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Antiamnesic Properties of the Sesquiterpene Lactone Azerin

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Terpene compounds include the so-called sesquiterpene lactones, isolated from plants. These substances have a broad spectrum of biological and pharmacological activity, including an effect on the brain [1,6,8,10]. In the present investigation the effect of the sesquiterpene lactone azerin was estimated during the conditioning and performance of the passive avoidance response after a short period of memory training.

Azerin (empirical formula $C_{20}H_{24}O_5$) was obtained at the Institute of Botanics of the Azerbaidzhan Academy of Sciences [3] from plants of a species of *Ferula*.

The antiamnesic properties of azerin were compared with those of the nootropic drug nootropyl (piracetam), which is widely used in clinics for the improvement of mnestic-intellectual abilities [5].

MATERIALS AND METHODS

The experiments were carried out on 70 noninbred male rats, weighing 250-300 g, which were trained in the passive avoidance response (PAR) by a method described previously [4]. All the animals were divided into three groups. The first two groups comprised

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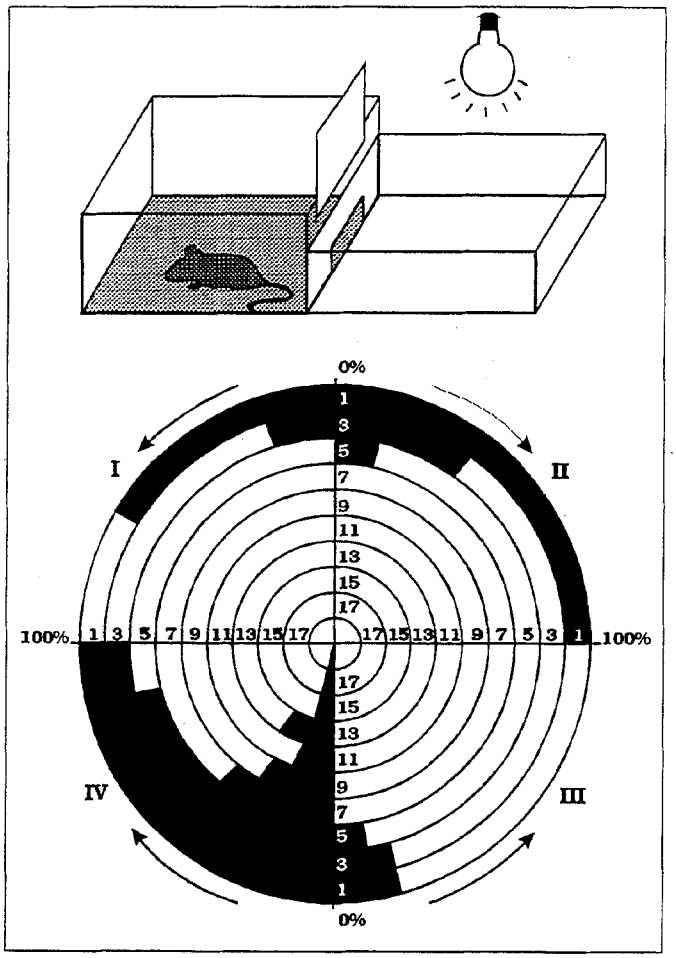

Fig. 1. Percentage of rats staying in the light compartment during PAR test in the control subgroups of animals (I, III) and in the subgroups of animals receiving nootropyl (II) and azerin (IV) before conditioning. Numbers along the radius show the day of PAR test. An angle of 90° means 100% of rats without amnesia. Reading direction is shown by the arrows. Top: schematic view of the conditioned response box used for PAR elaboration.

two control subgroups (10-11 rats) and two experimental subgroups (10-11 rats). The third group comprised a single control subgroup (10 rats) and two experimental subgroups (8-10 rats).

After PAR conditioning, the animals of the first two groups were returned immediately to the "home" cages. The third group of rats was returned to the "home" cages after receiving transcorneally an amnesive electroshock (ES) (20 mA, 500 msec) [5]. PAR was tested 24 h after training and then every 48 h. The test period lasted 900 sec. Memory traces of PAR were judged by the following parameters: the latent period of the first movement from the light into the dark compartment through a small hole in the conditioning box; the total period when the rats stayed in the illuminated compartment irrespective of visits to the dark compartment throughout the period of the PAR test; the percentage of animals staying in the illuminated compartment throughout the 900 sec. The level of activity and preservation of PAR were characterized by the first two parameters, while the third parameter showed the number of rats exhibiting no signs of amnesia.

Nootropyl (Poland) and the sesquiterpene lactone azerin were dissolved in olive oil and introduced per os (1 ml) into the rats in a dose of 100 mg/kg.

The animals received nootropyl and azerin on four consecutive days. The last injection was given on the day of PAR conditioning. The same volume of pure olive oil was introduced into the rats of the control subgroups following the same schedule.

The results were subjected to statistical analysis using the Student and Wilcoxson-Mann-Whitney tests.

TABLE 1. Effect of Nootropyl and Azerin on PAR Performance 24 h after Brief Conditioning ($M \pm m$)

Experimental conditions	Latent Period of entering dark compartment, sec	
	before conditioning	24 h after conditioning
Control I	8.5±2	683±98
Nootropyl	10.1±2	900±00*
Control II	10.6±1	451±94
Azerin	8.9±3	900±00*

Note: * – $p<0.05$ by Student test.

RESULTS

It had been established previously that neither nootropyl nor azerin disturbed the inborn instinct of rats to prefer a dark to a light space before training in an inescapable situation. This was demonstrated by the invariability of the latent period of the first en-

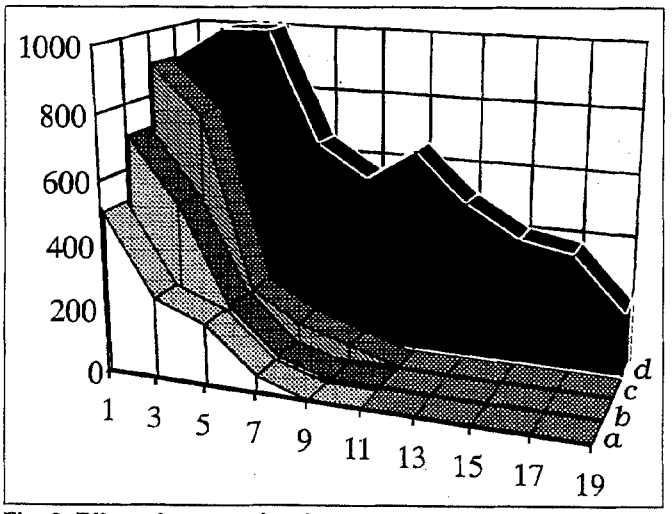

Fig. 2. Effect of nootropyl and azerin on activity and preservation of PAR assessed by the total period when the rats stayed in the light compartment throughout the 900 sec. Abscissa: days of PAR performance; *a, b*) control subgroups; *c*) experimental subgroup receiving nootropyl; *d*) experimental subgroup receiving azerin. Ordinate: total period when rats stayed in the light, sec.

TABLE 2. Antiamnesic Activity of Nootropyl and Azerin Assessed by Latent Period and Percentage of Animals with Amnesia among Rats Receiving an Amnesive ES ($M \pm m$)

Experimental conditions	Days of PAR test									
	day 1		day 3		day 5		day 7		day 9	
	latent period, sec	% of rats with amnesia	latent period, sec	% of rats with amnesia	latent period, sec	% of rats with amnesia	latent period, sec	% of rats with amnesia	latent period, sec	% of rats with amnesia
Control	28±4	100	14±2	100	32±7	100	41±3	100	26±4	100
Nootropyl	495±38	62.5*	174±14	100	116±8	100	57±6	100	32±9	100
Azerin	819±17*	20*	682±30*	50*	375±32*	80	308±33*	80	187±24	100

Note. *) $p<0.05$ by Wilcoxson–Mann–Whitney test

try into the dark compartment in unconditioned rats (Table 1).

During PAR testing 24 h after a short training period the maximum antiamnesic activity of nootropyl and azerin was observed under the given conditions. It was shown (see Table 1 and Figs. 1, 2) that the rats receiving nootropyl and azerin stayed in the light compartment throughout the whole 900 sec. Later on, however, significant differences were revealed in the antiamnesic properties of these two preparations. Nootropyl allowed for PAR preservation for 5-7 days; in the control subgroups total amnesia set in on the 2nd-3rd day, whereas the rats receiving azerin preserved the habit of avoiding the dark compartment during 17-19 days (see Figs. 1, 2).

Since the test substances were administered before conditioning, their influence on the mechanisms of memory and learning could probably be explained by interference with the process of consolidation [9].

For confirmation of this assumption and a study of the antiamnesic properties of the test drugs, the animals of the third group received a transcorneal ES immediately after conditioning [5,7]. This caused a total retrograde amnesia in the control subgroup of rats tested for PAR 24 h later. On the other hand, in rats receiving the drugs an improvement of PAR performance and a reduced percentage of animals with amnesia were noted (Table 2). The results thus show a positive effect of the test preparations on the process of consolidation. The data on nootropyl are in agreement with the results of other investigations [11]. In the present experiments too, however, the mnestic and antiamnesic properties of azerin were significantly superior to those of nootropyl. The differences between azerin and nootropyl activity were observed on the first day of the PAR test, and, especially, later on. On the first day nootropyl induced amnesia in 63% of rats, while azerin induced amnesia in just 20%; just 48 h later, amnesia developed in all rats receiving nootropyl, whereas an incomplete amnesia with an average latent period of 187±24, sec indicating the presence of PAR traces, was observed only on the 9th day in rats receiving azerin (see Table 2).

The ability of azerin, inherent in some of the sesquiterpene lactones, to raise the cAMP level [8] and to cause solubilization of membrane proteins by binding to SH-groups [2], provides a possible explanation for its high antiamnesic activity.

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