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Behavioral and Pharmacological Assessment of Butyrylcholinesterase in Rats[1]

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GENOVESE, R. F. AND B. P. DOCTOR. *Behavioral and pharmacological assessment of butyrylcholinesterase in rats.* PHARMACOL BIOCHEM BEHAV **51**(4) 647-654, 1995.—Advances in the treatment of organophosphorus (OP) toxicity have focussed on the use of exogenous cholinesterases to act as scavengers for the OP agent. To further investigate the feasibility of the scavenger approach, we evaluated the effects of highly purified horse serum butyrylcholinesterase (HS-BChE) on performance in rats. HS-BChE (5000 U, IP) produced substantial increases in blood enzyme activity for up to 72 h after injection. HS-BChE (5000 U, IP) had no effect on acquisition or retention of a passive avoidance task. In contrast, atropine sulfate (10 mg/kg) impaired retention when tested 168 h after administration. When examined for 10 days following administration, HS-BChE (7500 U, IP) had no effect on either total daily motor activity or circadian pattern of activity. HS-BChE (5000 U, IM) also had no acute or prolonged effects on the rate of lever pressing maintained by a VI56 s schedule of food reinforcement. HS-BChE (7500 U, IM) was observed to confer significant, but partial, protection against response rate decreases produced by the OP, MEPQ, under the VI56 s schedule of reinforcement. These results suggest that, in rats, HS-BChE, at doses that attenuate OP toxicity, may be devoid of cognitive or motor effects.

Butyrylcholinesterase MEPQ Motor activity Operant behavior Organophosphorus Passive avoidance

ORGANOPHOSPHORUS (OP) toxicity results largely from the inhibition of cholinesterases leading to severe and widespread increases in cholinergic activity. Treatment following OP exposure typically includes administration of several compounds including anticholinergics, oxime reactivators, and anticonvulsants. In certain instances, pretreatment with a reversible cholinesterase inhibitor, such as pyridostigmine, has also been used. Current therapies are, to a certain extent, limited in that detrimental effects of the OP frequently remain unabated (8,19).

A novel approach to treatment of OP toxicity, currently under development, involves administration of cholinesterases (6). Exogenous cholinesterases act as scavengers, attaching to, and neutralizing the OP agent before target organs can be affected. In this regard, studies have shown that fetal bovine acetylcholinesterase provides significant protection against OP toxicity induced by potent agents such as soman and VX, in rhesus monkeys and mice (7,18,21,27). Similarly, protection against OP agents is conferred by human and equine butyrylcholinesterase in rats, mice, and rhesus monkeys (4,5,22,27,28).

The effectiveness of an enzyme therapy is primarily limited by a) the amount of enzyme available, and b) the rate of reactivity with the OP agent. It is advantageous to administer exogenous cholinesterases prophylactically, because a maximal amount of enzyme can be present upon OP exposure. There is, however, a paucity of studies that have investigated the potential of exogenous cholinesterases to produce adverse effects in the absence of OP exposure. Any detrimental effects produced by the enzyme treatment would compromise the desirability of prophylactic administration and, thus, restrict the value of the approach. Therefore, we evaluated the potential of horse serum butyrylcholinesterase (HS-BChE) to produce cognitive or motor impairment in rats.

[1] In conducting the research described in this report, the investigators adhere to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

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EXPERIMENT 1: TIME COURSE FOR BLOOD ENZYME ACTIVITY ELEVATION

In Experiment 1 we determined the extent and duration of elevated blood BChE activity following HS-BChE administration (IP). Blood was sampled before and 12 times (0.5–192 h) after injection. Each blood sample was assayed for enzyme activity.

METHOD

Subjects

Adult male Sprague–Dawley rats (Charles River, Wilmington, MA) were used. Rats were individually housed under a 12L : 12D cycle (lights on at 0600 h) and water was always available in the home cages. Food (Agway Pro Lab Rodent Chow) was restricted to maintain body weights at approximately 320 g.

Pharmacological Procedure

Horse serum butyrylcholinesterase (HS-BChE; EC 3.1.1.8) (Sigma, St. Louis, MO) was dissolved in a sodium phosphate buffer (pH 8.0). The solution was purified to homogeneity using affinity chromatography (procainamide column eluted with 100 mM decamethonium bromine). The resulting eluate was then subjected to an ammonium sulfate cut (70%) to concentrate the solution. Pellets resulting from centrifugation (13,000–15,000 rpm, refrigerated) were dissolved in 50 mM sodium phosphate buffer (pH 8.0) and dialyzed to remove any remaining elution buffer and/or ammonium sulfate. The final solution was then assayed for enzyme activity, using the method of Ellman (10), immediately before injection. A 50-mM sodium phosphate buffer (pH 8.0) was used for vehicle injections. HS-BChE used in Experiments 2–5 was prepared in the same manner as in Experiment 1.

BChE activity is expressed as units of activity per ml of blood. One unit of BChE will hydrolyze 1.0 μmol of butyrylthiocholine to thiocholine and butyrate per min at pH 8.0 at 25°C. One milligram of the purified HS-BChE is approximately equal to 750 U.

Rats were injected with 5000 U of HS-BChE (n = 6) or vehicle (n = 5). Injections were given IP in a volume of approximately 0.7 ml. Blood was sampled 24 h before injection and 12 times after injection (0.5, 1, 2, 4, 8, 12, 16, 24, 48, 72, 96, 192 h). Blood samples were obtained by making a small incision in the tail vein and withdrawing 50–100 μl of blood using a pipette. Collected blood was diluted 10-fold with distilled water and 5–20-μl samples were assayed using the procedure of Ellman (10).

RESULTS AND DISCUSSION

Figure 1 presents blood BChE activity before and after injection of HS-BChE or vehicle. HS-BChE (5000 U) rapidly increased blood BChE activity in all rats, and the observed average peak concentration of approximately 55 U/ml occurred 4 h after injection. Blood BChE activity remained at nearly peak concentrations for 4–24 h after injection (Fig. 1, inset) and was substantially elevated for over 72 h following injection. In contrast, blood BChE activity in rats injected with vehicle remained essentially unchanged, compared to preinjection levels (0.5–2.0 U/ml), during the entire 192-h sampling period. These results complement and extend previous studies by Raveh et al, (22) demonstrating that human plasma butyrylcholinesterase, injected IM into rats and mice, produced substantially elevated blood BChE activity for 72 h following administration. HS-BChE has also been found to have a prolonged time course in rhesus monkeys (4).

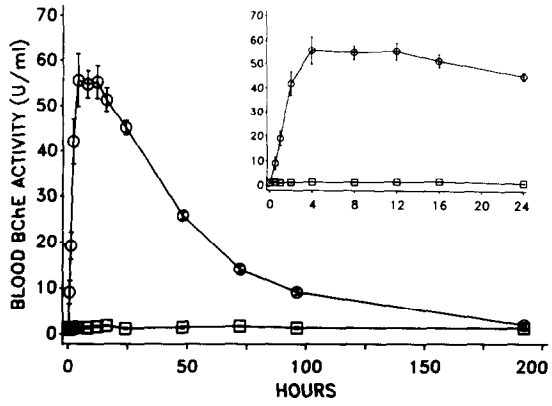

FIG. 1. Average BChE levels in rats injected IP with 5000 U HS-BChE (n = 6, circles) or vehicle (n = 7, squares) from blood sampled during 192 h (inset 24 h) following administration. Vertical lines about each point represent ± SEM. Points above time 0 represent endogenous BChE levels measured 24 h before injection.

EXPERIMENT 2: PASSIVE AVOIDANCE PERFORMANCE

Experiment 2 evaluated putative effects of HS-BChE on memory performance using a passive avoidance procedure [for reviews see (24,25)]. Based on results from Experiment 1, we administered HS-BChE before training so that blood enzyme activity levels would be elevated during the initial acquisition of avoidance learning. Additionally, we tested retention of avoidance learning at times during and after the elevation of blood enzyme activity levels. Thus, we attempted to maximize the possibility of observing an enzyme-induced cognitive deficit. For comparison, we also evaluated a dose of atropine, because anticholinergics, including atropine, have been shown to disrupt performance under similar procedures.

METHOD

Subjects

Adult male Sprague–Dawley rats as described in Experiment 1 were used.

Procedure

Sessions were conducted using a computerized shuttle-box system (model PACS-30, Columbus Instruments, Columbus, OH). The stainless steel cage is divided, by a motorized door, into two compartments, each measuring 54.6 × 33 × 48.3 cm. One compartment could be illuminated by a house light located on the compartment ceiling. A scrambled, constant-current, electric shock stimulus (1.0 mA, 1.0 s) could be presented through the grid floor of the second compartment. During training, rats were put inside the illuminated compartment and, after 1 min had elapsed, the sliding door was opened, allowing entry into the dark compartment. Upon entry into the dark compartment, the sliding door was closed and the electric shock stimulus was presented. The rat was then removed from the chamber and returned to the home cage. Subsequently, retention tests were conducted at 24, 72,

and 168 h posttraining. During retention tests, rats were introduced into the illuminated compartment and after 1 min the sliding door was opened, allowing entry into the dark compartment. Upon entry into the dark compartment, the sliding door was closed and the rat was removed from the compartment and returned to the home cage. If the rat failed to enter the dark compartment after 5 min, the session was terminated, and the transfer time was assigned a value of 5 min. HS-BChE (5000 U) (n = 5) or vehicle (n = 7) was injected (IP) in a volume of approximately 0.7 ml, 4 h before training. Atropine sulfate (10 mg/kg) (n = 10) was dissolved in 0.9% saline and injected (IP) in a volume of 1.0 ml/kg body weight, immediately after training.

Data Analysis

Average transfer times between drug and vehicle treatment groups during each of the three retention tests were evaluated with Dunnett's t-tests as calculated using the General Linear Models procedure of the SAS (Cary, NC) statistical software package.

RESULTS AND DISCUSSION

Figure 2 presents transfer times for drug and vehicle treatment groups during testing and each of three retention tests. All rats rapidly transferred into the dark compartment during the training session. Retention of avoidance learning was observed as maximal (300 s) or nearly maximal transfer times when tested 24 h after training, regardless of treatment. When tested 72 h after training, rats administered vehicle or BChE did not transfer into the dark compartment during the entire 300-s testing interval. On average, rats treated with atropine transferred into the dark compartment before the maximum testing interval at 72 h. The difference in transfer times for atropine- and vehicle-treated rats at the 72-h test, however, was not statistically significant. When tested 168 h after training, average transfer times for all three groups of rats were less than the 300 s testing interval. Atropine-treated rats, but not HS-BChE-treated rats, were significantly different from vehicle controls ($p < 0.05$) in this respect.

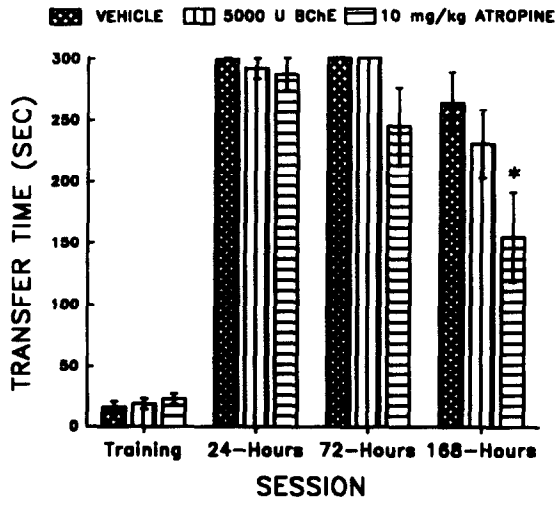

FIG. 2. Transfer times for rats under the passive avoidance procedure during acquisition (training) and for retention tests conducted 24, 72, and 168 h following training. Rats received 5000 U HS-BChE (n = 5) or vehicle (n = 10) 4 h before training, and 10 mg/kg atropine sulfate (n = 10) immediately after training. Vertical lines about each bar represent ± SEM. *Statistically significant difference between vehicle group, $p < 0.05$.

HS-BChE (5000 U) failed to disrupt either acquisition or retention of passive avoidance learning. Detection of impaired acquisition of avoidance learning was maximized because training was conducted when blood enzyme activity approached peak levels (based on the results of Experiment 1). Furthermore, it is unlikely that any disruption of performance was masked by state-dependent learning [e.g., (20)] because the 168-h test occurred at a time when, as indicated by the results of Experiment 1, blood enzyme activity was at or near normal levels. In contrast to the effects of HS-BChE, atropine disrupted retention of avoidance learning. Previous studies have reported similar effects of atropine on passive avoidance tasks (1,2,16,26).

EXPERIMENT 3: MOTOR ACTIVITY

Measurement of spontaneous motor activity is an established technique for evaluating the behavioral and toxicological effects of a wide variety of compounds [for review see (23)]. In Experiment 3 we evaluated the possibility that HS-BChE might adversely affect spontaneous motor activity. One advantage of the procedure is that activity can be monitored continuously over a period of several days. Thus, performance was assessed before, during, and after periods of elevated blood enzyme activity.

METHODS

Subjects

Adult male Sprague-Dawley rats as described in Experiment 1 were used except that food was always present in the home cage after the beginning of the experiments.

Procedure

Sessions were conducted using a cage rack activity system (San Diego Instruments, San Diego, CA). Rats were housed individually in cages (45 × 25 × 18 cm) that were mounted with three infrared emitter/detector pairs. The activity cages were located in an isolated temperature- and humidity-controlled environmental chamber. A computer monitored and recorded single and consecutive beam breaks in 5-min intervals. Recording was conducted continually during each day of testing except for brief daily periods, during which rats were weighed and visually examined. Rats were first allowed several days to habituate to the free-feeding environment before compounds were administered. HS-BChE (7500 U, n = 4) or vehicle (n = 4) was injected, IP, in a volume of 1.1 cc, approximately 4 h before the onset of the dark period. The time of administration was chosen so that the dark period would start approximately at the same time as the beginning of the peak increase in blood BChE activity (as observed in Experiment 1). Motor activity was then monitored for the next 10 days.

Data Analysis

Because measures of single and consecutive beam breaks were found to be strongly correlated, only consecutive beam breaks were used as an index of activity. Activity counts were collected in hourly intervals and were totalled by 24-h period. Circadian activity was measured by calculating the proportion of total activity occurring in the dark period. For purposes of

statistical analysis, these data were converted to a percentage of the average values calculated from the 5 days prior to HS-BChE or vehicle administration. Differences between groups were assessed using two-factor (treatment × time) repeated-measures ANOVAs calculated with the General Linear Models procedure of the SAS (Cary, NC) statistical software package.

RESULTS AND DISCUSSION

Upon acclimatization to the motor activity chambers and free food, relatively stable activity counts per 24-h period were observed in all rats. Activity during the dark period was considerably greater than during the light period and typically accounted for 80%, or more, of the activity observed during a 24-h period. Body weight increased during the course of experimentation and rats weighed 360–425 g at the time of HS-BChE or vehicle injection. No disruption in body weight gain was observed following HS-BChE or vehicle treatment (data not shown).

Figure 3 shows the average activity counts (per hour) 12 h before and 60 h after HS-BChE (top) or vehicle (bottom) was administered. Results show the difference in activity during dark and light periods for HS-BChE- and vehicle-treated rats. However, no evidence of disruption in the pattern of activity was observed following HS-BChE injection during, and after, the time when peak blood BChE levels would be expected to occur. Figure 4 presents the average daily total activity counts during dark and light periods during the 5 days before, and 10 days after, HS-BChE or vehicle administration. Differences in activity during dark and light periods are also evident from Fig. 4, and the circadian pattern of activity is clearly maintained after HS-BChE and vehicle administration. ANOVA revealed no significant main effects for treatment or time for measures of total daily activity or circadian pattern of activity. Similarly, no significant treatment by time interaction was found for either measure.

We did not observe any disruption in motor activity produced by the administration of 7500 U HS-BChE in any rats.

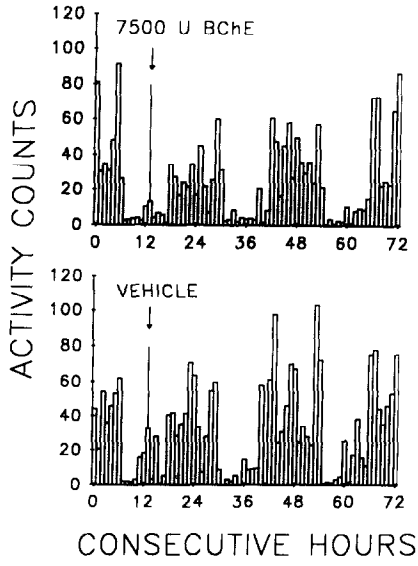

FIG. 3. Spontaneous motor activity in rats before and after injection of 7500 U BChE (top, n = 4) or vehicle (bottom, n = 4). Each bar represents the average number of activity counts for 1 h. Vertical line and arrow indicates the time of HS-BChE or vehicle injection.

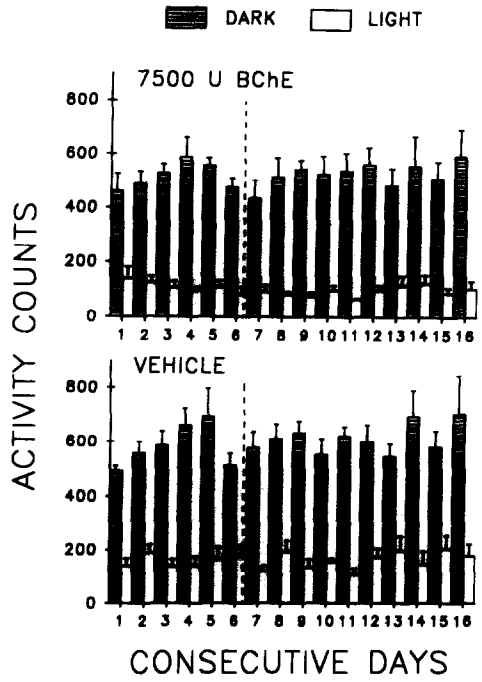

FIG. 4. Spontaneous daily motor activity during dark and light periods in rats before and after injection of 7500 U HS-BChE (top, n = 4) or vehicle (bottom, n = 4). Dashed vertical lines represent the time of injection. Vertical lines about each bar represents + SEM.

Total daily activity as well as the circadian pattern of activity remained essentially unchanged over a period of 10 days following administration. It is notable that the time period of observation included the interval before, during, and after blood BChE activity would be expected to increase. Therefore, HS-BChE had no effect on spontaneous motor activity.

EXPERIMENT 4: SCHEDULE-CONTROLLED BEHAVIOR

Experiment 4 evaluated the effects of HS-BChE on performance under a VI56 s schedule of food reinforcement. Schedule-controlled behavior has been shown to be sensitive to a wide variety of compounds (9). We have previously shown that several cholinergic compounds disrupt performance under schedules of reinforcement (12–14). We were interested in examining the possibility that HS-BChE might cause both immediate and prolonged or delayed effects on performance. Thus, we measured performance for 10 days following administration.

METHOD

Subjects

Adult male Sprague–Dawley rats as described in Experiment 1 were used.

Procedure

Sessions were conducted in standard rodent operant conditioning chambers (model # E-10-10, Coulbourne Instruments, Lehigh Valley, PA), housed in ventilated, light- and sound-attenuating cubicles. Each chamber contained two response

levers and a food trough that could be illuminated and was attached to a food dispenser capable of delivering 45-mg food pellets (Bio-Serv, Frenchtown, NJ). Each chamber also contained a house light mounted on the ceiling and two stimulus lights mounted above each of the response levers. A response was considered to occur when either lever was pressed with a downward force of at least 0.3 N. Experimental events were controlled and monitored by a DEC, PDP-11/73 computer, using the SKED-11 (State Systems, Kalamazoo, MI) software system.

All rats were initially trained to lever press for food pellets under a continuous schedule of reinforcement. Although two levers were present in each chamber, only one lever produced food reinforcement. When lever pressing was maintained by food presentation, all rats were trained to lever press under a VI56 s schedule of food reinforcement. The schedule specifies that the first lever press following an average interval of 56 s produces food reinforcement (i.e., a single food pellet). Interval values for the schedule were chosen pseudorandomly, without replacement, from normal distributions generated using the procedure of Fleshler and Hoffman (11). The range of intervals was 2.44–198.23 s. The house light and the stimulus lights above both levers were illuminated during the sessions that were 60 min in duration and were conducted at approximately the same time, Monday–Friday.

When responding under the VI56 s schedule of reinforcement appeared stable (as judged by inspection of cumulative response records), rats were assigned to three groups, each containing four rats. Groups were matched on the basis of rate of responding with the restriction that each group was balanced with respect to the position of the active response lever. Rats were then administered a single injection (IM) of HS-BChE (500 or 5000 U) or vehicle, approximately 3 h before the start of the daily session.

Data Analysis

When a response under the schedule of reinforcement occurred, the elapsed time within the session was recorded. From these data, the total number of responses and the rate of responding (responses per minute) were calculated for each rat. The response rate data were converted to a percentage of the average values obtained during 10 sessions before injection (baseline). Additionally, the number of pellets earned and the rate of nonreinforced responding (i.e., responses on the lever that never produced food) were calculated. To assess the difference in rate of responding between groups, a two-factor (treatment × time) repeated-measures ANOVA was calculated using the General Linear Models procedure of the SAS statistical software package.

RESULTS AND DISCUSSION

Responding maintained by the VI56 s schedule of reinforcement was characterized by a relatively constant rate of responding throughout the 60-min session in all rats. Table 1 presents the average rate of responding and number of food pellets earned during preinjection control sessions for each of the three treatment groups. Responding on the inactive lever was minimal or nonexistent in all rats for the duration of the experiment. Figure 5 presents the rate of responding (as a percentage of control performance) for the three treatment groups during the 10 sessions following injection. Response rates for all three groups remained near baseline values across the 10 sessions following treatment. ANOVA revealed that there were no significant differences in the rate of responding during these sessions between groups treated with vehicle, 500 U, or 5000 U of HS-BChE. ANOVA also revealed no significant treatment by time interaction. Similarly, the number of pellets earned during the posttreatment sessions was observed to be very similar to values observed during control sessions for all three treatment groups during all of the 10 posttreatment sessions (data not shown). In the present experiment, HS-BChE was administered IM, and previous research has shown that the time course for blood enzyme activity elevation following BChE injection is prolonged—with substantial elevation observed for 72 h after administration (22). Thus, we assessed performance during periods of elevated blood enzyme activity (i.e., days 1–4) as well as during periods when blood enzyme activity would be expected to return to normal (i.e., days 7–10).

TABLE 1

BASELINE RESPONDING UNDER THE VI56 S SCHEDULE OF REINFORCEMENT

Group	*N*	Response Rate	Pellets
500 U BChE	4	32.3 ± 3.2	57.7 ± 0.4
5000 U BChE	4	29.6 ± 3.2	56.9 ± 0.4
Vehicle	4	33.4 ± 5.8	56.5 ± 0.6

Values represent the mean ± SEM from 10 sessions preceding treatments. Response rate values are as responses per minute.

HS-BChE (500 or 5000 U) did not have any acute or long-term effects on performance maintained by a VI56 s schedule of food reinforcement. This result is consistent with, and extends, a previous experiment in which we demonstrated that repeated administration of 500 U of HS-BChE did not disrupt responding of rats on a similar schedule of reinforcement (15). In contrast, we have previously demonstrated that a variety of cholinergic compounds (e.g., oxotremorine, atropine, physostigmine) can produce substantial or complete response suppression in rats under similar or identical schedules of reinforcement (12–14).

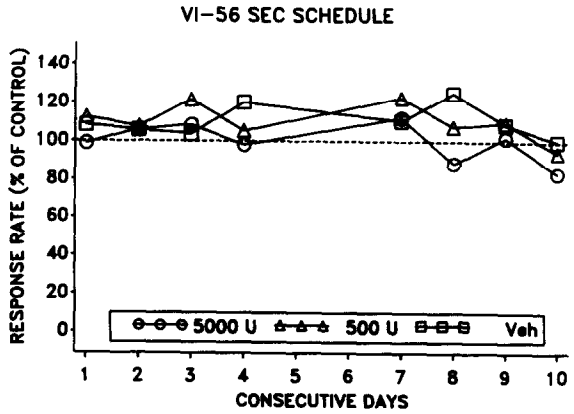

FIG. 5. Rate of responding by rats under a VI56 s schedule of food reinforcement. HS-BChE (5000 U, circles; 500 U, triangles) or vehicle (squares) was injected before session 1. Response rate is expressed as a percentage of the average values obtained during 10 control sessions preceding injection. Each point represents the mean of four rats.

EXPERIMENT 5: HS-BCHE PROTECTION AGAINST OP TOXICITY

Experiment 5 investigated the ability HS-BChE to protect against OP-induced performance deficits under the VI56 s schedule of reinforcement. MEPQ [7-(methylethoxyphosphinyloxy)-1-methylquinolinium iodide] is a short-acting, quaternary cholinesterase inhibitor (17). We evaluated response suppression produced by a single dose of MEPQ in rats pretreated with HS-BChE or vehicle. Additionally, we sampled blood from rats receiving HS-BChE, before and after MEPQ administration, to assess changes in blood enzyme activity level.

METHODS

Subjects

Adult male Sprague-Dawley rats as described in Experiment 1 were used.

Procedure

Rats were trained to lever press for food under a VI56 s schedule of food reinforcement as described in Experiment 4. When responding was stable, rats were divided into two groups, each containing five rats. Groups were matched on the basis of rate of responding with the restriction that each group was balanced with respect to the position of the active response lever. Rats were then administered a single injection (IM) of HS-BChE (7500 U) or vehicle in a volume of approximately 0.7 ml, and approximately 17.5 h later all rats were injected (IP) with 32 μg/kg MEPQ. MEPQ was dissolved in 0.9% saline and injected in volume of 1.0 ml/kg body weight, 10 min before the start of behavioral sessions. Blood was sampled from rats receiving HS-BChE at various intervals following injections.

Data Analysis

Response rate data as described for Experiment 4 were collected. Student's *t*-tests were used to evaluate the difference between groups in response rate on the day that MEPQ was administered. Additionally, repeated-measures *t*-tests were used to evaluate the difference in response rate, for each group, between the day before MEPQ was administered and the day MEPQ was administered.

RESULTS AND DISCUSSION

As in Experiment 4, responding maintained by the VI56 s schedule of reinforcement was characterized by a relatively constant rate of responding throughout the 60-min session in all rats. The average rates of responding (± SEM) on the day before MEPQ administration were 54.74 ± 8.74 and 54.52 ± 10.93 responses per minute for the HS-BChE and vehicle treated rats, respectively. Figure 6 presents the rate of responding following MEPQ administration as a percentage of the previous day's performance. MEPQ produced a complete or nearly complete suppression of responding in all rats pretreated with vehicle. By comparison, MEPQ produced significantly less response suppression ($p < 0.05$) in rats pretreated with HS-BChE. Thus, HS-BChE attenuated the toxic effects of MEPQ. Attenuation, however, was incomplete because responding in rats pretreated with HS-BChE was significantly less ($p < 0.05$) on the day that MEPQ was administered than the previous day. These results are consistent with previous studies reporting that BChE reduces MEPQ-induced lethality in mice (6). These results are also similar to previous studies demonstrating that BChE protection against OP toxicity in rats and monkeys, although significant, is incomplete (3,5).

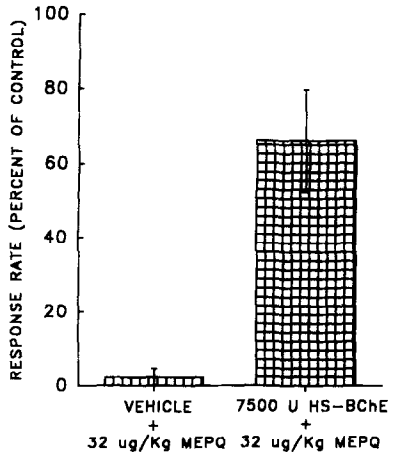

FIG. 6. Rate of responding under a VI56 s schedule of food reinforcement following administration of MEPQ in rats pretreated with vehicle or 7500 U HS-BChE. Response rate is expressed as a percentage of the values obtained during the session immediately prior to injections. Each bar represents the mean of five rats. Vertical lines about each bar represent ± SEM.

Table 2 contains BChE activity in rats receiving HS-BChE, measured from blood sampled before and after MEPQ injection. BChE activity was observed to decrease when measured 90 min after MEPQ administration, suggesting that the observed protection resulted directly from inhibition of HS-BChE by MEPQ. When blood was again sampled 64 and 88 h after HS-BChE administration, however, BChE activity increased. This result is somewhat surprising and may suggest that some HS-BChE continued to be mobilized into the blood. Based upon the 1 : 1 stoichiometry between MEPQ and BChE that has been determined in vitro (6), enough HS-BChE was present in blood to neutralize all of the administered MEPQ. That only partial protection was observed suggests that the rate of reaction of HS-BChE with MEPQ was not sufficient to completely neutralize the MEPQ before some endogenous enzyme was inhibited. It is, however, notable that both HS-BChE and MEPQ were administered systemically. Therefore, it is also possible that local concentrations of MEPQ were excessive, relative to BChE, and produced sufficient enzyme inhibition to produce a deficit in performance.

TABLE 2

BLOOD BChE ACTIVITY BEFORE AND AFTER MEPQ

	Hours Postinjection				
	10	17.5*	19	64	88
U/ml	34.8	53.1	41.6	58.7	43.2

*MEPQ was injected immediately after blood was sampled at 17.5 h posttreatment.

GENERAL DISCUSSION

The present series of experiments examined the effects of several doses of highly purified HS-BChE on performance using three distinctly different behavioral tasks. We first determined that systemic administration of HS-BChE produced prolonged increases in blood BChE activity. To maximize the possibility of detecting a disruption in performance, each assessment task sampled behavior over a period of time that included intervals during and after increased BChE activity.

We observed that, unlike the cholinergic receptor antagonist atropine, HS-BChE did not disrupt the acquisition or retention of passive avoidance learning. HS-BChE also failed to disrupt the rate or pattern of responding controlled by a VI56 s schedule of food reinforcement. Additionally, we observed no effects from HS-BChE on either total daily motor activity or the circadian pattern of motor activity. Thus, our results indicate that, in rats, relatively large doses of exogenous BChE are not behaviorally active. It is, however, notable that a previous study reports that HS-BChE suppressed responding by rhesus monkeys on a serial recognition task (5). Therefore, important species differences may exist in the effects of exogenous administration of BChE. HS-BChE used in the latter study, however, was not purified to the extent as in the present study. Additionally, the latter study administered HS-BChE IV, whereas we used IP and IM routes of administration. Further research is needed to delineate the factors that may result in HS-BChE-induced performance impairment.

HS-BChE, within the range of doses shown to be devoid of behavioral effects, provided significant protection against performance degradation produced by the OP, MEPQ. Moreover, blood BChE activity was observed to decrease and then increase following MEPQ exposure. This result is consistent with the expected reaction of MEPQ and HS-BChE. Similar results have been obtained previously using MEPQ and other highly toxic OP agents (3-6,22,28). Protection afforded by HS-BChE, however, was incomplete even though blood BChE activity was increased to levels expected to be sufficient to neutralize the amount of MEPQ administered. This result indicates a limitation in the efficacy of a scavenger therapy when both enzyme and OP are administered systemically.

Taken together, the results of the present series of experiments indicate that BChE may be valuable for the treatment of OP toxicity. In this respect, our results are consistent with, and extend, the results of previous studies. Because many OP agents rapidly and irreversibly inhibit cholinesterases, the efficacy of a scavenger therapy is maximized when administration precedes exposure. The observed lack of effect of HS-BChE on behavior in the present series of experiments suggests that exogenous BChE may, potentially, be administered prophylactically without disrupting behavior.

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