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Interaction of Blockers of Ionotropic NMDA Receptors and Metabotropic Glutamate Receptors in a Working Memory Test in Rats

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Glutamate, the main excitatory neurotransmitter in the mammalian CNS, acts via ionotropic and metabotropic receptors. Results from in vitro studies demonstrating tight interactions between ionotropic NMDA receptors and subtype 5 metabotropic glutamate receptors (mGlu5) have shown that blockade of mGlu5 receptors increases the behavioral effects of NMDA receptor antagonists. The aim of the present work was to study the actions of the highly selective mGlu5 receptor antagonist MTEP alone and in combination with MK-801, a blocker of the NMDA receptor-associated ion channel, on performance of a delayed selection task (a test of working memory) in rats. MK-801 (0.1 mg/kg) induced a specific impairment to working memory, with proactive interference (degradation of the ability to remember current information because of the effects of previously learned material). Administration of MTEP (5.0 mg/kg) combined with both solvent and with MK-801 had no significant effects, demonstrating the small or nonexistent involvement of mGlu5 receptors in the mechanisms of working memory.

KEY WORDS: schizophrenia, working memory, proactive interference, NMDA receptors, MK-801, metabotropic glutamate receptors, MTEP, rats.

Glutamate is the main excitatory neurotransmitter in the central nervous system and acts via two classes of receptor: ionotropic and metabotropic [6].

Binding of glutamate to ionotropic glutamate receptors affects intracellular homeostasis by altering membrane permeability for cations. The N-methyl-D-aspartate (NMDA) subtype of glutamate receptors is one of the main subtypes of ionotropic glutamate receptors, playing a role in many behavioral processes, including the mechanisms of memory and attention [18].

Metabotropic glutamate (mGlu 1–8) receptors are divided into three groups on the basis of their common properties: structure, mechanism of neurotransmission, and pharmacological sensitivity. Receptors of the first group of metabotropic glutamate receptors (subtypes 1 and 5) activate phospholipase C via G-proteins [3]. In vitro studies have demonstrated that mGlu5 receptor agonists potentiate the activity of NMDA receptors in various parts of the brain [8], which led to the suggestion that mGlu5 receptors play a modulatory role in relation to NMDA receptors.

The expression of mGlu5 receptors is maximal in the hippocampus, prefrontal cortex, and some areas of the striate body [19]. As these areas of the brain take part in learning and memory processes and the formation of emotions and motivations, blockade of mGlu5 receptors has come to be regarded as a possible supplementary mechanism of memory and learning impairment.

In fact, results from animal studies support the hypothesis that the actions of NMDA receptor antagonists are increased by combined administration with mGlu5 receptor antagonists. For example, the mGlu5 receptor antagonist

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2-methyl-6-(phenylethynyl)pyridine (MPEP) enhanced the increase in motor activity and amplified the impairment to prepulse inhibition induced by phenylcyclidine, a blocker of NMDA receptor-associated ion channels, in rats [10]. In studies of the properties of working memory, combined administration of MPEP and phenylcyclidine at doses which are ineffective when given alone increased the number of erroneous responses in animals in a delayed selection task [2]. In another study [11], combined administration of MPEP and MK-801, a blocker of NMDA receptor-associated ion channels, decreased the number of correct responses in a spontaneous alternation test in rats.

Apart from affinity for mGlu5 receptors, MPEP binds to the noradrenaline transporter [9], monoamine oxidase A [5], and NMDA receptors [15], which hinders interpretation of experimental results obtained using this substance. The aim of the present work was to perform a detailed investigation of the effects of blockade of mGlu5 receptors using the highly selective antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl] pyridine (MTEP). Working memory was analyzed using a delayed selection task [7]. This method allows one of the key characteristics of impairments to working memory, the phenomenon of proactive interference [13], to be identified and described in patients with schizophrenia [16]. Proactive interference is manifest as degradation of the ability to remember current information because of the effects of previously learned (interfering) material. On repeated presentation of similar stimuli (for example, left and right pedals for pressing), the previous information is overlaid on the current, leading to incorrect performance of the task.

METHODS

Experiments were performed on nine male Wistar rats weighing 220–260 g (from the Rappolovo supplier, Leningrad Region). Animals were kept in individual plastic cages with a 24-h light regime (12 h day, 12 h night, light switched on at 09:00), a controlled temperature (22 ± 2°C) and air humidity (40–70%), and free access to water but restricted food (14 g/day).

Operant behavior was developed in standard Skinner boxes (MED Associates Inc., USA) fitted with two pedals positioned on each side of an automated device delivering 45-mg granules of feed mix (MLab Rodent Tablets, USA), with a sound emitter and light source (2.8 W) located above. The boxes were placed in a ventilated soundproofed chamber fitted with compact light bulbs (2.8 W).

The animals were trained to press one of the levers (the left pedal in half the rats, the right pedal in the other half) to obtain food reinforcement. Delivery of feed mix granules to the feeder was accompanied by a sound signal (3 kHz, 70 dB, 1 sec) and a flash of the light bulb above the feeder.

After successful acquisition of the pedal-pressing skill, task complexity was increased: the animal had to press the pedal and look into an opening located on the opposite wall of the operant box in order to obtain the reinforcement. This was followed by presentation to the animal of the other pedal of the experimental box, and it was only after pressing this pedal that the animal's action was reinforced. Daily sessions consisted of 100 trials, performance of which in 30 min was used as the criterion for transferring the animal to the next stage.

At the following stage, the animal was trained to a sample dissimilarity selection rule. The animal was presented with one of the two pedals of the box (the sample pedal) in random order. After pressing the pedal and looking into the opening, both pedals of the box were presented. The animal had to press the pedal (pedal selection) on the side opposite to the sample pedal in order to obtain the food. If the rat did not press the pedal within 20 sec of the beginning of presentation, the attempt was defined as a pass. The interval between trials was 5 sec. Daily sessions consisted of 96 trials, excluding passes. The learning criterion was 90% or more correct trials with fewer than five passes per session. Each pedal had to be pressed in at least 40% of trials, to demonstrate the absence of any marked preference for either side. When this criterion was reached, the animals were progressed to the final stage of training to the delayed sample-dissimilarity selection task.

Unlike the situation in the previous task, after pressing the sample pedal, the animal could look into the opening and move to the phase of selecting the reinforced pedal with a variable delay (0, 8, 16, or 32 sec). The criterion for successful performance of the test was >95% correct responses in trials with the minimum delay, >50% correct responses in trials with the maximum delay, <5 passes, and 40–60% of presses on the left pedal. Proactive interference was detected by comparing each combination of the preceding and current trials in one session. If the sample pedal in the preceding trial was on the same side as that in the current trial, then the current trial was designated a "repeat." If the sample pedal in the preceding trial was on the opposite side, then the current trial was designated a "substitution." Analysis of the results included determination of the proportion of correct responses for each time of trial (repeat/substitution) with delays of different durations.

The study used the following pharmacological substances: (+)MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine maleate, Sigma-Aldrich, Germany) and MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (Merz Pharmaceuticals, Germany). Agents were dissolved in 0.9% sodium chloride solution. MK-801 solution was given i.p. in 1 ml/kg; MTEP solution was given i.p. in 2 ml/kg. Doses were given 15 min before experimental sessions.

Experimental sessions were performed on six days per week. Proactive interference was detected by giving the animals MK-801 (0.1 mg/kg) once weekly for five weeks [14]. The sequence of tests using combinations of study pharma-

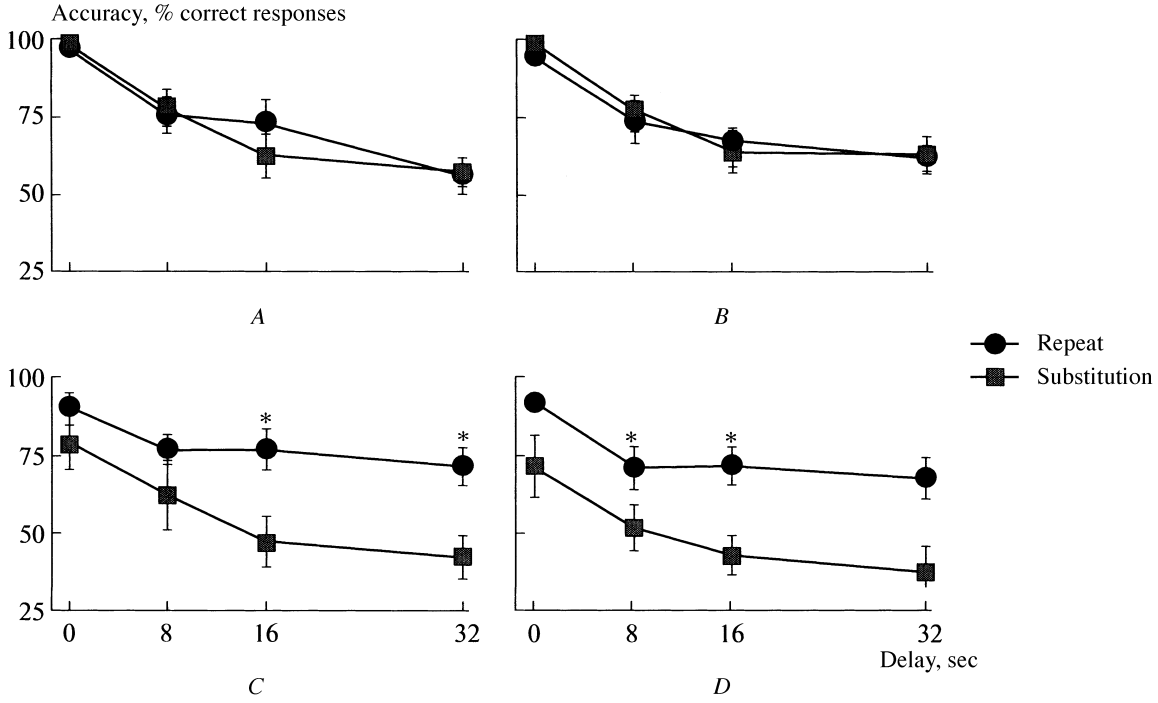

Fig. 1. Effects of combined administration of MK-801 (0.1 mg/kg, i.p.) and MTEP (5.0 mg/kg, i.p.) or their solvents on the accuracy with which the animals performed a delayed sample-dissimilarity selection task. *A*) Solvent + solvent; *B*) 5.0 mg/kg MTEP + solvent; *C*) solvent + 0.1 mg/kg MK-801; *D*) 5.0 mg/kg MTEP + 0.1 mg/kg MK-801. Data are shown as percentages of correct responses ($M \pm m$) on presentation of the selection pedal with delays of 0, 8, 16, and 32 sec. Repeat trials were those in which the sample pedal was presented on the same side as the current pedal. Substitution trials were those in which the sample pedal was presented on the side opposite to the side on which the sample pedal had been presented in the preceding trial. *Significant differences (Bonferroni test) compared with substitution of the sample pedal presentation side, $p < 0.05$. Each group consisted of $n = 9$ animals.

TABLE 1. Effects of Combined Administration of MK-801 (0.1 mg/kg, i.p.) and MTEP (5.0 mg/kg, i.p.) or Their Solvents on Measures of Performance of the Delayed Selection Task ($M \pm m$)

Parameter	Number of passes	Latent period of pressing sample pedal, sec	Latent period of approaching feeder, sec
Solvent + solvent	4.6 ± 1.4	5.8 ± 1.2	0.32 ± 0.03
Solvent + 0.1 mg/kg MK-801	6.4 ± 2.1	6.3 ± 1.4	0.35 ± 0.05
5.0 mg/kg MTEP + solvent	9.3 ± 3.3	7.7 ± 1.4	0.32 ± 0.03
5.0 mg/kg MTEP + 0.1 mg/kg MK-801	4.4 ± 1.3	5.1 ± 0.8	0.34 ± 0.03

ceuticals was determined using the Latin squares method. At least two sessions without drug treatment were performed between pharmacological tests.

Data were analyzed using the following variables: the proportion of correct responses at each of the delays, the number of passes, the latent periods of pedal presses, the latent periods of approaching the feeder when responses were reinforced. Experimental results were analyzed using the statistical program suite SPSS (SPSS for Windows version 14.0). The statistical significance of substance effects was evaluated by two-factor analysis of variance adapted for repeat measurements (independent variables): 1) trial type (repeat/substitution) and 2) combination of study agents. Intergroup comparisons were made using an a posteriori test with the Bonferroni correction.

RESULTS

During the last stage of training to the delayed sample-dissimilarity selection task, performance of the task by the animals stabilized at a level of about 100% correct respons-

es in trials with the minimum delay and at the random selection level (50%) in trials with the maximum delay. Animals passed in no more than five trials per session. A relationship between correct task performance and delay duration was seen in every experimental session.

After administration of MK-801 at a dose of 0.1 mg/kg, there was a marked reduction in the accuracy of task performance when the sample pedal in the previous trial was located on the opposite side to that in the current trial ($F_{2,52} = 3.44$, $p < 0.05$). Administration of MTEP (5.0 mg/kg) had no effect on task performance by the experimental animals and did no enhance the reduction in task performance accuracy induced by MK-801 in the situation in which the sample pedal in the preceding trial was located on the opposite side as compared with the current trial (Fig. 1).

No combination of drug treatments had any significant influence on the latent period of pressing the sample pedal ($F_{2,35} = 32.02$, $p = 0.73$), the latent period of approaching the feeder when responses were reinforced ($F_{2,35} = 0.22$, $p = 0.8$), or the number of passes ($F_{2,35} = 1.80$, $p = 0.18$) (Table 1).

DISCUSSION

The results obtained here show that decreases in glutamatergic neurotransmission resulting from administration of a blocker of NMDA receptor-associated ion channels impaired the performance of a delayed selection task. Different types of memory are involved in performing the delayed selection task: instantaneous perceptual memory (recognition of the current stimulus), short-term memory (current/past stimulus), and long-term referential memory (selection rule). The delay between the stimuli complicates the task as it requires information to be held in perceptual memory during a time period. Retention of the trace in short-term memory can be detected as the phenomenon of proactive interference, when information on a previous selection influences the next selection, leading to decreases in task performance accuracy.

Proactive interference, manifest as a reduction in task performance accuracy when the sample pedal in the preceding trial was located on the opposite side as compared with the current trial, was seen after administration of MK-801, a blocker of NMDA receptor-associated ion channels. The MK-801 dose (0.1 mg/kg) was selected on the basis of a preliminary experiment whose results showed that higher a dose of MK-801 (0.3 mg/kg) produced marked impairment to movement coordination and the appearance of stereotypy, such that the animals were unable to perform the task. At the same time, a lower MK-801 dose (0.03 mg/kg) had no significant effect on task performance. MK-801 at a dose of 0.1 mg/kg induced moderate increases in the animals' motor activity without decreasing the level of correct performance of the task, though it did produce the proactive interference effect.

The MTEP dose of 5.0 mg/kg was selected as effective in potentiating the action of MK-801 on motor activity [17]. Administration of the mGlu5 receptor antagonist MTEP (5.0 mg/kg) had no effect on the animals' performance of the delayed selection task: MTEP did not decrease task performance accuracy when combined with solvent and did not enhance the impairments induced by MK-801.

Despite the fact that many studies [2, 11] have demonstrated potentiation the effects of blockers of NMDA receptor-associated ion channel by the mGlu5 receptor antagonist MPEP, enhancement of the effects of MK-801 on the delayed selection task was not seen in the present study when the mGlu5 receptor antagonist MTEP was combined with MK-801. One of the most likely reasons for this contradiction consists of differences in the mechanisms of action of MTEP and MPEP. Both substances are selective mGlu5 receptor antagonists, though MPEP also has affinity for the noradrenaline transporter [9] and monoamine oxidase A [5]. Furthermore, as has been demonstrated in electrophysiological studies on cell cultures, MPEP (200 μM) has affinity for NMDA receptors, acting as an antagonist [15]. This latter mechanism of action of MPEP (antagonism of NMDA receptors) may make the decisive contribution to enhancing the deleterious effects of blockers of NMDA receptor-associated ion channels.

In vivo and in vitro studies have shown that MTEP is a highly selective mGlu5 receptor antagonist with no significant effects on other mGlu receptor subtypes [1]. Furthermore, MTEP has less effect than MPEP on other receptor systems. Thus, MTEP produces minimal inhibition of the calcium currents in recombinant NR1A_2B receptors (an NMDA receptor subtype) induced by stimulation of these receptors with glutamate [4, 12].

The second possible reason that MTEP does not have a potentiating action on the effects of MK-801 is the prior repeated administration of MK-801 (0.1 mg/kg for five weeks [14]), which may alter the sensitivity of NMDA receptors. As mGlu5 receptors have a modulatory role in relation to NMDA receptors [8], the possibility that changes in the sensitivity of NMDA receptors produced the functional resistance to the modulatory effects of mGlu5 receptors cannot be excluded.

CONCLUSIONS

Thus, administration of MK-801, a blocker of NMDA receptor-associated ion channels, induced a specific impairment to short-term memory, i.e., the phenomenon of proactive interference. The fact that MTEP had no effect on the phenomenon of proactive interference induced by MK-801 in the delayed selection task is evidence that mGlu5 recep-

tors do not play any significant role in this type of impairment to short-term memory.

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