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Prevention of Soman-Induced Cognitive Deficits by Pretreatment With Human Butyrylcholinesterase in Rats

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BRANDEIS, R., L. RAVEH, J. GRUNWALD, E. COHEN AND Y. ASHANI. *Prevention of soman-induced cognitive deficits by pretreatment with human butyrylcholinesterase in rats.* PHARMACOL BIOCHEM BEHAV 46(4) 889–896, 1993.—This study examined the ability of pretreatment with human serum butyrylcholinesterase (HuBChE) to prevent soman-induced cognitive impairments. Behavioral testing was carried out using the Morris water maze task evaluating learning, memory, and reversal learning processes. Pretreatment with HuBChE significantly prevented the memory and reversal learning impairments induced by soman. A small deficiency in performance was observed only during part of the learning period in HuBChE-treated rats after administration of soman. Results support the contention that pretreatment alone with HuBChE is sufficient to increase survival and to prevent impairment in cognitive functioning following exposure to soman.

Soman Human butyrylcholinesterase (HuBChE) Morris water maze Cognitive deficits Rats Pretreatment Protection Prophylaxis

ORGANOPHOSPHATE (OP) nerve agents are considered to be among the most potent chemical warfare agents. Manifestation of toxic symptoms is due to inhibition of acetylcholinesterase (AChE), which leads to accumulation of acetylcholine (ACh) both at central and peripheral synapses (22,44).

Exposure to either acute high doses or chronic low doses of potent anticholinesterases, in particular pinacolylmehtylphosphonofluoridate (soman), had recently been demonstrated to result in severe brain pathology. Thus, neuronal degeneration and necrosis of piriform and entorhinal cortex, neocortex, amygdaloid nuclei, dentate gyrus, hippocampus, septum, and various thalamic nuclei have been frequently reported following exposure to soman (8,9,24,27,29,30,32, 33,45). Furthermore, persistent severe alterations in behavior have been observed in OP-exposed animals (14,16,23,28,35, 38,47,48). Cognitive incapacitation in humans (10,36,39) and animals (7,17,18,26,34), especially impairment of learning and memory, was described following poisoning with anti-ChE such as soman, isopropylmethylphosphono fluoridate (sarin), diisopropylfluorophosphate (DFP), and diethyl *S*-2-ethylthioethyl phosphorodithioate (disulfoton).

Present treatment for OP intoxication consists of traditional multidrug regimen that contain pyridostigmine, atropine, oxime reactivator, and anticonvulsant drugs (12,21, 25,37,41). Although most of these regimens have been demonstrated to increase survival among experimental animals challenged with OPs, they could not prevent postexposure symptoms such as convulsions and behavioral deficites (12,21).

A possible strategy to prevent toxic manifestations following exposure to OPs is to sequester anti-ChE toxic compounds in the blood and thereby to detoxify them before they can inhibit AChE at physiologically important targets (1,11). For this strategy, enzyme scavengers such as AChE (11) or butyrylcholinestertase (BChE) (5,11) have been demonstrated to be promising single prophylactic drugs for OP toxicity. Since enzymes from human source appear to be most suitable scavenging antidotes, we initiated a study aimed at the evaluation of BChE purified from human plasma (HuBChE) as a prophylactic scavenger candidate. Initial study with partially purified HuBChE has been demonstrated by us to increase significantly survival among mice and rats challenged with the potent anti-ChE soman, without the need for postexposure treatment (2).

A most significant injury caused by OP intoxication is neuronal degeneration of the hippocampus. The latter is associated with spatial learning and memory. Therefore, it was

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important to asses the efficacy of HuBChE not only to protect animals, but also to prevent impairment of cognitive functions following exposure to OPs. To this end, the Morris water maze (MWM) task, which is considered to be most sensitive to hippocampal lesions (3,15,31,42) and to cholinergic manipulations (4,6,19,43), was chosen as a spatial orientation task.

We report here on the ability of pretreatment with highly purified HuBChE alone, to prevent soman-induced deficits in cognitive functioning in rats by assessing spatial learning, spatial memory, and reversal learning. The study was divided into two stages. Stage I was conducted to determine the appropriate time interval following soman exposure for the detection of behavioral impairments. In stage II the potential prophylactic ability of HuBChE was evaluated.

METHOD

Subjects

Male Sprague–Dawley rats (3 months old) of two consecutive shipments were supplied several weeks apart, from Charles River Breeding Laboratories, UK. The average body weight (±SD) was 259 ± 15 and 250 ± 7 g. Animals were housed five per cage, in a temperature-controlled environment (22 ± 1°C) with a 12L : 12D normal cycle. The rats had free access to food and water. Behavioral testing was carried out between 0800 and 1300 h, 3 days a week.

Rats were maintained in accordance with the principles enunciated in the *Guide for Care and Use of Laboratory Animals*, NIH publication

No. 85-23, 1985 revision.

Materials

Electrophoretically homogenous HuBChE was purified from outdated human plasma by a technique to be published elsewhere. One milligram pure enzyme contains 11 nmol active sites with a specific activity of 750 units/mg (butyrylthiocholine as substrate). Enzyme activity was assayed by the procedure of Ellman et al. (13) using butyrylthiocholine as substrate. Soman (>98% purity) was prepared in our laboratory. The same batch was used throughout the study.

Drug Administration

Stage 1. In this stage, behavioral changes over time elapsed after injection of soman were examined.

The IV LD_{50} of soman (at 95% confidence limits; body weight 259 ± 15 g) obtained for two separate determinations were 49.2 (45.8–52.9) and 55.5 (50.6–62.0). Two groups of rats were challenged with 45–60 μg/kg soman IV (0.9–1.1 × LD_{50}). The equitoxic dose, assuming an average body weight of 259 g, was 65–86 nmole/rat. Surviving rats that displayed severe symptoms of OP poisoning were selected for behavioral testing. Two control groups were treated with saline. One soman-treated group and one control group were tested 1 week after exposure and a second pair of soman- and saline-treated groups was assesed 4 weeks postinjection. Rats were randomly assigned to the different treatment groups. Each group consisted of nine animals.

Stage 2. In this experiment, the effect of pretreatment with HuBChE was evaluated both in soman-exposed and saline-injected sham-handled rats, 1 week after soman challenge. This stage was performed immediately following stage I.

The IV LD_{50} of soman in rats weighing 250 ± 7 g was 46.5 μg/kg (43.1–50.1). Rats were randomly divided into two treatment groups (n = 9). One group was treated IV with 4 mg (44 nmol) of purified HuBChE/rat and 10 min later rats were challenged with 70 μg/kg soman IV (1.5 × LD_{50}), which is equivalent to an averege of 97 nmol/rat for animals weighing 250 g. The second group was treated with HuBChE only. Both groups were tested 1 week following treatment.

Apparatus

Rats were tested in a circular metal water maze (diameter: 1.4 m, height: 50 cm) that was painted white and was filled to a height of 25 cm with water (26 ± 1°C) in which powdered milk was dissolved. A white metal platform (12 × 12 cm) covered by wire mesh was present inside the pool; its top surface was 20 mm below the surface of the water. Thus, the platform was invisible to a viewer inside the pool. The pool surface was divided into four quadrants of equal area, NE, NW, SE, and SW. The platform was placed midway between the center and rim of the pool in any of the four quadrants.

The maze was brightly lit and surrounded by well-lit, salient objects, which were held constant throughout training. Performance in the maze was monitored by a tracking system consisting of an overhead video camera linked to a TV monitor and an image analyzer (CIS-2) coupled to a microcomputer (system designed and produced by Galai Laboratories, Ltd., Migdal Ha-Emek, Israel).

Procedure

Training. Each rat was trained for 2 consecutive days, eight trials (two blocks)/day, in which the platform position remained constant and was located in the center of the southeast quadrant of the pool. Within each block of four trials, each rat started at each of the starting locations, but the sequence of locations was randomly selected. A trial consisted of placing a rat by hand into the water facing the wall of the pool at one of four starting locations, i.e., north, south, east or west around the pool's perimeter. Prior to training, the rat was placed on the platform for 60 s. If, on a particular trial, a rat found the platform, it was permitted to remain on it for 60 s. A trial was terminated after 120 s if a rat failed to find the platform, and the rat was placed on the plaftorm for additional 60 s before starting the next trial. Escape latency (the time to find the platform), path length (the distance travelled by the rat), and speed (the swimming rate of the rat) were recorded on each trial by the monitoring system.

Transfer test. Three minutes following the last training trial (trial 16), the platform was entirely removed from the pool (a probe trial). In this trial (trial 17), the rat was placed into the water for a limited period (60 s), and its spatial bias was measured by recording the relative distribution of escape latency and path length over the four quadrants of the pool.

Reversal test. During trials 18–21 (third day), the platform position was changed to the northwest quadrant, opposite to the training quadrant. Thus, during reversal learning, the platform location was moved relative to the configuration of objects within the room, but the pool occupied the same place within the room throughout the entire experiment. Rats were evaluated as described above.

RESULTS

Stage 1

Clinical observations. Manifestations of symptoms in soman-poisoned animals included tremors, fasciculations,

tonic-clonic convulsions, salivation, dyspnea, ataxia, and piloerection. Although rats were fed with wet pellets inside the cages, a significant loss of weight (10–15%) occurred during the first 10 days following intoxication. Later on a normal gain of weight was observed. Most of soman-treated rats were hyperactive and in two rats clonic convulsions were observed even 1 month after exposure.

Training. For each rat, the escape latency, path length, and swimming speed of every four trials in each of the training days were grouped into blocks (two blocks for each day). Scores were analyzed by a three-way ANOVA (2 × 2 × 4) with one repeated variable (blocks) and two nonrepeated variables (challenge—soman/saline, and time following challange—week/month). Specific comparisons were performed using the simple main effects contrasts analysis (46), which is specifically suited for testing significant interactions when some of the variables involved are of repeated measurement type.

Escape latency and path length. The interaction between challenge and blocks was found significant [$F(3, 96) = 6.21$, $p < 0.001$ and $F(3, 96) = 8.31$, $p < 0.001$, for escape latency and path length, respectively]. Both soman-challenged groups showed significantly longer escape latencies and path lengths (indicating an impaired performance) than control rats during the whole training period (see Figs. 1 and 2). Furthermore, for the escape latency measure, soman-treated groups did not show any learning curve while the control groups displayed a significant learning ($p < 0.001$) toward the second block of training and maintained the same level of performance thereafter. Path length measurements are consistent with escape latency data. Thus, the soman-treated groups did not show any pattern of learning; moreover, these groups showed a further decrease in performance at the beginning of the second day of training ($p < 0.001$, block No. 3).

The control groups showed a significant learning toward the second block of training ($p < 0.001$) and a further increase in performance toward the fourth block ($p < 0.05$). It should be pointed out that for both measures (i.e., escape latency and path length) no significant difference was found between soman-challenged animals tested 1 and 4 weeks after exposure.

Swimming speed. The interaction between challenge and blocks was significant, $F(3, 96) = 14.21$, $p < 0.001$. Both soman-treated groups showed significantly lower swimming speeds than control rats only during the first day of training (see Fig. 3). These differences diminished during the remaining trials.

Transfer trial. The escape latency and path length for the transfer trial (trial No. 17) were analyzed by a three-way ANOVA (2 × 2 × 4) with one repeated variable (quadrant in the pool) and two nonrepeated variables (challenge—soman/saline, and time following challenge—week/month). Specific comparisons were performed using the simple main effects contrasts analysis.

The interaction between challenge and quadrant in the pool was found significant for both measures [$F(3, 96) = 8.72$, $p < 0.001$ and $F(3, 96) = 7.77$, $p < 0.001$ for escape latency and path length, respectively]. In both measures, the two soman-challenged groups did not show any spatial bias during the transfer trial. In that respect, no difference was found between these two groups. Figure 4 shows that soman-treated rats spent an equal time in each of the four quadrants of the pool, while Fig. 5 shows that the total distance covered by

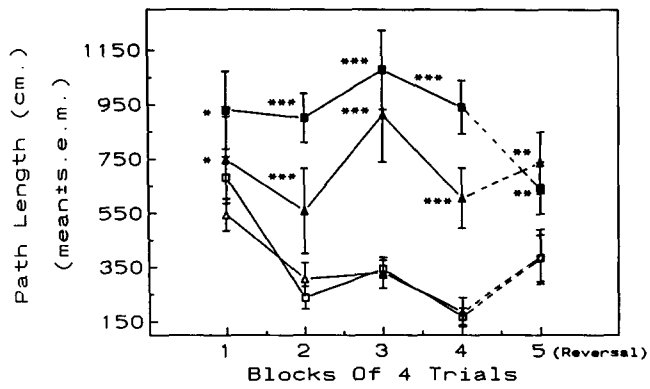

FIG. 2. Path length of rats 1 week and 4 weeks following soman challenge. Filled squares: soman, 1 week; filled triangles: soman, 4 weeks; open squares: saline, 1 week; open triangles: saline, 4 weeks. $*p < 0.05$, $**p < 0.005$, $***p < 0.001$ compared to respective control group.

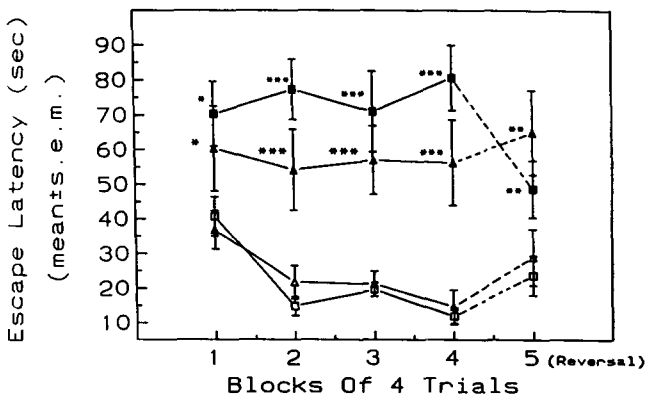

FIG. 1. Escape latency of rats 1 week and 4 weeks following soman challenge. Filled squares: soman, 1 week; filled triangles: soman, 4 weeks; open squares: saline, 1 week; open triangles : saline, 4 weeks. $*p < 0.01$, $**p < 0.005$, $***p < 0.001$ compared to respective control group.

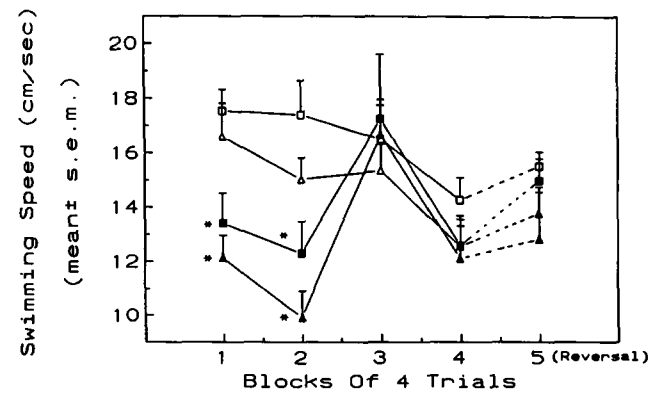

FIG. 3. Swimming speed of rats 1 week and 4 weeks following soman challenge. Filled squares: soman, 1 week; filled triangles: soman, 4 weeks; open squares: saline, 1 week; open triangles: saline, 4 weeks. $*p < 0.001$ compared to respective control group.

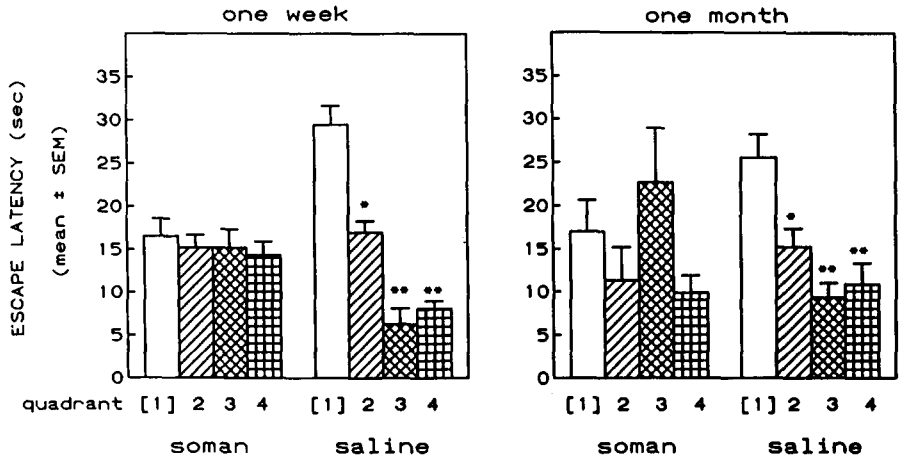

FIG. 4. Distribution of escape latency during transfer trial 1 week and 4 weeks following soman challenge. $*p < 0.01$, $**p < 0.001$ compared to quadrant No. 1 in control group.

swimming was equal in each of the quadrants of the pool except for quadrant No. 4, in which the distance swum was significantly shorter ($p < 0.05$) than that of quadrant No. 1.

In contrast to rats intoxicated with soman, control rats spent significantly more time and swam significantly longer distance in the training quadrant relative to the three other quadrants of the pool.

Reversal test. For each rat, the escape latency, path length, and swimming speed of the reversal test (trials No. 18–21) were grouped into one block. All three measures were analyzed by a two-way ANOVA (2 × 2) (challenge—soman/saline, and time following challenge—week/month). Specific comparisons were performed using the simple main effects contrasts analysis.

The effect of challenge was found significant [$F(1, 32) = 11.96$, $p < 0.005$ and $F(1, 32) = 9.31$, $p < 0.005$, for the escape latency and path length, respectively]. Both soman-challenged groups showed significantly longer escape latencies and longer path lengths (indicating an impaired performance) than control rats (see Figs. 1 and 2).

It is important to point out that no significant difference was found between soman-treated and control rats in swimming speed measure.

Stage 2

Clinical observations. Rats pretreated with HuBChE alone did not show any clinical symptoms. The gain in body weight was normal. After IV exposure to 70 μg/kg soman (ca. 1.5 × LD_{50}), most HuBChE-treated rats (6/9) displayed mild symptoms of intoxication (e.g., slight tremors, mild salivation, and general weakness). Rats returned to normal after 2 h. In the first 48 h after the exposure to soman, a loss in body weight was observed; however, rats showed normal gain in body weight beginning on the third day after the injection of soman. It should be pointed out that animals challenged with

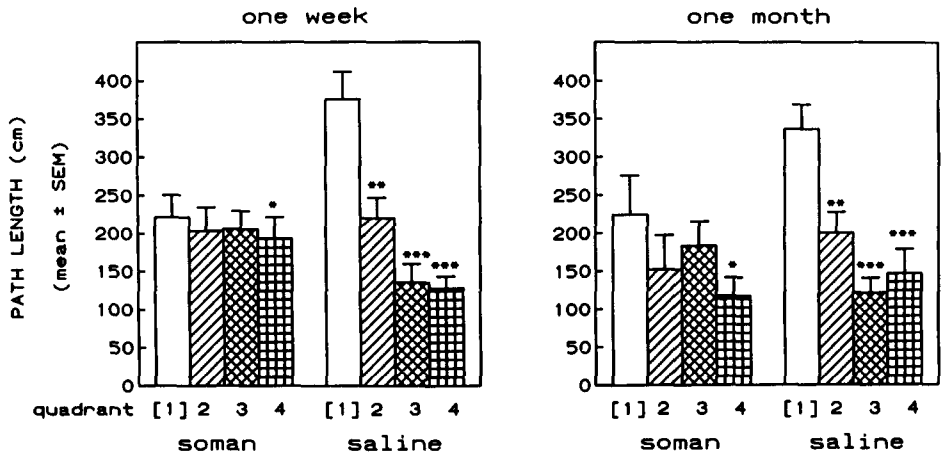

FIG. 5. Distribution of path length during transfer trial 1 week and 4 weeks following soman challenge. $*p < 0.05$ compared to quadrant No. 1 in soman group. $**p < 0.01$, $***p < 0.001$ compared to quadrant No. 1 in control group.

70 μg/kg of soman both in stage I and stage II died within a few minutes (n = 14).

Blood level of HuBChE before and after soman challenge (an average of 97 nmol/rat) was 43.1 ± 0.6 nmol/rat and 18.2 ± 1.0 nmol/rat, respectively. In a different set of experiments an increase of 10% in the initial level of blood HuBChE prevented completely manifestation of toxic signs in animals exposed to the same dose of soman. These rats were not subjected to behavioral tests.

It should be noted that the time course of HuBChE following an intravenous injection displayed biphasic exponential decay with a half-life of 53 h for the slow elimination process (not shown).

Behavioral testing. Stage II was conducted immediately following stage I, in the same experimental and environmental conditions. To establish a hard standard for detailed comparison between the two stages, we statistically compared the results of the saline control group of stage I with the results of three additional saline-treated groups evaluated in our laboratory during the same period.

No significant differences were found between the four groups, either in training [$F(3, 32) = 2.34$, NS and $F(3, 32) = 1.24$, NS, for the escape latency and path length measures, respectively], transfer test [$F(3, 32) = 0.65$, NS and $F(3, 32) = 1.78$, NS], or reversal test [$F(3, 32) = 0.42$, NS and $F(3, 32) = 0.62$, NS]. Since the four control groups did not differ from each other, a hard comparable point on which the two stages could be compared had been met. Indeed, the same effects and trends were obtained irrespective of the control group used for the statistical analyses.

In addition, in view of the high dose of soman administered to HuBChE-pretreated rats of stage II (70 μg/kg), and the large number of animals that were required to obtain nine rats that will survive a dose of 0.9–1.1 × LD_{50} soman, the soman-treated animals of stage I were used to analyze the efficacy of HuBChE on the alleviation of soman-induced behavioral deficits in rats. Consequently, results are analyzed below with respect to the saline control and nonprotected soman-exposed groups that were tested 1 week after the challenge with soman (see Stage I). Thus, four groups of rats are referred to as: I, saline only (stage I); II, HuBChE only (stage II); III, saline followed by soman (stage I); IV, HuBChE followed by soman (stage II).

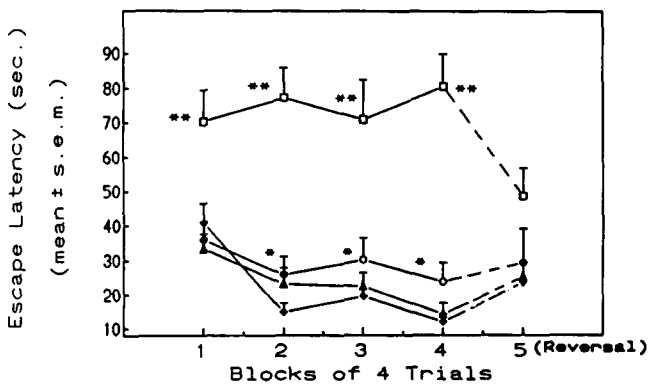

FIG. 6. Escape latency of soman and control rats treated with HuBChE. Open squares: soman; open circles: soman + HuBChE; filled triangles: HuBChE; filled diamonds: saline. *$p < 0.05$ compared to saline group. **$p < 0.001$ compared to all other groups.

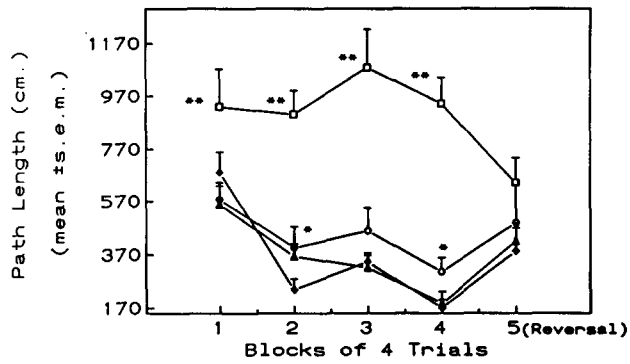

FIG. 7. Path length of soman and control rats treated with HuBChE. Open squares: soman; open circles: soman + HuBChE; filled triangles: HuBChE; filled diamonds: saline. *$p < 0.05$ compared to saline group. **$p < 0.001$ compared to all other groups.

Training. For each rat, the escape latency, path length, and swimming speed of every four trials in each of the training days were grouped into blocks (two blocks for each day). Scores were analyzed by a three-way ANOVA (2 × 2 × 4) with one repeated variable (blocks) and two nonrepeated variables (challenge—soman/saline, and treatment—HuBChE/saline). Specific comparisons were performed using the simple main effects contrasts analysis.

Escape latency and path length. The interaction between challenge and treatment was found significant [$F(1, 32) = 24.08$, $p < 0.001$ and $F(1, 32) = 22.12$, $p < 0.001$, for escape latency and path length, respectively]. Nonprotected rats exposed to soman (III) showed throughout the whole training period a significantly impaired performance compared to the three other groups (see Figs. 6 and 7). HuBChE + soman group (IV) performed significantly better than nonprotected rats challenged with soman (III).

The interaction among challenge, treatment, and blocks was also found significant [$F(3, 96) = 2.83$, $p < 0.05$ and $F(3, 96) = 4.94$, $p < 0.005$ for escape latency and path length, respectively]. HuBChE + soman group, when compared to saline control animals (I), displayed small impairment during three blocks of training (escape latency measure) and during two blocks (path length measure). A characteristic computer depiction of the paths travelled by the various groups is shown in Fig. 8.

Swimming speed. The interaction among challenge, treatment, and blocks was found significant, $F(3, 96) = 4.59$, $p < 0.01$. Nonprotected rats injected with soman (III) showed a significantly slower swimming speed than the other three groups only during the first day of training (see Fig. 9). This difference diminished during the remaining trials. In one case, HuBChE-treated rats (II) displayed a somewhat slower swimming speed during the third block of training ($p < 0.02$), whereas in the other groups such an effect was not detected.

Transfer trial. The escape latency and path length measures for the transfer trial (trial No. 17) were analyzed by a three-way ANOVA (2 × 2 × 4) with one repeated variable (quadrant in the pool) and two nonrepeated variables (challenge—soman/saline, and treatment—HuBChE/saline). Specific comparisons were performed using the simple main effects contrasts analysis.

Escape latency and path length. The interaction among challenge, treatment, and quadrants was significant [$F(3, 96) =$

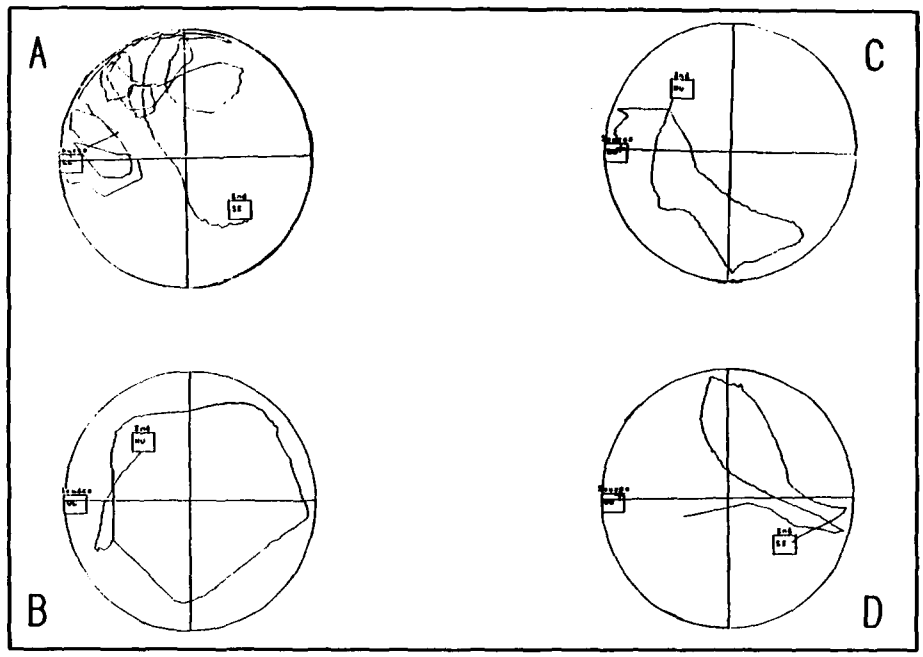

FIG. 8. A characteristic computer depiction of the path travelled by soman and control rats treated with HuBChE. (A) soman group; (B) saline group; (C) soman + HuBChE group; (D) HuBChE group.

4.19, $p < 0.01$ and $F(3, 96) = 5.08$, $p < 0.005$, for escape latency and path length measures, respectively].

Nonprotected rats treated with soman (III) did not show any spatial bias during the transfer trial (see Figs. 10 and 11). The time spent and the distance swum were equal in each of the four quadrants of the pool. In marked contrast, the other three groups spent more time and swam a longer distance in the training quadrant than in the three other quadrants of the pool. Thus, nonprotected rats did not show any tendency to search for the platform during the probe trial, while HuBChE + soman group (IV) displayed a spatial bias similar to the control animals (I and II).

Reversal test. For each rat, the escape latency, path length, and swimming speed of the reversal test (trials No. 18–21) were grouped into one block. All three measures were analyzed by a two-way ANOVA (2 × 2) (challenge—soman/saline, and treatment—HuBChE/saline). Specific comparisons were performed using the simple main effects contrasts analysis.

Nonprotected rats injected with soman (III) showed an impaired performance relative to the other three groups as indicated by both escape latency and path length measures; however, this difference was not statistically significant (see Figs. 6 and 7). The performance of group IV (HuBChE + soman) was very similar to that of the control group.

The interaction between challenge and treatment for the swimming speed measure was significant, $F(1, 32) = 5.25$, $p < 0.05$. The swimming speed of group IV (HuBChE + so-

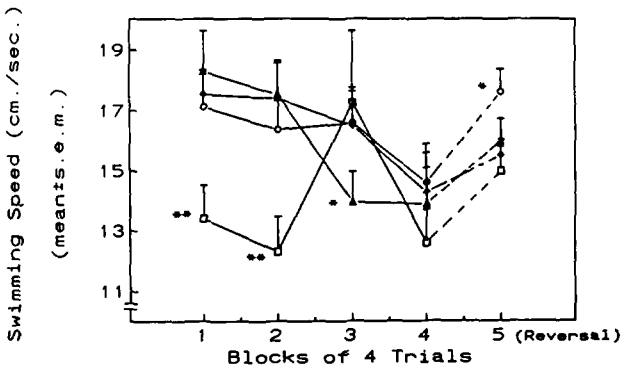

FIG. 9. Swimming speed of soman and control rats treated with HuBChE. Open squares: soman; open circles: soman + HuBChE; filled triangles: HuBChE; filled diamonds: saline. $*p < 0.02$, $**p < 0.001$ compared to all other groups.

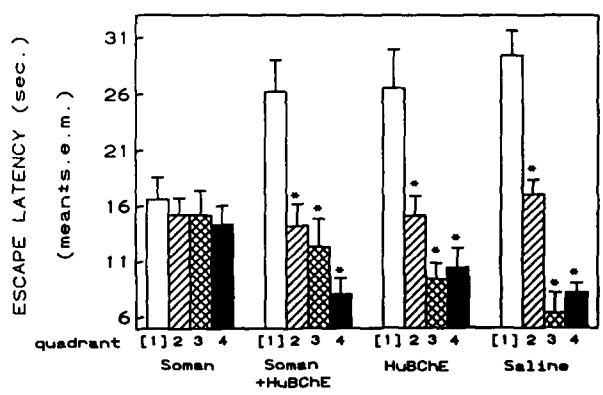

FIG. 10. Distribution of escape latency during transfer trial for soman and control rats treated with HuBChE. $*p < 0.001$ compared to quadrant No. 1 in the respective group.

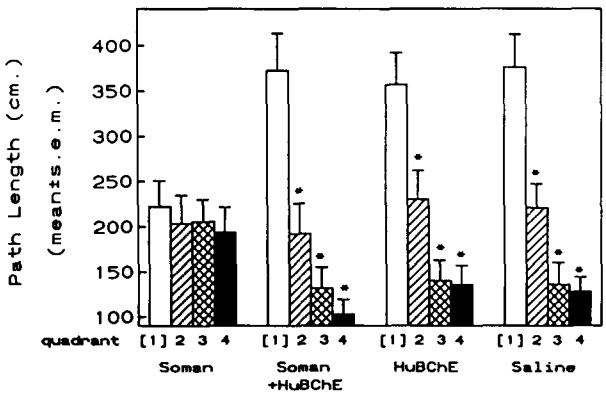

FIG. 11. Distribution of path length during transfer trial for soman and control rats treated with HuBChE. *$p < 0.001$ compared to quadrant No. 1 in the respective group.

man) was significantly higher than that of the other three groups (see Fig. 9).

DISCUSSION

Cognitive functioning in rats was significantly impaired in our study following IV administration of 0.9–1.1 × LD_{50} of soman. The deficit in cognitive functioning was clearly pronounced during acquisition and retention, either 1 or 4 weeks following soman challenge. The deterioration in reversal learning was smaller, albeit not statistically different, 1 week after soman challenge. Reversal learning, as an experession of the ability to shift strategies to task demands (40), is a higher level, more complex cognitive function than learning or memory; therefore, this ability might be impaired after a progressive neuronal change that took place a few weeks later. In this respect, it might be pointed out that the neuropathological damage in the hippocampus caused by OPs is intensified with time (20).

Our results support previous conclusions regarding the nonspecific effects of OPs (17). Thus, reference memory and working memory [see (17) and literature cited therein] were seriously injured following soman intoxication. Further, aquisition and reversal learning were also significantly impaired.

HuBChE significantly prevented the development of soman-induced cognitive decrements. No significant differences were displayed during both retention and reversal learning between soman-challenged animals pretreated with HuBChE and control saline-treated rats. However, during part of the training period, the HuBChE + soman group was somewhat deficient in performance compared to the control (saline) group. These findings are consistent with previous results that had shown that both cognitive functions, retention and reversal learning, when compared to acquisition, are especially sensitive to cholinergic manipulations (19,40).

HuBChE treatment alone was devoid of any impairments in behavioral performance, either motor or cognitive. In that respect, it seems that HuBChE has no undesirable performance decrements.

Nonspecific motor coordination effects could neither explain the behavioral deficits of soman-challenged rats nor the preventive effects of HuBChE, since no such effects were demonstrated in the swimming ability of the rats. While specific cognitive impairments were observed along the whole behavioral testing period in soman-challenged rats, the swimming speed of these rats was lower only during the first day. Furthermore, the swimming ability of HuBChE-treated rats was not different from that of the control rats.

As far as we now know, the MWM task has not been utilized to study soman-challenged rats. This task has been demonstrated to be a very useful tool in the research of the mechanisms underlying learning and memory (3), being very sensitive to hippocampal lesions. The impairments in spatial cognitive functioning found in the present study are consistent with the brain pathology, especially the neural degeneration of the hippocampus, demonstrated as a result of OPs toxicity (8,9,24,29,30,33,45).

In conclusion, results shown here support the concept that pretreatment alone with a scavenger such as HuBChE is sufficient to increase not only survival but also to alleviate deficits in cognitive functioning after exposure to a potent nerve agent such as soman.

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