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Effect of a Prolyl Endopeptidase Inhibitor, JTP-4819, on Radial Maze Performance in Hippocampal-Lesioned Rats

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MIYAZAKI, A., K. TOIDE, Y. SASAKI, Y. ICHITANI AND T. IWASAKI. *Effect of a prolyl endopeptidase inhibitor, JTP-4819, on radial maze performance in hippocampal-lesioned rats.* PHARMACOL BIOCHEM BEHAV **59**(2) 361–368, 1998.—The effect of a novel prolyl endopeptidase (PEP) inhibitor, (S)-2-2[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-N-(phenylmethyl)-1-pyrrolidinecarboxamide (JTP-4819), on spatial learning deficits in rats with dorsal hippocampal (DH) lesions was examined using an eight-arm radial maze. The correct performance remained at chance levels even after 18 acquisition trials in rats with DH lesions. JTP-4819 (3.0 mg/kg, p.o.) significantly ameliorated this learning impairment after 34–41 days of treatment. When DH lesions were created in rats after achievement of learning, postoperative performance deteriorated prominently, but gradually recovered with the repetition of trials. JTP-4819 (3.0 mg/kg, p.o.) significantly decreased the number of trials needed to reattain learning criterion. After the behavioral experiment, the choline acetyltransferase (ChAT) activity and [^{3}H]-pirenzepine binding (K_d, B_{max}) in the residual hippocampus and cerebral cortex were analyzed. Neither parameter was significantly affected by JTP-4819. In conclusion, JTP-4819 can improve both learning and relearning deficits of spatial memory in DH-lesioned rats, postulating that enhancement of neuropeptide activity via PEP inhibition may be involved in the mechanism of action of JTP-4819. © 1998 Elsevier Science Inc.

Prolyl endopeptidase JTP-4819 Eight-arm radial maze Spatial memory Hippocampal lesion Choline acetyltransferase Muscarinic-1 receptor rat

DESPITE extensive worldwide efforts, there are still no drugs available that are satisfactorily effective for Alzheimer's disease (AD). To date, the most prominent neurochemical deficit characterized in AD patients is degeneration of the cholinergic neurons projecting to the hippocampus and cerebral cortex (1,5,23,31,47). Postmortem studies have also provided evidence that several types of neuropeptide-containing neurons are pathologically altered in AD (2,8,14,32), suggesting that neuropeptide dysfunction also seems to underly the memory deficits in this disease. Importantly, there is considerable evidence that these neuropeptides, including substance P (SP), arginine-vasopressin (AVP), and thyrotropin-releasing hormone (TRH), can enhance memory and learning in rodents (7,12,15,26,33,37,40). In addition, SP, AVP, and TRH also possess a neurotrophic effect, which may be related to neuronal plasticity (3,4,16,17,22,34). These findings have served as an impetus to develop a therapeutic agent positively modulating neuropeptides in AD. Neuropeptides such as SP, AVP, and TRH that participate in memory and learning contain proline residues, and the C-terminals of these residues are probably metabolized by prolyl endopeptidase (PEP, EC.3.4.21.26) (21). PEP is a serine protease, which was first discovered as an oxytocin-degrading enzyme in the human uterus (45), and was found to be a monomer with a molecular weight of about 7.6 kD (49). This enzyme is widely distributed throughout the body, including the brain, and is believed to exert diverse physiological actions (18,20,38,46,48,49). (S)-2-2[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-N-(phenylmethyl)-1-pyrrolidinecarboxamide (JTP-4819, Fig. 1) is a strong and specific PEP inhibitor (40). JTP-4819 potently inhibits PEP (40), and thus blocks the degradation of SP, AVP, and TRH (39,40) in the brain. The decrease of intracerebral

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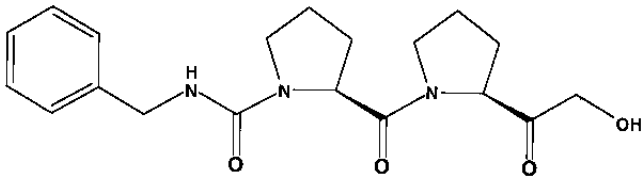

FIG. 1. Chemical structure of JTP-4819.

SP (42) and TRH immunoreactivity (35) in aged rats is reversed by both single and repeated doses of JTP-4819. JTP-4819 also increases acetylcholine (ACh) release in the frontal cortex and hippocampus of young and aged rats (40), and repeated treatment of aged rats normalizes their cholinergic imbalance by correcting choline acetyltransferase (ChAT) activity in the cerebral cortex and choline uptake in the hippocampus (43). Behavioral studies have shown that JTP-4819 antagonizes scopolamine-induced amnesia in rats performing the passive avoidance task (40) and improves Morris water maze learning deficits in aged rats (43) and in rats with middle cerebral artery occlusion (36).

It is well known that the septo-hippocampal pathway, together with the nucleus basalis of Meynert (nbM)-cortical pathway, is a major projection of cholinergic neurons in the brain (5,25), and these pathways characteristically show degeneration in patients with AD (1,5,23,31,47). As the hippocampus seems to be crucial for spatial memory (27,29), lesions of the dorsal hippocampus (DH) with cholinergic deafferentiation could be regarded as an animal model of the memory deficit in AD. In the present study, we examined the effect of JTP-4819 on learning and relearning deficits in DH-lesioned rats performing the eight-arm radial maze task. ChAT activity and [^{3}H]-pirenzepine binding (muscarinic-1 (M1) receptor binding) in the residual hippocampus and cerebral cortex were also analyzed to investigate the biochemical actions of JTP-4819.

METHOD

Animals

Wistar–lmamichi male rats (Imamichi Institute for Animal Reproduction; body weight: 320–360 g for Experiment 1, and 240–280 g for Experiment 2) were used. Each rat was individually housed in a wire-net cage in a temperature- and humidity-controlled room (23 ± 2°C and 60 ± 5%) under a 12-h light/dark cycle (lights on at 0800 h). Throughout the radial maze experiment, the body weight was maintained at approximately 80–85% of the freely feeding weight.

Drugs and Treatments

JTP-4819 (synthesized at our Research Institute; molecular weight; 359.43) was dissolved in distilled water.

In Experiment 1, drug administration was started the day after surgery and was continued daily. In Experiment 2, treatment was given daily from the day after the first relearning trial. Animals were orally given either the active drug or distilled water (1 ml/kg) 1 h before the beginning of each of the trials, which was carried out on alternate days. Administration was done in the evening (1700–2000) on the days when no behavioral trials were performed. Treatment was done in a blinded fashion.

For the biochemical study, acetyl-CoA (Sigma), choline chloride (Sigma), physostigmine sulfate (Sigma), isopropylhomocholine (Eicom, Kyoto), tetramethylammonium (Wako Pure Chemical Industries, Osaka), sodium 1-decanesulfonate (Tokyo Chemical Industry, Tokyo), [^{3}H]-pirenzepine (New England Nuclear, Boston), and atropine sulfate (Tokyo Chemical Industry) were used.

Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg IP), and fixed on a stereotaxic instrument (David Kopf). According to the atlas of Paxinos and Watson (30), an electrode (diameter: 0.7 mm; exposed tip: 1.5 mm) was inserted into the dorsal hippocampus (AP: −3.8 mm from the bregma; ML: ±1.4 mm and ±3.4 mm; DV: −3.0 mm and −2.8 mm from the dura surface). Bilateral lesions were produced by maintaining a tissue temperature of 58°C for 60 s with a lesion generator (Radionics RFG-4). Control rats underwent a sham operation, consisting of craniotomy and dura perforation. Figure 2 illustrates the location of typical DH lesions.

Apparatus

The radial maze of Olton et al. (28) was slightly modified for the present experiments, as described by Hiraga and Iwasaki (13). In brief, the apparatus consisted of a central platform (diameter: 37 cm) and eight arms (length: 60 cm; width: 12 cm; height from the floor: 50 cm). At the far end of each arm, a hole 3 cm in diameter and 1 cm deep was made to contain a food pellet (45 mg) as a reward. Each arm and the central platform were partitioned by a transparent acryl guillotine door, which the experimenter could move up and down arbitrarily with a fishing line. The maze was placed in a 350 × 250 cm room, where there were a table and a flusher as visual cues. The illumination over the central platform was 130 lx.

Behavioral Procedures

Experiment 1 (effect on learning). Rats (n = 34) were randomly assigned to the following five groups: 1) a sham group treated with the vehicle (n = 4), 2) a DH-lesioned group treated

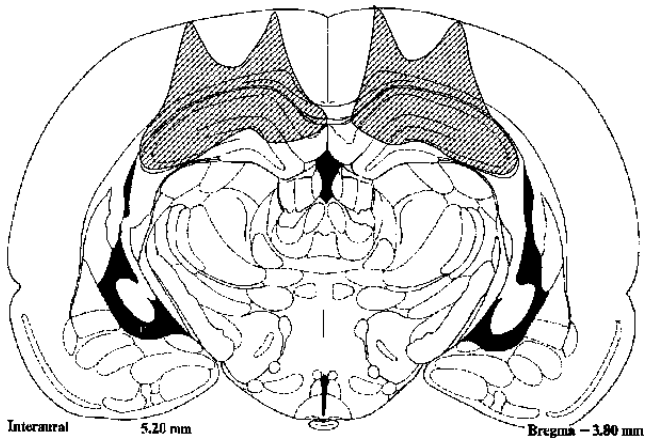

FIG. 2. Schematic drawing of a representative dorsal-hippocampal lesion in a coronal section approximately at the center of the anterior–posterior level of the lesion. This drawing was made according to the atlas of Paxinos and Watson (30).

with the vehicle (n = 7), 3) a DH-lesioned group treated with JTP-4819 at 0.3 mg/kg (n = 8), 4) a DH-lesioned group treated with JTP-4819 at 1.0 mg/kg (n = 8), and 5) a DH-lesioned group treated with JTP-4819 at 3.0 mg/kg (n = 7). Drug administration was started from the day after surgery. Each rat was handled for 5 min before the habituation trial. Four rats were simultaneously given a 10-min habituation trial daily, for 3 days starting from 3 days after creation of the DH lesions. During habituation trials, all guillotine doors were left open and food pellets were placed at sites other than the food well in each arm and on the central platform. Each rat was given 18 daily acquisition trials, on alternate days starting from the sixth day after surgery. At the beginning of each acquisition trial, one food pellet was placed in the food well of each arm with all guillotine doors closed and the rat was placed on the center of the platform facing in an arbitrary direction. Each trial was started by opening all the guillotine doors. The first entry into an unselected arm was defined as a correct choice and repeated entry was classed as an error. Whenever the rat returned to the platform, all the guillotine doors were closed, and after a 5-s delay the doors were reopened for the next choice. Each trial was completed when the rat consumed all eight pellets or when a 10-min time limit had been reached. The number of correct choices in the first eight choices was recorded as a parameter of memory. The time spent in each choice ([the time required for trial—(5 s (delay) $\times$ (total number of choices - 1))]/total number of choices) was calculated as a general behavioral parameter, reflecting locomotion, alertness, and/or hesitation. Each parameter was analyzed using data from blocks of two trials.

Experiment 2 (effect on relearning). After 3 days of habituation trials, each rat was started on preoperative acquisition trials. When the number of correct choices in the first eight choices totaled 35 or more on five consecutive trials (including no trials with five or less correct choices, and less than one trial with six correct choices), the rat was judged to have reached the learning criterion. One or 2 days after reaching the criterion, DH lesioning was performed as described above. For 2 days after surgery, food and water were given ad lib. Body weight was then reduced to 80–85% of the freely feeding weight again.

Each rat was given 19 daily reacquisition trials on alternate days, starting on the sixth day following the operation by the same method as the preoperative acquisition trials. The rats (n = 39) were assigned to the following five groups: 1) a sham group treated with the vehicle (n = 4), 2) a DH-lesioned group treated with the vehicle (n = 9), 3) a DH-lesioned group treated with JTP-4819 at 1.0 mg/kg (n = 9), 4) a DH-lesioned group treated with JTP-4819 at 3.0 mg/kg (n = 9), and 5) a DH-lesioned group treated with JTP-4819 at 10.0 mg/kg (n = 8). Assignment was based on the number of correct choices in the first reacquisition trial, the number of trials to reach the learning criterion preoperatively (these parameters determined in the last five preoperative acquisition trials), and the interval from the day of reaching the learning criterion to DH lesion surgery. Drug administration was started from the day after the first reacquisition trial. In the reacquisition trials, the number of correct choices and the time spent in each choice were recorded and analyzed as described in Experiment 1.

Biochemical Study

Rat brain dissection. On the day after the final reacquisition trial (Experiment 2), each rat was decapitated 1 h after medication. The brain was removed, and the cerebral cortex and hippocampus were dissected on ice (11). The cerebral cortex and hippocampus were stored at −80°C until ChAT activity and [^{3}H]-pirenzepine-binding were measured.

Assay of ChAT activity and [^{3}H]-pirenzepine-binding. ChAT activity was measured according to the method of Kaneda and Nagatsu (19) with some modifications. Frozen brain tissue was homogenized in three volumes of 25 mM phosphate buffer containing 0.5% Triton X-100 (pH 7.4), and centrifuged at 12,500 $\times$ g for 60 min at 4°C. Then the supernatant was diluted in 15 volumes of 25 mM phosphate buffer

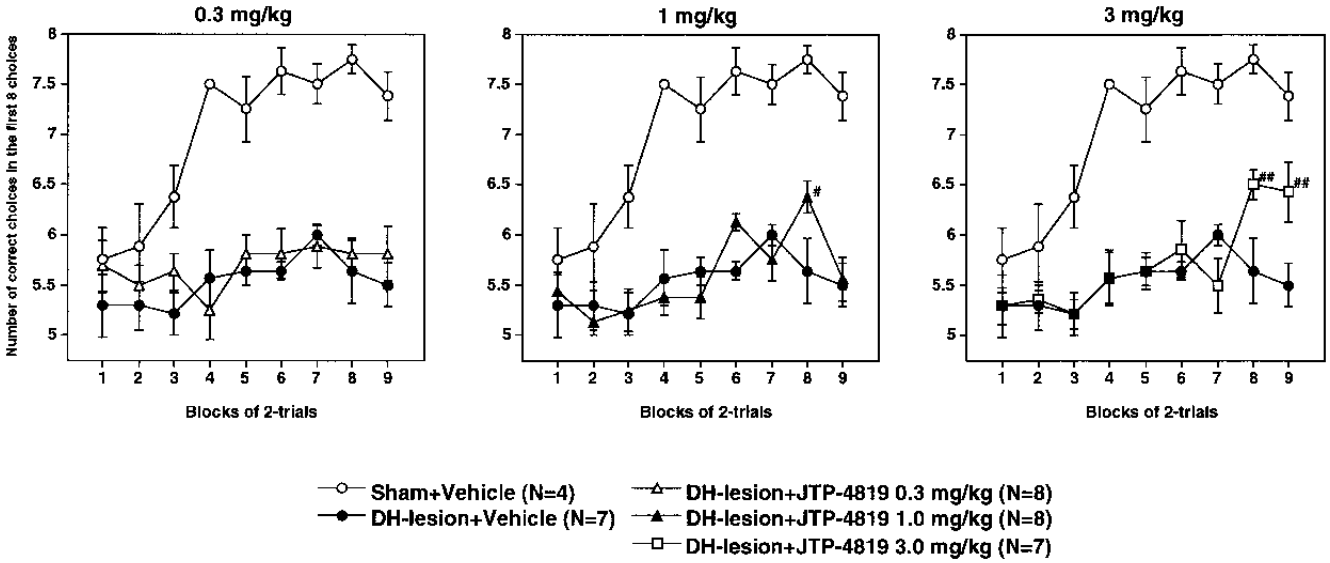

FIG. 3. Effect of JTP-4819 on the learning deficit in rats with pretraining dorsal hippocampal (DH) lesions in the eight-arm radial maze. Each point represents the number of correct choices (mean ± SE) in the first eight choices. #$p < 0.05$, ##$p < 0.01$ vs. DH-lesion + vehicle group.

(pH 7.4), and used as the enzyme solution. To 100 μl of this enzyme solution, 100 μl of a substrate solution (10 mM choline chloride, 0.4 mM acetyl-CoA, 0.2 mM physostigmine sulfate, 0.3 mM sodium chloride, and 20 mM EDTA-Na in 0.1 M sodium phosphate buffer, pH 7.4) was added to initiate the reaction. After incubation for 20 min at 37°C, the reaction was stopped by addition of 50 μl of 1 M perchloric acid. After being let stand for 10 min, 10 μl of 0.1 mM isopropylhomocholine (the internal standard) was added, and the mixture was centrifuged at 1,600 × *g* for 10 min at 4°C. An aliquot of the supernatant (10 μl) was injected into a high-performance liquid chromatography column with an electrochemical detector (Eicom, Kyoto).

For the assay of [^{3}H]-pirenzepine binding, 50 μl of [^{3}H]-pirenzepine was added and the final concentration was adjusted to 1–32 nM in cell membrane mixture (containing 25 mM Tris (pH 7.4), 2 mM $MgCl_2$, and 150 mM NaCl). To assay nonspecific binding, atropine was added and the final concentration was adjusted to 10 μM. Then the mixture was incubated at 30°C for 1 h and the reaction was terminated by filtration using a cell harvester (Brandel). Subsequently, the radioactivity remaining on the filter was measured with a liquid scintillation counter Wallac 1410 (Wallac, Finland), and the K_d and B_{max} values were calculated using a Scatchard plot.

The protein content was measured by the method of Lowry et al. (24).

Statistical Analysis

Behavioral testing. The number of correct choices in the first eight choices and the time spent in each choice were analyzed by repeated measures two-way ANOVA; the simple main effect was tested in each block. When the treatment × block interaction was not significant, the effect of treatment throughout the entire trial was tested by one-way ANOVA. The number of trials needed to reattain the learning criterion was compared by one-way ANOVA. In each analysis, Fisher's PLSD test was used as a post hoc multiple comparison test.

Biochemical study. Student's *t*-test was used to compare the sham-operated group with the vehicle-treated DH-lesioned group. Comparison between the DH-lesioned groups was done by one-way ANOVA, followed by Duncan's multiple comparison test.

RESULTS

Experiment 1 (Effect on learning)

Number of correct choices. DH-lesioned rats showed a low level of acquisition, nearly at chance level, even after 18 trials, indicating that DH lesions produced marked impairment of the acquisition process (Fig. 3). Two-way ANOVA revealed significant effects of treatment, $F(4, 29) = 28.10$, $p < 0.01$, block, $F(8, 232) = 10.30$, $p < 0.01$, and interaction, $F(32, 232) = 2.09$, $p < 0.01$). The multiple comparison test revealed that the values for all DH-lesioned groups were significantly lower than those for the sham-operated group in the third and subsequent blocks (the significant differences are not illustrated). At a dose of 3.0 mg/kg, JTP-4819 significantly ameliorated the DH lesion-induced learning deficit in the eighth and ninth blocks ($p < 0.01$). while a dose of 1.0 mg/kg, only achieved improvement in the eighth block ($p < 0.05$).

Time for each choice. As training went on, the time spent in each choice gradually shortened in all groups, while DH-lesioned rats exhibited paticularly low values throughout the trials (Fig. 4). Two-way ANOVA revealed significant effects of treatment, $F(4, 29) = 5.35$, $p < 0.01$, block, $F(8, 232) = 16.82$, $p < 0.01$, and interaction, $F(32, 232) = 1.67$, $p < 0.05$. The multiple comparison test indicated that DH lesioning significantly shortened the time spent in each choice in the seventh and subsequent blocks ($p < 0.01$) compared with the sham-operated group (Fig. 4). At a dose of 3.0 mg/kg, JTP-4819 significantly reversed the DH lesion-induced decrease in the seventh ($p < 0.05$) and subsequent blocks ($p < 0.01$).

Experiment 2 (effect on relearning)

Number of correct choices. Figure 5 shows the number of trials to reattain the learning criterion in each group. Sham-operated rats required just five trials (including the criterion trials) to reach the learning criterion again. DH lesioning markedly increased the number of trials required. The multiple comparison test following one-way ANOVA revealed that JTP-4819 (3.0 mg/kg) significantly decreased the number of trials required to reach the criterion ($p < 0.05$), indicating facilitation of the relearning process.

Figure 6 shows the relearning curves for each group, The number of correct choices was significantly decreased in all DH-lesioned groups at the first acquisition trial (no drug treatment), in comparison with that in the sham-operated

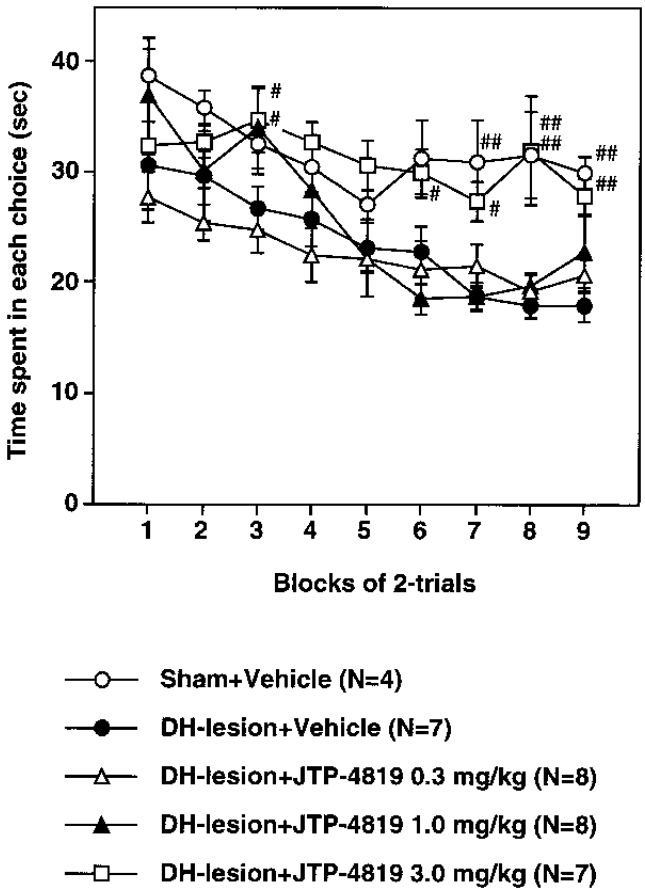

FIG. 4. Effect of JTP-4819 on the time spent in each choice of the radial maze in rats with pretraining dorsal hippocampal (DH) lesions. Each column represents the mean ± SE of the number of animals indicated in parentheses. #$p < 0.05$, ##$p < 0.01$ vs. DH-lesion+vehicle group.

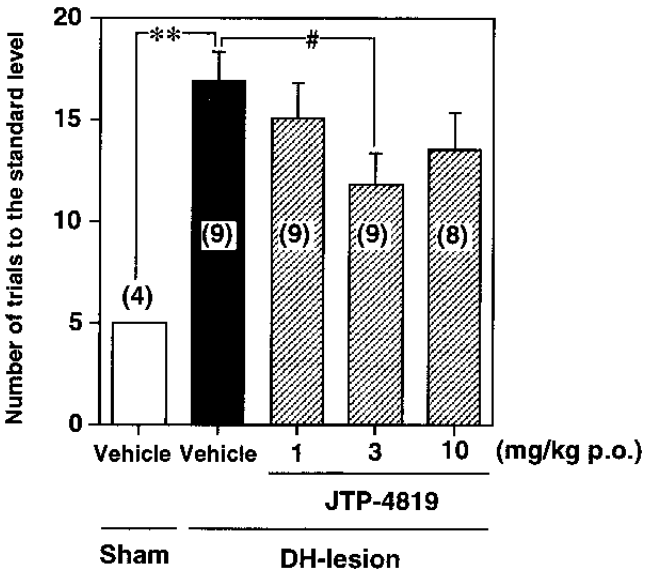

FIG. 5. Effect of JTP-4819 on the trials to reattain the learning criterion in rats with posttraining dorsal hippocampal (DH) lesions in the eight-arm radial maze. Each column represents the mean ± SE of the number of animals indicated in parentheses. **DH-lesion+vehicle vs. sham+vehicle ($p < 0.01$); #DH-lesion+JTP-4819 vs. DH-lesion+vehicle ($p < 0.05$).

rats, indicating an obvious memory deficit, However, all DH-lesioned rats gradually recovered thereafter. Two-way ANOVA revealed significant effects of treatment, $F(4, 29) = 16.92$, $p < 0.01$, and block, $F(8, 272) = 21.52$, $p < 0.01$, while the interaction, $F(32, 272) = 1.03$, was not significant, The multiple comparison test showed that DH lesioning significantly decreased the number of correct choices, compared with sham operation ($p < 0.01$). JTP-4819 (3.0 mg/kg) did not significantly attenuate the decrease, although some improvement was seen at a dose of 3.0 mg/kg ($p < 0.10$).

Time for each choice. The time spent in each choice in the relearning process (Fig, 7) was shorter than that in the learning process (Fig. 4) in the sham-operated rats. Neither treatment, $F(4, 34) = 0.54$, nor block, $F(8, 272) = 0.47$, had a significant effect on relearning.

ChAT Activity and [^{3}H]-Pirenzepine Binding

Table 1 shows the ChAT activity and [^{3}H]-pirenzepine binding of the residual hippocampus and cerebral cortex. The residual hippocampal ChAT activity was significantly reduced by DH lesioning to 67% of the level in the sham-operated group ($p < 0.01$). This reduction was not significantly affected by JTP-4819 (1.0–10.0 mg/kg). Cortical ChAT activity was not altered by DH lesioning or drug treatment. [^{3}H]-pirenzepine binding (represented by the K_d and B_{max} values) in the hippocampus and cerebral cortex was also not significantly affected by either DH lesioning or the drug.

DISCUSSION

The present study demonstrated that DH lesions caused deterioration of both learning and relearning in the eight-arm radial maze task, and impaired the original learning process more severely than the relearning process. These findings suggest that an intact hippocampus is essential for original learning, while relearning may occur through the activity of the residual hippocampus and/or compensation by other brain regions. Accordingly, reference memory may at least partly survive once it has been acquired before lesioning. Repeated administration of JTP-4819 (3.0 mg/kg) significantly improved

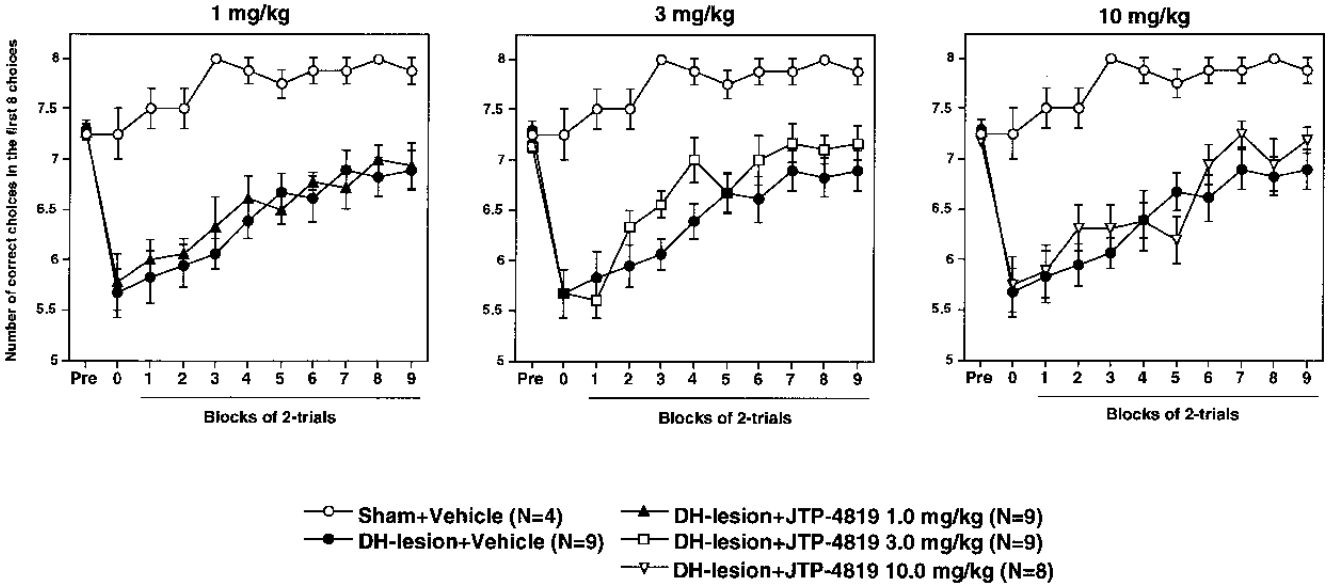

FIG. 6. Effect of JTP-4819 on the relearning deficit in rats with posttraining dorsal hippocampal (DH) lesions in the eight-arm radial maze. Each point represents the number of correct choices (mean ± SE) in the first eight choices. Pre: the last (criterion) five trials in the original learning. 0: first requisition trial (no drug treatment).

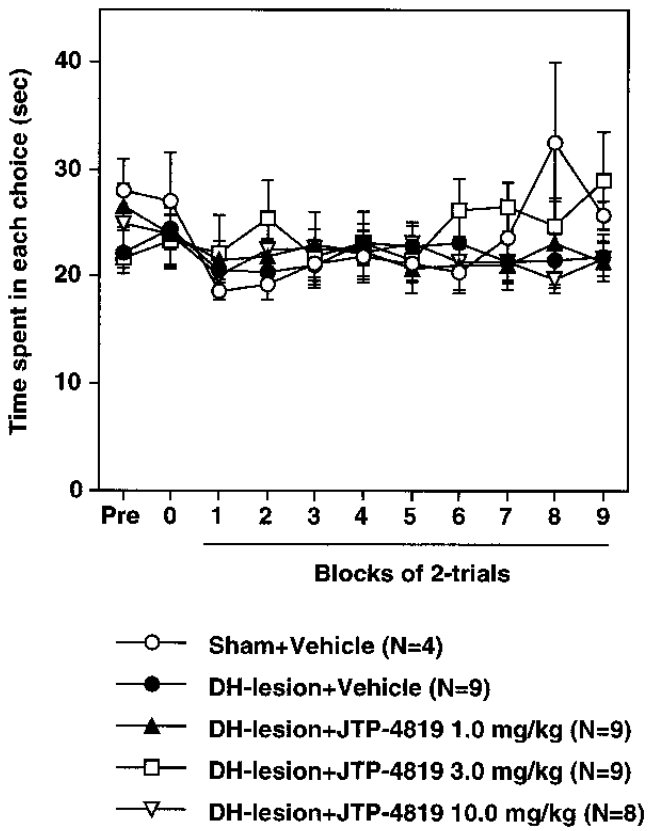

FIG. 7. Effect of JTP-4819 on the time spent in each choice of the radial maze in rats with posttraining dorsal hippocampal (DH) lesions. Each point represents the mean ± SE of the number of animals indicated in parentheses. Pre: the last (criterion) five trials in the original learning. 0: first reacquisition trial (no drug treatment).

original learning as well as relearning in this model (Figs. 3 and 6). The relatively slower improvement of original learning may be attributed to the necessity for rats to acquire the basic "win-shift" rule (i.e., reference memory) in this task.

In addition to creating learning deficits, the time spent in each choice was shortened during the original learning process by DH lesioning (Fig. 4), and this shortening was reversed by repeated administration of JTP-4819 (3.0 mg/kg). However, the time for each choice was not shortened by DH lesioning during relearning, perhaps because it was relatively short in sham-operated rats (Fig. 4 and 7). Alternatively, DH-lesioned rats might have shown a decreased emotional response to an unfamiliar environment (6) (Fig. 4). whereas they might not show such a response after becoming sufficiently accustomed to the maze (Fig, 7). Therefore, JTP-4819 (3.0 mg/kg) might compensate for hippocampal dysfunction, and thus normalize the decreased emotional response of the DH-lesioned rats. We have previously found that even a high oral dose of 100 mg/kg of JTP-4819 has little influence on spontaneous locomotor activity in mice (unpublished data).

The effects of JTP-4819 on ChAT activity and M1-binding were also examined in an attempt to clarify the biochemical mechanisms underlying the improvement of learning impairment by this drug. A significant reduction of ChAT activity was observed in the residual hippocampus but not in the cerebral cortex of DH-lesioned rats, indicating that the damage produced by lesioning involved the residual hippocampus. On the other hand, M1-binding was not affected by DH lesioning. After repeated administration of JTP-4819, no significant changes were observed in either ChAT activity or M1-binding. However, our previous neurochemical studies have demonstrated that administration of JTP-4819 for 3 weeks significantly reverses the age-related increase of ChAT activity in the cerebral cortex and the decrease of high-affinity [^{3}H]-choline uptake in the hippocampus (43). In addition, JTP-4819 increases ACh release in the cerebral cortex and hippocampus of young and aged rats (40), probably by enhancing the ACh-

TABLE 1

EFFECT OF REPEATED ADMINISTRATION OF JTP-4819 ON THE HIPPOCAMPAL AND CORTICAL ChAT ACTIVITIES AND [^{3}H]-PIRENZEPINE BINDING IN RATS WITH DORSAL HIPPOCAMPAL LESIONS

			[^{3}H]-Pirenzepine Binding	
Drugs	Daily dose (mg/kg, p.o.)	ChAT activity (nmol/h/mg protein)	B_{max} (fmol/mg protein)	K_d (nM)
Hippocampus				
Sham		159.3 ± 3.8 (4)	645.5 ± 56.4 (4)	11.2 ± 1.3 (4)
Vehicle		106.1 ± 8.1* (9)	619.5 ± 52.2 (8)	12.8 ± 0.9 (8)
JTP-4819	1.0	113.8 ± 5.9 (9)	606.9 ± 64.7 (8)	14.4 ± 1.2 (8)
	3.0	110.6 ± 8.0 (9)	523.4 ± 39.6 (9)	15.4 ± 2.5 (9)
	10.0	115.8 ± 5.5 (8)	618.1 ± 52.4 (7)	12.7 ± 1.2 (7)
Cerebral cortex				
Sham		181.0 ± 28.8 (4)	605.7 ± 87.2 (4)	18.3 ± 2.8 (4)
Vehicle		147.5 ± 2.2 (9)	552.5 ± 28.8 (9)	18.3 ± 1.5 (9)
JTP-4819	1.0	151.2 ± 4.7 (9)	534.1 ± 41.6 (9)	17.9 ± 1.8 (9)
	3.0	143.6 ± 2.8 (9)	577.5 ± 29.9 (9)	19.7 ± 2.2 (9)
	10.0	148.6 ± 4.5 (8)	595.4 ± 26.0 (8)	17.9 ± 1.7 (8)

Each parameter was estimated after 38 days of JTP-4819 administration and rats were killed 60 min after the final drug dose. Values are means ± SE of the number of animals indicated in parentheses.

*Sham vs DH-lesion + Vehicle ($p < 0.01$, Student's t-test).

releasing effects of TRH (10,44) and SP (41) via inhibition of PEP. Thus, it remains possible that enhancement of central cholinergic function may be involved in the ameliorating effect of JTP-4819 despite the fact that cholinergic parameters (ChAT and M1-binding) were unaltered in the present study. Further studies seem to be needed to determine the effect of JTP-4819 on ACh release in this model.

Alternatively, a direct action on the neuropeptidergic system may have contributed to the ameriolative effect of JTP-4819 in this study. It has been reported that nonspecific deterioration of the basal forebrain cholnergic system in combination with the loss of other related neurons is needed to produce spatial learning impairment (9). As the hippocampal radiofrequency lesions using in this study were not selective for cholinergic neurons, non-cholinergic denervation may also have contributed to the learning deficits in DH-lesioned rats, and direct or compensatory enhancement of the noncholinergic system by JTP-4819 might have occured. In our previous studies, we demonstrated that the deficits of memory-related behavior in several animal models were improved through the enhancement of neuropeptidergic function secondary to PEP inhibition by JTP-4819. For instance, coadministration of JTP-4819 and SP, AVP, or TRH improved the retention time of rats with scopolamine-induced amnesia in the passive avoidance response, suggesting that JTP-4819 mediated the increase of SP, AVP, and TRH levels in the brain through inhibition of the degradation of these peptides by PEP. Furthermore, JTP-4819 has been demonstrated to improve Morris water maze learning deficits in aged rats (43), possibly suggesting the activation of brain SP (42) and TRH (35) function. More recently, we also found that JTP-4819 improved Morris water maze learning deficits in rats with middle cerebral artery occlusion and restored cortical TRH levels in this model (36). Accordingly, JTP-4819 may exhibit a compensatory effect on learning deficits in DH-lesioned rats via direct activation of neuropeptides by inhibition of PEP, although further studies on neuropeptidergic neurons in this model are needed to support such a mechanism.

In conclusion, the present study showed that JTP-4819 ameliorated impairment of both learning and relearning in DH-lesioned rats performing the eight-arm radial maze test. In light of our previous findings, it seems that these effects of JTP-4819 may be due to enhancement of neuropeptide function via inhibition of PEP.

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