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Short communication

D-Cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats

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Abstract

D-Cycloserine, a partial agonist at the glycine binding site on the NMDA receptor/channel complex, did not affect the number of errors (attempts to pass through two incorrect panels of the three-panel gates at four choice points) in the working memory task with a three-panel runway setup, when injected bilaterally at 1 or 10 μg/side into the dorsal hippocampus. Intrahippocampal administration of the muscarinic receptor antagonist scopolamine (3.2 μg/side) significantly increased the number of working memory errors. The increase in working memory errors induced by intrahippocampal 3.2 μg/side scopolamine was significantly reduced by concurrent infusion of 1 and 10 μg/side D-cycloserine. These results suggest that positive modulation of the NMDA receptor/channel through activation of the glycine site can compensate dysfunction of hippocampal cholinergic neurotransmission involved in working memory function.

Keywords: D-Cycloserine; Glycine site; NMDA receptor; Acetylcholine; Hippocampus; Working memory

1. Introduction

It has been reported that administration of both competitive and noncompetitive NMDA receptor antagonists disrupts memory performances of rats and mice in some behavioral paradigms that depend on the hippocampal function (Butelman, 1989; Davis et al., 1992; Parada-Turska and Turski, 1990; Ward et al., 1990), suggesting the importance of NMDA receptor-mediated neurotransmission in the memory processes in the hippocampus. Also, glycine modulation at the strychnine-insensitive glycine site on the NMDA receptor complex is required for acquisition of spatial memory in the Morris water maze task, since this behavior is sensitive to blockade of the glycine site by 7-chlorokynurenic acid (Watanabe et al., 1992). On the other hand, the central cholinergic system is thought to play a critical role in learning and memory (Smith, 1988); lesions or degeneration of the cholinergic nervous system and pharmacological antagonism of acetylcholine receptors produce cognitive deficits in both experimental animals and humans. Recent behavioral studies demonstrated that D-cycloserine, a partial agonist at the glycine modulatory site of the NMDA receptor/channel complex, attenuated the failure of learning and memory performance of rats induced by the muscarinic acetylcholine receptor antagonist scopolamine in the water maze task and in the T-maze alternation paradigm (Fishkin et al., 1993; Sirviö et al., 1992). D-Cycloserine has also been shown to attenuate scopolamine-induced memory impairements in monkeys (Matsuoka and Aigner, 1996a) and in humans (Jones et al., 1991). Furthermore, D-cycloserine was effective in reversing impairment of spatial working memory in hippocampal-lesioned rats, as assessed by the eight-arm radial maze task (Schuster and Schmidt, 1992). These findings suggest that the hippocampus is a candidate structure responsible for the interactive processes between cholinergic and glutamatergic systems associated with the mnemonic function. We previously demonstrated, using a three-panel runway task, that intrahippocampal administration of scopolamine or the competitive NMDA receptor antagonists disrupted working memory performance of rats, i.e., acquisition of new and variable information that was useful only within a session (Ohno et al., 1992), indicating that this behavior was suitable for the investigation of memory processes mediated by hippocampal muscarinic and NMDA receptors. The purpose of the present study was to clarify the hippocampal muscarinic/glutamatergic interaction in regulating working memory function, by investigating the effects of in-

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trahippocampal injection of D-cycloserine on the working memory failure caused by scopolamine.

2. Materials and methods

2.1. Three-panel runway task

Eight- to ten-week-old male rats of the Wistar strain (Japan SLC) were placed on a deprivation schedule to maintain their weights at approximately 80% of the free-feeding level (230–250 g) prior to the experiment. Working memory was assessed with a three-panel runway apparatus, as described in our previous reports (Ohno and Watanabe, 1996a; Ohno et al., 1992). In brief, this apparatus (175 × 36 × 25 cm) was composed of a start box, a goal box and four consecutive choice points intervening between them. Each choice point consisted of a gate with three panels (12 × 25 cm). The rats were prevented from passing through two of the three panels in the gate by front stoppers, and were prevented from returning to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all the gates. When the rats reached the goal box, they received two food pellets (about 50 mg each; Muromachi Kikai) as positive reinforcement. The rats were made to run the task in six consecutive trials (defined as one session) per day with removal of the front stopper of only one of the three-panel gates (the correct panel gate) at each choice point. Trials were run at 2-min intervals, and water was freely available between trials in the home cage. The locations of the correct panel gates were held constant within a session, but were changed from one session to the next (working memory procedure). Twelve different patterns of correct panel gate locations were used, as described previously (Ohno and Watanabe, 1996a).

The number of times an animal attempted to pass through an incorrect panel gate (defined as errors) and the time required for the animal to obtain food pellets (defined as latency) were recorded for each rat during each trial of a session. Since repetitive attempts to enter the same incorrect panel gate were counted as one error, the maximal level was two errors at each choice point, and thus eight errors per trial. The number of errors and latency recorded in the first trial were presented separately, and those parameters in the second to the sixth trial of a session were summed together for the evaluation of working memory function. The learning criterion was less than eight errors summed from the second to sixth trials (working memory errors). A rat was used in the experiment, if it achieved this criterion in three consecutive sessions.

2.2. Surgery and experimental procedures

Five animals that achieved the learning criterion were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and were implanted bilaterally with guide cannulae for microinjection of drugs into the hippocampus, as described previously (Ohno and Watanabe, 1996a; Ohno et al., 1992). The position of the injection cannula tip, which protruded 1.0 mm below the tip of the guide cannula, was aimed at the dorsal hippocampus (3.8 mm posterior to the bregma, 2.2 mm lateral to the midline, 3.2 mm ventral to the surface of the skull measured at the bregma) according to the brain atlas of Paxinos and Watson (1982). The rats were allowed at least 5 days of postoperative recovery before runway sessions were resumed. The rats were used after it was confirmed that they met the learning criterion following the surgical manipulation.

D-Cycloserine (Research Biochemicals International, Natick, MA, USA) and scopolamine hydrobromide (Sigma, St. Louis, MO, USA) were dissolved in saline. Two microliters of the drug solution or saline was injected into the dorsal hippocampus through the injection cannula, which was connected to a 5-μl Hamilton syringe via a polyethylene tube. The rate of injection was 0.5 μl/min. The injection cannula was left in place for 1 min after completion of the injection, to facilitate diffusion of the drug. Rats received the runway test 10 min after intrahippocampal injection of the drug was completed. The five animals used were given microinjections repeatedly with the sequence of drug and dose administration varying on a random basis; a minimum of 3 days was allowed between the microinjections. Performance on the runway task during non-injected sessions was not affected by repeated injections, and met the learning criterion.

After completion of behavioral testing, each rat was deeply anesthetized with ether and then perfused transcardially with saline, followed by 4% paraformaldehyde solution. The brains were removed from the skull and post-fixed for 48 h in paraformaldehyde solution. Thereafter, 50-μm thick sections were stained with Cresyl violet to verify the injection site histologically, as described previously (Ohno and Watanabe, 1996a; Ohno et al., 1992).

2.3. Statistical analysis

The significance of differences between the groups was determined by a one-way analysis of variance (ANOVA) followed by Dunnett's test when F ratios reached significance ($P < 0.05$).

3. Results

In the three-panel runway task, the random performance level was four errors per trial, or 24 errors per session. In the working memory task, the number of errors made from the second to the sixth trial (working memory errors) markedly decreased with repeated training, whereas the errors in the first trial remained constant at approximately four. Approximately 20–30 training sessions were required

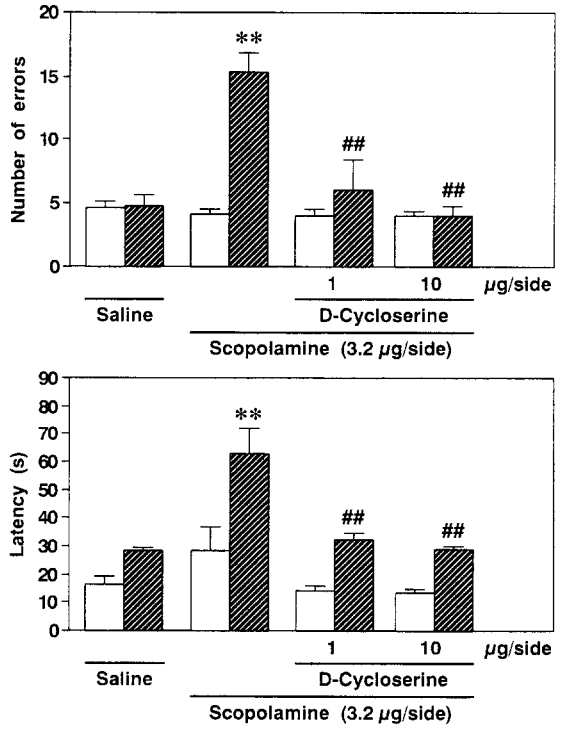

Fig. 1. Effects of concurrent injection of D-cycloserine on increases in working memory errors and latency induced by intrahippocampal administration of scopolamine. Rats received the runway test 10 min after drugs were administered. Each column represents the mean ± S.E.M. of errors and latencies for 5 animals recorded in the first trial (open columns), and those summed from the second to the sixth trial within a session (hatched columns). The significance of differences from the saline-injected group (** $P < 0.01$) and from the scopolamine-injected group (## $P < 0.01$) was determined by a one-way ANOVA followed by Dunnett's test.

for the rats to reach the criterion of less than eight working memory errors. Latency was also reduced during repeated sessions and was stable from the 10th session on.

D-Cycloserine at 1 or 10 μg/side, administered bilaterally into the dorsal hippocampus, did not affect the number of errors in the working memory task or the latency to obtain food pellets placed in the goal box (Table 1).

Scopolamine at 3.2 μg/side significantly increased the number of working memory errors when administered intrahippocampally ($F(1,8) = 40.13$, $P < 0.01$), although it had no effect on the number of errors made in the first trial (Fig. 1). Intrahippocampal administration of 3.2 μg/side scopolamine also significantly prolonged the latency to obtain food pellets from the second to sixth trials of a session ($F(1,8) = 14.05$, $P < 0.01$). The increase in working memory errors induced by intrahippocampal injection of 3.2 μg/side scopolamine was significantly reduced by concurrent administration of 1 and 10 μg/side D-cycloserine ($F(2,12) = 12.99$, $P < 0.01$). D-Cycloserine at 1 and 10 μg/side was also effective in decreasing the prolonged latency resulting from administration of 3.2 μg/side scopolamine into the hippocampus ($F(2,12) = 11.57$, $P < 0.01$).

4. Discussion

We previously reported that working memory performance on the three-panel runway task was sensitive not only to hippocampal NMDA receptor blockade but also to antagonism of the glycine modulatory site by intrahippocampal injection of 7-chlorokynurenic acid (Ohno et al., 1992, 1994), suggesting that this mnemonic behavior depended upon positive modulation of the NMDA receptor channel via the glycine binding site. In the present study, intrahippocampal administration of D-cycloserine, a partial agonist at the glycine site associated with the NMDA receptor complex, did not affect working memory performance of rats on the three-panel runway task. Some studies showed that D-cycloserine was effective in facilitating acquisition and memory retrieval processes assessed by various behavioral paradigms, such as the T-maze spatial learning task, passive avoidance test and eye-blink conditioning task (Flood et al., 1992; Monahan et al., 1989; Thompson et al., 1992). Concerning a possible explanation for the ineffectiveness of intrahippocampal D-cycloserine in this experiment, it is most likely that the rapid acquisition performance of rats during the working memory session does not allow us to detect the facilitatory effect of D-cycloserine on the normal memory function.

In addition to the reversal by systemic administration of D-cycloserine of memory failure caused by the muscarinic receptor antagonist scopolamine (Fishkin et al., 1993; Jones et al., 1991; Matsuoka and Aigner, 1996a; Sirviö et al., 1992), the interactive mechanism between cholinergic and glutamatergic systems in memory processes was recently evidenced by the fact that the behaviorally subthreshold doses of scopolamine and the noncompetitive NMDA receptor antagonist MK-801 ((+)-5-methyl-10,11-dihydro-

Table 1
Effects of intrahippocampal injection of D-cycloserine on the number of errors and latency in a working memory task

Drug	μg/side	n	Number of errors		Latency (s)	
			Trial 1	Trial 2–6	Trial 1	Trial 2–6
Saline	–	5	4.6 ± 0.5	4.8 ± 0.9	16.6 ± 2.5	28.2 ± 1.4
D-Cycloserine	1	5	4.4 ± 0.2	5.2 ± 1.8	14.4 ± 1.5	28.4 ± 1.4
	10	5	4.8 ± 0.4	4.4 ± 1.1	14.8 ± 1.3	28.2 ± 1.1

Rats received the runway test 10 min after D-cycloserine was injected. Values are means ± S.E.M. of errors and latencies recorded in the first trial, and those summed from the second to the sixth trial of a session.

5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) significantly impaired visual recognition memory of rhesus monkeys in a delayed nonmatching-to-sample task when both drugs were administered systemically and concomitantly (Matsuoka and Aigner, 1996b). We also found that the ineffective dose of MK-801 aggravated scopolamine-induced disruption of inhibitory avoidance learning in rats (Ohno and Watanabe, 1996b). The present study showed that the working memory failure induced by local infusion of scopolamine into the hippocampus was reversed by concurrent injection of D-cycloserine. We recently reported that cholinergic activation by the cholinesterase inhibitor physostigmine was also effective in preventing the working memory deficit by intrahippocampal scopolamine (Ohno et al., 1996). These results suggest that the septo-hippocampal cholinergic activity is necessary for working memory performance, i.e., acquisition process of new information within a session, and that positive modulation of NMDA receptor-mediated neurotransmission via activation of the glycine site can compensate the hippocampal muscarinic deficiency in terms of working memory function. It is, however, important to note that physostigmine does not affect impairment of working memory caused by intrahippocampal administration of the competitive NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) (Ohno et al., 1996), a finding suggesting that the cholinergic activation is insufficient to support the memory processing through specific hippocampal glutamatergic pathway when NMDA receptor-mediated transmission is fully blocked. This finding forms a contrast to the action of D-cycloserine on the scopolamine disruption of memory performance in the present study, showing that the cholinergic and glutamatergic systems are differentially involved in working memory function. It is conceivable that glutamatergic neurotransmission via NMDA receptors rather than the cholinergic mechanism functions as a final mediator of the memory processes in the hippocampus.

It is possible that glutamatergic regulation of hippocampal acetylcholine release contributes to neural mechanisms by which intrahippocampal D-cycloserine reverses the scopolamine-induced deficit in working memory. In fact, activation of NMDA receptors can enhance acetylcholine release in certain brain structures, such as the medial septum, cerebral cortex and striatum (Moor et al., 1996; Nishimura and Boegman, 1990; Ulus et al., 1992). It is probable that systemically administered D-cycloserine enhances the opening of the NMDA receptor channel on cholinergic neurons to stimulate acetylcholine release, and thereby attenuates scopolamine-induced memory impairments (Fishkin et al., 1993; Matsuoka and Aigner, 1996a). However, NMDA receptor activation fails to increase [^{3}H]acetylcholine release from brain slices of the hippocampus (Ulus et al., 1992) or in vivo acetylcholine release measured in the hippocampus by microdialysis (Moor et al., 1996). It seems, therefore, unlikely that intrahippocampal administration of D-cycloserine reverses the scopolamine disruption of working memory by increasing acetylcholine release at the level of cholinergic nerve terminals in the hippocampus.

Alternatively, postsynaptic interactions between cholinergic and glutamatergic systems in the hippocampus may account for the reversal by intrahippocampal D-cycloserine of scopolamine-induced working memory failure. Markram and Segal (1990) demonstrated, using hippocampal slices, that acetylcholine amplified NMDA receptor-mediated responses through acting on muscarinic receptors 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. The cholinergic facilitation of glutamate-mediated synaptic transmission in the hippocampus can be considered to underlie the known role of the cholinergic system in mnemonic processes. The present finding of reversal by D-cycloserine of working memory failure resulting from hippocampal muscarinic blockade provides direct evidence that a link between NMDA and muscarinic receptor-mediated neurotransmission in the hippocampus contributes to the regulation of memory processes, although the precise mechanism for mediating such functional interactions between the two neuronal systems remains to be clarified.

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