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Effect of a Novel Prolyl Endopeptidase Inhibitor, JTP-4819, on Spatial Memory and Central Cholinergic Neurons in Aged Rats

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K. TOIDE, M. SHINODA, T. FUJIWARA AND Y. IWAMOTO. *Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on spatial memory and central cholinergic function in aged rats.* PHARMACOL BIOCHEM BEHAV **56**(3), 427–434, 1997.—The effects of a novel prolyl endopeptidase inhibitor (PEP), (S)-2-[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-*N*-(phenylmethyl)-1-pyrrolidinecarboxamide (JTP-4819), on performance of the Morris water maze task and on central cholinergic function were investigated in aged rats. Spatial memory (escape latency, path length, and swimming speed to the platform) was impaired in aged rats performing the Morris water maze task when compared to young rats. Administration of JTP-4819 (1 mg/kg, p.o.) for 14 days improved this memory deficit in aged rats, as shown by the decrease in escape latency and path length. In addition, when JTP-4819 (at doses of 1 and 3 mg/kg, p.o.) was administered for 3 wk, it reversed the age-related increase of ChAT activity in the cerebral cortex and the decrease of ^{3}H-choline uptake in the hippocampus. These data suggest that JTP-4819 ameliorates age-related impairment of spatial memory and partly reverses central cholinergic dysfunction, possibly due to the enhancement of neuropeptide function by inhibition of PEP mediated degradation of substance P, arginine-vasopressin, and thyrotropin-releasing hormone. **Copyright © 1997 Elsevier Science Inc.**

Prolyl endopeptidase inhibitor (S)-2-[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-*N*-(phenylmethyl)-1-pyrrolidine-carboxamide (JTP-4819) Morris water maze Spatial memory ChAT activity ^{3}H-choline uptake Acetylcholine and choline content Aged rat Alzheimer's disease

CURRENTLY, new drugs are required that can improve memory and learning or delay the neurodegenerative process in conditions such as Alzheimer's disease (AD). The most marked and consistent neurochemical abnormality found in AD is the degeneration of basal forebrain cholinergic neurons innervating the cerebral cortex and hippocampus (2,4,21, 29,49). Accordingly, elucidation of the role played by damage to the forebrain cholinergic system has been a central part of attempts to understand the cognitive deficits associated with this disease and the treatment of AD has been primarily based on the use of cholinergic agents (32).

Recently, several studies have been published on the abnormalities of neuropeptides and their related proteases in AD, providing evidence that some neuropeptide-containing neuron populations are pathologically altered in this disorder (1,3,6,12,14,31). Many biologically active peptides contain proline within their amino acid sequence (46,48). An enzyme that hydrolyses peptide bonds at the carboxyl terminus of L-proline residues was first found as an inactivator of oxytocin in the human uterus (47), and this enzyme was subsequently purified and named prolyl endopeptidase (PEP) (48). PEP has since been isolated from various tissues in mammals (16,38,51,54, 55). Because of its high specificity for proline residues (20,47, 48,54), PEP has been proposed to play a role in the metabolism of proline-containing neuropeptides such as substance P (SP), arginine-vasopressin (AVP), thyrotropin-releasing hormone (TRH), neurotensin, angiotensin II, oxytocin, and bradykinin (10,18,50,51,53). It has also been shown that AVP, SP, and TRH are capable of improving the performance of animals in learning and memory tasks (5,11,13,33,37). However, there

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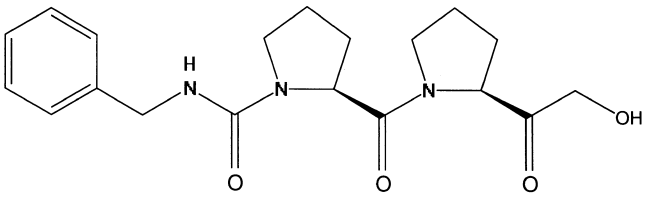

FIG. 1. Chemical structure of JTP-4819.

have been contradictory reports concerning PEP activity in the brains of AD patients (1,7,14,39), and it still remains unclear whether the activity of this enzyme is reduced or increased in AD.

On the basis of the above findings, we have attempted to develop a PEP inhibitor for the treatment of AD. Among the various compounds tested, we found a novel PEP inhibitor, (S)-2-[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-*N*-(phenylmethyl)-1-pyrrolidinecarboxamide (JTP-4819, Fig. 1) (19). JTP-4819 shows strong and highly specific inhibition of PEP, thus inhibiting the degradation of proline-containing neuropeptides such as AVP, SP, and TRH, reversing scopolamine induced amnesia in the passive avoidance test (42), ameliorating memory deficits in rats with middle cerebral artery occlusion in Morris water maze (34), and increasing the cortical and hippocampal levels of SP- and TRH-like immunoreactivity in aged rats (35,44). In the present study, we evaluated the effects of JTP-4819 on spatial memory (Morris water maze task) and on central cholinergic function in aged rats.

METHODS

Animals

The animals used were male Fisher (F344) rats (3 months old: 250–310 g; 24 mo old: 360–550 g), housed at a temperature of 23 ± 1°C and a relative humidity of 55 ± 5% under a 12-h light-dark cycle.

1. Morris Water Maze

Drug administration. JTP-4819 dissolved in distilled water (0.1 ml per 100 g body) or distilled water of the same volume were administered once daily through a stainless steel stomach tube. Administration was done for 20 days at 60 min before each test session.

Apparatus. The Morris water maze task (28) was used with the modifications described below. The water maze consisted of a circular pool (diameter: 144 cm, height: 45 cm) with a featureless blue inner surface. The pool was filled to a depth of 25 cm with 23°C (±1°C) water. The hidden platform was a clear Plexiglass stand (diameter: 10 cm) submerged 2 cm below the water surface so that it was invisible at water level. A video Image Motion Analyzer (Neuroscience AXIS 30, Tokyo) was used to measure the escape latency and swimming path length to the platform.

Habituation. At the beginning of the experiment, the rats were allowed to swim freely for 1 min to become habituated to the apparatus.

Training and testing. From the day after habituation, each animal underwent one acquisition trial per day. The platform position and the start point, which was on the opposite side to the platform at an angle of 160°, were kept constant during the acquisition trials. The animals were given 120 sec to find the platform during each acquisition trial, and were allowed to rest on the platform for 30 sec after they found it. The trial was terminated after 120 sec if the rat failed to find the platform, and the animal was placed on the platform for 30 sec by the experimenter. The latency was recorded as 120 sec when a rat failed the trial.

Testing of the rats and observation were done in the same way as during training. Namely, the path length (the distance traveled by the rats), escape latency (the time to find the platform), and velocity (the swimming rate of the rat) were recorded during each trial using the monitoring system.

2. Determination of ChAT Activity, ^{3}H-choline Uptake and ACh and Choline Levels

The day after completing the behavioral studies, we examined the effects of JTP-4819 on cortical and hippocampal cholinergic function in the young and aged rats which had been used in the Morris water maze task.

2-1. ChAT Activity

One hour after the completion of JTP-4819 administration for 21 days, rats were decapitated and their brains were quickly removed and the cerebral cortex and hippocampus were dissected from the fresh brains (9). The left cerebral cortex and hippocampus of 4 and 8 rats per each group were frozen and stored at −80°C until the determination of ChAT activity. The ChAT assay was described as follows (17). Namely, an enzyme solution for the measurement of ChAT activity was prepared from frozen brain tissue by homogenization in 3 volumes of 25 mM phosphate buffer containing 0.5% triton (pH 7.4) per g wet weight, followed by centrifugation at 20,000 g for 60 min at 4°C. Then the supernatant obtained by dilution in 15 volumes of 25 mM phosphate buffer (pH 7.4) was used as the enzyme solution. The reaction was initiated by adding 100 μl of substrate solution containing 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) to 100 μl of enzyme solution in 25 mM sodium phosphate buffer (pH 7.4) at 37°C for 20 min, and the reaction was stopped by adding 50 μl of 1 M perchloric acid and placing the mixture in an ice-bath. After 10 min, 10 μl of 0.1 mM isopropylhomocholine (the internal standard) was added and the reaction mixture was centrifuged at 1,600 g for 10 min at 4°C. After the supernatant was filtered through a 0.45 μm millipore filter, a 10 μl aliquot was injected into a high-performance liquid chromatography with an electrochemical detector (HPLC/ECD). Measurements were performed at an applied potential of 450 mV and a flow rate of 1.0 ml/min, using a platinum electrode 3.0 mm in diameter. The mobile phase was 0.1 M phosphate buffer (pH 8.5) containing 0.6 mM tetramethylammonium and 1.23 mM sodium 1-decanesulfonate, which was degassed by passage through a 0.45 μm millipore filter before use. The protein content of samples was determined by the method of Lowry (22).

2-2. High Affinity Choline Uptake (HACU)

The right cerebral cortex and hippocampus of 4 rats per each group were rinsed in ice-cold 0.32 M sucrose before the HACU assay (52). Synaptosomes were prepared by homogenizing tissue samples in 3 ml of cold 0.32 M sucrose buffered with 5 mM Tris-HC1 (pH 7.4). The homogenate was centrifuged at 1,000 g for 10 min to remove cellular debris, after which the resulting pellet was washed once with buffered sucrose and recentrifuged. The supernatants were combined

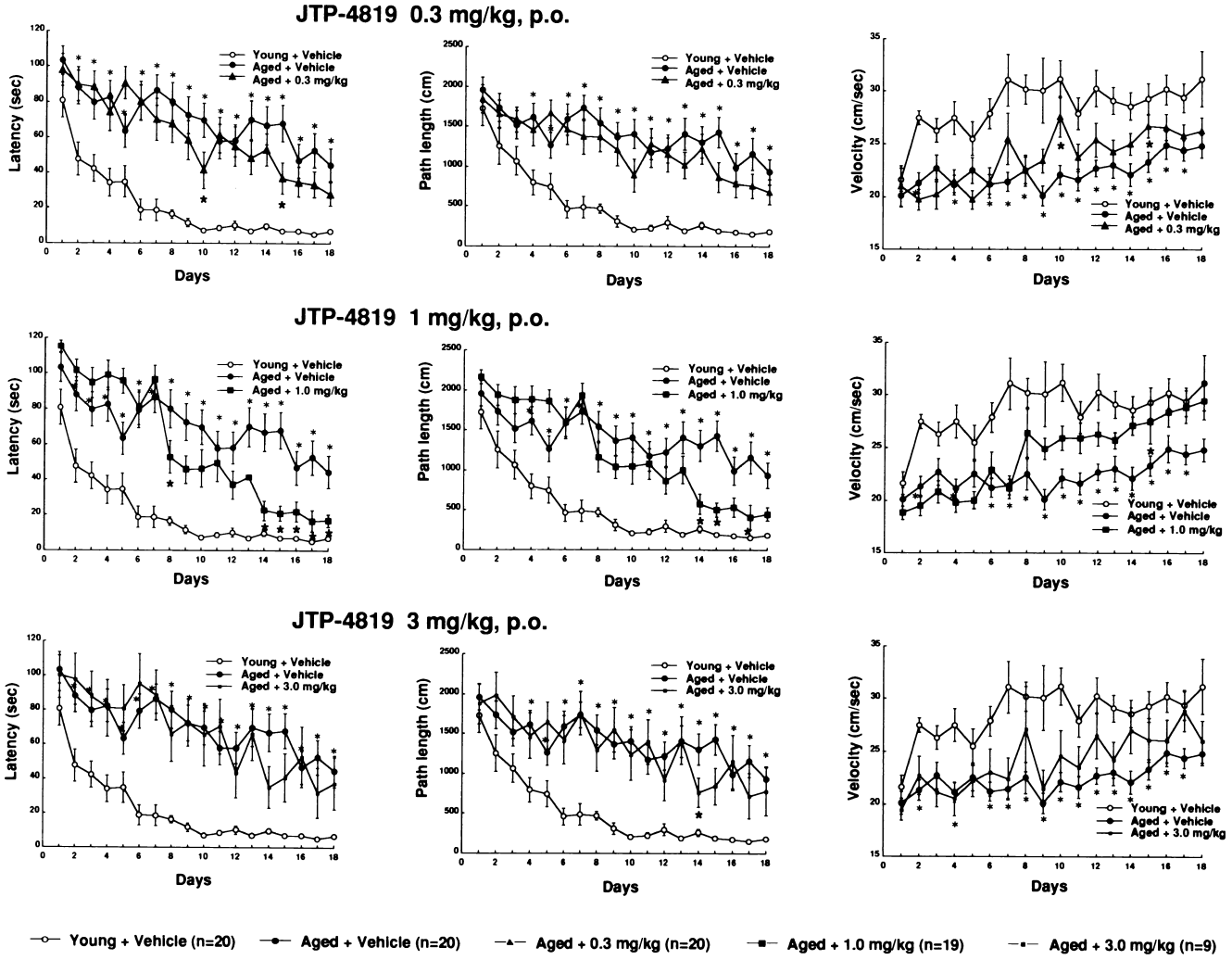

FIG. 2. Effect of JTP-4819 on the spatial memory deficit of aged rats in the Morris water maze. Ordinate: escape latency (left panel), path length (middle panel) and velocity (right panel) to find the hidden platform are shown. Abscissa: each session indicated occurred at 60 min after JTP-4819 administration. Each point represents the mean ± S.E. of the number of animals indicated in parentheses. *$p < 0.05$ versus young control rats. Star = $p < 0.05$ versus aged control rats (one-factor ANOVA followed by Duncan's multiple comparison test).

and centrifuged at 12,000 g for 20 min to yield a crude synaptosomal pellet. This was resuspended in 3 ml of HEPES-buffered Krebs-Ringer solution (in mM: NaCl, 124; KCl, 5; $CaCl_2$, 1.5; $MgCl_2$, 1.3; HEPES-NaOH (pH 7.4), 20; and glucose, 10) containing 0.5 μM choline chloride. Aliquots of each sample (0.1 ml) were mixed with Krebs-Ringer solution (0.8 ml) and were equilibrated at 37°C. Then 0.1 ml of [^{3}H]-choline (6 μCi/ml) was added and incubation was continued for an additional 20 min. Choline uptake was terminated by centrifuging the sample rapidly in the cold and washing it twice with cold Krebs-Ringer solution to remove excess radioactivity. The protein content of samples was determined by the method of Lowry (22).

2-3. Determination of ACh and Choline Levels in the Cerebral Cortex and Hippocampus

The assay of ACh and choline was performed by the method described previously (40) with some modifications. After 5 rats per each group were killed by microwave irradiation (9.7 KW, 1.5 s), their brains were removed and dissected to remove the cerebral cortex and hippocampus. The left cortex and hippocampus were separately homogenized in 10 volumes of 0.1 M perchloric acid containing 10^{-4} M ethylhomocholine as the internal standard. After cooling on ice for 30 min, the samples were centrifuged at 10,000 g for 15 min at 4°C, after which ACh and choline were extracted from the supernatant as follows. The supernatant was adjusted to pH 3.5–4.0 with 0.2 M $KHCO_3$, let stand for 30 min, and then subjected to centrifugal filtration through a 0.45 μm millipore filter. Then 10 μl of the filtrate was injected into an HPLC/ECD apparatus. The mobile phase and HPLC/ECD conditions were the same as for the ChAT assay.

Chemicals

JTP-4819 (mol.wt. 359.43) was synthesized at our Research Institute. JTP-4819 is colorless needles with melting point 139°C and specific rotation $[\alpha]_D = -125°$ (c 1.01, methanol). The compound is fairly soluble in methanol, soluble in ethanol,

methylene chloride and distilled water, slightly soluble in tetrahydrofuran, and extremely insoluble in ether. The following agents were obtained commercially: [^{3}H]-choline (NEN), acetyl-CoA (Sigma), choline chloride (Sigma), scopolamine hydrobromide (Sigma), and physostigmine sulfate (Sigma). For all aqueous solutions, the water used was purified by a Milli-Q system (Milli-RX 12: RO membrane, ELIX module; Milli-Q SP UF; Q-Pak cartridge, ultrafiltration membrane, 0.22 mM Milli-Pak filter, Millipore, USA).

Statistical Analysis

Significant differences in Morris water maze task performance were assessed in two ways. First, two-factor (group X trial) analysis of variance (ANOVA), with repeated measure over trial, was used. Second, one-factor ANOVA followed by Duncan's multiple comparison test was used on the data of each trial. For analysis of the differences in neurochemical data between young and aged rats, Student's t-test was used, while changes in the aged rats due to JTP-4819 administration were assessed by one-factor ANOVA followed by Duncan's multiple comparison test.

RESULTS

1. Effect of JTP-4819 on Spatial Memory in the Morris Water Maze Task

Vehicle-treated aged rats significantly showed longer both path length and escape latency [$F(5, 92) = 30.385$, $p = 0.001$], and shorter swim speed [$F(5, 92) = 10.228$, $p = 0.001$] when compared with vehicle-treated young rats (Fig. 2). These deficits tended to be improved by oral JTP-4819 at a dose of 0.3 mg/kg, although the differences in path length and escape latency between the rats with and without JTP-4819 administration did not always reach statistical significance. At a dose of 1.0 mg/kg orally, JTP-4819 significantly shortened both path length and escape latency from day 14 of administration onwards. However, the improvement was less marked when the dose was increased to 3 mg/kg, indicating that the dose-response curve was bell-shaped with the maximum effect at 1 mg/kg. JTP-4819 only showed significant but not consecutive increases at a dose of 1.0 mg/kg, p.o. on day 10 and at doses of 0.3 and 1.0 mg/kg, p.o. on day 15, respectively (Fig. 2).

2. Effect of JTP-4819 on ChAT Activity in the Cerebral Cortex and Hippocampus

Both cortical and hippocampal ChAT activity were significantly increased in vehicle-treated aged rats compared with young rats. Oral administration of JTP-4819 at doses of 1 and 3 mg/kg for 21 days significantly reversed the age-related increase of ChAT activity in the cerebral cortex, although it had almost no effect on ChAT activity in the hippocampus (Fig. 3).

3. Effect of JTP-4819 on Cortical and Hippocampal High-Affinity ^{3}H-Choline Uptake

Cortical ^{3}H-choline uptake was significantly increased in vehicle-treated aged rats compared with young rats, while hippocampal uptake was decreased. Oral administration of JTP-4819 (1 and 3 mg/kg, p.o.) for 21 days significantly reversed the age related decrease of hippocampal ^{3}H-choline uptake, although it had almost no effect on cortical uptake (Fig. 4).

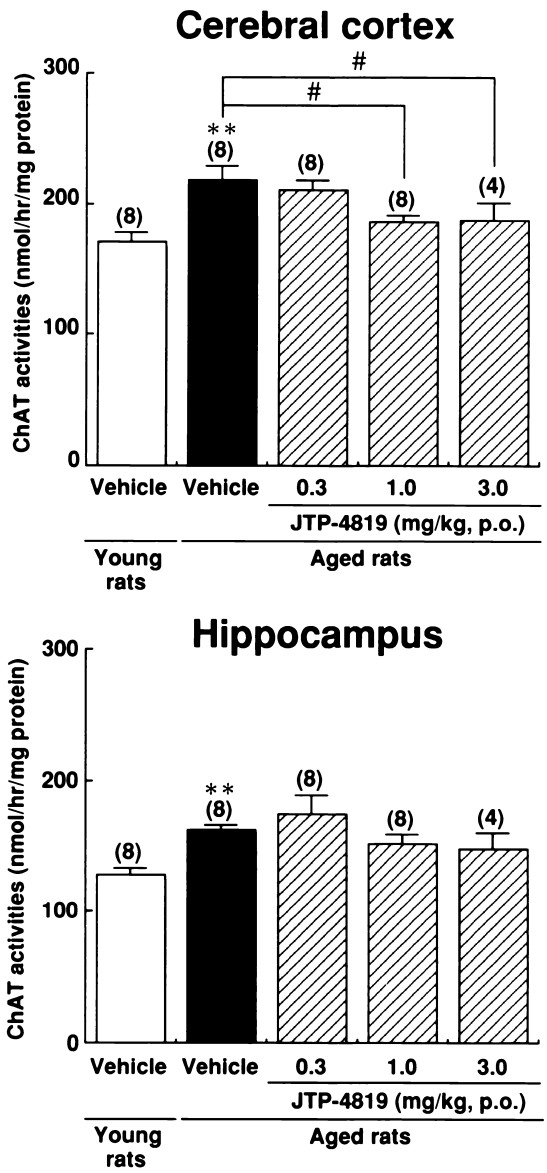

FIG. 3. Effect of repeated administration of JTP-4819 on the cortical and hippocampal ChAT activity in aged rats. ChAT activity was estimated after 21 days of JTP-4819 administration and rats were killed 60 min after the final drug dose. Columns and bars represent the mean ± S.E. from four and eight rats. $^{**}p < 0.01$ versus young control rats (Student's *t*-test). $\#p < 0.05$ versus aged control rats (one-factor ANOVA followed by Duncan's multiple comparison test).

4. Effect of JTP-4819 on ACh and Choline Levels in the Cerebral Cortex and Hippocampus

The cortical and hippocampal ACh levels were similar in vehicle-treated aged rats and young rats. Oral administration of JTP-4819 (0.3–3.0 mg/kg, p.o.) for 21 days did not significantly alter cortical or hippocampal ACh and choline levels in aged rats (Fig. 5).

DISCUSSION

In the present study, we investigated whether inhibition of PEP by JTP-4819 could ameliorate spatial memory deficits in

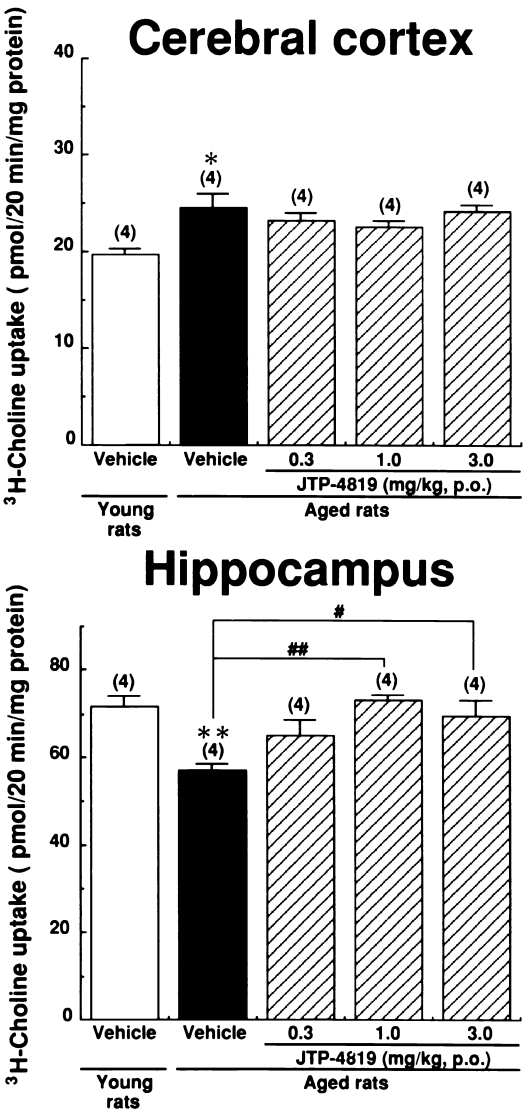

FIG. 4. Effect of repeated administration of JTP-4819 on the cortical and hippocampal ^{3}H-choline uptake in aged rats. Choline uptake was estimated after 21 days of JTP-4819 administration and rats were killed 60 min after the final drug dose. Columns and bars represent the mean ± S.E. from four rats. $*p < 0.05$ and $**p < 0.01$ versus young control rats (Student's *t*-test). $\#p < 0.05$ and $\#\#p$ 0.01 versus aged control rats (one-factor ANOVA followed by Duncan's multiple comparison test).

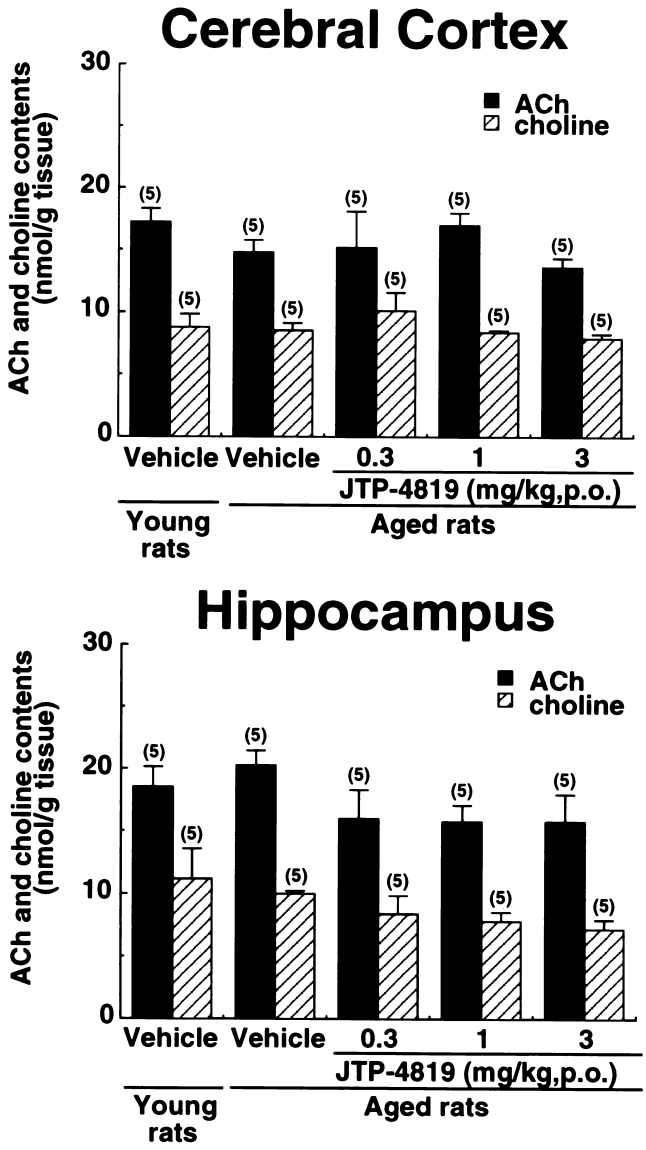

FIG. 5. Effect of repeated administration of JTP-4819 on the ACh and choline contents of the cerebral cortex and hippocampus in aged rats. ACh and choline contents were estimated after 21 days of JTP-4819 administration and rats were killed 60 min after the final drug dose. Data represent the mean ± S.E. for groups of five rats. Black columns: ACh, Striped columns: choline.

aged rats performing a memory-related behavior task (Morris water maze), as well as the effects of JTP-4819 on central cholinergic function in aged rats performing this task.

Compared to young rats, vehicle-treated aged rats showed clear impairment of path length, escape latency, and swimming speed in the Morris water maze task with a hidden platform, suggesting that these age-related changes were possibly due to spatial memory deficits. JTP-4819 (1.0 mg/kg, p.o.) reversed the age-related decrease of both path length and escape latency from day 14 of administration onwards, suggesting the amelioration of spatial memory. The swimming speed showed no significant amelioration in JTP-4819-treated rats, indicating that the amelioration of the water maze performance deficits by JTP-4819 was unlikely to be mediated via an effect on sensorimotor function. However, it has been reported that learning in rats released from the same point may involve the acquisition of a habitual response mediated via the caudate nucleus, as opposed to the formation of a true spatial representation in the hippocampus (26,36). Therefore, further experiments involving the release of rats from different starting points need to carried out using the Morris water maze to confirm our findings. It is also necessary to carry out experiments using both a visual platform version and a probe test in order to eliminate the influence of age-induced sensorimotor deficits and habitual responses.

JTP-4819 showed a bell-shaped dose-response curve in the

Morris water maze task. In contrast, its inhibitory effect on PEP and its effect on the brain levels of neuropeptides (SP, AVP, and TRH) did not show such a bell-shaped dose-response curve (41,42). Therefore, further studies are needed to clarify the mechanism involved (e.g., to investigate the effects of second messenger systems on postsynaptic neuropeptide function in memory related-behavior).

We have already demonstrated that administration of JTP-4819 either before the acquisition or retention trial has an anti-amnesic effect in scopolamine-treated rats performing the passive avoidance task (42), suggesting that JTP-48 19 may also possess the ability to enhance the acquisition and retrieval processes of memory. More recently, we found that JTP-4819 could improve spatial memory in rats with middle cerebral artery occlusion performing the Morris water maze task (34) and dorsal hippocampal-lesioned rats performing the radial arm maze task (27). These observations suggest that JTP-4819 has potent and wide-ranging effects on performance deficits in various experimental models of memory-related performance.

After the behavioral study, we examined the effects of JTP-4819 on central cholinergic function. ChAT activity was increased in the cerebral cortex and hippocampus of vehicle-treated aged rats compared with vehicle-treated young rats, while ^{3}H-choline uptake was increased in the cortex and decreased in the hippocampus of the aged rats. In addition to the previously reported decrease in the cortical and hippocampal levels of SP and TRH (35,44), these cholinergic changes found in aged rats suggest that the age-related deficit of spatial memory was at least partly based on both cholinergic (2,30) and peptidergic disturbances. Our findings regarding cortical and hippocampal ChAT activity, ^{3}H-choline uptake, and the brain ACh content in aged rats are partly in agreement with those of previous investigations, with the discrepancies probably being due to differences in the species and age of the animals used (23,24,30,52). Repeated administration of JTP-4819 for 21 days normalized ChAT activity in the cerebral cortex of aged rats as well as ^{3}H-choline uptake by the hippocampus, although a single dose had no effect (data not shown). The question arises as to why JTP-4819 improved the cholinergic imbalance in the brain after repeated administration. In contrast to these findings, our previous microdialysis study demonstrated that a single dose of JTP-4819 enhanced ACh release in the frontal cortex and hippocampus of both young and aged rats (42). Therefore, the primary effect of JTP-4819 on cholinergic neurons may be the enhancement of ACh release from nerve terminals, followed by normalization of ^{3}H-choline uptake and ChAT activity in aged rats during repeated administration, suggesting that functional improvement may occur through the activation of cholinergic neurotransmission by continuous promotion of ACh release.

Regarding ACh release, our previous report and another study have already demonstrated that TRH increases ACh release in the cerebral cortex and hippocampus (8,45). In addition, we recently found that ACh release was increased in the frontal cortex and hippocampus by SP (43) and AVP (unpublished data). At present, enhancement of ACh release by JTP-4819 is thought to be probably due to the increase of TRH, SP, and/or AVP in the brain secondary to PEP inhibition, although we have not assessed the effect of other proline-containing neuropeptides such as neurotensin and angiotensin. In addition, we have already demonstrated that administration of JTP-48 19 reversed the age-related decrease of cortical and hippocampal SP and TRH levels in rats, possibly due to an inhibitory effect on PEP (35,44). Taken together, these findings suggest that JTP-4819 initially inhibits PEP activity in the brain, resulting in an increase of proline-containing neuropeptides like SP and TRH secondary to prevention of their degradation (42), and consequently reverses central cholinergic dysfunction. These neuropeptides may also have several neuroprotective actions (15,25,52), together with a positive effect on memory and learning (5,11,13,33,37). Thus, the deficit of spatial memory and the cholinergic dysfunction that occur with senescence may have been reversed by the protective effect of these neuropeptides following repeated administration of JTP-4819.

In conclusion, JTP-4819 ameliorated the deficits of water maze performance shown by aged rats and reversed the age-related changes of central cholinergic neurons, possibly due to activation of peptidergic neurons via PEP inhibition. These findings support the concept that JTP-4819 may be of some value for treating deficits in cognitive function associated with AD.

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REFERENCES

1. Aoyagi, T.; Wada, T.; Nagai, M.; Kojima, F.; Harada, S.; Takeuchi, T.; Takahashi, H.; Hirokawa, K.; Tsumita, T. Deficiency of kallikrein-like enzyme activities in cerebral tissue of patients with Alzheimer's disease. Experientia 46:94–97; 1990.
2. Bartus, R. T.; Dean III, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
3. Beal, M. F.; Mazurek, M. F. Substance P-like immunoreactivity is reduced in Alzheimer's disease cerebral cortex. Neurology 37:1205–1209; 1987.
4. Coyle, J. T.; Donald, L. P.; Delong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
5. De Wied, D.; Gaffori, O.; Ree, J. M.; Jong, W. Central target for the behavioral effects of vasopressin neuropeptides. Nature 308:276–280; 1984.
6. Ferrier, I. N.; Cross, A. J.; Johnson, J. A.; Robrets, G. W.; Crow, T. J.; Corsellis, J. A. N.; Lee, Y. C.; O'Shaughnessy, D.; Adrian, T. J.; McGregor, G. P.; Baracesehamilton, A. J.; Bloom, S. R. Neuropeptides in Alzheimer type dementia. J. Neurol. Sci. 62:159–170; 1983.
7. Gibson, A. M.; Edwardson, J. A.; McDermott, J. R. Post mortem levels of some brain peptidases in Alzheimer's disease: Reduction in proline endopeptidase activity in cerebral cortex. Neurosci. Res. Commun. 9:73–81; 1991.
8. Giovannini, M. G.; Casamenti, F.; Nistri, A.; Paoli, F.; Pepeu, G. Effect of thyrotropin releasing hormone (TRH) on acetylcholine release from different brain areas investigated by microdialysis. Br. J. Pharmacol. 102:363–368; 1991.
9. Glowinski, J.; Iversen, L. L. Regional studies of catecholamine in the rat brain. I: The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. J. Neurochem. 13: 655–669; 1966.
10. Griffiths, E. C.; Baris, C.; Visser, T. J.; Klootwijk, W. Thyrotropin-

releasing hormone inactivation by human postmortem brain. Regul. Peptides 10:145–155; 1985.
11. Hasenohrl, R. U.; Huston, J. P.; Schuurman, T. Neuropeptide substance P improves water maze performance in aged rats. Psychopharmacology 101:23–26; 1990.
12. Husain, M. M.; Nemeroff, C. B. Neuropeptides and Alzheimer's disease. J. Am. Geriatr. Soc. 38:918–925; 1990.
13. Huston, J. P.; Hasenohrl, R. U.; Gerhardt, P.; Schwarting, R. K. W. Sequences-specific effects of neurokinin substance P on memory, reinforcement and brain dopamine activity. Psychopharmacology 112:147–162, 1993.
14. Ichai, C.; Chevallier, N.; Delaere, P.; Dournaud, P.; Epelbaum, J.; Hauw, J. J.; Vincent, J. J.; Checler, F. Influence of region-specific alterations of neuropeptidase content on the catabolic fates of neuropeptides in Alzheimer's disease. J. Neurochem. 62:645–655; 1994.
15. Iwasaki, Y.; Kinoshita, M.; Ikeda, K.; Takamiya, K.; Shiojima, T. Tropic effect of various neuropeptides on the cultured ventral spinal cord of rat embryo. Neurosci. lett. 101:316–320; 1989.
16. Kalwant, S.; Porter, A. G. Purification and characterization of human brain prolyl endopeptidase. Biochem. J. 276:237–244; 1991.
17. Kaneda, N.; Nagatsu, T. Highly sensitive assay for choline acetyltransferase activity by high-performance liquid chromatography with electrochemical detection. J. Chromatogra. 341:23–30; 1985.
18. Kato, T.; Nakano, T.; Kojima, K.; Nagatsu, T.; Sakakibara, S. Changes in prolyl endopeptidase during maturation of rat brain and hydrolysis of Substance P by the purified enzyme. J. Neurochem. 35:527–535: 1980.
19. Kobayashi, K.; Abe, H.; Toide, K.; Uchida, I. Novel prolyl endopeptidase inhibitors: JTP-3399 and JTP-4819. Design, Synthesis and pharmacological activity on central nervous system. XIIIth Inter. Symp. Med. Chem. Abstr. 06; 1994.
20. Koida, M.; Walter, R. Post-proline cleaving enzyme. J. Biol. Chem. 251:7593–7599; 1976.
21. Lippa, A. S.; Pelham, R. W.; Beer, B.; Critchett, D. J.; Dean, R. L.; Bartus, R. T. Brain cholinergic dysfunction and memory in aged rats. Neurobiol. Aging 1:13–19; 1980.
22. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
23. Luciano, A.; Alema, G. S.; Imperato, A.; Massimo, C.; Ramacci, M. T.; Scrocco, M. G. Aging of the cholinergic synapse in the rats hippocampus: Effect of acetyl-L-carnitine, in: Cholinergic basis for Alzheimer Therapy, In Becker, R.; Giacobini, E. Eds. Boston, Birkhauser; 1991: 134.
24. Matsuoka, N.; Maeda, N.; Yamazaki, M.; Ohkubo, Y.; Yamaguchi, I. Effect of FR121196, a novel cognitive enhancer, on the memory impairment of rats in passive avoidance and radial arm maze tasks. J. Pharmacol. Exp. Ther. 263:436–444; 1992.
25. Mattioli, R.; Schwarting, R. K.; Huston, J. P. Recovery from unilateral 6-hydroxydopamine lesion of substantia nigra promoted by the neurotachykinin substance P 1-11. Neuroscience 48:595–605; 1992.
26. McDonald, R.; White, N. M. Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. Behav. Neural. Biol. 61:260–270; 1994.
27. Miyazaki, A.; Toide, K.; Sasaki, Y.; Ichitani, Y.; Iwasaki, T. Effects of a prolyl endopeptidase inhibitor, JTP-4819, on radial maze performance in hippocampal-lesioned rats. Jpn. J. Psychopharmacol. 15:522, 1995.
28. Morris, R. G. M. Spatial localization does not require the presence of local cues. Learn. motiv. 12:239–260; 1981.
29. Perry, E. K. The cholinergic hypothesis. Ten years on. Br. Med. Bull. 42:63–69; 1986.
30. Quirion, R.; Wilson, A.; Rowe, W.; Aubert, I.; Richard, J.; Doods, H.; Parent, A.; White, N.; Meaney, M. J. Facilitation of acetylcholine release and cognitive performance by an M_2-muscarinic receptor antagonist in aged memory-impaired rats. J. Neurosci. 15:1455–1462; 1995.
31. Rossor, M. N.; Emson, P. C.; Dawbarn, D.; Mountjoy C. Q.; Roth, M. Neuropeptide and dementia. Progr. Brain. Res. 66:143–159; 1986.
32. Sarter, M. Taking stock of cognition enhancers. Trends Pharmacol. Sci. 12:456–461; 1991.
33. Schlesinger, K.; Lipsitz, D. U.; Peck, P. L.; Pelleymounter, M. A.; Stewart, J. M.; Chase, C. N. Substance P enhancement of passive and active avoidance conditioning in mice. Pharmacol. Biochem. Behav. 19:655–661; 1983.
34. Shinoda, M.; Matsuo, A.; Toide, K. Pharmacological studies of a novel prolyl endopeptidase inhibitor, JTP-4819, in rats with middle cerebral artery occlusion. Eur. J. Pharmacol. 305:31–38; 1996.
35. Shinoda, M.; Okamiya, K.; Toide, K. Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on thyrotropin-releasing hormone-like immunoreactivity in the cerebral cortex and hippocampus of aged rats. Jap. J. Pharmacol. 69:273–276; 1995.
36. Sutherland, R. J.; Rudy, J. W. Place learning in the Morris place navigation task is impaired by damage to the hippocampal formation even if the temporal demands are reduced. Psychobiology 16:157–163; 1988.
37. Taira, K.; Kaneto, H. Experimental models for studying the avoidance response in mice and the anti-amnesic effect of prolyl endopeptidase inhibitors. Folia Pharmacol. Japan. 89:243–252; 1987
38. Taylor, W. L.; Dixon, J. E. Catabolism of neuropeptides by a brain proline endopeptidase. Biochem. Biophy. Res. Commun. 94:9–15; 1980.
39. Terwel, D.; Markerink, M.; Jolles, J. Peptidases are affected differently in neocortical regions of brains from patients with Alzheimer's disease. Dementia 3:108–113; 1992.
40. Toide, K. Effects of aniracetam on one-trial passive avoidance tests and cholinergic neurons in discrete brain regions of rats. Arch. int. Pharmacodyn. 298:25–37; 1989.
41. Toide, K.; Fujiwara, T.; Iwamoto, Y.; Shinoda, M.; Okamiya, K.; Kato, T. Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on neuropeptide metabolism in the rat brain. Naunyn-Schmied. Arch. Pharm. 353:355–362; 1996.
42. Toide, K.; Iwamoto, Y.; Fujiwara, T.; Abe, H. JTP-481 9: A novel prolyl endopeptidase inhibitor with potential as a cognitive enhancer. J. Pharmacol. Exp. Ther. 274:1370–1378; 1995.
43. Toide, K.; Iwamoto, Y.; Fujiwara, T.; Okamiya, K. A novel prolyl endopeptidase (PEP) inhibitor, JTP-4819, activates peptidergic-cholinergic neurotransmission as a cognitive enhancer: Implications and mechanism of action. In: Louilot, A.; Durkin, T.; Spampinate, U.; Cador, M., eds. Proceedings of the 6th International Conference in Neuroscience Monitoring Molecules in Neuroscience, Acheve d'imprimer sur les presses, 273–274; 1994.
44. Toide, K.; Okamiya, K.; Iwamoto, Y.; Kato, T. Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on PEP activity and substance P- and arginine-vasopressin-like immunoreactivity in the brains of aged rats. J. Neurochem. 65:234–240; 1995.
45. Toide, K.; Shinoda, M.; Takase, M.; Iwata, K.; Yoshida, H. Effects of a novel thyrotropin-releasing hormone analogue, JTP-4819, on extracellular acetylcholine and choline levels in the rat frontal cortex and hippocampus. Eur. J. Pharmacol. 233:21–28; 1993.
46. Turner, A. J. Processing and metabolism of neuropeptides. Essays Biochem. 22:69–119; 1986.
47. Walter, R.; Shlank, H.; Glass, J. D.; Schwartz, I. L.; Kerenyi, T. D. Leucylglycinamide released from oxytocin by human enzyme. Science (Wash., DC) 173:827–829; 1971.
48. Walter, R.; Simmons, W. H.; Yoshimoto, T. Proline specific endo- and exopeptidases. Mol. Cell. Biochem. 30:111–127; 1980.
49. Whitehouse, P. J.; Price, D. L.; Struble, R. G.; Clark, A. W.; Coyle, J. T.; Delong, M. R. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. Science 215:1237–1239; 1982.
50. Whitty, C. J.; Kapatos, G.; Bannon, M. J. Neurotropic effects of substance P on hippocampal neurons in vitro. Neurosci. Lett. 164:141–144; 1993.
51. Wilk, S. Minireview prolyl endopeptidase. Life Sci. 33:2149–2157; 1983.
52. Williams, L. R.; Rylett, R. J. Exogenous nerve growth factor

increases the activity of high-affinity choline uptake and choline acetyltransferase in brain of Fisher 344 male rats. J. Neurochem. 55:1042–1049; 1990.
53. Yoshimoto, T. Proline specific peptidases and their specific inhibitors. Yakugaku Zasshi 111:345–358, 1991.
54. Yoshimoto, T.; Nishimura, T.; Kita, T.; Tsuru, D. Post-proline cleaving enzyme (Prolyl endopeptidase) from bovine brain. Biochemistry 94:1179–1190; 1983.
55. Yoshimoto, T.; Ogita, K.; Walter, R.; Koida, M.; Tsuru, D. Synthesis of a new fluorogenic substrate and distribution of the endopeptidase in rat tissues and body fluids of man. Biochim. Biophys. Acta. 569:184–192; 1979.