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NMDA but Not AMPA Receptor Antagonists Impair the Delay-Interposed Radial Maze Performance of Rats

HONG-BIN LI,* KINZO MATSUMOTO,* MINORU YAMAMOTO† AND HIROSHI WATANABE*[1]

*Department of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan
†Clinical Pharmacology Research Laboratory, Yamanouchi Pharmaceutical Co. Ltd., Tokyo 174, Japan

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LI, H. B., K. MATSUMOTO, M. YAMAMOTO AND H. WATANABE. *NMDA but not AMPA receptor antagonists impair the delay-interposed radial maze performance of rats*. PHARMACOL BIOCHEM BEHAV **58**(1) 249–253, 1997.—The effects of the *N*-methyl-D-aspartate (NMDA) receptor antagonists CGS19755 and MK801 and the 2-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor antagonist YM90K on spatial working memory were investigated by using a delay-interposed radial-arm maze (RAM) task in rats. CGS19755 and MK801, at the largest dose that had no effect on the performance in the nondelayed RAM task, significantly decreased the initial correct response after the 5-min delay in the delay-interposed RAM task. In contrast, YM90K had no effect on the initial correct response and arm reentries in both the delay-interposed and nondelayed RAM task. CGS19755, MK801 and YM90K, at all doses tested, did not alter the running time in either the delayed or the nondelayed RAM tasks. These results suggest that spatial working memory can be impaired by a blockade of NMDA receptor function and that such impairment is particularly sensitive to delay interposition. The lack of effect of the AMPA receptor antagonist provides additional evidence of the importance of the NMDA subtype of the glutamate receptors in cognitive processes. © 1997 Elsevier Science Inc.

NMDA antagonist AMPA antagonist MK801 CGS19755 YM90K Delay-interposed radial maze task Spatial working memory

GLUTAMATE receptors are thought to be predominantly responsible for rapid excitatory neurotransmission and learning/memory in the central nervous system of vertebrates (8–10). These receptors are divided into the ionotropic and metabotropic glutamate receptors, based on whether they gate the ion channels or are coupled to G-proteins. Of the ionotropic receptors, the *N*-methyl-D-aspartate (NMDA) subtype is implicated in the induction of long-term potentiation (LTP) and long-term depression (LTD) and in the conduction of NMDA-receptor-dependent cognitive processes. Blockade of the NMDA subtype impairs the ability to learn maze tasks and eliminates LTP induction in rats (6,16). Another ionotropic receptor, the α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) subtype, plays diverse roles in a variety of physiological and pathological processes. Activation of the AMPA receptor modulates fast excitatory signal transmission, memory consolidation, epilepsy, neurodegenerative disorders and more (4,13,21,23).

The most distinguishable difference between the NMDA receptor and the AMPA receptor concerning learning and memory is that the NMDA receptor plays a critical role in the induction, maintenance and expression of LTP, which is believed to be a synaptic model of memory in the hippocampal formation (5). Blocking the LTP induction by administration of NMDA receptor antagonists disrupts learning and memory processes (16,20). In contrast, stimulation of the AMPA receptor or suppression of the rapid desensitization of this receptor can promote the induction of LTP and enhance the encoding of memory across tasks involving different sensory cues and performance requirements (14,25,30). These findings suggest that the AMPA receptor is also important in the development of cognitive function. However, the action of AMPA

[1]To whom requests for reprints should be addressed.

receptor antagonists on spatial cognitive processes has not been studied fully. In addition, although studies have demonstrated the detrimental effect of NMDA receptor antagonists on the spatial working memory processes, the difference between competitive NMDA receptor antagonists and NMDA receptor channel blockers in the spatial working memory process has not been completely elucidated.

Spatial working memory is one of the hippocampal-dependent cognitive processes (3,17), and the radial-arm maze (RAM) task has been a useful method for assessing brain lesion or drug effects on the hippocampal-dependent and -independent spatial memory processes (20). Delay interposition is used frequently to evaluate the abilities of animals to make a choice or differential response based on previous cues when the delay interval is inserted (27). In the present study, we investigated the effects of the NMDA receptor antagonists MK801 and CGS19755 and the AMPA antagonist YM90K on the spatial working memory process by using a delay-interposed RAM paradigm.

MATERIALS AND METHODS

Subjects

Subjects were 85 male Wistar rats (SLC Co., Shizuoka, Japan), weighing 230–240 g upon arrival in the laboratory. The rats were housed in groups of 4–5 per cage in constant room temperature (25 ± 1°C), 55 ± 5% relative humidity and a 12-hr light/dark cycle (lights on: 0730–1930) with free access to water. The animals were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free-feeding level.

Apparatus and Procedures

The eight-arm radial maze was used as described in previous reports (15,18). Each arm (50 × 12 cm) extends from an octagonally shaped central hub (30 cm across). The platform is elevated 40 cm above the floor. Small black Plexiglas caps (3 cm in diameter and 1 cm deep) mounted at the end of each arm serve as receptacles for reinforcers (45-mg pellets; Bio-Serv, Frenchtown, NJ). Guillotine doors surround the hub and control the animals' entrance to and exit from the central hub.

Prior to maze training, each animal was handled for 15 min daily for several days and given 10 min to adapt to the apparatus for 3 days. During the adaptation period, food pellets were scattered in the maze. Following this adaptation period, one daily training trial was conducted for each rat, and a food pellet was placed in each food cup at the end of the arms. A session was judged complete when the rat had chosen all 8 baited arms or had spent 10 min in the maze. Entries into arms that had not yet been visited were recorded as correct responses, and subsequent reentries were scored as errors. The number of correct responses before committing the first error (number of initial correct), the number of errors and the running time were used as the indices of RAM performance. When a rat made no errors or only one error after the seventh choice, for 5 consecutive days, the delay-interposed and nondelayed tasks were employed for drug testing. We used this overlearned RAM procedure so that choice accuracy could be measured more clearly against a stable baseline with minimal changes due to additional learning.

In the delay-interposed RAM task, a 5-min delay period was inserted between the third and fourth choices, and during this delay period the rat was returned to its home cage. In this task, the initial correct response was defined as the number of correct choices before the rat reentered an arm previously visited after the delay interposition. To minimize the within-task difference, the nondelayed RAM task was carried out after the completion of delayed-interposed RAM task.

Drug Administration

The following drugs were used in the present experiment: CGS19755 and MK801 (Research Biochemicals Inc., Natick, MA) and YM90K (Yamanouchi Pharmaceutical Co., Tokyo, Japan). CGS19755 and MK801 were dissolved in saline. YM90K was dissolved in saline by adding 1 N NaOH (pH 8.4). Drug solutions were freshly prepared and administered intravenously into the tail vein of rats at a constant volume (1 ml/kg) 15 min before starting the maze test. The drug administration schedule was designed to avoid giving the same rat the same dose at different injections. Rats with incomplete administration of test drug were excluded from the statistical analysis. Each rat was injected with a drug no more than four times except that for saline (vehicle) treatment, and each rat was given a minimum washout period of 1 week between consecutive treatments.

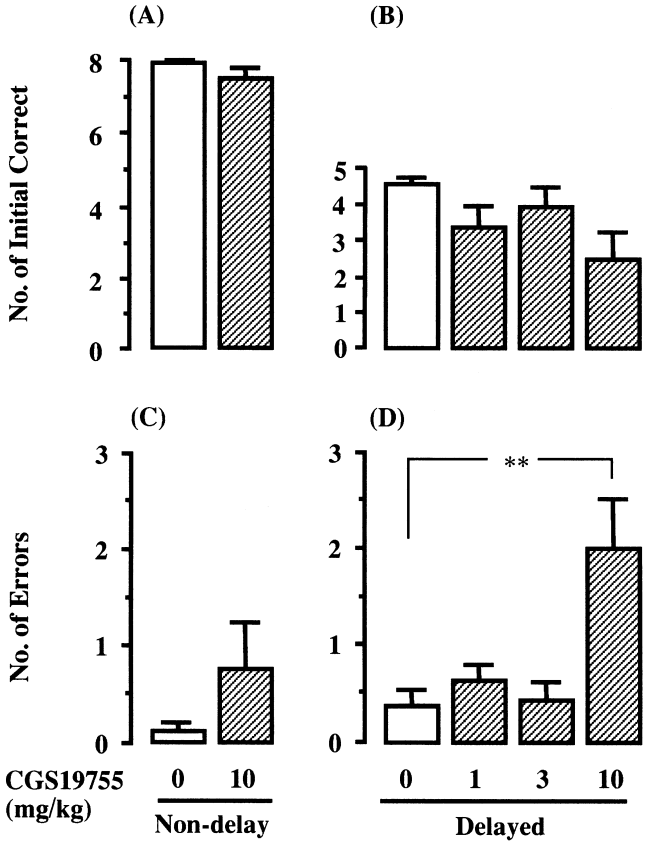

FIG. 1. Effect of CGS19755 on performance in the delay-interposed and nondelayed RAM tasks by rats. CGS19755 was administered 15 min before starting the experiments. In the delayed RAM task, the "No. of Initial Correct" response is defined as the number of correct choices before committing the first error. Open bars = control groups; shaded bars = CGS19755-treated groups. Each value is the mean ± SEM of 7–8 rats. $^{**}p < 0.01$ vs. vehicle-treated rats in the delayed interposed RAM task.

Statistic Analysis

Data obtained from drug- and saline-treated groups in the delayed RAM task were analyzed by one-way repeated measures analysis of variance. Significant main effects were analyzed further by the Student-Newmann-Keuls test. The initial correct response and error numbers in the nondelayed task and the running time were analyzed by the Mann-Whitney rank-sum test. The significance level was set at $p < 0.05$.

RESULTS

Effects of MK801 and CGS19755 on the Nondelayed and the Delay-Interposed RAM Task Performances

In the nondelay task, the vehicle-treated rats did not show impairment of radial maze performance. Treatment with CGS19755 (10 mg/kg) or MK801 (0.3 mg/kg) had no influence on the initial correct response and error numbers ($p > 0.05$; Figs. 1A,C, 2A,C). This result clearly suggests that, without delay interposition, these NMDA receptor antagonists do not impair the spatial working memory in rats.

In the delay-interposed RAM task, the vehicle-treated rats made no error when selecting the first three arms before the delay interposition but produced errors in the postdelay period. Treatment with CGS19755 at doses of 1–10 mg/kg did not alter the initial correct response [Fig. 1B; $F(3,30) = 2.2$, $p = 0.118$, $n = 8$), whereas treatment with 10 mg/kg CGS19755 produced notably more errors during the postdelay period [Fig. 1D; $F(3,30) = 5.05$, $p < 0.01$, $n = 8$]. Administration of 0.3 mg/kg of the NMDA channel blocker MK801 significantly decreased the initial correct response [Fig. 2B; $F(3,30) = 11.6$, $p < 0.01$, $n = 8$] and increased the number of errors compared with the vehicle-treated counterparts [Fig. 2D; $F(3,30) = 4.82$, $p < 0.05$, $n = 8$].

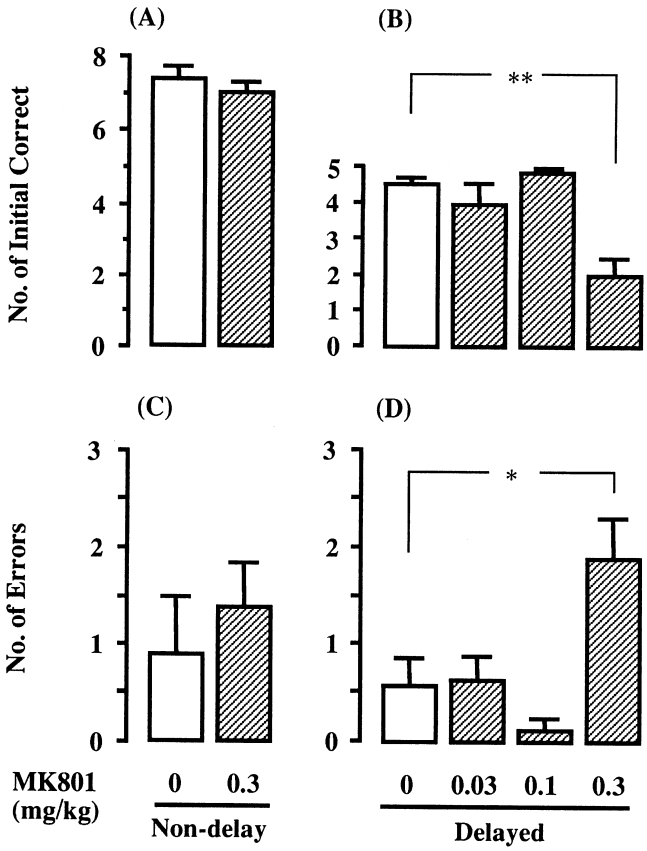

FIG. 2. Effect of MK801 on performance in the delay-interposed and nondelayed RAM tasks by rats. MK801 was administered 15 min before starting the experiments. In the delayed RAM task, the "No. of Initial Correct" responses were recorded as described in Fig. 1. Open bars = control groups; shaded bars = MK801-treated groups. Each value is the mean ± SEM of 8–9 rats. *$p < 0.05$ and **$p < 0.01$ vs. vehicle-treated rats in the delayed-interposed RAM task.

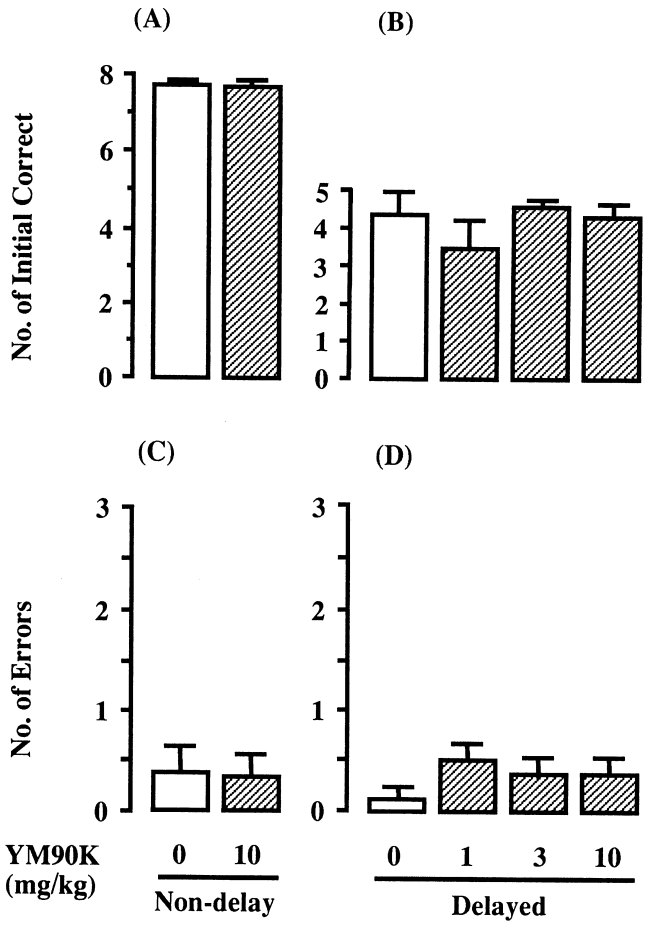

FIG. 3. Effect of YM90K on performance in the delay-interposed and nondelayed RAM tasks by rats. YM90K was administered 15 min before starting the experiments. The "No. of Initial Correct" responses were measured as described in Fig. 1. Open bars = control groups; shaded bars = YM90K-treated groups. Each value is the mean ± SEM of 8–9 rats.

Effect of AMPA Antagonist YM90K on the Nondelayed and the Delay-Interposed RAM Task Performances

In the nondelay-interposed RAM task, rats treated with YM90K presented no impairment when compared with their vehicle-treated counterparts (Fig. 3A,C, $p > 0.05$). In the delay-interposed RAM task, the administration of YM90K at doses of 1–10 mg/kg produced no effect on the initial correct response [Fig. 3B; $F(3,32) = 0.05$, $p = 0.059$] or on the number of errors in the postdelay period (Fig. 3D; $F(3,32) = 0.05$, $p = 0.505$; $n = 8$).

TABLE 1

EFFECT OF MK801, CGS19755 AND YM90K ON THE RUNNING TIME OF RATS IN THE DELAY- AND NONDELAY-INTERPOSED RAM TASKS

Drugs	Dose (mg/kg)	Running Time (s)	
		Without Delay	With 5-min Delay
CGS19755	0	125 ± 12	119 ± 21
	10	107 ± 17	117 ± 10
MK801	0	104 ± 13	130 ± 17
	0.3	124 ± 16	130 ± 25
YM90K	0	93 ± 15	91 ± 5.2
	10	97 ± 8.8	100 ± 7.3

To calculate the running time, the 5-min delay period was excluded from the total running time in the delay-interposed task. Each value is the mean ± SEM of 7–9 rats.

Effects of CGS19755, MK801 and YM90K Treatments on the Running Time of Rats in the Nondelayed and Delay-Interposed RAM Tasks

MK801, CGS19755 and YM90K at the doses tested failed to cause changes in the running time in either the nondelay or the delay-interposed RAM tasks (Table 1).

DISCUSSION

The present results demonstrate that CGS19755, a competitive NMDA receptor antagonist, and MK801, an NMDA open-channel blocker, produce disruption of maze performance in the delay-interposed but not in the nondelayed RAM task in rats. These findings suggest that NMDA receptor antagonists impair the later stages of the encoding processes of the spatial working memory but not the near-instantaneous use of spatial information (6).

Experimental evidence has indicated the important role of the hippocampal formation in physiological and pathological processes (5,17,20). In vivo and in vitro studies have shown that NMDA receptor antagonists inhibit the formation of the hippocampal LTP, which may mediate learning and working memory (3,10). Thus, the disruption of RAM performance caused by the NMDA antagonists in the present delay-interposed RAM task may have been due to the suppression of LTP formation. However, this possibility needs further investigation because the doses of MK801 used in the present study are reportedly insufficient to prevent the development of LTP in rats (1).

Suppression of neuronal activity by NMDA receptor antagonists in the prefrontal cortex during the delay period was probably involved in the disruption of RAM performance in the delay-interposed RAM task. Electrophysiological studies of single-neuron recordings from the forebrain lobe of rats and monkeys indicate that, in spatial delayed tasks, neurons of the prefrontal cortex become activated during the delay period, with different neurons showing different levels of activity depending on the cues (7,9,19,29). The failure of these neurons to maintain their activity during the delay period is associated invariably with errors in behavioral performance (11,12,20). These findings and those of the present study suggest that, rather than terminating the LTP formation, NMDA receptor antagonists impair the spatial working memory by suppressing the activity levels of these specific neurons during the delay period.

The present findings disagree with those of a report in which the administration of MK801 produced no serious disruption of maze performance by rats in a delayed RAM task (28). The inconsistency may be due to the different experimental conditions, such as the length of the delay interval, drug administration method and predelay time course. In the present study, CGS19755 and MK801 were administered 15 min before the start of the maze task, and detrimental effects were observed; in the other study (28), drugs were administered 1 h prior to the start of the task.

Arai and Lynch (2) have demonstrated that the facilitation of the AMPA receptor-mediated synaptic responses in the hippocampal slices reduces the amount of afferent stimulation needed to induce a maximal degree of LTP and proposed that this facilitation may enhance the memory processes. This proposal is supported by the data that applications of the AMPA receptor agonists not only increase the field excitatory postsynaptic potentials in hippocampal slice but also enhance the encoding of memory in tasks such as the two-odor discrimination, water maze and RAM (25). However, whether blockade of AMPA receptor is detrimental to learning and memory processes is not yet established. In the present study, the AMPA receptor antagonist YM90K showed no effect on the maze performance in both the delay-interposed and nondelayed RAM task. These results suggest that, unlike the actions of the NMDA receptor subtype, the contribution of the AMPA receptor subtype to the spatial cognitive processes is minor. This notion is supported by the findings that (a) blockade of the AMPA receptor affects neither the maintenance of LTP nor the field potentials in the hippocampus (22), and (b) NBQX, an AMPA receptor antagonist, has no effect on the spatial working memory of rats in the Morris water maze task (26).

We reported previously that the NMDA receptor antagonists MK801 and CGS19755 but not the AMPA receptor antagonist YM90K augment the scopolamine-induced disruption of RAM performance (15). Taking this observation and the results of the present study into consideration, the NMDA receptor and the AMPA receptor may not contribute equally to the cognitive functions, and dysfunction of the NMDA receptor may disrupt the spatial working memory.

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