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Alleviation of Anisomycin-Induced Amnesia by Pre-Test Treatment with Lysine-Vasopressin[1]

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JUDGE, M E AND D QUARTERMAIN *Alleviation of anisomycin-induced amnesia by pre-test treatment with lysine-vasopressin* PHARMAC BIOCHEM BEHAV 16(3) 463–466, 1982 —Amnesia in mice for a passive avoidance response induced by anisomycin injection immediately after training was reversed by 40 micrograms of lysine-vasopressin given one hour before testing Control groups receiving non-contingent shock instead of training were used to demonstrate that the effects of vasopressin were due to memory of shock received in a particular place, rather than non-specific suppression of locomotion The effects of vasopressin on retention were not mimicked by either pentylenetetrazol or epinephrine suggesting that the enhanced latencies were probably not the result of increases in fear or arousal These data support the hypothesis that the retrieval of memory can be facilitated by vasopressin The possibility of a relationship between the effects of vasopressin and those of catecholamine manipulations on memory is discussed

Amnesia Mice Passive avoidance Retrieval Vasopressin Anisomycin

MANY studies with both human and animal subjects have shown that the pituitary hormone vasopressin can enhance retention when it is administered both before and after acquisition as well as prior to testing [1, 2, 3, 4, 9, 10, 11, 14, 18] The empirical generalization which has emerged from these studies is that the hormone influences both the encoding and retrieval stages of memory processing [8] Most of the data which support a role for vasopressin in facilitating retrieval are derived from studies in which amnesias for inhibitory avoidance can be attenuated by pre-testing administration of the hormone For instance it has been demonstrated that amnesias induced by CO_2 anesthesia [14], the norepinephrine synthesis inhibitor diethyldithiocarbamate [2] and the protein synthesis inhibitor puromycin [9] can be reversed if animals have been treated with vasopressin before retention is tested While it is clear that administration of the hormone significantly increases test latencies in these studies, some ambiguity exists regarding the explanation of the facilitated avoidance because most experiments have not employed a control group to assess the possibility that acute vasopressin treatment enhances generalized fear The specificity of the hormone-induced enhancement of avoidance is usually evaluated by treating non-shocked animals with the agent [14] or by showing that the hormone does not influence locomotor activity measured in a pre-shock session [2] The use of such procedures would not differentiate between long test latencies resulting from enhancement of non-specific fear of the punishment shock and those occurring as a consequence of drug induced facilitation of a specific response-shock contingency In order to be sure that vasopressin treatment results in the enhancement of retrieval of a specific avoidance response it has to be demonstrated that animals which receive punishment shock in a different environment do not exhibit increased test latencies when tested in the experimental apparatus This non-contingent control procedure has been conspicuous by its absence in most of the published studies

The intention of this experiment was to determine whether a robust amnesia induced by the protein synthesis inhibitor anisomycin (ANI) could be alleviated by facilitating retrieval processes with lysine-vasopressin Non-contingently trained animals were included to evaluate non-specific effects of the hormone on performance, and stimulant-injected groups were included to control for apparent retrieval facilitation due to non-specific arousal

METHOD

Subjects

One hundred twenty male Swiss Webster mice weighing 35±5 grams were used in this experiment Animals were obtained from a commercial breeder (West Jersey Biological Supply) at ten weeks of age and housed in groups of 15 per cage for one week before the experiment was begun Two hours before training, pairs of mice were transferred to small cages (22×11×11 cm) where they remained for the duration of the experiment Water and food were available at all times

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Apparatus

The test chamber was a shuttle box 9 cm wide×11 cm high and 23 cm long, divided into two equal sections by means of a wall which contained a 4 cm square opening in the center which could be blocked by a vertically sliding door The acrylic walls of one side of the apparatus were white with a clear acrylic lid and on the other side walls and lid were black The floor was made of stainless steel rods (0 3 cm diameter, 7 cm between rods) perpendicular to the long axis of the box and held in a frame which pivoted at the center of the apparatus, activating a microswitch to indicate which side the mouse was on Electro-mechanical circuitry was used to record responses and latencies, and to activate the Coulbourn Instruments solid state shocker/distributor which was connected to the floor bars of the black side

Drugs

Immediately after training, mice were injected subcutaneously with 0 01 ml/gram of either 0 9% saline [S] or the protein synthesis inhibitor anisomycin (A) at 200 mg/kg which had been dissolved in acidified saline and pH adjusted to 7 0 On the test day all mice were injected subcutaneously with 0 2 ml one hour before being placed in the apparatus Injections consisted of either 0 9% saline (S), Lysine-Vasopressin (Diapid®, Sandoz) diluted with saline to final concentrations of 10, 25, or 40 micrograms in 0 2 ml (V10, V25, or V40), Pentylenetetrazol (PTZ) 10 or 20 mg/kg, or Epinephrine Hydrochloride (EPI) 0 1 or 1 0 mg/kg, both dissolved in saline

Procedure

On the training day individual mice were placed in the white side of the apparatus for response-contingent shock training (CS), the door was opened and simultaneously the latency timer was started When an animal crossed to the black side the latency timer stopped, the latency to cross was automatically recorded, and a 0 1 mA shock was delivered to the floor bars until the mouse escaped to the safe (white) side At this point a clock began to time a 60 second interval Subsequent entries into the dark side were punished in the same manner, resetting the 60 second timer The door was closed and the animal removed from the safe side after it failed to enter the dark side for one minute or after a maximum of 5 shocks Mice failing to enter the dark side twice within 3 minutes were discarded from the experiment (approximately 3% of subjects) In order to control for possible non-specific effects on retrieval resulting from interaction of shock and drug treatments, one group of mice (N=9) received a non-contingent shock (NCS) These animals were not placed in the test apparatus on the training day but were instead placed in small yellow box, 7 6 cm wide×10 2 cm long×9 8 cm high with 0 3 cm diameter floor bars spaced 1 3 cm apart They remained there while a CS animal was being trained, receiving the same shock number, duration and intensity as the CS mouse by means of yoked floor bars

Testing took place 48 hours after training All animals were injected with 0 2 ml of saline or vasopressin, one hour before being tested The nine animals which were injected with saline after training received a second saline injection before testing The anisomycin injected animals were divided into four groups, with approximately equal numbers of training shocks received by each group, and received either saline or one of the three doses of vasopressin The NCS group was injected with the highest dose of vasopressin (40 micrograms)

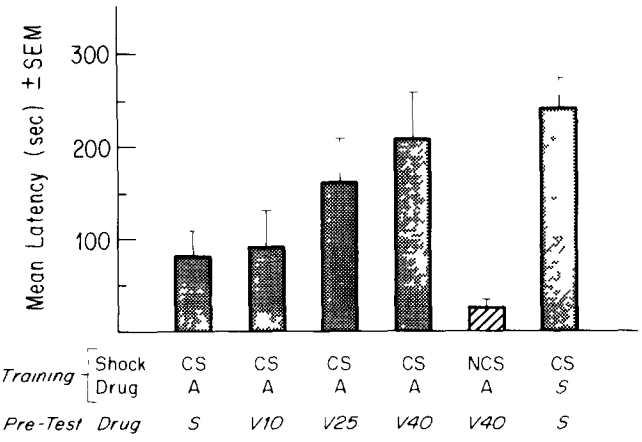

FIG 1 Group latencies to enter the compartment in which the training shock occurred Training Shock treatment CS=Contingent Shock NCS=Non-Contingent Shock Training Drug treatment S=Saline, A=Anisomycin Pre-Test Drug Treatments S=Saline V=Vasopressin, 10, 25, 40=dose in micrograms/mouse

One hour after injection, each mouse was placed in the white side of the apparatus, the door was opened and the latency timer started, as in training Animals were allowed a maximum of 300 seconds to cross to the dark side No shock was delivered in this session

RESULTS

The results are summarized in Fig 1 The group which received contingent shock and saline injection (CS, S-S) exhibited good memory with high latencies to enter the dark side where they had previously been shocked (Mean=241 2 sec, SEM=±34 5 sec, N=9) Those animals which received Ani after training and saline before testing (CS, A-S) were amnesic, crossing into the dark side in significantly less time than their saline injected counterparts (Mean=80 6±27 7 N=9 t vs (CS S-S)=3 6334, $p<0$ 004) These groups served as reference standards against which to assess the effects of vasopressin treatment

Vasopressin produced a dose-dependent reversal of the ANI-induced amnesia The lowest dose of 10 micrograms (CS, A-V10) was ineffective and did not reverse the amnesia (Mean=90 8±40 6, N=9 t vs (CS, A-S)=0 2075, $p>0$ 05, not significant) Animals receiving 25 micrograms of vasopressin (CS A-V25) displayed an intermediate latency (Mean =161 8±46 6, N=9) and were not significantly different from either the (CS, A-S) group ($t=1$ 4973, NS) or the (CS, S-S) group ($t=1$ 3689, NS) Forty micrograms of vasopressin effectively reversed the amnesia ((CS, A-V40) Mean=208 5±46 0, N=8 t vs (CS, A-S)=2 4475 $p<0$ 03, t vs (CS, S-S)=0 5771, NS) The non-contingent control animals (NCS, A-V40) had extremely low latencies to enter the dark side (Mean=26 2±4 5, N=9) and were significantly faster to enter it than controls ((NCS, A-V40) vs (CS, A-V40) $t=4$ 1953, $p<0$ 001)

An alternative interpretation of these data is possible Treatment with vasopressin might induce an increase in

TABLE 1

LATENCIES OF MICE TREATED WITH VASOPRESSIN (V), SALINE (S), PENTYLENETETRAZOL (PTZ), OR EPINEPHRINE (EPI) BEFORE TESTING

Group	N	Mean ± SEM
1 ANI-S	10	51 7 ± 23 9
2 ANI-V* (40 μg)	8	208 5 ± 46 0
3 ANI-PTZ (20 mg/kg)	10	51 8 ± 30 1
4 ANI-PTZ (10 mg/kg)	10	29 3 ± 11 5
5 ANI-EPI (1 0 mg/kg)	9	81 1 ± 35 7
6 ANI-EPI (0 1 mg/kg)	10	20 9 ± 8 2
7 ANI-S (NC)	9	17 7 ± 8 4
8 ANI-V (NC)	9	26 2 ± 4 5

*From Fig 1 Of groups 1 through 6, only group 2 (ANI-V 40 μg) is significantly different from group 1 (ANI-S) ($t=3$ 208, $df16$, $p<0$ 006) There is no significant difference between the Non-Contingent Shock groups (Group 7, ANI-S (NC) vs Group 8, ANI-V (NC) $t=0$ 892)

arousal or produce a state of generalized fear which could summate with the behavioral response most appropriate for the training situation (CS or NCS), resulting in test latencies similar to those in Fig 1 Increased arousal or fear might be expected to enhance avoidance in the contingently trained mice and enhance escape for the non-contingent animals This interpretation would attribute the effect of vasopressin to enhanced motivation rather than to facilitated retrieval In order to evaluate this interpretation we trained additional groups of mice One group was given non-contingent training and injected with Saline prior to testing According to an arousal-fear interpretation, these animals should have significantly slower crossover latencies than the non-contingent vasopressin group of Fig 1 Other groups were given conventional training followed by ANI and injected before testing with (a) Pentylenetetrazol (PTZ), a general CNS stimulant which increases arousal and in addition has anxiogenic properties [15], or (b) Epinephrine (EPI), which mimicks some of the hormonal consequences of shock-induced stress Our intention was to determine whether either of these agents induced test latencies comparable to those of the vasopressin treated animals These groups and their test latencies are shown in Table 1 Neither of the doses of EPI or PTZ produced significant increases (relative to ANI-SAL scores) in test latencies Furthermore, latencies of non-contingent mice treated with Saline and Vasopressin were not significantly different, indicating that vasopressin did not enhance escape behavior Other unpublished data from this laboratory have shown that amnesia induced by ANI for an inhibitory avoidance response cannot be alleviated by pre-test treatment with the adrenergic agonists clonidine and isoproterenol, or by the general stimulant strychnine Taken together with the results presented above, these data indicate that increased arousal or fear are not by themselves sufficient conditions for the enhancement of performance following amnesia The present findings are consistent with previous data which indicates that vasopressin facilitates retrieval processes

DISCUSSION

The results of this research demonstrate that vasopressin can reverse an anisomycin induced amnesia for passive avoidance training, and generally confirm the contention that vasopressin can enhance memory This conclusion is strengthened by a comparison of independent groups of mice both receiving the effective dose of vasopressin and differing only in their training procedure Only mice receiving vasopressin before testing *and* a specific pairing of shock with the dark side of the passive avoidance apparatus during training show a high latency to enter that side during the test session, indicating that vasopressin did not non-specifically suppress behavior

Research into the biological basis of memory has demonstrated that experimentally induced amnesias can be reversed by pre-test injections of drugs targeted to the central nervous system Agents stimulating the catecholaminergic system are particularly effective in reversing the frequently studied amnesias produced by injecting the protein synthesis inhibitors cycloheximide or anisomycin after training [12] This finding stimulated research ultimately demonstrating that not only do these agents significantly inhibit norepinephrine synthesis [5] and release [6], but the amnesia they produce can be mimicked by post-training inhibition of catecholamine synthesis with diethyldithiocarbamate [13] This evidence has led to the inference that catecholamine release is a significant factor in the retrieval of information from storage in the brain, a concept which receives additional support from neuro-physiological experiments showing that norepinephrine applied iontophoretically enhances the signal processing capacity of neurons [19]

Pituitary peptides such as vasopressin are also capable of reversing experimental amnesias shown to involve the catecholamines and in addition, antivasopressin serum is capable of inducing an amnesia for passive avoidance training when injected into the brain [17] This information, coupled with evidence that vasopressin effects the catecholamine systems in the brain [8,16], suggests that the functional effect of endogenous vasopressin on mammalian behavior may be to enhance the ability of an animal to retrieve and utilize stored information This idea has encouraged research in both animal models [1, 2, 3, 4, 9, 14] and humans [10, 11, 18] to attempt to improve memory with vasopressin Human research has yielded positive but tenative results, since experiments are limited by the need to use small numbers of subjects, the inability to run carefully matched controls, and stringent dose restrictions Animal models are not subject to these limitations, but can only provide information about relatively simple memories However, these memories concern highly significant environmental stimuli and the demonstration of drug effects in animal systems should readily generalize to more subtle aspects of mammalian memory

While the notion that vasopressin improves memory is attractive because of its potential applications, the data collected to date leaves a number of critical questions unanswered about the nature of vasopressin's effects on test performance The premise that the degree of similarity of the testing and training stimuli positively influences the probability of information retrieval is routinely used in the design of memory experiments A body of evidence has accumulated to support the concept that internal stimuli must also be considered as potent retrieval cues [7] In order to infer that a drug active during testing facilitates the retrieval

of learned information from the training situation, it must be demonstrated that the drug is not simply altering behavior by mimicking the internal stimuli arising from noxious environmental events experienced previously Thus, an animal injected with vasopressin might fail to move from one compartment of a passive avoidance apparatus to the other simply because the internal consequences of the injection evoked suppression of locomotor activity in response to generalized fear, rather than due to specific memory of shock received in the other compartment Recent experiments demonstrating apparent reversals of amnesias by vasopressin fail to distinguish between these possibilities since they lack the control groups necessary to show that the combination of noxious stimuli experienced in training were not sufficient to produce the results [2,14] The use of a Non-Contingent Shock control in the present experiment shows that in order to exhibit the high latencies characteristic of memory for passive avoidance training, mice must experience an associative pairing of shock with the testing apparatus These data thus support the contention that vasopressin can enhance an experimentally weakened memory

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