Acetoxycycloheximide-Induced Transient Amnesia: Protective Effects of Adrenergic Stimulants

(memory/rats/metaraminol/amphetamine/norepinephrine)

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ABSTRACT It has previously been shown that rats trained 5 hr after intracerebral injection of acetoxycycloheximide show a transient amnesia at 24 hr after training. We have tested the possibility that adrenergic stimulants might provide protection from the amnesia. Metaraminol, given either before or after training or before testing, prevents the drug-induced amnesia. D-Amphetamine, injected soon after training, also prevents the amnesia. A model is presented to explain the drug-induced amnesia in terms of both the assumed effect of acetoxycycloheximide on the adrenergic system and prevention of the amnesia by metaraminol and D-amphetamine.

The protein synthesis inhibitors, puromycin and acetoxycycloheximide (AXM), have been used in a number of experiments designed to determine whether protein synthesis is necessary for the formation of a long-term memory (1, 2). Although puromycin does indeed act to produce a reliable and long-lasting amnesia, it has become clear that the amnesia can be attributed to factors other than the temporary cessation of protein synthesis. Thus, a mixture of puromycin and AXM, which produces a more profound inhibition of protein synthesis than puromycin alone, has no amnesic effect (3).

Furthermore, the puromycin-induced amnesia can be reversed by various treatments, including the injection of drugs acting on the adrenergic system. It appears that the puromycin-induced amnesia may be the result of puromycin-peptides that block norepinephrine receptor sites at synapses (4). This hypothesis is in accord with the apparent need for adequate concentrations of norepinephrine both at the time of testing (5) and at the time of consolidation immediately after training (6).

Various amnesic effects of AXM (or cycloheximide) have been reported. Barondes and Cohen have found a long-lasting amnesia that is induced in minimally trained mice when AXM or cycloheximide is injected before or immediately after training (7). A transient amnesia has been observed in mice both by Flexner and Flexner (8) and by Quartermain et al. (9). Rats trained 5 hrs after intracerebral injections of AXM develop a transient amnesia at 24 hrs after training (10). Possibly AXM, like puromycin, has effects on the norepinephrine system that are responsible for the amnesic effects of the drug. If this were the case, adrenergic stimulants might provide protection from amnesia. We have found that the temporary amnesia induced in rats by injection of AXM is prevented by an injection of metaraminol given either before or after training or before testing. D-Amphetamine injected soon after training also prevents the AXM-induced amnesia. These results with D-amphetamine are similar to those reported by Barondes and Cohen (11).

MATERIALS AND METHODS Male Buffalo or Fisher rats (Microbiological Ass., 250-300 g) were used. 5 Hr before training (except in one control group injected with saline) they received bitemporal injections of AXM containing 20 μg of AXM in 50 μl of 0.15 M NaCl per site. Stereotaxic injection coordinates were: A = 3.5, L = 3.0, D = 3.0 (12). The injection procedure was performed under halothane (Ayerst) anesthesia administered by mask. AXM was the gift of the John L. Smith Memorial for Cancer Research, Pfizer and Co.

Rats were trained in a shock-avoidance, brightness-discrimination task in a Y-maze to a criterion of six out of seven correct responses, under described conditions (10). 30 Min before training or at various times between training and testing, rats received an intraperitoneal injection of 0.3 mg of metaraminol bitartrate (Invenex), 2.5 mg of D-amphetamine sulfate (Smith, Kline, and French), or 0.15 M NaCl. Volume of all intraperitoneal injections was 0.3 ml. Behavioral testing was done in the same maze at 24 hr or 6 days after training.

The first group of experiments was done to test the reproducibility of the previously described transient amnesia that followed training in the presence of AXM. In the second group, metaraminol was injected 0.5 hr before training to evaluate its effect on the memory of rats that had been injected intracerebrally with saline. The remaining three groups all consisted of AXM-treated rats; in the first, metaraminol was injected 0.5 hr before training; in the second, metaraminol was given for up to 23.5 hr after training; in the final group, D-amphetamine was administered within 2 hr after training.

RESULTS After treatment with metaraminol, rats were slightly lethargic in their cages but performed normally in the maze. There was no significant difference (Students' t-test; P > 0.1) in the number of trails to reach criterion on initial training between these rats and those that were not treated with metaraminol (mean ± SE = 15.8 ± 4.2 and 15.6 ± 2.6, respectively). By contrast, D-amphetamine caused marked behavioral excitation, which lasted 1-2 hr and seriously affected maze performance; its use was limited to a relatively short interval after training.

Abbreviations: AXM, acetoxycycloheximide.
The effects on memory of the control and experimental procedures are given in Table 1, where the results are expressed as \( \% \) savings of discrimination errors to criterion (ETO): \( \frac{\text{ET}_{\text{Train}} - \text{ET}_{\text{Test}}}{\text{ET}_{\text{Train}}} \times 100 \). Rats injected with AXM 5 hr before training and tested either 24 hr or 6 days after training showed, respectively, loss and retention of memory (Groups 1a and 1b). The difference between the scores of these two subgroups is highly significant (\( P < 0.001 \)), in agreement with reported results (10). Rats injected intracerebrally with saline 5 hr before training and with metaraminol 0.5 hr before training had a high level of retention of memory at 24 hr (Group 2) that was not significantly different (\( P > 0.1 \)) from that of an untreated control group studied previously (10).

Group 3 received AXM 5 hr before training. Group 3b, given metaraminol 0.5 hr before training, had significantly greater retention 24 hr after training (\( P > 0.01 \)) than its control injected with saline (Group 3d).

Group 4 (treated with AXM 5 hr before training) received metaraminol at various times between training and testing. The controls for this group, in which saline was injected 0.5 hr before testing, had a low level of retention when tested at 24 hr (Group 4a). By contrast, rats that received metaraminol up to 2 hr after training (Group 4b) had significantly greater retention (\( P < 0.01 \)) as did those treated with metaraminol 0.5–2 hr before testing (\( P < 0.001 \)). Metaraminol was without significant effect on savings when administered 2.5–21.5 hr after training (Group 4c; \( P > 0.1 \)). Not shown in the table are the results for three rats injected with metaraminol from 2–2.5 hr after training. Percent savings (mean ± SE) for this group is 51 ± 6. Also not shown in the table are results for three rats injected with metaraminol from 2 to 2.5 hr before testing.

Percent savings for this group is 46 ± 4. These values are intermediate between the values for retention and amnesia found in the groups shown in the table.

Treatment with d-amphetamine up to 2 hr after training (Group 5) led to significantly greater retention (\( P < 0.001 \)) that found in controls (Group 4a).

**DISCUSSION**

Metaraminol acts as a false transmitter and is a powerful agent for the displacement of norepinephrine and inhibition of its uptake. When administered intraperitoneally, it penetrates the central nervous system to only a limited extent (13), and so might produce the effects that we observed by peripheral action. However, other work (14) has shown that prior treatment with cycloheximide in the rat significantly alters blood–brain barrier permeability to peripherally-administered cocaine, resulting in increased accumulation of cocaine in brain tissue. Reasoning by extension, under conditions of prior treatment with AXM, increased accumulation of metaraminol in the central nervous system may occur as a result of an alteration of blood–brain barrier permeability to metaraminol. D-Amphetamine, which has potent effects on the central adrenergic system, was used in additional experiments. The two drugs injected after training had such similar effects on the AXM-induced amnesia that it appears reasonable to ascribe the results obtained with both to a central action. These observations indicate that agents that increase the availability of norepinephrine at the time of training, or for the first 2 hr during consolidation, or at the time of testing, protect against the transient amnesia. This finding suggests that AXM produces its effect by reducing the amount of available norepinephrine, a considerable departure from an

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**Table 1: Effects of metaraminol and d-amphetamine on AXM-induced amnesia**

<table>
<thead>
<tr>
<th>Group no. (n)</th>
<th>Injection (bitemporal)</th>
<th>Injection (IP) or train</th>
<th>Train or injection (IP)</th>
<th>Test</th>
<th>Mean savings (( % )) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a (5) AXM</td>
<td>5 hr 5 hr</td>
<td>Train hr</td>
<td>Test hr</td>
<td>6.6 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>1b (3) AXM</td>
<td>4.5 hr 4.5 hr</td>
<td>Train days 24 hr</td>
<td>Test 24 hr</td>
<td>20.3 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>2 (4) Saline</td>
<td>4.5 hr 4.5 hr</td>
<td>Train hr</td>
<td>Test hr</td>
<td>87.0 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>3a (5) AXM</td>
<td>4.5 hr 4.5 hr</td>
<td>Train hr</td>
<td>Test hr</td>
<td>22.6 ± 10.5</td>
<td></td>
</tr>
<tr>
<td>3b (6) AXM</td>
<td>4.5 hr 4.5 hr</td>
<td>Train hr</td>
<td>Test hr</td>
<td>79.3 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>4a (5) AXM</td>
<td>5 hr 5 hr 23.5 hr 0.5 hr</td>
<td>Train hr 22-24 hr Saline 5 hr</td>
<td>Test 17.6 ± 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4b (17) AXM</td>
<td>5 hr 5 hr 2.5-21.5 hr 5 hr</td>
<td>Train hr 22-24 hr MA 2.5-21.5 hr</td>
<td>Test 85.8 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4c (11) AXM</td>
<td>5 hr 5 hr 0.5-2 hr 5 hr</td>
<td>Train hr 22-24 hr AMP 22-24 hr</td>
<td>Test 21.6 ± 7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4d (5) AXM</td>
<td>5 hr 5 hr 0.5-2 hr 5 hr</td>
<td>Train hr 22-24 hr AMP 22-24 hr</td>
<td>Test 79.6 ± 5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (5) AXM</td>
<td>5 hr 5 hr</td>
<td>Train hr 22-24 hr AMP 22-24 hr</td>
<td>Test 77.0 ± 15.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers above the arrows refer to time intervals in hours.
AXM = acetoxycycloheximide; MA = metaraminol; AMP = d-amphetamine; (IP) = intraperitoneal.
Buffalo rats were used in Groups 1a, 1b, and 2; Fisher rats were used in the remaining groups.
earlier view based on assumed roles in memory of messenger RNA and protein.

The possibility has been considered that motivation or other factors present at the time of training or of retention-testing (and not part of the memory process itself) might account for the results. These less-specific factors seem to be ruled out by the finding that metaraminol and D-amphetamine given up to 2 hr after training protect memory and, additionally, that this protection is lost when the injection of metaraminol is delayed beyond 2 hr after training and up to 2 hr before testing.

The following model seems sufficient to account for our results: (a) norepinephrine released during the time of training and consolidation leads to facilitatory changes in the specific network of synapses that are associated with the training situation; (b) norepinephrine released at the time of testing causes preferential reactivation of this previously facilitated network; (c) injection of AXM leads to a fall in the amount of norepinephrine released and to a correlative fall in the degree of facilitation; (d) at 24 hr, norepinephrine is still not available in sufficient quantities to reactivate the network, resulting in a "failure of expression of memory"; (e) at 6 days, the norepinephrine system has recovered and the memory is expressed.

According to the above model amphetamine and metaraminol could prevent amnesia as follows: (a) When they are given within 2 hr of training, adequate facilitation would occur and the quantity of norepinephrine available at the time of testing would be sufficient to reactivate the fully facilitated network. (b) When they are given within 2 hr of testing, the quantity of norepinephrine would be greater during the test and sufficient to reactivate even the partly facilitated network. (c) When they are given between these critical periods, the transient elevation of the available norepinephrine concentration would have no effect.

This model requires an undefined process referred to as "facilitation," which occurs during the training and consolidation period.

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