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β-Adrenergic dysfunction exacerbates impairment of working memory induced by hippocampal NMDA receptor blockade in rats

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Abstract

To clarify the interactions between hippocampal glutamatergic and adrenergic systems in the working memory function of rats, the effects of hippocampal NMDA receptor blockade combined with noradrenaline depletion or α- and β-adrenoceptor blockade on this behavior were examined with a three-panel runway task. Intrahippocampal administration of the potent and competitive NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) at a dose of 32 ng/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 3.2 ng/side dose of CPP did not affect working memory errors. Administration of the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) at 50 mg/kg i.p. produced marked reductions in hippocampal and cortical noradrenaline contents, but it had no effect on working memory errors. Intrahippocampal administration of 3.2 ng/side CPP, the behaviorally ineffective dose in intact rats, significantly increased the number of working memory errors in the noradrenaline-depleted rats. The α-adrenoceptor antagonist phentolamine (3.2 mg/kg i.p.) did not affect working memory errors whether administered alone or in combination with intrahippocampal CPP (3.2 ng/side). The β-adrenoceptor antagonist propranolol (10 mg/kg i.p.) also had no effect on working memory errors. However, propranolol (10 mg/kg) produced a significant increase in working memory errors when administered together with intrahippocampal CPP (3.2 ng/side). These results suggest that hippocampal NMDA/β-adrenergic interactions are involved in neural processes mediating working memory function of rats.

Keywords: Noradrenaline; β-Adrenoceptor; Propranolol; NMDA receptor; Hippocampus; Working memory

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; Collingridge and Bliss, 1987), and that administration of both competitive and noncompetitive NMDA receptor antagonists disrupts the memory performance of rats in some learning tasks that depend on hippocampal functions (Butelman, 1989; Davis et al., 1992; Morris, 1989; Ohno et al., 1992; Parada-Turska and Turski, 1990; Staubli et al., 1989; Tonkiss et al., 1988; Ward et al., 1990). Application of noradrenaline has been found to induce long-lasting enhancement of synaptic transmission in the hippocampal dentate gyrus (Dahl and Sarvey, 1989; Lacaille and Harley, 1985; Neuman and Harley, 1983; Stanton and Sarvey, 1985). This phenomenon, termed noradrenaline-induced long-lasting potentiation, is also likely to require activation of NMDA receptors because NMDA receptor antagonists, such as D-(−)-2-amino-5-phosphonovaleric acid (D-AP5) and (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), block its induction (Burgard et al., 1989; Dahl and Sarvey, 1990). These findings suggest that the noradrenergic system facilitates NMDA receptor activation leading to hippocampal long-term potentiation, whereas little is known about the interaction between glutamatergic and noradrenergic systems in the regulation of memory function.

We previously reported that working memory in a three-panel runway task, acquisition of new and variable information that was useful only within a session, was disrupted by intrahippocampal administration of the selective and competitive NMDA receptor antagonists CPP, CGS 19755 and D-AP5 (Ohno et al., 1992), suggesting that this behavior was suitable for the investigation of

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memory processes mediated by hippocampal NMDA receptors. The purpose of the present study was to clarify the hippocampal glutamatergic/adrenergic interaction in the working memory performance of rats, by investigating the effects of intrahippocampal injection of CPP combined with noradrenergic depletion or receptor blockade on this behavior.

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, 1996; Ohno et al., 1992, 1994). 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, 1996).

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 are presented separately, and those parameters in the second to the sixth trial of a session are summed together for the evaluation of working memory function. The learning criterion was fewer 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

The rats 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 CPP into the hippocampus, as described previously (Ohno and Watanabe, 1996; Ohno et al., 1992, 1994). 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.

The drugs used in this study were (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP; Research Biochemicals International), *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride (DSP-4; Sigma Chemical), phentolamine hydrochloride (Sigma Chemical) and DL-propranolol hydrochloride (Sigma Chemical). CPP was dissolved in saline, after which the pH was adjusted to approximately 7.4 with an appropriate amount of NaOH. Other drugs were dissolved in saline. Two microliters of the CPP 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. The runway test was given 10 min after the CPP injection. Phentolamine and propranolol were administered i.p. 30 min before the runway test. Other rats received i.p. administration of 50 mg/kg DSP-4 or saline. It has been reported that DSP-4-induced depletion of brain noradrenaline is permanent, whereas peripheral changes are transient and are followed by a gradual recovery of noradrenergic deficits (Jaim-Etcheverry and Zieher, 1980; Ross, 1976). Thus, the rats were given the runway test 14 days after DSP-4 treatment, when decreased noradrenaline levels in peripheral organs were expected to revert to normal, and they received no training session during this period. When rats received phentolamine, propranolol or DSP-4 combined with CPP, a behaviorally subthreshold dose of intrahippocampal CPP (3.2 ng/side), which by itself had no significant effect on working memory performance, was used.

2.3. Histology and biochemistry

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, 1996; Ohno et al., 1992, 1994). Fig. 1 shows an example of cannula tracks in the hippocampus. The stained sections revealed that damage associated with the guide cannulae was restricted to the overlying cortex, and that all of the tips of the injection cannulae were successfully located in the dorsal hippocampus.

To determine the extent of noradrenaline depletion produced by DSP-4, 50 mg/kg DSP-4 was administered to five behaviorally naive animals. Fourteen days later, the animals were decapitated after stunning, and the brains were rapidly removed and dissected on ice into the hippocampus and cortex. Samples were then assayed for contents of noradrenaline, dopamine and serotonin, using a high-performance liquid chromatography system equipped with an electrochemical detector (Eicom, Kyoto, Japan).

2.4. Statistics

The 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.

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

CPP, administered bilaterally at 3.2 and 32 ng/side into the dorsal hippocampus, increased the number of working memory errors ($F(2,15) = 31.99$, $P_z < 0.01$), an effect that was significant only for the 32 ng/side dose, while it had no effect on the number of errors made in the first trial (Fig. 2). DSP-4 at a dose of 50 mg/kg, given i.p. 14 days before testing, did not affect the number of working memory errors. Intrahippocampal administration of 3.2 ng/side CPP, 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 ($F(1,8) = 14.52$, $P < 0.01$). Intrahippocampal CPP administration or treatment with DSP-4 did not affect significantly the latency to obtain

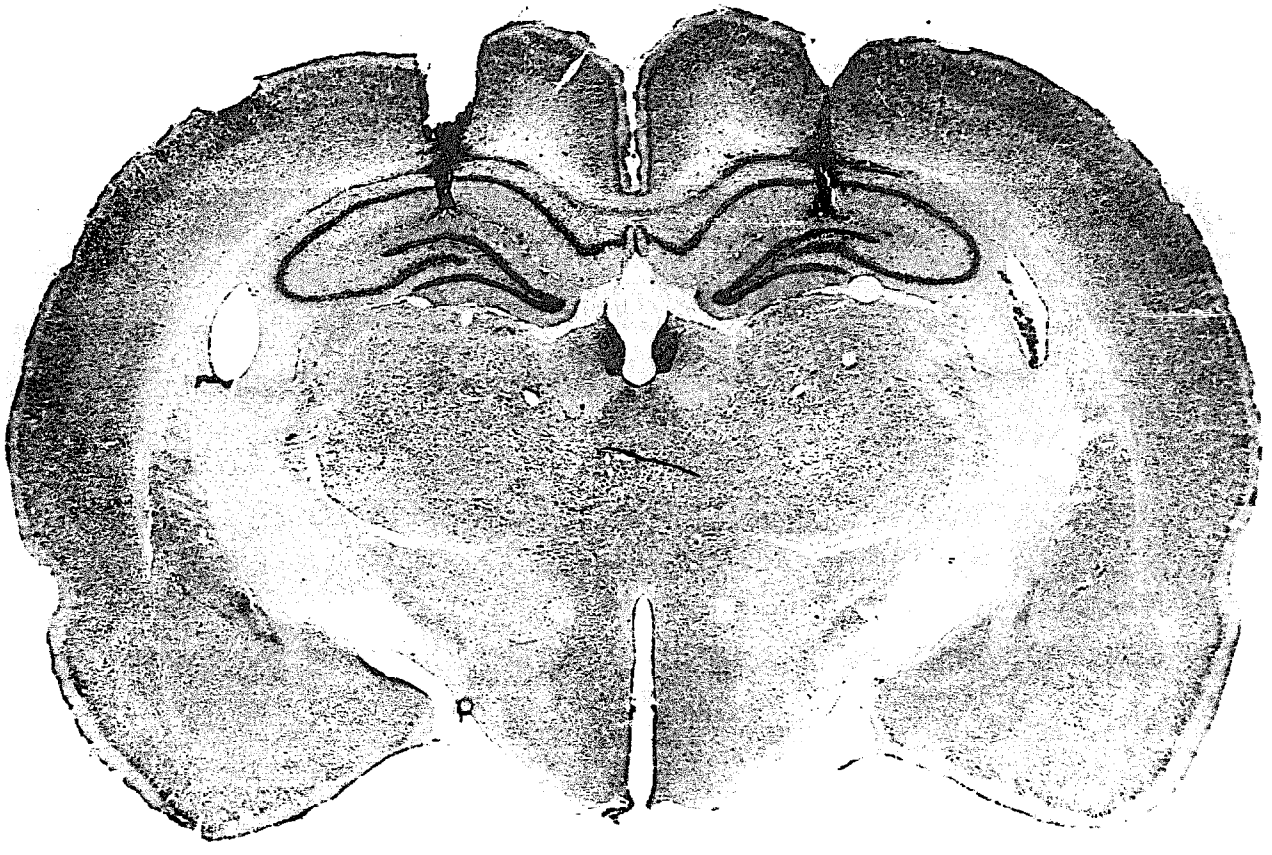

Fig. 1. Photomicrograph of a coronal section stained with Cresyl violet showing typical placements of cannulae in the hippocampus.

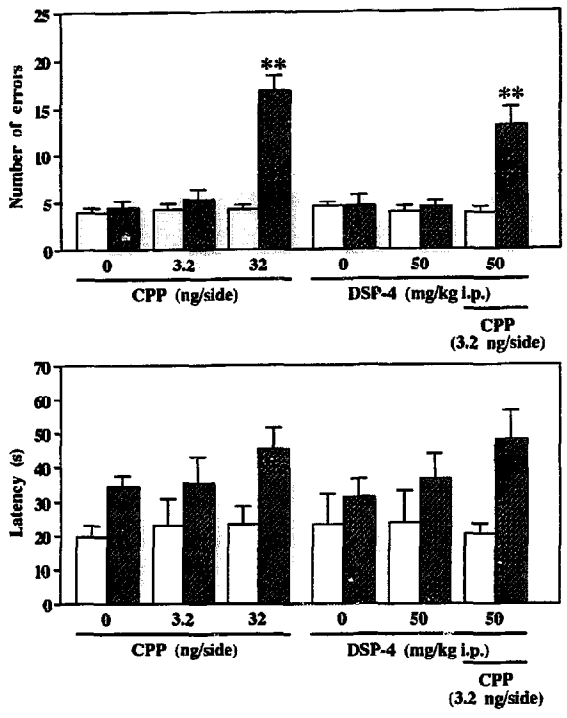

Fig. 2. Effects of intrahippocampal injection of CPP on the number of working memory errors and latency in intact and DSP-4-treated rats. The runway test was given 14 days after DSP-4 was administered. Rats received CPP injection 10 min before testing. Each column represents the mean ± S.E.M. of errors and latencies for 5–6 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 was determined by a one-way ANOVA followed by Dunnett's test, ** $P < 0.01$.

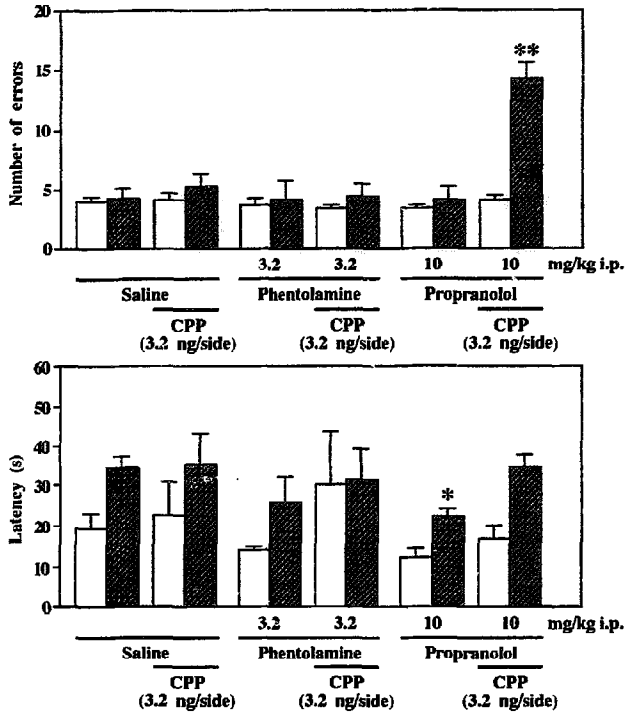

Fig. 3. Effects of intrahippocampal injection of CPP and i.p. administration of phentolamine and propranolol on the number of working memory errors and latency. The runway test was given 30 min after phentolamine and propranolol were administered. Rats received CPP injection 10 min before testing. Each column represents the mean ± S.E.M. of errors and latencies for 5–6 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 was determined by a one-way ANOVA followed by Dunnett's test, * $P < 0.05$, ** $P < 0.01$.

food pellets placed in the goal box, irrespective of whether the drugs were given independently or in combination.

Compared with the saline-treated control rats, in the 50 mg/kg DSP-4-treated rats, the content of noradrenaline in the hippocampus and cortex decreased to approximately 2.9 and 7.4%, respectively (Table 1). Treatment with 50 mg/kg DSP-4 had no significant effect on dopamine levels in both brain regions, but slightly reduced the content of serotonin in the cortex without affecting hippocampal serotonin levels.

As shown in Fig. 3, i.p. administration of 3.2 mg/kg phentolamine or 10 mg/kg propranolol did not affect the number of working memory errors. The latency to obtain food pellets was not affected by 3.2 mg/kg phentolamine. The latency from the second to the sixth trial, but not that in the first trial, was slightly reduced in 10 mg/kg propranolol-treated rats as compared with saline-treated rats ($F(1,9) = 9.88$, $P < 0.05$). Propranolol (10 mg/kg) produced a significant increase in working memory errors when administered in combination with intrahippocampal injection of 3.2 ng/side CPP ($F(1,10) = 37.11$, $P < 0.01$), which by itself had no effect on errors. Phentolamine (3.2 mg/kg) combined with intrahippocampal CPP (3.2 ng/side) did not affect the number of working memory errors. The latency was not affected by either 3.2 mg/kg phentolamine or 10 mg/kg propranolol administered together with intrahippocampal CPP. Rats treated with a higher dose of 10 mg/kg phentolamine in combination with intrahippocampal injection of 3.2 ng/side CPP

Table 1
Catecholamine and serotonin concentrations in the hippocampus and cerebral cortex of saline- and DSP-4-treated rats

Treatment	n	Hippocampus			Cortex		
		Noradrenaline	Dopamine	Serotonin	Noradrenaline	Dopamine	Serotonin
Saline	5	414.0 ± 38.4	38.0 ± 7.3	716.5 ± 49.8	316.2 ± 16.6	54.2 ± 9.8	779.9 ± 60.6
DSP-4 (50 mg/kg i.p.)	5	12.0 ± 3.6 [b]	20.0 ± 2.9	593.0 ± 72.9	23.4 ± 4.2 [b]	52.0 ± 7.4	592.5 ± 40.7 [a]

Animals were killed 14 days after saline or DSP-4 was administered. Values are means ± S.E.M. of contents of noradrenaline, dopamine and serotonin expressed as ng/g wet weight tissue. [a] $P < 0.05$, [b] $P < 0.01$ vs. the saline-treated group (Student's t-test).

showed severe sedation and failed to perform the runway task.

4. Discussion

The three-panel runway task allows us to assess the hippocampal-dependent working memory function of rats, as this behavior is significantly disrupted by dorsal hippocampal lesions (Kitajima et al., 1992). In the present study, working memory performance on the runway task was significantly impaired by intrahippocampal administration of the NMDA receptor antagonist CPP, but was not affected by brain noradrenaline depletion following DSP-4 treatment or by administration of the α-adrenoceptor antagonist phentolamine and the β-adrenoceptor antagonist propranolol. The performance of rats on a radial-arm maze task or a T-maze alternation task is related to spatial working memory function and is also sensitive to disruption by hippocampal lesions (Brito and Thomas, 1981; Olton et al., 1979) and blockade of NMDA receptor-mediated neurotransmission (Butelman, 1989; Parada-Turska and Turski, 1990; Ward et al., 1990). The present results are consistent with reports that neither pharmacological blockade of adrenergic neurotransmission (Hiraga and Iwasaki, 1984), DSP-4-induced noradrenaline depletion (Chrobak et al., 1985) nor lesions of the dorsal noradrenergic bundle with 6-hydroxydopamine (Decker and Gallagher, 1987; Pisa and Fibiger, 1983) affect the working memory performance in these learning tasks. Taken together, these findings suggest that hippocampal glutamatergic neurotransmission via NMDA receptors plays a critical role in working memory function, i.e. acquisition of new information within a session, whereas neither α- nor β-adrenergic neurotransmission contributes to mediating working memory processes when NMDA receptors can be fully activated under normal conditions.

The present study showed that noradrenergic DSP-4 lesions combined with a behaviorally ineffective dose of intrahippocampal CPP caused impairment of working memory, suggesting that the noradrenergic system participated in regulating working memory function when NMDA receptor-mediated glutamatergic neurotransmission declined in the hippocampus. In addition, it was found that β-adrenoceptor blockade by propranolol, but not α-adrenoceptor blockade by phentolamine, profoundly aggravated the disruption of working memory resulting from hippocampal NMDA receptor blockade. It is, therefore, conceivable that working memory processes involve functional interactions between hippocampal noradrenergic and glutamatergic systems through mediation of β-adrenoceptors and NMDA receptors, respectively. Application of noradrenaline induced long-lasting potentiation of synaptic transmission via β-adrenoceptors in the hippocampal dentate gyrus in slice preparations and in vivo, because the noradrenaline-induced potentiation was completely blocked by β-adrenoceptor antagonists, including propranolol, but was not affected by phentolamine (Lacaille and Harley, 1985; Stanton and Sarvey, 1985). Burgard et al. (1989) and Dahl and Sarvey (1990) reported that the NMDA receptor antagonists D-AP5 and CPP were effective in suppressing β-adrenoceptor agonist-induced hippocampal long-lasting potentiation, suggesting that this phenomenon, regarded as a candidate mechanism for learning and memory, also required activation of NMDA receptors. The results of the present study suggest that excitatory β-adrenergic neurotransmission contributes to supporting working memory processes via the hippocampal glutamatergic pathway when NMDA receptor-mediated transmission decreases, although further study is necessary to elucidate the precise neural mechanisms underlying such functional interactions.

It has been found that simultaneous deficits in multiple neurotransmitter systems, such as glutamatergic, noradrenergic and cholinergic systems, occur in the brains of patients with Alzheimer's disease (Araujo et al., 1988; Bondareff et al., 1982; Greenamyre and Maragos, 1993; Hardy et al., 1985, 1987). It is reasonable to speculate that numerous populations of neurons and neurotransmitter systems damaged simultaneously in the brains of such patients may be a causal factor in the profound memory impairment seen in this disease. With regard to the role of cholinergic function, we previously reported that intrahippocampal administration of scopolamine, a muscarinic receptor antagonist, was effective in disrupting working memory in the three-panel runway task (Ohno et al., 1992). Furthermore, we recently demonstrated that DSP-4-induced noradrenaline depletion or β-adrenergic blockade by propranolol, but not α-adrenergic blockade, exacerbated working memory deficits produced by systemic and intrahippocampal administration of scopolamine (Kobayashi et al., 1995; Ohno et al., 1993b, 1996). However, deficits in the peptidergic system, especially reductions in the somatostatinergic activity, are known to be one of the most consistent neurochemical changes observed in the brains of patients with Alzheimer's disease (Davies et al., 1980; Reinikainen et al., 1987). We also found that cysteamine-induced depletion of brain somatostatin did not affect working memory function, but it significantly potentiated the scopolamine disruption of working memory in the three-panel runway task (Ohno et al., 1993a). Taken together, our findings may provide evidence that the deficiency of β-adrenergic and somatostatinergic neurotransmission contributes in some manner to the severity of memory decline associated with glutamatergic and/or cholinergic deficits in the brains of patients with Alzheimer's disease.

References

Araujo, D.M., P.A. Lapchak, Y. Robitaille, S. Gauthier and R. Quirion, 1988. Differential alteration of various cholinergic markers in cortical

and subcortical regions of human brain in Alzheimer's disease, J. Neurochem. 50, 1914.

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

Bondareff, W., C.Q. Mountjoy and M. Roth, 1982, Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus ceruleus) in senile dementia, Neurology 32, 164.

Brito, G.N.O. and G.J. Thomas, 1981, T-maze alternation, response patterning, and septo-hippocampal circuitry in rats, Behav. Brain Res. 3, 319.

Burgard, E.C., G. Decker and J.M. Sarvey, 1989, NMDA receptor antagonists block norepinephrine-induced long-lasting potentiation and long-term potentiation in rat dentate gyrus, Brain Res. 482, 351.

Butelman, E.R., 1989, A novel NMDA antagonist, MK-801, impairs performance in a hippocampal-dependent spatial learning task, Pharmacol. Biochem. Behav. 34, 13.

Chrobak, J.J., D.L. DeHaven and T.J. Walsh, 1985, Depletion of brain norepinephrine with DSP-4 does not alter acquisition or performance of a radial-arm maze task, Behav. Neural Biol. 44, 144.

Collingridge, G.L. and T.V.P. Bliss, 1987, NMDA receptors – their role in long-term potentiation, Trends Neurosci. 10, 288.

Dahl, D. and J.M. Sarvey, 1989, Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus, Proc. Natl. Acad. Sci. USA 86, 4776.

Dahl, D. and J.M. Sarvey, 1990, β-Adrenergic agonist-induced long-lasting synaptic modifications in hippocampal dentate gyrus require activation of NMDA receptors, but not electrical activation of afferents, Brain Res. 526, 347.

Davies, P., R. Katzman and R.D. Terry, 1980, Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia, Nature 288, 279.

Davis, S., S.P. Butcher and R.G.M. Morris, 1992, The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro, J. Neurosci. 12, 21.

Decker, M.W. and M. Gallagher, 1987, Scopolamine-disruption of radial arm maze performance: modification by noradrenergic depletion, Brain Res. 417, 59.

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.

Hardy, J., R. Cowburn, A. Barton, G. Reynolds, E. Lofdahl, A.-M. O'Carroll, P. Wester and B. Winblad, 1987, Region-specific loss of glutamate innervation in Alzheimer's disease, Neurosci. Lett. 73, 77.

Hiraga, Y. and T. Iwasaki, 1984, Effects of cholinergic and monoaminergic antagonists and tranquilizers upon spatial memory in rats, Pharmacol. Biochem. Behav. 20, 205.

Jaim-Etcheverry, G. and L.M. Zieher, 1980, DSP-4: a novel compound with neurotoxic effects on noradrenergic neurons of adult and developing rats, Brain Res. 188, 513.

Kitajima, I., T. Yamamoto, M. Ohno and S. Ueki, 1992, Working and reference memory in rats in the three-panel runway task following dorsal hippocampal lesions, Jpn. J. Pharmacol. 58, 175.

Kobayashi, M., M. Ohno, T. Yamamoto and S. Watanabe, 1995, Concurrent blockade of β-adrenergic and muscarinic receptors disrupts working memory but not reference memory in rats, Physiol. Behav. 58, 307.

Lacaille, J.-C. and C.W. Harley, 1985, The action of norepinephrine in the dentate gyrus: beta-mediated facilitation of evoked potentials in vitro, Brain Res. 358, 210.

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.

Neuman, R.S. and C.W. Harley, 1983, Long-lasting potentiation of the dentate gyrus population spike by norepinephrine, Brain Res. 273, 162.

Ohno, M. and S. Watanabe, 1996, Concurrent blockade of hippocampal metabotropic glutamate and *N*-methyl-D-aspartate receptors disrupts working memory in the rat, Neuroscience 70, 303.

Ohno, M., T. Yamamoto and S. Watanabe, 1992, Effects of intrahippocampal injections of *N*-methyl-D-aspartate receptor antgonists and scopolamine on working memory and reference memory assessed in rats by a three-panel runway task, J. Pharmacol. Exp. Ther. 263, 943.

Ohno, M., S. Shibata, T. Yamamoto and S. Watanabe, 1993a, Working memory deficits following muscarinic blockade combined with depletion of brain somatostatin in rats, Brain Res. 610, 348.

Ohno, M., T. Yamamoto, M. Kobayashi and S. Watanabe, 1993b, Impairment of working memory induced by scopolamine in rats with noradrenergic DSP-4 lesions, Eur. J. Pharmacol. 238, 117.

Ohno, M., T. Yamamoto and S. Watanabe, 1994, Blockade of hippocampal M_1 muscarinic receptors impairs working memory performance of rats, Brain Res. 650, 260.

Ohno, M., A. Yoshimatsu, M. Kobayashi and S. Watanabe, 1996, Noradrenergic DSP-4 lesions aggravate impairment of working memory produced by hippocampal muscarinic blockade in rats, Pharmacol. Biochem. Behav. in press.

Olton, D.S., J.T. Becker and G.E. Handelmann, 1979, Hippocampus, space, and memory, Behav. Brain Sci. 2, 313.

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.

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Pisa, M. and H.C. Fibiger, 1983, Evidence against a role of the rat's dorsal noradrenergic bundle in selective attention and place memory, Brain Res. 272, 319.

Reinikainen, K.J., P.J. Riekkinen, J. Jolkkonen, V.-M. Kosma and H. Soininen, 1987, Decreased somatostatin-like immunoreactivity in cerebral cortex and cerebrospinal fluid in Alzheimer's disease, Brain Res. 402, 103.

Ross, S.B., 1976, Long-term effects of *N*-2-chloroethyl-*N*-ethyl-2-bromobenzylamine hydrochloride on noradrenergic neurons in the rat brain and heart, Br. J. Pharmacol. 58, 521.

Stanton, P.K. and J.M. Sarvey, 1985, Blockade of norepinephrine-induced long-lasting potentiation in the hippocampal dentate gyrus by an inhibitor of protein synthesis, Brain Res. 361, 276.

Staubli, U., O. Thibault, M. DiLorenzo and G. Lynch, 1989, Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory, Behav. Neurosci. 103, 54.

Tonkiss, J., R.G.M. Morris and J.N.P. Rawlins, 1988, Intra-ventricular infusion of the NMDA antagonist AP5 impairs performance on a non-spatial operant DRL task in the rat, Exp. Brain Res. 73, 181.

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.