Effect of a Novel Prolyl Endopeptidase Inhibitor, JTP-4819, on Thyrotropin-Releasing Hormone-Like Immunoreactivity in the Cerebral Cortex and Hippocampus of Aged Rats

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ABSTRACT—We investigated the effects of a novel prolyl endopeptidase inhibitor, JTP-4819 ((S)-2-[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-N-(phenylmethyl)-1-pyrrolidinecarboxamide), on thyrotropin-releasing hormone (TRH)-like immunoreactivity (TRH-LI) in the cerebral cortex and hippocampus of aged rats. The TRH-LI content of both brain regions in aged rats was significantly lower than that in young rats. A single oral dose of JTP-4819 (3 mg/kg) restored the cortical TRH-LI content in aged rats, while doses of 0.3 - 3 mg/kg restored it in the hippocampus. Repeated oral administration of JTP-4819 at a dose of 1 mg/kg for 21 days produced a significant increase of TRH-LI in the cerebral cortex, while it did so in the hippocampus at doses of 0.3 and 1 mg/kg. Our findings suggest that JTP-4819 may improve the functioning of TRHergic neurons, which deteriorate with senescence.

Keywords: JTP-4819, Prolyl endopeptidase, Thyrotropin-releasing hormone-like immunoreactivity

Many studies have shown that various neuropeptides such as thyrotropin-releasing hormone (TRH), substance P (SP) and arginine-vasopressin (AVP) play an important role in the central nervous system as neurotransmitters and neuromodulators and that deficiencies in these peptides have been linked to a variety of behavioral abnormalities and to the decline in cognitive ability (1-3). More recently, TRH has been shown to have an ameliorating effect on amnesia and memory deficits caused by aging (4).

TRH has a proline residue at its carboxy-terminal and is metabolized by a serine protease, prolyl endopeptidase (PEP, EC 3.4.21.26) (5), which is present at high levels in the cytosolic fraction of the brain. It has also been reported that TRH-deaminase purified from bovine brain is identical to PEP (6), suggesting that this enzyme may degrade TRH in brain regions such as the cerebral cortex and hippocampus, which are closely associated with memory and learning. It has been found that PEP activity is elevated in the brains of patients with Alzheimer’s disease (AD), suggesting an association with the pathogenesis of this disease (7).

We have developed a novel PEP inhibitor, (S)-2-[(S)-2-(hydroxyacetyl)-1-pyrrolidinyl]carbonyl]-N-(phenylmethyl)-1-pyrrolidinecarboxamide (JTP-4819, Fig.1) that exhibits potent and specific in vitro inhibition of PEP activity in both young and aged rats, at concentrations in the low nanomolar range. JTP-4819 also exhibits concentration-dependent inhibition of the PEP-induced degradation of TRH, with an IC50 value of approximately 10.7 nM (8, 9). In addition, JTP-4819 significantly improved age-related memory impairment and reversed the decrease of brain SP-LI content (9, 10). Following our previous findings, the present study was undertaken to investigate the effects of JTP-4819 on the TRH-like immunoreactivity (TRH-LI) content of the cerebral cortex and hippocampus of aged rats.

Fig. 1. Chemical structure of JTP-4819.
> 99%, identified by TLC and HPLC). The other agents used were obtained from the following sources: TRH from Peptide Institute, Inc. (Osaka), anti-TRH antiserum from UCB-Bioproducts (Bruxelles, Belgium), $^{125}$I-TRH from NEN (Boston, MA, USA), Amerlex-M from Amer-sham (Tokyo) and bovine serum albumin from Sigma (St. Louis, MO, USA).

JTP-4819 was dissolved in distilled water, it was administered in either a single dose or repeated doses for 21 days per os (p.o.) at a volume of 0.1 ml per 100 g body weight. Repeated administration of JTP-4819 had no effects on body weight and food and water consumption, as compared to those of the vehicle administered groups.

Rats were killed by microwave irradiation (young rats: 9.0 kW, 1.0 sec; aged rats: 9.0 kW, 1.5 sec) 60 min after the single administration (0.3–3 mg/kg, p.o.), or after the repeated administration (0.1–1 mg/kg, p.o.) for 21 days. Their brains were rapidly removed, and the cerebral cortex and hippocampus were dissected out on ice. Each brain region was separately homogenized in 10 volumes of 2 M acetic acid. After cooling on ice for 30 min, the samples were centrifuged at 11,500 x g and 4°C for 50 min, and the supernatant was stored at −80°C until assayed. Freeze-dried samples were suspended in 20 mM phosphate-buffered saline (pH 7.4) containing 0.5% bovine serum albumin and 0.03% Tween 20. Then 0.1 ml each of standard TRH or sample, anti-TRH antiserum (according to the supplier, this antiserum has no cross-reactivity with other peptides such as Pyro-Glu-His, Pyro-Glu-His-Pro-COOH and His-Pro-diketopiperazine) and $^{125}$I-TRH were mixed and incubated for 22 hr at 4°C. After incubation, 0.5 ml of Amerlex-M was added, the mixture was allowed to stand for 30 min and then centrifuged at 1,500 x g for 10 min. Finally, the radioactivity in the precipitate was measured by a γ-counter (1470 Wizard; Wallac, Finland).

All data are expressed as means ± S.E.M. Differences between the young and aged control rats were compared by Student’s t-test, and the significance of differences compared with the aged control rats was assessed by Duncan’s multiple comparison test.

The TRH-LI content was significantly decreased by 40%–65% in the cerebral cortex (Figs. 2 and 3, upper panels) and by 50%–60% in the hippocampus (Figs. 2 and 3, lower panels) of aged rats compared with those of young rats. Single administration of JTP-4819 dose-dependently restored the age-dependent decrease in TRH-LI content, with a significant restoration in the cerebral cortex observed at a dose of 3 mg/kg JTP-4819 (Fig. 2). Oral doses of 0.3–3 mg/kg significantly increased the TRH-LI content, which was higher than that of young rats in the hippocampus (Fig. 2). Repeated administration of JTP-4819 also dose-dependently restored the TRH-LI content; and a dose of 1 mg/kg for 21 days significantly increased the TRH-LI content in the cerebral cortex of aged rats, while doses of 0.3 and 1 mg/kg did so in the hippocampus (Fig. 3).

There are many reports indicating that brain levels of proline-containing neuropeptides, such as SP and AVP, are significantly reduced in patients with AD, which is the most common neurodegenerative disorder and affects a
In addition, it has been shown that the TRH content tends to be decreased in the brain and cerebrospinal fluid of AD patients (12, 13). We previously found that repeated administration of JTP-4819 (1 mg/kg, p.o.) ameliorated age-related spatial memory deficits in rats performing the Morris water maze task (10), and we recently showed that repeated administration of this drug restored the decreased hippocampal SP-LI content, which was not changed by a single administration in aged rats (9).

As shown in Figs. 2 and 3, TRH-LI was significantly decreased in the cortex and hippocampus of aged rats, a finding in agreement with previously published observations (14). JTP-4819 increased the TRH-LI content of the cerebral cortex and hippocampus in aged rats after both single and repeated administration. Interestingly, the effective dose of JTP-4819 for increasing cortical TRH-LI content was reduced by repeated administration, in accordance with our previous findings on the hippocampal SP-LI content in aged rats (9). In the previous study, the hippocampal SP-LI content could also be restored by repeated administration of JTP-4819 at a lower dose than that of single administration (9). On the other hand, we have already observed that the TRH-LI content did not change by the single administration of JTP-4819 in young rats (data not shown). Taken together, the data may suggest that JTP-4819 ameliorates the functional decline of both TRHergic and SPergic neurons with senescence. Thus, our findings seem to indicate that memory deficits in aged rats are related to a reduction in brain levels of neuropeptides such as TRH and SP.

The TRH-LI contents attained by repeated administration of JTP-4819 at doses of 0.3 - 3 mg/kg were lower than those by single administration. One possible explanation of the responsible mechanism is that repeated administration of JTP-4819 may accelerate the turnover rate of TRH due to release from nerve terminals much more than the single administration. Thus, clonic acceleration of TRH release may lead to a reduction in the intracellular TRH-LI content. Further studies on TRH releasing activity from nerve terminals and the extracellular TRH levels are clearly required.

Griffiths et al. (15) reported that TRH was metabolized to inactivate TRH-OH by PEP in the cytosol, but not in the particulate fraction of brain. We also recently found that TRH-OH levels in the brain cytosolic fraction were decreased by JTP-4819 (data not shown). These data suggest that the increase of TRH-LI produced by JTP-4819 in the brains of aged rats may be due to inhibition of TRH degradation by brain PEP.

In conclusion, our present study suggest that the cerebral and hippocampal TRH-LI contents are increased by JTP-4819 via the inhibition of PEP. This agent may eventually lead to a novel treatment of AD, since memory deficits in aged rats appear to be partly due to hypofunction of TRHergic neurons.

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