ELSEVIER

European Journal of Pharmacology 305 (1996) 31–38

ejp

Pharmacological studies of a novel prolyl endopeptidase inhibitor, JTP-4819, in rats with middle cerebral artery occlusion

Masahiko Shinoda *, Akira Matsuo, Katsuo Toide

Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1–1, Murasaki-cho, Takatsuki, Osaka 569, Japan

Received 23 October 1995; revised 26 February 1996; accepted 27 February 1996

Abstract

We studied behavioral and pharmacological effects of a novel prolyl endopeptidase inhibitor, (S)-2-[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-*N*-(phenylmethyl)-1-pyrrolidine-carboxamide (JTP-4819), in rats with middle cerebral artery occlusion. Administration of JTP-4819 (0.1 and 1 mg/kg p.o. for 7 days) significantly prolonged passive avoidance latency, while the latency of rats with middle cerebral artery occlusion receiving the vehicle was significantly shorter than that of sham-operated rats. The prolonged escape latency in the Morris water maze task in rats with middle cerebral artery occlusion was also significantly reduced by administration of JTP-4819 (0.3 and 1 mg/kg p.o.). Interestingly, administration of JTP-4819 (0.3–3 mg/kg p.o. for 15 days) restored the decreased cortical thyrotropin-releasing hormone (TRH)-like immunoreactivity content of rats with middle cerebral artery occlusion but did not affect the cortical and hippocampal substance P- or arginine vasopressin-like immunoreactivity content. These results suggest that JTP-4819 ameliorates memory impairment due to middle cerebral artery occlusion by restoring the cortical TRH content.

Keywords: JTP-4819; Prolyl endopeptidase; Morris water maze task; Passive avoidance task; TRH (thyrotropin-releasing hormone); Middle cerebral artery occlusion

1. Introduction

Rats with middle cerebral artery occlusion show infarcts in the cerebral cortex and caudate putamen on the ischemic side. Atrophy and shrinkage are generated in the infarcts about 2 weeks after the occlusion. The volume and the pathological states of the infarcts are similar to clinical findings (Tamura et al., 1981, 1990). These are several reports that rats with middle cerebral artery occlusion show memory and learning impairment in the passive avoidance response and active avoidance response with no change in spontaneous movement (Hirakawa et al., 1994; Tamura et al., 1985; Yamamoto et al., 1988), so that these animals could be a useful model for investigating memory and learning disturbances following focal cerebral ischemia.

Prolyl endopeptidase (EC 3.4.21.26) (Turner, 1986; Walter et al., 1980; Yoshimoto et al., 1983) is an enzyme involved in the metabolism of proline-containing neuropeptides, such as substance P, arginine vasopressin and thyrotropin-releasing hormone (TRH), in the brain (Kato et al., 1980; Wilk, 1983; Griffiths et al., 1985). Many studies have shown that these neuropeptides play an important role in the CNS as neurotransmitters and neuromodulators, and deficiencies of the peptides have been linked to a variety of behavioral abnormalities and to decreased cognitive ability (De Wied et al., 1984; Huston et al., 1993; Rossor et al., 1986). Interestingly, many reports indicate that the contents of these neuropeptides in the cerebral cortex and hippocampus were significantly reduced in patients with senile dementia, such as Alzheimer's disease (Husain and Nemeroff, 1990; Mazurek et al., 1985; Biggins et al., 1983). In this point of view, proline-containing neuropeptide contents in the brain might be closely related to memory and learning function.

We have developed a novel prolyl endopeptidase inhibitor, (S)-2-[[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]-carbonyl]-*N*-(phenylmethyl)-1-pyrrolidine-carboxamide (JTP-4819; Fig. 1) that exhibits potent and specific in vitro inhibition of prolyl endopeptidase activity (Toide et al., 1995a), and significantly increases the contents of substance P-like immunoreactivity, arginine vasopressin-like immunoreactivity and TRH-like immunoreactivity in the

* Corresponding author. Tel.: (81) (726) 81-9700; fax: (81) (726) 81-9722.

PII S0014-2999(96)00173-2

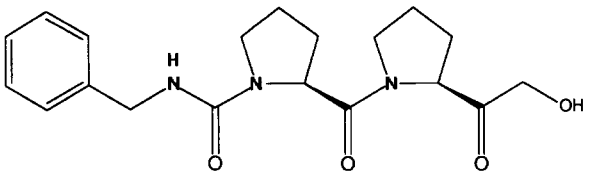

Fig. 1. Chemical structure of JTP-4819.

cerebral cortex and/or hippocampus, in intact rats (Toide et al., 1996). Moreover, administration of this drug improves memory-related disorders, such as scopolamine-induced amnesia in passive avoidance performance (Toide et al., 1995a) and age-related spatial memory deficits in Morris water maze task in rats (Toide et al., submitted). In the present study, we investigated the effects of JTP-4819 on learning behaviors and brain regional neuropeptide contents in rats with middle cerebral artery occlusion as a model for memory impairment induced by focal cerebrovascular ischemia.

2. Materials and methods

These studies were approved by the Animal Experiment Committee of our laboratory.

2.1. Subjects

The subjects were male Sprague-Dawley rats (Charles River Japan), 10-week-old and weighing 300–350 g at the start of the experiments. They were housed with a 12-h light/dark cycle at a temperature of 23 ± 3°C and a relative humidity of 55 ± 15% and were allowed free access to food and water.

JTP-4819 (M_r 359.43) was synthesized at our Research Institute. The other agents used were obtained from commercial sources.

2.2. Surgical procedure

Anesthesia was induced with 4% halothane (Hoechst Japan, Tokyo) and maintained with 2% halothane in 30% oxygen, 10% N_2O and 60% nitrogen. The left middle cerebral artery of each rat was occluded at the proximal portion. The method of middle cerebral artery occlusion, which was originally developed by Tamura et al. (1981), was slightly modified. In brief, each animal was placed in the lateral position, and skin and muscle incisions were made between the left eye and the left external ear. The temporalis muscle was then retracted on either side of the midline of the muscle without removal of the muscle and zygomatic arch. A small burr hole was opened in the basal surface of the temporal bone between the orbital tissue and the foramen ovale. The left middle cerebral artery was occluded with a bipolar electrocoagulator. In the sham-operated group, the middle cerebral artery was exposed but not occluded.

2.3. Passive avoidance task

The passive avoidance test was performed as described by Toide et al. (1995a). In brief, the step-through apparatus used consisted of a two-chambered box separated by a guillotine door; one compartment of the box was well-lit and the other remained dark. For the adaptation trial, the rat was placed in the bright compartment ($25 \times 12 \times 12$ cm), which served as the start box. The door was opened after 5 s, the rat was allowed to enter the dark compartment ($25 \times 12 \times 30$ cm) with a grid on the floor. Once the four legs were on the grid, the guillotine door was immediately shut. Until the acquisition trial after the adaptation trial, we kept the animals in their home cage in a dimly-lit room. After 1 h of adaptation trial, the acquisition trial was carried out in the same manner, except that a scrambled foot-shock (0.4 mA, 1 s) was delivered to the grid after the door was shut. In the retention trial, 24 h after the acquisition trial, the rat was again placed in the bright compartment and the response latency to enter the dark compartment was measured. The behavior observation period was maximally 300 s in the trial. The latency of rats which did not enter the dark compartment during the observation period was calculated as 300 s. The acquisition trial was performed at 13 days after middle cerebral artery occlusion, the retention trials were conducted the day after the acquisition trial. JTP-4819 was given orally once a day for 15 days starting 7 days after the occlusion. On days when the passive avoidance task was performed, drug administration was carried out after acquisition or retention trials.

2.4. Spontaneous movement

Spontaneous movements of each rat were measured using a Scanet counter (Tokyo Medical) for 300 s 24 h after the retention trial (15 days after occlusion).

2.5. Morris water maze task

The Morris water maze task was begun 7 days after middle cerebral artery occlusion. The method was essentially based on the technique described by Morris (1981). In brief, the swim tank was monotone blue, 144 cm in diameter and 45 cm in height, with a hidden platform 10 cm in diameter and 30 cm in height made of clear Plexiglas and placed 35 cm from the wall in the middle of one of the quadrants. The platform was submerged 2 cm below the surface of the water held at 23 ± 1°C. On the first day of acquisition, the rats were given a pretraining session in which they were allowed to swim freely in the pool for 60 s without the platform. The pretraining session was followed immediately by the first session of the acquisition trial. If the rat failed to find the platform within 120 s, the

animal was placed on it for 30 s. The start and platform positions were kept constant during the acquisition trials. The animal's performance was recorded using an overhead videocamera. Each subject was scored for latency to find the platform, the distance swum to the platform and average swimming speed by AXIS 30 (Neuroscience, Tokyo). JTP-4819 was given orally once a day for 15 days after the daily acquisition trial.

2.6. Measurements of substance P-, arginine vasopressin- and TRH-like immunoreactivity in rat brain tissue

The methods for measuring substance P- and arginine vasopressin-like immunoreactivity, and the method for TRH-like immunoreactivity were essentially based on the technique described by Cantor (1986) and Shinoda et al. (1995), respectively, with slight modifications. In brief, 2 days after the final acquisition trial in the Morris water maze task (22 days after the occlusion), the rats were killed by microwave irradiation (9.0 kW, 1 s). The brain of each rat was rapidly removed and the whole cerebral cortex and hippocampus were dissected out on ice. Each brain region was homogenized separately in 10 vols. of 12% acetic acid. After cooling on ice for 30 min, the samples were centrifuged at 11 500 × g and 4°C for 50 min, and the supernatant was stored at −80°C until assay. After freeze-drying, the samples were suspended in 20 mM phosphate-buffered saline (pH 7.4) containing 0.5% bovine serum albumin and 0.03% Tween 20. From each sample or each substance P, arginine vasopressin or TRH standard (Peptide Institute), 0.1 ml was mixed with anti-substance P antiserum, anti-arginine vasopressin antiserum (ZENECA), or anti-TRH antiserum (UCB-Bioproducts), and [^{125}I]substance P, [^{125}I]arginine vasopressin or [^{125}I]TRH (NEN). The mixture was incubated for 22 h at room temperature for the assay of substance P-, or arginine vasopressin-like immunoreactivity contents, or at 4°C for the TRH-like immunoreactivity content. After incubation, 0.5 ml of Amerlex-M (Amersham) for assays of arginine vasopressin-like immunoreactivity and TRH-like immunoreactivity, or 0.1 ml of IgG and 0.5 ml of anti-IgG diluted with 0.1 M borate buffer (pH 8.6) containing 0.1% bovine serum albumin and 4% polyethylene glycol 6000 was added for assay of substance P-like immunoreactivity. The mixture was allowed to stand for 30 min, and was centrifuged at 1500 × g for 10 min for the former, and 50 min for the latter. Radioactivity in the precipitate was counted using a gamma-counter (WALLAC). According to the suppliers, the antiserums we used had less than 0.3% cross-reactivity with neurokinin A and neurokinin B for anti-substance P, less than 0.1% cross-reactivity with oxytocin, neurotensin and mixed porcine neurophysins for anti-arginine vasopressin, no cross-reactivity with Pyro-Glu-His, Pyro-Glu-His-Pro-COOH and His-Pro-diketopeperazine for anti-TRH.

2.7. Histopathological examination

The histopathological study was conducted as follows. The rats were decapitated a week after spontaneous movement measurement (22 days after the occlusion); the brains were preserved in buffered 10% formalin. The tissues were embeded in paraffin wax and sections were cut at 4 μm and stained with Hematoxylin-Eosin.

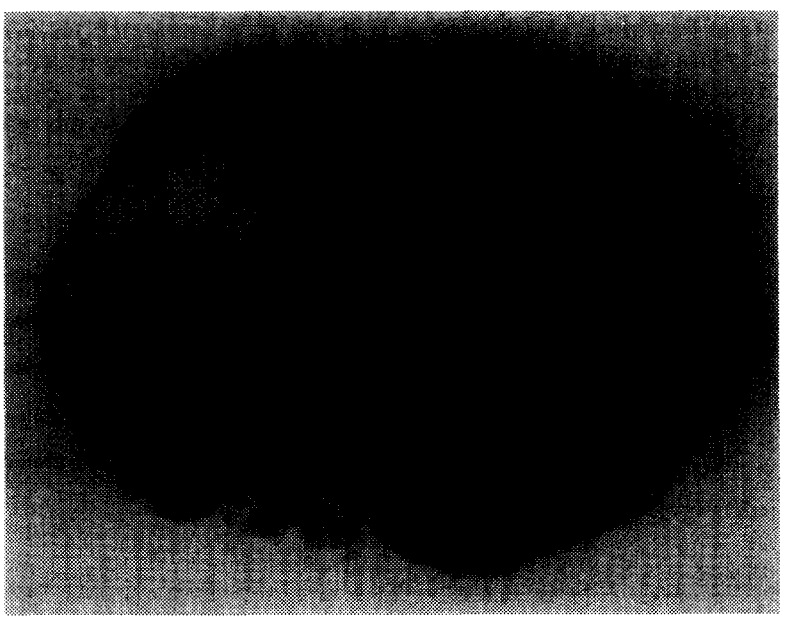

Fig. 2. Photograph of the coronal section showing a cystic infarct affecting the cerebral cortex and the caudate putamen 22 days after middle cerebral artery occlusion. This specimen exhibited marked ischemic damage.

2.8. Statistics

The significance of differences in passive avoidance response was assessed with the Mann-Whitney *U*-test. Latency in the Morris water maze task was analysed in two ways. First, a two-factor (group × trial) ANOVA, with repeated measures over trial, was used. Second, a one-factor ANOVA followed by Duncan's multiple comparison test was used for the data from each trial. Spontaneous movement and the velocities in the water maze were analysed using one-factor ANOVA. Substance P-like immunoreactivity, arginine vasopressin-like immunoreactivity and TRH-like immunoreactivity were analysed using Student's *t*-test and one-factor ANOVA followed by Duncan's multiple comparison test.

3. Results

3.1. Neuropathology

Rats with middle cerebral artery occlusion exhibited a remarkable pattern of infarcts. Ischemic damage as a very serious histological loss was observed in the cerebral cortex of the frontal, auditory areas and the lateral segment of the caudate putamen (Fig. 2). No effects on the ischemic infarct or atrophy size were produced by oral administration of JTP-4819 (data not shown).

On the other hand, body weight was decreased in middle cerebral artery-occluded rats after surgery, but it recovered gradually from day 10 onwards (data not shown).

3.2. Effect of JTP-4819 on passive avoidance task

The latency of the passive avoidance task was significantly shorter in middle cerebral artery-occluded rats than in the sham-operated rats. Administration of JTP-4819 (0.1 and 1 mg/kg p.o. for 7 days) prolonged the latency in a dose-dependent manner (Fig. 3).

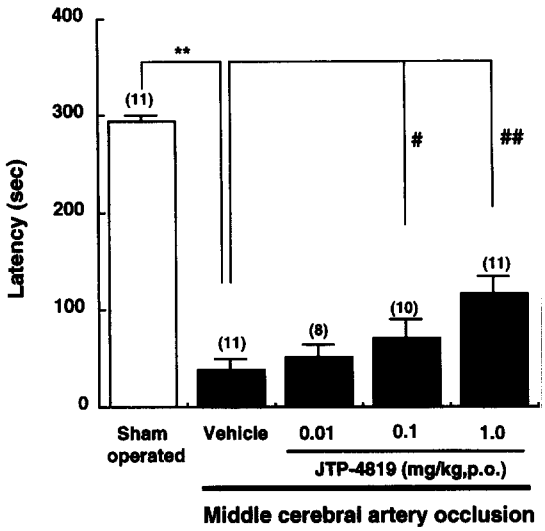

Fig. 3. Effect of JTP-4819 on passive avoidance response in rats with middle cerebral artery occlusion. Rats were trained at 13 days after surgery (6 days after the start of administration) and retention trials were performed at 14 days after occlusion. The drug was orally administered once a day from 1 week after the occlusion. Number of animals is indicated in parentheses. ** $P < 0.01$ vs. sham-operated rats, $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ vs. vehicle control rats (Mann-Whitney's *U*-test).

3.3. Effect of JTP-4819 on Morris water maze task

There were significant differences in aquisition between the groups [$F(5,46) = 30.203$, $P = 0.001$]. Namely, escape latency to the platform was decreased in a time-dependent manner in sham-operated rats, it was less than 10 s on and after the 10th day, which indicated completion of spatial learning. The latency of rats with middle cerebral artery occlusion was significantly longer than that of the sham-operated rats. The latency was significantly shortened by the administration of JTP-4819 (0.3 and 1 mg/kg p.o.), especially at a dose of 1 mg/kg as compared to the group given vehicle. This significant shortening lasted for 6 days after day 9 (Fig. 4). In contrast, the dose of 3 mg/kg produced little effect on this learning task.

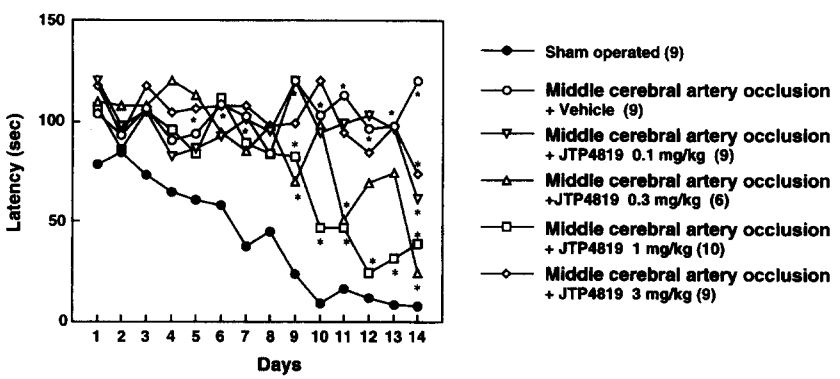

Fig. 4. Effect of JTP-4819 on the spatial memory deficit in rats with middle cerebral artery occlusion subjected to the Morris water maze. The drug was administered orally once a day for 15 days starting 1 week after occlusion. Number of animals is indicated in parentheses. There were significant differences in aquisition between the six groups [$F(5,46) = 30.203$, $P = 0.001$]. * $P < 0.05$ vs. sham-operated rats, * $P < 0.05$ vs. middle cerebral artery-occluded control rats (Duncan's multiple comparison test).

3.4. Effect of JTP-4819 on spontaneous movement and swimming velocity

24 h after the retention trial, the spontaneous movement of each rat was measured. There were no significant differences between the groups (Table 1). The velocities in the groups did not differ, and remained almost constant from the first day (day 1) until the last day (day 14) (Table 2).

3.5. Effect of JTP-4819 on substance P-, arginine vasopressin- and TRH-like immunoreactivity contents

The contents of three kinds of neuropeptides in the cerebral cortex and hippocampus after the Morris water maze task were measured using a RIA. In the cerebral cortex and hippocampus, the substance P-like immunoreactivity and arginine vasopressin-like immunoreactivity contents were not changed in rats with middle cerebral artery occlusion as compared with the sham-operated rats. Oral administration of JTP-4819 had no effect on these contents. The TRH-like immunoreactivity content was more than 70% reduced in the cerebral cortex of rats with middle cerebral artery occlusion. Administration of JTP-4819 (0.3–3 mg/kg p.o.) significantly reversed the decrease in TRH-like immunoreactivity content in the cerebral cortex but not in the hippocampus, where no histological change followed the occlusion (Fig. 5).

4. Discussion

In the present study, we investigated the memory impairment induced by middle cerebral artery-occlusive focal ischemia and the ameliorating effects of a novel prolyl endopeptidase inhibitor, JTP-4819, on learning behavior and brain regional neuropeptides contents. We demonstrated that, as compared with sham-operated rats, rats with middle cerebral artery occlusion had a significantly shortened step-through latency for the passive avoidance response, in accordance with previously published observations regarding this model (Tamura et al., 1985; Yamamoto et al., 1988). We found that oral administration of JTP-4819 (0.1 and 1 mg/kg) improved the latency shortened by middle cerebral artery occlusion. Administration of JTP-4819 (0.3 and 1 mg/kg) significantly shortened the escape latency in the Morris water maze, which had been prolonged by middle cerebral artery occlusion, while JTP-4819 had no effects on spontaneous movements and swimming velocity. In addition, neither ischemic infarct nor atrophy size about 3 weeks after middle cerebral artery occlusion were affected by repeated administration of JTP-

Table 1
Effect of JTP-4819 on spontaneous movement in rats with middle cerebral artery occlusion

Groups	Number of animals	Mean ± S.E. (counts)
Sham operation	11	1565.5 ± 194.7
Middle cerebral artery occlusion + vehicle	11	1979.2 ± 182.9
Middle cerebral artery occlusion + JTP-4819 (mg/kg)		
0.01	8	1431.4 ± 149.9
0.1	10	1745.6 ± 306.7
1	11	1663.0 ± 230.5

Count for each rat was measured for 300 s 24 h after retention trial (15 days after occlusion).

Table 2
Effect of JTP-4819 on velocity of rats with middle cerebral artery occlusion in Morris water maze task

Groups		Number of animals	Mean ± S.E. (cm/s)
Sham-operated	Day 1	10	25.6 ± 1.72
	Day 14	9	29.1 ± 2.66
Middle cerebral artery occlusion + vehicle	Day 1	11	29.6 ± 1.70
	Day 14	9	25.0 ± 2.09
Middle cerebral artery occlusion + JTP-4819 (mg/kg)			
0.1	Day 1	10	25.6 ± 1.93
	Day 14	9	25.6 ± 1.64
0.3	Day 1	9	30.2 ± 1.30
	Day 14	6	25.8 ± 3.77
1	Day 1	12	29.8 ± 1.41
	Day 14	10	29.6 ± 1.79
3	Day 1	10	29.8 ± 0.94
	Day 14	9	26.2 ± 1.39

4819. Therefore, the pharmacological effects of JTP-4819 on passive avoidance response and Morris water maze may be due to ameliorating effects on memory and learning functions per se.

To elucidate the background of the ameliorating effect of this drug, we studied the neuropeptide contents in the brain after the Morris water maze exposure. We found that only the cortical TRH-like immunoreactivity content among the three neuropeptides was decreased by middle cerebral artery occlusion. Strikingly, JTP-4819 significantly countered the decrease of TRH-like immunoreactivity content. We have already found that JTP-4819 increased the brain regional proline-containing neuropeptide contents by inhibiting prolyl endopeptidase activity (Toide et al., 1995b), and that JTP-4819 could enhance the ameliorating effect of TRH on the passive avoidance response in rats (Toide et al., 1995a). Yamamoto et al. (1991) and Lathamn et al. (1985) also reported that administration of TRH ameliorated the disturbance in passive avoidance learning and cortical somatosensory-evoked potential in rats with middle cerebral artery occlusion, respectively. Therefore, our present data suggested that the memory improvement caused by JTP-4819 might be based partly on restoration and enhancement of cortical TRH function, secondary to strong and specific prolyl endopeptidase inhibition (Toide et al., 1995a), decreased by focal brain ischemia, as JTP-4819, even at the very high oral dose of 100 mg/kg, had little influence on spontaneous motor activity, pentobarbital sleeping time, body temperature, maximal-electroshock and pentylenetetrazol-induced convulsions, the electroencephalogram, and other behavioral parameters (unpubl. data). In addition, JTP-4819 (0.3–3 mg/kg p.o.) had no antidepressant activity in the forced swimming test, and had no effect on the levels of acetylcholine, dopamine, serotonin and noradrenaline in the cerebral cortex, hippocampus and/or corpus striatum of rats (unpubl. data).

Dalmaz et al. (1986) and Dresdner et al. (1982) have demonstrated that prolyl endopeptidase is not localized in/on the plasma membrane but is found in the soluble fraction, and it is regarded as a soluble cytoplasmic enzyme. Moreover, Griffiths et al. (1985) reported that TRH was metabolized to TRH-OH by prolyl endopeptidase in the cytosol, but not in the particulate fraction. We also recently found that TRH-OH levels in the brain cytosolic fraction were decreased by JTP-4819 (Toide et al., 1996). These data suggest that the TRH-like immunoreactivity increase produced by JTP-4819 in the brain of rats is due to the inhibition of TRH degradation by brain prolyl endopeptidase. However, TRH is also metabolized by pyroglutamyl aminopeptidase II in the particulate fraction

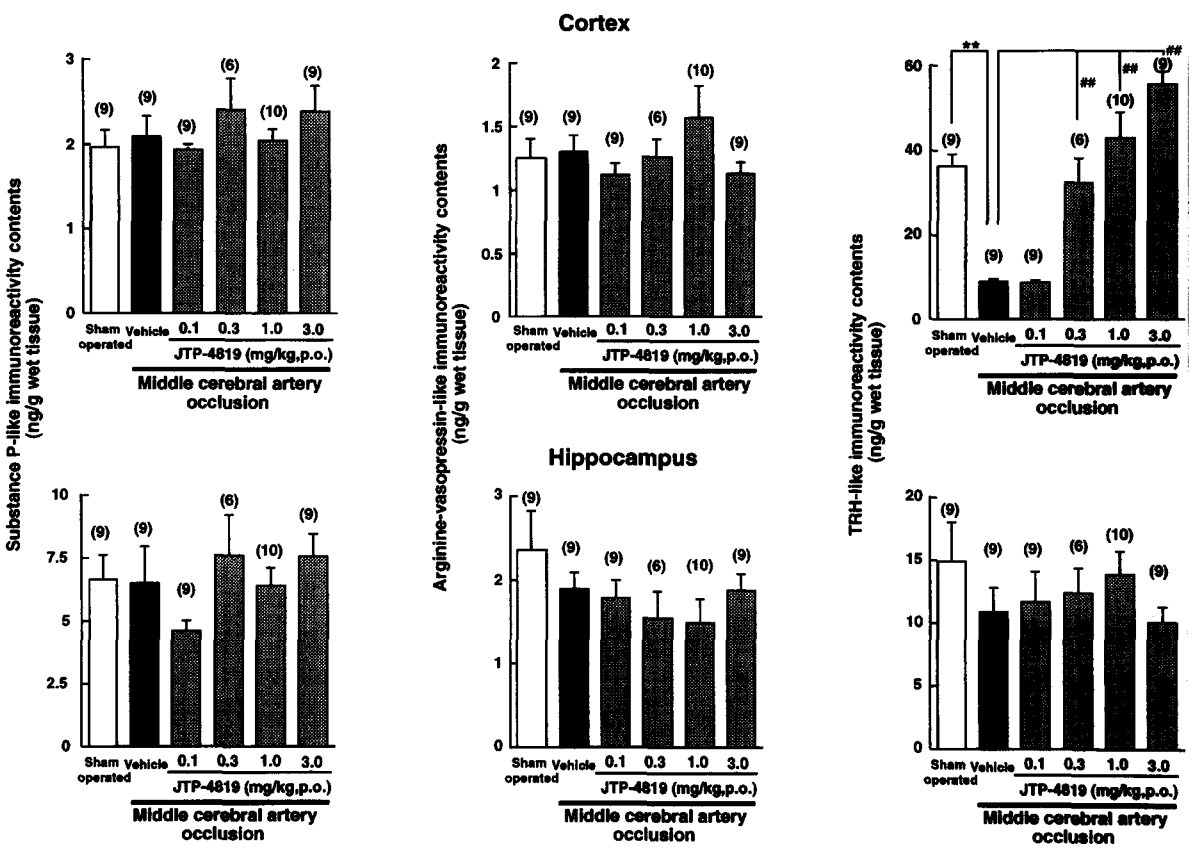

Fig. 5. Effects of JTP-4819 on substance P-, arginine vasopressin- and TRH-like immunoreactivity contents in the cerebral cortex and hippocampus in rats with middle cerebral artery occlusion. Rats were killed by microwave irradiation 22 days after middle cerebral artery occlusion. ** $P < 0.01$ vs. sham-operated rats (Student's t-test), $^{\#\#}$ $P < 0.01$ vs. vehicle control rats (Duncan's multiple comparison test).

(Griffiths et al., 1985), so further study of the role of these peptidases in TRH degradation is needed.

On the other hand, the contents of other neuropeptides, such as substance P- and arginine vasopressin-like immunoreactivity, were unaffected by occlusion or drug administration. It is unclear how these treatment-related differences between TRH-like immunoreactivity, and substance P- and arginine vasopressin-like immunoreactivity contents could be explained. In addition, our previous findings indicated that there were no changes in cortical and hippocampal arginine vasopressin-like immunoreactivity contents with senescence or drug administration (Toide et al., 1995b). In contrast, our most recent observation demonstrated that the cortical TRH-like immunoreactivity content of 24-month-old (aged) rats was significantly lower than that of 3-month-old (young) controls. The decrease was significantly restored by administation of JTP-4819 (Shinoda et al., 1995). Thus, JTP-4819 might possess selectively ameliorating effects on the hypofunctional animals with decreased TRH-like immunoreactivity content, such as aged and middle cerebral artery-occluded rat with memory deficits.

In the present experiments, the highest dose of JTP-4819 (3 mg/kg) did not affect escape latency in the Morris water maze. Our previous findings also indicated that the escape latency of aged rats in the Morris water maze was significantly shortened by oral administration of a dose of 1 mg/kg, but was not affected by the 3 mg/kg dose (Toide et al., submitted). Moreover, the ameliorating effect of JTP-4819 on scopolamine-induced amnesia in the passive avoidance response was reduced at a high dose (>3 or 10 mg/kg) (Toide et al., 1995a). Thus, the improvement by JTP-4819 of deficits in memory and learning behavior exhibited a bell-shaped dose-response curve, as is generally displayed by this type of drugs. However, our present results indicated that the cortical TRH-like immunoreactivity content decreased by middle cerebral artery occlusion was increased dose-dependently by administration of JTP-4819. The TRH-like immunoreactivity content attained at a dose of 3 mg/kg was much higher than that of sham-operated rats. A previous study showed that the ameliorating effect of TRH on memory impairment also showed a bell-shape pattern (Yamamura et al., 1991). One possible explanation is that increased neuropeptide levels in the brain secondary to prolyl endopeptidase inhibition by JTP-4819 act on postsynaptic receptors and thus activate second-messenger systems; i.e. the second-messenger systems may be responsible for the bell-shaped dose-response curve of JTP-4819 in relation to memory-related behavior. Another reason for no ameliorating effect being found with administration of JTP-4819 at a dose of 3 mg/kg may be the participation of some factors other than TRH in the cerebral cortex.

Several reports support the possibility that cholinergic agents have a cognition-enhancing effect on memory impairment after cerebral ischemia (Saito et al., 1985; Ono et al., 1993; Yamamoto et al., 1993). We have found that administration of JTP-4819 increases acetylcholine release in the frontal cortex and hippocampus in rats (Toide et al., 1995a), and stimulates high-affinity choline uptake in the hippocampus (Toide et al., submitted). Therefore, the activation of the cholinergic neuron system, in addition to TRHergic neurons, by JTP-4819, might have led to enhancement of the memory improvement. There are several reports that TRH possesses facilitatory effects on cholinergic neuron in the CNS (Yamamoto and Shimizu, 1989; Toide et al., 1993). Therefore, the ameliorating effects of JTP-4819 on memory impairment in rats with middle cerebral artery occlusion may occur through the facilitation of cholinergic function by TRH increases secondary to prolyl endopeptidase inhibition. Investigation of the effects of JTP-4819 on cholinergic function in rats with middle cerebral artery occlusion is needed to explain the complete mechanisms of action of this drug.

In conclusion, our present data suggest that amelioration of amnesia and spatial memory learning in rats with middle cerebral artery occlusion by the administration of JTP-4819 might be based in part on improvement of the hypofunction of TRHergic neurons. JTP-4819 would be a therapeutic drug with a novel mechanism for the treatment of patients with memory and learning deficits after focal cerebral ischemia.

Acknowledgements

We would like to thank T. Iwasaka and M. Ono for preparing the histopathological specimens.

References

Biggins, J.A., E.K. Perry, J.R. Mcdermott, A.I. Smith, R.H. Perry and J.A. Edwardson, 1983, Post mortem levels of TRH and neurotensin in the amygdala in Alzheimer's disease schizophrenia and depression, J. Neurol. Sci. 58, 117.

Cantor, P., 1986, Evaluation of a radioimmunoassay for cholecystokinin in human plasma, Scand. J. Clin. Lab. Invest. 46, 213.

Dalmaz, C., C.A. Netto, N. Volkmer, R.D. Dias and I. Izquierdo, 1986, Distribution of prolyl endopeptidase activity in sub-synaptosomal fractions of rat hypothalamus, Braz. J. Med. Biol. Res. 19, 685.

De Wied, D., O. Gaffori, J.M. Van Ree and W. De Jong, 1984, Central target for the behavioral effects of vasopressin neuropeptides, Nature 308, 276.

Dresdner, K., L.A. Barker, M. Orlowski and S. Wilk 1982, Subcellular distribution of prolyl endopeptidase and cation-sensitive neutral endopeptidase in rabbit brain, J. Neurochem. 38, 1151.

Griffiths, E.C., C. Baris, T.J. Visser and W. Klootwijk, 1985, Thyrotropin-releasing hormone inactivation by human postmortem brain, Regul. Pept. 10, 145.

Hirakawa, M., A. Tamura, H. Nagashima, H. Nakayama and K. Sano, 1994, Disturbance of retention of memory after focal cerebral ischemia in rats, Stroke 25, 2471.

Husain, M.M. and C.B. Nemeroff, 1990, Neuropeptides and Alzheimer's disease, J. Am. Geriatr. Soc. 38, 918.

Huston, J.P., R.U. Hasenohrl, P. Gerhaedt and R.K.W. Schwarting, 1993,

Sequence-specific effects of neurokinin substance P on memory, reinforcement and brain dopamine activity, Psychopharmacology 112, 147.

Kato, T., T. Nakano, K. Kojima, T. Nagatsu and S. Sakakibara, 1980, Changed in prolyl endopeptidase during maturation of rat brain and hydrolysis of Substance P by the purified enzyme, J. Neurochem. 35, 527.

Lathamn, A., R.H. Lye, J. Schrewsbury-Gee and P. Slater, 1985, Evaluation of the effects of two TRH analogues on cerbral ischemia (Abstract), Regul. Pept. 13, 80.

Mazurek, M.F., M.F. Beal and J.B. Martin, 1985, Vasopressin in postmortem Alzheimer brain, Ann. Neurol. 18, 143.

Morris, R.G.M., 1981, Spatial localization does not require the presence of local cues, Learn. Motiv. 12, 239.

Ono, S., K. Kitamura, M. Maekawa, K. Hirata, M. Ano, W. Ueki, T. Yamafuji and H. Narita, 1993, Protective effect of R(−)-1-(benzo[b]thiophen-5-yl)-2-[2-(*N*,*N*-diethylamino)ethoxy]-ethanol hydrochloride (T-588), a novel cerebral activator, against experimental cerebral anoxia, Jpn. J. Pharmacol. 62, 81.

Rossor, M.N., P.C. Emson, D. Dawbarn, C.Q. Mountjoy and M. Roth, 1986, Neuropeptides and dementia, Progr. Brain Res. 66, 143.

Saito, K., S. Honda, A. Toba and I. Yanagiya, 1985, Effects of bifemelane hydrochloride (MCI-2016) on acetylcholine level reduced by scopolamine, hypoxia and ischemia in the rats and Mongolian gerbils, Jpn. J. Pharmacol. 38, 375.

Shinoda, M., K. Okamiya and K. Toide, 1995, Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on thyrotropin-releasing hormone-like immunoreactivity in the cerebral cortex and hippocampus of aged rats, Jpn. J. Pharmacol. 69, 273.

Tamura, A., D.I. Graham, J. McCulloch and G.M. Teasdale, 1981, Focal cerbral ischemia in the rat. 1: Description of technique and early neuropathological consequences following middle cerbral artery occlusion, J. Cereb. Blood Flow Metab. 1, 53.

Tamura, A., M. Miyamoto, M. Shimizu, T. Kirino and K. Sano, 1985, Behavioral change after focal cerebral ischemia in the rat, J. Cereb. Blood Flow Metab. 5 (Suppl. 1), s379.

Tamura, A., T. Kirino, K. Sano, K. Takagi and H. Oka, 1990, Atrophy of the ipsilateral substantia nigra following middle cerebral artery occlusion in the rat, Brain Res. 510, 154.

Toide, K., M. Shinoda, M. Takase, K. Iwata and H. Yoshida, 1993, Effects of a novel thyrotropin-releasing hormone analogue, JTP-2942, on extracellular acetylcholine and choline levels in the rat frontal cortex and hippopcampus, Eur. J. Pharmacol. 233, 21.

Toide, K., Y. Iwamoto, T. Fujiwara and H. Abe, 1995a, JTP-4819: a novel proryl endopeptidase inhibitor with potential as a cognitive enhancer, J. Pharmacol. Exp. Ther. 274, 1370.

Toide, K., K. Okamiya, Y. Iwamoto and T. Kato, 1995b, 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.

Toide, K., T. Fujiwara, Y. Iwamoto, M. Shinoda, K. Okamiya and T. Kato, 1996, Effect of a novel prolyl endopeptidase inhibitor, JTP-4819, on neuropeptide metabolism in the rat brain, Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 355.

Turner, A.J., 1986, Processing and metabolism of neuropeptides, Essays Biochem. 22, 69.

Walter, R., W.H. Simmons and T. Yoshimoto, 1980, Proline specific endo- and exopeptidases, Mol. Cell. Biochem. 30, 111.

Wilk, S., 1983, Mini review prolyl endopeptidase, Life Sci. 33, 2149.

Yamamoto, M. and M. Shimizu, 1989, Effects of a new analogue of thyrotropin-releasing hormone on pentobarbital-induced sleeping time in rodents, Neuropharmacology 28, 863.

Yamamoto, M., A. Tamura, T. Kirino, M. Shimizu and K. Sano, 1988, Behavioral changes after focal cerebral ischemia by left middle cerebral artery occlusion in rats, Brain Res. 452, 323.

Yamamoto, M., A. Tamura, T. Kirino, M. Shimizu-Sasamata and K. Sano, 1991, Effects of thyrotropin-releasing hormone on behavioral disturbances in middle cerebral artery-occluded rats, Eur. J. Pharmacol. 238, 117.

Yamamoto, T., M. Ohno, I. Kitajima, S. Yatsugi and S. Ueki, 1993, Ameliorative effects of the centrally active cholinesterase inhibitor, NIK-247, on impairment of working memory in rats, Physiol. Behav. 53, 5.

Yamamura, M., H. Kinoshita, H. Nakagawa and R. Ishida, 1991, Pharmacological study of TA-0910, a new thyrotropin-releasing hormone (TRH) analogue (IV): effects on experimental memory impairment in mice and rats, Jpn. J. Pharmacol. 55, 241.

Yoshimoto, T., T. Nishimura, T. Kita and D. Tsuru, 1983, Post-proline cleaving enzyme (prolyl endopeptidase) from bovine brain, J. Biochem. 94, 1179.