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Diazepam Impairs Retention of Spatial Information Without Affecting Retrieval or Cue Learning

JORGE D. BRIONI[1] AND MARIA PIA AROLFO

Department of Pharmacology, Faculty of Chemical Sciences, National University of Cordoba Sucursal 16, CC61, 5016 Cordoba, Argentina

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BRIONI, J. D. AND M. P. AROLFO. *Diazepam impairs retention of spatial information without affecting retrieval or cue learning*. PHARMACOL BIOCHEM BEHAV **41**(1) 1–5, 1992.—We further investigated the effect of diazepam on the processing of spatial information in a water maze task. Diazepam significantly impaired the retention of spatial information in a group of rats trained to locate a hidden platform. In a free swim trial carried out after training, diazepam-treated rats showed no bias to the target quadrant. There was no effect of diazepam on retrieval of spatial information in well-trained rats, and diazepam was devoid of any effect on cue learning in the water maze. However, diazepam blocked latent place learning during cue training in the water maze. Our results indicate that the GABA-BZD receptor modulates spatial information processing and that diazepam specifically impairs the retention of spatial information without affecting retrieval or cue learning.

Diazepam GABA Memory Spatial learning

ON the basis of electrophysiological and receptor binding studies, GABA (gamma-aminobutyric acid) receptors have been classified into GABA-A and GABA-B (3, 14, 18, 27). The GABA-A receptor contains a variety of binding sites for therapeutically relevant drugs which allosterically interact with the GABA binding site or the associated chloride channel. These groups of drugs include benzodiazepines (BZD), barbiturates and beta-carbolines among others (10).

Diazepam, a prototypical BZD, not only exerts anxiolytic, anticonvulsant and muscle relaxant effects in man, but also produces anterograde amnesia (8,19). Diazepam and other BZD induce anterograde amnesia in a wide number of animal tasks including aversive and nonaversive paradigms, and most evidence points to an interference with acquisition and/or posttraining information processing (9,32). The evidence for the effect of BZD on spatial tasks is unclear. An impairment of place learning in the water maze has been observed (22), but no drug effect was reported in another study using the radial arm maze (15). We have recently reported that diazepam impairs place learning in the water maze, without affecting the acquisition of spatial information (1).

In view of the well-known amnesic effect of diazepam in humans and recent evidence supporting the role of central GABA-BZD receptors in learning and memory processes (5, 17, 21), we further evaluated the effect of diazepam on different associative and nonassociative processes during place learning in a water maze.

METHOD

Animals

Male Wistar rats (90 days old) from our colony were used. They were housed in groups of six–seven in wire-mesh cages and maintained on a 12-h light-dark period (lights on 7:00 a.m.) with food and water available ad lib.

Apparatus

The water maze used was a circular, galvanized-steel swimming pool measuring 1.80 m in diameter and 0.50 m in height, filled to a depth of 20 cm with 25°C water. Attached to the rim of the tank along 1.70 m of its circumference was a white strip that extended 20 cm from the rim, that served as a salient cue. Other cues were also available in the environment surrounding the tank. Four points equally spaced around the perimeter of the tank were arbitrarily designated to serve as starting locations. On this basis, the tank was divided into four equal quadrants in a clockwise order (target, adjacent, opposite and adjacent). Located in the center of one of these quadrants was a $14 \times 14 \times 19$ cm (w. $\times$ l. $\times$ h.) Plexiglas platform (i.e., its surface was 1 cm below the water level). Following each day of training the tank was drained and cleaned.

Place Training Procedure

A trial began when the rat was placed in the pool facing the wall of the tank at one of the starting positions which varied

[1]Requests for reprints should be addressed to Jorge D. Brioni, Neuroscience Department, Pharmaceutical Products Division, Building AP-10 (47W), Abbott Laboratories, Abbott Park, IL 60064.

from trial to trial in a quasirandom order. The rat was allowed to swim until it located and climbed onto the escape platform. If the rat did not locate the platform within 90 s, it was gently guided to it. The rat remained on the platform for 20 s before being removed. The following trials were conducted after a 5–10-s delay. After the third trial the rat was returned to the home cage. The rats received 3 trials per day during the 4 consecutive days of training. The animals were injected with saline or diazepam 30 min before each session. The platform remained in a fixed location throughout the training period (reference memory). Escape latencies were measured as the time after being released in the pool until the rat escaped onto the platform. Subjects were videotaped through a wide-angle lens attached to a camera mounted above the tank.

Free Swim Trial

Twenty-four h after the training phase, a trial without the escape platform was carried out for 30 s. In previous studies we have used a 60-s trial but we decided to reduce it to 30 s. Since random trained animals can find the platform in 40 s, searching beyond this time would not reflect spatial bias (4). The 4 possible positions of the platform and the limits of the 4 quadrants were marked on the video screen to indicate its exact surface area. From video tapes made during this free swim it was possible to calculate the quadrant times (the number of seconds spent by the rat in each of the four quadrants).

Retrieval

In order to evaluate the effect of diazepam on the retrieval of spatial information, four groups of 8 rats were trained to locate a hidden platform according to the place training procedure outlined above. Animals received 18 trials of training under saline and were able to escape in less than 10 s during the last three trials. One day after the training was completed, animals were subjected to a 30-s free swim trial under the influence of saline or diazepam. In this design the effect of diazepam on retrieval of spatial information on well-trained rats was evaluated.

Cue Training

Four groups of 7 rats were trained to escape to a black platform protruding 1 cm above the water surface. Rats were trained according to our general procedure. They were injected with saline or diazepam doses 30 min before the training session on each of the 4 consecutive days. Although they were released from different starting positions on each trial, the cue platform remained in a fixed location during training. A free swim trial would be expected to indicate significant spatial bias in saline-treated rats since this method of cue training produces a high degree of latent place learning (24,31).

Visual Discrimination in the Pool

Rats were trained to discriminate a rigid black platform that protruded 1 cm above the water surface, from a black and white floating one. Rats were given 6 trials per day on each of 10 consecutive days. Animals were injected with saline or diazepam doses 30 min before each session. On each trial the platforms were placed in any of 4 possible locations to avoid the use of spatial cues to locate the correct one. The 2 platforms were equidistant from the starting position on all trials. To avoid the use of a praxis strategy the rats were placed in the water starting in each trial from a different position. They were allowed to swim until they reached the rigid platform and climbed on it. If the rat did not find the rigid platform in 90 s, it was gently guided to the platform and left there for additional 20 s. They were immediately submitted to the next trial, and after 6 trials returned to the home cage. A response was considered correct when the rat climbed onto the rigid platform without touching the floating one.

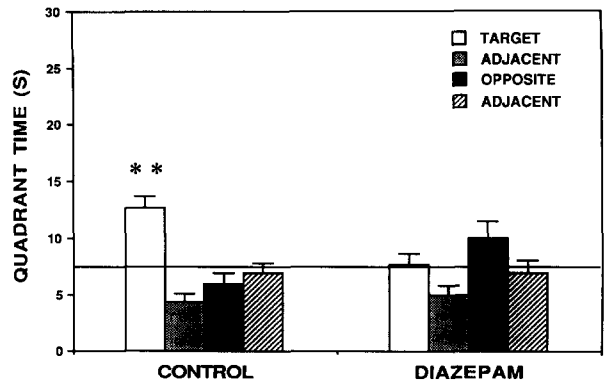

FIG. 1. Effect of diazepam on place learning. Free swim data corresponding to rats trained under saline or 1 mg/kg diazepam. No platform was present and animals received no drug injection. Quadrants were designed in a clockwise order. Data represent the mean ± SEM time spent in the 4 quadrants of the pool (n = 9). The horizontal line represents chance performance. $^{**}p<0.01$, as compared to the time spent by control rats in the remaining quadrants.

Drugs

Diazepam was prepared in saline solution with Tween 80. It was injected IP, 30 min before the session.

Statistics

The data were analyzed by one-way or two-way analysis of variance (ANOVA) with repeated measures where appropriate, followed by the Fisher PLSD test for individual mean comparisons. When quadrant time data was analyzed, the numerator degrees of freedom was reduced by one (24).

RESULTS

Effect of Diazepam on Place Learning

In a group of rats trained to locate a hidden platform in the pool, 1 mg/kg diazepam significantly impaired place learning. The free swim trial carried out 24 h after the training phase (Fig. 1) showed that only the animals in the control group exhibited bias to the target quadrant. The analysis of the time spent in the four quadrants revealed a significant quadrant effect, $F(2,54) = 8.4$, $p<0.01$, and a significant drug × quadrant interaction, $F(3,54) = 5.0$, $p<0.05$. The time spent by control rats in the target quadrant was significantly longer than the time they spent in the remaining quadrants, $F(2,24) = 10.0$, $p<0.01$, and also longer than the time diazepam-treated rats spent in the target quadrant, $F(1,16) = 9.7$, $p<0.01$.

Effect of Diazepam on Retrieval

Well-trained rats consistently escaped onto the hidden platform in less than 10 s. They were injected with saline or diazepam doses, and 30 min later evaluated in a free swim trial.

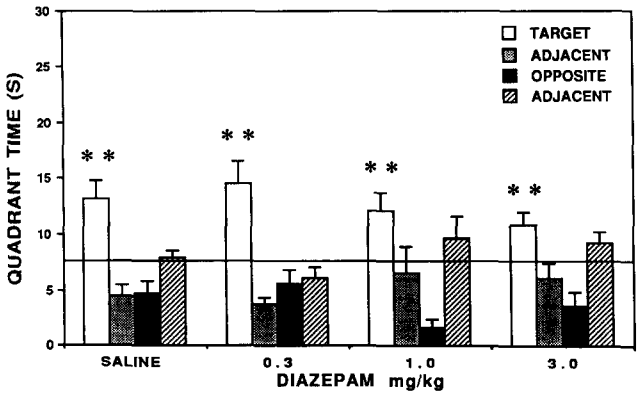

FIG. 2. Effect of diazepam on retrieval. Animals were subjected to 18 trials of place training. On the day of the test they were injected with saline or diazepam and 30 min later released into the pool. Data represent the mean ± SEM time in seconds spent in the 4 quadrants of the pool (n = 8). The horizontal line represents chance performance. $^{**}p<0.01$, as compared to the time spent by the same rats in the other quadrants.

Figure 2 shows that there was no effect of diazepam on retrieval of spatial information, $F(3,28) = 1.3$, NS. There was a significant quadrant effect, $F(2,84) = 24.6$, $p<0.001$, and no drug × quadrant interaction, $F(9,84) = 1.3$, NS. The time spent in the target quadrant was similar across all groups, $F(3,34) = 0.8$, NS. If each group is individually analyzed, a significant bias towards the target quadrant is observed in all the groups (all p's<0.01.)

Effect of Diazepam on Cue Training

As an impairment of performance in the hidden platform task (place learning) may be due to the effect of diazepam in nonassociative factors, we specifically investigated the effect of diazepam on animals that were trained to locate a visible platform in the pool. Figure 3 shows that diazepam did not affect cue learning. A day-by-day analysis revealed no drug effect, $F(3,24) = 2.0$, NS, a significant repeated measure effect, $F(3,72) = 173$, $p<0.0001$, and no interaction, $F(9,72) = 1.3$, NS.

Effect of Diazepam on Latent Spatial Learning

Figure 3 shows the time the rats spent in the target and opposite quadrant during a free swim trial conducted 24 h after cue training in the pool. Control rats and the 0.3 mg/kg diazepam group exhibited bias towards the target quadrant, $F(2,27) = 4.3$, $p<0.05$; $F(2,27) = 5.4$, $p<0.05$, while the other diazepam groups did not ($F = 0.7$ and 0.6, NS). Consistent with the impairing effect of diazepam on retention of spatial information, diazepam also impaired place information latently learned during cue training.

Effect of Diazepam on Visual Discrimination

Diazepam did not affect the two-platform visual discrimination task, $F(3,24) = 0.4$, NS. There was a significant trial effect, $F(4,96) = 1.92$, $p<0.0001$, as all groups increased the number of correct responses during training, but no drug × trial interaction, $F(12,96) = 1.3$, NS, was observed. All groups learned the task with similar efficiencies and there was no difference between saline and the diazepam groups (data not shown).

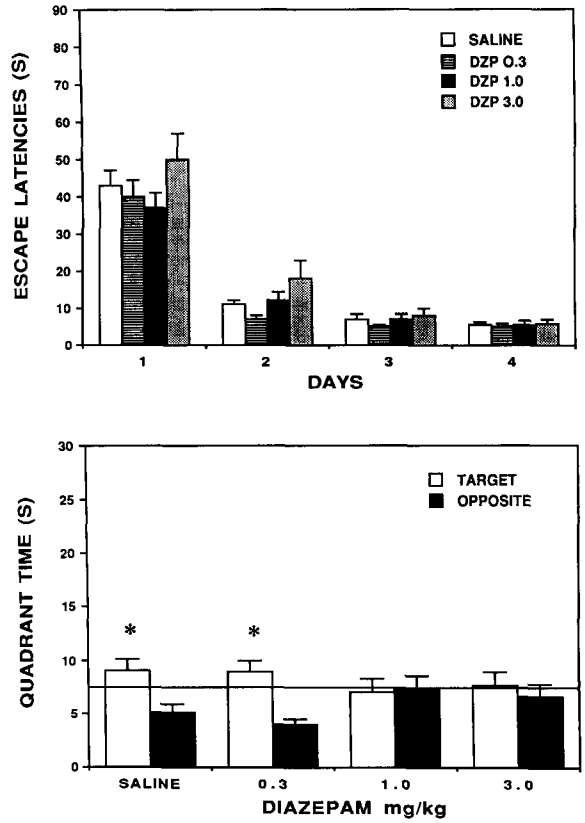

FIG. 3. Effect of diazepam on cue learning. Data represent the mean ± SEM escape latencies averaged over days (n = 7). Rats were subjected to 3 trials per day during 4 days. Saline or diazepam doses were injected IP, 30 min before each training day (upper graph). Effect of diazepam on latent place learning. A free swim trial was carried out 24 h after the cue training. Data represent the mean ± SEM time in seconds spent in the target and opposite quadrants (n = 7). The horizontal line represents chance performance. $^{*}p<0.05$, as compared to the time spent by the same rat in the opposite quadrant (lower graph).

DISCUSSION

Alzheimers's disease is a neurodegenerative process associated with severe cognitive deficiencies. Among the neurochemical alterations present in Alzheimer's patients are the loss of cortical GABAergic terminals (13), and a significant reduction of GABA-A and GABA-B receptors (6,7). Pharmacological studies have demonstrated the effect of GABAergic agonists and antagonists on memory. They have provided extensive evidence that posttraining administration of GABAergic antagonists, including picrotoxin and bicuculline, enhance retention, while GABAergic agonists impair retention (21). These effects are found when the drugs are injected into the amygdaloid complex, suggesting that the GABAergic system in the amygdala is one of the central areas involved in the modulation of memory storage (5). Diazepam and other benzodiazepines induce anterograde amnesia in humans (8), as well in experimental animals trained to perform a variety of memory tasks (9,32).

In a previous study we reported that diazepam significantly impaired the performance of rats in the water maze. A trial-by-trial analysis indicated that 1 mg/kg diazepam did not affect acquisition of spatial information, but there was no bias to the target quadrant in the diazepam-treated rats in the free swim trial

carried out 24 h after the training phase (1). We replicated these previous findings in the present study as there was no spatial bias in animals that received 1 mg/kg diazepam. This impaired performance could not only be related to a deficit in the retention processes but also to an effect of diazepam on retrieval. Data from clinical studies demonstrate that cognitive priming and novelty can carry memory scores of amnesic patients to normal values, suggesting that amnesia could be due to a deficit of retrieval not of storage (33). Similarly, memories could also become weak or inaccessible to retrieval as a result of a drug effect (16). We specifically investigated this hypothesis in experimental animals and it is clear that diazepam did not affect retrieval of spatial information in well-trained rats (Fig. 2). Thus diazepam effects on spatial tasks cannot be explained by a drug-induced retrieval deficit. An effect of diazepam on the consolidation of spatial information is the hypothesis that we would favor at this point of our studies, as the consolidation of the memory trace is an important phase that has been demonstrated with the aid of many drugs and hormones (16,20).

The effect of diazepam on place learning could be due to nonassociative factors such as an effect on motivation or motor skills. Although diazepam can affect muscle tone and coordination (11), there was no effect of diazepam on cue learning in the present study, suggesting that motivational or motor effects of diazepam did not account for the impaired place learning we observed. Training in a cue version of the water maze with the platform always in the same quadrant is a procedure that produces a high degree of place latent learning (24,31). Diazepam blocked latent place learning in animals performing under cue training conditions in the water maze. This result provides strong support to the notion that diazepam specifically impairs place learning since the rats were performing as well as controls throughout the cue task but were unable to process information about the distal cues on the environment.

Prompted by clinical data obtained from amnesic patients like H.M. (29), animal models of amnesia have tried to dissociate the types of learning impaired by lesions to specific regions of the brain like the amygdala or the hippocampus (23, 26, 28). In this regard, spatial tasks are highly dependent on hippocampal function while visual discrimination tasks are not (2, 25, 26). We investigated the generality of the effects of diazepam in a visual discrimination task in the pool, using the same diazepam dose range that impaired performance in a spatial task. Diazepam did not affect visual discrimination on the pool as experimental rats were as efficient as control rats throughout the test. Considering that the septo-hippocampal cholinergic system is critical for place learning (12) and that muscimol and chlordiazepoxide injections in the septum impair spatial learning at the same doses that reduced hippocampal high-affinity choline uptake (4,30), the cholinergic septo-hippocampal system may be the central target for the effects of diazepam on spatial processing.

In summary, our data demonstrate that diazepam specifically impairs retention of spatial information without affecting retrieval or cue learning but rather impairs retention by affecting postacquisition neurobiological processes. These results also indicate the participation of the GABA/BZD receptor during consolidation of spatial information.

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