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BRIEF COMMUNICATION

Prevention of Memory Loss Following Puromycin Treatment[1]

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HALL, M. E., K. SCHLESINGER AND E. STAMM. *Prevention of memory loss following puromycin treatment.* PHARMAC. BIOCHEM. BEHAV. 4(3) 353–355, 1976. – Female C57BL/6J mice were trained on a one trial passive avoidance response. Twenty-four hours later, they were treated with puromycin in combination with either 2.0 or 10.0 mg/kg of amphetamine, 0.3 mg/kg of strychnine, or 20.0 or 50.0 mg/kg of pentylenetetrazol. Tests one week after training revealed that treatment with these stimulant drugs prevented the memory loss characteristic of puromycin; an exception being those animals injected with the low dose of amphetamine. Biochemical determination of amino acid incorporation into protein revealed that none of the stimulant drugs used significantly altered the extent or the duration of protein synthesis inhibition induced by puromycin. These results are interpreted as showing that the amnesic effects of puromycin can be counteracted by a state of heightened nervous system excitation.

Puromycin Amnesia Arousal Stimulant drugs

IT HAS repeatedly been demonstrated that the protein synthesis inhibiting drugs puromycin, cycloheximide and acetoxycycloheximide can impair memory. This impairment has been taken as evidence that protein synthesis is required for permanent memory formation [1]. Recently, there have been reports that the memory impairment normally associated with protein synthesis inhibition can be prevented by the administration of stimulant drugs. Amphetamine and corticosteroids have been found effective in preventing the memory loss normally resulting from treatment with cycloheximide (CXM) [1] while amphetamine and metaraminol have been shown effective in preventing acetoxycycloheximide (AXM) induced amnesia [9]. Furthermore, the protective effect of amphetamine was not associated with any reduction in the extent of protein synthesis inhibition induced by CXM [1].

One question arising from this research is whether these stimulant drugs prevent memory loss by antagonizing the amnesic properties of the protein synthesis inhibiting drug employed, or whether they act by strengthening the memory trace directly. Many stimulant drugs, including amphetamine, strychnine and picrotoxin, have been shown capable of retroactively facilitating memory storage [5,6]. Barondes and Cohen [1] reported that in mice not treated with CXM, treatment with amphetamine did not enhance recall as measured seven days later. It is nonetheless possible that amphetamine did have some facilitative effect and that while this effect was obscured in animals with normal recall, it was quite significant in mice whose recall had been weakened by CXM.

In the present study, we have examined the effects of puromycin, administered in combination with several stimulant drugs, on the recall of a passive avoidance response. Puromycin, unlike CXM or AXM, is reliably effective at inducing amnesia when administered 24 hr after training [2,8]. Consequently, the training experience and the administration of stimulant drugs were separated by 24 hr, greatly reducing the possibility of retroactive facilitation of the memory trace.

METHOD

Animals

Two hundred female C57BL/6J mice were used in this experiment. All mice were 50–70 days of age throughout the experiment. These experimental animals were bred in our laboratory from breeding stocks obtained from the Jackson Laboratory, Bar Harbor, Maine. All animals were

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maintained under standard laboratory conditions (temperature 74° F ± 3°) with ad lib access to Purina Mouse Breeder Chow and tap water.

Training Apparatus

The apparatus was a two compartment passive avoidance box. The small compartment, which served as the start box, was 20 cm long, 18 cm high and 2.5 cm wide. The large compartment, which served as a shock box, was 15 cm square and 18 cm high. The walls of both boxes were of translucent white Plexiglas, and the floor of both boxes was made of stainless steel rods, 1.5 mm in dia., spaced 6 mm apart. The two compartments were separated by a guillotine door, and the shock box was covered with a removable clear Plexiglas top.

Training Procedure

Training consisted of placing each animal in the start box facing the open guillotine door. Following entry into the shock chamber, the guillotine door was lowered into place, preventing re-entry into the start box. Within 3 sec of entering the shock box, a scrambled 1.6 mA footshock of two seconds duration was delivered through the grid floor. The animal was then promptly returned to its home cage. Training scores for each mouse consisted of the latency, in seconds, measured between the time the mouse was placed in the start box and the moment when the mouse placed all four paws inside the shock chamber. Retention of the avoidance response was tested by returning the mouse to the start box one week after training, and recording the latency to enter the shock box. Mice failing to enter within 3 min were removed and given a score of 180 sec. Memory scores were calculated by subtracting the mean training score from the mean test score for each group.

Drug Regimen

Puromycin dihydrochloride, neutralized to pH7 by the addition of 1N NaOH, was injected bilaterally at a depth of 2 mm, at sites 2 mm anterior to bregma and 2 mm lateral to the midsaggital suture. All injections were done under light ether anesthesia 24 hr after training. The dose was 100 μg per site in a volume of 10 μl/site, administered at a rate of 10 μl/min. Control animals received similar bitemporal injections of saline. In addition, all animals were given intraperitoneal (IP) injections of either saline, 2.0 mg/kg of d-amphetamine, 10 mg/kg of d-amphetamine, 0.3 mg/kg of strychnine, 20 mg/kg of pentylenetetrazol or 50 mg/kg of pentylenetetrazol. All IP injections immediately followed puromycin or saline treatment and were given while the animal was still anesthetized. All drugs given IP were dissolved in saline and injected in a volume of 20 mg/kg of body weight.

Biochemical Determination

In order to determine the extent and duration of protein synthesis inhibition, additional mice were treated in the following ways: (a) injected both intracranially and intraperitoneally with saline; (b) injected intracranially with puromycin and intraperitoneally with saline; (c) injected intracranially with puromycin and intraperitoneally with either amphetamine (10 mg/kg), (d) strychnine (0.3 mg/kg) or (e) pentylenetetrazol (50 mg/kg).

Mice subjected to the above treatments were decapitated either 2 hr (5 mice/group) or 20 hr (3 mice/group) after treatment. In all cases, decapitation was preceded 30 min earlier by an intraperitoneal injection of 0.2 ml of radioactive lysine (l-lysine-4, 5-H^3, New England Nuclear, specific activity 30 c/mM diluted to 3c/mM and injected as 100 μc/ml).

Following decapitation, all brains were quickly removed, weighed, and homogenized. After complete homogenization, aliquots were taken from each sample and the protein precipatated with cold trichloracetic acid. The acid insoluble protein was collected following the procedure of Kennel [4] and measured using a liquid scintillation counter.

RESULTS

Table 1 shows the net mean latencies and standard error of the mean for the 13 groups of animals. An overall Kruskal-Wallis analysis of variance by ranks [3] revealed a significant difference among group means ($H = 84$, $df = 12$, $p<0.001$). A multiple post hoc comparison of means (Duncan multiple range test with $p<0.05$) revealed the following effects. (1) Puromycin was highly effective in inducing amnesia, as puromycin-plus-saline treated mice (Group 1A) did not differ significantly from mice receiving no footshock at all (Group 7B). (2) These animals differed significantly from mice receiving footshock but no puromycin (Group 1B).

Of the 6 groups treated with puromycin plus a stimulant drug, only Group 2A, the puromycin plus 2.0 mg/kg of amphetamine, differed significantly from the saline-plus-saline control group (Group 1B), while on the other hand, only Group 2A failed to differ significantly from the puromycin-plus-saline control group (Group 1A).

Finally, when comparisons are made between groups identical in terms of intraperitoneal drug treatment but differing in bitemporal drug treatment (e.g., Group 2A vs. Group 2B), only in the case of the two groups receiving 2.0 mg/kg of amphetamine is there a significant difference between such groups.

Results of the biochemical determinations for all puromycin-treated mice were expressed as the percent of protein synthesis relative to the nonpuromycin treated control mice. Protein synthesis was significantly impaired in all puromycin treated mice, while the extent of inhibition was the same for all puromycin treated mice, regardless of whether they were injected with saline or one of the three stimulant drugs. An overall analysis of variance revealed that the various stimulant drug treatments did not produce any significant differences in the extent of protein synthesis inhibition either 2 hr ($F = 1.19$) or 20 ($F = 0.19$) after puromycin treatment.

DISCUSSION

With the exception of the lower dose of amphetamine, all stimulant drug treatments resulted in an apparent prevention of the memory loss characteristic of puromycin treatment. It is considered highly unlikely that the long latencies seen in these groups were due to any direct facilitation of memory superimposed over a puromycin-induced memory deficit. Studies of retrograde facilitation of memory by stimulant drugs generally reveal a gradient of effectiveness much shorter than 24 hr [5]. Even if such long term facilitation had occurred, one would expect mice

TABLE 1

EFFECTS OF STIMULANT DRUGS ON PUROMYCIN-INDUCED AMNESIA

Intraperitoneal injections (mg/kg)	Net Mean Latency (± standard error of the mean) Group A puromycin injected intracranially	Group B saline injected intracranially
1. Saline	20.87 ± 7.58†‡	145.0 ± 11.6*
2. Amphet. (2.0)	71.0 ± 15.5†‡	152.0 ± 4.78*
3. Amphet. (10.0)	109.0 ± 18.3*	121.0 ± 21.0*
4. Strych. (0.3)	92.9 ± 26.8*	92.4 ± 20.4*
5. Pentylenetetrazol (20)	112.9 ± 16.3*	90.1 ± 18.5*
6. Pentylenetetrazol (50)	141.0 ± 12.5*	128.0 ± 16.4*
7. Saline (No Footshock)	—	4.2 ± 2.19

*Differ from group 1A by $p<0.05$.
†Differ from group 1B by $p<0.05$.
‡Groups A and B differ by $p<0.05$.

treated with saline plus a given stimulant to have longer latencies than mice treated with puromycin plus the same stimulant; the difference reflecting the degree of impairment due to puromycin. No such differences were seen except in the case of the 2.0 mg/kg dose of amphetamine. Furthermore, entertaining the possibility that retroactive facilitation does occur, but is only detectable in conjunction with a weak memory trace, additional mice were trained on the passive avoidance task with a weaker (0.16 mA) footshock, to produce mice with a weak avoidance response. Such training resulted in a net mean latency of 40 sec (n = 10), significantly shorter than that of mice trained using a 1.6 mA footshock. Amphetamine or strychnine administered to these mice 24 hr after training did not result in longer test latencies than those seen in mice similarly trained but treated with saline. Therefore it seems reasonable to conclude that, in this experiment, the stimulant drugs used prevented puromycin-induced amnesia by virtue of some interaction with the puromycin treatment, rather than by strengthening the memory trace directly.

From the data on the degree and duration of protein synthesis inhibition induced by puromycin in conjunction with the stimulant drugs, it seems clear that the stimulants did not prevent amnesia by decreasing the extent or duration of inhibition. Therefore, the source of the ameliorative effects of these stimulant drugs must be sought elsewhere.

The finding that puromycin-induced amnesia can be prevented by a wide range of stimulant drugs suggests that the ameliorative factor may be, as originally suggested by Barondes and Cohen [1], a heightened state of arousal. This suggestion must be tempered, however, by the realization that these stimulant drugs were administered to etherized mice just following puromycin treatment. Possible drug interactions may have altered the normal biochemical effects of these stimulants. Still, the similarity of results using three stimulants with such different modes of action supports the suggestion that a general increase in nervous system excitation can prevent puromycin-induced amnesia. Consistent with this is our recent finding (Hall, unpublished observation, 1974) that puromycin-induced amnesia can also apparently be blocked by stressing mice with cold or immobilization, or by amphetamine treatment, administered 24 hrs after puromycin treatment.

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