Cerebral protein synthesis inhibition and discrimination training: effects of D-amphetamine

LARRY R. SQUIRE

Veterans Administration Hospital, San Diego 92161 and Department of Psychiatry, University of California School of Medicine, La Jolla, Calif. 92093 (U.S.A.)

(Accepted July 26th, 1979)

Inhibition of cerebral protein synthesis before or shortly after training blocks the formation of long-term memory. This finding is based on studies with 3 classes of inhibitors, several species and a wide variety of behavioral tasks, and has most commonly been interpreted as evidence that brain protein synthesis during or shortly after training is an essential step in the formation of long-term memory.

Recently, this view has been complicated by reports that the amnesic effects of brain protein synthesis inhibition can sometimes be reversed by drugs that affect brain catecholamine metabolism. These findings have been interpreted to mean that CXM interferes with retrieval rather than with the formation of long-term memory, and that the action of CXM might be mediated by catecholamines rather than by inhibition of brain protein synthesis. In the present study we have explored the effect of D-amphetamine on the amnesia produced by protein synthesis inhibition. We found that D-amphetamine reversed amnesia, but this effect was due to facilitation of performance during retention testing, rather than to a specific improvement in retrieval of previously unavailable information. These results are consistent with the view that CXM inhibits protein synthesis required for the formation of long-term memory.

Male, Swiss, albino mice (25–35 g) were obtained from Simonson Laboratories and trained to perform an object discrimination in the automated Deutsch Carousel, as described previously. Mice were trained for 20 trials, which required about 10 min, and tested for retention 24 h later by giving an additional 20 trials in an identical fashion. When mice were trained 30 min after an injection of CXM (n=153, 120 mg/kg, 0.30 ml/25 g of body weight) or saline (n=230), performance significantly improved during the brief 20-trial training session (F=9.5, P < 0.01) Mice given CXM accumulated slightly more trials correctly (mean=8.1) than mice given saline (mean=7.6) (F=5.2, P < 0.02), although this difference amounted to no more than a 5% separation between groups in any single block of 4 trials. In previous studies CXM had no measurable effect on acquisition during brief training.

To evaluate the amnesic effects of training during a 1–2 h period of 90–95% brain protein synthesis inhibition, mice were retested 24 h after training by giving them
Fig. 1. A: Retest scores. Mice were trained for 20 trials and then retested 24 h later by giving them another 20 trials on the same task. The drugs indicated above the bars were given 30 min before training. The drugs indicated below each bar were given 1 h before retest. B: Reversal Scores. Mice were trained for 20 trials and then retested 24 h later by giving them another 20 trials of reversal training. The drugs indicated above the bars were given 30 min before training. The drugs indicated below each bar were given 1 h before retest (SAL, saline; CXM, cycloheximide, 120 mg/kg; D-AMP, d-amphetamine, 1 mg/kg).

an additional 20 trials. Seventy-seven of the mice given CXM and 98 of the mice given saline before training were given d-amphetamine (1.0 mg/kg) 1 h before the retention test. The remaining mice were given saline. Fig. 1A shows the retest scores for all 4 groups. The drug given before training significantly affected 24 h retention (saline vs CXM, $F = 23.0, P < 0.01$). Mice given CXM did exhibit measurable savings at retest, but were markedly amnesic. Additional groups tested 5 days after training indicated that this amnesic effect of CXM was persistent (saline, 5 day mean $= 10.4 \pm 0.3$; CXM, 5 day mean $= 8.9 \pm 0.3$, $P < 0.01$).

The drug given before retest also affected retention (saline vs d-amphetamine, $F=11.1, P < 0.01$). The facilitatory effect of d-amphetamine extended both to mice trained after saline ($F=4.9, P < 0.05$) and also to mice trained after CXM ($F=7.0, P < 0.05$). d-Amphetamine could have facilitated the 24 h retention of normal and amnesic mice by specifically facilitating retrieval. By this view, mice trained after injection of CXM stored some information about the task, but could not achieve a normal level of retention. d-Amphetamine facilitated retrieval and thereby allowed the retention score of CXM-mice to approach that of saline-mice. An alternative possibility is that d-amphetamine facilitated performance during the 20-trial retest, but had no effect on retrieval of previously acquired information. By this view, d-amphetamine increased arousal or improved attention in a way that facilitated relearning. These two possibilities were evaluated in 3 ways.

1. Performance early and late in retention testing. If the facilitatory effect of d-amphetamine were due to improved retrieval, then the benefit of improved retrieval should have been apparent at the beginning of retesting. Alternatively, if d-amphetamine facilitated relearning during the retesting session, then this effect should have become apparent only during the course of the retesting session. To evaluate these two alternatives, scores were compared from the first and last 8 trials of retesting. In
Fig. 2. Prolonged training. Mice were given 69 trials 1 h after injection of saline or D-amphetamine. Amphetamine facilitated acquisition of the two-choice discrimination task ($P < 0.01$), particularly during the middle third of training. Performance began at 30% correct because of a strong preference for the incorrect object.

The first 8 trials, the effect of CXM was highly significant ($F=16.1$, $P < 0.01$; saline, mean=4.4 trials correct vs CXM, mean=3.7 trials correct), but the facilitatory effect of D-amphetamine fell short of significance ($F=3.2$, $0.1 > P > 0.05$; saline, mean=3.9 trials correct vs D-amphetamine, mean=4.2 trials correct). In the last eight trials of retention testing, both the effect of D-amphetamine and the effect of CXM were significant ($P<0.01$). Thus, the amnesic effect of CXM was apparent throughout the 20-trial retest session, but the facilitatory effect of D-amphetamine was not clearly apparent until after the first 8 trials.

2. Reversal testing. A more direct way to evaluate the effect of D-amphetamine is to train mice for 20 trials in the normal way and then to retest them 24 h later by giving them 20 trials of reversal testing in which the previously incorrect object is used as the correct object. The results are shown in Fig. 1B. Control mice had difficulty acquiring the reversal task because of their memory for the competing habit. Since mice given CXM before training were amnesic, and remembered the competing habit more poorly than control mice, this group performed better on reversal testing than control mice (trials correct/20: 11.2 vs 9.9, $F=8.7$, $P < 0.01$).

If D-amphetamine had acted to reverse amnesia by improving retrieval, then memory of the original habit should have improved and interfered with performance during reversal testing. Yet, mice given CXM before training and D-amphetamine before reversal testing performed better than mice given CXM before training and saline before reversal testing (12.2 vs 11.2, $F=4.6$, $P < 0.03$). This finding provides strong evidence that the facilitatory effect of D-amphetamine cannot be attributed to improved retrieval. It seems more reasonable to suppose that the drug's effect is due to facilitation of performance during the 20-trial retesting session.
3. Performance during prolonged training. If the effect of D-amphetamine were due to facilitation of performance during the second 20-trial session, then D-amphetamine should be capable of facilitating initial acquisition of the discrimination task during prolonged training, at least during trials 21–40. Mice were given 69 training trials, beginning 1 h after injection of saline (n = 44) or 1 mg/kg D-amphetamine (n = 42). Acquisition was more rapid after D-amphetamine (P < 0.01), particularly during the middle third of training. Previous work has also demonstrated that D-amphetamine can facilitate acquisition.

A behavioral analysis of the effects of D-amphetamine on CXM-produced amnesia has indicated: (1) the effect of D-amphetamine was not apparent during the early trials of the retention test, but was clearly apparent during the later trials; (2) on a reversal test D-amphetamine improved performance rather than increasing interference from the originally learned habit; and (3) D-amphetamine facilitated acquisition of the discrimination task during prolonged training. Taken together, the results indicated that D-amphetamine produced recovery from amnesia, but this recovery could not be explained as the return of memory for previously acquired information. Recovery from amnesia was due to facilitation of performance during the 20-trial retesting session.

The results presented here differ from the results of a previous study in which D-amphetamine reversed the amnesic effects of CXM. In that study recovery of memory for a food-motivated discrimination task was investigated using a reversal test like the one described here, and the facilitatory effects of D-amphetamine appeared to be due to a specific recovery of memory, not to an acceleration of relearning. One possibly important feature of that study was that the amnesia produced by CXM was transient, so that amphetamine's effect was to accelerate spontaneous recovery of memory. It seems possible that when amnesia is long-lasting or permanent, as with the present task, treatment with D-amphetamine does not reverse the amnesia. D-Amphetamine might temporarily permit a weakly formed memory to be expressed, but recovery of memory should not be long-lasting, unless there has been an opportunity for relearning during the retention test. In other studies involving the passive avoidance task, the MAO inhibitors catron and pargyline seemed to antagonize the amnesic effects of CXM, but D-amphetamine was not effective. It was not clear whether the amnesia associated with CXM was transient or long-lasting.

D-Amphetamine and other stimulants have also been reported to prevent amnesia when given shortly after training. These results have usually been interpreted to mean that D-amphetamine increases the effectiveness of a short-term, protein synthesis-independent memory process which has been proposed to hold information while a protein synthesis-dependent long-term memory process is developing. It should be clear that this phenomenon is consistent with the view that cerebral protein synthesis is required for long-term memory. Cerebral protein synthesis is presumably only one of several essential steps that must occur for memory formation to occur, and it is possible that catecholaminergic mechanisms also have an important role.

In summary, two kinds of effects have been reported by which D-amphetamine
can oppose the amnesic action of CXM on memory. In one case, D-amphetamine given shortly after training prevented the amnesic effects of CXM. In the other case, D-amphetamine given shortly before retest appeared to reverse the amnesic effects of CXM. In the present study, the ability of D-amphetamine to reverse amnesia has been evaluated and found to reflect an acceleration of relearning during retention testing, and not a specific recovery of lost memory. Thus, in the case of long-lasting amnesia produced by giving CXM before training, the available data suggest that memory cannot be recovered later by giving D-amphetamine prior to retest. The evidence therefore supports the conclusion that CXM and other inhibitors of cerebral protein synthesis cause amnesia by blocking the formation of long-term memory.

I thank Stanley St. John and Mary Fox for research assistance.

Supported by the Veterans Administration, by NIMH Mental Health Clinical Research Center Grant 1P50 30914 01 and by the Spencer Foundation.

16 Squire, L. R. and Barondes, S. H., Memory impairment during prolonged training in mice given inhibitors of cerebral protein synthesis, Brain Research, 56 (1973) 215–225.
