Effect of ZTTA, a Prolyl Endopeptidase Inhibitor, on Memory Impairment in a Passive Avoidance Test of Rats with Basal Forebrain Lesions

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INTRODUCTION

Prolyl endopeptidase (PEP, EC 3.4.21.26) is a unique intracellular endopeptidase that exhibits a high substrate specificity for the proline residues in peptides. It is distributed in the testes, liver, skeletal muscle and brain of rats, and in human body fluids (1). Arginine-vasopressin (AVP) has been reported to be one of the major substrates of PEP (2); thyrotropin-releasing hormone (TRH) is also regarded as major substrates, and they are neuropeptides which play important roles as neurotransmitters and/or modulators for acetylcholine (ACh). There is thus a possibility that these neuropeptides enhance and regulate the cholinergic function. The development of a PEP inhibitor as a possible treatment for senile dementia of Alzheimer's type (SDAT) has been considered (3). Significant reductions in the activity of choline acetyltransferase (ChAT), the enzyme that synthesizes ACh and is regarded as a specific cholinergic marker were observed in the cortex and hippocampus of patients who died of SDAT (4). Electrolytic or excitotoxic lesions of the basal forebrain (BF) cause marked reductions in the activities of ChAT and acetylcholinesterase (5,6). The BF-lesioned rat model used in the present study was chosen on the basis of the central cholinergic hypothesis; this model is also a generally accepted animal model of SDAT.

In this study, we investigated the effects of ZTTA, a specific inhibitor of PEP (Tsuru, D., personal communication) (7), on (a) the performance of BF-lesioned rats on the step-through type passive avoidance test, and (b) the ChAT activity in the rat anterior cortex.

MATERIALS AND METHODS

Animals

Seven-week-old male rats of the Wistar strain (Seiwa Experimental Animals, Fukuoka, Japan) were maintained in temperature-controlled (23 ± 2°C) animal quarters, and given food and water ad libitum. The rats were housed in groups of four per cage under a 12 hr light/dark cycle. The animal experiments were performed in accordance with *the Guideline for Animal Experiments of Yakult Central Institute.*

Basal Forebrain Lesion

A rat was anesthetized with sodium pentobarbital (40 mg/kg i.p.) and then fixed in a stereotaxic apparatus. The 2 holes necessary for the insertion of the electrode were made at appropriate locations on the skull with a dental drill, and bilateral high-frequency electrolytic lesions were induced with an insulated electrode, which was bare 1 mm from the tip, 10 mA being delivered for 15 sec with a Research RF lesion generator system (Model RFG-4A; Radionics Inc., Burlington, MA). The coordinates for the electrolytic pallidal lesions were axial, −0.5 mm; lateral, ±2.6 mm; and ventral, 7.8 mm, with the bregma, midline and dura as 0, according to the atlas of Paxinos and Watson (8). The electrode was left for 2 min after completion of the delivery of the current, then carefully withdrawn, and the scalp was apposed with sutures. Sham-operated rats underwent a similar surgical procedure with holes drilled in the skull and insertion of the electrode, but they did not undergo delivery of the current.

Drugs

The main drug used in this study was N-benzyloxycarbonyl-thioprolylthioprolinal-dimethylacetal (ZTTA; MW 412.53; Ki 2.9 μM; Yakult Inc., Tokyo, Japan; Fig. 1) (7). It was suspended in a 4% Arabic gum solution, and was administered orally in a volume of 0.5 ml per 100g body weight from the 6th day to the 9th day after the surgery. Previous studies using BF-lesion model suggested that continuous or daily administration would be more effective for BF-lesion model than single administration (9,10). So concerning the experimental schedule of the present study, we followed the precedents. The administration of drugs on the 9th day was done 1 h before the acquisition trial of the passive avoidance test. The 4% Arabic gum solution was administered as the vehicle. Eserine sulfate (physo-

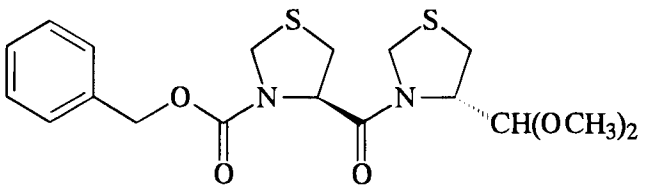

Fig. 1. Chemical structure of ZTTA (N-benzyloxycarbonyl-thioprolyl-thioprolinal-dimethylacetal).

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stigmine; Sigma Chemicals, St. Louis, MO, USA), a cholinesterase inhibitor, was dissolved in saline, and administered intraperitoneally in a volume of 0.1 ml per 100g body weight with the same schedule as that used for ZTTA.

Passive Avoidance

The passive avoidance test apparatus was comprised of a dark compartment with a grid floor and a bright compartment illuminated from above by a 20-W daylight lamp, the two chambers being separated by a guillotine-type door (Muromachi Kikai Inc., Tokyo, Japan). On the 9th day after the surgery, the rats were subjected to an acquisition (i.e., learning) trial. The acquisition trial consisted of placing a rat in the bright compartment, opening the door after 5 sec, and shutting the door as soon as all four feet of the rat had reached the dark compartment. The rat was then subjected a 0.5-mA foot shock via the grid floor for 10 sec 6 times, with 3 sec intervals. After 1 hr, the retention test was performed, consisting of placing the rat in the bright compartment and then recording the time taken to enter the dark compartment (step-through latency; STL). A maximum latency of 300 sec was used in the retention test, and the numbers of rats passively avoiding the dark compartment until this STL cut-off time were compared among the groups.

Choline Acetyltransferase Activity

After the end of this behavioral test, each rat was decapitated, and its brain was removed rapidly and dissected into the anterior cortex and posterior cortex. Choline acetyltransferase (ChAT) activity was measured by the method of Fonnum (11) with slight modifications.

Histology

Sections of the ventral hemispheres of the brains, 450 μm in thickness, were cut using a tissue chopper, and the locations and extent of the lesions were verified.

Data Analysis

For statistical analysis, Wilcoxon's t-test, Dunnett test and Fisher's test were used.

RESULTS

The lesion sites in the BF are shown schematically in Fig. 2. They are illustrated based on the lesion area of each coronal brain level from all lesioned animals. The electrolytic lesions of the BF involved the substantia innominata, medial forebrain bundle, ventral pallidum and globus pallidus.

The BF-lesions significantly decreased the ChAT activity in the anterior cortex but not in the posterior cortex (Table I). Therefore, biochemically, it was observed that the lesions produced a relatively selective loss of ChAT activity in the rostral half of the cortex. The decreased ChAT activity was not affected by the consecutive administration of ZTTA for 4 days (Table I).

In the preliminary experiments, the effect of various doses of ZTTA (0.1, 1 and 10 mg/kg, p.o.) on passive avoidance test of intact rats was investigated, and we found that ZTTA at 10 mg/kg significantly prolonged the intact rats' STL values (mean ± SD) from 100 ± 43 to 228 ± 44 ($p < 0.05$), and increased the number of rats achieving the STL cut-off time (n/N; n, number of rats achieving the cut-off time in the retention test;

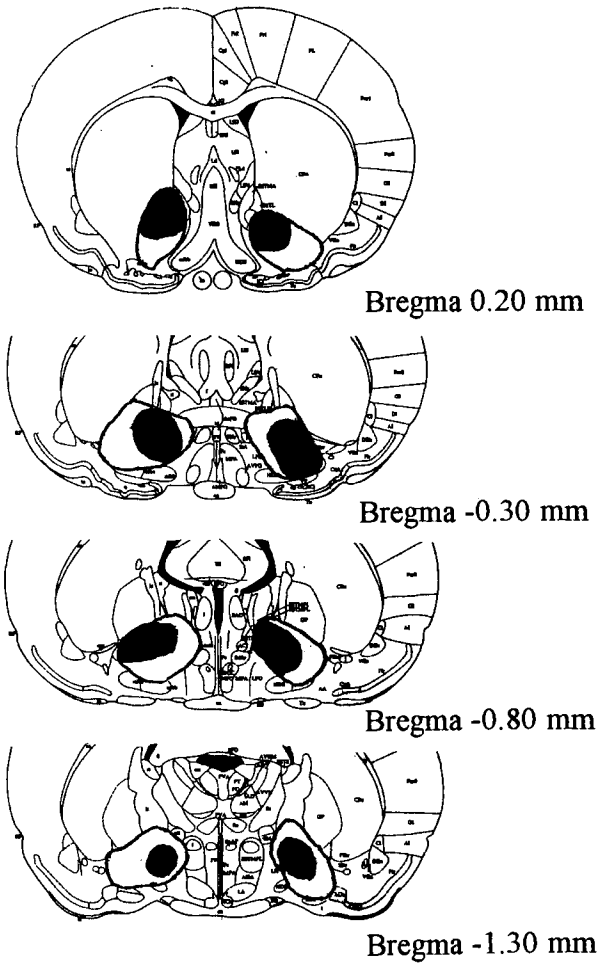

Fig. 2. Schematic representation of the extent of the bilateral lesions of the posterior level of the basal forebrain according to the atlas of Paxinos and Watson[18]. (■), minimum lesion area; (□), maximum lesion area.

Table I. Effects of Basal Forebrain (BF)-Lesions on Choline Acetyltransferase Activity in the Rat Anterior and Posterior Cerebral Cortex[a]

Treatment	Choline acetyltransferase (nmoles/mg of protein/hr)	
	Anterior cortex	Posterior cortex
Intact	14.4 ± 3.1 (100)	11.7 ± 2.9 (100)
Sham	16.0 ± 0.6 (111)	N.D.
BF-lesioned	7.7 ± 1.6 (53)[b]	12.5 ± 2.0 (107)
+ZTTA (10 mg/kg)	8.2 ± 1.3 (57)	N.D.

[a] Bilateral electrolytic lesions were induced in rats; the locations of the lesions are shown in Fig. 2. After behavioral testing, the enzyme activity was measured in the anterior and posterior cerebral cortex. The values are means ± S.D. for at least four animals, and the percentages as to the intact group are given in the parentheses. N.D., not determined.

[b] $p < 0.01$ vs. intact group.

N, number of rats tested) from 2/8 to 6/8 in the retention test. So we investigated the effect of ZTTA at 10 mg/kg on passive avoidance test of rats with BF lesions. There was no significant difference between the intact and sham-operated groups in the numbers of rats achieving the STL cut-off time in the retention test (Table II). The STL of the intact and sham-operated groups were 282 ± 59 vs. 258 ± 104 s. There was also no significant difference between the sham-operated and ZTTA-treated groups or between the sham-operated and physostigmine-treated groups (Table II). In contrast, the number of rats achieving the STL cut-off time in the BF-lesioned group was significantly decreased compared with that of the sham-operated-group ($p < 0.05$, Fisher's test, Table II). The STL of the BF-lesioned group was also significantly short compared with that of the sham-operated group ($p < 0.05$, Wilcoxon's t-test, Table II). In the ZTTA-treated group, the number of rats achieving the STL cut-off time in the retention test was significantly increased ($p < 0.05$, Fisher's test, Table II) and the STL also tended to be increased (Table II). In the physostigmine-treated group, the number of rats achieving the STL cut-off time was also increased ($p < 0.01$, Fisher's test, Table II), and the STL also tended to be increased (Table II).

DISCUSSION

Our findings suggest that the ZTTA treatment had an activating effect on impaired functions of learning and memory caused by the decrease of ACh function in the central nervous system. Sedative or muscle-relaxing effects of test drugs sometimes influences the result in a passive avoidance test. But, in the present study, 1) there was no significant difference between the sham-operated and ZTTA-treated groups in locomotor activity in the home cages just before the retention test, and 2) there was no significant difference between the sham-operated and ZTTA-treated groups in STL values in the acquisition test (5 ± 2 vs. 5 ± 4). These observations indicate that ZTTA did not have a sedative or muscle-relaxing effect.

ZTTA is a PEP inhibitor, so it is reasonable to speculate that several kinds of neuropeptides which are substrates of PEP may be responsible for the activating effect of ZTTA on learning and memory functions. For example, TRH accelerates the cholinergic transmission in the brain (12), and the AVP fragment AVP_{4-9} enhances the ACh release from the rat hippocampal region (13). But, on the other hand, we observed that the BF-lesions caused a decrease of ChAT activity in the anterior cortex, as previous groups have reported (9), and this decrease was not reversed by ZTTA (Table I) or physostigmine (data was not shown). These results indicate that ZTTA has no direct effect on the synthesis of ACh in the cholinergic neurons, at least, projecting from the BF to the cortex, and suggest the possibilities that another cholinergic neurons projecting form the septum to the hippocampus, or some other non-cholinergic neurons play an important role in the beneficial effect of ZTTA. For example, catecholaminergic neurons are thought to play an essential role in various memory processes and they are modulated by neuropeptide AVP (14). However, the mechanism underlying the effect of ZTTA observed in the present study certainly remains a matter of debate.

In conclusion, ZTTA attenuated the deficit in the passive avoidance performance caused by the BF-lesions. The beneficial effect of ZTTA may thus be mediated by the action of several kinds of neuropeptides. To elucidate the mechanism of action of ZTTA in detail, further experiments should be conducted to determine the effects of ZTTA on the neuropeptide contents and functions of central nervous system.

Table II. Effects of ZTTA and Physostigmine (PHY) on the Step-Through Type Passive Avoidance Test in Rats with Sham- and Electrolytic Basal Forebrain (BF)-Lesions[a]

Treatment	n/N	(%)	STL
Intact	9/10	90	282 ± 59
Sham	5/ 6	83	258 ± 104
+PHY (0.1 mg/kg)	5/ 7	71	228 ± 124
+ZTTA (10 mg/kg)	5/ 7	71	226 ± 127
BF-lesioned	4/20	20*	126 ± 124$
+PHY (0.1 mg/kg)	6/ 7	86 ##	266 ± 89
+ZTTA (10 mg/kg)	7/10	70 #	217 ± 134

[a] n, number of rats achieving the cut-off time in the retention test. N, number of rats tested. The cut-off time was set at 300 s. * $p < 0.05$ vs. sham group, # $p < 0.05$, ## $p < 0.01$ vs. BF-lesioned group (Fisher's test), $ $p < 0.05$ vs. sham group (Wilcoxon's t-test).

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