An α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Antagonist and an *N*-Methyl-D-aspartate (NMDA) Channel Blocker Synergistically Impair Spatial Memory in Rats

Hongbin LI, Kinzo MATSUMOTO, and Hiroshi WATANABE*

Department of Pharmacology, Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194 Japan. Received June 4, 1998; accepted August 10, 1998

The interaction of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist YM90K with the competitive *N*-methyl-D-aspartate (NMDA) antagonist CGS19755 or with the NMDA channel blocker MK801 on the spatial memory of rats was examined by the 4-arm-baited radial maze task. When administered alone, these drugs had no effect on the spatial memory. The combination of YM90K and MK801 synergistically disrupted working and reference memories, whereas the combination of YM90K and CGS19755 had no effect. These results indicate that the blockade of the AMPA receptor and NMDA channels produces a synergistic impairment of spatial memory and suggest that interaction between the AMPA and NMDA receptors plays a role in cognitive function.

Key words spatial memory; *N*-methyl-D-aspartate (NMDA) antagonist; α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist

The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) subtypes of the glutamate receptor mediate excitatory synaptic transmission and play a crucial role in modulating learning and memory.^{1,2)} These receptors have different profiles on cognitive functions^{3,4)} and interact with other receptors in primates and rodents.^{2,5)} Recent molecular investigations indicate that the NMDA receptor has distinct binding sites for endogenous agonist glutamate, co-agonist glycine and channel blockers.⁶⁾ The AMPA receptor is co-localized with the NMDA receptor at the postsynaptic site of the excitatory synapses, and they respond simultaneously to their endogenous transmitter glutamate released from excitatory nerve endings.⁷⁻⁹⁾ These findings raise the possibility that AMPA and NMDA receptors may interact with each other and influence the cognition process. Thus, the functional significance of the co-localization of these receptor subtypes in the brain is of great interest. So far, no report is available concerning the interaction between AMPA and NMDA receptors on the cognitive process. In this study, we addressed this issue by testing how an AMPA antagonist with an NMDA channel blocker or with a competitive NMDA receptor antagonist affected spatial working and reference memories using the 4-arm-baited radial maze task.

MATERIALS AND METHODS

Procedure Male Wistar rats (SLC, Shizuoka, Japan) were housed and raised as previously described.²⁾ The maze consisted of an octagonal platform and eight arms radially extending from the platform with guillotine-type doors. Small black plastic cups (3 cm in diameter, 1 cm deep), mounted at the end of each arm, served as receptacles for reinforcers (45 mg food pellet, Bio-Serve, Frenchtown, NJ, U.S.A.). The animals were trained using a win-shift version of the task,¹⁰⁾ whereby 4 arms of the maze were baited with a reinforcer at the start of the trial. For each rat, 4 arms were randomly assigned to serve as the baited set. This set remained unchanged throughout the whole experiment for any

given rat. In each trial, the rat was placed in the central platform and then all of the doors were opened to allow it to enter arms freely. The trial was judged complete when the rat had entered all baited arms or had spent 10 min on the maze. Since the reinforcer was not replaced during the trial, only the first entry into each baited arm was recorded as a correct choice and subsequent re-entry as a working memory error. Entry into the unbaited arms was defined as reference memory error. We also calculated the running time that a rat spent to complete an arm choice as an index of motor activity of the animal during the maze performance. To calculate the running time, the total running time was divided by the total number of arm entries.

Drugs Drugs for maze tests were: CGS19755 [*cis*-4-(phosphonomethyl)-piperidine-2-carboxylic acid], a competitive NMDA receptor antagonist; MK801, an NMDA open-channel blocker; YM90K [6-(1-imidazolyl)-7-nitroquinoxaline-2,3-(1*H*,4*H*)-dione], a selective AMPA receptor antagonist.¹¹⁾ MK801 or CGS19755 was dissolved in saline, while YM90K was dissolved in saline by adding 1 N NaOH. Either saline or YM90K was administered intraperitoneally (i.p.) 10 min before the i.p. injection of MK801 or CGS19755. Thirty minutes after YM90K injection, the rat was put in the maze for the spatial memory test. Drug solutions were freshly prepared before the start of the experiments.

Statistics Data were analyzed by two-way analysis of variance followed by Student-Newman-Keuls test. Differences with $p < 0.05$ were considered significant.

RESULTS

When administered alone, two doses of MK801, CGS19755 or YM90K had no effect on spatial working and reference memories (Figs. 1 and 2). Two-way ANOVA revealed that administration of MK801 after YM90K significantly impaired the spatial working ($F_{\text{MK801} \times \text{YM90K}}(2,53) = 4.71$, $p = 0.0131$, Fig. 1A) and reference memories ($F_{\text{MK801} \times \text{YM90K}}(2,53) = 3.77$, $p = 0.0296$, Fig. 1B) in rats. The animals administered with both YM90K and 0.2 mg/kg MK801 com-

* To whom correspondence should be addressed.

Fig. 1. Synergistic Impairment of Spatial Working and Reference Memory Performance Caused by MK801 and YM90K in Rats

Either saline or YM90K was administered i.p. 10 min before the i.p. injection of MK801. Thirty minutes after YM90K, the radial maze performance was tested and the numbers of working memory errors (A), reference memory errors (B) and running time (C) were recorded. Each column represents the mean $\pm$ S.E.M. ($n=8-10$). $*p<0.05$ (the Student-Newman-Keuls test).

mitted significantly more working and reference memory errors than those which received vehicle, MK801 or YM90K alone. Though the combined use of MK801 and YM90K increased the maze running time, no synergistic impairment on locomotion was found ($F_{\text{MK801} \times \text{YM90K}}(2,53)=0.76$, $p=0.472$, Fig. 1C).

In contrast to the synergistic action of MK801 and YM90K, the administration of CGS19755 after YM90K had no effect on either spatial working ($F_{\text{CGS19755} \times \text{YM90K}}(2,47)=0.014$, $p=0.986$, Fig. 2A) or reference memory ($F_{\text{CGS19755} \times \text{YM90K}}(2,47)=0.696$, $p=0.504$, Fig. 2B) but significantly increased the maze running time in a synergistic manner ($F_{\text{CGS19755} \times \text{YM90K}}(2,47)=3.44$, $p=0.040$, Fig. 2C).

DISCUSSION

The present study showed that concurrent blockade of the NMDA receptor channel and AMPA receptor impaired spatial memory, while concurrent blockade of the NMDA receptor competitive binding site and AMPA receptors did not, demonstrating that NMDA and AMPA receptors, to some extent, have synergistic interactions.

It is generally accepted that spatial memory depends on hippocampal function during the task performance. Disruption of hippocampal function and blockade of the hippocampal NMDA receptor induces spatial cognitive deficits in rats.^{2,4,10,12} In a previous study,⁴ the AMPA receptor antagonist YM90K (3–10 mg/kg, i.p.) or the NMDA antagonists MK801 (0.03–0.3 mg/kg, i.p.) and CGS19755 (3–10 mg/kg, i.p.), at low doses (YM90K: 3–10 mg/kg, i.p.; MK801: 0.03–0.3 mg/kg, i.p.; CGS19755: 3–10 mg/kg, i.p.), did

Fig. 2. Effects of CGS19755, YM90K and Their Combination on Spatial Working and Reference Memories

Either saline or YM90K was administered i.p. 10 min before the i.p. injection of CGS19755. Thirty minutes after YM90K, the radial maze performance was tested and the numbers of working memory errors (A), reference memory errors (B) and running time (C) were recorded. Three rats in the CGS19755 and YM90K combined group could not finish the maze running and were excluded from the study. Each column represents the mean $\pm$ S.E.M. ($n=8-10$). $*p<0.05$ (the Student-Newman-Keuls test).

not impair spatial memory in a conventional maze task, while the NMDA antagonists (0.3 mg/kg MK801 and 10 mg/kg CGS19755) caused a significant impairment of the performance in the delay-interposed maze task, suggesting that the AMPA receptor contributes slightly, if not at all, to spatial memory compared to the NMDA receptor. The present results that the use of NMDA antagonists MK801 (0.1 or 0.2 mg/kg, i.p.) or CGS19755 (5 or 10 mg/kg, i.p.) separately, failed to impair spatial memory in the conventional maze task agree with our previous findings.

It is likely that, at least at the doses used in the present and previous studies, the levels of MK801 and CGS19755 in the brain, especially in the hippocampus, were not high enough to block the signal transduction needed for spatial memory. However, it should be noted that at the same dose, MK801, but not CGS19755, impaired spatial memory in rats pretreated with 10 mg/kg (i.p.) YM90K. Although no report is available on the intrinsic inhibitory activity of AMPA receptor antagonists on the maze performance of rats, it is likely that a synergistic interaction of NMDA and AMPA receptors exists in the spatial memory process and that blockade of AMPA receptor by YM90K augments the inhibitory effect of MK801 on NMDA receptor in the hippocampus, resulting in apparent impairment of spatial cognition. MK801 blocks the NMDA receptor-gated cation channels when the channel is in the open state, while CGS19755 competes with glutamate at the binding site on the receptor and selectively prevents the activation of the NMDA receptor.^{6,13} Recently, Medina *et al.*¹⁴ reported that activation of the Ca^{2+} permeable AMPA receptors induces an inactivation of the adjacent NMDA re-

ceptor current and that the AMPA receptor blockage prevents the development of NMDA receptor inactivation, yielding a postsynaptic integration of excitatory input. Thus, such a mechanism may be responsible for the synergistic memory deficit found in the present study.

In contrast to the effect of the YM90K and MK801 combination, the combined administration of YM90K and CGS19755 did not synergistically impair the spatial memory. It is unlikely that the difference in the effects may be due to the insufficient dose of CGS19755 since CGS19755, in the same dose range, disrupted the delay interposed radial maze task.⁴⁾ Sharkey *et al.*¹⁵⁾ reported that whilst both MK801 and CGS19755 increased cerebral blood flow beyond that required to meet underlying metabolic demand in the conscious rat, MK801 but not CGS19755 produced focal disturbances in the relationship between the blood flow and glucose metabolism, suggesting a difference in the pharmacological profile between competitive and non-competitive NMDA receptor antagonists. Moreover, in the present study a synergistic increase of the running time was found in the rats treated with YM90K and CGS19755 but not with YM90K and MK801, suggesting changes in the motor activity of the animals following the administration of test drugs. Although the mechanism underlying the synergistic action of YM90K and CGS19755 on maze running remains unclear, these findings suggest different pharmacological actions between an NMDA channel blocker and a competitive antagonist. Thus, one might infer that very likely the distinct interactions with the AMPA receptor may be, at least in part, to these different pharmacological action profiles. These speculative conclusions require further investigation.

In conclusion, the present results indicate that blockade of

the AMPA receptor and the NMDA cation channel synergistically impairs spatial cognition, while blockade of the AMPA receptor and the NMDA competitive binding site did not. These findings support the hypothesis that NMDA and AMPA receptors are crucial in learning and memory.

REFERENCES

- 1) Monaghan D. T., Bridges R. J., Cotman C. W., *Annu. Rev. Pharmacol. Toxicol.*, **29**, 365—402 (1989).
- 2) Li H.-B., Matsumoto K., Tohda M., Yamamoto M., Watanabe H., *Brain Res.*, **725**, 268—271 (1996).
- 3) Staubli U., Rogers G., Lynch G., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 777—781 (1994).
- 4) Li H. B., Matsumoto K., Yamamoto M., Watanabe H., *Pharmacol. Biochem. Behav.*, **58**, 249—253 (1997).
- 5) Moghaddam B., Adams B., Verma A., Daly D., *J. Neurosci.*, **17**, 2921—2927 (1997).
- 6) McBain C. J., Mayer M. L., *Pharmacol. Rev.*, **74**, 723—760 (1994).
- 7) Nakanishi S., *Science*, **258**, 597—603 (1992).
- 8) Bekkers J. M., Stevens C. F., *Nature (London)*, **341**, 230—233 (1989).
- 9) Nusser Z., Mulvihill E., Streit P., Somogyi P., *Neuroscience*, **61**, 421—427 (1994).
- 10) Olton D. S., Samuleson R. J., *J. Exp. Psychol.: Animal Behav. Processes*, **2**, 97—116 (1976).
- 11) Ohmori J., Sakamoto S., Kubota H., Shimizu-Sasamata M., Okada M., Kawasaki S., Hidaka K., Togami J., Furuya T., Murase K., *J. Med. Chem.*, **37**, 467—475 (1994).
- 12) Morris R. G. M., Anderson E., Lynch G., Baudry M., *Nature (London)*, **319**, 774—776 (1986).
- 13) Jahr C. E., *Science*, **255**, 470—472 (1992).
- 14) Medina J., Filippova N., Barbin G., Ben-Ari Y., Bregestovski P., *J. Neurophysiol.*, **72**, 456—465 (1994).
- 15) Sharkey J., Ritchie I. M., Butcher S. P., Kelly J. S., *Brain Res.*, **651**, 27—36 (1994).