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Recovery from Amnesia Induced by Pre-test Injections of Monoamine Oxidase Inhibitors[1,2]

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BOTWINICK, C. Y. AND D. QUARTERMAIN. *Recovery from amnesia induced by pre-test injections of monoamine oxidase inhibitors.* PHARMAC. BIOCHEM. BEHAV. 2(3) 375–379, 1974. – Amnesia was induced in C57BL6J male mice by pre-training injections of cycloheximide (CYC) in a one-trial passive avoidance task. This amnesia was reversed by pre-testing injections of two monoamine oxidase inhibitors (MAOI's) catron and pargyline. The results of 2 non-contingent control groups indicated that mice injected with cycloheximide but given foot shock in a place different from the training apparatus did not show increased latency following treatment with catron and pargyline. This indicates that recovery is specific for training in the passive avoidance task. Depletion of norepinephrine (NE) by diethyldithiocarbamate (DEDTC), a dopamine beta hydroxylase inhibitor, resulted in an amnesia similar to that induced by CYC. DEDTC-induced amnesia was also reversed by catron and pargyline.

Amnesia Cycloheximide Diethyldithiocarbamate Memory recovery Monoamine oxidase inhibitors

A NUMBER of recent studies on experimentally induced amnesia have focused on the issue of the permanence of the effect. These experiments have attempted either to determine whether memory recovers spontaneously some time following amnesic treatment, or to induce recovery by reminder treatments or by pharmacological means. Spontaneous recovery of memory has been observed in a number of experiments [13, 17, 23], but has not occurred in some others [3, 6, 12]. The results of reminder induced recovery experiments are more consistent in showing positive findings [7, 10, 11, 14, 15, 16], although some failures have been reported [2].

Recently, evidence has been presented which indicates that a reminder shock may not be an adequate means to evaluate the permanence of retrograde amnesia. Haycock *et al.* [9] and Gold *et al.* [8] have shown that non-contingent foot-shock improves performance on the retention test because it summates with the weak habit of partially amnesic animals, rather than because it recovers the original memory. Whether this explanation is adequate to account for all the reminder recovery findings remains to be determined.

Evidence that pharmacological manipulation may lead to recovery from amnesia induced by protein synthesis inhibition has recently appeared. Roberts, Flexner and Flexner [21] have shown that puromycin induced amnesia could be reversed by pretesting injections of imipramine, tranylcypromine and d-amphetamine. Serota *et al.* [22] have demonstrated that amnesia induced by acetoxycycloheximide (AXM) can be prevented by post-training injections of metaraminol or d-amphetamine. Since all of these drugs increase tissue concentrations of catecholamines, it has been suggested that protein synthesis inhibition may be producing amnesia by depleting concentrations by biogenic amines most likely norepinephrine [5,21]. Some direct evidence for this hypothesis comes from a recent experiment which shows that both cycloheximide (CYC) and AXM inhibit the activity of tyrosine hydroxylase and thus reduces the functional pool of catecholamines [5].

The object of the present study was to determine if amnesia for a one-trial passive-avoidance response induced by CYC could be reversed by pretesting injections of agents which increase intraneuronal levels of catecholamines. A second objective was to determine whether amnesia induced by norepinephrine depletion could also be reversed by increasing brain catecholamine levels.

EXPERIMENT 1

The aim of this experiment was to determine whether memory of a one-trial inhibitory avoidance response lost following CYC administration could be restored by pretesting injections of two monoamine oxidase inhibitors (MAOI's), catron (CAT) and pargyline (PAR). Catron and pargyline, by preventing the oxidative deamination of norepinephrine (NE) and dopamine (DA), raise the intraneuronal levels of these transmitters and might be expected to attenuate CYC-induced amnesia if CYC is exerting its

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effect via a depletion of intraneuronal central catecholamines.

METHOD

Animals

The animals were male C57BL/6J mice, 25 g in weight.

Apparatus

The apparatus was a two compartment passive-avoidance box. The small compartment, made of clear Plexiglas, was 10 cm long and 7 cm wide. A circular hole, 5 cm in dia., served as an entrance to the large compartment which had black Plexiglas sides and was 15 cm long and 10 cm wide. The floor was made of steel rods 3 mm in dia. and set 13 cm apart. A black Plexiglas guillotine door separated the 2 compartments. The entire apparatus was 20 cm high and both compartments were covered with separate hinged lids.

Design

The basic design was as follows. Mice were injected with either CYC (120 mg/kg sc) or physiological saline and 30 min later given a training trial in the passive avoidance apparatus. For each group that received a foot-shock (FS) on the training day, there was a corresponding control group which did not receive foot-shock (NFS). The effect of the MAOI's in recovering memory was evaluated 24 hr later on the test day. Animals which did not receive FS on the training day served to evaluate the effects of the MAOI's alone, on step out latencies. In addition, 2 non-contingent control groups were employed. The design of those groups was as follows. One Day 1, animals were injected with CYC and given foot-shock in an apparatus different from the passive avoidance box. On the test day, mice were injected with the MAOI's and tested in the passive avoidance apparatus. This control was designed to determine whether increases in latency following treatment with MAOI's is specific to passive avoidance training. All of the groups used in Experiment 1 are shown in Table 1.

TABLE 1

DIFFERENT TREATMENT GROUPS IN EXPERIMENT 1

Group	N	Training Day FS/NFS	Training Day Drug	Test Day Drug
1	15	FS	SAL	SAL
2	10	NFS	SAL	SAL
3	23	FS	CYC	SAL
4	10	NFS	CYC	SAL
5	10	FS	SAL	CAT
6	10	NFS	SAL	CAT
7	10	FS	CYC	CAT
8	10	NFS	CYC	CAT
9	10	FS	CYC	PAR
10	10	NFS	CYC	PAR
11	15	FS	SAL	PAR
12	10	NFS	SAL	PAR

Procedure

Thirty min following CYC injection, mice were placed in the small compartment and after they had entered the large compartment, the door was closed and the FS groups received a 2.0 sec 0.2 mA shock automatically delivered through the bars. The NFS groups were left in the large compartment for 2.0 sec. Latencies to enter the large compartment were recorded on the training trial and on the retention test trial. On Day 2, mice were injected interperitoneally with either catron (10 mg/kg) dissolved in 0.2 cc solution of physiological saline, pargyline (75 mg/kg), or physiological saline. Catron was injected 1 hr prior to testing and pargyline 2 hr. These dose levels and injections times were selected so that greater than 95% inhibition of MAO was achieved at the time of testing. Retention was tested by placing mice back into the passive-avoidance apparatus using the same procedure as employed on Day 1, except that no shock was given. Animals that failed to enter the large compartment within 300 sec were removed and given a score of 300+.

RESULTS

There were no significant differences in training latency among the groups. Mean latency was 17.04 with a range from 15.34 sec to 18.61 sec. Retention test latencies are shown in Fig. 1. In the NFS groups, neither catron nor pargyline significantly increased test latencies of CYC or saline injected animals. There were no significant differences between CYC-SAL-FS and CYC-SAL-NFS groups. The effects of the different drug treatments are revealed in the groups which received FS on the training day. CYC-saline injected groups showed a significant retention deficit 24 hr later when compared with the saline-saline group (t = 5.80, $p<0.001$). Both the CYC-catron group and CYC-pargyline group had significantly longer latencies than the CYC-saline group (CYC-pargyline, t = 8.18, $p<0.001$; CYC-catron, t = 5.41, $p<0.001$). Neither catron nor pargyline significantly influenced latencies of saline injected mice.

In order to be sure that the increased latencies of the CYC-catron and CYC-pargyline groups were specific to the passive avoidance response, 2 non-contingent control groups were employed. On Day 1, 30 mice were injected with CYC as previously described. Thirty min later, they were placed in a box different from the training apparatus and given a 0.2 mA foot-shock for 2.0 sec. The box, which was 21 cm long, 11-1/2 cm wide, and 13-1/2 cm high, was constructed of Plexiglas. The floor was made of brass rods 3 mm in dia. and set 3-1/2 cm apart. The inside was gray and the entire box was covered with a clear Plexiglas lid. On Day 2, mice were divided into 3 groups; Group A (N = 10) was injected with catron (10 mg/kg), Group B (N = 10) with pargyline (75 mg/kg) and Group C (N = 10) with saline. Group A was tested 1 hr and Group B 2 hr following injection. Half of the mice in Group C were injected 1 hr

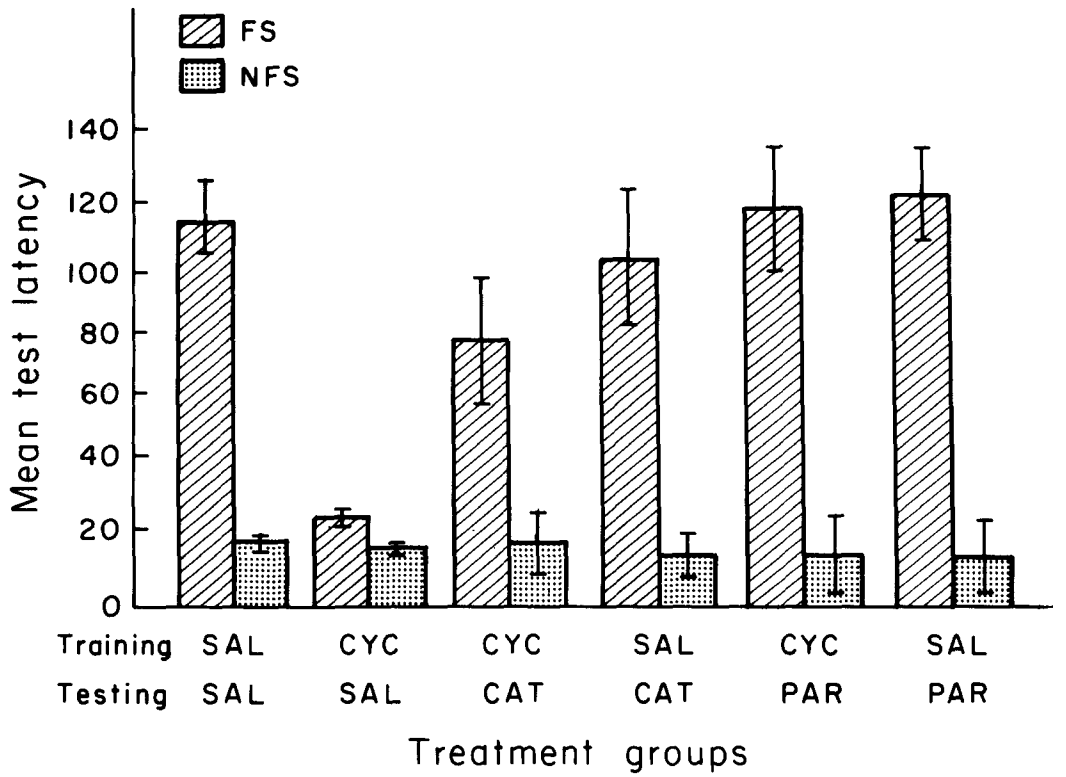

FIG. 1. Mean test latencies for groups in Experiment 1. Drug treatment on the Training and Testing day is indicated below each group.

and the other half 2 hr following injection of saline. Testing consisted of placing each mouse in the small compartment of the passive avoidance box and recording latencies to enter the large compartment. No FS was given. Mean latency for the 3 groups was as follows: saline, 17.0 sec; catron, 17.2 sec; and pargyline, 17.1 sec. All of these groups had significantly shorter latencies than the corresponding contingent shock groups (Fig. 1), (saline, $t = 7.91$, $p<0.001$; catron, $t = 3.59$, $p<0.01$; pargyline, $t = 6.0$, $p<0.001$). These results indicate that the MAOI's are not increasing test latencies because of non-specific effects. Rather, these data show that increased latencies in CYC-catron and CYC-pargyline groups are specific to the passive avoidance training and thus probably reflect restoration of some part of the original memory.

EXPERIMENT 2

We have previously shown that administration of DEDTC, a compound which inhibits synthesis of dopamine beta hydroxylase and thus reduces the functional pool of norepinephrine, produces retrograde anmnesia in the passive avoidance task [19]. The aim of the present experiment was to determine whether this amnesia could be attenuated by pre-testing treatment of monoamine oxidase inhibitors.

METHOD

Apparatus

Apparatus was the same as described in Experiment 1.

Design and Procedure

In this experiment, our basic procedure was identical to that previously described. Mice were injected with DEDTC before training on the passive-avoidance task and injected with catron or pargyline before testing. On Day 1, 35 mice were injected with DEDTC (250 mg/kg subcutaneously in 0.3 cc saline solution) and 15 mice were saline 3 hr prior to training. We have previously shown that the most effective inhibition of dopamine-beta-hydroxylase in brain was measured by conversion of (^{14}C) dopa to (^{14}C) norepinephrine was apparent between 90 min and 4.5 hr after administration of DEDTC [19]. On the following day, DEDTC-treated mice were ordered into the following groups: Group 1 (N = 13) was injected with saline (0.3 cc ip) 1 hr prior to testing; Group 2 (N = 10) was injected with catron (10 mg/kg ip in a 0.2 cc solution of saline) 1 hr prior to testing; Group 3 (N = 12) with pargyline (75 mg/kg ip in 0.2 cc saline solution) 2 hr prior to testing; and Group 4 (N = 15) was injected with saline both before training and 1 hr prior to testing.

RESULTS

Results of this experiment are shown in Table 2. DEDTC-saline injected mice showed the expected amnesia when compared with the saline-saline group ($t = 8.80$, $p<0.001$). Both catron and pargyline attenuated DEDTC induced amnesia. The DEDTC-saline group had significantly shorter latencies than both the DEDTC-catron ($t = 8.10$, $p<0.001$) and the DEDTC-pargyline groups ($t = 6.14$,

TABLE 2

MEAN LATENCY SCORES FOR BOTH TRAINING AND TESTING IN EXPERIMENT 2

Group	N	Drug on Training Day	Drug on Test Day	Mean Training Latency (sec)	Mean Test Latency (sec)
1	13	DEDTC	Saline	18.3	18.8
2	10	DEDTC	Catron	18.6	97.1
3	12	DEDTC	Pargyline	18.0	89.0
4	15	Saline	Saline	19.0	112.6

$p<0.001$). Test latencies of DEDTC-catron and DEDTC-pargyline were not significantly different from those of the saline-saline group. It has previously been demonstrated in Experiment 1 that neither catron nor pargyline significantly influence latencies of saline injected mice. These data indicate that amnesia resulting from inhibiton of norepinephrine biosynthesis is not permanent and suggest that, like CYC, DEDTC is interfering with memory retrieval rather than preventing memory storage. This is supported by the results of a recent study [18] which showed that DEDTC induced amnesia in a food motivated discrimination reversal task could be reversed by pre-testing injections of pargyline.

DISCUSSION

The results of Experiment 1 demonstrate that amnesia for a one-trial inhibitory avoidance response induced by CYC can be attenuated by administration of MAOI's. The increased latencies following administration of catron and pargyline are specific to animals that were shocked in the training apparatus and thus cannot be accounted for in terms of drug induced lethargy or general debilitation. These increased latencies probably reflect recovery of some part of the original memory of the avoidance response. This contention is supported by the behavior of the mice during testing. CYC treated animals injected with catron and pargyline exhibited behavior similar in a number of respects to saline controls. When placed in the small compartment on the test trial, they showed increased activity and pronounced approach-avoidance behavior. When they finally entered the large compartment, they did so in an extremely hesitant fashion. This behavior was in sharp contrast to CYC-saline treated animals who entered the large compartment without hesitation. Saline injected mice frequently defecated and urinated while in the small compartment but CYC treated mice were never observed to do so. Defecation and urination was also frequently observed in CYC mice treated with catron and pargyline. These observations provide additional support for the view that MAOI's, by increasing intraneuronal levels of brain catecholamines, restore the animals' ability to retrieve the memory of the avoidance response learned during protein synthesis inhibition.

Similar observations have recently been made in a food motivated discrimination reversal task. Quartermain and Botwinick [18] have shown that amnesia induced by CYC can be reversed by pre-test injections of catron, pargyline, and d-amphetamine. We have shown that these compounds do not influence retention scores of saline treated animals, and that the improved performance of CYC animals treated with these agents cannot be accounted for in terms of generalized enhancement of learning.

The exact nature of the relationship between CYC-induced amnesia and depletion of brain catecholamines is not clear at the present time. It does not seem likely that CA's are still significantly depleted 24 hr after injection, although no biochemical data at this time point are presently available. Flexner *et al.* [5] have shown that maximum inhibition of tyrosine hydroxylase following CYC treatment occurs 2 hr after injection and, by 4 hr, recovery is beginning to occur. This assay however was based on large samples of cerebral hemisphere, and the possibility exists that specific regions critical to memory retrieval may have been more profoundly depleted. It seems likely from the behavioral data that CYC has effects on the utilization and mobilization of CA's which are still apparent 24 hr following treatment. Additional biochemical studies will be necessary to determine the nature of these effects.

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