RAPID INHIBITION OF EXPLORATORY MOVEMENTS AS A TEST OF NOOTROPIC ACTIVITY

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The study of nootropic drugs is based mainly on evaluation of their effects on formation, consolidation and recall of conditioned reflexes. It follows from the definition of nootropics as drugs selectively activating integrative brain functions that it is also worth while studying other parameters of higher nervous activity in this connection. One widely used form of behavior is habituation, also known as "negative learning," for it consists of extinguishing rather than acquiring behavioral reactions [8, 10]. One manifestation of habituation is weakening of exploratory behavior (EBR) in response to a new situation assessed by an animal as not biologically meaningful. Since it could be considered that nootropics, which facilitate associative processes lying at the basis of learning, may also accelerate the formation of this evaluation, there appeared to be a need to study the effect of standard nootropic and of nootropics of a new type which we have described, namely peptide and topologic analogs of piracetam based on pyroglutamate [2, 5] and proline [3], on extinction of EBR. Of the methods currently used to evaluate nootropics, the most informative is considered to be the conditioned passive avoidance reflex (CPAR) [1, 7]. The aim of the investigation described below was to compare activity of these compounds in the habituation test with data obtained previously relating to their activity in the CPAR test.

It was suggested previously that repeated recording of motor activity at intervals of 1-7 days be used to study forgetting [9]. We used a new approach, namely to study the time course of inhibition of EBR during one recording session. The experiments were conducted on nonimbred male albino mice weighing 18-25 g. A group of 10 animals was kept for 30 min in the chamber of an "Optovarimex" multichannel motor activity recorder (USA). The experiments were carried out between 10 a.m. and 1 p.m., from May through October. The indicator of habituation was the ratio of the total number of runs performed in the last 5 min of recording and the number of movements in the first 5 min, expressed in per cent. The specific activity of piracetam and its analogs was determined. A 0.9% solution of sodium chloride (control group) or one of the test substances was injected 15 or 45 min before recording began. Two controls were set up daily — at the beginning and end of the experiment. Each dose of the test substance was injected into at least four groups of animals. The significance of differences between the control and experimental samples was determined by the Wilcoxon—Mann—Whitney U test, Besides standard nootropics (piracetam, meclofenoxate, pyritinol), we also studied peptide and topologic analogs of piracetam based on pyroglutamic acid: L-pyroglutamine-L-asparaginamide (TGS-34), L-pyroglutamyl-D-asparaginamide (TGS-33), L-pyroglutamyl-glycinamide (TGS-4), D-pyroglutamyl-glycinamide (TGS-4), D-pyroglutamyl-glycinamide (TGS-24), and L-pyroglutamyl-D-alaninamide (TGS-20), and based on proline: N-acetyl-L-prolinamide (TGIS-6), N-acetyl-D-prolinamide (TGS-86), and cyclopropylglycine (TGS-8). Experiments with each nootropic began with a dose effective in the CPAR test, after which the dose was reduced to the threshold level for the habituation test. The preliminary data were published previously [6].

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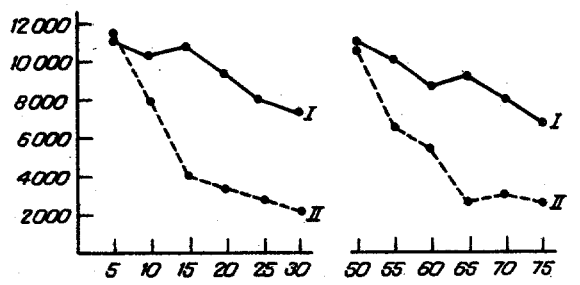

Fig. 1. Effect of piracetam on dynamics of extinction of EBR in mice. Ordinate, number of locomotions in group of 10 mice; abscissa, time after placing in "Optovarimex" recorder (in min). I) 0.9% Sodium chloride solution, 0.1 ml/10 g; II) piracetam, 50 mg/kg; on left — injection 15 min, on right — 45 min before recording.

EXPERIMENTAL RESULTS AND DISCUSSION

Comparison of absolute values of initial motor activity of the different groups of animals shows that these parameters were quite variable: the number of runs in the first 5 min varied on different days from 6000 to 15,000. Nevertheless, evaluation of the dynamics of the change in the number of movements with time in all the groups tested revealed a similar tendency: after a period of active EBR, lasting 5-10 min, there was a gradual decrease in the number of movements, down to 4000-9000 in the different groups by the 30th minute of recording (Fig 1, I). The same tendency naturally was found by calculation of the relative parameter which we used, but the scatter between the experiments was significantly smaller: in different groups this parameter amounted to 60-75% (Table 1).

Piracetam injected 15 min before the beginning of recording did not change the initial motor activity. At the same time it significantly altered the dynamics of habituation reduction of motor activity developed sooner, and it was more marked by the end of recording than in animals of the control groups (Fig 1, II). Analogs of piracetam based on pyroglutamate and proline likewise did not change the initial motor activity, but accelerated extinction of EBR (Table 1). Since inhibition of motor activity toward the end of recording could be the result of the depressant effect of the various nootropics, which develops in the course of time, additional experiments were carried out in which the drugs were injected 45 min and not 15 min before recording In this case initial activity did not differ from the control, but depression of activity in the next 30 min again took place more rapidly than in the control.

Meclofenoxate and pyritinol also accelerated the development of extinction of EBR, but their action differed from that of piracetam and its analogs: even in the smallest of the doses studied (25 mg/kg) these drugs reduced initial motor activity, and this masked habituation.

Comparison of these data with those obtained previously in relation to nootropic activity in the CPAR test demonstrated definite similarity between these tests This applies in particular to relative activity of the compounds studied. We showed previously that, judging by the level of threshold doses in the CPAR test, peptide derivatives of piracetam based on pyroglutamate and proline are 2-4 orders of magnitude more active than piracetam itself [2, 7]. Similar relationships were found in a study of extinction of EBR; differences in effectiveness of these compounds were see particularly clearly on calculation of the specific activity (Fig. 2). For some nootropics studied previously we showed that the dose-effect curve in the CPAR test is dome-shaped. For example, the maximal antiamnesic effect of piracetam corresponded to a dose of 300 mg/kg, and with an increase or decrease in the dose the effect diminished [7]; compound TGS-4 gave the maximal effect in the CPAR test in a dose of 0.5 mg/kg [2]. In the study of extinction of EBR the peak of activity of these compounds also was found with doses of 300 and 0.5 mg/kg respectively (Table 1).

TABLE 1. Effect of Standard Nootropics and Peptide Analogs of Piracetam on Habituation in Extinction of EBR Test

Drug	Dose, mg/kg	Parameter of habituation, %	Drug	Dose, mg/kg	Parameter of habituation, %
Piracetam	25	33/60*	TGS-4	0,3	41/85*
	50	22/70*		0,5	11/65*
	300	11/63*		10,0	27/70*
	500	34/73*	TGS-24	0,1	3,8/54**
	800	39,9/59		0,5	6,4/62**
Meclofenoxate	25	30/75*		1,0	5,2/62**
	50	20/61*			
			TGS-20	0,25	20/67*
Pyritinol	25	6/55**		0,5	24/80*
	50	13/65*		1,0	17/69**
TGS-34	0,01	11/66*	TGIS-6	1,0	23/60**
	0,1	29/66*		7,0	6,6/80**
	10,0	19/66*		14,0	13,3/61*
TGS-33	0,01	22/68*	TGIS-86	14,0	17/46*
	0,1	11/64*	T	1,0	27/68
	10,0	29/67*	TGS-8	1,0	27/68
				10,0	10/82*

Legend. Each number is the result of averaging data for 4-6 groups, each containing 10 mice; numerator gives data for groups of mice receiving the corresponding nootropic, denominator gives averaged controls for the same days. Levels of significance by Wilcoxon—Mann—Whitney U test: $*p \leq 0.05$, $**p \leq 0.01$.

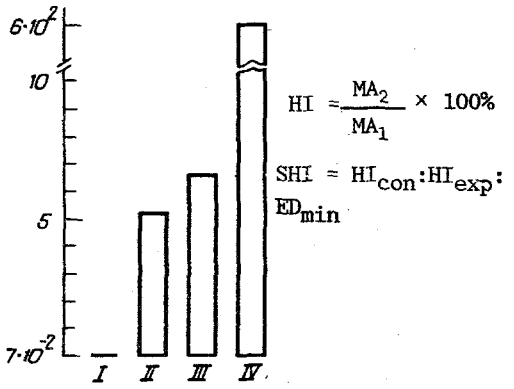

Fig. 2. Comparison of piracetam and its peptide analogs for relative activity. I) Piracetam, 25 mg/kg; II) TGS-4, 0.5 mg/kg; III) TGS-20, 1 mg/kg; IV) TGS-34, 0.01 mg/kg. HI) (Habituation index) — ratio of motor activity (MA) during last 5 min of recording to MA in first 5 min of recording, expressed in per cent. SHI (specific habituation index) — ratio of HI in control to HI under the influence of test substance, divided by threshold dose. Ordinate, value of SHI.

Meanwhile definite differences were found in the action of the nootropics in the two tests. First, the threshold doses were much lower for our suggested habituation test and for the CPAR; for example, for piracetam the minimal doses in which it accelerates extinction of EBR were 12.5-25 mg/kg, whereas the threshold dose in the CPAR test was 200 mg/kg. For pyritinol and meclofenoxate these doses were 25 and 100 mg/kg respectively. The same shift of the threshold doses also was found for peptide analogs of piracetam, for example, for the pyroglutamate derivative TGS-20 the threshold dose for extinction of EBR was 0.25 mg/kg, whereas for conditioned reflex learning it was 1 mg/kg, and for the proline derivative TGS-6 these doses were 1 and 7-14 mg/kg respectively. A significant difference between the two tests relates to their sensitivity to stereoisomers; whereas in the CPAR test only derivatives of L-pyroglutamate and L-proline, and not their

tamate (TGS-24, for example) and D-proline (TGS-86) derivatives.

Specific sensitivity of CPAR to L-isomers, together with a number of other features, suggested that the action of piracetam on learning processes is receptor (peptidergic) in character [2]. It has been shown that depending on the structure of the C-terminal amino acid, the pyroglutamyl-dipeptides studied differ in their effect on different phases of processing of the memory engram. L-pyroglutamyl-Dalaninamide, for example, facilitates primary information processing, fixation, and withdrawal, whereas L-pyroglutamyl-L-asparaginamide facilitates only the first phase [4, 6]. It has been suggested that peptidergic receptor mechanisms involved in the realization of different forms of memory are heterogeneous. Physiological data enabling habituation to be identified with a particular stage of memory engram processing during learning are not available. The results of the pharmacologic analysis described above can evidently be taken to indicate a difference in principle between habituation and all known forms of memory involved in conditioned-reflex learning.

It follows from the material described above that the suggested modification of the extinction test is an adequate model for evaluation of nootropics, and that the clearest effect is given by "selective" nootropics, free from any psychosedative action. The suggested test has certain advantages over the main test usually used to evaluate nootropics, namely the study of CPAR: it is technically simple and does not give rise to stress, not even the aversive learning stimulus used to develop an avoidance reaction, i.e., it is closer to natural conditions. Extinction of the exploratory reaction also is more sensitive than the CPAR to nootropics.

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