AZIDOTHYMIDINE-INDUCED DISTURBANCE OF LONG-TERM MEMORY IN MICE

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The problem of the molecular mechanisms of learning and memory remains unsolved. According to one hypothesis, memory consolidation is dependent on the reverse transcription process in nerve cells [3-5]. If this is true, if synthesis of the DNA formed as a result of reverse transcription during learning is blocked, this ought to prevent long-term memory formation.

This hypothesis was tested in the present investigation, for which the recently discovered property of 3'-azido-3'-dioxythymidine (AZT) of blocking highly effectively DNA synthesis catalyzed by reverse transcriptase, but not affecting DNA synthesis by other types of DNA-polymerases [2], was used. The effect of AZT on passive avoidance learning in mice was studied.

EXPERIMENTAL METHOD

Male CBLW mice weighing 20-24 g, kept under conditions of free access to food and water, were used. The animals were taught passive avoidance in a chamber with an electrode floor, consisting of dark and lit compartments measuring 12 × 12 cm, connected by a "guillotine" door. On the day of training the mice were put in the lit compartment of the chamber and the latent period of their entering the dark compartment was recorded. Two electric shocks, by a current of 1 mA and lasting 2 sec, were applied to the animals in the dark compartment 180 sec after it was placed in the chamber. The second shock was applied when the door between the compartments was open. After escaping the mouse was returned to its cage. Testing consisted of replacing the animal in the lit compartment of the chamber for 180 sec and recording the latent period of its entering the dark compartment, the length of its stay in the dark and lit compartment, and the number of animals not entering the dark compartment. The AZT solution and physiological saline were coded and injected intraperitoneally. The results were subjected to statistical anslysis by the Wilcoxon–Mann–Whitney test [1].

EXPERIMENTAL RESULTS

There were three series of experiments.

In series I the effect of various doses of AZT (2.5, 5, 10, 20, 40, and 80 mg/kg), injected 1 h before training, on formation of passive avoidance skill was investigated, with testing carried out one week after training. The experiments showed that AZT disturbed the skill considerably. The amnesic action of AZT was exhibited when it was given in a dose of 5 mg/kg, and reached a maximum with a dose of 20 mg/kg. A further increase in the dose of the drug did not potentiate the effect. In the subsequent experiments, AZT was therefore given in a dose of 20 mg/kg. Special tests showed that this dose of AZT did not change the latent period of the animals' entering the dark compartment on the day of training, and likewise did not affect the animals' behavior in the open field test or the level of pain sensitivity measured 1 h after injection of the compound.

In the experiments of series II the time of appearance of memory disturbances due to administration of AZT was studied. The compound was injected 1 h before training in a dose of 20 mg/kg. Preservation of the skill was tested 1, 2, 3, and 7 days after training. Passive avoidance behavior tested after 24 h in mice receiving AZT was the same as in the control animals. An effect appeared only on the 2nd day after training, and it reached a maxi-

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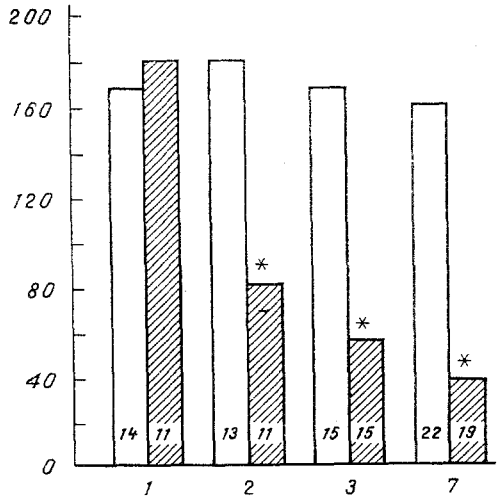

Fig. 1

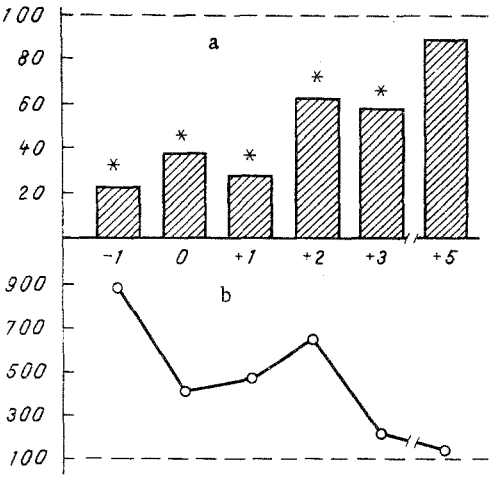

Fig. 2

Fig. 1. Effect of AZT (20 mg/kg) on latent period of entering dark compartment of chamber by mice when tested at different times after training. Abscissa, time of testing (in days); ordinate, latent period of entering dark compartment (in sec). Unshaded columns — control, shaded — AZT. Numbers inside columns indicate number of animals in group. *p < 0.001 compared with control.

Fig. 2. Effect of AZT (20 mg/kg) injected at various times before and after training on passive avoidance behavior tested 1 week after training. a) Latent periods of entering dark compartment by mice (control — 100%); b) number of animals (in percent) in experimental group which entered dark compartment during testing (number of control mice entering dark compartment — 100%). Abscissa, time (in h) of injection of AZT (0 h — time of ending training). *p < 0.001 compared with control.

mum on the 7th day (Fig. 1). The latent periods of entering the dark compartment by the control animals were virtually unchanged at different times of testing.

In the experiments of series III AZT (20 mg/kg) was injected at different times before and after training, and preservation of the skill was tested 7 days later, AZT was found to have an amnesic action when injected 1 h before training, immediately after training, and 2 and 3 h after training; injections 2 and 3 h after training, however, was much less effective (Fig. 2). When AZT was injected 5 h after training it had no amnesic action.

Injection of AZT thus disturbed the formation of long-term memory in mice. Its action differs in its temporal characteristics from that of blockers of RNA and protein synthesis on memory. These are known to give an amnesic effect only if injected during the first 30-60 min after the end of training, and disturbances of long-term memory are already apparent a few hours after training [6, 7].

These differences suggest that in the course of a few hours after the end of training a process essential for long-term memory formation, and differing from RNA and protein synthesis, must take place in the brain cells. The need for its product becomes apparent a few days after training. In our opinion this process may be DNA synthesis in the nerve cells by reverse transcription.

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ANTICONVULSANT PROPERTIES OF THE CEREBROSPINAL FLUID DUE TO ANTIEPILEPTIC SYSTEM ACTIVATION

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Electrical stimulation of structures of the antiepileptic system [1] is known to inhibit epileptic activity (SpA) of separate foci [2] and of epileptic complexes [4] and also generalized EpA [5]. It can be tentatively suggested that endogenous substances formed or secreted intensively in response to activation of the antiepileptic system are involved in the realization of antiepileptic effects arising during electrical stimulation of the structures of that brain system.

The aim of this investigation was to study the effect of the cerebrospinal fluid (CSF) of animals subjected to chronic electrical stimulation of the cerebellar cortex, which plays an important role in suppression of EpA [1, 4] and also of the CSF of animals subjected to electroshock stimulation (seizures) of focal and generalized EpA.

EXPERIMENTAL METHOD

Acute experiments were carried out on cats of both sexes weighing 2.5-3.5 kg and on male Wistar rats weighing 250-300 g.

Donor cats were anesthetized with pentabarbital (40 mg/kg) and stimulating electrodes were implanted into the cortex of the vermis cerebelli (lobes V-VII). Single daily electrical stimulations (ES) began 5-7 days after the operation (100 Hz, 0.5 msec, 5-10 V, duration of stimulation 5 sec; total number of sessions 30-40). Under ether anesthesia, tracheotomy was performed on cats of another group, and stimulating electrodes were implanted in the frontal zones of the cerebral cortex. When 2.5-3 h had elapsed after the end of ether administration electroshock stimulation began (60 Hz, 5.0 mA, duration 2 sec). After 3 to 10 seizures and 0.5-1.5 min after the next stimulation, CSF was withdrawn. Stimulating electrodes were implanted into the cerebellum and cerebral cortex of the animals of the control group, but an electric current was not applied. The CSF of the experimental and control cats was obtained by suboccipital puncture, and treated with gordox (Gedeon Richter, Hungary) in a dose of 1000 U/ml or with bacitracin in a dose of 1 mg/ml to inhibit proteolysis.

Under hexobarbital anesthesia (100 mg/kg) cannulas were inserted into the lateral ventricle of the recipient rats, at coordinates (AP = −0.8; L = 1.2; H = 3.5) taken from the atlas [6]. The animals were used in the experiments 7-10 days after the operation. CSF was injected in a dose of 10 µl by means of a microinjector over a period of 1.5-2 min. Metrazol in a dose of 40 mg/kg was injected intraperitoneally 10 min after injection of the CSF. The animals were kept under observation for 10 min after the injection of metrazol. The seizure response was assessed in points of the following scale: 0) no seizure response; 1 point) paroxysmal twitching; 2 points) clonic spasms of the trunk; 2 points) clonic spasms of the forelimbs, the animals rising on their hind limbs (kangaroo posture); 4 points) marked clonicotonic convulsions with the animal falling on its side; 5 points) repeated

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