Pharmacology Biochemistry & Behavior, Vol. 7, pp. 51–54.

Effect of Lysine Vasopressin on Pentylenetetrazol-Induced Retrograde Amnesia in Rats[1]

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(Received 23 December 1976)

BOOKIN, H. B. AND W. D. PFEIFER. *Effect of lysine vasopressin on pentylenetetrazol-induced retrograde amnesia in rats.* PHARMAC. BIOCHEM. BEHAV. 7(1) 51–54, 1977. – Lysine vasopressin (1 μg/rat SC) administered 1 hr prior to either the acquisition trial or 24 hr retention trial facilitated passive avoidance retention. Amnesia was produced when a single 50 mg/kg (IP) injection of pentylenetetrazol was given immediately following the passive avoidance acquisition trial. A single injection of lysine vasopressin (1 μg/rat SC) administered 1 hr prior to either the acquisition trial or 24 hr retention trial antagonized the amnesia.

Lysine vasopressin Pentylenetetrazol Amnesia Memory consolidation Memory retrieval

THE STUDY OF learning and memory has been aided by various pharmacological agents that either facilitate or interfere with learning and memory processes. Treatment of animals with puromycin [6], CO_2 [12], electroconvulsive shock [10] or pentylenetetrazol [11] given in association with training produce amnesic effects. Treatment with such agents as adrenocorticotrophic hormone [16], melanocyte stimulating hormone [17] and vasopressin [1, 16, 17] when given in conjunction with training or testing, may facilitate performance at learning and/or memory tasks.

The memory suppressive action of puromycin can be blocked in mice, if the animals are adrenalectomized prior to training [4]. If corticotropin gel is given up to 3 days prior to training or within 16 hr following training the puromycin effect is also blocked [5]. These results suggested that ACTH may be the effective agent in blocking puromycin-induced amnesia. However, when highly purified corticotropin was given, the anti-amnesic effect was not demonstrated [8]. Since corticotropin gel contains several pituitary peptides which have been shown to delay extinction of an avoidance response [16], its antagonistic effect on puromycin-induced amnesia was postulated to be due to a vasopressin impurity. This hypothesis was validated by the finding that desglycinamide lysine vasopressin (DG-LVP) was effective in blocking the puromycin amnesia [8]. Similar effects have been observed using lysine or arginine vasopressin and these hormones are somewhat more effective than DG-LVP [14].

More recently, it has been reported that the amnesia effects of CO_2 can be blocked by either ACTH or DG-LVP [13]. While ACTH reduced the CO_2 amnesia only when administered before the test trial, DG-LVP reduced the amnesia when administered either before learning or before the testing of an avoidance response.

We now report that lysine vasopressin (LVP) can also block pentylenetetrazol (PTZ) induced amnesia, the effect occurring whether administered prior to the acquisition or test trial of a passive avoidance response.

EXPERIMENT 1

The purpose of Experiment 1 was to determine whether preacquisition treatment of rats with LVP can effectively block amnesia produced by PTZ in a passive avoidance task.

METHOD

Animals

Forty-eight male Sprague-Dawley derived rats attained from the Charles River Breeding laboratories in three separate shipments were used. The rats were 6–7 weeks of age and weighed between 180–210 g at the start of the experiment. A 12 hr day–night cycle from 6:00 a.m. to 6:00 p.m. was in effect and room temperature was maintained at 21°C–23°C. Animals were housed in pairs in wire mesh cages (37 × 18 × 23 cm) with food and water available ad lib. All animals were kept under these

[1] This research was supported by funds from the Connecticut College Psychology Department.

conditions for 8 days prior to training. Six hr prior to the acquisition trial, all animals were food deprived in an attempt to equalize absorption of PTZ. Training was conducted between 5:00 p.m. and 9:00 p.m.

Apparatus

A modified step-through passive avoidance apparatus described by Ader, Weijnen and Moleman [2] was used. Passive avoidance conditioning took place in a dark room with a white noise (10dB) background. The apparatus consisted of a 40 × 40 × 40 cm Plexiglas chamber with black walls and a grid floor. Rats entered this chamber through a 6 × 6 cm guillotine-operated door in the center of a 6 × 25 cm mesh covered elevated start box. The distance from the bottom of the door to the grid floor was 3 cm. Only the start box was illuminted using a 25 W lamp fixed 40 cm above its center. The start box had additional illumination provided by two Archer Exiter relay system photocells, used to measure latency to enter the shock chamber.

Procedure

Each rat was given a single passive avoidance training trial. One minute after the animal was placed in the start box, the door to the darkened chamber was opened, automatically starting a timer that measured step-through latency time. During the time the animal was in the start box, a photocell beam was interrupted by the animal. When the rear legs of the rat passed into the shock chamber, photocell contact was made, automatically stopping the timer. After a 1 sec delay (to allow further movement into the chamber), the grid floor was electrified. Scrambled shock was delivered by a Grason-Stadler Model 700 shock generator set to deliver a shock of intensity of 0.25 mA for 3 sec. All animals were removed from the shock chamber 7 sec after the termination of the shock. Animals failing to step-through within 60 sec in this single acquisition trial were eliminated from the experiment.

The total sample was randomly divided into 4 groups of 12 animals. In all groups, either 0.5 ml saline (SAL) or 0.5 ml synthetic lysine vasopressin (LVP) was injected SC 1 hr prior to the acquisition trial.

LVP (105 U/mg Ferring batch No. 0857) was dissolved in a hydrochloric acid solution of pH 3.8. This solution was diluted with acidified saline (pH 3.8) to a concentration of 2 μg/ml. The saline and both pentylenetetrazol solutions were adjusted to pH 3.8.

Immediately on termination of the acquisition trial, Groups 1 (LVP-PTZ) and 2 (SAL-PTZ) were given the amnesic treatment (PTZ: pentylenetetrazol, 50 mg/kg). Convulsions from this treatment were observed in a 60 × 50 × 30 cm stainless steel box for 5 min. Prior to this experiment, pilot studies indicated that LVP did not reduce the occurrence of convulsions from PTZ.

Groups 3 (LVP-SAL) and 4 (SAL-SAL) were given sham-amnesic treatment (0.5 ml saline, pH 3.8, IP) and placed in the stainless steel box for 5 min following the acquisition trial.

Twenty-four and 48 hr after the acquisition trial, each animal was again placed in the start box for 1 min. Step-through latency to enter the shock chamber was measured as before, to a maximum of 300 sec. Animals entering the shock chamber during the test trials were removed after 10 sec. During the 24 hr test, any animal reaching the maximum latency was placed in the shock chamber for 10 sec. Shock was not presented during either test.

TABLE 1

STEP-THROUGH LATENCIES FOR TEST TRIALS IN EXPERIMENT 1.

Group	Treatment	N	MEDIAN RETEST LATENCY (Interquartile Range) 24 hr	48 hr
1	LVP-PTZ	12	165.6 (43.3 – 258.3)	300.0 (45.4 – 300.0)
2	SAL-PTZ	12	17.5 (8.3 – 35.2)	27.8 (18.2 – 45.0)
3	LVP-SAL	12	300.0 (300.0 – 300.0)	300.0 (174.1 – 300.0)
4	SAL-SAL	12	211.4 (46.6 – 300.0)	127.9 (21.2 – 300.0)

RESULTS

One animal was eliminated from the experiment due to an acquisition latency greater than 60 sec and replaced by another animal. Two animals in the SAL-PTZ group and four animals in the LVP PTZ group did not exhibit convulsions and were replaced by other animals.

The median step-through latencies for the training trial were less than 10.8 sec in all groups. Groups 1 and 3, pretreated with LVP, did not differ in step-through latency response during the acquisition trial from Groups 2 and 4, pretreated with saline ($U = 259$, not significant).

Median step-through latencies during the test trials are presented in Table 1. A Friedman 2-way Analysis of Variance indicated that passive avoidance conditioning occurred in the placebo group (SAL-SAL) with a significant increase in response latency 24 hr after the presentation of shock ($\chi^2 = 12.0$, $p < 0.001$). A trend showing extinction of the response was observed on the 48 hr retention test (Table 1).

Group by group comparisons were made by means of Mann-Whitney U tests. Pretreatment with LVP (LVP-SAL) facilitated the avoidance response compared to the SAL-SAL group: $U = 31$, $p < 0.02$, during the 24 hr test. (Because of the arbitrary cutoff point of 300 sec and the large number of ties occurring, we have also analyzed the data using contingency tables and the chi-square tests. Response latencies were divided into 3 classes: (1) 0–30.0 sec; (2) 30.1–299.9 sec; (3) 300 sec. A 3 classes × 6 treatment conditions contingency table analysis was significant. Separate 3 response classes × 2 treatment conditions contingency table analyses were done also. The same statistically significant results were established by the chi-square analyses as with the Mann-Whitney tests.)

Convulsions occurred in both the SAL-PTZ and LVP PTZ groups. These convulsions consisted of tonic, clonic and cataleptic phases. Onset of overt seizures occurred between 55 sec and 150 sec after injection. No differences were observed between the two groups exhibiting convulsions.

Compared to the SAL-SAL group, the SAL-PTZ group showed amnesia at both the 24 hr ($U = 16$, $p < 0.002$) and

48 hr ($U = 37$, $p < 0.05$) retention tests. Amnesia in the group was almost complete.

LVP administration 1 hr prior to the amnesic treatment (LVP-PTZ) led to a reduction of amnesia on both retention tests (compared to the SAL-PTZ group: $U = 0$, $p < 0.002$). At the 24 hr test, this reduction of amnesia was incomplete as a comparison with the LVP–SAL group yielded a significant difference ($U = 16$, $p < 0.002$). At the 24 hr test the LVP-PTZ group was not significantly different from the SAL-SAL group ($U = 60$, not significant). At the 48 hr test, there was no significant difference between the LVP-PTZ group and either the LVP-SAL or SAL-SAL group.

EXPERIMENT 2

The results of Experiment 1 indicated that LVP suppresses PTZ-induced amnesia when administered 1 hr prior to the acquistion trial. This may indicate that LVP is able to promote memory consolidation. It has been postulated that PTZ-induced amnesia may be the result of interference with memory retrieval [7] and that LVP may also exert its effect via a retrieval mechanism [13]. The purpose of Experiment 2 was to investigate the possibility that both LVP and PTZ influence retrieval rather than the consolidation of memory.

METHOD

Animals

Forty male Sprague-Dawley derived rats of the same description as in Experiment 1 were used. The animals weighed between 225–295 g at the start of the experiment.

Apparatus and Procedure

The apparatus and training procedure were the same as those in Experiment 1 except that a shock intensity of 0.3 mA was used due to the heavier body weight of these animals.

The total sample was randomly divided into 4 groups of 10 animals. Immediately on termination of the acquisition trial, Group 1 (PTZ–LVP) and 2 (PTZ–SAL) received the amnesic treatment (PTZ: pentylenetetrazol, 50 mg/kg). Groups 3 (SAL-LVP) and 4 (SAL-SAL) were given sham-amnesic injections (0.5 ml saline). One hr prior to the 24 hr retention test (23 hr after acquisition), Groups 1 and 3 received 0.5 ml LVP (2 μg/ml) and Groups 2 and 4 received 0.5 ml saline. Testing procedures were identical to those used in Experiment 1.

RESULTS

No statistically significant differences were found between groups for the acquisition trials. The median step-trhough latencies were less than 9.5 sec in all groups.

Median step-through latencies during the test trials are presented in Table 2. Latencies were analyzed in the same manner as in Experiment 1.

A Friedman 2-way Analysis of Variance indicated that passive avoidance conditioning again occurred in the placebo group (SAL–SAL) with a significant increase in response latency 24 hr after the presentation of shock ($\chi^2 = 6.4$, $p < 0.02$). Extinction of the response was observed at 48 hr ($\chi^2 = 6.4$, $p < 0.02$).

In those animals that received LVP 23 hr after acquisition (SAL-LVP), a facilitation of the avoidance response was observed during the 24 hr ($U = 22.5$, $p < 0.05$) and 48 hr ($U = 20.0$, $p < 0.05$) retention tests, compared to the SAL-SAL group.

TABLE 2

STEP-THROUGH LATENCIES FOR TEST TRIALS IN EXPERIMENT 2

Group	Treatment	N	MEDIAN RETEST LATENCY (Interquartile Range) 24 hr	48 hr
1	PTZ-LVP	10	171.1 (38.4 – 300.0)	300.0 (71.5 – 300.0)
2	PTZ-SAL	10	9.0 (3.0 – 38.3)	15.9 (6.6 – 54.2)
3	SAL-LVP	10	300.0 (202.8 – 300.0)	300.0 (96.2 – 300.0)
4	SAL-SAL	10	152.1 (73.1 – 300.0)	99.3 (47.8 – 218.5)

Convulsions occurred in both the PTZ-LVP and PTZ–SAL groups. These convulsions were similar to those described in Experiment 1. The PTZ-SAL group showed amnesia at both the 24 hr ($U = 2$, $p < 0.002$) and 48 hr ($U = 11$, $p < 0.02$) retention tests, compared to the SAL-SAL group.

LVP administration 1 hr prior to the 24 hr retention test led to reduction of PTZ-induced amnesia on both the 24 hr ($U = 8$, $p < 0.002$) and 48 hr ($U = 10$, $p < 0.002$) retention tests. The reduction of amnesia was complete during the 48 hr test as a significant difference existed between the PTZ-LVP group and the SAL-SAL group ($U = 23$, $p < 0.05$). There was no significant difference between the PTZ-LVP group and the SAL-LVP group during either retention test.

DISCUSSION

The administration of LVP 1 hr prior to the acquisition trial or 1 hr prior to the test trial in a passive avoidance task, facilitated passive avoidance responding. Previous studies have reported a facilitatory effect by LVP, but conclusions regarding its action were confounded as passive avoidance responding was not demonstrated in the control groups [1, 3, 15]. In the present report, the facilitatory effect of the hormone was demonstrated; the control group in both experiments exhibited conditioning. The passive avoidance responses in these control groups were consistent with the findings of Ader *et al.* [2].

Amnesia was produced using a convulsive dose of pentylenetetrazol (50 mg/kg). LVP administration 1 hr prior to the acquisition trial and amnesic treatment produced a reduction of PTZ-induced amnesia. This result is consistent with other findings using puromycin [8,14] and CO_2 [13] amnesic treatments.

PTZ has been postulated to be exerting its amnesic effect by interfering with memory consolidation processes. The finding in Experiment 1 that LVP suppresses PTZ-induced amnesia when administered prior to the acquisition trial is consistent with the hormone exerting its effect through memory consolidation. LVP may facilitate the consolidation process itself, or protect the consolidation process from the disruptive action of PTZ.

However, the results of Experiment 2 make this hypothesis untenable. As LVP was administered 1 hr prior to the test session, a period of 23 hr after training and PTZ

treatment, it is unlikely that the hormone could affect memory consolidation. It is also unlikely that the results of Experiment 1 could be due to the hormone altering the animals' sensitivity to footshock. It appears that the suppression of PTZ-induced amnesia by LVP is mediated via a retrieval process, either by a facilitation of the retrieval process or by a suppression of PTZ-induced interference of retrieval.

This hypothesis is consistent with the interpretation that convulsive doses of PTZ effect retrieval rather than consolidation [7]. It is possible that the preacquisition administration of LVP may influence later retrieval by the same mechanism.

The findings of the present study and others that have shown peptide hormones to be effective blockers of amnesic agents when administered prior to training or testing [8, 13, 14], raise important questions not only concerning the mechanisms of hormones and amnesic agents, but also regarding the current dual-process theory of memory [9]. While we view our results as being consistent with a retrieval hypothesis, a full understanding of the theoretical construct awaits our elucidation of the complex neurophysiological and neurochemical events that occur as a function of the learning task and the pharmacological agent.

ACKNOWLEDGEMENT

We gratefully acknowledge Steve Gunn for his help in statistical analysis, Neal Meyer, Otello Desiderato and John MacKinnon for helpful criticisms, and Mrs. Dolores Desrosiers for help in preparing the manuscript.

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