Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 465 (2003) 97–103
www.elsevier.com/locate/ejphar

Effects of T-82, a novel acetylcholinesterase inhibitor, on impaired learning and memory in passive avoidance task in rats

Kazuo Isomae[a,*], Shinichi Morimoto[a], Hiroshi Hasegawa[a], Kayo Morita[b], Junzo Kamei[b]

[a] *Central Research Laboratories, SSP Co. Ltd., 1143 Nanpeidai, Narita, Chiba 286-8511, Japan*
[b] *Department of Pathophysiology and Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa, Tokyo 142-8501, Japan*

Received 22 January 2003; received in revised form 11 February 2003; accepted 14 February 2003

Abstract

Effects of 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumarate (T-82), a new quinoline derivative, on drug- and basal forebrain lesion-induced amnesia models were examined in rats. Scopolamine (0.5 mg/kg, i.p.) and cycloheximide (1.5 mg/kg, s.c.) shortened the step-through latency in the passive avoidance task. T-82 significantly ameliorated amnesia induced by scopolamine or cycloheximide at the dose of 0.03, 0.1 and 0.3 mg/kg, p.o., and 0.3 and 1.0 mg/kg, p.o., respectively. Basal forebrain lesions with ibotenic acid shortened the step-through latency in passive avoidance task. An acute (0.1 and 0.3 mg/kg, p.o.) or subacute (0.03–0.3 mg/kg, p.o., for 7 days) treatment of T-82 significantly reversed the shortened latency. These results suggest that T-82 may ameliorate the impairment of memory induced by acetylcholinergic dysfunction.

Keywords: T-82; Scopolamine; Basal forebrain lesion; Passive avoidance task; (Rat)

1. Introduction

It is well known that the central acetylcholinergic systems may play an important role in learning and memory (Watts et al., 1981; Bartus et al., 1982; Murray and Fibriger, 1986). Alzheimer's disease is characterized by acetylcholinergic dysfunction in neocortex and hippocampus, which correlates with the severity of impaired learning and memory in patients with Alzheimer's disease (Perry et al., 1978; Wilcock et al., 1982). These findings lead to search for the agents that increase acetylcholine level in the brain. The cholinesterase inhibitors and the choline acetyltransferase activators may compensate for the decreased acetylcholine level in the brain of a patient with Alzheimer's disease. Cholinesterase inhibitors were developed as an alternative drug therapy for Alzheimer's type senile dementia after the cholinergic hypothesis upon the etiology of the disease was proposed in 1980. Among them, cholinesterase inhibitors do not delay the progress of Alzheimer's disease. Recently, it was reported that tacrine and donepezil (E2020) ameliorate memory deficits induced by drugs and brain lesions in rats (Nabeshima et al., 1991a; Poorheidari et al., 1998; Cheng et al., 1996). Tacrine acts through inhibition of acetylcholinesterase, and shows the ameliorating effect on cycloheximide- and basal forebrain lesion-induced memory deficits in rats (Nabeshima et al., 1991a). Summers et al. (1986) and Farlow et al. (1992) reported that tacrine significantly improves cognition in clinical studies with Alzheimer's disease patients. E2020 also inhibits acetylcholinesterase and ameliorates the deficits in patients with Alzheimer's disease (Rogers et al., 1996). It has been suggested that tacrine and E2020 act through central acetylcholinergic systems, such as inhibition of acetylcholinesterase activity and regulation of acetylcholine release (Suzuki et al., 1994; Svensson et al., 1996). However, in clinical trials using physostigmine or tacrine, some problems arose in continuing the drug therapy because of short duration of action and/or hepatic dysfunction. Then, focus on structural modification of tacrine created some candidates as derivatives with lower side effects (Dejmek, 1990; Shutske et al., 1989).

* Corresponding author. Tel.: +81-476-27-1511; fax: +81-476-26-7948.
E-mail address: Kazuo.Isomae@ssp.co.jp (K. Isomae).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01487-0

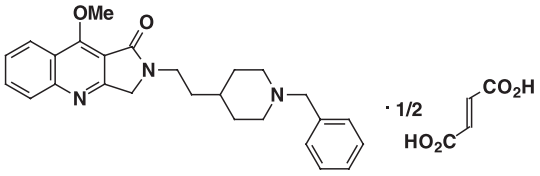

Fig. 1. Chemical structure of T-82.

In an attempt to find a new type of acetylcholinesterase inhibitor that selectively inhibits acetylcholinesterase in the brain, we have synthesized a new quinoline derivative, 2-[2-(1-benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumarate (T-82) (Fig. 1). We recently reported that T-82 inhibits the acetylcholinesterase activity in the rat cerebral cortex with an IC_{50} value of 109.4 nM, whereas T-82 hardly inhibits butyrylcholinesterase, a nonselective cholinesterase located in the blood and peripheral organs. The inhibition activity of T-82 on acetylcholinesterase in human plasma was 322-fold higher than that on butyrylcholinesterase. In addition, we also observed that T-82 potently increased extracellular acetylcholine concentration in rat brain (Isomae et al., 2002). Based on these results, we suggested that T-82 is a selective acetylcholinesterase inhibitor. Therefore, in the present study, we examined the effects of T-82 on experimental amnesia models using the step-through type passive avoidance task in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats were purchased from Clea Japan (Tokyo, Japan). Eight- or nine-week-old rats were used for the tests on scopolamine and cycloheximide-induced amnesia. Twelve- or thirteen-week-old rats were used for the test on basal forebrain lesion-induced amnesia. All the animals were housed in a room controlled at 23 ± 2 °C with 55 ± 10% humidity and maintained under at alternating 12-h light/dark cycle (light automatically on at 7:00 a.m.). Food and water were given ad libitum. This study was carried out in accordance with the guidelines for the care and use of laboratory animals of The Japanese Pharmacological Society.

2.2. Passive avoidance task

A two-compartment step-through passive avoidance task apparatus was used. The apparatus consists of an illuminated compartment (30 × 15 × 25 cm) separated by a sliding door from a dark compartment (30 × 30 × 30 cm) equipped with a grid floor. In the acquisition trial, each rat was placed in the light compartment, and the sliding door was opened 10 s later. The time required for each rat to enter the dark compartment was recorded. As soon as the rat entered the dark compartment, the sliding door was closed and an electric foot shock (1.0 mA) was applied to the floor grid for 3 s. The animal was then returned to its home cage until the retention trial. The retention trial was carried out 24 h after the acquisition trial. Each rat was again placed in the light compartment, the sliding door was opened 30 s later, and the latency until the rat entered the dark compartment was measured (step-through latency). If the animal did not enter the dark compartment within 600 s, the animal was given a ceiling step-through latency of 600 s.

T-82 and E2020 were orally administered 30 min prior to the acquisition trial. Rats were intraperitoneally treated with scopolamine (0.5 mg/kg) 15 min prior to the acquisition trial. In the case of cycloheximide (1.5 mg/kg), a protein synthesis inhibitor, rats were subcutaneously treated immediately after the acquisition trial.

2.3. Neurosurgery

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). In bilateral basal forebrain lesions, stereotaxic coordinates referred to the atlas of Paxinos and Watson (Paxinos and Watson, 1986). Bilateral neurotoxic lesions of the basal forebrain were produced by infusion of ibotenic acid. A pair of stainless injection needles connected via polyethylene tubing to a 10-μl microsyringe mounted on a microdrive injector was inserted into the basal forebrain bilaterally. The coordinates were (Bregma A: 1.4 mm, L: −2.6 mm, H: 7.0 mm) and (Bregma A: 1.4 mm, L: 2.6 mm, 7.0 mm). Ibotenic acid was dissolved in phosphate-buffered saline (pH 7.4) at a concentration of 10 μg/μl and then infused with a volume of 0.75 μl during 3 min. The injection needles were left in place for 5 min to ensure that the drug had diffused away from the needle tips. Sham-operated rats were also infused with the phosphate-buffered saline (pH 7.4). The acquisition trial in the passive avoidance task was carried out two weeks after the surgical operation.

2.4. Drugs

2-[2-(1-Benzylpiperidin-4-yl)ethyl]-2,3-dihydro-9-methoxy-1*H*-pyrrolo[3,4-*b*]quinolin-1-one hemifumarate (T-82) and (±)-2-[(1-benzylpiperidin-4-yl)methyl]-5, 6-dimethoxy-indan-1-one hydrochloride (E2020) were synthesized at Central Research Laboratories, SSP (Narita, Japan). These drugs were dissolved in distilled water and administered orally. Scopolamine (Sigma-Aldrich, St. Louis, MO, USA) and cycloheximide (Sigma-Aldrich) were dissolved in physiological salt solution and administered intraperitoneally. Ibotenic acid (Sigma-Aldrich) was dissolved in phosphate-buffered saline (pH 7.4) and injected into basal forebrain bilaterally.

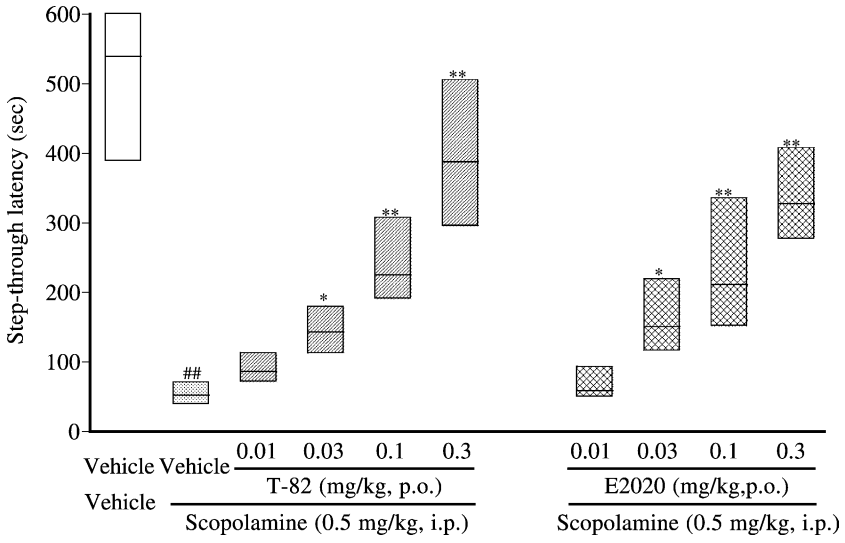

Fig. 2. Effects of T-82 and E2020 on scopolamine-induced amnesia in the passive avoidance task in rats. T-82 and E2020 were administered orally 30 min prior to the acquisition trial. Scopolamine was administered intraperitoneally 15 min prior to the acquisition trial. The retention trial was performed 24 h after the acquisition trial. Each box represents median and interquartile range of eight to ten animals. $^{\#\#}P<0.01$ vs. vehicle-treated group. $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle and scopolamine-treated group.

2.5. Statistical analyses

The results were represented as medians and interquartile ranges. Statistical comparisons were made with the Mann–Whitney *U*-test for two groups and with the Kruskal–Wallis test, a nonparametric one-way analysis of variance, followed by a Dunnett's test for multiple comparisons. Differences with *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of T-82 on scopolamine-induced impairment of passive avoidance task in rats

Scopolamine (0.5 mg/kg, i.p.) significantly impaired the acquisition of learning when given 15 min before the acquisition trial (Fig. 2). T-82, at doses ranging from 0.03 to 0.3 mg/kg, p.o., administered 30 min before the acquis-

Table 1
Effects of T-82 and E2020 on the step-through latency in the acquisition trials in passive avoidance tasks in rats

Treatment	No. of animals	Step-through latency in acquisition trial (s)	Treatment	No. of animals	Step-through latency in acquisition trial (s)
Vehicle	10	14.1 ± 1.1	Vehicle	7	5.6 ± 0.8
Scopolamine	10	11.5 ± 1.6	Cycloheximide	9	3.9 ± 0.4
T-82 0.01 mg/kg + Scopolamine	8	14.8 ± 1.7	T-82 0.03 mg/kg + Cycloheximide	9	3.4 ± 0.2
T-82 0.03 mg/kg + Scopolamine	8	10.2 ± 1.6	T-82 0.1 mg/kg + Cycloheximide	9	3.1 ± 0.2
T-82 0.1 mg/kg + Scopolamine	8	10.6 ± 1.6	T-82 0.3 mg/kg + Cycloheximide	9	4.1 ± 0.4
T-82 0.3 mg/kg + Scopolamine	8	10.5 ± 1.1	T-82 1.0 mg/kg + Cycloheximide	9	4.3 ± 0.5
E2020 0.01 mg/kg + Scopolamine	8	12.3 ± 1.7	E2020 0.03 mg/kg + Cycloheximide	8	3.5 ± 0.3
E2020 0.03 mg/kg + Scopolamine	8	13.0 ± 2.0	E2020 0.1 mg/kg + Cycloheximide	8	4.2 ± 0.6
E2020 0.1 mg/kg + Scopolamine	8	8.6 ± 0.7	E2020 0.3 mg/kg + Cycloheximide	8	3.3 ± 0.2
E2020 0.3 mg/kg + Scopolamine	8	13.6 ± 2.6	E2020 1.0 mg/kg + Cycloheximide	8	3.7 ± 0.4
Sham	7	8.4 ± 1.9	Sham	7	9.7 ± 1.5
BF lesion	8	8.1 ± 1.1	BF lesion	8	6.1 ± 0.9
BF lesion + T-82 0.01 mg/kg	7	8.8 ± 1.5	BF lesion + T-82 0.01 mg/kg/day	7	7.9 ± 1.1
BF lesion + T-82 0.03 mg/kg	7	7.6 ± 1.0	BF lesion + T-82 0.03 mg/kg/day	7	7.5 ± 1.2
BF lesion + T-82 0.1 mg/kg	8	8.2 ± 1.6	BF lesion + T-82 0.1 mg/kg/day	7	5.6 ± 1.1
BF lesion + T-82 0.3 mg/kg	8	6.7 ± 1.1	BF lesion + T-82 0.3 mg/kg/day	7	6.8 ± 1.1
BF lesion + E2020 0.01 mg/kg	7	7.6 ± 0.8	BF lesion + E2020 0.01 mg/kg/day	7	8.3 ± 1.5
BF lesion + E2020 0.03 mg/kg	7	8.4 ± 1.5	BF lesion + E2020 0.03 mg/kg/day	7	9.6 ± 2.6
BF lesion + E2020 0.1 mg/kg	7	4.7 ± 0.4	BF lesion + E2020 0.1 mg/kg/day	7	6.5 ± 1.2
BF lesion + E2020 0.3 mg/kg	7	7.1 ± 1.0	BF lesion + E2020 0.3 mg/kg/day	7	5.6 ± 0.4

Each value represents the mean ± S.E.M. Scopolamine (0.5 mg/kg) was administered intraperitoneally 15 min prior to the acquisition trial. Cycloheximide (1.5 mg/kg) was administered subcutaneously immediately after the acquisition trial. Sham: sham operation; BF: basal forebrain.

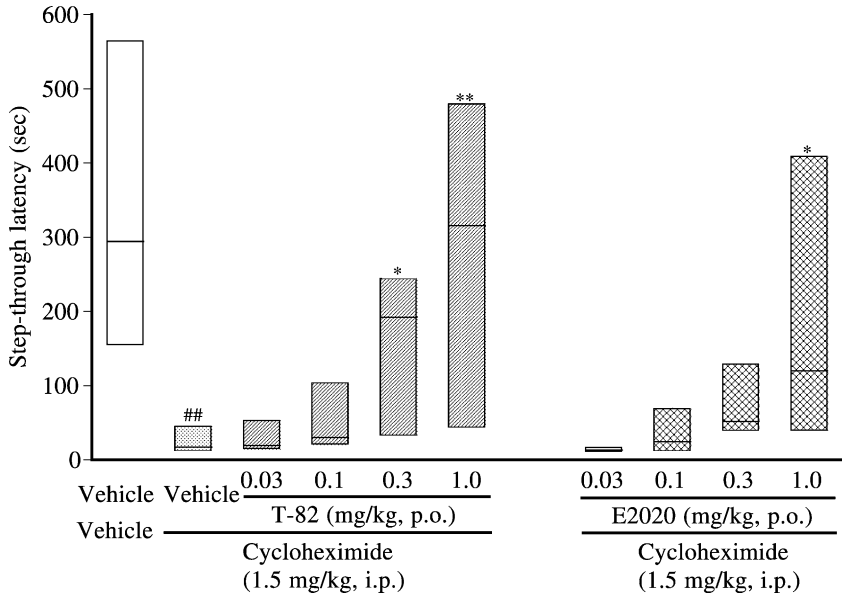

Fig. 3. Effects of T-82 and E2020 on cycloheximide-induced amnesia in the passive avoidance task in rats. T-82 and E2020 were administered orally 30 min prior to the acquisition trial. Cycloheximide was administered subcutaneously immediately after the acquisition trial. The retention trial was performed 24 h after the acquisition trial. Each box represents median and interquartile range of seven to nine animals. $^{\#\#}P<0.01$ vs. vehicle-treated group. $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle and cycloheximide-treated group.

ition trial, dose-dependently and significantly reversed the scopolamine-induced reduction of step-through latency, whereas a lower dose of 0.01 mg/kg, p.o., showed no such effect (Fig. 2). E2020, at doses ranging from 0.03 to 0.3 mg/kg, p.o., also significantly attenuated the impairment of learning and memory induced by scopolamine in rat when administered 30 min before acquisition (Fig. 2). T-82 and E2020 themselves administered 30 min before acquisition trial had no effect on learning and memory when administered alone (Table 1).

3.2. Effect of T-82 on cycloheximide-induced impairment of passive avoidance task in rats

Cycloheximide (1.5 mg/kg, s.c.) significantly impaired the acquisition of learning when given immediately after the acquisition trial (Fig. 3). T-82, at doses of 0.3 and 1.0 mg/kg, p.o., administered 30 min before the acquisition trial, dose-dependently and significantly reversed the cycloheximide-induced reduction of step-through latency, whereas lower doses of 0.03 and 0.1 mg/kg, p.o., showed no such

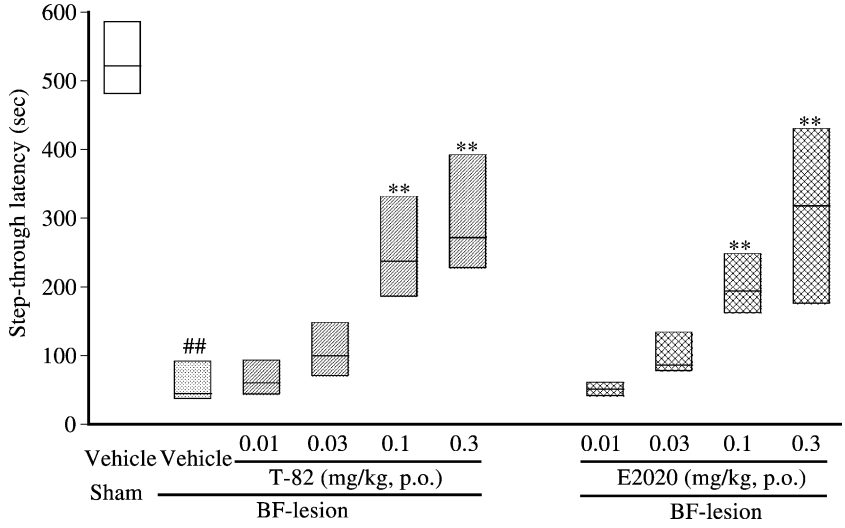

Fig. 4. Effects of single administration with T-82 and E2020 on basal forebrain (BF)-lesion-induced amnesia in the passive avoidance task in rats. T-82 and E2020 were administered orally 30 min prior to the acquisition trial. The retention trial was performed 24 h after the acquisition trial. Each box represents median and interquartile range of seven to eight animals. $^{\#\#}P<0.01$ vs. vehicle-treated sham group. $^{**}P<0.01$ vs. vehicle-treated BF-lesion group.

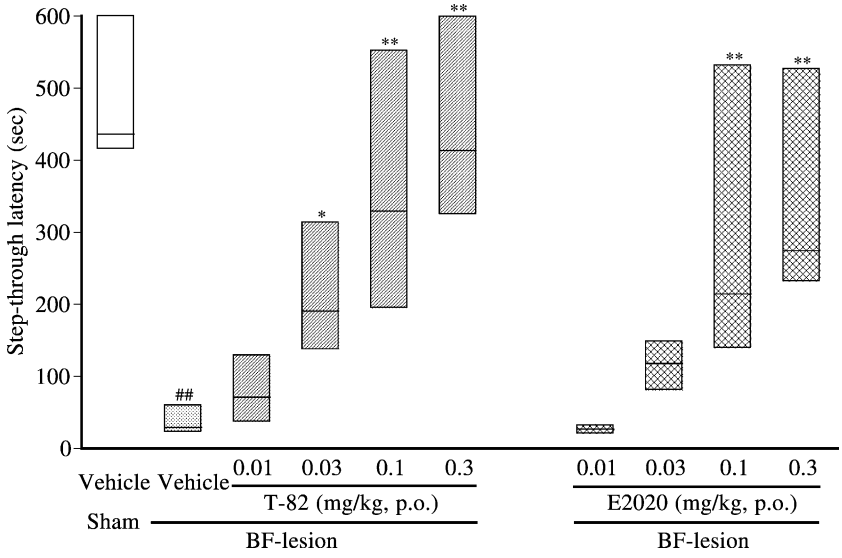

Fig. 5. Effects of subacute (7 days) administration with T-82 and E2020 on basal forebrain (BF)-lesion-induced amnesia in the passive avoidance task in rats. T-82 and E2020 were administered orally once a day for 7 days before training and once 30 min prior to the acquisition trial. The retention trial was performed 24 h after the acquisition trial. Each box represents median and interquartile range of seven to eight animals. $^{\#\#}P<0.01$ vs. vehicle-treated sham group. $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle-treated BF-lesion group.

effect (Fig. 3). E2020, at a dose of 1.0 mg/kg, p.o., also significantly attenuated the impairment of learning and memory induced by cycloheximide in rat when administered 30 min before acquisition (Fig. 3).

3.3. Effect of T-82 on basal forebrain lesion-induced impairment of passive avoidance task in rats

The basal forebrain lesion significantly impaired the acquisition of learning. Indeed, the step-through latency in the basal forebrain-lesioned rats was significantly shorter than that in sham-operated rats (Figs. 4 and 5). Acute treatment with T-82 (0.1 and 0.3 mg/kg, p.o.) partially but significantly attenuated the impairment of learning and memory induced by basal forebrain lesion. E2020, at doses of 0.1 and 0.3 mg/kg, also partially but significantly attenuated the impairment of learning and memory induced by basal forebrain lesion.

Subacute treatment with T-82, at doses ranging from 0.03 to 0.3 mg/kg/day, once a day for 1 week, markedly attenuated the impairment of learning and memory induced by basal forebrain lesion. Furthermore, subacute treatment with E2020, at doses of 0.1 and 0.3 mg/kg/day, once a day for 1 week, also significantly attenuated the impairment of learning and memory induced by basal forebrain lesion.

4. Discussion

It has been suggested that central acetylcholinergic activities influence the cognitive function. In the present study, we examined the effects of T-82 on two different drugs and basal forebrain lesion-induced experimental amnesia in passive avoidance tasks in rats. It is well known that systemic administration of muscarinic acetylcholinergic antagonist such as scopolamine impairs the performance of experimental animals in a wide variety of learning and memory tasks, including passive avoidance. The acute treatment with T-82, at doses of 0.03–0.3 mg/kg, p.o., dose-dependently ameliorated the scopolamine-induced amnesia. Furthermore, we also observed that there was no significant difference between the potencies of T-82 and E2020 in reversing scopolamine-induced impairment in rats performing a passive avoidance task. It is well known that the ameliorative effect of E2020 on scopolamine-induced amnesia is due to inhibition of acetylcholinesterase in the brain. We recently reported that T-82 inhibits the acetylcholinesterase activity in the rat cerebral cortex with an IC_{50} value of 109.4 nM, whereas T-82 hardly inhibits butyrylcholinesterase, a nonselective cholinesterase located in the blood and peripheral organs. In addition, we also observed that T-82 potently increased extracellular acetylcholine concentration in rat brain (Isomae et al., 2002). Thus, it is possible that the ameliorating effects of T-82 and E2020 on scopolamine-induced amnesia are likely due to the inhibition of acetylcholinesterase activity in the brain. On the other hand, since the nontoxic dose of T-82 was estimated to be 70 mg/kg/day in repeated oral administration tests in male rats (personal communication), the anti-amnesic dose of T-82 did not have any effect on the spontaneous behavior in the passive avoidance task.

In the present study, cycloheximide, a protein synthesis inhibitor, induced amnesia in rats. Indeed, cycloheximide markedly shortened the step-through latency to enter the dark compartment in passive avoidance test. It has been suggested that reduction of acetylcholinergic neuronal

activity, such as inhibition of biosynthesis of receptor protein, is responsible for the cycloheximide-induced amnesia in the passive avoidance task, since cycloheximide decreases the density of muscarinic receptors (Nabeshima et al., 1991b) and cycloheximide-induced amnesia is ameliorated by acetylcholinesterase inhibitors, such as physostigmine and tacrine in the passive avoidance task (Nabeshima et al., 1991a) in rats. In the present study, we observed that the acute treatment with T-82, at doses of 0.3 and 1.0 mg/kg, p.o., dose-dependently ameliorated the cycloheximide-induced amnesia. Therefore, it is possible that T-82 ameliorates the cycloheximide-induced amnesia through the activation of the acetylcholinergic neuronal system subsequence to inhibit acetylcholinesterase activity in the brain. Acute oral administration of E2020 also ameliorated the cycloheximide-induced impairment, but a dose of >1.0 mg/kg was required for significant effect. Thus, the present results indicate that the effect of T-82 is relatively more potent than E2020 in ameliorating the cycloheximide-induced amnesia.

Within the basal forebrain is a core of acetylcholinergic neurons divided into several regions that include the nucleus basalis magnocellularis or nucleus basalis meynert in primates (Dubois et al., 1985; Flicker et al., 1983). The basal forebrain is an important region for memory function, since there are many reports describing basal forebrain lesion-induced amnesia (Dubois et al., 1985; Flicker et al., 1983; Hepler et al., 1985; Miyamoto et al., 1987; Smith, 1988). In the present experiment, we confirmed that ibotenic acid-induced basal forebrain lesion severely impaired memory function in the passive avoidance task. Acute treatment with T-82 or E2020, at doses of 0.1 and 0.3 mg/kg, dose-dependently and significantly ameliorated basal forebrain lesion-induced amnesia in this task. Nabeshima et al. (1991a) reported that basal forebrain lesion decreases choline acetyltransferase activity and impairs memory function in the passive avoidance task. Furthermore, they also reported that tacrine, an acetylcholinesterase inhibitor, ameliorates the basal forebrain lesion-induced amnesia in passive avoidance task, although it fails to ameliorate the decrease of choline acetyltransferase activity. Based on these results, they suggest that the tacrine-induced amelioration of basal forebrain lesion-induced amnesia in the passive avoidance test may be interpreted as an inhibition of acetylcholinesterase activity. Therefore, it is possible that the ameliorating effect of T-82 on basal forebrain lesion-induced amnesia is likely due to the inhibition of acetylcholinesterase activity in the brain. On the other hand, in the present study, we observed that subacute (once a day for 7 days) treatment with T-82 or E2020 more clearly ameliorated basal forebrain lesion-induced amnesia in passive avoidance task. Furthermore, subacute treatment with T-82 for 7 days even at a low dose (0.03 mg/kg), but not E2020, was effective for improving basal forebrain lesion-induced amnesia. Thus, the effect of T-82 is relatively more potent than E2020 in ameliorating basal forebrain lesion-induced amnesia.

Our results of the present study indicate the possibility that T-82 ameliorates the scopolamine-, cycloheximide- and basal forebrain lesion-induced amnesia through the activation of the acetylcholinergic neuronal system subsequence to inhibit acetylcholinesterase activity in the brain. However, we recently demonstrated that the potency of T-82 at inhibiting acetylcholinesterase activity was about ninefold lower than that of E2020. Interestingly but unexpectedly, in the present study, we observed that the anti-amnesia effects of T-82 in passive avoidance tasks were as potent as those of E2020. These results suggest that some mechanisms other than the inhibition of acetylcholinesterase activity in the brain might be involved in the anti-amnesia effects of T-82. Recently, Toma et al. (2002) reported that T-82 shows high affinity for σ_1 receptors labeled by (+)-[^{3}H]pentazocine and moderate affinity for musarinic M_1 receptors. Senda et al. (1996) reported that selective σ_1 receptor agonist SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride) significantly ameliorates the scopolamine-induced and basal forebrain lesion-induced memory impairment in passive avoidance task. These results suggest that the anti-amnesic effect of T-82 may be mediated through the activation of the acetylcholinergic neuronal system subsequence to not only inhibit acetylcholinesterase activity, but also have a direct effect on muscarinic M_1 receptors and σ_1 receptors. However, further studies are necessary before this issue can be resolved with greater certainty.

In conclusion, T-82 improved memory deficits in various experimental amnesia models induced by acetylcholinergic dysfunction, suggesting that T-82 may be effective for therapy in patients with a dementia such as Alzheimer's disease.

References

Bartus, R.T., Dean, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction critical review. Science 217, 408–414.

Cheng, D.H., Ren, H., Tang, X.C., 1996. Huperizine A, a novel promising acetylcholinesterase inhibitor. NeuroReport 8, 97–101.

Dejmek, L., 1990. 7-MEOTA. Drugs Future 15, 126–129.

Dubois, B., Mayo, W., Agid, Y., Le Moal, M., Simon, H., 1985. Profound disturbance of spontaneous and learned behaviors following lesions of the nucleus basalis magnocellularis in rats. Brain Res. 338, 249–258.

Farlow, M., Gracon, S.I., Hershey, L.A., Lewis, K.W., Sadowsky, C.H., Dolan-Ureno, J., 1992. A controlled trial of tacrine in Alzheimer's disease. J. Am. Med. Assoc. 268, 2523–2529.

Flicker, C., Dean, R.L., Watkins, L., Fisher, S.K., Bartus, R.T., 1983. Behavioral and neurochemical effects following neurotoxic lesions of a major cholinergic input to the cerebral cortex in the rats. Pharmacol. Biochem. Behav. 18, 973–981.

Hepler, D.J., Wenk, G.L., Cribbs, B.L., Olton, D.S., Coyle, J.T., 1985. Memory impairments following basal forebrain lesions. Brain Res. 346, 8–14.

Isomae, K., Ishikawa, M., Ohta, M., Ogawa, Y., Hasegawa, H., Kohda, T., Kamei, J., 2002. Effects of T-82, a new quinoline derivative, on cholinesterase activity and extracellular acetylcholine concentration in rat brain. Jpn. J. Pharmacol. 88, 206–212.

Miyamoto, M., Kato, J., Narumi, S., Nagaoka, A., 1987. Characteristics of

memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 419, 19–31.

Murray, C.L., Fibriger, H.C., 1986. Pilocarpine and physostigmine attenuate spatial memory impairments produced by lesions of the nucleus basalis magnocellularis. Behav. Neurosci. 100, 23–32.

Nabeshima, T., Maruyama, E., Katoh, A., Kameyama, T., 1991a. The effect of tacrine (THA) on cycloheximide- and basal forebrain lesion-induced memory deficit in rats. Jpn. J. Pharmacol. 57, 311–319.

Nabeshima, T., Tohyama, K., Murase, K., Ishihara, S., Kameyama, T., Yamasaki, T., Hatanaka, S., Kojima, H., Sakurai, T., Takasu, Y., Shiotani, T., 1991b. Effects of DM-9384, a cyclic derivative of GABA, on amnesia and decreases in $GABA_A$ and muscarinic receptors induced by cycloheximide. J. Pharmacol. Exp. Ther. 257, 271–274.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, San Diego.

Perry, E.K., Tomlinson, B.E., Blessed, G., Bergman, K., Gibson, P.H., Perry, R.H., 1978. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2, 1457–1459.

Poorheidari, G., Stanhope, K.J., Pratt, J.A., 1998. Effects of the potassium channel blockers, apamin and 4-aminopyridine, on scopolamine induced deficits in the delayed matching to position task rats—a comparison with the cholinesterase inhibitor E2020. Psychopharmacology 135, 242–255.

Rogers, S.L., Friedhoff, L.T., Apter, J.T., Richter, R.W., Hartford, J.T., Walshe, T.M., Baumel, B., Linden, R.D., Kinney, F.C., Doody, R.S., Borison, R.L., Ahem, G.L., 1996. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US multicenter, randomized, double-blind, placebo-controlled trial. Dementia 7, 293–303.

Senda, T., Matsuno, K., Okamoto, K., Kobayashi, T., Nakata, K., Mita, S., 1996. Ameliorating effect of SA4503, a novel σ_1 receptor agonist, on memory impairments induced by cholinergic dysfunction in rats. Eur. J. Pharmacol. 315, 1–10.

Shutske, G.M., Pierrat, F.A., Kapples, K.J., Cornfeldt, M.L., Szewczak, M.R., Huger, F.P., Bores, G.M., Haroutunian, V., Davis, K.L., 1989. 9-Amino-1,2,3,4-tetrahydroacridin-1-ols: synthesis and evaluation as potential Alzheimer's disease therapeutics. J. Med. Chem. 32, 1805–1813.

Smith, G., 1988. Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Res. Rev. 13, 103–118.

Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K., Kling, A., 1986. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. 315, 1241–1245.

Suzuki, T., Nonaka, H., Fujimoto, K., Kawashima, K., 1994. Tacrine increases stimulation-evoked acetylcholine release from rat hippocampal slices. Jpn. J. Pharmacol. 65, 337–342.

Svensson, A.L., Zhang, X., Nordberg, A., 1996. Biphasic effect of tacrine on acetylcholine release in rat brain via M1 and M2 receptors. Brain Res. 726, 207–212.

Toma, K., Yamada, S., Kimura, R., Ito, O., Yano, K., 2002. In vitro and ex vivo binding properties of T-82, a novel acetylcholinesterase inhibitor, to neurotransmitter receptors in rat brain. Jpn. J. Pharmacol. 88 (Suppl. I), 191P.

Watts, J., Stevens, R., Robinson, C., 1981. Effects of scopolamine on radial maze performance in rats. Physiol. Behav. 26, 845–851.

Wilcock, G.K., Esiri, M.M., Bowen, D.M., Smith, C.C.T., 1982. Alzheimer's disease: correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J. Neurol. Sci. 57, 407–417.