE activity levels remained above baseline levels for a minimum of 2 days postinjection. By day 3, two subjects' activity levels had fallen below baseline. Only one animal's enzyme level was above baseline on the seventh day postinjection, and all had returned to normal enzyme activity by day 14 postinjection.

To represent the in vivo environment as closely as possible, the antibodies were not purified, but were measured in a dilution of whole blood (mg/ml). The analysis of antibodies to the Eq-BuChE provided a quantitative measure of antibody development by binding only BuChE antibodies to the highly purified BuChE used to coat the ELISA plates. Following the first injection of Eq-BuChE, anti-BuChE levels began to rise after 7 days postinjection, just after average enzyme activity decreased to near baseline levels. On day 14 postinjection an increase in anti-BuChE (mg/ml) was detected ($p = 0.05$). Days 28 and 56 postinjection were also above baseline levels ($p = 0.05$). This response in anti-BuChE levels, however, was less than 0.3 mg/ml in all animals.

The 21-week interval between the first and second injection was sufficient for the enzyme activity in blood to stabilize at baseline levels. Figure 4A shows the enzyme activity detected in blood following the injection of 54,000 IU Eq-BuChE. In one monkey (Gamma), BuChE activity increased to approximately 30 U/ml (only slightly higher than the increase following the first injection). This animal's blood enzyme activity returned to normal levels by 1 day postinjection. Enzyme activity in the remaining three monkeys exceeded 40 U/ml 1 h after the second injection, and were above baseline on day 3 post injection. By day 7, all subjects' blood enzyme activity levels returned to normal.

Antibodies detected in blood samples taken immediately before the second injection were low (median = 0.1520 mg/ml), but remained slightly but significantly elevated compared to original levels ($p = 0.05$). Antibodies in samples taken at 1, 4, and 8 h, and 1, 2, and 3 days after the second injection were neither significantly above the original nor the second baseline reading ($ps > 0.05$). Therefore, the original baseline value was used to detect increases in anti-BuChE levels from baseline following the second injection. As mentioned previously, one-tailed Wilcoxon signed-rank tests of the median were used to detect increases from baseline. Figure 4B illustrates the rise in antibodies to the enzyme after the third day following the second injection, in contrast to antibody development after the seventh day following the first injection. On days 7, 14, 28, 56, 84, 112, and 140 postinjection, the anti-BuChE levels were greater than original levels ($p = 0.05$).

In the two animals having the greatest amount of antibody development following the first injection, the peak antibody levels were high after the second injection (Gamma, 1.8 mg/ml; Lambda, 2.8 mg/ml). In the other two monkeys, the response to the second injection was markedly lower, 0.3 mg/ml or less. Accordingly, those monkeys had the highest amount of enzyme activity. A one-tailed Wilcoxon signed-rank test of medians revealed that the development of antibodies in all monkeys occurred earlier and rose to a higher level after the second injection than after the first ($ps = 0.05$).

DISCUSSION

The SPR task generated well-controlled responding in all subjects, with high accuracy on both matching and nonmatching trials, and stable, moderate latencies. Accuracy as a function of serial position increased slightly with items later in the list, but was clearly above chance levels at all list positions. This performance is characteristic of rhesus monkeys under similar SPR procedures (13).

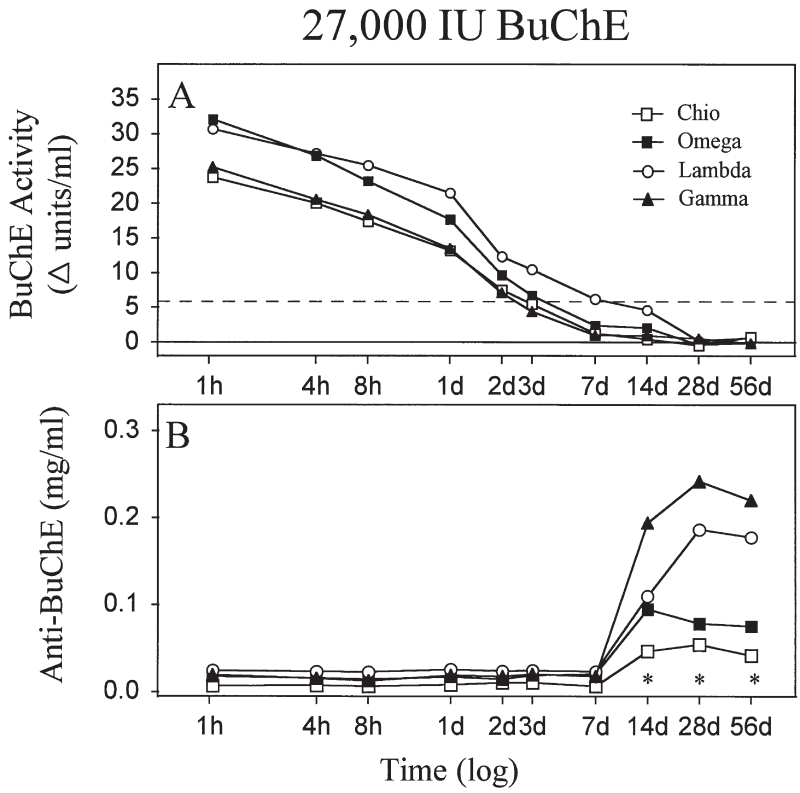

FIG. 3. Enzyme in circulation and immunological response to the first Eq-BuChE injection (IV). A given data point represents an average of two to four assays of a sample from an individual monkey. Blood was taken immediately before and 1, 4, and 8 h, and 1, 2, 3, 7, 14, 28, and 56 days after injection. (A) BuChE activity (units/ml) after injection (baseline levels taken immediately before injection were subtracted from the determinations of whole-blood BuChE). The dashed line represents the upper limit of a 95% confidence interval for the average baseline enzyme activity. (B) Antibody development to injected BuChE. Antibody (mg/ml) levels were measured as whole-blood lysates by ELISA using purified horse serum BuChE as the antigen and interpolating values from a monkey IgG standard curve. *Denotes $p = 0.05$, Wilcoxon signed-rank test.

Administration of 27,000 and 54,000 IU of Eq-BuChE did not cause any observable gross behavioral effects in the animals. Likewise, no effect of enzyme administration was detected in cognitive performance as measured by percent correct at each serial list position on the SPR task. The unimpaired performance was observed despite 7- to 18-fold increases in baseline levels of BuChE in circulation. BuChE activity not only increased but also remained elevated in circulation for a period spanning several days, during which time responding to the task remained unaffected. Repeated administration of the enzyme did produce a heightened immunogenic response, although this increase had no apparent effect on the monkeys' behavior (i.e., compare immunological and behavioral data 3 days post-54,000 IU BuChE). Although it is possible that other tasks may have revealed a behavioral effect of the enzyme, the SPR task used presently is widely accepted as a reliable method to detect change in cognitive function in nonhuman primates (3–6,13,15,24). It is also conceivable that higher doses than used in the present study would produce a negative behavioral effect. The highest dose in this study, however, was double that which afforded protection against two LD_{50} of OP in monkeys (3). Thus, it is unlikely that higher doses than used in this study would be administered for therapeutic purposes.

The lack of behavioral effect of Eq-BuChE in the current study conflicts with those effects reported previously by other researchers using an SPR task in nonhuman primates (3,5). Prior accounts of Eq-BuChE's behavioral effects have emphasized either the enzyme itself (3), or its nonpurified status (9), in generating adverse reactions that disrupted task performance. The results of the present study suggest that even a high dose (double the doses used in previous studies) of unpurified exogenous enzyme causes no obvious negative behavioral effect. Despite the nonpurified enzyme used in the present study, the monkeys continued to work for food with no change in either response latency or accuracy, relative to control. Hence, whatever the source of the discrepancy between the current lack of effect and previously reported effects, it is clear that the enzyme itself was not responsible. One could argue that the lack of effect found here is due to administration of an indeterminate or negligible amount of pure enzyme. Conversely, the relatively large increases in enzyme activity indicate a substantial amount of enzyme was administered.

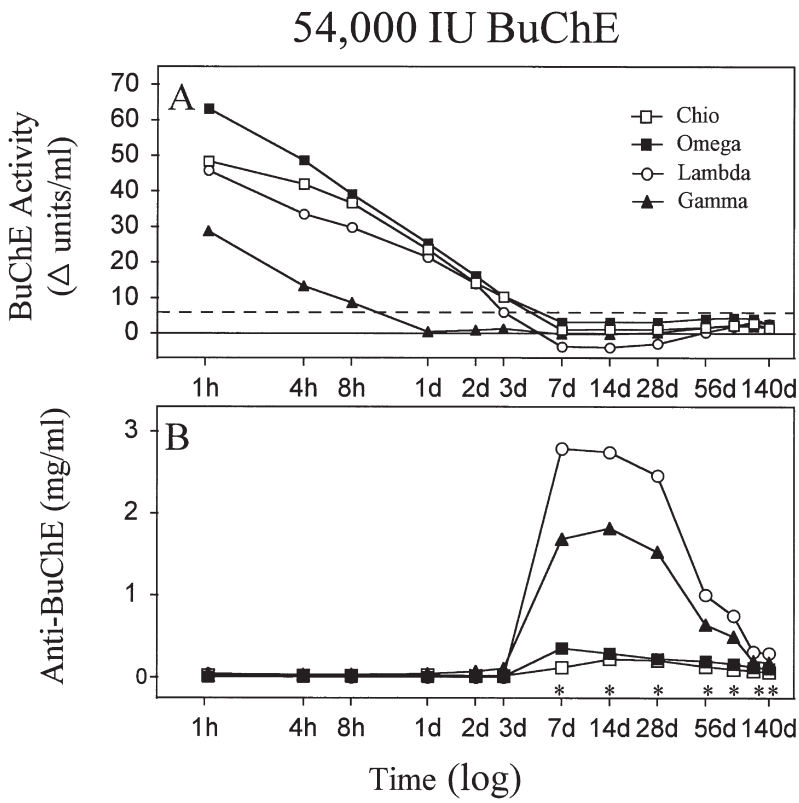

FIG. 4. Effects of 54,000 IU BuChE on blood enzyme activity and antibody levels (data were recorded as described in Fig. 3). The second injection occurred 21 weeks following the first. Blood was taken immediately before and 1, 4, and 8 h, and 1, 2, 3, 7, 14, 28, 56, 84, 112, and 140 days after injection. Plotting characteristics are similar to those in Fig. 3. *Denotes $p = 0.05$, Wilcoxon signed-rank test.

Butyrylcholinesterase produces little behavioral effect on motor coordination as measured by performance under the PEP task (22). It has also been reported to produce little effect on a spatial discrimination task (19); however, this claim is more difficult to evaluate. That task involved repeated acquisition during each session. Five of 12 response panels were randomly selected each day as "correct." Pressing a correct panel briefly illuminated a light, and pressing the fifth produced light and food. Pressing incorrect panels, or making multiple presses on any panel, produced no consequences and were treated as errors. Data were presented in the form of daily total errors for each subject, and were not insubstantial (several hundred errors during daily 50-trial sessions). From this presentation, unfortunately, it is impossible to determine whether subjects learned the discrimination at all (i.e., that the number of errors on successive trials decreased within the session). Further, it is unclear whether the similar large number of errors evidenced after Eq-BuChE administration represents a similar degree of behavioral control relative to baseline, or in fact, a behavioral disruption. That is, it is conceivable that performance was well controlled under baseline, with the vast majority of errors occurring during trials early in the session and subsequently declining, but that Eq-BuChE administration resulted in fewer early-session errors, but a less rapid decline (i.e., slower acquisition of the discrimination). The above considerations, thus, undermine any conclusions about the effect(s) of BuChE on spatial discrimination.

In contrast, the present study provides clear evidence of discriminative control over responding, in that baseline choices were highly accurate (over 80%). These accuracy levels were fostered despite the fact that the stimuli controlling responding were no longer physically present, and in some cases had been presented at least 9 s previously (i.e., items at the beginning of the list). Such accurate responding provides clear opportunity for disruption by BuChE, yet after doses six times or more than that used in the spatial discrimination study mentioned above (19), responding on this complex cognitive task remained unaffected, overall and at each serial position.

The immunological effects of repeated exposures to exogenous BuChE in rhesus monkeys have to date not been reported. In rabbits, a series of four exposures to purified Eq-BuChE caused an increasingly greater immune response after each exposure, while the amount of active enzyme in circulation declined more rapidly to baseline levels after each trial (10). In the present study, the immune response followed a similar pattern. The increase in anti-BuChE levels was more pronounced, and the amount of BuChE in circulation declined more rapidly, after the second injection than after the first. These patterns demonstrate that enhanced development of antibodies with repeated enzyme exposure decreases the circulating level of active enzyme. Although the present study does not describe the specific nature of the antibodies, it does provide a measure of the total concentration of antibody development. The concentration of antibodies is an essential con-

sideration in predicting effectiveness of enzyme as a prophylactic antidote because the amount of enzyme available to sequester the nerve agent will depend on the rate at which it is inactivated by antibodies. The total concentration of antibodies is, therefore, more relevant in the context of the present study than knowing the particular class of antibodies. This information, in practice, may be largely moot, in that an immunologically and physiologically compatible enzyme (i.e., human plasma BuChE delivered to humans) would be indicated.

In addition to lacking behavioral side effects and adverse immune responses, a relatively long in vivo half-life is also an important aspect of pretreatment regimens for use in applied situations (7). The analysis of blood samples after injections of 27,000 IU and 54,000 IU showed elevated enzyme levels for at least 48 h in all animals after the first injection and 72 h in three of four monkeys after the second injection. This suggests that BuChE would effectively protect against OP exposure for a relatively long period, although the amount of OP exposure must be taken into account when determining the amount of protection afforded by the enzyme. To be effective, the prophylactic should be active in circulation for a relatively long time to avoid impractical, multiple administrations, as was the case with pyridostigmine use (11).

The current results add to accumulating evidence that ChEs, and BuChE in particular, have potential for use in humans as a prophylactic measure against toxicity arising from OP pesticides or chemical warfare agents. This approach avoids inevitable problems associated with introducing agents that themselves produce detrimental effects. The present demonstration of minimal behavioral effects on a complex cognitive task in rhesus monkeys (despite very high doses of enzyme), as well as a relatively long duration of availability in vivo, strongly support the recommendation that BuChE, acting as a biological scavenger for highly toxic OPs, may effectively replace current pretreatment regimens.

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