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Noradrenergic DSP-4 Lesions Aggravate Impairment of Working Memory Produced by Hippocampal Muscarinic Blockade in Rats

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OHNO, M., A. YOSHIMATSU, M. KOBAYASHI AND S. WATANABE. *Noradrenergic DSP-4 lesions aggravate impairment of working memory produced by hippocampal muscarinic blockade in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 257–261, 1997.—To clarify the interactions between hippocampal cholinergic and adrenergic systems in working memory function of rats, the effects of hippocampal muscarinic receptor blockade combined with noradrenaline depletion on this behavior were examined with a three-panel runway task. Intrahippocampal administration of the muscarinic receptor antagonist scopolamine at a dose of 3.2 μg/side significantly increased 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, whereas the 0.32 μg/side dose of scopolamine did not affect working memory errors. Administration of the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) at 50 mg/kg IP caused a marked reduction in hippocampal noradrenaline concentration, but it had no effect on working memory errors. Intrahippocampal administration of 0.32 μg/side scopolamine, the behaviorally ineffective dose in intact rats, significantly increased the number of working memory errors in the noradrenaline-depleted animals. These results suggest that hippocampal muscarinic/noradrenergic interactions are involved in neural processes mediating working memory function of rats. © 1997 Elsevier Science Inc.

Noradrenaline Acetylcholine Hippocampus DSP-4 (*N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine) Scopolamine Working memory

MULTIPLE neurotransmitter deficits occur in the brains of patients with Alzheimer's disease (AD) (12,13), and may contribute to the severity of memory decline in that disease. In fact, Bondareff et al. (3,4) suggested that a subgroup of AD patients with noradrenergic dysfunction characterized by a reduced number of locus coeruleus (LC) neurons exhibited more severe cognitive decline than AD patients with LC neuronal counts comparable to those of age-matched control subjects. Consistently, we demonstrated, using a three-panel runway task, that both DSP-4-induced noradrenaline depletion and β-adrenergic blockade by propranolol exacerbated impairment of working memory induced by systemic administration of the muscarinic receptor antagonist scopolamine (18,22). Intrahippocampal administration of scopolamine or the M_1-selective muscarinic receptor antagonist pirenzepine was also effective in disrupting working memory performance of rats, i.e., acquisition of new and variable information that was useful only within a session (21,23), indicating that this behavior depended on septohippocampal cholinergic function. These findings suggest that the hippocampus is one candidate structure for mediating the interactions between cholinergic and noradrenergic systems associated with working memory. The purpose of the present study was to clarify the hippocampal muscarinic/adrenergic interaction in regulating working memory function, by investigating the effects of intrahippocampal injection of scopolamine combined with noradrenergic depletion on working memory performance.

METHOD

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

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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 (20,21,23). 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 (20).

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.

Surgery and Experimental Procedures

The rats that achieved the learning criterion were anesthetized with sodium pentobarbital (40 mg/kg IP), and were implanted bilaterally with guide cannulae for microinjection of scopolamine into the hippocampus, as described previously (20,21,23). 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 (27). 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.

(−)-Scopolamine hydrobromide (Sigma Chemical Co.) was dissolved in saline. Two microliters of the scopolamine 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. On the test day, rats received a single injection of the drug, and then they were given a test session, starting from 10 min after the intrahippocampal injection was completed. As microinjections were made repeatedly into each rat (two doses of scopolamine and saline), a minimum of three days were allowed between microinjections. Performance on the runway task during non-injected sessions was not affected by repeated injections, and met the learning criterion.

N-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4; Sigma Chemical Co.) was prepared in saline immediately before use. After the effects of intrahippocampal scopolamine were examined, the animals were treated with DSP-4 (50 mg/kg IP). It has been reported that DSP-4-induced depletion of brain noradrenaline was permanent, whereas peripheral changes were transient and were followed by a gradual recovery of noradrenergic deficits (17,29). Thus, the rats received the runway test 14 days after the DSP-4 treatment when decreased noradrenaline levels in peripheral organs were expected to revert to normal, and no training session was given during this period. Thereafter, the DSP-4-treated animals received intrahippocampal scopolamine injection followed by the runway test.

Histology and Biochemistry

After completion of behavioral testing, each rat was deeply anesthetized with ether and then perfused transcardially with saline, followed by a 4% paraformaldehyde solution. The brains were removed from the skull and postfixed 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 (20,21,23). Furthermore, in our previous experiments, we also examined the diffusion of dye that was injected intrahippocampally at a volume of 2 μl/side, and confirmed that the dye injections were confined to the dorsal hippocampus (20,21,23).

To determine the extent of noradrenaline depletion produced by DSP-4, 50 mg/kg DSP-4 was administered to four behaviorally naive animals. Fourteen days later, the animals were decapitated after stunning and the brains were rapidly removed, and then the hippocampus was separated from the brains on ice. Samples were then assayed for contents of noradrenaline, dopamine and serotonin, using the HPLC-ECD system.

Statistics

The statistical significance of differences between the groups was determined by Student's *t*-test or by a one-way analysis of variance (ANOVA) followed by Dunnett's test.

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 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.

Scopolamine, administered bilaterally at 0.32 and 3.2 μg/side into the dorsal hippocampus, increased the number of working memory errors [$F(2, 12) = 24.67$, $P < 0.01$], an effect that was significant only for the 3.2 μg/side dose, while it had no effect on the number of errors made in the first trial (Fig. 1). Intrahippocampal scopolamine had a tendency to prolong the latency to obtain food pellets in trials 2–6 [$F(2, 12) = 3.72$,

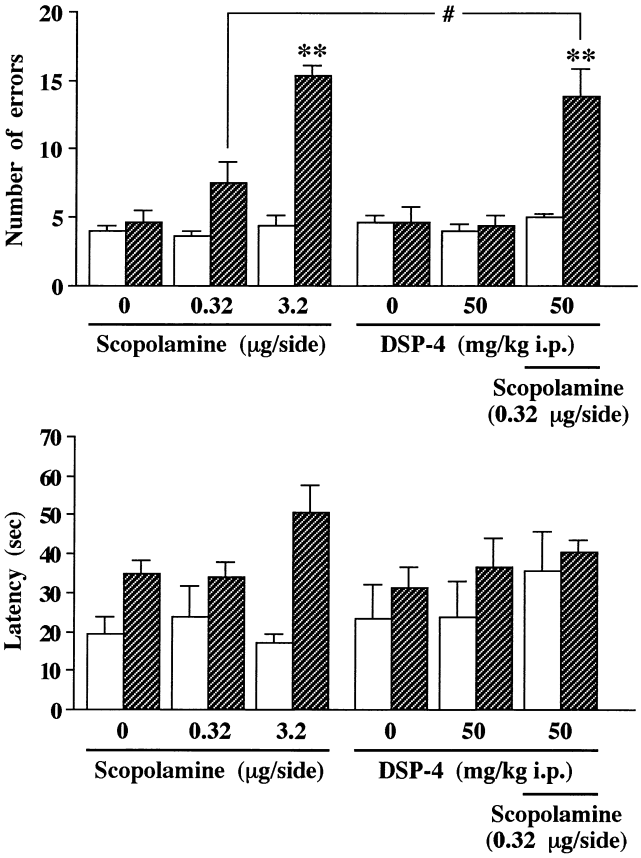

FIG. 1. Effects of intrahippocampal injection of scopolamine on the number of working memory errors and latency in intact and DSP-4-treated rats. The runway test was given 14 days after DSP-4 was administered. Rats received scopolamine injection 10 min before testing. Each column represents the mean ± SEM 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 0.32 μg/side scopolamine-injected group (#$P < 0.05$) was determined by a one-way ANOVA followed by Dunnett's test.

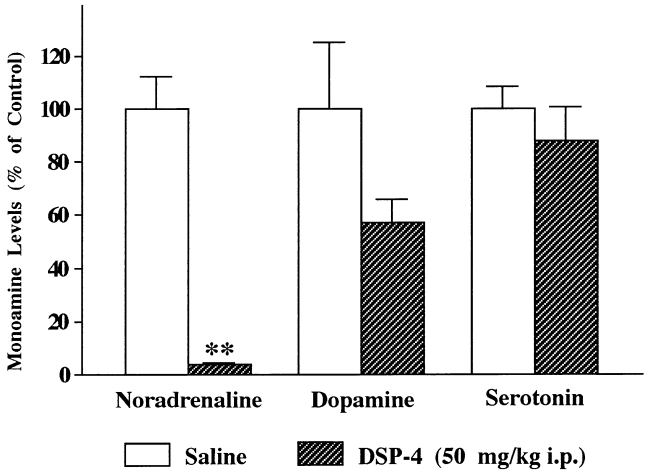

FIG. 2. Effects of DSP-4 on the concentrations of noradrenaline, dopamine and serotonin in the hippocampus. Animals were sacrificed 14 days after saline or 50 mg/kg DSP-4 was administered. Each column represents the mean ± SEM of contents of monoamines for 4 animals expressed as percentage of those of the saline-treated group. The significance of differences from the saline group was determined by Student's *t*-test, **$P < 0.01$.

$P < 0.1$], but this effect did not reach significance. DSP-4 at a dose of 50 mg/kg, given IP 14 days before testing, did not affect the number of working memory errors. Intrahippocampal administration of 0.32 μg/side scopolamine, which had no effect on errors in intact rats, caused a significant increase in working memory errors in the 50 mg/kg DSP-4-treated rats [$F(1, 8) = 14.85$, $P < 0.01$]. The number of working memory errors in animals given combined treatment with 50 mg/kg DSP-4 and intrahippocampal 0.32 μg/side scopolamine was significantly higher than that of animals treated with 0.32 μg/side scopolamine alone [$F(1, 8) = 5.82$, $P < 0.05$]. Rats treated with DSP-4 did not show the prolonged latency to obtain food pellets placed in the goal box, irrespective of whether they received intrahippocampal 0.32 μg/side scopolamine or not.

The concentrations of noradrenaline, dopamine and serotonin in the hippocampus of saline-treated rats were 407.5 ± 48.8, 37.8 ± 9.5 and 696.7 ± 59.0 ng/g wet weight tissue (means ± SEM), respectively. Treatment with 50 mg/kg DSP-4 almost completely depleted noadrenaline content in the hippocampus without affecting the level of serotonin (Fig. 2). DSP-4 slightly reduced the content of dopamine in the hippocampus, which was not a statistically significant effect.

DISCUSSION

In the present study, working memory performance on the three-panel runway task was significantly impaired by intrahippocampal administration of the muscarinic receptor antagonist scopolamine, but was not affected by brain noradrenaline depletion following DSP-4 treatment. In our previous study, administration of the α-adrenergic receptor antagonist phentolamine or the β-adrenergic antagonist propranolol had no effect on working memory (18,24). Neither pharmacological blockade of adrenergic neurotransmission nor lesions of the forebrain noradrenergic projection system affected performance of rats on radial-arm maze or T-maze alternation task (9,11,16,28), a behavior related to spatial working memory and sensitive to disruption by hippocampal lesions (5,26) and muscarinic receptor blockade (2,6,16,25). Taken together, these findings suggest that hippocampal cholinergic neurotransmission via muscarinic receptors, but not noradrenergic neurotransmission, plays a critical role in working memory function, i.e., acquisition of new information within a session. The major finding of the present study was that noradrenergic DSP-4 lesions combined with a behaviorally ineffective dose of intrahippocampal scopolamine administration impaired working memory, suggesting that the noradrenergic system participated in regulating working memory function when muscarinic receptor-mediated neurotransmission declined in the hippocampus. This result extends our previous findings that DSP-4 and propranolol potentiated the disruptive effect of systemically administered scopolamine on working memory in the runway task (18,22). Similarly, the disruptive effect of scopolamine on the performance in the radial maze working memory task was augmented in rats with dorsal noradrenergic bundle lesions (11). Also, Harrell et al. (15) reported that β-adrenergic blockade by propranolol aggravated the impaired working memory performance of medial septal-lesioned rats in the 8-arm radial maze. The present experi-

ment, which utilized a microinjection technique, provides direct evidence that the hippocampus is a site of functional interactions between the cholinergic and noradrenergic systems involved in working memory processes.

With regard to long-term potentiation (LTP), a neurobiological mechanism hypothesized to be involved in memory formation, Bröcher et al. (7) reported that coapplication of noradrenergic and cholinergic agonists facilitated, in a synergistic manner, induction of LTP in slices of the rat visual cortex when combined with tetanic stimulation; this effect was mediated by activation of β-adrenergic and muscarinic receptors. They found that this facilitation was completely attenuated by blockade of *N*-methyl-d-aspartate (NMDA) receptors, suggesting that β-adrenergic and muscarinic stimulation facilitated LTP induction possibly by enhancing depolarizing responses to tetanic stimulation and thus increasing Ca^{2+} entry through NMDA receptors (7). In the hippocampal dentate gyrus, it has been demonstrated that application of noradrenaline in the absence of tetanic stimulation can induce long-lasting potentiation of synaptic transmission by stimulating β-adrenergic receptors (19,30), a phenomenon which is also prevented by NMDA receptor blockade (8,10). We previously reported that intrahippocampal injections of selective and competitive NMDA receptor antagonists, CPP, CGS 19755 and D-AP5, were able to disrupt working memory in the three-panel runway task (21). Furthermore, we recently found that DSP-4-induced noradrenaline depletion or β-adrenergic blockade by propranolol also exacerbated the impairment of working memory resulting from hippocampal NMDA receptor blockade (24). Thus, it is conceivable that postsynaptic interactive cascades following hippocampal noradrenergic and muscarinic/NMDA receptor-mediated neurotransmission may be involved in regulating working memory function, although the precise neural mechanisms underlying such interactions remain to be clarified.

Numerous populations of neurons and neurotransmitter systems, including the cholinergic, noradrenergic and glutamatergic systems, are damaged simultaneously in the AD brain (1,3,4,12–14), and such damage to multiple systems may be a factor in causing the profound memory impairment in this disease. Thus, our findings provide evidence that deficits in noradrenergic neurotransmission contribute in some manner to the severity of memory decline associated with cholinergic/glutamatergic deficits in the AD brain.

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