Effect of Nebracetam on the Disruption of Spatial Cognition in Rats

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ABSTRACT — Central cholinergic hypofunction causes the disruption of spatial cognition, while cholinomimetics improve this disruption in rats. Scopolamine (0.5 mg/kg, i.p.) has also been reported to disrupt radial maze performance in rats. Nebracetam (WEB 1881 FU), a new nootropic candidate, was able to correct this scopolamine-induced disruption of spatial cognition at the dose of 10 mg/kg, p.o. Furthermore, nebracetam enhanced oxotremorine-induced tremors in mice. These results indicate that nebracetam has a cholinergic enhancing effect. The scopolamine-induced disruption of spatial cognition has been previously reported to improve not only by cholinomimetics but also by brain noradrenergic drugs such as L-*threo*-DOPS and amantadine. Nebracetam reversed the change of brain noradrenaline contents in the frontal cortex and hippocampus in which the noradrenaline content decreased by treatment with scopolamine. Nebracetam also decreased the Δ^9-tetrahydrocannabinol (6 mg/kg, i.p.)-induced disruption of spatial cognition, which was reported to be related to the lymbic noradrenergic function. These results suggest that the cognitive enhancing effect of nebracetam involves not only cholinergic mechanisms but also involves lymbic and hippocampal noradrenergic mechanisms.

In cerebrovascular or Alzheimer's disease, cholinergic hypofunction generally causes dementia such as memory loss or disorientation (1, 2). Tetrahydroaminoacridine, a cholinergic enhancer, has been shown to improve these symptoms in patients with dementia (3). From these cholinergic hypotheses, cholinergic antagonist scopolamine is now widely used in animal experiments for memory disturbance. Scopolamine has been shown to disrupt passive avoidance performance (4, 5) and maze performance in rats (6). Cholinergic enhancers such as physostigmine (5) tetrahydroaminoacridine (7) and oxotremorine (8) have also been found to improve these deficits of memory performance.

Furthermore, our previous report suggested that the scopolamine-induced disruption of spatial cognition was rectified not only by cholinergic drugs but also by noradrenergic drugs such as L-*threo*-DOPS and amantadine (9). These effects may be due to noradrenergic α_1-receptor stimulation, because the retrieval effects of L-*threo*-DOPS or amantadine were inhibited by the α_1-blocker prazosin (9). Biochemical studies also indicated the noradrenergic involvement of spatial cognition on 8-arm radial maze performance in rats. Scopolamine decreased the brain NA contents in the frontal cortex and the hippocampus in accordance with the disruption of spatial cognition (9, 10).

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In addition, Δ^9-tetrahydrocannabinol (THC), one of the major constituents of marihuana, also disrupted the spatial cognition of rats (11). the THC-induced disruption of spatial cognition was improved by either noradrenaline (NA)- or serotonin-related compounds but not by antidepressant drugs. From these findings, the THC-induced disruption of spatial cognition was thought to be more related to the lymbic monoamine system than to the cholinergic system (11).

Nebracetam (4-aminomethyl-1-benzylpyrrolidine-2-one-hemifumarate: WEB 1881 FU) is being developed as a novel compound that possesses both nootropic and cholinomimetic properties in animal studies (12). Nebracetam also possesses cholinergic receptor affinity, especially towards M_1 receptors (12, 13). In the present study, to investigate whether nebracetam possesses an ameriorative effect on the disruption of spatial cognition and cholinergic and noradrenergic involvement, the drug's affect on scopolamine and THC-induced disruption of spatial cognition were also examined using an 8-arm radial maze performance test in rats. In this experiment, the effect of nebracetam was compared with that of aniracetam, which is a benzylpyrrolidine derivative similar to nebracetam.

MATERIALS AND METHODS

Experiment 1: Eight arm radial maze task

Male Wistar rats weighing 200–250 g, supplied by Kyu-Do Co., Ltd. (Saga, Japan), were used for this experiment and were kept on a constant light-dark cycle (light 0700–1900) with a restricted diet (CE-2, Clea, Japan) in an air conditioned room (23 ± 1°C, 60% humidity). Each rat was placed on a platform (25 cm in diameter) in the middle of an 8-arm radial maze, in which each arm had been baited with a food pellet. Performance of the animal in each session was assessed by three parameters: the number of correct choices, the number of errors which was defined as choosing arms that had already been visited, and the time elapsed before the animal ate all 8 pellets. The behavioral observation was discontinued after 10 min even if the animal did not finish the task.

In the drug test phase, we used only rats that had acquired spatial cognition; i.e., the rats that had made 7 or more correct choices and either one or no errors during the first 8 choices in each of 3 consecutive sessions. Significantly decreased correct choices in the presence of significantly increased errors were considered to demonstrate a disruption of spatial cognition. Spatial cognition in the affected animals was considered to be improved when the drug-state rats made significantly increased correct choices and significantly decreased errors as compared with untreated affected animals.

Nebracetam (Boehringer Ingelheim, Germany, dissolved in water) was orally administered 60 min before each session. Aniracetam (Nippon Roche, Japan, suspended in 0.5% CMC-Na) was also orally administered 60 min before the session. Scopolamine (Sigma, USA, dissolved in physiological saline) was intraperitoneally administered 30 min before the session. THC, isolated from cannabis by Prof. I. Nishioka and Y. Shoyama (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushyu University), was suspended in 1% Tween 80 and then intraperitoneally administered 60 min before the session.

Wilcoxon's rank sum test was used for the data analysis in these experiments.

Experiment 2: Effects on brain NA levels

Only male Wistar rats that had been trained in the 8-arm radial maze task and had sucessfully performed this task were used in this experiment. Brain NA levels were studied in rats whose spatial cognition in the 8-arm radial maze was disrupted by scopolamine and whose scopolamine-induced disruption of spatial cognition was then improved by nebracetam using high performance liquid chromatography with electrochemical detection (HPLC-ECD).

Following the observation of maze performance, the rats were sacrificed by decapitation and the brain was immediately removed. The

brain was cut into 1-mm coronal cryosections and punched out on an ice-cooled glass stage (Fig. 1). The regional samples were homogenized in 200 μl of 0.5 M-PCA containing 0.05% Na_2EDTA and 0.1% $Na_2S_2O_5$ (All reagents for HPLC studies were purchased from Sigma, USA). Following centrifugation, the supernatant was injected into a HPLC-ECD. The HPLC system (Waters Assoc., Milford, MA) utilized a Shodex ODS column (Showa Denko, Tokyo, Japan) set at a potential of +0.75 V versus the reference electrode. The HPLC mobile phase was 0.1 M citrate-phosphate buffer containing 1.5 mM sodium octyl sulphate (PIC-B_8) and 11% methanol with 20 μM Na_2EDTA (14). The flow rate was maintained at 0.9 ml/min. Brain NA contents were quantified by calculating the area under the curves using an integrater (Waters Model 730, Waters Assoc.), and their contents were determined based on standard curves.

Either Student's *t*-test or the Cochran-Cox test were used to determine the significances in NA contents.

Experiment 3: Oxotremorine-induced tremors

To investigate whether nebracetam reverses the disruption of spatial cognition by stimulating cerebral acetylcholinergic pathways or some other mechanisms, nebracetam was studied for its possible potentiating effect on tremors induced by oxotremorine which acts directly on muscarinic acetylcholine (ACh) receptors.

Five-week-old male ddY mice weighing 20–25 g, supplied by Seiwa Experimental Animals (Fukuoka, Japan), were used in this experiment. They were kept under the same conditions as described above. Oxotremorine (Sigma, USA) was dissolved in 0.9% physiological saline and was intraperitoneally administered. Nebracetam was first orally administered for 50 min and then intraperitoneally administered for 20 min before the oxotremorine treatment. Observations for tremors was made at 5-min intervals starting 30 min after the oxotremorine treatment. The severity of tremors was scored according to 5 grades: 0: absent, 1: mild, 2: moderate, 3: marked, and 4: severe.

The Mann Whitney-*U* test was used for this experiment.

Experiment 4: Spontaneous motor activity and behavioral toxicity

Male Wistar rats were used for this experiment. Spontaneous motor activity was measured with Hall's open-field apparatus (60-cm bottom diameter) (15), and the numbers across the line were measured for 3 min. In testing the behavioral toxicity of nebracetam, the incidence of muscle relaxation was measured by a digital traction meter (Model F-1, Muromachi Kikai, Japan). When each rat was placed on the sliding table of the apparatus and was slowly pulled by his tail, forepaw resistance was detected by a sensor. The motor

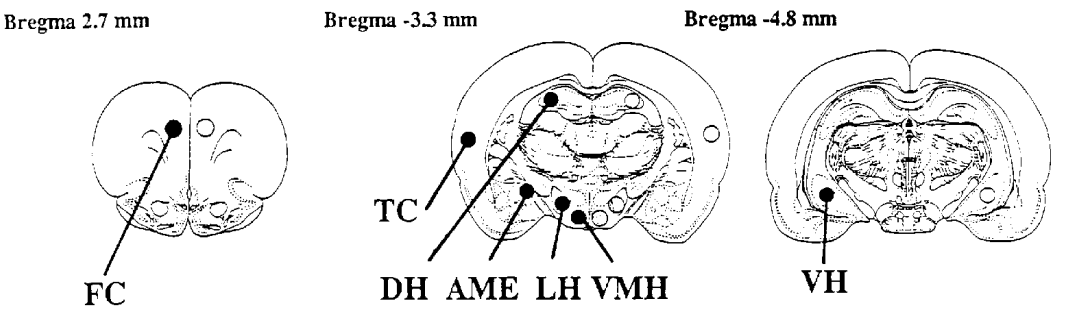

Fig. 1. Brain dissections and punch-out site. Abbreviations: FC, frontal cortex; TC, temporal cortex; DH, dorsal hippocampus; VH, ventral hippocampus; AME, medial amygdala; LH, lateral hypothalamus; VMH, ventromedial hypothalamus.

co-ordination was also measured by a rota-rod apparatus (Muromachi Kikai, Japan). The rats were tested for the incidence of catalepsy by placing both forepaws on the horizontal bar (12-cm high), and the cataleptogenic effect was assessed as positive when the rat maintained this position for longer than 1 min. The rectal temperature was measured by a digital thermometer.

The Mann Whitney-*U* test and Fisher's exact probability test were used to analyze the data from these experiments.

RESULTS

Experiment 1: Eight arm radial maze task

Effects on scopolamine-induced disruption of spatial cognition: The rats which had acquired spatial cognition in the 8-arm radial maze made approximately 8 correct choices and 0 errors in the first 8 choices (N = 21). Scopolamine at 0.5 mg/kg, i.p. significantly decreased the number of correct choices and significantly increased errors (N = 21), indicating that disruption of spatial cognition had occurred.

The effects of the oral administration of the test drug nebracetam on the scopolamine-induced disruption of spatial cognition were investigated at doses of 5 to 50 mg/kg (N = 7–9). At 10 mg/kg (N = 9), nebracetam significantly increased the number of correct choices and significantly decreased the errors in scopolamine-treated rats, suggesting that spatial cognition disruption was decreased. However, at a dose of 20 mg (N = 8) or 50 mg/kg (N = 7), nebracetam did not improve this disruption of spatial cognition (Fig. 2). Aniracetam significantly decreased the scopolamine-induced disruption of spatial cognition at a dose of 50 mg/kg, p.o. (N = 9, Fig. 3).

Effects on THC-induced disruption of spatial cognition: In rats that had acquired spatial cognition, the intraperitoneal administration of THC at 6 mg/kg significantly decreased the number of correct choices while significantly increasing the errors (N = 11), suggesting a disruption of the spatial cognition. The THC-induced disruption was expressed differently from the scopolamine-induced one: some rats hesitated to enter the first arm, while others just went up and down whatever arm they first encounterd.

The effects of nebracetam at 20 to 200 mg/kg, p.o. (N = 3–10) on these characteristic features in the disruption of spatial cognition were studied. A significant improvement in spatial cognition was observed in the animals treated with 100 mg/kg (N = 10, Fig. 4).

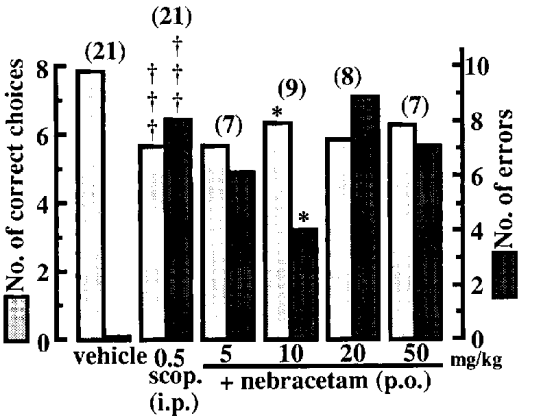

Fig. 2. Effect of nebracetam on the scopolamine-induced disruption of spatial cognition in rats. scop.: scopolamine; $^{*}P < 0.05$ vs. scop.; $^{\dagger\dagger\dagger}P < 0.001$ vs. vehicle.

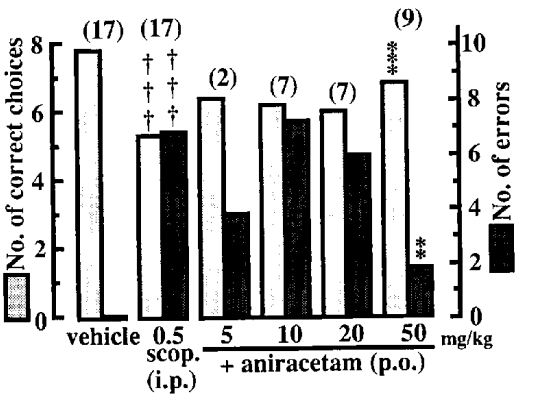

Fig. 3. Effect of aniracetam on the scopolamine-induced disruption of spatial cognition in rats. scop.: scopolamine; $^{**}P < 0.01$, $^{***}P < 0.001$ vs. scop.; $^{\dagger\dagger\dagger}P < 0.001$ vs. vehicle.

Aniracetam at 10 and 20 mg/kg, p.o. improved the THC-induced disruption of spatial cognition (N = 8, Fig. 5).

Experiment 2: Effects on brain NA levels

In this experiment, the detection limit of NA was about 10 pg according to the calibration curves (data not shown), which still enough to detect the content in small NA samples from brain tissue.

In non-trained intact control rats, one hour after the oral administration of nebracetam at 10 mg/kg (N = 5), the NA levels in the ventral region of the hippocampus were significantly higher than those of the corresponding controls (Fig. 6).

Brain NA levels in the frontal cortex, dorsal and ventral hippocampus, lateral hypothalamus and medial amygdala (N = 13) were significantly decreased in rats with scopolamine-induced disruption of spatial cognition (N = 11). The oral administration of nebracetam (10 mg/kg, p.o.) improved spatial cognition while brain NA levels in the frontal cortex, dorsal and ventral hippocampus as well as the amygdala were normalized to normal control levels (N = 6, Fig. 7).

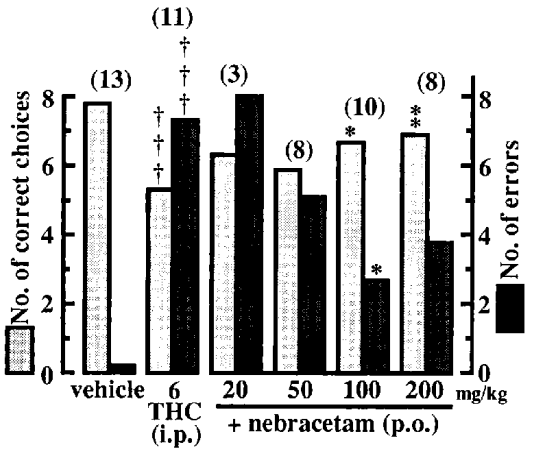

Fig. 4. Effect of nebracetam on the THC-induced disruption of spatial cognition in rats. THC: Δ^9-tetrahydrocannabinol; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. THC; $^{\dagger\dagger\dagger}P < 0.001$ vs. vehicle.

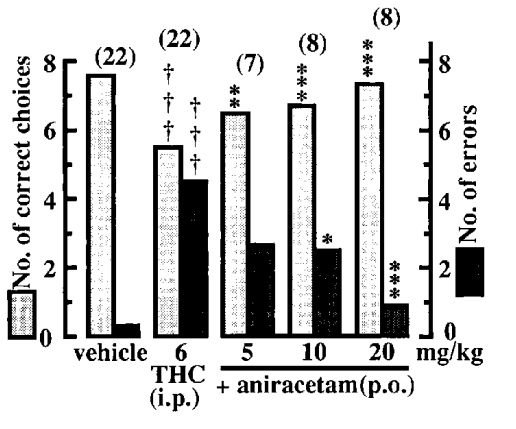

Fig. 5. Effect of aniracetam on the THC-induced disruption of spatial cognition in rats. THC: Δ^9-tetrahydrocannabinol; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. THC; $^{\dagger\dagger\dagger}P < 0.001$ vs. vehicle.

Experiment 3: Oxotremorine-induced tremors

The mice that were intraperitoneally treated with oxotremorine at 0.5 mg/kg, i.p. developed tremors which became most marked after 10 to 15 min and then later subsided with time (N = 10). Nebracetam at a dose of 10 mg/kg, i.p. significantly potentiated the oxotremorine-induced tremors from 10 min on (N = 10, Fig. 8B), while the oral administration of nebracetam did not potentiate oxotremorine-induced tremors (N = 9–10, Fig. 8A). This suggests that nebracetam possesses a weak central cholinergic enhancement at a narrow dose range.

Experiment 4: Spontaneous motor activity and behavioral toxicity

Ambulation of the rats measured at 60 min after administration by an open-field test was not affected by nebracetam at doses from 20 to 100 mg/kg when compared with the control (N = 8, Fig. 9). In addition, aniracetam also did not affect ambulation in this experiment at 20 to 100 mg/kg (N = 8, Fig. 9). The frequency of rearing decreased by nebracetam at 50 mg/kg, p.o., but not at the other doses tested. Aniracetam also did not affect ambulation in this experiment at 20 to 100 mg/kg (N = 8,

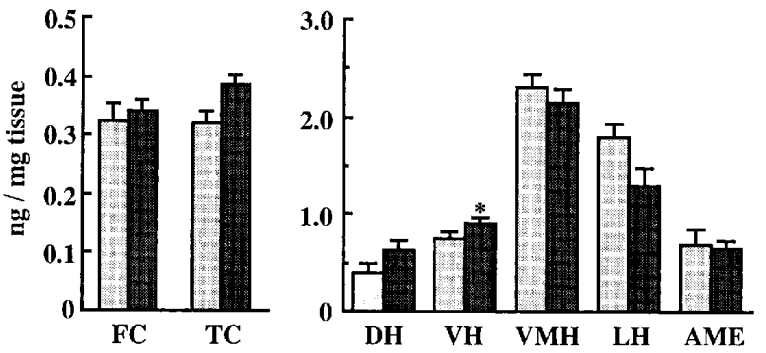

Fig. 6. Brain noradrenaline changes following nebracetam administration in normal control rats. ▒ vehicle; ■ nebracetam, 10 mg/kg, p.o. Abbreviations: FC, frontal cortex; TC, temporal cortex; DH, dorsal hippocampus; VH, ventral hippocampus; VMH, ventromedial hypothalamus; LH, lateral hypothalamus; AME, medial amygdala. Mean ± S.E. $^{*}P < 0.05$ vs. vehicle.

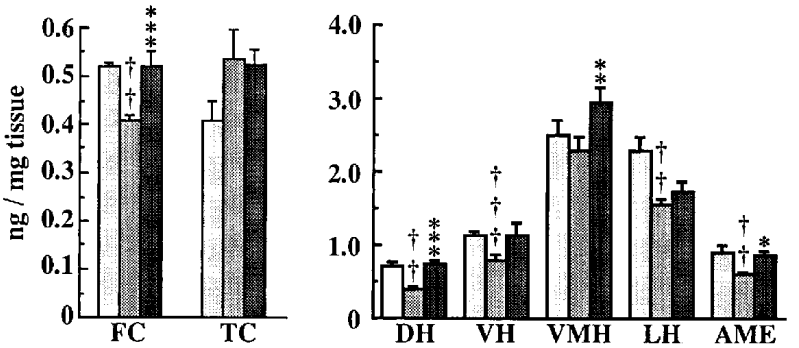

Fig. 7. Changes in brain noradrenaline levels following nebracetam administration in scopolamine-treated disrupted rats. ▒ vehicle; ▒ scopolamine, 0.5 mg/kg, i.p.; ■ scopolamine + nebracetam, 10 mg/kg, p.o. Abbreviations: FC, frontal cortex; TC, temporal cortex; DH, dorsal hippocampus; VH, ventral hippocampus; VMH, ventromedial hypothalamus; LH, lateral hypothalamus; AME, medial amygdala. Mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. scopolamine; $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. vehicle.

Fig. 9). To confirm the behavioral toxicity of the drug, motor coordination, muscle tone, incidence of catalepsy and rectal temperature were all tested at larger drug doses than those used for the cognitive experiments. Both nebracetam and aniracetam caused neither motor uncoordination nor catalepsy (N = 8, data not shown). These drugs also had no affect on muscle tone (N = 8, Fig. 9). Nebracetam raised the rectal temperature at 100 and 200 mg/kg, p.o., but did not cause any hypothermia (N = 8, Fig. 9).

DISCUSSION

Alzheimer's senile dementia is frequently accompanied by hypofunction of the brain acetylcholinergic neural systems (16, 17). This hypofunction is expressed by an impairment of some mental activities including speech, memory, visual space perception, personality and cognition (abstract ability, calculation ability and judgement) (16). Among some individuals, an impairment of time and spatial orientation also appears at an initial stage.

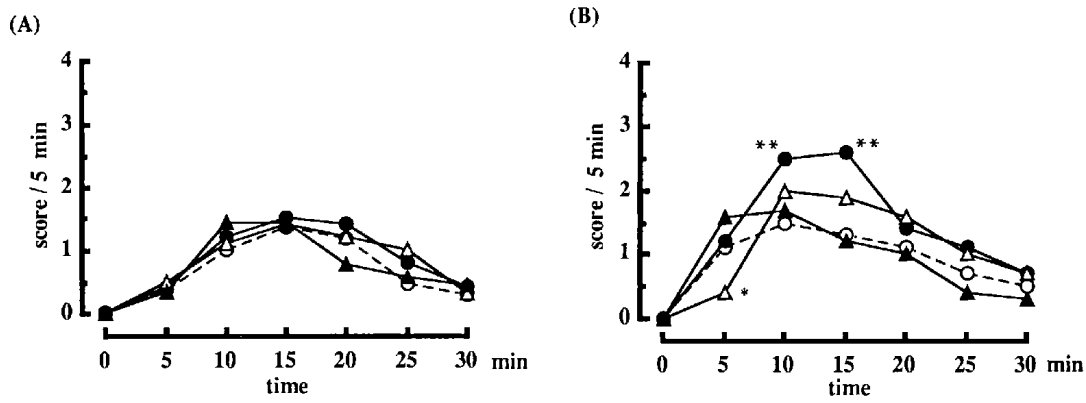

Fig. 8. Potentiating effect of nebracetam on oxotremorine-induced tremors in mice. (A) oral administration, (B) intraperitoneal administration. ···○··· oxotremorine, 0.3 mg/kg, i.p.; —●— oxotremorine + nebracetam, 10 mg/kg; ···△··· oxotremorine + nebracetam, 20 mg/kg; —▲— oxotremorine + nebracetam, 50 mg/kg; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. oxotremorine control.

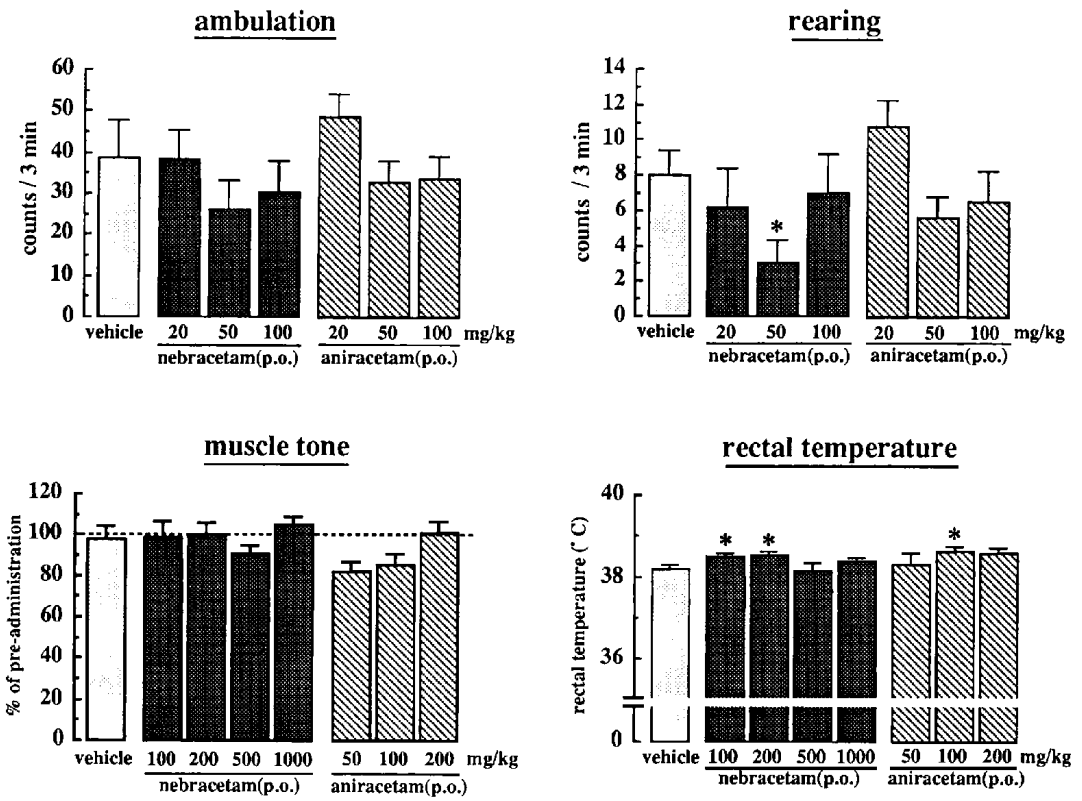

Fig. 9. Influences of nebracetam and aniracetam on general behavior of rats. Mean ± S.E. $^{*}P < 0.05$ vs. vehicle.

It is well-known that scopolamine, a muscarinic ACh receptor blocker, markedly disrupts spatial cognition such as that acquired by rats in this study. Scopolamine also disrupts passive avoidance performance in rats and mice (18). The cholinergic involvement of memory and learning was well-demonstrated because cell loss in the nucleus basalis of Meynert, a source of the extrinsic cholinergic input to the cortex and amygdala, and cell loss in the medial sept/diagonal band complex, a source of cholinergic input to the hippocampus, are characteristic of human aging and occur to a much greater extent in Alzheimer's disease (19). In animal studies, lesions of either the septal area or nucleus basalis also impaired memory and learning (20). Scopolamine-induced disruption was shown to be markedly reversed by the ACh esterase inhibitor physostigmine or catecholaminergic drugs such as amantadine (9). Furthermore, our previous report indicated that the ameliorative effect of amantadine is inhibited by prazosin, a noradrenergic α_1-receptor blocker (9). Noradrenergic involvement in memory and learning was also suggested because it shows an age-related decline, with moderate to severe additional deterioration in Alzheimer's disease (21, 22). Furthermore, the cell numbers of the locus coeruleus, which provides noradrenergic innervation of the cortex and hippocampus, appears to be particularly affected by age and disease (23). Thus, the available data suggest the importance of the brain noradrenergic system as well as the cholinergic nervous system in learning and memory.

Therefore, we first studied the effect of nebracetam on scopolamine-induced disruption of spatial cognition in rats and compared it with that of aniracetam. Nebracetam reversed the scopolamine-induced disruption of spatial cognition. This improvement effect was also considered to be due to the stimulation of the acetylcholinergic systems because nebracetam had an affinity for muscarinic M_1-receptors in vitro and can potentiate ACh synthesis (12). Furthermore, to observe whether nebracetam had a cholinergic stimulating effect, the effect of nebracetam on the tremor induced by oxotremorine which had a stimulating effect on central muscarinic-receptors (24, 25) was examined using mice. Nebracetam potentiated oxotremorine-induced tremors at a dose of 10 mg/kg, i.p. because this dose should stimulate both the M_1-receptors and ACh synthesis. However, nebracetam did not potentiate this tremor at higher doses. This diminishing effect of the higher dose of the drug might be due to a negative feed-back mechanism by the increased ACh release (25).

The retrieval effect of nebracetam on the disruption of spatial cognition in rats, however, was in an inverted-U dose-dependent manner. This mode of action was also observed in the treatments with noradrenergic, serotonergic and cholinergic agonists (9–11). This means that the optimal dose must be used to obtain an improvement of cognitive disruption. Furthermore, multiple neurotransmitter balances may also play an important role in the performance of spatial cognitions such as higher brain functions. From this evidence, higher doses of nebracetam are suggested to induce imbalances of multiple neurotransmitter interactions including interactions between the cholinergic and noradrenergic systems.

Aniracetam improved the scopolamine-induced disruption of spatial cognition and this ameliorative effect of aniracetam might be due to the same mechanism as that of nebracetam.

In the above-described experiments, we investigated whether nebracetam acted through a noradrenergic mechanism to decrease scopolamine-induced disruption of spatial cognition. Previous reports have suggested that nebracetam had noradrenergic antidepressant profiles because of its antagonism of tetrabenazine-ptosis, distress call activation and central β-receptor down regulation (12). In addition, at higher doses, nebracetam tended to increase NA release and/or turnover (26). Our previous report also indicated that antidepressants such as desipramine or imipramine improve scopolamine-induced disruption of spatial cognition at a dose that had

no anti-cholinergic effects (9, 10).

In the present biochemical studies, brain NA contents in various brain regions were studied using HPLC-ECD. In rats with impaired spatial cognition induced by scopolamine, brain NA levels were decreased in the frontal cortex, dorsal and ventral hippocampus and the amygdala complex. Nebracetam normalized the decreased NA levels in these area. One of the mechanisms in spatial cognition was also reported to be present in the brain noradrenergic system. Amantadine or L-*threo*-DOPS, which have no cholinergic enhancing effects, improved the disruption of spatial cognition (9). These results may also be due to brain noradrenergic function. Interestingly, in the present study, the normalizing effect of nebracetam on brain NA levels was observed only in the areas that are considered to be important for memory and learning. Nebracetam, as well as these drugs, may ameliorate the disrupted spatial cognition via noradrenergic systems in either the frontal cortex or other lymbic systems.

In addition, THC, which is known to cause a marked orientation impairment in humans, remarkably impaired the radial maze performance of rats as well. The characteristic features of THC-induced disruption of spatial cognition were reported to be different from those of the scopolamine-induced one (11). The THC-induced disruption of spatial cognition was also improved by noradrenergic or serotonergic compounds, while antidepressants such as desipramine etc. did not affect the THC-induced spatial disruption (11). This impairment was reversed by nebracetam as well as physostigmine and amantadine which had previously been reported to be highly effective (9). These results also suggest that nebracetam also operates through a noradrenergic mechanism to reverse spatial disruption. Aniracetam also decreased the THC-induced disruption of spatial cognition like nebracetam, but was more effective.

Finally, the present study on behavioral toxicity suggested that nebracetam, even in high doses, did not affect spontaneous motor activity and did not have motor toxicity, including muscle-relaxation and other cataleptgenic effects.

From the present results, it is considered that nebracetam is more effective than aniracetam in the ameliorative effect on spatial disruption induced by cholinergic deficiency and that nebracetam might be effective in the treatment of hypofunction of brain ACh and NA systems in dementia. Furthermore, nebracetam might be a useful drug for the treatment of dementia without any adverse effects.

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REFERENCES

1 Bartus, R.T., Dean, R.L., Beer, B. and Lippa, A.S.: The cholinergic hypothesis of geriatric memory dysfunction: A critical review. Science **217**, 408–414 (1982)

2 Davis, K.L. and Yamamura, H.I.: Cholinergic underacting in human memory disorders. Life Sci. **23**, 1729–1734 (1978)

3 Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K. and Kling, A.: Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. **315**, 1241–1245 (1986)

4 Rush, D.K.: Scopolamine amnesia of passive avoidance: A deficit of information acquisition. Behav. Neural Biol. **50**, 255–274 (1988)

5 Dilts, S.L. and Berry, C.A.: Effect of cholinergic drugs on passive avoidance in the mouse. J. Pharmacol. Exp. Ther. **158**, 279–285 (1976)

6 Eckerman, D.A., Gordon, W.A., Edwards, J.D., Macphail, R.C. and Gage, M.I.: Effect of scopolamine, pentobarbital and amphetamine on radial arm maze performance in rat. Pharmacol. Biochem. Behav. **12**, 595–602 (1980)

7 Hodges, H., Ribeiro, A.M., Gray, J.A. and Marchbanks, R.M.: Low dose tetrahydroaminoacridine (THA) improves cognitive function but does not affect brain acetylcholine in rats. Pharmacol. Biochem. Behav. **36**, 291–298 (1990)

8 Brioni, J.D. and Izquierdo, I.: Retention enhancement by pre-test β-endorphine and oxotremorine and its reversal by scopolamine. Behav. Neural

Biol. **50**, 251–254 (1988)

9 Fujiwara, M., Iwasaki, K. and Matsumoto, Y.: Involvement of brain noradrenaline in spatial cognition in rats. Japan. J. Pharmacol. **46**, Supp. 18P (1987)

10 Fujiwara, M., Matsumoto, Y., Iwasaki, K., Kataoka, Y. and Ueki, S.: A new nootropic drug, minaprine, improved the scopolamine-induced spatial memory disruption. Japan. J. Pharmacol. **45**, Supp. 226P (1987)

11 Fujiwara, M., Matsumoto, Y., Iwasaki, K., Kataoka, Y. and Ueki, S.: THC impaired the spatial learning of rats in the radial arm maze task. Japan. J. Pharmacol. **45**, Supp. 79P (1987)

12 Kuhn, F.J., Schingnitz, G., Lehr, E., Montagna, E., Hinzen, H.D. and Giacheiti, A.: Pharmacology of WEB 1881-FU, a central cholinergic agent, which enhances cognition and cerebral metabolism. Arch. Int. Pharmacodyn. Ther. **292**, 13–34 (1988)

13 Kitamura, Y., Hayashi, S. and Nomura, Y.: Effect of WEB 1881 FU, a novel nootropic on cholinergic and adrenergic receptors in the rat brain: Action on M_1-muscarinic receptors. Japan. J. Pharmacol. **52**, 597–607 (1990)

14 Iwasaki, K., Fujiwara, M., Shibata, S. and Ueki, S.: Changes in brain catecholamine levels following olfactory bulbectomy and the effect of acute and chronic administration of desipramine in rats. Pharmacol. Biochem. Behav. **24**, 1715–1719 (1986)

15 Hall, C.S.: Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. **18**, 385–403 (1934)

16 Coyle, J.T., Price, D.L. and DeLong, M.R.: Alzheimer's disease: a disorder of cortical cholinergic innervation. Science **219**, 1184–1189 (1983)

17 Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W. and DeLong, M.R.: Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science **215**, 1237–1239 (1983)

18 Kuribara, H. and Tadokoro, S.: Differences in cholinergic agonist- and cholinergic antagonist-induced changes in avoidance responses between dd mice and Wistar rats. Japan. J. Psychopharmacol. **3**, 159 (1980)

19 McGeer, P.L., McGeer, E.G., Suzuki, J., Dolman, C.E. and Nagai, T.: Aging, Alzheimer's disease and the cholinergic system of the basal forebrain. Neurology **34**, 741–745 (1984)

20 Miyamoto, M., Kato, J., Narumi, S. and Nagaoka, A.: Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nuclei in rats. Brain Res. **419**, 19–31 (1987)

21 Vijayashankar, N. and Brody, H.A.: A quantitative study of the pigmented neurons in the nucleus locus coeruleus and subcoeruleus in man as related to aging. J. Neuropathol. Exp. Neurol. **38**, 490–498 (1979)

22 Adolfsson, R., Gottfries, G.G., Roos, B.E. and Winblad, B.: Changes in brain catecholamines in patient with dementia of Alzheimer's types. Br. J. Psychiat. **135**, 216–223 (1979)

23 Marcyniuk, B., Mann, D.M.A. and Yates, P.O.: Loss of nerve cells from locus coeruleus in Alzheimer's disease is topographically arranged. Neurosci. Lett. **64**, 247–252 (1986)

24 Messer, W.S., Jr., Ellerbrock, B.R., Price, M.A. and Hoss, W.: Autoradiographic analysis of agonist binding to muscarinic receptor subtypes. Biochem. Pharmacol. **38**, 837–850 (1989)

25 Hoss, W., Messer, W.S., Jr., Monsma, F.J., Jr., Miller, M.D., Ellerbrock, B.R., Scranton, T., Ghodsi-Hovsepian, S., Price, M.A., Balan, S., Mazloum, Z. and Bohnett, M.: Biochemical and behavioral evidences for muscarinic autoreceptors in the CNS. Brain Res. **517**, 195–201 (1990)

26 Bechtel, W.D. and Mierau, J.: WEB1881, a novel antidepressant stimulates noradrenaline release in vivo and in vitro. Psychopharmacology (Berlin) **96**, Supp. 274 (1988)