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Protein Synthesis and Amnesia: Studies with Emetine and Pactamycin

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DUNN, A. J., H. E. GRAY AND P. M. IUVONE. *Protein synthesis inhibitors and amnesia: studies with emetine and pactamycin.* PHARMAC. BIOCHEM. BEHAV. 6(1) 1–4, 1977. – Two antibiotic inhibitors of protein synthesis, emetine and pactamycin, have been tested for their effects on cerebral and peripheral protein synthesis and amnesia. Peripherally administered emetine but not pactamycin inhibited cerebral protein synthesis, although this inhibition was lower than that observed with cycloheximide or anisomycin. Pactamycin had a lesser effect on adrenal protein synthesis than emetine. This was reflected in the ability of emetine but not pactamycin to block ACTH-induced corticosteroidogenesis. Anisomycin and cycloheximide caused amnesia in a passive avoidance task, whereas pactamycin and emetine did not. These results are inconsistent with the amnesia being due to inhibition of protein synthesis in a peripheral organ. They are also inconsistent with the amnesia being due to the suppression of an adrenocortical response as previously suggested. No obvious correlation between amnesia and the mechanism of protein synthesis was observed. The most parsimonious explanation is that inhibition of cerebral protein synthesis is necessary for amnesia.

Amnesia Protein synthesis inhibitors Adrenal cortex Anisomycin Cycloheximide Emetine Pactamycin

A LARGE number of reports have indicated that inhibitors of protein synthesis can be amnestic in a variety of behavioral tasks [1, 2, 3]. In order to produce amnesia the inhibition of cerebral protein synthesis must be very high, generally 80–90 percent or more [2]. At the doses required to produce such inhibitions no drug is likely to be absolutely specific, thus the only way to strengthen the hypothesis that cerebral protein synthesis is essential to the formation of long-term memory, is to test other protein synthesis inhibitors.

To date, most studies have been performed using puromycin, cycloheximide or acetoxycycloheximide. More recently, several studies have used anisomycin, a drug similar to cycloheximide but having fewer side effects [11, 12, 20]. One preliminary report also used emetine and streptovitacin A [4]. We have studied the effects of emetine and of another drug, pactamycin, on both the production of amnesia and on the inhibition of protein synthesis in the brain and other tissues. Both drugs have previously been shown to inhibit protein synthesis in rat liver [7, 16, 18].

A problem with many protein synthesis inhibitors is their inability to inhibit brain protein synthesis following peripheral administration, presumably because they do not pass the blood-brain barrier. Unfortunately, direct intracerebral administration of the drugs is undesirable since intracranial injections can themselves be amnestic [6]. However, the use of inhibitors that do not penetrate the brain, of which pactamycin is an example, has permitted us to examine whether the amnestic action of protein synthesis inhibitors is on the brain or on some peripheral tissue. In particular, Nakajima [17] has argued that the amnestic action of cycloheximide is adrenocortically mediated, since the amnesia can be reversed by corticosterone administration. Thus we have measured adrenal protein synthesis and the ability of the adrenal cortex to respond to ACTH following administration of the protein synthesis inhibitors.

The results of these studies suggest that inhibition of cerebral protein synthesis is necessary for amnesia and that adrenocortical effects are not responsible for the amnesia.

METHOD

Male ICR mice were obtained from Flow Laboratories, Dublin, Va. Emetine and cycloheximide were obtained from Sigma Chemical Co., St. Louis, Missouri; anisomycin was a gift from Mr. N. Belcher, Pfizer, Inc., Groton, Connecticut; pactamycin was a gift from Dr. G. Whitfield, Upjohn Co., Kalamazoo, Michigan; and synthetic ACTH 1-24 was a gift from Organon Inc., New Jersey. L-[4,5-^{3}H] Lysine was obtained from Amersham-Searle, Inc., Arlington Heights, Illinois. All drugs were injected subcutaneously at the back of the neck in physiological saline at the stated doses and times.

Protein synthesis was assayed by the incorporation of [^{3}H] lysine into protein following a subcutaneous injection

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of 30 μCi. Mice were sacrificed 30 min later and the brains, and where stated the adrenal glands and a sample of the liver, rapidly excised and weighed. After rinsing in cold saline the brain and liver were rapidly homogenized in 5 ml of 0.05 M borate buffer. The radioactivity in free and protein-bound lysine was assayed as previously described [19]. The adrenal glands were cleansed of adhering fat tissue, rinsed and homogenized in 1 ml of borate buffer, and transferred to a centrifuge tube. The homogenizer pestle and tube were washed with a further 1 ml of buffer, and the washings were combined with the homogenate. Proteins were precipitated by the addition of 0.2 ml of 50% trichloroacetic acid (TCA) and the precipitates collected by filtration on Whatman glass-fiber filters (GF/C). The filter was washed with 5% TCA and the radioactivity in the residue and filtrate determined [8]. Results on protein synthesis are expressed as the relative radioactivity (RR = protein radioactivity/free lysine radioactivity), which corrects for alterations of amino acid uptake (see Ref. [19]).

To examine the response to ACTH, mice were injected with 1 unit (10 μg) of ACTH 1-24 30 min after the protein synthesis inhibitor or saline. Mice were sacrificed by decapitation 15 min after the injection of ACTH and blood was collected in a heparinized tube. Plasma corticosterone was assayed fluorometrically [14].

The amnestic activity of the drugs was tested in a step-through task as previously described [5,15]. To initiate training or testing the mice were placed on a small brightly lit platform connected with a dark enclosed box. When the mice entered the box in the training trial, a footshock of 0.3mA was automatically activated and remained on until the animal retreated from the box. The latency to step-through (STL) into the box was recorded on both training and testing. Mice that did not step through into the dark compartment within 100 sec upon training were discarded from the study. Testing occurred 24 hr after training and animals were allowed to remain on the platform for 300 seconds longer than their initial step-through latency or until they stepped through, whichever occurred first. Retention is expressed as the change in STL (testing minus training); a low ΔSTL reflects poor retention and a high ΔSTL good retention. Differences between groups were analyzed using a Mann-Whitney U-test.

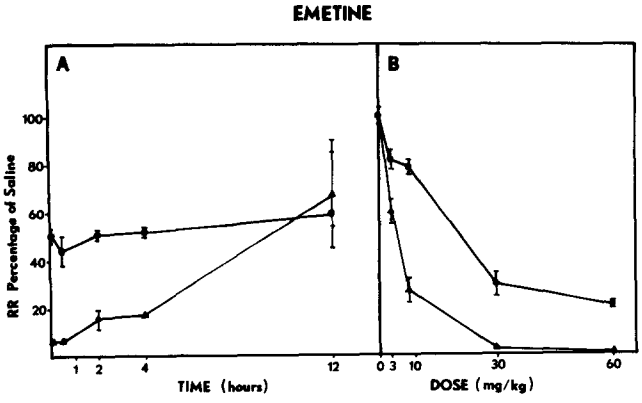

FIG. 1. Emetine inhibition of mouse brain and adrenal protein synthesis. Male ICR mice (4 per group) were injected subcutaneously with emetine or saline and protein synthesis assayed using a 30 min pulse of [^{3}H]lysine. Results are expressed as the mean ± s.e.m. of the relative radioactivity (protein dpm/free lysine dpm) as a percentage of saline-control. A. 30 mg/kg of emetine was injected 2, 30, 60, 240 or 720 min before [^{3}H]lysine. B. 3, 7.5, 30 or 60 mg/kg of emetine was injected 30 min before [^{3}H]lysine. Circles – brain; triangles – adrenal glands. All inhibitions were statistically significant by Student's t-test ($p < 0.05$).

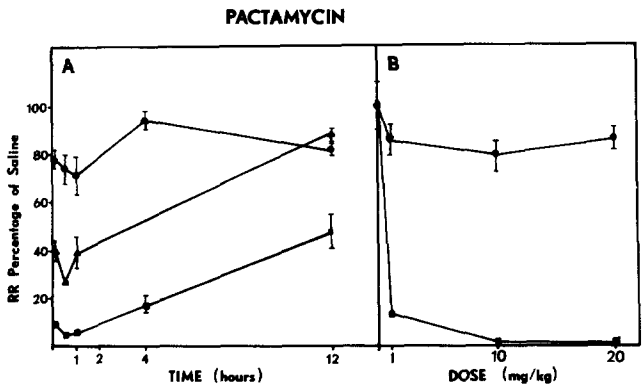

FIG. 2. Pactamycin inhibition of mouse brain, liver and adrenal protein synthesis. Male ICR mice (4 per group) were injected subcutaneously with pactamycin or saline and protein synthesis assayed using a 30 min pulse of [^{3}H]lysine. Results expressed as in Fig. 1. A. 2 mg/kg of pactamycin was infected 2, 30, 60, 240 or 720 min before [^{3}H]lysine. B. 1, 10 or 20 mg/kg of pactamycin was injected 15 min before [^{3}H]lysine. Circles – brain; squares – liver; triangles – adrenal glands. All inhibitions were statistically significant ($p < 0.05$, Student's t-test) except for the brain at 4h and the adrenals at 12h (A), and the brain at 1 mg/kg (B).

RESULTS

Emetine inhibited cerebral and adrenal protein synthesis in a dose-dependent manner (Fig. 1B). At a dose of 30 mg/kg the onset of the inhibition was very rapid and remained relatively constant in the brain at about 50% throughout a 12 hr period (Fig. 1A). In the adrenals the inhibition was initially much greater, but steadily declined to about 50% by 12 hr after the drug administration.

In contrast to emetine, pactamycin (Fig. 2) had very little effect on cerebral protein synthesis; however, the inhibitions at the two higher doses (10 and 20 mg/kg) were statistically significant. In the liver the inhibition was much more dramatic and severe at the lowest dose tested (1 mg/kg). At a relatively low dose (2 mg/kg), protein synthesis by the liver recovered slowly throughout the 12 hr period studied. These results in the liver are similar to those observed by others [7]. Cerebral protein synthesis was only slightly depressed, but this depression was statistically significant at all except for the 4 hr time points. Adrenal protein synthesis was only partly impaired, and slow recovery occurred as in the liver (Fig. 2B).

Inhibition of adrenocortical protein synthesis has been shown to block the steroidogenic response to ACTH [13], and the lack of this response has been claimed to be the mechanism of the amnestic effect of cycloheximide [17]. Thus we tested various antibiotics for their ability to block steroidogenesis following ACTH. These results are shown in Table 1. Saline injection alone significantly elevated plasma corticosterone levels presumably due to secretion of endogenous ACTH. Injection of ACTH produced a dramatic increase of corticosterone relative to saline. Injection of cycloheximide or anisomycin prior to the ACTH not only prevented the corticosterone response to ACTH but also decreased plasma corticosterone below quiet-control levels.

TABLE 1

Plasma corticosterone following protein synthesis inhibitors and ACTH

First injection	Second injection	Plasma corticosterone (Mean ± s.e.m.) μg/100 ml
None	None	26.6 ± 2.7
Saline	Saline	38.7 ± 4.4[a]
Saline	ACTH	59.0 ± 5.8[b]
Cycloheximide (120 mg/kg)	ACTH	9.5 ± 0.9[d]
Anisomycin (25 mg/kg)	ACTH	13.2 ± 1.9[d]
Pactamycin (2 mg/kg)	ACTH	55.5 ± 4.2
Emetine (30 mg/kg)	ACTH	36.8 ± 3.8[c]

[a]Significantly different from quiet $p < 0.025$

[b]Significantly different from Saline-Saline $p < 0.001$

[c]Significantly different from Saline-ACTH $p < 0.01$

[d]Significantly different from Saline-ACTH $p < 0.001$

Male ICR mice were either uninjected or injected with saline or drug. 30 min. later they were injected with saline or ACTH (1 unit per mouse). Mice were sacrificed and plasma corticosterone determined 15 min. later.

This suggests that steroidogenesis due to both endogenous and exogenous ACTH was effectively inhibited. Emetine significantly decreased the response to ACTH, although this effect was not as dramatic as that due to anisomycin or cycloheximide. Pactamycin did not significantly decrease plasma corticosterone in response to ACTH treatment. This result is consistent with the low inhibition of adrenal protein synthesis observed with pactamycin as compared to emetine (Figs. 1 and 2).

Finally we tested the effects of the inhibitors on the retention of passive avoidance behavior. Drugs were injected 3 min (pactamycin) or 30 min (emetine) prior to training and mice were tested 24 hr later. The results are shown in Fig. 3 in which the bars represent median values of the change in step-through latency (ΔSTL). All mice exhibiting an initial STL of greater than 100 sec were rejected and of those thus selected there were no significant differences in initial STL between the groups. However, in the emetine-treated group, 14 out of 27 mice failed to reach the initial STL criterion, and for this reason immediate posttrial injection of this drug was also tested.

Both anisomycin (ANI) and cycloheximide (CXM) significantly decreased the ΔSTL, indicating amnesia. Neither pactamycin (PACT) nor emetine (EME) either pre- or post-training (EME*) significantly altered the ΔSTL. Pretraining treatment with emetine did result in appreciable numbers of mice showing low ΔSTL's. Since, as mentioned above, emetine-treated animals frequently did not step through on Day 1, there may be a performance deficit. However, posttraining treatment caused no appreciable amnesia. It is notable that in the cycloheximide group significant numbers of mice showed high retention, and we have observed this in other unpublished experiments. We also recently observed that pentylenetetrazol (Metrazol) produced a bimodal distribution in C57Bl/6J mice, with animals generally showing either very high or very low ΔSTL's [15]. The significance of these results is unclear.

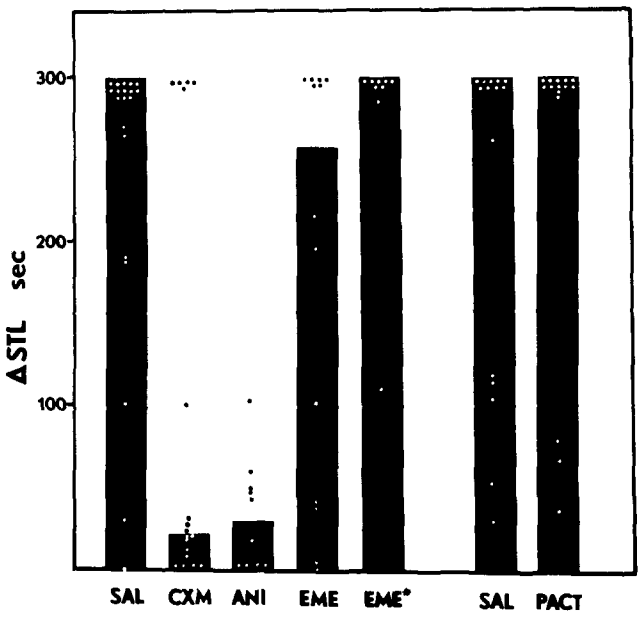

FIG. 3. Retention of passive avoidance behavior following cycloheximide, anisomycin, emetine or pactamycin. Bars are the median increment in step-through latency (ΔSTL) between training and testing. All individual values are presented as points. SAL-saline, CXM-cycloheximide (120 mg/kg), ANI-anisomycin (25 mg/kg) injected subcutaneously 30 min prior to training. EME*-emetine (30 mg/kg) injected immediately post-training. Right hand columns: SAL-saline, PACT-pactamycin (2 mg/kg) injected 3 min before training. Mean initial step-through latencies (sec): SAL, 30; CXM, 20; ANI, 26; EME, 24; EME*, 31; SAL, 26; PACT, 28. None of these initial STL's was significantly different from saline. The left-hand columns are the combined results of experiments performed on five separate days within a one month period.

DISCUSSION

Neither pactamycin nor emetine were particularly effective inhibitors of cerebral protein synthesis. The result with emetine is consistent with that previously found by Bennett *et al.* [4] in rats. In previous studies we have observed much more profound inhibitions of cerebral protein synthesis with cycloheximide (ca. 90% at 30 or 150 mg/kg, Ref. [9]) or anisomycin (92% at 25 mg/kg, Zornetzer, Appleton and Dunn, manuscript submitted). The effects on adrenal protein synthesis of emetine and pactamycin were markedly different, the former drug being much more effective than the latter. These results correlate with their efficacy in suppressing adrenal corticosteroidogenesis, which is unaffected by pactamycin but impaired by emetine. Nevertheless, neither drug suppressed basal corticosterone secretion as judged by plasma corticosterone as effectively as cycloheximide or anisomycin.

The effects on the retention of passive avoidance are fairly clear; whereas cycloheximide and anisomycin were amnestic, pactamycin and emetine were not. At the simplest level this result correlates with the effectiveness of the drugs in inhibiting cerebral protein synthesis. However, in our hands, anisomycin was very effective in eliciting amnesia, whereas cycloheximide was less so, with significant numbers of mice exhibiting good retention. This correlated with the observation that even cycloheximide-treated mice that stepped through on testing often showed fear responses such as defecation and tail-rattling. The

results with emetine could be regarded as superficially similar to those with cycloheximide. This could be related to the 50% inhibition of cerebral protein synthesis by emetine or to the blockade of adrenal steroidogenesis. However, close inspection of the passive avoidance data shows that the distribution of ΔSTL's was quite different for CXM and EME; a very few animals treated with emetine showed complete amnesia, most having intermediate ΔSTL's. Thus emetine is not producing the same behavioral effects as cycloheximide. This is consistent with an earlier report in abstract form [4].

The major conclusion is that peripherally administered pactamycin or emetine produces a relatively low inhibition of cerebral protein synthesis and is not amnestic. However, both drugs inhibit protein synthesis in the periphery, and emetine blocks the adrenocortical response to ACTH. The inhibition of peripheral protein synthesis is comparable to that produced by cycloheximide in mouse tissues [21]. We may conclude that the amnestic effects of cycloheximide and anisomycin are unlikely to be due to inhibition of protein synthesis in any peripheral organ, since otherwise emetine and pactamycin should have been amnestic. Furthermore, the results with emetine suggest that inhibition of adrenocortical steroidogenesis is not amnestic. This is inconsistent with Nakajima's hypothesis that such a block is responsible for the amnesia [17]. It is consistent with our previous results that aminoglutethimide, which blocks adrenal corticosteroidogenesis, dexamethasone, which blocks pituitary ACTH release, and cortexolone, which blocks corticosterone receptors, are not amnestic in a passive avoidance task [10]. It seems most likely then that the amnesia produced by cycloheximide, anisomycin and puromycin is caused by inhibition of cerebral protein synthesis.

There seens to be no obvious relationship between the mechanism of inhibition of protein synthesis and the amnesia. Pactamycin and cycloheximide both inhibit the binding of initiating tRNA's to ribosomes; emetine and cycloheximide inhibit ribosome movement along the mRNA; anisomycin prevents aminoacyl-tRNA binding to the transpeptidase; cycloheximide inhibits peptide bond formation and puromycin causes premature release of synthesized peptides [18]. Thus the amnesia most probably reflects inhibition of protein synthesis, regardless of mechanism.

ACKNOWLEDGEMENTS

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