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Long-Term Suppression of the Cerebral Spread of a Memory: Effects of Idazoxan and Clonidine

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CHURCH, A. C., L. B. FLEXNER, J. B. FLEXNER AND E. E. REYNOLDS. *Long-term suppression of the cerebral spread of a memory: Effects of idazoxan and clonidine.* PHARMACOL BIOCHEM BEHAV **32**(3) 749–756, 1989.—Bitemporal injections of puromycin consistently induce amnesia of aversive maze-learning in mice when administered within 3 days of training. These bitemporal puromycin injections lose their amnestic effectiveness if the latency between training and injection is extended beyond 6 days. Consistent with other evidence, we believe that memory (in our task) "spreads" during the 6 days following training. Since previous experiments have indicated that the central noradrenergic system is involved in this process of "memory spread," we have examined the effect of stimulation or blockade of the α_2-receptor. To this end, we administered a single dose of the α_2-adrenoceptor antagonist, idazoxan, or the α_2-agonist, clonidine. Idazoxan (1 mg/kg, SC) had no effect on engram spread. Clonidine (25 μg–125 ng/kg, SC), by contrast, suppressed engram spread for at least 30 days after treatment. When mice were tested at 60 and 90 days after treatment, spontaneous recovery (i.e., engram spread) was evident in only about 50% of the clonidine treated mice. Coadministration of idazoxan with clonidine blocked the effects of clonidine on "memory spread."

Memory Clonidine Idazoxan Puromycin α_2-Adrenoceptors

WE have previously noted evidence (13), derived from studies on man (46), monkey (29), cat (44) and rodents (19, 22, 32, 34), that supports the view that an effective memory trace of aversive maze-training in mice spreads over time from the hippocampal-entorhinal area to widespread forebrain areas. Although it is not possible to distinguish between the possibility that memory is initially dependent on the hippocampal-entorhinal area rather than being actually located there, we have chosen the location hypothesis based upon the studies cited above and because we find it to be conceptually simpler. Under the working hypothesis that we have used, this "spread of memory" is revealed by a loss of the amnestic efficacy of bitemporal injections of puromycin (see Rationale subsection under the Method section for more details). In order to produce amnesia following this "spread of memory," a combination of bitemporal plus biventricular plus bifrontal (T+V+F) injections must be used. In our experimental design, the process of "memory spread" (loss of vulnerability to *bitemporal* injections of puromycin) normally occurs within 6 days following training (19). In the interests of clarity, we will henceforth refer to the lack of amnestic actions of bitemporally administered puromycin as providing evidence of "memory spread."

An important role of the central noradrenergic system in this process of putative "memory spread" has emerged. Three consecutive daily (posttraining) injections of one or another dopamine β-hydroxylase inhibitor (FLA-63 or U-14624) was found to suppress "memory spread" for a period of 30 days (12). Similar, but longer lasting effects (60–90 days of suppression) were obtained when a single dose of propranolol was administered one day following training (14). These effects of propranolol were determined to be stereoselectively produced by the levorotatory isomer which most potently blocks β-adrenergic receptors. Further experiments led to the conclusion that propranolol's actions were due largely to the blockade of the subtype of β-receptor known as the β_1-receptor (13). Finally, through the use of BAAM (bromoacetyl-alprenolol-menthane), a nonselective, irreversible β-receptor antagonist that does not cross the blood-brain barrier, we found that blockade of peripheral β-receptors fails to reproduce the propranolol effect; i.e., that "memory spread" is critically but perhaps not solely dependent on its blockade of central nervous system β-receptors (7). Thus, based on the foregoing experiments, it appears clear that certain drugs that interfere with the integrity of central noradrenergic transmission produce a long-lasting suppres-

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sion of the process of "memory spread."

The α_2-adrenoceptor system is an additional component of the central noradrenergic system. This receptor is believed to be located on noradrenergic nerve terminals (2, 27, 45) as well as on postjunctional sites (2, 41, 43). Moreover, it has been noted that activation of α_2-receptors in several membrane systems (including brain) inhibits membrane-bound adenylate cyclase (10). The prevalent, though not unanimous (24,31), view of the physiological role of the α_2-system is that the presynaptic receptor provides feedback inhibition of the synthesis and release of norepinephrine (NE). α_2-Agonists have been shown to decrease NE turnover and release, while α_2-antagonists appear to enhance NE turnover and release (23, 26, 27, 36). In the following experiments we have used the α_2-agonist, clonidine (CLO), and the selective α_2-antagonist, idazoxan (IDA) (9,20), in tests of their effects on "memory spread." In these experiments, two basic questions were posed:

1) Does IDA or CLO modify the basic amnestic properties of puromycin;

2) If these properties are unaffected, then does treatment with IDA or CLO extend the period of vulnerability to bitemporal puromycin and so suggest that they suppress "memory spread?"

METHOD

Rationale of Test for "Memory Spread"

Our test for evidence of "memory spread" is based upon the following observations. Bitemporal injections (19) of puromycin (90 μg/12 μl injection; total—180 μg) that primarily affect the hippocampal-entorhinal area (19) consistently induce amnesia of aversive maze-learning in mice if they are administered within 3 days of maze training. These same bitemporal injections of puromycin become consistently ineffective when administered 6 or more days after maze training. Induction of amnesia at these later times requires a modification to the injection protocol such that six injections (T+V+F; 30 μg/12 μl injection; total—180 μg) are required. These six injections affect widespread neocortical sites in addition to the temporal regions and to a lesser extent impinge upon the corpus striatum and the thalamus (15). It should be noted that the same total dosage of puromycin was used in both the 2 and 6 injection protocols (8). A peptidyl conjugate of puromycin appears to be the agent that causes both amnesia and a difficulty in relearning. As evidenced from subcellular fractionation studies, it appears that the site of action of the peptidyl-puromycin is at synaptic sites in the nervous system (16).

Our findings with intracerebral injections of puromycin are interpreted to indicate that the locus of the effective engram normally spreads from the hippocampal-entorhinal area (bitemporal injection zone) to widespread forebrain areas (six injection zone) within 6 days. Accordingly, we conclude that the effective memory tract is limited to the temporal brain regions when bitemporal injections of puromycin induce amnesia, but that a more widespread effective engram is present when bitemporal injection fail to induce amnesia.

Behavioral Procedures

Male and female Swiss-Webster mice (30–35 g) from our closed colony were housed 4 to a cage at room temperature and were placed in individual cages the day before use (14). They were trained in a single session in a Y-maze (17) to a criterion of 9 out of 10 correct responses. The maze was constructed of wood painted dull gray, and consisted of 3 equally sized arms, 20 cm long, 11 cm wide and 15 cm deep, joined to an equilateral triangularly-shaped center compartment with sides of 11 cm. The floor of the maze was composed of brass rods, 3 mm in dia., spaced 1 cm apart. Each compartment could be separated from the center by a guillotine door. Each of the arms had a hinged lid of clear Plexiglas. Intermittent foot-shock, manually applied (0.2–0.4 mA from a DC source; 2 sec on, 2 sec off), was given for failure to move from the stem of the Y within 5 sec and for errors of left-right discrimination. Shock was adjusted with individual mice to the minimal level (not less than 0.2 mA) that produced the desired behavioral response. After entering the correct arm of the maze and remaining there for 10 sec, the mouse was allowed to climb up a ladder and was returned to its home cage for 30 sec before starting the next trial. The same procedure was used in tests for retention of memory of the training experience without knowledge of the specific treatment given to each mouse (testing). All mice were in excellent condition following treatment with IDA or CLO. Tests for retention of memory were delayed however, until a subject had normal cage and maze behavior, namely about 10–15 days after the intracerebral injections of puromycin. The waiting period ensured that the mice had recovered from the acute toxic effects of the puromycin (lethargy, aphagia, adipsia, excitability on handling, but no convulsions). Thus, the mice ran normally in the maze and 90% of the testing errors were errors of discrimination.

Total errors were the sum of failures to make a choice within 5 sec plus incorrect choices, i.e., all mistakes were added until the mouse performed correctly in 9 out of 10 consecutive trials. The Mann-Whitney U-test was used for statistical comparisons between groups and thus median scores are represented in all graphic illustrations of behavioral results. A Kruskal-Wallis one-way analysis of variance was performed on the training scores of mice treated with CLO and IDA and used in experiments to assess "memory spread." The test confirmed that initial training scores were not associated with subsequent testing scores, $H(8) = 11.06$, $p > 0.10$.

Surgical Procedures

The intracerebral injections were administered to mice that had been lightly anesthetized with Evipal [150 mg/kg, IP; described more fully in (19)]. The scalp was incised and then reflected, and a fine hole was bored by hand using an awl containing a steel needle. Intracerebral injections were made with a 27-gauge needle clamped into the rack and pinion of a stereotaxic apparatus. Bilateral injections (all at a depth of 2 mm from the surface of the skull) were made through holes placed:

(1) just above the angle between the caudal sutures of the parietal bones and the origin of the temporal muscles—referred to as bitemporal injections.;

(2) 2 mm lateral to the sagittal suture and 2 mm rostral to the caudal sutures of the parietal bones—referred to as biventricular injections; and

(3) 4 mm rostral to these last mentioned holes and 1 mm lateral to the sagittal suture—referred to as bifrontal injections.

When only bitemporal injections of puromycin were made, each injection contained 90 μg of puromycin dihydrochloride (ICN Pharmaceuticals) dissolved in 12 μl of distilled water and brought to pH 7 with NaOH. When bitemporal plus biventricular plus bifrontal (T+V+F) injections were made, each injection contained 30 μg per 12 μl of distilled water. It should be noted here that we did not use intracerebral saline controls for puromycin. This has long been common practice among investigators in this field [reviewed in (5)].

IDA (kindly supplied by Reckitt and Colman, Hull, England)

and CLO (Sigma) were dissolved in distilled water and 0.1–0.2 ml was injected SC. All mice survived these treatments in excellent condition.

Receptor Binding Assays

We have used an ex vivo receptor binding assay largely as described (35). In this assay subjects are treated in vivo with a receptor ligand and the degree of receptor occupancy is then assessed in vitro.

Mice were sacrificed by cervical dislocation. The cerebral hemispheres were rapidly removed, frozen in liquid nitrogen, and stored at −70°C. Subsequently, the tissue was homogenized in 8 vol. of ice-cold 50 mM Na-K phosphate buffer (pH 7.4 at 27°C) (42) with 10 strokes of a motor-driven Teflon/glass homogenizer. The crude membranes were then used immediately without washing. Between 3 and 4 mg of the membranes were incubated at 27°C with 1 nM [^{3}H]IDA (Amersham; 40 Ci/mmol) for 30 min in a final volume of 1 ml of buffer. Samples were filtered under reduced pressure with Whatman GF/B filters and then washed 4 times with 4 ml of ice-cold assay buffer. Specific binding was defined as the difference in the amount of [^{3}H]IDA bound in the absence and presence of 2 μM yohimbine (Sigma). All samples were run in duplicate.

EXPERIMENTS

In all experiments, mice received a single SC injection of IDA or CLO. In order to attain a high level of receptor occupancy by IDA, we used a dose of 1 mg/kg. In our first studies of CLO however, we used the relatively low dose of 25 μg/kg, because of evidence that showed that this dose was sufficient to depress cerebral NE synthesis (3). These first studies with CLO were followed later by dose response studies which examined the behavioral response to increasingly lower doses of this potent agonist.

Experiment 1

This experiment included several behavioral and puromycin controls. Specifically, tests were made to determine:

(a) whether IDA or CLO affects maze learning or relearning;

(b) if, after treatment with these drugs, bitemporal injections of puromycin induce amnesia within 3 days after learning as they do in untreated mice;

(c) most importantly, if either IDA or CLO modifies the sensitivity of the brain to puromycin such that bitemporal injections of puromycin now induce amnesia when given 6 or more days after learning;

(d) if IDA or CLO alters the sensitivity of the brain to puromycin such that 6 intracerebral injections of puromycin (T+V+F) fail to produce amnesia 6 or more days after learning.

(a) Reversal experiments were only used in this experiment in order to test the effects of the two drugs on learning and relearning. As shown in Fig. 1, the mice were treated with either IDA or CLO one day after being trained to run to one arm of the Y-maze. One day after drug treatment, they were reverse trained to run to the opposite arm of the maze. After an additional 7-day period, the mice were tested for memory of the reversal training. Similar to both the results obtained presently from saline controls (train, 9.5 errors; reverse train, 7.5 errors; test, 0 errors; relearning savings, 100%) as well as those noted in past experiments with untreated mice (19), mice treated with IDA or CLO made fewer errors during reversal training than during initial training and had high reversal relearning savings (IDA, 83.3%; CLO, 100%).

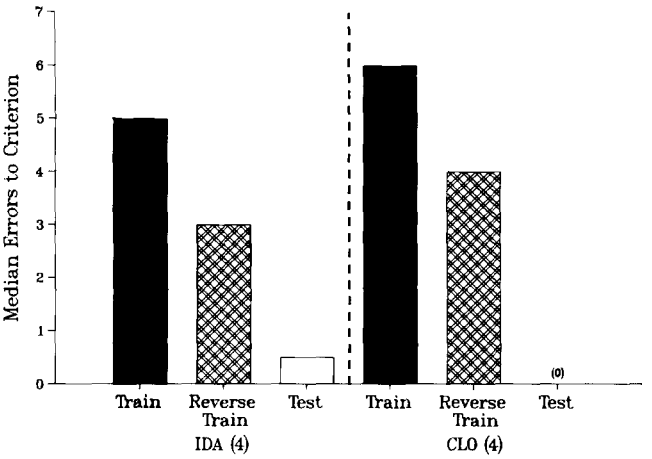

FIG. 1. Effect of idazoxan (IDA) or clonidine (CLO) on learning (reverse training) and subsequent recall (test). IDA (1.0 mg/kg) or CLO (25 μg/kg) were administered SC 1 day following training, and mice were then reverse trained 1 day later. Memory of the reverse training was tested 7 days later.

Conclusion

The results show that neither drug affected learning or memory as measured by reversal training in the Y-maze.

(b) The results of tests to determine whether IDA or CLO interfere with the amnestic actions of puromycin injected bitemporally within 3 days after learning, are presented in Table 1. Groups 1a and 1b, respectively, were injected with IDA or CLO on the day after maze training, and then given puromycin 1 day after the α_2-drugs. In both groups puromycin induced profound amnesia of the maze training.

(c) The results of tests to assess whether IDA or CLO treatment

TABLE 1

LACK OF INTERACTION BETWEEN IDAZOXAN (1 mg/kg) OR CLONIDINE (25 μg/kg) ON THE EFFECTS OF PUROMYCIN

Drug Treatment Timing	Training Errors	Testing Errors	Memory Spread
1. Prior to Engram Spread			
a. Train —1—> IDA —1—> Puro (4)	6.5	11.5	–
b. Train —1—> CLO —1—> Puro (4)	5.5	10.5	–
2. Following Engram Spread			
a. Train —9—> IDA —10—> Puro (4)	6.5	1.5	+
b. Train —9—> CLO —10—> Puro (7)	5.5	0.0	+
c. Train —9—> IDA —10—> Puro (4) T+V+F	6.0	9.5	NA
d. Train —9—> CLO —10—> Puro (4) T+V+F	7.0	10.0	NA

Bitemporal injections of puromycin were administered except as indicated. NA = not applicable.

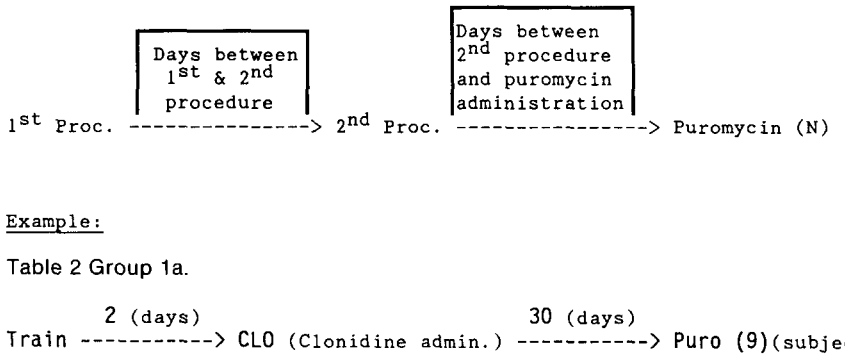

FIG. 2. Guide for interpretation of tables of behavioral results.

enhance the actions of puromycin administered 6 or more days after training are presented in part 2 of Table 1. Groups 2a and 2b were injected with IDA or CLO respectively, 9 days after maze training to allow ample time for the process of "memory spread." Ten days following the drug treatment, bitemporal injections of puromycin were administered. This interval between drug treatment and puromycin equalled the delay to be used in the behavioral tests described below. Consistent with past results from untreated mice (19,32), puromycin failed to induce amnesia.

Conclusions

We conclude from the results of (b) and (c) that neither IDA nor CLO interfered with or enhanced the amnesic properties of bitemporally administered puromycin.

(d) Studies were conducted to determine whether IDA or CLO modify the sensitivity of the brain to puromycin, to the extent that T+V+F injections of puromycin fail to induce amnesia. The results of these tests are presented in Table 1 (see Fig. 2 for guide to tables). As in (c) above, treatment with IDA or CLO was delayed until 9 days after training to allow ample time for "memory spread." Puromycin (T+V+F) followed 10 days after the drug treatment. Both the IDA and CLO groups developed profound amnesia in contrast to their insensitivity to bitemporal injections of puromycin.

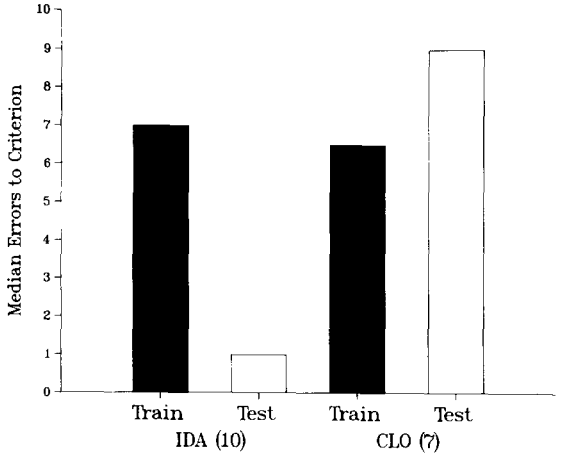

FIG. 3. Effect of idazoxan (IDA) or clonidine (CLO) on "memory spread." Mice were trained, injected SC with IDA (1.0 mg/kg) or CLO (25 μg/kg) 1 day later, and then injected bitemporally with puromycin 10 days after drug treatment. Memory was tested ~12 days after puromycin.

Conclusion

We conclude that neither IDA or CLO affected either learning or memory (testing) of the Y-maze, nor did either drug modify the amnestic effects of puromycin. Consistent with its normal effect, puromycin bitemporally injected two days after training (1 day after IDA or CLO) induced profound amnesia. When drug treatment was delayed until 9 days after learning to allow time for development of "widespread memory trace," bitemporal puromycin, as in normal, failed to induce amnesia, while T+V+F injections induced profound amnesia.

Experiment 2

We next tested the effect of IDA (1 mg/kg) or CLO (25 μg/kg) on "memory spread." The schedule of training, drug treatment and testing was identical to that used with the β-adrenoceptor antagonists (13); i.e., the drugs were given 2 days after training and then, to allow ample time for the normal process of "memory spread," puromycin was injected 10 days later.

As shown in Fig. 3, those mice treated with IDA made very few errors during testing and thus had a high level of savings (85.7%). In contrast, the mice treated with CLO showed no memory of the training and had negative savings (−38.5%).

Conclusions

From these findings it is evident that the two α_2-agents produced very different effects on test performance with highly significant ($p<0.001$) group differences in test scores. Thus, in the dosages used here, we conclude that IDA had no effect on "memory spread," whereas CLO potently suppressed it.

Experiment 3

The purpose of this experiment was to determine the degree and duration of receptor occupancy produced by 1 mg/kg of IDA and by several of the low doses of CLO found to suppress "memory spread." Consistent results were limited to assays with IDA. Our highly inconsistent results with CLO were likely related to the low dosage with which we were concerned and to the essential manipulations used in the ex vivo method. As shown in Fig. 4, IDA reduced specific binding of 1 nM [^{3}H]IDA in preparations of cerebral hemispheres by about 80% over the first 30 min following treatment. Full recovery to control values of

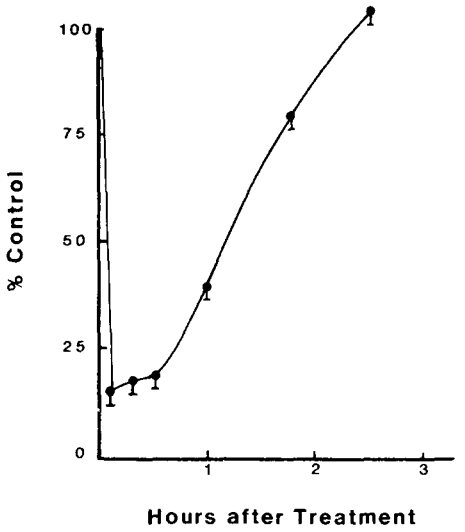

FIG. 4. Effect of a single dose of idazoxan (IDA, 1.0 mg/kg, SC) on specific binding of ^{3}H-IDA (1 nm) in homogenates of cerebral hemispheres. Values derived from means ± SEM of ^{3}H-IDA (pmole/g wet weight of tissue) specifically bound. Control values = 4.90 ± 0.23. For each group, N = 3–4.

[^{3}H]IDA binding sites occurred 2.5 hr after IDA treatment.

Conclusions

These results suggest that the dose of 1 mg/kg of IDA was adequately large to test the effect of α_2-blockade on "memory spread" and that increased dosage was to be avoided because of the possibility of introducing side effects (38).

Experiment 4

In Experiment 2, we found that posttraining administration of CLO (25 μg/kg) prolonged the normal period during which bitemporal injections of puromycin induce amnesia. The following experiment was directed at testing whether we could sufficiently lengthen the interval between post or pretraining treatment with CLO and the subsequent bitemporal injections of puromycin, such that puromycin would fail to induce amnesia. These studies, then, were aimed at determining how long a single exposure to CLO could prolong the normal period of sensitivity to bitemporal puromycin. The results are presented in Table 2.

When CLO (25 μg/kg) was given 2 days after training, bitemporal injections of puromycin were administered after an interval that varied from 30 to 90 days. Group 1a of Table 2 shows that puromycin consistently induced profound amnesia 30 days after treatment with CLO. At 60 days (group 1b), however, the action of puromycin was inconsistent. Six of the 10 mice of this group had high testing savings scores (83.3%) while the remaining 4 mice had negative savings scores (−15.4%) that are characteristic of amnesia. At 90 days (group 1c) treatment with puromycin again led to inconsistent results. Five of the 10 mice of this group had high (100%) and 5 had negative (−17.4%) savings scores. Thus, the 60- and 90-day posttreatment CLO groups did not differ significantly from each other.

Similar results were obtained when treatment with CLO (25 μg/kg) preceded training. In mice trained 30 days after CLO treatment, bitemporally injected puromycin consistently induced amnesia (group 2a). Inconsistent results were obtained when the drug treatment was extended to 60 (group 2b) or to 90 days (group 2c) before training. With these schedules approximately half the mice in each group had profound amnesia (savings ≤ −70%) following treatment with puromycin, while the remainder demonstrated a high level of savings (100%) during testing. The training and testing performance of the 60- and 90-day pretreatment CLO groups did not differ significantly. Likewise, the performance of these pretreatment groups did not differ from that of the corresponding posttreatment CLO groups.

TABLE 2

DURATION OF ACTION OF CLONIDINE (CLO) ON THE INHIBITION OF MEMORY SPREAD

Drug Treatment Timing		Training Errors	Testing Errors	Memory Spread
1. CLO Prior to Engram Spread				
a. Train —2→ CLO —30→	Puro (9)	6.0	9.0	−
b. Train —2→ CLO —60→	Puro (10)	6.0	3.5	±
	Retained Memory (6)	6.0	1.0	+
	Amnesic (4)	6.5	7.5	−
c. Train —2→ CLO —90→	Puro (11)	6.0	8.5	±
	Retained Memory (6)	6.0	0.0	+
	Amnesic (5)	7.0	12.0	−
d. Train —2→ CLO —90→	Test (8)	6.0	1.0	NA
2. CLO Prior to Training				
a. CLO —30→ Train —10→	Puro (4)	5.0	8.0	−
b. CLO —60→ Train —10→	Puro (10)	6.0	8.5	±
	Retained Memory (4)	6.5	0.0	+
	Amnesic (6)	5.0	8.5	−
c. CLO —90→ Train —10→	Puro (11)	6.0	8.5	±
	Retained Memory (5)	6.0	0.0	+
	Amnesic (6)	7.0	15.0	−

NA = not applicable.

Conclusions

We interpret these findings to indicate that a single SC treatment with 25 μg/kg of CLO administered either after or before training suppressed "memory spread" in all mice for at least 30 days. At 60 days following CLO treatment, spontaneous recovery of "memory spread" appeared to have occurred in only about half the mice and there was no evidence of further recovery at 90 days following treatment with CLO. Neither the number of errors to criterion on initial training nor the sex of a mouse was significantly related to the recovery of "memory spread." Our results cannot be attributed to normal forgetting, for as shown in Table 2 (group 1d), mice treated with CLO, but not with puromycin, had consistently high testing scores 92 days after training.

Experiment 5

The purpose of this experiment was to determine if pretreatment with IDA would prevent the CLO-induced suppression of "memory spread." Two days after training, 5 mice were injected with IDA (1 mg/kg) and 10 min later with CLO (25 μg/kg).

Puromycin was injected bitemporally 10 days after these treatments. The median testing savings in these mice was 86.0% and the number of errors to criterion made during training were normal (6.5).

Conclusions

We conclude from these results that pretreatment with IDA completely prevented CLO's suppression of "memory spread," and that consequently, CLO's suppression of "memory spread" is critically but perhaps not exclusively dependent upon its interaction with α_2-adrenoceptors.

Experiment 6

The purpose of this experiment was to determine the effect on "memory spread" of increasingly low doses of CLO which varied in steps of one order of magnitude from 12.5 μg to 1.25 ng/kg. A total of 32 mice received injections of clonidine in this experiment. As shown in Fig. 5, bitemporal puromycin injected 10 days after posttraining treatment with CLO induced profound amnesia at all doses of CLO down to and including 125 ng/kg. Mice treated with 12.5 or 1.25 ng/kg of CLO had, like the saline controls, a high level of relearning savings following bitemporal puromycin. Experiments with 125 ng/kg of CLO were continued to determine the duration of its effect on the abnormal induction of amnesia by bitemporal puromycin (Table 3). When CLO was given 2 days after training and followed 30 days later with bitemporal puromycin all mice were amnesic (group 1a) and the same result, with one exception, was found in the group treated with CLO 30 days before training (group 2a). The proportion of mice that escaped amnesia increased when the interval between treatment with CLO and treatment with puromycin was substantially lengthened (groups 1b,c and 2b,c). In the absence of puromycin those mice treated with CLO 2 days after training had excellent retention of memory 90 days later (group 1d).

Conclusions

We conclude from the dose response data that "memory spread" is vulnerable to a remarkably low dose of CLO. Relearning savings of those groups of mice treated with 25 μg/kg of CLO either before or after training did not differ significantly ($p>0.05$) from the corresponding groups treated with 125 ng/kg of CLO.

DISCUSSION

Our observations in these experiments lead to the following conclusions: 1) Both CLO and IDA, as we have used them, had no effect on learning in the Y-maze or, in the absence of puromycin, on relearning. 2) A single treatment with 25 μg/kg (or as little as 125 ng/kg) of CLO given either before or 2 days after training suppressed "memory spread" in 29 out of 30 mice for at least 30 days. At both 60 and 90 days after treatment, "memory spread" occurred irregularly, varying from 30 to 70% of the subjects within a group. 3) By contrast, administration of IDA 2 days after training, at a dose (1 mg/kg) that appeared to block over 80% of cerebral α_2-receptors, had no effect on "memory spread." 4) Pretreatment with IDA prevented the CLO-induced suppression of "memory spread."

As stated above, "memory spread" appears to be critically if not solely dependent on the central adrenergic system. In other words, the central adrenergic system is important in maintaining this aspect of mnemonic plasticity just as it is in maintaining plasticity in the visual system (25,30). This conclusion was based on our observations that subcutaneous injections of propranolol or betaxolol that blocked both peripheral and central β-receptors

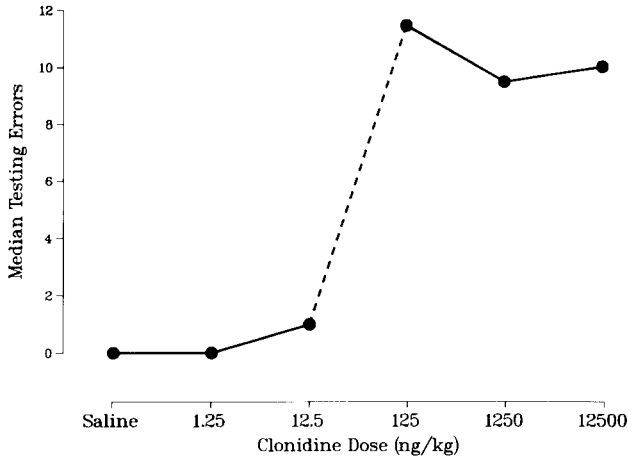

FIG. 5. Dose response curve of clonidine's effect on "memory spread." Mice were trained, injected with clonidine 2 days later, injected bitemporally with puromycin 10 days later, and tested for memory ~12 days after puromycin. Values shown represent 6–8 mice in each group.

suppressed the spread of memory in contrast to the ineffective subcutaneous injections of BAAM which blocked only peripheral β-receptors. Although the actions of CLO are complex, its primary effect is that of a centrally acting α_2-agonist (33). A further indication that the adrenergic system is importantly involved in CLO's suppression of memory spread is the observation that IDA, an α_2-antagonist, blocked the action of CLO on "memory spread." We are left with the possibility among others that changes in both the central and peripheral adrenergic systems

TABLE 3

EFFECT OF A NEAR-THRESHOLD DOSE OF CLONIDINE (CLO) ON THE INHIBITION OF MEMORY SPREAD

Drug Treatment Timing		Training Errors	Testing Errors	Memory Spread
1. 125 ng/kg CLO Following Training				
a. Train —2→ CLO —30→ Puro (6)		5.5	9.5	−
b. Train —2→ CLO —60→ Puro (6)		8.0	11.0	±
	Retained Memory (1)	7.0	2.0	+
	Amnesic (5)	7.5	12.0	−
c. Train —2→ CLO —90→ Puro (6)		6.0	10.5	±
	Retained Memory (2)	6.5	1.5	+
	Amnesic (4)	6.0	10.5	−
d. Train —2→ CLO —90→ Test (4)		6.0	0.5	NA
2. 125 ng/kg CLO Prior to Training				
a. CLO —30→ Train —10→ Puro (5)		7.0	8.0	−
b. CLO —60→ Train —10→ Puro (5)		6.0	7.0	±
	Retained Memory (2)	6.5	1.5	+
	Amnesic (3)	6.0	8.0	−
c. CLO —90→ Train —10→ Puro (7)		7.0	3.0	±
	Retained Memory (4)	7.0	2.0	+
	Amnesic (3)	6.0	9.0	−

Values shown are group medians. NA = not applicable.

may be necessary for suppression of "memory spread." To gain further insight into the respective roles played by central versus peripheral adrenergic systems, the effects of intracerebrally injected ligands should be studied. Unfortunately, certain requirements of our current puromycin injection protocol make such studies impractical. This is because our standard treatment with puromycin frequently fails to induce amnesia for at least 60 days following a procedure in which the skull (and dura mater) is pierced by even a small needle hole. In the presence of previous penetration into the brain, intracerebrally administered puromycin is lost more rapidly from the brain tissue as has been shown with the tritiated compound (11).

As noted above, the α_2-agonist CLO has been found to reduce both the rate of synthesis and the release of NE, and to inhibit NE-stimulated adenylate cyclase in brain. CLO has also been reported to be a highly potent inhibitor of the spontaneous firing of the NE neurons of the locus coeruleus (LC), probably by acting upon α_2-receptors located on or near LC neuronal cell bodies (1). Finally, CLO has been found to inhibit the firing of serotonergic (5-HT) neurons of the raphe nuclei, an effect apparently mediated by α_2-receptors (α_2-heteroreceptors) located on 5-HT nerve endings (28,37). It is uncertain which of these 4 actions of CLO is most important in producing the long-term changes observed in the present experiments. It may be suggested, however, that the LC is likely to be importantly involved since very small doses of CLO have been shown both to inhibit the firing of LC neurons (37) and to elicit behavioral changes (4). Furthermore, it has been demonstrated in mice that a unilateral lesion of the LC, made shortly after learning, dramatically extended the duration of labile memory during which electroconvulsive shock produces retrograde amnesia (47).

Our experimental data are insufficient to resolve several additional questions:

(1) We routinely test for "engram spread" 10 days after a single treatment. If the "memory spread" is found to be suppressed, this means that the single treatment must have produced a basic modification of the "memory spread" system that endures for at least several days. Because we have avoided repetitive treatments in order to minimize side-effects, we do not know whether multiple drug treatments might produce effects quite different from a single treatment. In our experiments, we are limited to the conclusion that the function of NE neurons involved in "memory spread" is far more sensitive to the agonist CLO, than to the antagonist IDA.

(2) It must be emphasized that our hypothesis linking NE to "memory spread" does not exclude a similar role for other neurotransmitters. Interference with other systems may be equally effective, and such findings would lead to the conculsion that persistent neurotransmitter imbalance is the basis of long-term suppression of "memory spread."

(3) As previously stated in the introduction and in a previous paper (14), available evidence does not permit distinction between the possibility that development of the independently effective widespread engram is a) entirely dependent upon temporal region programming of forebrain sites, or b) that a widespread engram is immediately present but that its retrieval is dependent upon temporal region processing.

In this series of studies, we have found that a relatively brief pharmacological stimulus can result in long-term changes in the processing of an aversively conditioned memory. By analogy, a similar pattern of results has been obtained in physiological studies of neural information storage, where a relatively brief period of cellular stimulation results in a long-term change in cellular responsiveness (40). Two such examples, in the mammalian brain, are long-term potentiation (LTP), demonstrated in the hippocampus and other brain regions (39), and long-term depression (LTD), demonstrated in the cerebellum (21). In LTP, an increase in excitability of postsynaptic cells persists for days or weeks (6,39) following brief tetanization of appropriate pathways; in LTD, brief stimulation of suitable pathways leads for an hour to diminution of the Purkinje cell response to vestibular nerve stimulation (21).

Our studies appear to demonstrate that one of the functions of the brain, namely, the process of "memory spread," can be similarly affected by a brief period of pharmacological depression of NE transmission. All of the treatments to date, that have proved effective in our experimental conditions, are treatments that diminish NE transmission, either presynaptically (inhibition of tyrosine hydroxylase; stimulation of α_2-receptors), or postsynaptically (nonspecific inhibition of β-receptors; inhibition of β_1-receptors). It seems reasonable to suppose that identification of the mechanisms by which a brief suppression of NE activity can produce such long-lasting effects will contribute to an understanding of how the brain organizes itself in response to the adaptive demands of the environment. We have previously briefly discussed the possible involvement in this process of persistent conformational alterations of the allosteric proteins of receptors and/or their coupled systems (13).

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