Pharmacological Study of TA-0910, a New Thyrotropin-Releasing Hormone (TRH) Analog (IV): Effects on Experimental Memory Impairment in Mice and Rats

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ABSTRACT—The effects of a new TRH analog, TA-0910, orally administered, on experimental memory impairments for the one-trial passive avoidance response in anoxic mice (light-dark box), active avoidance response in basal forebrain (BF)-lesioned rats (shuttle box), and delayed alternation task in scopolamine-treated rats (T-maze) were studied. In mice, TA-0910 (3–30 mg) administered 60 min before the retention trial dose-dependently prolonged the passive avoidance response latency reduced by CO2-exposure that was given immediately after the acquisition trial, but not when it was given 60 min before the acquisition or just after the anoxic treatment. In rats, TA-0910 (0.3–3 mg/kg) administered 40–60 min before the test trial, dose-dependently prevented the reduction in mean avoidance rate caused by BF-lesioning and elevated the scopolamine (0.1 mg/kg, i.p.)-induced reduction in percent correct choice level in the alternation task. TRH (30–300 mg/kg), on the other hand, produced no improvements in any of the above tests. These results suggest that TA-0910 improves impaired memory by correcting the retrieval process of memory.

Thyrotropin-releasing hormone (TRH) is a hypothalamic hormone that stimulates secretion of thyrotropin and prolactin. However, radioimmunoassay has demonstrated that two-thirds of the total TRH content of the brain is distributed widely in areas other than the hypothalamus (1). Also, autoradiographic studies have revealed that TRH receptors of the brain are concentrated in the cerebral cortex and the limbic system (2, 3). Based on these findings, TRH is considered to play an important role in the brain in the maintenance of consciousness and emotional or intellectual functions (4). Moreover, Yarbrough and Pomara (5) recommended the use of TRH for the treatment of dementia of the Alzheimer type (DAT) from the functional relationship between TRH and central cholinergic nervous system and the effectiveness of TRH in the treatment of dementia of patients with amyotrophic lateral sclerosis complicated by degeneration of cholinergic nerves, and by Metcalf (6) and Griffiths (7) from the effect of TRH to improve disturbance of consciousness (8) and its antidepressant effect (9, 10).

Recently, improvements in mental symptoms (such as disturbances in impressibility, emotional disturbances, reduced ability to think, deduced spontaneity, inertia, and apathy) were reported in patients with Alzheimer’s disease (AD) and cerebrovascular dementia after administration of TRH (11, 12). Tem-
Temporary improvements in the arousal level, mood, and semantic memory have also been noted in AD patients intravenously administered high doses of TRH (13). However, TRH has a number of properties inconvenient for the treatment of DAT such as the short duration of action (14), very weak effect on the central nervous system by oral administration (15), and inevitable hormonal effects (16). Therefore, the development of orally administrable TRH analogs having a long action time and weak hormonal effects has been attempted (17, 18). TA-0910, [1-methyl-(S)-4,5-dihydroorotyl-L-histidyl-L-prolinamide], is a TRH analog that fulfills these conditions. Namely, it is about 100 times more potent than TRH in the central nervous system (CNS) action and its duration of action is about 8 times longer than that of TRH in mice and rats (19, 20). However, its thyrotropin releasing activity is about 50 times less potent than that of TRH in rats (19).

Many pharmacological studies suggest that central cholinergic system plays an important role in memory processes. For example, anticholinergic drugs such as scopolamine produce memory impairment in humans and experimental animals, while cholinomimetics such as physostigmine, oxotremorine and arecoline enhance memory formation (21–24). We have already reported that the effects of TA-0910 on the CNS is considered to be partly mediated by activation of the cholinergic neurons (20, 25). Therefore, TA-0910 is expected to have an ameliorating effect on scopolamine-induced memory impairments for various learning tasks in rodents. Moreover, TA-0910 is considered to be effective on memory impairments induced by CO2-exposure and/or the basal forebrain (BF)-lesioning, because cholinergic drugs are also reported to show improving effects on them (26–29). In this study, from the above-mentioned points of view, the effects of oral administration of TA-0910 on the memory impairments in anoxic mice, BF-lesioned rats and scopolamine-treated rats were examined by the passive avoidance response, active avoidance response and delayed alternation task, respectively, using TRH as a reference drug.

MATERIALS AND METHODS

Materials
Male Slc: ddY mice (28–32 g) and male Slc: Wistar rats (190–240 g) were used. The animals were maintained as follows. Twenty-five mice each were housed in a plastic cage (42W × 26D × 15H cm), and each rat was housed in one compartment (15W × 25D × 14H cm) of a stainless steel 5-compartment wire-mesh cage (mesh: 6 mm, 75W × 25D × 14H cm). All animals were kept in the animal room maintained at 23 ± 1°C with 55 ± 5% humidity, and illuminated for 12 hr (6:30–18:30). The mice and rats were allowed free access to a pellet diet (CRF-1, Oriental Yeast, Co., Ltd.) and tap water. TA-0910 (1-methyl-(S)-4, 5-dihydroorotyl-L-histidyl-L-prolinamide tetrahydrate; Lot No. 503010) and TRH (L-pyroglutamyl-L-histidyl-L-prolinamide L-tartrate monohydrate; Lot No. N11419A) used were synthesized in the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd. Other drugs used for the study were pentobarbital sodium (Nacalai Tesque) and scopolamine hydrobromide (Sigma). TA-0910 and TRH were dissolved in distilled water and administered orally at 10 ml/kg in mice and at 2 ml/kg in rats.

Methods
One-trial passive avoidance response
A learning box consisting of two rooms, one light (white opaque resin, 9W × 7D × 14H cm) and the other dark (black opaque resin, 14W × 14D × 14H cm), prepared on the basis of the light-dark box of Bammer and Chesher (30) for the mouse, was used. A guillotine door opening, 3-cm high and 3-cm wide, was made on the floor in the center of the partition between the two rooms. Stainless steel grids (3 mm in diameter) were placed at 7-mm intervals (distance between the centers of grids) on the floor of the dark room to produce foot shocks (FS) through a solid-state
shocker/scrambler (Model 111-33, Lehigh Valley Electronics). The rooms were illuminated with a 15 W white fluorescent rod light placed 25 cm above the learning box along its long axis. The box was enclosed in a ventilated sound-attenuating chamber (100W X 60D X 60H cm), with the noise of the ventilator used for masking (55 horn). The guillotine door at the path was opened in advance, and mice were gently placed in the light room with their heads away from the path. When the mice had put all four limbs in the dark room, the guillotine door was closed, and a 0.5 mA was applied immediately for 3 sec (acquisition trial). The mice were immediately transferred to an air-tight glass container (0.7 l) supplied with 100% CO2 gas at a flow rate of 3 l/min and kept anoxic for 20 sec. The mice were taken out of the container and then ventilated artificially by massaging on the chest until spontaneous respiration was resumed. When spontaneous motions were provoked by touch stimulation after restoration of the righting reflex, the mice were exposed to CO2 gas for 15 sec under the same conditions as above. After these treatments, the mice were housed in cages in groups of 4 - 6 and were given food and water ad libitum. Twenty-four hours after the acquisition trial, the mice were placed again in the light room in the same manner as the day before, and the time until their four limbs completely entered the dark room (latency of response) was measured (retention trial). The latencies were measured to a maximum of 300 sec, and those exceeding this level were regarded as 300 sec. The drugs were administered orally before the acquisition trial, immediately after the anoxic treatment, or before the retention trial. The conditions of FS in normal mice without the anoxic treatment were 0.25 mA and 3 sec, because the FS of 0.5 mA and 3 sec is too strong for evaluation of the effects of drugs in normal mice. Several groups receiving no FS with or without CO2-exposure were run in parallel.

Active avoidance response
The active avoidance response was exam-ined with a two-way shuttle box (RSC-001, Muromachi Kikai; 46W X 19.5D X 20H cm) consisting of two identical compartments. A buzzer (2.8 kHz, 85 - 90 db) of 5-sec duration was given with an alarm generator placed on the ceiling in the center of the box as a conditioned stimulus (CS). If the animal did not move to the other compartment during this period, a 0.8 mA electric stimulus was applied for a maximum of 5 sec via the flooring grid (3 mm in diameter, 11 mm apart from one another) connected to the shock generator/scrambler (SGS-001, Muromachi Kikai) as an unconditioned stimulus (UCS). The inter-trial interval (ITI) was varied in a range of 20 - 60 sec (variable interval). The conditioned avoidance response was considered to be positive when the rat moved to the other compartment by CS alone and, thus, avoided the electric shock (UCS). This avoidance rate was calculated. The inter-trial responses or spontaneous migration of the rat to the other compartment between trials were also evaluated. The shuttle box was enclosed in a ventilated sound-attenuating chamber (MC-050, Muromachi Kikai; 80W X 60D X 60H cm), and control of the test schedule, measurement of avoidance responses, and mathematical procedures were performed with an automatic data-processing system (Muromachi Kikai, DAC-104). Each rat received twenty trials/session of avoidance response daily for 3 consecutive days. On the last day of the trial, animals showing an avoidance rate of 70% or above were selected, and their bilateral BF's (a region including the ventral globus pallidus, substantia innominata, and preoptic area) were lesioned on the next day. The animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and fixed to the stereotaxic apparatus.

Electrolytic lesions of the BF were produced by application of heat (65°C, 2 min) produced with a lesion generator (RFG-4A, Muromachi Kikai) via an electrode (0.25 mm in diameter, with insulation coating except for 0.5 mm from the tip), inserted into the BF (A:6.4, L:2.8, H:-1.0) according to the atlas of De Groot (31). Test trials were carried out 11 days after
production of the BF lesion. TA-0910 and TRH were administered orally 50 min and 20 min before the test trials, respectively. After completion of all tests, the brain of the rats was fixed by perfusing 10% formalin under pentobarbital (40 mg/kg, i.p.) anesthesia. The size and location of the BF lesion were examined microscopically for necrosis, loss of magnocellular neurons and the presence of gliosis. Figure 1 shows a typical photomicrograph of the BF lesion.

![Photomicrographs showing a typical lesioned area of the rat bilateral basal forebrain according to the atlas of De Groot.](image)

**Fig. 1.** Photomicrographs showing a typical lesioned area of the rat bilateral basal forebrain according to the atlas of De Groot.

**Delayed alternation task**

A corridor type T-maze prepared on the basis of the report of Stanton et al. (32) and Overton (33) was used for the study. A T-maze was placed in a sound-isolated room with a masking white noise of about 70 db. One light bulb positioned in the upper part of the room provided a constant illumination of about 40 lux. A T-maze consisted of three compartments: the starting box (15W × 12D cm), selection box (stem, 46W × 12D cm), and left and right arms room (60W × 12D cm). Each compartment was partitioned from the next by a wall with a guillotine door, and the height of the wall of all compartments was 25 cm. A feeding plate was placed 5 cm above the floor at the end of each arm, and a food pellet (about 45 mg) for rats was placed as a reward at each trial. During the study period, the body weight of the rats were controlled by limited feeding so that their weights were maintained at 70–80% of those with free access to food. For the first 2 days, the rats were allowed to freely explore the apparatus for 10 min a day for adaptation to handling and the apparatus. For the following 2 days, they were trained to run from the starting box to the left and right arms room with motivation of food 3 times on the first day and 4 times on the second day. The animals were trained in the delayed alternation task for 3 days from the day after the training run. One trial of this task consists of a forced run and a free-choice run. In the forced run, the animals were forced to enter only one of the two arms, and food was given as a reward when they entered it. The rat was immediately returned to the starting box and promptly (with a delay of 5 sec) allowed to begin a free-choice run by opening the guillotine door. On the free-choice run, the animals could enter either the left or right arm, but the reward for the correct choice was given only when they entered the arm different from the forced run. If the rats selected the same arm as the forced run, it was considered an error response, and they were immediately allowed to have free choice repeatedly until they made the correct choice. The arm in which the reward was placed on the forced run was determined according to the Gellerman series (34), and the left and right arms were used for forced runs at the same number of times within a day. This training was performed 10 times a day with a delay of 5 sec and an ITI of 1 min. Only the animals which showed percent correct choice (number of trials with correct choices/10 trials) of more than 85% on the third day of training were used in subsequent evaluations.

**Test 1) Correct choice-delay relationship:** Using 29 rats showing a percent correct choice of at least 85% in the above condition, a total of 10 trials of delayed alternation task were performed using a delay of 5, 20, 60, 180, and 540 sec (2 trials each) for 2 consecutive days (ITI = 1 min). The order of trials with the 5 different delays was randomized in individual animals and on each day. The animals were
placed in an opaque acrylic resin case (37W × 24D × 34H cm) during the delay. Trials were not repeated on error choices.

**Test 2) Effect of scopolamine:** Twelve other rats that met the above learning criterion (percent correct choice 85%) were used. They were divided into two groups (n = 6) with comparable mean percent correct choices, and a total of 8 trials were performed under delays of 5, 20, 60, and 180 sec (2 trials each) for 2 consecutive days (ITI = 1 min). Both groups were treated with scopolamine (0.1 mg/kg) and saline. Other procedures were the same as in Test 1. Scopolamine was administered i.p. 20 min before the trials.

**Test 3) Effect of TA-0910 and TRH on scopolamine-induced short-term memory impairment:** Twenty-three rats 2 weeks after completion of Test 1 were used. The same training as above was performed again for 3 days, and the animals were divided into 7 groups (3–4 animals in each group) with no significant difference in the mean percent correct choice on the third day. The 7 groups were subjected to the alternation task using a delay of 180 sec every 3–4 days 7 times from the next day (8 trials/day, ITI = 1 min). All groups were treated with the 7 doses of distilled water, TA-0910 (0.3, 1, 3 mg/kg) or TRH (30, 100 mg/kg) with scopolamine, and distilled water and saline in random order. TA-0910 and TRH were administered p.o. 40 min before the trial, and scopolamine was administered i.p. 20 min before the trial. Other procedures were identical with Test 2.

**Test 4) Effect of TA-9010 or TRH:** Twenty-one rats 10 days after completion of Test 3 were used. The same training as above was carried out for 2 days, and the animals were divided into 6 groups (3–4 animals in each group) with comparable percent correct choices on the second day. Delayed alternation tasks were given using a delay of 180 sec every 3–4 days 6 times from the next day (8 trials/day, ITI = 1 min). All groups were treated with the 6 doses of distilled water, TA-0910 (0.3, 1, 3 mg/kg), or TRH (30, 100 mg/kg) in random order. The procedures were the same as in Test 3 except that scopolamine was not used.

### Statistical analysis

1) The significance of differences in the latency of passive avoidance response was examined by the Mann-Whitney U-test (two-sided). 2) The significance of differences in the active avoidance response among groups was examined first by the Kruskal-Wallis H-test, followed by the Scheffé type multiple comparison test. 3) The significance of differences in the results of the delayed alternation task was examined first by the Kruskal-Wallis H-test, followed by the Tukey type multiple comparison test or the Mann-Whitney U-test. The difference in the percent correct choice as compared with the random choice level (50%) was examined by the one-sample critical ratio test.

### RESULTS

**One-trial passive avoidance response**

The mean latency of the passive avoidance response in the retention trial was significantly shortened in the anoxia group exposed to CO2 gas immediately after the acquisition trial as compared with the non-anoxia group (Tables 1 and 2). When TA-0910 (3, 10, and 30 mg/kg) was administered 60 min before theretention trial, the mean latency increased dose-dependently, and recovered at 30 mg/kg to a level comparable to that in the non-anoxia group (Table 1). However, when TA-0910 was administered 60 min before the acquisition trial (10 and 30 mg/kg) or immediately after the anoxic treatment (3, 10, and 30 mg/kg), the mean latency was not different from that in the anoxia group (Table 2).

When TRH (100 and 300 mg/kg) was administered 30 min before the retention trial, the mean latency was little different from that in the anoxia group (Table 1). When TA-0910 (30 mg/kg) was administered 60 min before the retention trial to mice not subjected to FS, the mean latency was little different from that in the group treated with distilled water re-
Table 1. Effect of TA-0910 and TRH on the latency in the retention trial of passive avoidance in anoxic mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg, p.o.</th>
<th>CO₂ exposure</th>
<th>No. of mice</th>
<th>Latency (sec, mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>40</td>
<td>195 ± 14 **</td>
</tr>
<tr>
<td>TA-0910</td>
<td>3</td>
<td>+</td>
<td>40</td>
<td>103 ± 12 *</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>40</td>
<td>150 ± 16</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>40</td>
<td>182 ± 20</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>32</td>
<td>198 ± 15 **</td>
</tr>
<tr>
<td>TRH</td>
<td>100</td>
<td>+</td>
<td>32</td>
<td>117 ± 14</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>+</td>
<td>32</td>
<td>138 ± 15</td>
</tr>
</tbody>
</table>

TA-0910 and TRH were administered to mice 60 min and 30 min before the retention trial, respectively. Foot-shock (0.5 mA, 3 sec), *without CO₂ exposure. *P < 0.05, **P < 0.01, compared with the respective control group with CO₂ exposure (Mann-Whitney U-test).

Table 2. Effect of TA-0910 administered orally pre- or post-acquisition trial on the latencies of retention trial in anoxic mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg, p.o.</th>
<th>CO₂ exposure</th>
<th>No. of mice</th>
<th>Latency (sec, mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Pre-acquisition trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>18</td>
<td>204 ± 16 **</td>
</tr>
<tr>
<td>TA-0910</td>
<td>10</td>
<td>+</td>
<td>19</td>
<td>126 ± 9 **</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>13</td>
<td>118 ± 21</td>
</tr>
<tr>
<td>II. Post-acquisition trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>22</td>
<td>239 ± 17 **</td>
</tr>
<tr>
<td>TA-0910</td>
<td>3</td>
<td>+</td>
<td>21</td>
<td>120 ± 19 **</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>21</td>
<td>133 ± 16</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>21</td>
<td>146 ± 18</td>
</tr>
</tbody>
</table>

TA-0910 was administered to mice 60 min before (I) or immediately after (II) the acquisition trial. Foot-shock (0.5 mA, 3 sec), *without CO₂ exposure. **P < 0.01, compared with the respective control group with CO₂ exposure (Mann-Whitney U-test).

Regardless of the presence or absence of the anoxic treatment (Table 3). In normal animals subjected to FS, but not to anoxia, the mean latency of the group administered TA-0910 60 min before the retention trial (3, 10, and 30 mg/kg) was not significantly different from that of the distilled water group (Table 3).
Table 3. Effect of TA-0910 on the latency of the retention trial in mice with or without CO2 exposure of foot-shock

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>CO2 exposure</th>
<th>FS</th>
<th>No. of mice</th>
<th>Latency (sec, mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>−</td>
<td>−</td>
<td>33</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>TA-0910</td>
<td>30</td>
<td>−</td>
<td>−</td>
<td>32</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>+</td>
<td>−</td>
<td>33</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>TA-0910</td>
<td>30</td>
<td>+</td>
<td>−</td>
<td>32</td>
<td>15 ± 6</td>
</tr>
</tbody>
</table>

TA-0910 was administered to mice 60 min before the retention trial. CO2 exposure was given twice immediately after the acquisition trial. FS: foot-shock (0.25 mA, 3 sec). *without CO2 exposure, **without foot-shock.

Active avoidance response

In the 85 rats that showed a percent avoidance of 70% or above after a 3-day training period of active avoidance response, the bilateral BF was electrically lesioned on the day after the last training. When test trials were performed 11 days after the BF lesion, the mean body weight of the rats had nearly returned to the pre-lesion level. The mean avoidance rate of the BF-lesioned control group (55.6 ± 5.60%) was significantly lower than that of the sham operation group (87.6 ± 2.23%). TA-0910 dose-dependently increased the mean avoidance rate to 58.5 ± 4.37, 62.6 ± 5.15, and 77.9 ± 2.15% at 0.3, 1, and 3 mg/kg, respectively. The mean avoidance rate in the group administered 3 mg/kg TA-0910 was significantly higher than that in the BF-lesioned control group. On the other hand, the mean avoidance rates of groups administered TRH (30, 100, and 300 mg/kg) were almost unchanged (Fig. 2).

The number of inter-trial responses in the sham operation group, BF-lesioned control group, TA-0910 group, and TRH group were 7–12, 6–8, 4–8, and 4–7, respectively. No significant difference was observed in the values among these groups.

Delayed alternation task

Test 1) Correct choice-delay relationship: The percent correct choice using a delay of 5, 20, 60, and 180 sec, decreased with prolongation of the delay to 81.9 ± 3.25, 74.1 ± 3.82, 64.7 ± 4.72, 61.2 ± 4.42, and 52.6 ± 3.79%, respectively. The percent correct choices under delays of 5 to 180 sec still were maintained significantly higher than the random choice level, 50%.

Test 2) Effect of scopolamine: In the saline group, the percent correct choice using a delay of 5, 20, 60, and 180 sec decreased with prolongation of the delay to 88.9 ± 3.14, 75.0 ± 4.81, 66.7 ± 4.11, and 63.9 ± 4.02%, respectively. These values were, however, all significantly higher than the random choice level. In the scopolamine group, the percent correct choice using a delay of 5, 20, 60, and 180 sec also decreased with prolongation of the delay to 72.2 ± 4.27, 67.5 ± 3.29, 63.9 ± 4.02, and 48.3 ± 5.46%, respectively. The difference in the percent correct choice as compared with the random choice level was not significant only when the delay was 180 sec (Fig. 3).

Test 3) Effect of TA-0910 and TRH on scopolamine-induced short-term memory impairment: In the distilled water group, the per-
Fig. 2. Effect of TA-0910 and TRH on memory impairment following basal forebrain (BF) lesions in test trial of rats. Groups of 14 to 17 rats were used. Experimental memory impairment was induced by BF lesions 24 hr after the 3rd training. The test trial was performed 11 days after lesioning. C: Control. Each value represents the mean ± S.E. avoidance rates per 20-trial daily session in columns. *P < 0.05, **P < 0.01, compared with the respective control group with BF lesion (Scheffe type multiple comparison test).

Fig. 3. Effect of scopolamine on the delayed alternation task in rats. Groups of 12 rats were used. Each rat received saline or scopolamine (0.1 mg/kg, i.p.). Scopolamine was given 20 min before the trial. Random choice level (50%) is represented by a horizontal broken line. ***P < 0.01, ****P < 0.001, compared with the value for random choice level (Mann-Whitney U-test). ••P < 0.05, •••P < 0.01, compared with the value for the respective saline group under each delay condition (Mann-Whitney U-test).

Test 4) Effect of TA-0910 and TRH: When alternation tasks under a delay of 180 sec were performed without scopolamine administra-
Fig. 4. Effect of TA-0910 and TRH on the 180 sec-delayed alternation task in rats treated with scopolamine. Groups of 23 rats were used. TA-0910 and TRH were given 40 and 20 min before the trial, respectively. Scopolamine was given 20 min before the trial. Random choice level (50%) is represented by a horizontal broken line. C: Control. * P < 0.05, **P < 0.01, compared with the value for scopolamine-treated control (Tukey type multiple comparison test).

DISCUSSION

Since the memory impairments induced by physical (cerebral ischemia, brain injury) or chemical (drugs, anoxic treatment) means in the mouse one-trial passive avoidance test are improved by the administration of various drugs including cholinergic drugs such as physostigmine and oxotremorine before the retention trial (28, 35–38), these memory impairments are considered to be due to disturbance of the retrieval process of memory rather than of the consolidation of memory (39). In a similar experiment in mice using a light-dark box, the shortened latency in the retention trial 24 hours after exposure to CO₂ gas immediately following the acquisition trial was dose-dependently prolonged by oral administration of TA-0910 60 min before the retention trial. However, no prolongation of the latency was observed when the drug was administered before the acquisition trial or immediately after the anoxic treatment. These results suggest that TA-0910 improves memory impairment by acting on the retrieval process of memory. In the passive avoidance response without FS, the latency of response in the group administered TA-0910 was not different from that in the distilled water group (61.9 ± 2.8%), with no significant difference.

In DAT patients with a primary symptom of registration impairment, marked decreases in the choline acetyltransferase (CAT) activity have been demonstrated in various areas of the cerebral cortex and the hippocampus (40, 41), and remarkable loss of neurons has been
noted in the Meynert's basal ganglia, which are the origins of cholinergic nerves projecting to the cerebral cortex (42). Therefore, rats with lesioned BF including the nucleus basalis magnocellularis, corresponding to Meynert's basal ganglia in humans, are frequently used as an animal model of DAT (28, 29, 43, 44).

In our study of the rat active avoidance response using a shuttle box, the oral administration of TA-0910 60 min before the test trial dose-dependently elevated the avoidance rate reduced by bilateral electrolytic lesion of BF, and it recovered the rate to a level comparable to that of the sham operation group at 3 mg/kg. Tamaki and Kameyama (45) noted that the acquisition of active avoidance response in Fischer344 rats was promoted by TRH (20 mg/kg, i.p.), but that this promotion, being correlated with the increase in the number of inter-trial responses, is ascribed to the locomotor stimulatory activity of TRH. In rats with bilateral electrolytic lesioned BF, the number of inter-trial responses was not affected by 3 mg/kg of TA-0910, a dose that can improve memory but one-tenth of that required to produce an increase in locomotor activity (M. Yamamura et al., unpublished data). Therefore, the increase in the motor activity is not a cause of the memory improving effect of TA-0910.

In the delayed alternation task, the percent correct choice decreased with prolongation of the delay and became 60% to 70% when the delay was 60 and 180 sec. Since these values were significantly higher than the random choice level (50%), the delay of 60 and 180 sec on the task is considered to reduce the short-term memory of rats but not to obliterate it completely. The correct choice levels in the scopolamine-group were lower than those in the saline-group. The correct choice levels with delays of 20 and 60 sec in the scopolamine-group were not significantly different from those in the saline-group, although the levels with delays of 5 and 180 sec were significantly lower than those in the saline-group. The reason for this lack of difference with delays of 20 and 60 sec is not clear, but it might be possibly due to an insufficient dose of scopolamine. However, 0.1 mg/kg of scopolamine was able to reduce the correct choice level with a delay of 180 sec to the random choice level. This scopolamine-induced impairment of short-term memory was reversed by the oral administration of 3 mg/kg of TA-0910 before the trial.

It should be noted that the effective dose of TA-0910, 3 mg/kg, in BF-lesioned rats and scopolamine-treated rats in one-tenth of that in anoxic mice. This discrepancy may be ascribed to differences in the task, means to induced memory impairments and animal species, because no change in gross behavior is observed in mice at a dose below 100 mg/kg of TA-0910 (K. Kinoshita et al., personal communication) and the CNS action of TA-0910 in mice has been confirmed to occur at the dose range of 1–30 mg/kg orally (19).

The latency of the passive avoidance response in normal mice not subjected to anoxia was not significantly different between the TA-0910 group and distilled water group. In the delayed alternation task, also, TA-0910 had little effect on the short-term memory of normal rats not treated with scopolamine. From these results, TA-0910 is considered to have no effect on the ability of learning and memory of normal mice and rats but to improve impaired memory.

The CNS effects of TRH were inferior to those of TA-0910 in potency and duration, but there were no qualitative differences between the CNS effect of TA-0910 and TRH (20). This suggests that TRH also has an improving effect on memory impairments. In fact, there are a number of reports that TRH improved the memory impairments in various learning tasks (26, 27, 46, 47). In the present study, oral administration of TRH (100 or 300 mg/kg) had no effect on memory impairments in anoxic mice or BF-lesioned rats and scopolamine-induced short-term memory impairment in rats. This discrepancy may be ascribed to differences in the task, means to induce memory impairments, dose, and route of administration, but most importantly to the
short duration of TRH action and its much weaker CNS actions by oral administration than by administration via other routes (15).

TA-0910 induces the recovery from the reduction of acetylcholine (ACh) turnover rate in the cerebral cortex and hippocampus of rats treated with pentobarbital to a normal level (K. Kawashima et al., personal communication). Furthermore, TA-0910 applied microiontophoretically to ACh-sensitive neurons of the rat cerebral cortex potentiated the excitation induced by ACh (25). These findings suggest an involvement of cholinergic nervous systems of the brain in the action mechanism of TA-0910. This possible mechanism is also supported by the following evidence: The memory impairment of BF-lesioned rats in the active avoidance response is ameliorated by pilocarpine or physostigmine (48), but deteriorated by scopolamine (49), and the scopolamine-induced short-term memory impairments on the delayed alternation task is improved by physostigmine (50). In addition, electrolytic lesioning of the BF causes a 30–40% decrease in CAT activity over the nearly entire cerebral cortex 1–4 weeks after the operation (44, 51). Therefore, the improvement of memory impairments of BF-lesioned rats by TA-0910 appear to be mainly due to its activating action on ACh neurons in the cerebral cortex. However, Araki et al. (52) noted not only a decrease in the CAT activity in the cerebral cortex but also decreases in norepinephrine, dopamine, and serotonin in the cerebral cortex, corpus striatum, and hippocampus in BF lesioned rats and suggested that monoaminergic nervous systems play a greater role than the cholinergic nervous systems in the memory impairment of these animals. In addition to the above-mentioned facilitating action on cholinergic nervous systems, TA-0910 also has the following stimulatory actions on the monoaminergic nervous systems. Namely, in the behavioral pharmacological studies, TA-0910 increases the locomotor activities in mice, and antagonizes the hypothermia in reserpinized mice (20). In neurochemical studies, TA-0910 potentiates the accumulations of 3-methoxytyramine in the nucleus accumbens and 5-hydroxytryptophan in the corpus striatum in rats treated with a monoamine oxidase inhibitor (pargyline) and an aromatic L-amino-acid decarboxylase inhibitor (NSD 1015), respectively (K. Kawashima et al., personal communication). Therefore, this increase in the activity of monoaminergic nervous systems is considered to contribute to the anti-amnesic effect of TA-0910.

These observations suggest that TA-0910 improves the memory impaired by anoxia, lesioning of BF or scopolamine by affecting the retrieval process of memory, and this effect of TA-0910 is considered to be due not only to enhanced control of the cholinergic nervous system but also to that of the monoaminergic nervous system.

REFERENCES


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