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Effects of Vasopressin on Active and Passive Avoidance Behavior

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Male rats were trained in an active avoidance and/or a "step-through" type of passive avoidance situation. Lysine vasopressin administration resulted in resistance to extinction of active avoidance behavior if it was injected 1 hr prior to the third and final acquisition session; peptide treatment 6 hr prior to this session did not affect extinction. Resistance to extinction of passive avoidance behavior was also obtained when lysine vasopressin was injected 1 hr prior to the first retention trial on Day 3 of training. Peptide administration 6 hr prior to this trial appeared to be ineffective.

If rats were trained in both the active and passive avoidance situation spaced 6 hr apart, lysine vasopressin only affected extinction of the particular behavior tested 1 hr after the single administration of peptide. The behavioral effects of lysine vasopressin evidently depend upon the time of treatment. No evidence of generalization was observed even though both behavioral responses were aversively motivated.

Rats subjected to posterior lobectomy are markedly less resistant to extinction of a shuttle box avoidance response than sham-operated control animals. Treatment with pitressin or lysine vasopressin as long-acting preparations restore normal behavior in these animals (de Wied, 1965). In intact rats the administration of long-acting pitressin increases resistance to extinction of a shuttle box avoidance response (de Wied and Bohus, 1966). Recently a similar effect of short-lasting lysine vasopressin was demonstrated (de Wied, 1971). The effect is of a long-term nature since a single injection of vasopressin at a critical period of time during avoidance training facilitates active avoidance behavior for a longer period than can be accounted for by the presence of the peptide in the organism.

Vasopressin is released in response to specific and nonspecific stimuli, and emotional stress effectively stimulates the discharge of vasopressin from

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the posterior pituitary (Nash, 1971). The nonspecific nature of the release of vasopressin raises the question as to whether the long term behavioral effect of this octapeptide is specific for the particular response acquired under the maximal influence of this peptide or whether generalization occurs to other aversively motivated behavioral responses. Since lysine vasopressin also increases resistance to extinction of a passive avoidance response (Ader and de Wied, 1972), it became possible to study the interaction between two fear-motivated responses, when only one of these responses was tested under the maximal influence of lysine vasopressin.

METHODS

Male rats of an inbred Wistar strain weighing 120-130 g were used. The animals were housed under ad lib. food and water conditions. In the animal colony, the lights were on from 5 AM to 7 PM. All observations were made between 9 and 12 AM and 2 and 5 PM.

Active avoidance behavior was studied in a pole-jumping situation as described in detail elsewhere (de Wied, 1966). Briefly, the rats were trained to avoid the unconditioned stimulus (US) of an electric floor shock (0.20 mA, AC) by jumping onto a pole located in the center of the conditioning apparatus. The US was applied if an avoidance response had not occurred within 5 sec of conditional stimulus (CS) presentation. A light on top of the box served as the CS. Ten acquisition trials were given daily for 3 days with intertrial intervals averaging 60 sec. Ten nonreinforced extinction trials per day were given on the following 2 successive days.

Passive avoidance behavior was studied in a simple "step-through" type of passive avoidance situation (Ader *et al.*, 1972). The apparatus consisted of a 40X40X40-cm Lucite chamber with black walls and a grid floor. Rats entered this chamber through a guillotine-operated door from a 6X25-cm mesh-covered elevated runway. The shock chamber remained dark while a 25 W lamp was fixed 40 cm above the center of the elevated runway. On the first day adaptation training (rats were placed in the shock chamber for 2 min) was followed by a single trial in which the rats were placed on the elevated runway facing away from the door and allowed to enter the dark chamber. Latency to enter was recorded. Three such trials were given on the next day with an intertrial interval of approximately 2 min. After the third of these trials, the rats received a single 1-sec unavoidable electric shock (0.25 mA AC as supplied by a shock scrambler) through the grid floor of the dark chamber. Animals with a response latency of 30 sec or more on any of these three training trials were eliminated from the experiments. Retention was tested 24, 48, and, in one experiment, 72 hr after the shock trial. The

animals were placed on the elevated runway and the latency to enter the dark chamber was recorded to a maximum of 300 sec.

In Expt I, 24 rats were trained in the pole-jumping situation: 12 in the morning and 12 during the afternoon. One-half milliliter of saline or 0.5 ml (2 μg/ml) of lysine vasopressin (synthetic lysine vasopressin, 60 U/mg, Ferring AB batch No. 12865) dissolved in saline was injected subcutaneously between 8 and 11 AM on Day 3, the last day of acquisition training–that is, 1 hr prior to the morning session or 6 hr before the afternoon session. Four animals were discarded because they did not meet the criterion of learning (five or more avoidance responses) on the second day of training.

Experiment II was performed with two groups of rats, 12 of each trained in the passive avoidance situation either in the morning or in the afternoon. One-half milliliter saline or 0.5 ml (2 μg/ml) lysine vasopressin (LVP) was injected subcutaneously between 8 and 11 AM on Day 3–that is, 1 or 6 hr prior to the first (24 hr) retention test (23 or 18 hr *after* the shock trial). A second retention test was run on Day 4 (48 hr).

In Expt III, 32 rats were trained in the active and in the passive avoidance situations. Sixteen rats were trained in the pole-jumping apparatus during the morning session, then subjected to the step-through procedure during the afternoon, while 16 rats were trained first in the passive avoidance test in the morning and subjected to the active avoidance test in the afternoon. On Day 3 of the experiment, 0.5 ml of saline or 0.5 ml (2 μg/ml) LVP was injected subcutaneously 1 hr prior to the morning session. Subsequently, the rats were run in the afternoon session, 6 hr after the injections, and tested for extinction on Days 4 and 5.

RESULTS

The data of Expts I and II are summarized in Tables 1 and 2. Rats trained in the active avoidance test and injected with saline or LVP 1 hr prior to the third and final acquisition session were resistant to extinction as shown by the number of avoidance responses during the two extinction sessions. Rats trained during the afternoon sessions showed no difference in avoidance performance on the third day, 6 hr after the injection and exhibited no resistance to extinction. Avoidance behavior of saline-treated controls was not affected by the time of training, i.e., whether the animals were trained in the morning or in the afternoon.

Rats trained in the passive avoidance situation and injected with saline 1 or 6 hr prior to the first retention trial (Day 3) showed no substantial increase in median response latency after a single shock trial. An increase in avoidance latency was observed in rats treated with LVP 1 hr prior to the first retention test. Latency was further increased on the second retention trial. If LVP was

TABLE 1

Effect of a Single Injection of Lysine Vasopressin Administered on the Third Day of Acquisition on the Subsequent Extinction of a Pole-Jumping Avoidance Response

Treatment and time of injection	Mean (± SE) number of conditioned avoidance responses in each block of ten trials		
	Third acquisition session	First extinction session	Second extinction session
1 hr before			
Saline (5)[a]	8 ± 0.4	6 ± 0.4	2 ± 0.5
Lysine vasopressin (1 μg) (5)	9 ± 0.4	9 ± 0.2	8 ± 0.5[b]
6 hr before			
Saline (5)	8 ± 0.0	6 ± 0.2	1 ± 0.2
Lysine vasopressin (1 μg) (5)	8 ± 0.2	7 ± 0.2	1 ± 0.6

[a] Number of animals.
[b] $p < 0.01$ (two-tailed t test).

TABLE 2

Effect of a Single Injection of Lysine Vasopressin Administered Prior to the First Retention Trial on Retention of a Passive Avoidance Response

Treatment and time of injection	Median response latency		
	Last training session (mean of three trials)	First retention trial	Second retention trial
1 hr before			
Saline (6)[a]	3.0	2.5	2.5
Lysine vasopressin (1 μg) (6)	9.0	77.5[b]	112.5[b]
6 hr before			
Saline (6)	4.0	3.0	2.0
Lysine vasopressin (1 μg) (6)	2.5	4.0	3.0

[a] Number of animals.
[b] $p < 0.01$ (Mann-Whitney U test).

given 6 hr prior to the first retention test, the median latency did not differ from that of saline-treated controls. Here again, response latencies were similar in saline-treated rats whether the rats were trained in the morning or in the afternoon.

The data from Expt III are shown in Fig. 1. Resistance to extinction of the active avoidance response was observed in rats injected with LVP 1 hr prior to the final acquisition session. LVP-treated rats made significantly ($p < 0.001$) more avoidance responses than controls during extinction sessions on Day 4 and on Day 5. Despite this persisting difference between the two

groups of animals in active avoidance behavior, no such difference was found in median avoidance latencies in the passive avoidance test given 6 hr later; neither LVP- nor saline-treated rats exhibited an increase in response latency during the retention trials (Fig. 1A). Conversely, relative to controls, a marked increase in response latency was observed in the passive avoidance situation in rats treated with LVP 1 hr prior to the first retention trial ($p < 0.01$ on each day), whereas the active avoidance behavior of LVP- and saline-treated rats did not differ when studied in the afternoon (Fig. 1B).

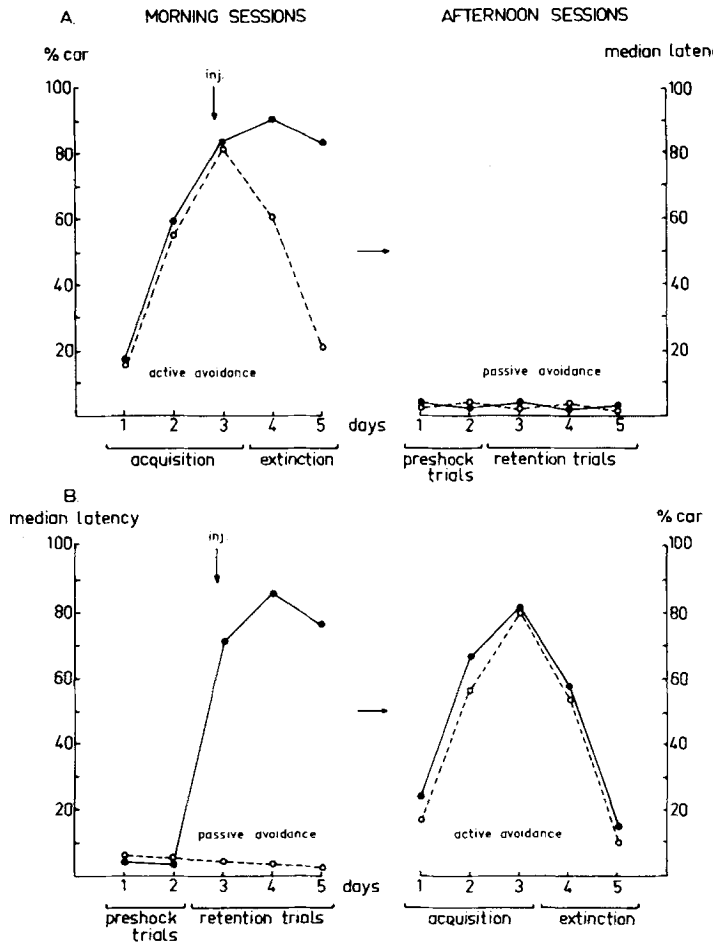

Fig. 1. Effects of a single injection of lysine vasopressin on active and passive avoidance behavior. The rats were trained in active avoidance in the morning session then in passive avoidance in the afternoon (A, upper figure), or in passive avoidance in the morning and active avoidance in the afternoon (B, lower figure). The arrows indicate the time of injection of lysine vasopressin (solid) or saline (broken lines).

A comparison of the avoidance indices of saline-treated rats of Expt III with those of Expts I and II indicated no interactions between morning and afternoon training sessions.

DISCUSSION

The present results, together with previous findings (de Wied, 1971), indicate that the resistance to extinction of an active avoidance response can be influenced by lysine vasopressin only if treatment is provided shortly before or after effective avoidance responding; treatment administered as much as 6 hr before or after avoidance training is apparently ineffective in modifying the behavior. Further, the results of Expt II suggest that a similar temporal relationship holds for the retention of passive avoidance behavior as well. The results of Expt II should, however, be interpreted with some caution since the saline-treated animals did not show an increased response latency following the single shock trial. There is no apparent reason for the failure of this particular group to display a passive avoidance response. Although the level of electric shock stimulation was low, two previous populations (Ader and de Wied, 1972; Ader *et al.*, 1972) did show an increase in response latency with this shock intensity and, as in the present experiments, the latency of the control group was exceeded by animals treated with LVP (Ader and de Wied, 1972).

Taken together, the results of the present study also suggest that the influence of lysine vasopressin on avoidance behavior is restricted to that behavior which is occurring during the period of optimal vasopressin influence; vasopressin affected avoidance behavior only when the peptide was administered 1 hr prior to the performance of that particular avoidance response. Presumably, the peptide is not present in appropriate quantities in the CNS when different training is introduced 6 hr after treatment. If the behavioral effect of vasopressin was due to a long-lasting general arousal of some CNS mechanism(s), changes in performance in any number of other, similar behavioral situations would have been likely. On the contrary, no evidence of generalization or transfer of the effects of vasopressin from one behavioral situation to another was observed; the peptide affected behavioral performance in only one of the avoidance situations, depending upon the time of treatment. Moreover, this specificity was evident even though both behavioral responses were similarly motivated—presumably, by the fear elicited by painful electric shock.

Although a short-acting preparation of lysine vasopressin was used, the present data, together with the results of de Wied (1971) on active avoidance and Ader and de Wied (1972) on passive avoidance, warrant the conclusion that vasopressin or related peptides of posterior pituitary origin affect the

retention of avoidance behavior such that there is a long-term preservation of the conditioned response. The mechanism(s) by which this occurs is not yet understood.

Our results clearly indicate the importance of a time-dependent association between exogenous vasopressin administration and specific environmental signals. The effect of exogenous vasopressin in maintaining conditioned behavior, then, may be related to the relative ease with which a given behavioral response is elicited when situation specific cues reoccur. If this is true, it might be that an association between the endogenous release of vasopressin or related peptides and specific environmental cues is of physiological significance in the maintenance of new behavior patterns in spite of the nonspecific nature of the release of this polypeptide (Nash, 1971). The fact that removal of the posterior pituitary in rats impairs the maintenance of a shuttle box avoidance response (de Wied, 1965) is in keeping with this hypothesis.

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