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Clonidine Reverses the Amnesia Induced by Dopamine Beta Hydroxylase Inhibition

LEWIS S. FREEDMAN, M. Z. BACKMAN AND DAVID QUARTERMAIN

Division of Behavioral Neurology, New York University School of Medicine
341 East 25th Street, New York, NY 10010

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FREEDMAN, L. S., M. Z. BACKMAN AND D. QUARTERMAIN. *Clonidine reverses the amnesia induced by dopamine beta hydroxylase inhibition.* PHARMAC. BIOCHEM. BEHAV. 11(3) 259–263, 1979.—The role of noradrenergic (NE) mechanisms in amnesia induced by the dopamine-β-hydroxylase (DBH) inhibitor, diethyldithiocarbamate (DEDTC) was examined by studying the antiamnestic characteristics of the alpha-NE receptor stimulator clonidine. DEDTC (250 mg/kg) administered 3 hr prior to training to C57BL/6J mice resulted in marked deficits when retention of a multiple trial food motivated spatial discrimination task was measured 24 hr after learning. Investigation of the temporal aspects of recovery indicated that the agonist was an effective antiamnestic agent when administered 0, 1, 3, 21 and 23 hr after training. No recovery was observed when the drug was administered 6 and 18 hr posttraining. A dose response study of the effectiveness of clonidine administered 1 hr prior to testing indicated recovery of memory at doses ranging from 10–500 μg/kg. The clonidine induced recovery was not a result of general performance facilitation, but specific to the memory tested. In addition, the clonidine effect was pharmacologically specific to its actions on NE receptors, as recovery was blocked by pre-treatment with the alpha-NE antagonist, phentolamine. No recovery from DEDTC induced amnesia was seen with post-training or pre-test injection of d-amphetamine.

Amnesia Diethyldithiocarbamate Clonidine Alpha-noradrenergic receptors

THERE is considerable evidence that pharmacological agents that either inhibit catecholamine (CA) synthesis at the tyrosine hydroxylase (TH) or dopamine-β-hydroxylase (DBH) steps, or block CA receptors directly, result in retention deficits [6, 9, 11]. These findings have generally been interpreted as providing support for Kety's [10] hypothesis that release of norepinephrine (NE) promotes consolidation of long term memory. Recent experiments indicate that CA's may also influence memory retrieval. For example, it has been shown that diethyldithiocarbamate (DEDTC) produced amnesias when given 30 min prior to a retention test 1, 3, 5, and 7 days after training [7]. In addition, it has been shown that amnesias induced by inhibition of both CA and protein synthesis can be alleviated by pre-testing administration of pharmacological agents which stimulate central CA mechanisms [2, 3, 11, 13, 14].

While these experiments appear to provide strong support for a role of CA's (particularly NE) in memory processing, they are not free of ambiguity. In the studies which show that inhibition of DBH disrupts retention performance, it is frequently difficult to determine whether the amnesia results from inhibition of the synthesis of NE or from one or more of the many possible side effects which are induced by high doses of copper chelating agents, such as DEDTC. The suspicion that the non-specific side effects of DEDTC may contribute to memory disruption is strengthened by the finding that other inhibitors of DBH, such as FLA-63, fusaric acid and U14-624 are reported not to induce amnesia for a single trial passive avoidance response when administered immediately posttraining [9].

On the other hand, studies which demonstrate that these amnesias can be reversed by NE stimulation suggest that DEDTC is influencing memory by a direct effect on NE synthesis. For example, amnesia induced by pre-training DEDTC can be reversed by intraventricular injections of NE up to 1 hr after training [19]. It has also been demonstrated that administration of dihydroxyphenylserine (DOPS), a direct NE precursor, prevented amnesia when it was administered 60 min prior to DEDTC [8]. Results from this laboratory have shown that amnesias produced by both DEDTC and FLA-63 can be reversed at the retrieval stage by administration of the monoamine oxidase inhibitors pheniprazine and pargyline [3,11].

The present set of experiments were designed to provide more direct evidence for the involvement of NE in DEDTC-induced amnesia by determining whether memory can be restored by administration of the specific alpha-noradrenergic stimulator, clonidine. An additional objective was to examine the effects of amphetamine, a CA releasing agent on DEDTC induced amnesia.

METHOD

Subjects

Animals used throughout these experiments were male mice of the C57BL/6J strain obtained from the Jackson Laboratory. Animals were 10–12 weeks old and weighed 20–23 g at the time of the experiments. Mice were individually housed throughout the experimental period and had free access to food except during deprivation periods. Water was available ad lib. At time of training, animals were 85–90% of initial body weight.

Apparatus

The behavioral test employed to measure retention was a multiple trial food motivation spatial discrimination task. The apparatus was a T-maze 3 in. wide and 3 1/2 in. high. The center alley was 11 1/4 in. long and each arm was 7 in. long. The initial 3 1/2 in. of the center alley served as a start box separated from the rest of the maze by a guillotine door. Guillotine doors at the start of each arm prevented retracing. The entire maze was painted flat black and was covered with clear Plexiglas lids.

Design and Procedure

On two consecutive days, each animal was given a 10 min adaptation session during which time each food cup contained ten 20 mg Noyes pellets. On the training day (24 hr after second adaptation session), mice were injected with DEDTC (250 mg/kg, SC) 3 hr prior to maze learning. We have shown that this dose blocks conversion of DA to NE and at 3 hr results in a 50% depletion of whole brain NE. Animals were trained to go to the right or left arm of the maze until a criterion of 7 out of 8 correct choices (noncorrection) was achieved. Retention was tested 24 hr later by retraining mice to the same criterion. Both treated and untreated animals usually reached criterion on training within 23–30 trials (Mean trials to criteria at training: Control 23.2, DEDTC 22.2). Control mice showed good retention (60–80% savings) 24 hr later. Percent savings was calculated by analysis of trials to criteria on training and testing sessions using the formula:

$$\frac{\text{Training}-\text{testing}}{\text{Training}} \times 100 = \text{Percent savings}$$

DEDTC treated animals show a well developed amnesia at 24 hr that spontaneously recovers by 48 hr after training. Clonidine-hydrochloride and d-amphetamine-sulfate were dissolved in 0.9% NaCl and appropriate dosages injected IP at varying times post-training. All drug solutions were prepared so that injection volume was 0.1 mg/10 g body weight.

EXPERIMENT 1

The purpose of the first experiment was to investigate the temporal course of effectiveness of clonidine as a recovery agent for a DEDTC induced amnesia, by injecting the agonist 0, 1, 3, 6, 18, 21, and 23 hr posttraining. The dose of clonidine employed, 0.5 mg/kg, has previously been shown effective in reversal of a cycloheximide induced amnesia [13].

Results

Results of this experiment are shown in Fig. 1. Analysis of variance of these data indicate a highly significant difference among various time course groups, $F(6,90)=28.9$, $p<0.001$. Clonidine is effective at 0, 1 and 3 hr posttraining and 1 and 3 hr pretesting but ineffective 6 hr posttraining and 6 hr preretention testing. A trend analysis indicates a highly significant quadratic, $F(1,90)=117.3$, $p<0.001$, and cubic, $F(1,90)=14.7$, $p<0.001$) component which confirms the biphasic nature of the gradient depicted in Fig. 1.

EXPERIMENT 2

Previous studies from our laboratory have demonstrated the effectiveness of various CA drugs as antiamnestic agents

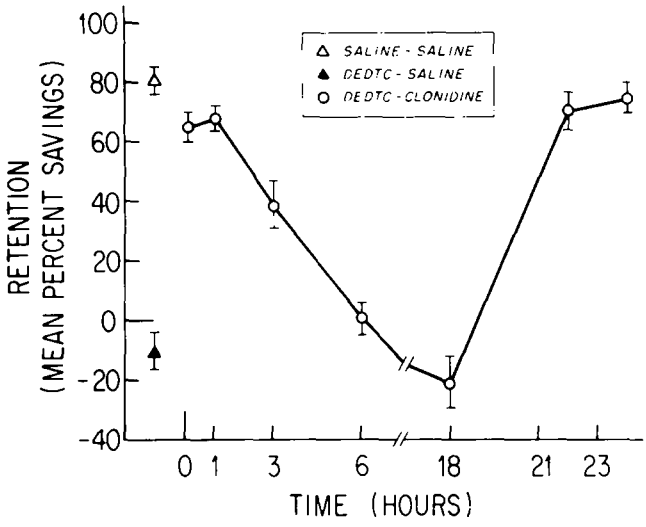

FIG. 1. The effect of clonidine (500 μg/kg) on retention in DEDTC-treated mice. Clonidine was injected to independent groups of animals (N=15) at various times (0–23 hr) post-training and animals tested for retention at 24 hr. Data expressed as mean percent savings ± SEM.

when administered prior to retention test. These data suggest that one site of action of CA agonists may be on retrieval mechanisms. The present experiment was designed to study the dose related characteristics of direct NE receptor stimulation on retrieval. Animals were injected with varying doses of clonidine (1–500 μg/kg) 1 hr prior to the 24 hr retention test.

Results

Results of this experiment are shown in Fig. 2. Analysis of variance of these data indicate a highly significant,

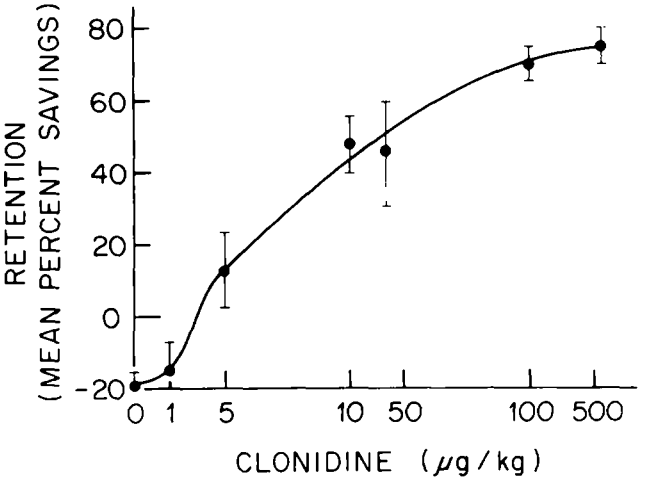

FIG. 2. The effect of varying doses of clonidine (1–500 μg/kg) on retention in DEDTC-treated mice. Clonidine was injected to independent groups of animals (N=12) one hour prior to testing. Abscissa: dose of clonidine in micrograms (μg) per kilogram (kg) of body weight on a log scale. Ordinate: retention expressed as mean percent savings ± SEM.

$F(5,65)=16.7$, $p<0.001$, dose effect. No recovery can be seen with 1 and 5 μg/kg, but enhanced performance is observed as the dose level is increased to 500 μg/kg at which point retention was comparable to that of saline controls. It is noteworthy that substantial recovery (48.7% savings) is demonstrated with a dose as small as 10 μg/kg of clonidine. Data from this experiment demonstrate that the alpha-noradrenergic receptor stimulator, clonidine, administered before testing reverses DEDTC amnesia in a dose dependent fashion.

In order to determine whether this facilitated retention is a reflection of recovery of a specific memory or merely the result of a general enhancement of performance, retention was tested in additional groups of animals using a reversal paradigm. Previous data from our laboratory [13] have shown that when retention is measured with a reversal test, saline-treated mice require significantly more trials to reach criterion when retrained to the same side. On the other hand, amnestic animals require fewer trials to reach criterion when retrained to opposite side than when tested to the same side. We would predict that if clonidine is recovering a specific memory, performance of DEDTC-clonidine treated mice would be similar to saline animals when tested to the opposite side. Groups of Saline-Saline, DEDTC-Saline and DEDTC-clonidine animals were trained to a criterion of 7/8 correct choices and tested for retention of either (1) side to which they were trained or (2) opposite side. Clonidine was injected at a dose of 0.5 mg/kg 1 hr prior to the retention test.

TABLE 1

BEHAVIORAL SPECIFICITY OF CLONIDINE-INDUCED RECOVERY

GROUP	MEAN % SAVINGS	
	Same Side	Opposite Side
Saline-Saline	78.8 (15)	−6.8 (13)
DEDTC-Saline	−12.5 (37)	10.4 (13)
DEDTC-Clonidine	68.2 (20)	−18.2 (13)

Data is expressed as mean percent savings. Number of animals per group is indicated in parenthesis.

These data are shown in Table 1. Analysis of variance indicated a highly significant effect both for drug groups, $F(2,80)=10.4$, $p<0.001$ and type of retention test, $F(1,80)=19.9$, $p<0.001$. The interaction between drug group and type of retention test was also significant, $F(2,80)=35.0$, $p<0.001$ indicating that the level of retention depended on the side to which animals were tested. Thus mice treated with clonidine exhibited enhanced performance relative to DEDTC-Saline group when tested to the side to which they were trained but impaired performance when tested to the opposite side. This latter impairment is presumably the result of proactive interference from the previously learned habit recovered by the drug. These data therefore indicate that clonidine-induced recovery is related to a specific memory rather than generalized enhancing performance.

If the clonidine induced recovery was working through its effects on alpha-NE mechanisms, we would expect to block the effect of ths agonist with the alpha antagonist phentolamine. Phentolamine (1.0 mg/kg) was administered 20 min prior to clonidine and animals tested for retention. These data are shown in Table 2. The Saline-Saline and DEDTC-Saline groups are taken from Experiment 2 and included for comparison. It can be seen that phentolamine almost totally blocks the antiamnestic effects of clonidine. Savings scores for the DEDTC-phentolamine-clonidine group are significantly different ($t=9.412$, $p<0.001$) from the DEDTC-clonidine group and are comparable to the DEDTC-Saline group. Thus, alpha-noradrenergic mechanisms seem to be involved in clonidine reversal of DEDTC induced amnesia. It is noteworthy that pilot studies indicated that higher doses of phentolamine (2.5, 5.0 and 10.0 mg/kg) were also effective antagonists of the clonidine effect but produced amnesia in saline treated mice. These results are consistent with the hypothesis that DEDTC induced amnesia is the result of functional impairment of noradrenergic neuronal activity and that direct activation of NE post synaptic receptors can restore impaired retention to normal levels.

TABLE 2

PHARMACOLOGICAL SPECIFICITY OF CLONIDINE-INDUCED RECOVERY

GROUP	MEAN % SAVINGS
Saline-Saline	78.8 ± 3.92 (15)†
DEDTC-Saline	−12.5 ± 5.58 (37)†
DEDTC-Saline-Clonidine	75.4 ± 5.48 (15)
DEDTC-Phentolamine-Clonidine	0.7 ± 3.99 (9)‡

Data are expressed as mean percent savings ± SEM. Number of animals per group is indicated in parenthesis.
†data from Expt. II for comparison
‡$t=9.614$ vs. DEDTC-Clonidine, $p<0.001$

EXPERIMENT 3

Amnesias induced by protein synthesis inhibitors can be reversed by either posttraining [1, 14, 18] or pretest [11] injections of d-amphetamine, an agent that releases catecholamines. Quinton and Bloom [14] have shown that d-amphetamine will reverse DEDTC induced amnesia for a single trial passive avoidance task when administered preretention test but not posttraining. This experiment was designed to study whether amphetamine could reverse a

TABLE 3

EFFECT OF AMPHETAMINE ON DEDTC-INDUCED AMNESIAS

Amphetamine Dose (mg/kg)	Mean % Savings
*Post-training**	
0.5	−15.5 ± 7.30 (9)
1.0	−14.6 ± 10.09 (8)
*Pre-Testing***	
1.0	−21.8 ± 5.72 (9)
5.0	−31.0 ± 10.94 (8)

Data is expressed as mean percent savings ± SEM. Number of animals per group is indicated in parenthesis.
*immediately posttraining
**30 min pretesting

DEDTC induced amnesia for a multiple trial discrimination task. Using the standard experimental design, animals were injected with DEDTC (250 mg/kg) 3 hr prior to training, trained to criterion (7/8 correct choices) and tested for retention at 24 hr. d-Amphetamine was administered immediately posttraining (0.5, 1.0 mg/kg) or 30 min prior to retention test (1.0, 5.0 mg/kg).

Results

Results expressed as percent savings are shown in Table 3. These data indicate that amphetamine at all times and doses was totally ineffective in reversing DEDTC induced amnesia. It should be noted that the animals injected prior to the retention test were not disoriented or grossly hyperactive and exhibited maze running performance comparable to that seen in DEDTC treated mice.

GENERAL DISCUSSION

The results of these experiments provide additional evidence that noradrenergic mechanisms are involved in amnesias induced by DEDTC. Previous data from our laboratory indicated that amnesias induced by DEDTC or FLA-63 could be reversed by pretest administration of the MAO inhibitors, pargyline or pheniprazine, two drugs that raise intraneuronal levels of monoamines [3,11]. Our present data indicate that clonidine, an alpha-noradrenergic receptor stimulator, is also an effective antiamnestic agent. The present results taken together with our previous findings indicate that activation of both pre- and postsynaptic adrenergic mechanisms can reverse memory loss induced by agents that block the synthesis of norepinephrine.

The demonstration that amnesia can be reversed by clonidine given 23 hr posttraining suggests that DEDTC may be disrupting retrieval rather than blocking the formation of the memory trace. However, an effect on memory storage mechanisms cannot be conclusively ruled out by these experiments. It is possible that in this task, the spontaneous recovery seen at 48 hr posttraining may reflect the emergence of a slowly consolidating memory trace [20]. It has been suggested that antiamnestic agents administered posttraining may be operating by accelerating the rate of consolidation of a partially formed memory. If this hypothesis is correct clonidine administered at any time before spontaneous recovery occurs should reverse the amnesia. The fact that clonidine is ineffective 6 and 18 hr after training rules against this interpretation.

Investigations of the temporal characteristics of recovery reveal that clonidine is effective in blocking amnesia when it is administered up to 3 hr after and also 3 hr before testing. The temporal gradient after training is consistent with the findings of other studies which have shown that the effectiveness of antiamnestic agents is inversely related to time of administration after training [5, 13, 19]. It has been hypothesized that neuronal stimulants administered posttraining increase the strength and durability of the short term memory trace, thereby providing a holding mechanism to maintain the trace until consolidation processes are reestablished [5]. There is, however, no direct evidence to support this hypothesis.

Reversal of amnesia by clonidine administered 1 hr prior to testing probably reflects the effects of the agonist on retrieval mechanisms. This is consistent with the findings of several recent studies which have shown that drugs administered before testing can alleviate memory loss induced by a variety of agents [13, 16, 17]. The mechanisms by which clonidine is influencing retention during testing is not known. One hypothesis [4] is that the enhanced retention performance is the result of strengthening of a memory trace which has been weakened as a result of NE depletion during learning. Evidence for this hypothesis is equivocal. It has been shown that amphetamine administered pre-testing will increase latencies of mice poorly trained in a passive avoidance task [4]. However, another study failed to demonstrate an increase in retention for a spatial habit in a T-maze when poorly trained mice were treated pre-testing with the dose of the MAOI pheniprazine which improved retention in amnestic animals [12]. The effects of pretesting administration of memory enhancing drugs to poorly trained animals is not well understood and more research will be necessary before the hypothesis can account for the improved retention of amnestic animals.

Another approach to determining whether drugs administered before testing to amnestic animals are improving performance by strengthening a weak habit is to examine the durability of the recovered memory. If the hypothesis is correct, recovered animals should continue to show retention on a subsequent test. On the other hand, if the amnesia returns when animals are retested without the drug, it would be unlikely that the recovery was a reflection of a strengthened habit. There are no published data which specifically examines this question. We do, however, have some preliminary results with amnesias induced by protein synthesis inhibition which are relevant. We have shown that mice made amnestic for an avoidance response by a posttraining injection of anisomycin show a complete recovery of memory if treated with amphetamine 30 min before testing. These animals, however, relapse into amnesia when tested without amphetamine. This finding suggests that the memory trace has been fully formed but stored in such a way that it is inaccessible without the aid of the drug. Further studies will be necessary to determine the generality of this finding.

The absence of recovery following both posttraining and pretesting administration of amphetamine is paradoxical if activation of CA mechanisms is necessary for recovery from DEDTC induced amnesia. Failure to find recovery with immediate posttraining amphetamine confirms a recent finding [14] and suggests that in DEDTC-treated animals there exists a deficit in presynaptic NE pools available for drug-induced release. The finding that at a time when NE synthesis and endogenous tissue levels of NE are normal, pretest administration of amphetamine also fails to reverse the amnesia is more puzzling. Thus, the data presented in this report indicate that postsynaptic NE receptors are responsive to activation whereas presynaptic release mechanisms are, for some yet unexplained reason, refractory to drug-induced stimulation. It should be noted that amphetamine is a highly effective recovery agent for amnesias induced by protein synthesis inhibitors. This suggests that amnesias induced by DEDTC and by protein synthesis inhibitors though behaviorally similar [11] are mediated by different neurochemical mechanisms.

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