Pharmacology Biochemistry & Behavior, Vol. 12, pp. 663–665. Printed in the U.S.A.

Two Sensitive Periods for the Amnesic Effect of Anisomycin[1]

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Received 28 December 1979

GRECKSCH, G. AND H. MATTHIES. *Two sensitive periods for the amnesic effect of anisomycin.* PHARMAC. BIOCHEM. BEHAV. 12(5) 663–665, 1980.—Impairment of retention of a brightness discrimination in rats was obtained when anisomycin (80 μg bilaterally into both hippocampi) was injected 10 min before and 80 min after training or 240 and 360 min after training. No amnesia was observed when anisomycin was injected 45 and 165 min post training. The two separate sensitive periods for the amnesic effect of the inhibitor obviously correspond to the two phases of increased protein synthesis during the consolidation of the same learning procedure. The results support the previous findings of the two independent and qualitatively different macromolecular processes. They also argue for the inhibition of protein synthesis as an important mechanism in the amnesic effect of anisomycin.

Amnesia Anisomycin Sensitive periods Protein synthesis

IN recent years many pharmacological and behavioral results have been presented to suggest that formation of memory is dependent on protein synthesis. Several inhibitors of protein synthesis such as puromycin [6], acetoxycycloheximide [2], cycloheximide [1, 3, 5, 7, 16] and anisomycin [4, 8, 17, 18] inhibit formation of long-term memory in various training tasks. Recently we have found an impairment of retention of brightness discrimination in rats by intrahippocampally applied anisomycin [9]. In this experiment anisomycin was injected immediately before and 80 minutes after training. In experiments investigating the macromolecular changes during training and consolidation of the brightness discrimination task it was found that two peaks of enhanced incorporation of ^{3}H-fucose and of ^{3}H-leucine into the proteins of the hippocampus after training do exist [10, 11, 14], the first during training and the second 5–9 hours after training. The two phases of enhanced protein synthesis after training differ qualitatively, the first being mainly characterized by the formation of soluble proteins, whereas the second peak showed an increased synthesis of membrane bound insoluble proteins. We investigated whether the two processes would be equally influenced by an inhibitor of protein synthesis, as well as whether in both cases an amnesic effect would result.

METHOD

All experiments were performed on 114 male rats of our own breeding stock. The animals received chronically implanted microcannulas in each hippocampus (coordinates according to Skinner: AP=3.1 mm, lateral=3.1 mm; vertical=3.1 mm). In each case 1 μl of solution per hippocampus was injected during approximately 30 sec.

Control animals received injections with artificial cerebrospinal fluid (ACF). Anisomycin (ANI) was dissolved in ACF under addition of equimolar HCl solution; the pH value of the solution was adjusted to 7.

Training Procedure

The training task used was a brightness discrimination in a semiautomatic Y-chamber motivated by electrical stimuli [12]. After the application of a starting stimulus (1.0 mA) to the grid floor the rat escaped from the starting compartment of the Y-chamber. In order to avoid the stimulus, the rat must run into the illuminated alley of the chamber. Entering the dark alley of the chamber is punished by footshock. A trial is evaluated as correct only when the animal runs directly into the illuminated alley after application of the starting stimulus.

To avoid position training, the direction of the illuminated alley was changed after every 3 trials. The training was terminated after 31 trials. The mean intertrial interval was 1 min (30–90 sec).

Retention Test

Twenty-four hours after training, retention was tested in a relearning procedure, which was performed in the same manner as the training procedure.

Injections

Hippocampal injections were performed according to Fig. 1B. 80 μg anisomycin/hippocampus were injected in group 2: 10 min before training and 2 hours after the first injection, i.e. approximately 80 min after training; group 4: 45 min and

[1]This work was supported by the Ministry of Science and Technology of the G.D.R.

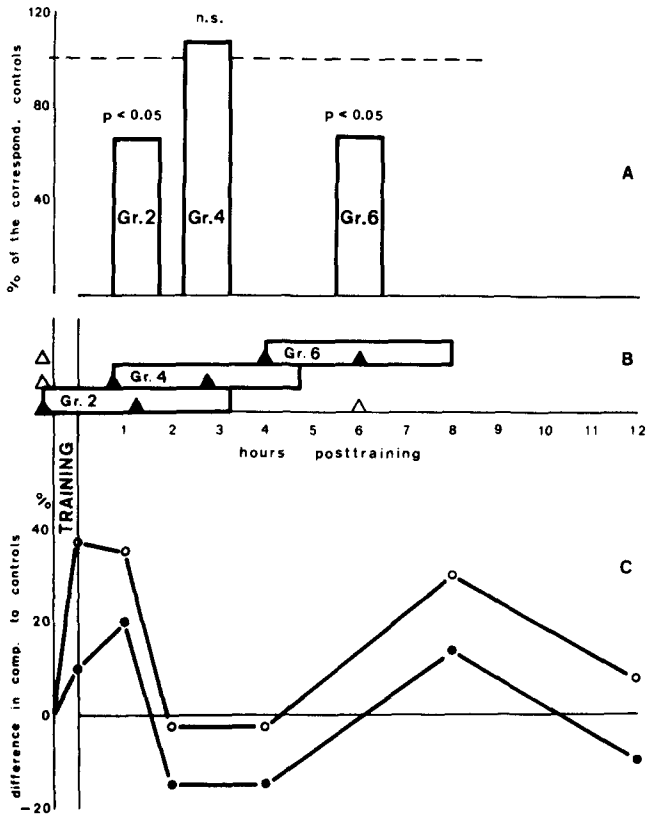

FIG. 1. (A) Effect of anisomycin on the retention of brightness discrimination is dependent on the time of protein synthesis inhibition. Retention of the anisomycin treated groups as percentage of the retention of the corresponding control groups. The retention of the corresponding control group was taken as 100% in each case. (B) Time period in which the protein synthesis inhibition was about 90%. Black triangles=injections of 80 μg anisomycin/hippocampus. White triangles=injections of ACF. (C) Labelling of hippocampal proteins by radioactive leucine during and after training. Ordinate=percentage deviation of the means of trained animals as compared to controls (zero line). Black circles=biochemical determination. White circles=microautoradiographical determination. This figure is from Pohle *et al.* [13]

165 min after training; group 6: 4 hours and 6 hours after training. The control groups 1, 3, and 5 received intrahippocampal injections with 1 μl ACF/hippocampus at the corresponding periods.

In order to handle all rats at the same time in relation to training, groups 3, 4, 5 and 6 received additional injections with ACF 10 min before training and the groups 1 and 2 six hours after training.

Evaluation

In each case the incorrect trials (errors) during training and relearning were ascertained; these values served as a basis to calculate the savings.

Savings percent=([training errors-relearning errors]/training errors)×100

Localization of the Cannulas

After the experiments the rats were killed and the brains fixed with 10% formaldehyde. Then the brains were sliced along the channel produced by the cannula and the position of the tip of the cannula was checked stereomicroscopically. Only rats with correct position of the cannulas in CA 1 of the dorsal hippocampus were considered for the evaluation of data.

Statistics

Statistical evaluation was performed using the Mann-Whitney U-test.

RESULTS

The hippocampal injection of anisomycin before training (group 2) exhibited no influence on the training performance. The effect of two anisomycin injections on retention of brightness discrimination depends on the injection time in relation to training (Fig. 1A). The bilateral intrahippocampal applications of ANI before and 80 min after training (group 2) caused a pronounced impairment of retention as we have found recently (Grecksch *et al.* in press). The injection of ANI four and six hours after training also produced a significant retention impairment (group 6) in comparison with the corresponding controls (group 5). On the other hand the injection of ANI 45 min and 165 min after training showed no effect on retention.

DISCUSSION

The present results support the assumption that intact protein synthesis in the hippocampus during and immediately after the training procedure is a necessary prerequisite for the formation of long-term memory. The impairment of retention by anisomycin injections before and 80 min after training can not be caused by a proactive effect of the protein synthesis inhibitor, since injections 45 and 165 min after training do not affect the retention performance. This is in accordance with results in the literature [8,17], where a retention impairment was no longer found when anisomycin was injected 30 or 105 min after training. On the other hand, anisomycin injected 4 and 6 hours after training again induced a significant impairment of retention. Therefore the effect of protein synthesis inhibition in the hippocampus shows a biphasic time course dependent on the inhibition time in relation to the training. This time course is much the same as the time course of the macromolecular changes observed during consolidation (Fig. 1C). Leucine incorporation in hippocampal proteins was enhanced at two times, first during training and second 5 to 9 hours after training [10,13]. In radiochemical [15] and autoradiographic [14] experiments using ^{3}H-fucose as a precursor for glycoproteins two peaks of incorporation in trained animals were also found. This biphasic pattern of protein synthesis in the hippocampus was seen only in trained animals compared to passive and active controls. Active controls were subjected to an equal number of foot-shocks and performed the same number of runs as the corresponding trained animals, but the illumination and foot-shock release were paired randomly so that the animals could not learn. The two periods of increased protein synthesis were not evident in these active control animals so that they cannot be artifacts of stress, foot-shocks, or arousal of the training procedure.

Since it is possible to impair retention in a brightness discrimination by protein synthesis inhibition 4 to 8 hours after training, at a time period when protein synthesis in trained animals normally is enhanced, we can suggest that

the second peak of protein labelling is likewise a neuronal expression of consolidation processes initiated by the training, as the first phase of increased macromolecular synthesis after the training procedure.

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