PSYCHOTROPIC EFFECTS OF DIETHYL GLUTAMATE IN MICE

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Much evidence has now been obtained to show that glutamatic and aspartic acids are involved in neurotransmission in excitatory synapses of mammalian brain structures, where they participate in the regulation of behavior [6]. However, the functional role of the glutamate-aspartatergic system of the CNS in animal behavior has not yet been explained, for most known antagonists of receptors of excitatory amino acids do not pass readily through the blood-brain barrier (BBB) [8]. In some investigations conducted in vitro or with intracentral injection of excitatory amino acids and blockers of their receptors it has been suggested that dicarboxylic amino acids play a role in the regulation of motor activity and of memory processes in animals [7, 10].

The aim of this investigation was to study the psychotropic effects of diethyl glutamate (DEG), a nonselective antagonist of excitatory amino acids, in mice, whose BBB is more permeable than that of rats [3].

EXPERIMENTAL METHOD

Experiments were carried out on 203 noninbred male albino mice aged 1.5 months and weighing 15-18 g. The effect of DEG on spontaneous motor activity and on orienting and investigative behavior of the animals was studied by the open field test. The test system consisted of a square area (28 × 28 cm), surrounded by Plexiglas walls 32 cm high. Four squares were marked out on the area, with four holes in them. The behavior of the mice in the open field was studied for 3 min with an intensity of illumination of 90 lx. The forced swimming test [11] was carried out in a modification developed for mice [12]. The effect of DEG on the various stages of formation of the memory trace was studied on a model of passive avoidance conditioning [5]. During reproduction of the passive avoidance conditioned reflex (PACR) the number of mice visiting the dark compartment during observation for 150 sec was recorded.

DEG was synthesized and generously provided by Candidate of Chemical Sciences A. A. Ozerov (Volgograd) and the structure of the substance was confirmed by PMR-spectroscopy (Tesla BS-567A). DEG was injected intraperitoneally into the animals in doses of 10-500 mg/kg 30 min before testing in 0.1 ml of isotonic sodium chloride solution. The results were subjected to statistical analysis by one-way analysis of variance (ANOVAR), Student's test [14], and Fisher's exact method.

EXPERIMENTAL RESULTS

No change in behavior of the mice in the open field was observed 30 min after intraperitoneal injection of DEG in doses of 10 and 100 mg/kg. Increasing the dose of the drug to 200 and 500 mg/kg caused diminution of motor activity (the number of squares crossed) and of orienting and investigative behavior (the number of whole inspections and of rearings on the hind limbs) of the animals (Table 1). DEG in doses of 100 and 200 mg/kg caused a dose-dependent shortening of the duration of immobilization of the mice in the forced swimming test (Fig. 1). Under the influence of the compound not only was the duration of uninterrupted swimming by the animals increased, but so also was the number of attempts to jump out of the water, which can be interpreted as an increase in investigative activity leading to avoidance of an aversive situation. To study the effect of DEG on memory processes, in three of experiments the compound was injected in doses of 100 and 200 mg/kg before training, after training, and in the period of reproduction of the PCAR 24 h later. When DEG was injected 30 min

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TABLE 1. Effect of Diethyl Glutamate on Behavior of Mice in Open Field Test

Dose, mg/kg	Number of squares crossed	Number of rearings on hind limbs	Number of inspections of holes	Grooming	Number of defecations
Control	20,5±2,6	15,9±3,2	3,6±0,5	0,8±0,2	0,1±0,1
10	18,6±3,4	13,3±3,4	5,1±2,1	1,0±0,2	
100	15,1±1,7	12,9±2,7	3,3±0,8	0,3±0,1	0,5±0,2
200	11,4±3,1*	5,7±2,0*	1,4±0,5*	0,5±0,2	0,1±0,1
500	9,1±2,5*	3,5±2,3*	1,4±0,4*	0,7±0,1	

Legend. *$p < 0.05$ (Student's t test) compared with control.

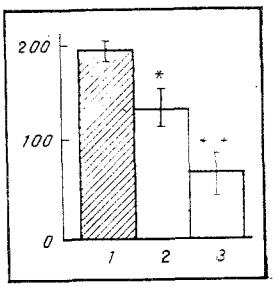

Fig. 1. Effect of DEG in doses of 100 mg/kg (2) and 200 mg/kg (3) intraperitoneally, after 30 min, on duration of immobilization of mice in forced swimming test. Physiological saline was injected in the control group (1). Ordinate, duration of immobilization (in sec); $p < 0.05$ (Student's t test) compared with control group 1; **$p < 0.05$ (Student's t test) compared with group 2.

before learning, formation of the PCAR was disturbed, as shown by an increase in the number of mice visiting the dark compartment during 150 sec of observation (Fig. 2a). DEG, injected in the above-mentioned doses, during the first few seconds after the mice had been trained in the PCAR, did not affect formation Of the memory trace (Fig. 2c). The amnesic effect of the excitatory amino acid antagonist was observed also when it was injected in a dose of 100 mg/kg 30 min before reproduction of the PCAR. Under these circumstances the number of mice not visiting the dark compartment was unchanged by DEG in a dose of 200 mg/kg, probably because of inhibition of motor activity (Fig. 2c).

The results relating to the psychoinhibitory action of DEG in doses of 200 and 500 mg/kg on behavior of mice in an open field suggests that receptors of excitatory amino acids participate in the control of motor activity and of the orienting and investigative activity of animals. Under the influence of DEG there was a marked decrease in vertical activity of the rodents, and this also is characteristic of selective antagonists of N-methyl-D-aspartate (NMDA) receptors [4]. However, blockers of NMDA receptors, unlike DEG, increase the horizontal activity of animals due to stimulation of the dopaminergic system [1]. In a state of "behavioral despair" (the forced swimming test), when the noradrenergic and dopaminergic systems of the brain are activated, DEG in doses of 100 and 200 mg/kg increases activity of the mice in the search for escape, indirect confirmation of interaction of DEG with NMDA receptors, linked with catecholaminergic systems. These data can be explained by reciprocal relations between dopaminergic and glutamatergic systems in the deep brain structures. We know that glutamic acid, which is secreted in endings of the corpus striatum, excites cholinergic strionigral pathways inhibiting the intrastriatal dopaminergic system [2]. Injection of the muscarinic cholinoceptive blocker atropinc, like DEC, leads to disinhibition of the catecholamiuergic systems and to shortening of the duration of immobilization in the forced swimming test [13].

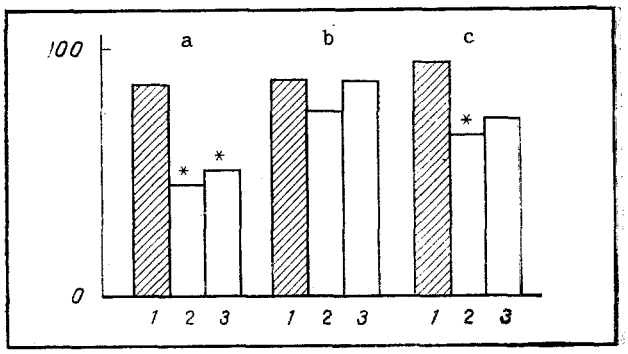

Fig. 2. Effect of DEG in doses of 100 (2) and 200 mg/kg (3) on performance of PCAR by mice when injected before training (a), after training (b), and before reproduction of reflex (c). Physiological saline was injected in control group (1). Ordinate, percent of mice not visiting dark compartment during 150 sec; $*p < 0.05$ (Fisher's exact method) compared with control group (1).

Injection of DEG before training or before reproduction of the PCAR by the mice caused amnesia of the skill, evidence that the glutamate-aspartatergic system of the brain is involved in fixation and recall of the memory trace. Our results are confirmed by studies of neurochemical mechanisms of long-term potentiation in the hippocampus. Most workers consider that plastic changes in neuronal transmission during long-term potentiation correspond to processes of memory trace formation in the whole brain and are realized through NMDA-receptors of excitatory amino acids [7, 9]. The problem of the effect of DEG on memory consolidation processes remains unsolved, for the results of injection of DEG immediately after training cannot be fully interpreted because the pharmacokinetic characteristics of this compound are not known.

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