ROLE OF DIFFERENT TYPES OF GLUTAMATE RECEPTORS IN SPATIAL MEMORY IN RATS

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UDC 612.821.2.06:[612.822.014.467:547.466.64].019:599.323.4

KEY WORDS: spatial memory; glutamate receptors; brain; rats

Dicarboxylic amino acids (glutamic and aspartic) are the main excitatory mediators in brain synapses and are involved in a whole range of mental functions in man and animals [1]. Recent investigations of glutamatergic neurotransmission have revealed several types of receptors, both pre- and postsynaptic in their location, and which determine the efficiency of synaptic excitation during the action of glutamate as neurotransmitter. These include, principally, receptors sensitive to N-methyl-D-aspartate (NMDA), to quisqualate or alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), and to 2-amino-4-phosphonobutyrate (2-APB). Each type of glutamate receptor is characterized by concrete features of its molecular organization, its specific localization in brain structures and, as a result of that, a different role in the realization of brain functions [11].

NMDA-receptors have been shown to play an important role in the formation of conditioned-reflex skills in animals and, in particular, during passive avoidance training and solution of spatial problems in rodents [12, 13]. Involvement of quisqualate/AMPA- and 2-APB-glutamate receptors in memory processes has not yet been adequately studied.

Accordingly we undertook a comparative study of the effect of antagonists of different types of glutamate receptors on spatial differentiation in rats when solving problems in a maze with food and aversive (water maze) reinforcement.

EXPERIMENTAL METHOD

Experiments were carried out on 58 noninbred male albino rats weighing 180-250 g.

The animals were taught a spatial skill in an 8-arm radial maze with food reinforcement [9]. The arrangements of the maze consisted of a central platform (25 cm in diameter) raised to a height of 70 cm, with 8 radial arms (42 × 9 cm) radiating from it, and with food reinforcement (cheese) at the end of each arm. In the course of 1 month, food-deprived animals were taught to obtain food in 4 of the 8 arms of the maze: testing began by placing the rats in the center of the maze and ended after the animals had visited all four reinforced arms, or after 180 sec if training was unsuccessful. Visiting an arm awaiting reinforcement was considered to be a mistake of long-term memory, whereas a second visit to an arm awaiting reinforcement was regarded as a mistake of short-term memory [14].

To study the effect of antagonists of glutamate receptors on spatial differentiation under aversive reinforcement conditions, we used a water maze [6]. This consisted of a rectangular basin (150 × 70 cm) containing water (16-18°C), and divided by a partition into blind and through compartments. In the course of nine days the animals were taught to seek the way out of the maze, and mistaken visits and the time taken from the start compartment to the finish compartment were recorded.

Research Institute of Pharmacology, Volgograd Medical Institute, Ministry of Health of Russia. (Presented by Academician of the Russian Academy of Medical Sciences O. S. Adrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 617-619, June, 1992. Original article submitted June 5, 1991.

TABLE 1. Effect of MK-801, DEG, and 2-APB in a Single Injection on Spatial Memory Parameters of Rats in an 8-Arm Radial Maze (M ± m)

Substance, dose, mg/kg	Number of observations	Error of long-term memory	Error of short-term memory	Time taken to pass through maze sec
Control	35	1,31±0,23	0,26±0,10	40,34±4,99
MK-801, 0,01	9	1,22±0,40	0,11±0,11	36,56±7,68
0,05	8	1,87±0,87	0,14±0,14	42,13±4,42
0,10	10	2,50±0,74*	0,80±0,80	56,80±17,34
0,30	6	4,20±1,31*	0,20±0,20	149,90±24,98*
Control	34	1,20±0,25	0,23±0,10	67,68±9,17
DEG 50	6	1,33±0,42	0,10±0,10	63,83±8,38
100	11	1,31±0,39	0,21±0,13	75,45±18,61
250	12	0,86±0,42	0,14±0,14	114,92±21,86*
500	6	1,33±0,21	0,16±0,16	135,51±21,45*
Control	25	1,48±0,26	0,20±0,10	55,72±7,45
2-APB 10	6	0,85±0,31	0,14±0,14	60,33±25,01
50	7	0,83±0,65	0,33±0,21	96,57±24,27*
100	8	1,02±0,57	0,00±0,00	157,87±14,64*

Legend. When MK-801 was used in dose of 0.30 mg/kg, testing time was increased to 300 sec. Asterisk indicates significant ($p < 0.05$) difference from control by Wilcoxon–Mann–Whitney test.

Animals which had learned to differentiate in the radial or water maze were randomized and divided into groups, which were given the following antagonists of glutamate receptors, dissolved in distilled water, and injected intraperitoneally: MK-801 30 min, diethyl ester of glutanlate (DEG) and 2-APB 1 h before the test. A commercial sample of MK-801 (from "Merck") was used, DEG was obtained by Candidate of Chemical Sciences A. A. Ozerov (Volgograd) and 2-APB was synthesized in the Department of Organic Chemistry, A. I. Gertsen Leningrad Pedagogic Institute. The results were subjected to statistical analysis and the significance of differences between groups was estimated by the nonparametric Wilcoxon–Mann–Whitney test.

EXPERIMENTAL RESULTS

Experiments with antagonists of glutamate receptors were preceded by a 30-day period of training of the animals, and as a result of it the rats developed a sufficiently firm habit of visiting the four arms of the 8-arm radial maze awaiting reinforcement. This was shown by the high value of long-term and short-term memory, namely 1.3 ± 0.2 and 0.3 ± 0.1 mistaken visits per session respectively. Administration of MK-801, a noncompetitive antagonist of NMDA-receptors, in doses of 0.01 and 0.05 mg/kg did not change the efficacy of performance of the skill in the maze, but higher doses of the drug (0.10 and 0.30 mg/kg) led to a significant increase in the number of mistakes of long-term memory and of the time required to solve the problem (Table 1). In rats receiving MK-801 in a dose of 0.3 mg/kg a characteristic behavioral syndrome was observed to arise in response to administration of noncompetitive NMDA-antagonists (excitation, sterotyped movements, ataxia). By contrast with MK-801, the competitive antagonists of quisqualate/AMPA- and 2-APB-receptors, namely DEG and 2-APB respectively, had no effect on the accuracy of solution of the spatial problem, as could be judged by the number of mistaken visits to the arm of the maze, although both substances in high doses did significantly delay the progress of the rats to the maze (Table 1). An increase in the task performance time under the influence of DE& in a dose of 250 mg/kg and of 2-APB in a dose of 50 mg/kg was not accompanied by any disturbances of movement, and signs of ataxia and motor discoordination were observed only after administration of the largest of the doses of glutamate receptor antagonists used in the study.

In the water maze, where a powerful aversive stimulus (cold water) was the reinforcement, it took 8-9 days for the animals to reach the asymptotic level of training. MK-801 led to marked disturbance of the spatial skill in the water maze, and did so in smaller doses (0.05 mg/kg) than in the 8-arm radial maze. Particularly marked amnesia developed in response to injection of this substance in doses of 0.10 and 0.30 mg/kg, for in the first case 50% of the animals, in the second, actually 75%, did not succeed in finding the way out of the maze. By contrast with MK-801, DEG and 2-APB did not give rise to any change in the parameters of spatial memory in the water maze, even in high doses, in which these substances delayed solution of the problem in the radial maze (Figs. 1 and 2).

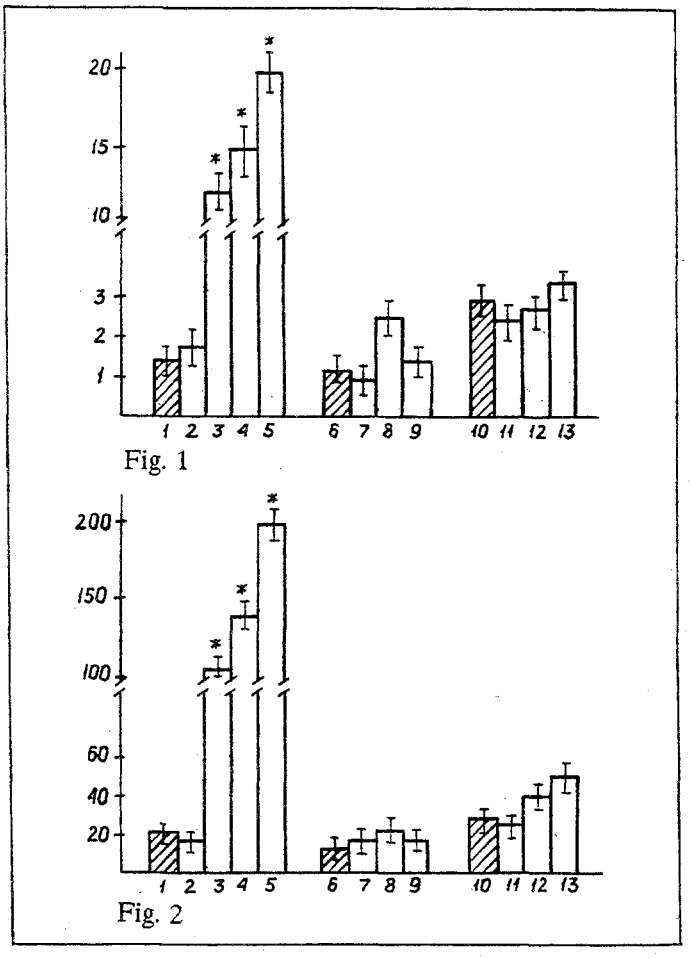

Fig. 1. Effect of glutamate receptor antagonists MK-801, DEG, and 2-APB on accuracy of task performance by rats in a water maze. Ordinate, number of mistakes per session. Legend: injection of physiological saline (1, 6, 10); of MK-801 in a dose of 0.01 (2), 0.05 (3), 0.10 (4), and 0.30 (5); DEG in a dose of 100 (7), 250 (8), and 500 (9); and 2-APB in a dose of 10 (11), 50 (12), and 100 mg/kg (13) mg/kg body weight. Values for which $p < 0.001$ compared with control are indicated by an asterisk.

Fig. 2. Effect of glutamate receptor antagonists MK-801, DEG, and 2-APB on task performance time by rats in a water maze. Ordinate, time (in sec). Legend as to Fig. 1.

Analysis of memory information during spatial differentiation is known to take place in the cortex and hippocampus, from which information on the choice made and the command signals are relayed along fibers running to n. accumbens to the motor systems [10]. Our findings are evidence that glutamate receptors are involved in different stages of the complex process of spatial differentiation. NMDA-receptors evidently take part in the extraction of information relative to memory processes themselves, for MK-801, an antagonist of receptors of this type, led to considerable disturbances of the accuracy of problem solving in the radial and water mazes.

In all probability, the switching of information to relay systems in fibers of the hippocampus and n. accumbens, takes place through quisqualate/AMPA- and 2-APB-receptors, for their antagonists, DEG and 2-APB, only delayed task performance without affecting the effectiveness of choice in the radial maze. This hypothesis can be strengthened by evidence of the close functional interaction between glutamate- and dopaminergic systems in the performance of locomotor function. In particular, quisqualate/AMPA receptors mediate excitation of neurons in n.

accumbens, exhibiting synergism with dopamine receptors that modulate neurons in this part of the brain [4]. As a result, agonists of quisqualate/AMPA-receptors and of dopamine receptors mutually potentiate (whereas antagonists weaken) the psychostimulating action of one on the other [5]. Like DEG, the dopamine receptor antagonist haloperidol did not affect the accuracy of choice in the radial maze, but sharply delays task performance [8]. Assuming that quisqualate/AMPA- and 2-APB-receptors, jointly with dopamine receptors, participate in the relaying of memory information, traveling from the hippocampus to the motor systems, it becomes clear that in the water maze, the aversive medium of which promotes activation of the dopaminergic system of the brain [7], DEG and 2-APB do not inhibit task performance.

The results showing that all three glutamate receptor antagonists studied caused no change in parameters of short-term memory were somewhat unexpected, although research demonstrating the role of glutamate receptors, at least those of NMDA-type, in processes of short-term memory during spatial differentiation, are known [2, 3]. These disagreements can be attributed to differences in the behavioral model used in this study, which is inferior to other experimental variants (for example, the introduction of delayed choice) for evaluation of short-term memory [14].

The authors are grateful to Professor L. Iversen (Harlow, England) and Candidate of Chemical Sciences A. A. Ozerov (Volgograd) for providing the MK-801 and the diethyl ester of glutamate.

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