Effects of lysine vasopressin on passive avoidance learning*

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On a single conditioning trial, a 1-sec electric shock (.125 or .250 mA) was administered when rats entered a darkened chamber from a lighted elevated runway. Latency to enter the chamber was recorded on retention trials given 24 and 48 h later. Animals received subcutaneous injections of varying doses of lysine vasopressin or a placebo solution immediately after the training trial or immediately before the first retention trial. Nonshocked control animals showed no increase in response latencies on successive trials, nor was there a difference between the placebo and vasopressin groups under the "no-shock" condition. Treatment with lysine vasopressin increased resistance to extinction, irrespective of the time of treatment.

A recent emphasis in studying pituitary-adrenal influences on behavior has been a concern with the effects of adrenocorticotrophic hormone on the acquisition and retention of conditioned avoidance responses (e.g., de Wied, 1969). For example, hypophysectomized animals are severely deficient in their ability to acquire an active avoidance response, but treatment with ACTH is capable of restoring this adaptive capacity (de Wied, 1964). Moreover, de Wied (1969) has shown that this effect is an extraadrenal influence of ACTH, since fragments of the ACTH molecule that are devoid of corticotropic activity (ACTH 4-10) are equally effective in restoring normal behavior to hypophysectomized animals. In contrast, removel of the posterior pituitary does not influence the acquisition of a conditioned avoidance response but does result in rapid extinction of the response. Treatment with ACTH-like peptides as well as with pitressin tannate in oil (a crude extract of the posterior and intermediate pituitary lobes) restores the normal pattern of extinction (de Wied, 1965). In normal intact rats, the administration of ACTH analogues as well as pitressin effect an increased resistance to extinction of an active avoidance response (de Wied & Bohus, 1966).

The mechanisms by which ACTH and pitressin affect the maintenance of an avoidance response appear to be different since the effect of pitressin is obtained whether treatment is given during the process of acquisition or extinction, whereas the effects of ACTH are confined to the relatively immediate period during which the treatment is administered (de Wied & Bohus, 1966). de Wied (1971) recently found evidence to suggest that vasopressin is the peptide in the pitressin preparation that is responsible for its relatively long-term effect on the maintenance of an active avoidance response. To the extent that the consolidation of learned responses in general may depend upon the presence of certain neurogenic peptides, it behooves one to demonstrate that the same phenomenon can be demonstrated in several learning situations. Levine & Jones (1965) have shown that ACTH will also retard the extinction of a passive avoidance response conditioned by presenting electric shock stimulation to deprived rats approaching water reinforcement. The present experiment represents an extension of such studies in investigating the effects of lysine vasopressin on passive avoidance behavior. To obviate the complications of an approach-avoidance paradigm involving a potential interaction between the effects of vasopressin and a state of deprivation, a simple "step-through" type of passive avoidance situation was used. Based on a parametric analysis of this situation (Ader, Weijnen, & Moleman, 1972), the intensity and duration of electric shock were chosen to provide the latitude to observe either an increase or decrease in resistance to extinction.

METHOD

Male Wistar rats, weighing 180 to 210 g, were used in all experiments. The animals, born and reared in our laboratory, were housed five per cage under ad lib food and water and a 12-h light-dark schedule. The lights were on from 0600 to 1800 h, and all observations were made between 1330 and 1630 h.

Passive avoidance conditioning took place in a dark, sound-attenuated room. The apparatus, described in detail elsewhere (Ader et al, 1972), consisted of a 40-cm^3 Lucite chamber with black walls and a grid floor through which electric shock could be introduced. A 6 x 25 cm mesh-covered elevated runway extended from the 6-cm^2 guillotine-operated door in the center of one wall. 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 of training (adaptation), the animals were placed inside the box for 2 min. This was followed by a single trial in which the rat was placed at the end of the elevated runway (facing away from the door) and allowed to enter the box. Three such trials were given on Day 2. The animals remained in the box for 10 sec and the intertrial interval was approximately 2 min. On the third of these trials, the animals received a single 1-sec electric shock through the grid floor of the cage. Control animals were similarly treated except that electric shock was omitted. Animals with a mean response latency exceeding 30 sec on these three training trials were eliminated from the experiment. Retention was tested on successive days when the animals were again placed on the elevated runway. Although no electric shock was presented, the auditory stimulation provided by the shock scrambler remained on. Latency to enter the darkened chamber (to a maximum of 300 sec) was recorded.

In the first experiment, animals received an electric shock of .250 mA, following which they were injected subcutaneously with 0.5 ml of saline (placebo) or with 0.3, 0.9, or 2.7 micrograms/ml of lysine vasopressin (LVP). A second population received placebo or LVP (0.9 or 2.7 micrograms/ml) treatment 1 h before the first retention trial (approximately 23 h after the shock trial).

The same procedures were followed in a second experiment, except that the animals received only .125 mA of electric shock and were treated with placebo or 2.7 micrograms/ml LVP. In both experiments, retention was tested 24 and 48 h after the shock trial. In a third experiment, animals treated with placebo or 2.7 micrograms/ml LVP immediately after the shock trial (.250 mA) were tested for retention, beginning at 48 h, without the intervening 24-h test.

RESULTS AND DISCUSSION

The data from Experiments 1 and 2 are given in Table 1. Animals that did

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Table 1
Effects of Lysine Vasopressin (LVP) on Retention of a Passive Avoidance Response

Shock Intensity	Time of Treatment	Group	N	Median Response Latency: Last Training Trial	Retention Trial: 24 H Sec	24 H Stat	48 H Sec	48 H Stat
.250	After Shock	Placebo	16	6.0	19.0		26.5	
		LVP (0.3 ug/ml)	16	9.7	17.5	H = 3.24	19.5	H = 7.96
		LVP (0.9 ug/ml)	15	9.3	44.0	n.s.	36.0	$p < .05$
		LVP (2.7 ug/ml)	17	7.0	60.0		58.0	
	Before Retention	Placebo	6	13.6	14.0	H = 2.87	14.5	H = 5.98
		LVP (0.9 ug/ml)	7	9.0	58.0	n.s.	78.0	$p < .05$
		LVP (2.7 ug/ml)	7	9.0	71.0		120.0	
.125	After Shock	Placebo	16	8.6	12.0	U = 63.5	12.0	U = 11.9
		LVP (2.7 ug/ml)	17	10.3	14.0	n.s.	22.0	n.s.
	Before Retention	Placebo	7	9.7	8.0	U = 15.5	20.0	U = 22.5
		LVP (2.7 ug/ml)	7	6.0	5.0	n.s.	41.0	n.s.
0	After Shock	Placebo	11	13.7	8.0	U = 33.5	16.0	U = 35.0
		LVP (2.7 ug/ml)	7	12.8	15.0	n.s.	9.0	n.s.

not experience electric shock showed no increase in their latency to enter the darkened chamber on successive days. Moreover, in the absence of shock, the animals treated with LVP did not differ from the placebo group, indicating that the treatment itself did not influence the performance measure in this situation. There were no differences between the randomly selected groups in response latency prior to receiving electric shock, and, when tested 24 h after shock, the median response latencies shown by the nonshocked placebo group, as well as the placebo-treated animals given .125 and .250 mA of electric shock, were virtually identical with those previously reported (Ader et al, 1972).

The arbitrary maximum response latency of 300 sec was exceeded by slightly over 10% of the animals on each of the retention tests, necessitating the use of nonparametric statistical analyses. The Kruskal-Wallis and Mann-Whitney tests were used where appropriate. Animals that received .250 mA of electric shock showed an increase in response latency 24 and 48 h after the trial on which they were shocked. Relative to the placebo group, treatment with LVP immediately after the shock trial resulted in a greater degree of avoidance of the darkened chamber, as evidenced by longer median response latencies, particularly on the 48-h retention test. There was a progressive increase in response latencies with increases in the dose of LVP, except for the animals treated with the lowest dosage; these rats did not differ from the placebo group. The same effect was observed when treatment was administered 23 h after the shock trial (1 h before the first retention trial). Animals treated with LVP showed a progressive increase in response latencies, as compared with placebo-treated animals, at 24 and 48 h, but, considering the high variability, only the difference on the 48-h test was statistically significant.

An electric shock intensity of .125 mA resulted in little or no resistance to extinction among the placebo-treated animals. The response latencies of the animals treated with LVP were somewhat greater than those of the placebo group (again, only at 48 h), but, again because of high variability, the differences did not reach a statistically significant level.

Animals in the third experiment were tested for retention, beginning 48 h after the shock trial, to determine if the significant differences observed at 48 h in the above experiments represented a cumulative effect of testing at 24 h. Placebo- and LVP-treated animals showed preshock latencies of 7.0 and 7.2 sec, respectively. The placebo group showed retention latencies of 11.0 and 21.0 sec, whereas animals treated with 2.7 micrograms/ml LVP had median latencies of 36.5 and 102.0 sec when tested 48 and 72 h after the training trial. With Ns of 4 and 5 per group, however, these differences were not statistically significant. Nonetheless, on the basis of these data, it does not appear likely that the differences noted at 48 h after training resulted from the intervening 24-h test.

Like treatment with ACTH (Levine & Jones, 1965), LVP increased resistance to extinction of a passive avoidance response in intact animals. This effect obtained whether the single injection of LVP was administered immediately after the learning trial or immediately before the first test for retention, 24 h later. Very recent data indicate that the same effect is obtained in an active avoidance situation (Bohus, Ader, de Wied, in preparation). Moreover, while it was previously shown that LVP injected 6 h after original learning had no effect on retention (de Wied, 1971), these recent data also found that LVP treatment given 6 h before the first retention test was also ineffective in altering resistance to extinction of either an active or a passive avoidance response. If, as hypothesized, the consolidation of learned responses depends upon the presence of specific neurogenic peptides, it is not surprising that there should be a critical period for the effects of LVP. However, it is difficult to understand how treatment with LVP 24 h after training could retroactively facilitate the consolidation of avoidance responses. On the contrary, the present data suggest the hypothesis that treatment just before, during, or immediately after training may influence the retention of learned responses by increasing the level or facilitating the formation of neurogenic peptides, while treatment just prior to the time when the organism is again challenged by the environment may exert a proactive effect by reinstituting some recognizable features of the organism's previous response to the demands of the environment.

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