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Lysine Vasopressin Attenuation of Diethyldithiocarbamate-Induced Amnesia[1]

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ASIN, K. E. *Lysine vasopressin attenuation of diethyldithiocarbamate-induced amnesia.* PHARMAC. BIOCHEM. BEHAV. 12(3)343–346, 1980.—The effect of lysine vasopressin on diethyldithiocarbamate-induced amnesia for a step-through passive avoidance task was studied in rats. It was determined that although the hormone attenuates the amnesia when it is administered prior to retrieval testing, it fails to do so when it is injected prior to training. These results are consistent with the reports of others which demonstrate the reversal of diethyldithiocarbamate-induced amnesia by catecholamine agonists.

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THE INFLUENCE of peptides on brain function has recently received widespread attention. In the area of learning and memory processes, vasopressin appears to have become a focus of interest. The hormone has been shown to influence both the acquisition and maintenance of a variety of tasks, including active and passive avoidance [1, 2, 7, 11, 24, 40] and the absence of the hormone may interfere with task retention [3, 9, 10, 36, 38].

Perhaps the most striking evidence favoring vasopressin involvement in memory processes is its ability to reverse the amnestic effects of a wide variety of agents. Thus, vasopressin (or its analogues) has been found to reverse electroconvulsive shock- (ECS), CO_2- and antibiotic-induced amnesia when it is administered within one hour of the testing trial, or one hour prior to, or immediately following, the training trial [13, 14, 22, 27, 31, 33, 34, 39], and has been found to promote recall in man in cases of accident-precipitated or naturally occurring memory deficits [23,26].

This study investigates the effects of lysine vasopressin (LVP) on diethyldithiocarbamate-induced (DDC) amnesia. DDC, a dopamine-β-hydroxylase inhibitor, has repeatedly been reported to induce amnesia for a passive avoidance task [6, 16, 25, 32, 35], presumably through interference with noradrenaline (NA) synthesis [35].

METHOD

Subjects

Adult, male Sprague-Dawley derived rats, obtained from the breeding colony maintained by the University of Illinois, served as subjects. Rats weighed 260 ± 15 g and were individually housed during the course of the experiment, with food and water freely available. Subjects were maintained on a reverse 14:10 hour light:dark cycle, with light onset at midnight. All training and testing procedures occurred between the hours of 4:00 and 7:00 p.m. to control for diurnal hormonal variations.

Apparatus

The passive avoidance chamber was divided into two compartments of unequal size and brightness, similar to that used by Lissak and Bohus [24]. The larger side (27×21×28 cm) was painted white and included a 5 W light bulb in series with a 1.5 kΩ resistor, located on the rear wall 20 cm from the floor. The smaller chamber was painted black except for the floor, which was composed of metal rods, 0.2 cm in dia. and spaced 0.5 cm apart. These rods were wired to a LeHigh Valley shock scrambler which was, in turn, connected to a Hunter power source, for an effective delivery of 90 V (short circuit). The compartment measured 25×15×28 cm. A metal guillotine door (7×8.5 cm) allowed passage from one side of the apparatus to the other when it was raised. The avoidance chamber supported two hinged lids and rested on a small table in the same room where the animals were maintained. A paper towel was placed under the smaller compartment to catch animal waste.

Time measurements were made by the experimenter with a stopwatch and all times were recorded to the nearest second.

Drugs

DDC (sodium salt) was purchased from Sigma Chemical Company, St. Louis, MO. The drug was dissolved in physiological saline in a concentration of 300 mg/cc and was in-

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jected in a dose of 1200 mg/kg. Pilot studies indicated that this drug dosage was necessary to assure reliable amnesia.

LVP (isolated from porcine hypothalamus) was the generous gift of Dr. C. Smith of the University of Illinois Medical Center. Its pressor activity was approximately 278 U/mg. The hormone was dissolved in saline prior to injection in a concentration of either 0.28 U/cc or 2.8 U/cc; subjects received 0.5 cc of the solution (approximately 0.5 and 5 μg, respectively) SC.

All drug solutions were prepared immediately prior to their use. Saline served as the control injection in volumes equivalent to those including the drugs. Plastic ware was used throughout the course of the experiment.

Procedure

All subjects were given at least one week to adapt to the light/dark schedule and were handled 5 min per day beginning two days prior to training. On the first day of training, animals were weighed and assigned to one of six experimental groups, which were matched for weight (see below). The animal was then gently placed in the larger, brighter compartment, facing away from the raised guillotine door. The subject was allowed to explore the apparatus for 5 min, following which the animal was returned to his home cage. The towel beneath the smaller chamber was changed between animals.

Twenty-three hours later, each animal was administered either LVP or saline and, one hour later, was placed in the lit compartment. Total time spent in each compartment was recorded for 5 min. At the end of this time, the guillotine door was lowered, preventing the animal from crossing over into the white compartment (the rat was invariably in the dark side). For the next 5 min, a 5 sec foot shock was delivered to the subject every 15 sec. Immediately following this, the animal was removed from the apparatus and was returned to his home cage. Fifteen minutes later, the rat was administered either saline or DDC. The avoidance chamber was aired for 5 min between animals and the paper towel beneath the unlit side was changed.

Retention testing was conducted 48 hr later. One hour prior to this, however, each animal was injected with either LVP or saline. The testing procedure was similar to that on the first day of training except that the animals were not weighed. The treatment groups were as follows: SSS: Saline prior to training, after training and prior to testing (n=10). SDS: Saline prior to training, DDC after training, and saline prior to testing (n=11). VDS1: 0.14 U LVP prior to training, DDC after training, and saline prior to testing (n=10). VDS2: Similar to VDS1 except 1.4 U LVP were given prior to training (n=7). SDV1: Saline prior to training, DDC after training, and 0.14 U LVP prior to testing (n=11). SDV2: Similar to VDS2 except 1.4 U LVP were given prior to testing (n=12). Two additional groups, VSS (n=11) and SSV (n=11), were given vasopressin (0.14 U) before training or before testing, respectively, and saline at all other times.

Tests of significance on the difference in mean times spent in the lit compartment between groups were conducted using unidirectional Students' *t*-tests for the second day of training and the day of testing.

RESULTS

A histogram of mean times (±SEM) spent in the lit compartment on the day of testing is shown in Fig. 1. The mean times for groups VSS (298.9 ± 0.6) and SSV (296.9 ± 2.1) were virtually identical to that for group SSS (298.5 ± 1.2) and are therefore not pictured. Furthermore, group VDS2 is not included since all rats given 1.4 U LVP prior to training and their DDC injection had died by the following day. Toxic effects of intracerebral injections of vasopressin have been reported [21] and it is possible that the dose of LVP used in this study interacted with the toxic effects of DDC, since rats receiving 1.4 U LVP prior to testing failed to experience any noticeable illness (i.e., group SDV2).

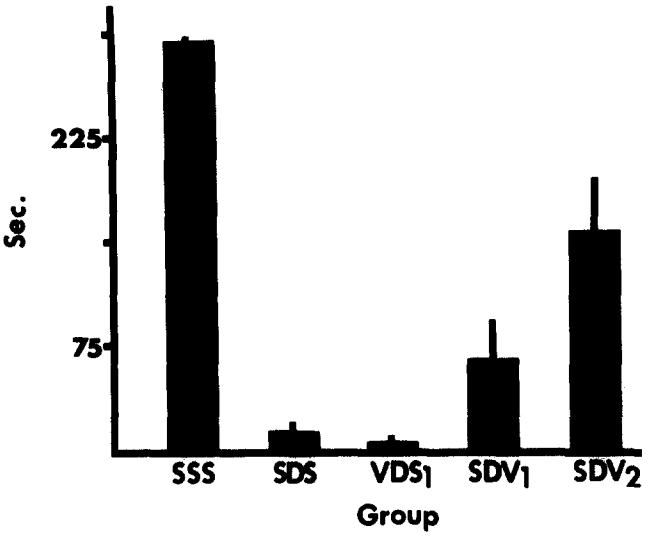

FIG. 1. Mean number of seconds (±SEM) spent in the lit compartment on the retrieval testing day. Groups recieving DDC after training and vasopressin prior to testing (SDV1 and SDV2) showed significantly better avoidance of the previously shocked compartment compared to groups receiving saline or vasopressin prior to training and DDC injections (SDS and VDS1, respectively). Rats receiving only vasopressin, prior to training or testing (not pictured), showed avoidance responding comparable to saline injected controls (SSS).

Animals in groups SDS and VDS1 showed highly significant amnesia for the passive avoidance task compared to groups SSS, VSS and SSV ($p<0.001$ for all comparisons) and failed to differ from one another. Groups receiving DDC and subsequent LVP (SDV1 and SDV2) showed significantly greater mean times spent in the lit compartment compared to the other groups receiving DDC (SDS and VDS1; $0.01<p<0.03$ for all comparisons). Furthermore, groups SDV1 and SDV2 differed from one another ($p<0.05$) and from groups SSS, VSS, and SSV ($p<0.01$).

Thus, animals receiving DDC after training and LVP prior to testing showed significantly greater retention for a passive avoidance task compared to those animals receiving either LVP or saline prior to training and DDC.

Although animals in groups SDV1 and SDV2 differed from rats in groups SSS, VSS and SSV, attenuation, rather than complete reversal, of amnesia has been reported following vasopressin, particularly for CO_2-induced amnesia [31]. It is possible that a larger dose of LVP would have restored the avoidance response completely (see [13]).

It is notable that average times spent in the lit compartment in the second day of training did not differ between groups receiving saline (17.0 ± 3.6) or vasopressin (22.2 ± 4.3). Thus it is unlikely that LVP attenuated the amnesia through a locomotor depressant action or through actions on the "innate dark preference" of rats.

DISCUSSION

Statistical analysis indicated that rats which had been injected with DDC after the training trial spent significantly less time in the lit compartment when compared to saline injected controls. This result is consistent with a number of other studies which have demonstrated the amnestic actions of DDC on active and passive avoidances [4, 12, 15, 20, 29, 32].

In the present study, DDC treated rats which were administered lysine vasopressin prior to testing spent significantly more time in the lit compartment compared to rats receiving DDC and saline. It is unlikely that vasopressin itself increases dark avoidance behavior since groups receiving the hormone prior to the second training session failed to differ in chamber preference from saline injected controls. This LVP attenuation of DDC-induced amnesia is similar to what has been reported following CO_2- or ECS-induced amnesia [27, 33, 34] and contributes additional evidence that DDC does not prevent the formation of the memory trace but may rather prevent its retrieval.

Unlike what has been reported to occur for CO_2-, ECS-, and antibiotic-induced amnesia [13, 14, 22, 27, 31, 33, 34, 39] pretraining administration of vasopressin was found to be ineffective in modifying DDC-induced amnesia for a passive avoidance task. One possible explanation for this failure to find a reversal might lie in the dose of LVP used in the current study. Although doses lower than those used here have been reported to affect response maintenance [8] it is possible that DDC amnesia requires a larger dose for its reversal. However, a higher dose of the hormone prior to training was precluded due to the high mortality rate at doses greater than 0.14 U, even when the peptide was injected 75 min prior to DDC. Although it is still possible that a higher dose of LVP prior to training might have promoted subsequent recall, it is notable that a dose of vasopressin which significantly attenuated DDC-induced amnesia when given before testing was unable to restore the avoidance response when given prior to training.

One possible explanation for the failure of pretraining, but not pretesting, injections of LVP to effectively modify the amnesia may be the mode of action through which vasopressin exerts its antiamnestic actions. It has been reported that vasopressin increases the reduction of NA levels following treatment with α-methyl-para-tyrosine [36], indicating increased NA turnover, and it is possible that memory enhancement following vasopressin stems from the activation of noradrenergic mechanisms.

Further evidence for NA involvement in the actions of vasopressin is provided by reports that pretreatment of animals with α-methyl-para-tyrosine prevents memory enhancement by vasopressin of a poorly learned task [19], as does 6-hydroxydopamine (6-OHDA) destruction of the dorsal noradrenergic bundle [18]. Additionally, Hoffman *et al.* [17] have reported that vasopressin's ability to maintain ethanol tolerance in mice can be blocked by pretreatment with 6-OHDA. Were NA involvement also necessary for the antiamnestic actions of vasopressin, it would not be expected to influence DDC-induced amnesia if it were present during the period of NA synthesis inhibition by DDC, but it would be expected to reinstate the avoidance response if it were given prior to testing, since NEP synthesis returns within 24 hr [5]. Such was found to be the case in the current study; injections of LVP prior to the retrieval trial, 48 hr after DDC injection, successfully restored the response in a dose dependent fashion but, when given prior to training, failed to affect avoidance performance.

Although this hypothesis for NA involvement in the antiamnestic actions of LVP requires further investigation, it is notable that the effects of LVP on DDC-induced amnesia are similar to those which have been reported by others for catecholamine agonists. Thus, amphetamine and monoamine oxidase inhibitors have been reported to reduce DDC amnesia if they are given prior to testing, but not if they are administered following training, in the presence of DDC [4, 28, 29, 30].

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