ELSEVIER

European Journal of Pharmacology 312 (1996) 145–147

ejp

Short communication

Interactive processing between glutamatergic and cholinergic systems involved in inhibitory avoidance learning of rats

Masuo Ohno *, Shigenori Watanabe

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University 62, Fukuoka 812-82, Japan

Received 23 May 1996; accepted 12 July 1996

Abstract

Rats were tested with a one-trial inhibitory avoidance paradigm in which the latency to enter the shock compartment served as a measure of memory retention. Pretraining administration of the non-competitive NMDA receptor antagonist MK-801 (0.3 mg/kg i.p.) significantly reduced the response latency during the retention test given 24 h after rats received a step-through inhibitory avoidance training. MK-801 at 0.1 mg/kg did not affect the retention latency. The muscarinic receptor antagonist scopolamine (1.0 mg/kg i.p.) also interfered with the inhibitory avoidance response in the retention test when administered before the training trial. The lower dose of 0.3 mg/kg scopolamine, which by itself was ineffective, significantly impaired inhibitory avoidance learning when administered simultaneously with the behaviorally subthreshold dose of 0.1 mg/kg MK-801 before the training trial. These results suggest that interactive mechanisms regulated by concurrent activation of NMDA and muscarinic receptors are involved in learning processes of inhibitory avoidance performance in rats.

Keywords: Glutamate; Acetylcholine; NMDA receptor; Learning; Memory; Inhibitory avoidance task

1. Introduction

It is well documented that mechanisms mediated by NMDA receptors are responsible for induction of long-term potentiation in the hippocampus which is hypothesized to be a neural basis of memory formation (Bliss and Collingridge, 1993), and that administration of both competitive and non-competitive NMDA receptor antagonists disrupts memory performances of rats and mice in some behavioral paradigms including an inhibitory avoidance task (Danysz et al., 1988; DeNoble et al., 1990; Morris, 1989; Parada-Turska and Turski, 1990; Venable and Kelly, 1990; Ward et al., 1990). On the other hand, the central cholinergic system is also known to play a critical role in learning and memory (Smith, 1988). For example, lesions or degeneration of the cholinergic nervous system and pharmacological antagonism of acetylcholine receptors produce cognitive deficits in both experimental animals and humans. In contrast to extensive evidence that the glutamatergic and cholinergic systems play individual roles in learning and memory processes, little is known about the possible interactive mechanisms between these neurotransmissions in the regulation of mnemonic processing. In the present study, we investigated the effects of combined administration of the non-competitive NMDA receptor antagonist MK-801 and the muscarinic receptor antagonist scopolamine on inhibitory avoidance learning of rats, and thereby clarified whether NMDA receptor-mediated glutamatergic neurotransmission regulated memory processes by interacting with the cholinergic system.

2. Materials and methods

The animals used were male rats of the Wistar strain (Japan SLC, Shizuoka, Japan) weighing 220–300 g. The rats were housed under a constant temperature (23 ± 2°C) and a 12-h light/dark cycle (light period 07.00–19.00 h), and were allowed free access to food and water. Behavioral tests were carried out between 10.00 and 15.00 h with a step-through inhibitory avoidance apparatus which consisted of two compartments separated by a sliding guillotine door. The starting compartment (25 cm long, 10 cm wide and 30 cm high) was illuminated with a 100 W incandescent light bulb placed 40 cm above the floor. The

* Corresponding author. Fax: +81 92 632 2752.

PII S0014-2999(96)00580-8

other compartment was a dark box which measured 30 × 30 × 30 cm and had a grid floor made of 23 parallel stainless steel rods (3 mm in diameter) spaced 1 cm apart. A shock generator (model SGS-002; Muromachi Kikai, Tokyo, Japan) was connected to the steel rods and provided scrambled footshock.

On the training trial, the rat was placed in the illuminated compartment of the inhibitory avoidance box. After a 10-s delay, the slide door was raised so that the rat could pass through the entrance (8 cm wide, 8 cm high) to the dark compartment. When the rat stepped completely into the dark compartment (with all four paws on the shock grid floor), the door was lowered, and after a 10-s delay, a 0.5 mA scrambled shock was applied to the grid floor for 5 s. The rats were immediately removed from the dark box and were returned to their home cage. The time to enter the dark compartment was measured (training latency). On the retention test given 24 h later, the rats were placed in the illuminated compartment. The retention trial proceeded in the same manner as the training trial except that no footshock was delivered when the rats entered the dark box. The response latency to enter the dark compartment (retention latency) was measured. During the retention test, the rats were allowed access to the dark compartment for a maximum of 300 s; the rat that did not enter the dark box within 300 s was assigned a retention latency of 300 s.

(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801; Research Biochemicals International) and (−)-scopolamine hydrobromide (Sigma Chemical Co.) were dissolved in saline, and were administered i.p. in a volume of 0.1 ml per 100 g body weight. The doses were expressed in terms of the salt. Rats were injected with MK-801 and scopolamine, respectively, 30 min and 20 min before they received the inhibitory avoidance training. The statistical significance of differences in step-through latencies between the groups was analyzed by means of the Mann-Whitney *U*-test.

3. Results

The latency to enter the dark compartment on the training trial for vehicle-treated control animals was 7.9 ± 1.3 s (mean ± S.E.M., $n = 10$). As shown in Fig. 1A and Fig. 2A, no significant effects of drug treatments on the training latencies were observed whether MK-801 and scopolamine were administered individually or in combination. On the retention trial tested 24 h later, the control rats exhibited the long step-through latency (286.2 ± 12.0 s, $n = 10$), indicating good acquisition and retention of the inhibitory avoidance response (Fig. 1B and Fig. 2B).

The rats that received 0.3 mg/kg MK-801 before the inhibitory avoidance training showed significantly shorter response latency during the retention test as compared with the control rats (Fig. 1B). At the lower dose of 0.1 mg/kg, however, MK-801 had no effect on the retention latency.

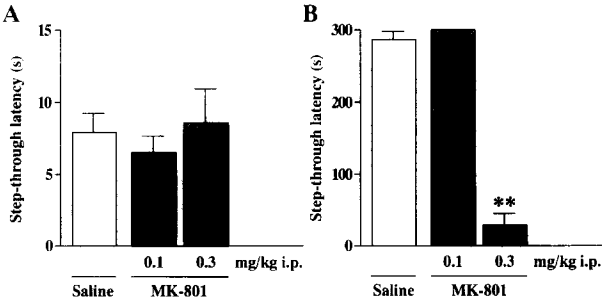

Fig. 1. Effect of pretraining administration of MK-801 on the inhibitory avoidance response in rats. Each column represents the mean ± S.E.M. of step-through latencies for 7–10 animals on the training trial (A) and those on the retention trial tested 24 h later (B). The significance of differences from the saline-injected group was determined by Mann-Whitney *U*-test (** $P < 0.01$).

Pretraining administration of 1.0 mg/kg scopolamine, but not that of 0.1 or 0.3 mg/kg scopolamine, produced a significant decrease in the latency to enter the dark compartment during the retention test (Fig. 2B). Scopolamine at 0.3 mg/kg, but not 0.1 mg/kg scopolamine, significantly reduced the retention latency when administered concurrently with the behaviorally ineffective dose of 0.1 mg/kg MK-801 before the inhibitory avoidance training. The response latency of rats that received combined administration of 0.3 mg/kg scopolamine and 0.1 mg/kg

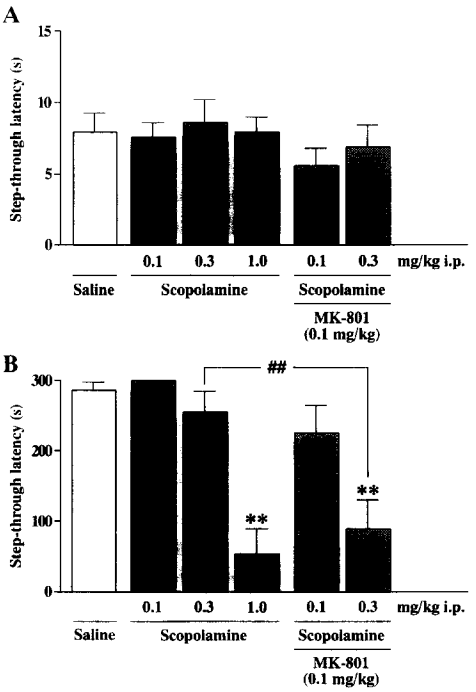

Fig. 2. Effects of pretraining administration of scopolamine and MK-801 on the inhibitory avoidance response in rats. Each column represents the mean ± S.E.M. of step-through latencies for 7–10 animals on the training trial (A) and those on the retention trial tested 24 h later (B). The significance of differences from the saline-injected group (** $P < 0.01$) and from the 0.3 mg/kg scopolamine-injected group (## $P < 0.01$) was determined by Mann-Whitney *U*-test.

MK-801 was significantly shorter than that of rats treated with 0.3 mg/kg scopolamine alone.

4. Discussion

Consistent with the previous reports showing that pharmacological blockade of NMDA receptors and muscarinic cholinergic receptors impaired inhibitory avoidance learning of rats (Danysz et al., 1988; DeNoble et al., 1990; Venable and Kelly, 1990), pretraining administration of the non-competitive NMDA receptor antagonist MK-801 or the muscarinic antagonist scopolamine produced a deficit of inhibitory avoidance response in the retention test. The major finding of the present study was that the behaviorally ineffective doses of MK-801 and scopolamine significantly impaired inhibitory avoidance learning when administered in combination before training, suggesting some interactive mechanism via NMDA and muscarinic receptors involved in memory processes. Likewise, Matsuoka and Aigner (1996) recently demonstrated that low doses of MK-801 and scopolamine, which were ineffective when given alone, disrupted visual recognition memory of rhesus monkeys in a delayed non-matching-to-sample task. D-Cycloserine, a partial agonist at the glycine site on the NMDA receptor/channel complex, has been reported to attenuate the scopolamine-induced deficits in spatial learning of rats in a Morris water maze task and those in memory performance of rats in a T-maze alternation paradigm (Fishkin et al., 1993). Although brain sites responsible for such functional interactions between glutamatergic and cholinergic systems in memory processes are still unknown, the hippocampus is an important structure in which the individual transmission plays a key role in mediating inhibitory avoidance learning of rats; the competitive NMDA receptor antagonist AP5 or scopolamine produces an amnesic effect when administered directly into the hippocampus immediately after training (Izquierdo et al., 1992; Jerusalinsky et al., 1992). Markram and Segal (1990) found, using hippocampal slices, that acetylcholine, acting on muscarinic receptors, amplified NMDA receptor-mediated responses and thereby caused a long-lasting facilitation of excitatory postsynaptic potentials, suggesting the cholinergic activity functioning to modulate the glutamatergic transmission postsynaptically in the hippocampus. Taken together, these findings suggest that a link between the NMDA and muscarinic receptor-mediated neurotransmission is involved in the regulation of memory processes, although the precise mechanism and candidate structures for mediating the functional interactions between the two nervous systems remain to be clarified.

Numerous populations of neurons and neurotransmitter systems, including the glutamatergic and cholinergic systems, are damaged simultaneously in the brain of patients with Alzheimer's disease (Greenamyre and Maragos, 1993; Hardy et al., 1985), and such deficits in multiple systems may be a factor in causing the profound memory impairment in this disease. Thus, the present finding provides evidence that the concomitantly damaged glutamatergic and cholinergic neurotransmission contributes in some manner to the severity of memory decline in Alzheimer's disease.

Acknowledgements

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

Bliss, T.V.P. and G.L. Collingridge, 1993, A synaptic model of memory: long-term potentiation in the hippocampus, Nature (London) 361, 31.

Danysz, W., J.T. Wroblewski and E. Costa, 1988, Learning impairment in rats by *N*-methyl-D-aspartate receptor antagonists, Neuropharmacology 27, 653.

DeNoble, V.J., K.W. Jones, C.L. Schaeffer and L.M. Bauerle, 1990, 3-((±)-2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and phencyclidine produce a deficit of passive avoidance retention in rats, Eur. J. Pharmacol. 175, 197.

Fishkin, R.J., E.S. Ince, W.A. Carlezon, Jr. and R.W. Dunn, 1993, D-Cycloserine attenuates scopolamine-induced learning and memory deficits in rats, Behav. Neural Biol. 59, 150.

Greenamyre, J.T. and W.F. Maragos, 1993, Neurotransmitter receptors in Alzheimer disease, Cerebrovasc. Brain Metab. Rev. 5, 61.

Hardy, J., R. Adolfsson, I. Alafuzoff, G. Bucht, J. Marcusson, P. Nyberg, E. Perdahl, P. Wester and B. Winblad, 1985, Transmitter deficits in Alzheimer's disease, Neurochem. Int. 7, 545.

Izquierdo, I., C. Da Cunha, R. Rosat, D. Jerusalinsky, M.B.C. Ferreira and J.H. Medina, 1992, Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat, Behav. Neural Biol. 58, 16.

Jerusalinsky, D., M.B.C. Ferreira, R. Walz, R.C. Da Silva, M. Bianchin, A.C. Ruschel, M.S. Zanatta, J.H. Medina and I. Izquierdo, 1992, Amnesia by post-training infusion of glutamate receptor antagonists into the amygdala, hippocampus, and entorhinal cortex, Behav. Neural Biol. 58, 76.

Markram, H. and M. Segal, 1990, Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine, J. Physiol. 427, 381.

Matsuoka, N. and T.G. Aigner, 1996, Cholinergic-glutamatergic interactions in visual recognition memory of rhesus monkeys, NeuroReport 7, 565.

Morris, R.G.M., 1989, Synaptic plasticity and learning: selective impairment of learning in rats and blockade of long-term potentiation in vivo by the *N*-methyl-D-aspartate receptor antagonist AP5, J. Neurosci. 9, 3040.

Parada-Turska, J. and W.A. Turski, 1990, Excitatory amino acid antagonists and memory: effect of drugs acting at *N*-methyl-D-aspartate receptors in learning and memory tasks, Neuropharmacology 29, 1111.

Smith, G., 1988, Animal models of Alzheimer's disease: experimental cholinergic denervation, Brain Res. Rev. 13, 103.

Venable, N. and P.H. Kelly, 1990, Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice, Psychopharmacology 100, 215.

Ward, L., S.E. Mason and W.C. Abraham, 1990, Effects of the NMDA antagonists CPP and MK-801 on radial arm maze performance in rats, Pharmacol. Biochem. Behav. 35, 785.