Effects of Bifemelane Hydrochloride (MCI-2016) on Experimental Amnesia (Passive Avoidance Failure) in Rodents

Akihiro TOBE, Tomoko YAMAGUCHI, Rie NAGAI and Mitsuo EGAWA
Biosciences Laboratory, Research Center, Mitsubishi Chemical Industries, Limited, 1000 Kamoshida-cho, Midori-ku, Yokohama 227, Japan
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Abstract—To further predict the possible activity on memory disorders, the effect of MCI-2016 (bifemelane hydrochloride) was examined using the passive avoidance (PAR) failure technique as an experimental model of amnesia. The amnesia was produced either by post training treatments of electroconvulsive shock (ECS), scopolamine (mice) and cycloheximide or by pre-test injection of scopolamine (rats). In ECS-PAR failure model, the retention test was carried out 3 hr (3 hr experiment) or 24 hr (24 hr experiment) after ECS. MCI-2016 showed a significant improvement when administered just after ECS (3 hr experiment, 30 mg/kg, i.p.) or 0.5 hr before the retention test (24 hr experiment, 10–30 mg/kg, i.p.). Ca hopantenate was only active in the 3 hr experiment (500 mg/kg, i.p.), and piracetam was rather active in the 24 hr experiment (60 mg/kg, i.p.). MCI-2016 (30 mg/kg, i.p.) prevented the scopolamine-induced PAR-failure. In this model, physostigmine (0.3 mg/kg, i.p.) exhibited a tendency to improve the failure. In another scopolamine-induced PAR failure model in mice, all of the test drugs showed a significant improvement at different dose levels. The effect of MCI-2016 (25–100 mg/kg, p.o.) was superior to those of piracetam, aniracetam and choline chloride. Higher doses of MCI-2016 were required to improve the cycloheximide-induced PAR failure. Considering the experimental conditions and results, it may be suggested that MCI-2016 ameliorates the amnesia possibly through its influence on memory consolidation and retrieval processes.

Memory loss and similar cognitive-associated dysfunctions are commonly recognized as being among the most serious symptoms of dementia and cerebrovascular diseases. In a previous investigation, we reported the improving effect of MCI-2016 on scopolamine-induced deficit of spontaneous alternation behavior (1), which has been proposed as one of the useful models to predict the potential of compounds for cognitive disorders (2). Since no conclusive animal models are yet available, the efficacy of compounds should be finally assured in clinical trials after confirming the activity in various animal models. MCI-2016 has a clinical reputation as being effective for the treatment of the decrease in spontaneity and cognitive and emotional disturbances accompanied by cerebrovascular diseases (3).

In laboratory investigation, the drug has been shown to potentiate the effects of physostigmine (antianoxic action, EEG activation and improvement of scopolamine-induced deficit of spontaneous alternation behavior) (4, 5) and to counteract the decrease of acetylcholine levels following ischemia, hypoxia and scopolamine injection (6). MCI-2016 also increases the turnover of norepinephrine which is partially antagonized by atropine (7). In the present study, we examined the effects of MCI-2016 on passive avoidance response (PAR) failures induced by electroconvulsive shock (ECS), scopolamine and cycloheximide in rodents.

Materials and Methods

Animals: Male Wistar rats (180–330 g, 7–9 weeks old, purchased from Japan
Laboratory Animals, Inc.) and male ddY strain mice (20–30 g, 4–5 weeks old, purchased from Shizuoka Laboratory Animal Center) were used. They were housed in groups of 5 for rats and 10 for mice, with a 12 hr diurnal light cycle.

Drugs: 4-(o-Benzylphenoxy)-N-methylbutylamine hydrochloride (bifemelane hydrochloride, MCI-2016), aniracetam and piracetam were synthesized in our laboratory. Other drugs used were calcium hopantenate (Ca-hopantenate, Tanabe), physostigmine sulfate (Sigma), choline chloride (Sigma), scopolamine hydrobromide (Sigma), and cycloheximide (Sigma).

Passive avoidance response failure in rats: a) Apparatus: The experimental box was a two compartment avoidance box. The large compartment (50×50×50 cm) was painted black and illuminated with a 100 W bulb. The small shock compartment (20×14×20 cm) was also painted black without a bulb. The floor was made of metal grids (12 mm apart), through which a foot shock (FS) could be applied. The FS was delivered by a shock scrambler generator (SGS-001, Muromachi-Kikai) with a shock intensity of 3 mA. The two compartments were separated by a guilotine door. b) ECS-induced PAR failure: One min of free exploration was permitted for each animal. As soon as the animals entered the small compartment, the door was closed and FS was delivered for 5 sec. The animals which did not enter the compartment within 60 sec were excluded from the experiments. Immediately after PAR training (acquisition), the animals were removed from the small compartment and received either ECS or non-ECS (sham-ECS). The ECS was administered through ear electrodes under the conditions of 60 mA, 1 msec, 200 Hz for 0.8 sec by a ECT-Unit (Ugo Basile, Italy). The time between FS and ECS never exceeded 10 sec. The retention test was performed in two different schedules. In the case of the retention test at 3 hr after the training, drugs were administered i.p. just after ECS. In the case of the retention test at 24 hr after ECS, drugs were administered 0.5 hr before the retention test. In both cases, the test period consisted of placing the animals in the large compartment and noting the latency to enter the small compartment and the time spent in the small compartment during a 3 min observation. c) Scopolamine-induced PAR failure: The training schedule was almost the same as in the case of b), except that the animals received no ECS. Scopolamine (1.5 mg/kg, i.p.) was administered 0.5 hr before the retention test. Drugs were administered simultaneously with scopolamine. In the test period, the latency to enter the small compartment was also noted. In b) and c), the number of animals which stayed longer than 90 sec in the large compartment were also checked as non-amnestic for gross comparison of the effects. d) Effects on motor function: To predict the influence of test drugs on motor function, rats that experienced no foot shock and no ECS were placed in the large compartment of the box, and the latency to enter the small compartment was checked. Drugs were administered 0.5 hr (in accordance with the dosing schedules for the 24 hr experiment of ECS-PAR failure and scopolamine PAR-failure) or 3 hr (in accordance with the dosing schedule for the 3 hr experiment of ECS-PAR failure) before the test.

Passive avoidance response failure in mice: a) Apparatus: The experimental box was 40×18×19 cm in size with a grid floor (7 mm apart). In the center of the floor, an acrylic square platform (5×5×0.5 cm) was placed. b) procedure: In the training session, the animals were placed individually on the platform. Every time the animals moved off the platform, continuous FS of 1.8–2 mA intensity was applied through the grid floor. During the 3 to 5 min training, most of the animals acquired PAR. Just after the training, amnestic agents were administered. In the case of the scopolamine-induced PAR failure test, scopolamine was administered 1 mg/kg, i.p., and the retention test was performed 1 hr thereafter. For the cycloheximide-induced PAR failure test, cycloheximide was administered 140 mg/kg, s.c., and the retention test was performed 24 hr thereafter. In both tests, drugs were administered simultaneously with the amnestic agents. In the test session, the latency to step down from the platform was measured. If the
animals stayed longer than 60 sec on the platform, they were considered non-amnestic. c) Effects on motor function: Mice that experienced no foot shock were placed on the platform, and the latency to step down from the platform was checked. Drugs were administered 1 hr (in accordance with the dosing schedule for scopolamine-PAR failure) or 24 hr (in accordance with the dosing schedule for cycloheximide-PAR failure) before the test.

Statistical analysis: Experimental values were given in terms of the mean±S.E. Difference between mean values was evaluated by Student's t-test and was considered to be significant if the P value was smaller than 0.05.

Results

ECS-induced PAR failure in rats (3 and 24 hr experiments): In both experimental conditions, the non-ECS control group exhibited a high degree of retention with the latency ranging from 130 to 180 sec during the 3 min observation. In contrast, the ECS-control group showed the amnestic behavior (PAR-failure) whose latency was significantly shortened compared with the non-ECS control group. 3 hr experiments: MCI-2016 prolonged the latency and shortened the time spent at doses of 3 to 30 mg/kg, i.p. Significant prolongation of the latency was observed at 30 mg/kg, i.p., of MCI-2016. As for reference drugs, Ca-hopantenate showed moderate but significant prolongation of the latency at 500 mg/kg, i.p. Only a tendency to prolong the latency was observed with piracetam (60 mg/kg, i.p.) and physostigmine (0.3 mg/kg, i.p.) (Table 1).

24 hr experiments: the MCI-2016 treated group performed a better PAR than the ECS-control group at 10 to 30 mg/kg, i.p. That is, the latency was significantly prolonged after 10 mg/kg, i.p., and the time spent was significantly shortened after 30 mg/kg, i.p. In this schedule, piracetam also showed a prolongation of the latency at 60 mg/kg, i.p. Although physostigmine prolonged the latency at 0.1 to 0.3 mg/kg, i.p., it did not reach a significant level (Table 2).

Table 1. Effects of MCI-2016 and other reference drugs on electroconvulsive shock-induced passive avoidance failure (ECS-PAR failure) in rats (3 hr experiments)

| Drugs           | Dose (mg/kg, i.p.) | Latency to enter small compartment (sec±S.E.) | Time spent in the small compartment (sec±S.E.) |
|-----------------|--------------------|-----------------------------------------------|-----------------------------------------------|
| Non ECS-control |                    |                                               |                                               |
| ECS-control     |                    |                                               |                                               |
| ECS-MCI-2016    | 3                  | 145.5±17.7 (7/10)                             | 22.5±13.1                                    |
|                 | 10                 | 58.5±15.3** (1/10)                            | 98.0±16.9**                                  |
|                 | 30                 | 80.0±19.8** (2/10)                            | 98.5±20.4**                                  |
|                 |                    | 77.5±18.1** (2/10)                            | 87.0±16.7**                                  |
|                 |                    | 128.9±25.7** (6/10)                           | 36.0±17.5**                                  |
| Non ECS-control |                    |                                               |                                               |
| ECS-control     |                    |                                               |                                               |
| ECS-Ca-hopantenate | 125               | 169.5±13.0 (9/10)                             | 5.5±5                                        |
|                 | 250                | 39.5±7.6** (1/10)                             | 114.0±16.7**                                 |
|                 | 500                | 56.8±17.2** (1/11)                            | 101.5±15.0**                                 |
|                 |                    | 80.5±22.0** (4/11)                            | 98.6±17.6**                                  |
|                 |                    |                                                | 74.1±19.9**                                  |
| Non ECS-control |                    |                                               |                                               |
| ECS-control     |                    |                                               |                                               |
| ECS-physostigmine | 0.1                | 173.5±6.5 (10/10)                             | 1.0±1                                        |
|                 | 0.3                | 61.0±20.4** (2/10)                            | 87.5±20.1**                                  |
|                 |                    | 30.0±6.5** (0/10)                             | 132.0±9.6**                                  |
|                 |                    | 90.0±20.4** (3/10)                            | 79.5±18.4**                                  |
|                 |                    |                                                | 67.5±18.8**                                  |
| ECS-piracetam   | 60                 | 75.5±19.7** (4/10)                            |                                               |

Drugs were administered just after ECS (3 hr prior to the retention test). Figures in parentheses indicate the number of animals staying in the large compartment more than 90 sec/number of animals used. *P<0.05 and **P<0.01 vs. the non ECS-control group. #P<0.05 vs. the ECS-control group.
Scopolamine-induced PAR failure in rats:
In the scopolamine treated group, the latency was shortened to approximately half that of the control, but the time spent was not prolonged significantly. Therefore, only the latency was used as an index of amnesia in the present experiment. MCI-2016 showed an improvement of latency at 30 mg/kg, i.p. Only 30% of the animals reached the criteria (percentage of animals which stayed in the large compartment longer than 90 sec) in the scopolamine treated group. After 30 mg/kg,

Table 2. Effects of MCI-2016 and other reference drugs on electroconvulsive shock-induced passive avoidance failure (ECS-PAR failure) in rats (24 hr experiments)

| Drugs                  | Dose (mg/kg, i.p.) | Latency to enter small compartment (sec±S.E.) | Time spent in the small compartment (sec±S.E.) |
|------------------------|--------------------|-----------------------------------------------|-----------------------------------------------|
| Non ECS-control        |                    |                                                |                                               |
| ECS-control            | 50.9±16.9**        | (4/18)                                        | 49.3±18.9**                                   |
| ECS-MCI-2016           | 10                 | 105.0±17.8*                                   | 98.6±17.8*                                   |
|                        | 30                 | 96.9±18.9*                                    | 86.6±18.9*                                   |
| Non ECS-control        |                    |                                                |                                               |
| ECS-control            | 140.7±19.4         | (9/12)                                        | 14.3±9.9                                     |
| ECS-piracetam          | 33.9±17.5**        | (1/10)                                        | 94.0±19.6**                                   |
|                        | 60                 | 98.6±8.6*                                     | 34.5±13.9*                                   |
| Non ECS-control        | 151.3±15.4         | (13/16)                                       | 14.1±9.9                                     |
| ECS-control            | 45.0±15.2**        | (2/14)                                        | 91.8±15.0**                                   |
| ECS-Ca-hopantenate     | 250                | 63.7±19.3**                                   | 69.3±17.8**                                   |
|                        | 500                | 24.7±4.9**                                    | 115.0±11.8**                                  |
| Non ECS-control        | 136.3±21.0         | (10/12)                                       | 12.9±10.7                                     |
| ECS-control            | 29.6±5.5**         | (0/12)                                        | 114.8±13.9**                                  |
| ECS-physostigmine      | 0.1                | 55.4±12.9**                                   | 88.5±14.7*                                   |
|                        | 0.3                | 50.4±17.2**                                   | 90.4±16.1**                                   |

Drugs were administered 23.5 hr after ECS (0.5 hr prior to the retention test). Figures in parentheses indicate number of animals staying in the large compartment more than 90 sec/number of animals used. *P<0.05 and **P<0.01 vs. the non ECS-control group. #P<0.05 vs. the ECS-control group.

Table 3. Effects of MCI-2016, physostigmine and piracetam on scopolamine-induced passive avoidance failure (scopolamine-PAR failure) in rats

| Drugs                  | Dose (mg/kg, i.p.) | Scopolamine 1.5 mg/kg, i.p. | Latency to enter small compartment (sec±S.E.) |
|------------------------|--------------------|-----------------------------|-----------------------------------------------|
| Control                |                    | 148.8±16.8                 | (13/16)                                       |
| Scopolamine control    | +                  | 83.8±17.5*                 | (5/16)                                        |
| MCI-2016               | 10                 | 96.1±18.1*                 | (7/16)                                        |
|                        | 30                 | 135.5±13.4*                | (12/16)                                       |
| Control                |                    | 135.0±13.8                 | (16/22)                                       |
| Scopolamine control    | +                  | 69.3±15.0*                 | (7/22)                                        |
| Physostigmine          | 0.3                | 102.4±16.0                 | (7/14)                                        |
| Piracetam              | 100                | 79.6±18.8*                 | (5/14)                                        |

Scopolamine was administered 0.5 hr prior to the retention test. Test drugs were administered simultaneously with scopolamine. Figures in parentheses indicate number of animals staying in the large compartment more than 90 sec/number of animals used. *P<0.05 and **P<0.01 vs. control group. #P<0.05 vs. scopolamine control group.
i.p., of MCI-2016, however, 75% of the animals were able to meet the criteria. Two reference drugs, physostigmine (0.3 mg/kg, i.p.) and piracetam (100 mg/kg, i.p.), exhibited a tendency to improve the PAR failure. There was no statistical significance in the latency between the control group and the physostigmine+scopolamine group (Table 3).

**Scopolamine-induced PAR failure in mice:** The control animals usually stepped down from the platform with the latency of 100 to 150 sec. The latency was significantly shortened to about 30–50 sec in the scopolamine treated group. Under these conditions, MCI-2016 significantly prolonged the latency at 25–100 mg/kg, p.o. As for other drugs, physostigmine (0.1 mg/kg, i.p.), choline chloride (200 mg/kg, i.p. or p.o.), piracetam (1000 mg/kg, p.o.) and aniracetam (100–200 mg/kg, p.o.) showed significant prolongation of the latency. Among them, the physostigmine treated group performed the best PAR (Table 4).

**Cycloheximide-induced PAR failure in mice:** The latency in the cycloheximide treated group was significantly shortened by about 70% of the control group. MCI-2016 showed no significant effect at 10 to 100 mg/kg, p.o. (data not shown). By increasing the doses up to 200 mg/kg, p.o., the drug came to exhibit a significant prolongation of the latency. Aniracetam also showed the similar effect at 200 mg/kg, p.o. (Table 5).

**Effects on motor function:** Rats: Test drugs were administered to the animals with no FS and no ECS at those doses which were shown to be active in ECS-PAR or scopolamine-PAR failure tests. None of the test drugs significantly changed the latency to enter the small compartment. Among them, 30 mg/kg, i.p., of MCI-2016 (0.5 hr pretreatment) and 500 mg/kg, i.p., of Ca-hopantenate (3 hr treatment) exhibited a tendency to slightly prolong the latency. As for scopolamine (1.5 mg/kg, i.p., 0.5 hr) treated animals, they showed a latency similar to that of control animals (Table 6).

### Table 4. Effects of MCI-2016 and other reference drugs on scopolamine-induced passive avoidance failure (scopolamine-PAR failure) in mice

| Drugs            | Dose (mg/kg) | Scopolamine 1 mg/kg, i.p. | Latency to step down from the platform (sec±S.E.) |
|------------------|--------------|---------------------------|--------------------------------------------------|
| Control          |              |                           | 107.8± 8.3 (31/39)                                |
| Scopolamine control | +           |                           | 38.8± 6.5** (8/39)                                |
| MCI-2016         | 25 p.o.      | i                         | 110.8±21.9## (8/12)                               |
|                  | 50           | +                         | 82.1±17.1* (6/12)                                |
|                  | 100          | +                         | 112.9±20.3## (8/12)                               |
| Physostigmine    | 0.1 i.p.     | +                         | 140.8±15.2## (6/6)                                |
| Choline chloride | 100 p.o.     | +                         | 70.8±22.7 (3/6)                                  |
|                  | 200          | +                         | 129.2±26.2## (5/6)                                |
|                  | 400          | +                         | 25.0±12.5** (1/6)                                 |
|                  | 200 i.p.     | +                         | 108.3±28.8* (5/6)                                 |
| Piracetam        | 300 p.o.     | +                         | 58.8±17.5* (4/12)                                |
|                  | 500          | +                         | 70.8±17.2 (6/12)                                 |
|                  | 1000         | +                         | 104.2±16.6## (8/12)                               |
| Aniracetam       | 50 p.o.      | -                         | 56.3±12.4## (8/18)                                |
|                  | 100          | -                         | 84.4±14.2## (10/18)                               |
|                  | 200          | +                         | 127.1±21.4## (9/12)                               |

Scopolamine and test drugs were administered just after PAR training. Figures in parentheses indicate number of animals staying on the platform more than 60 sec/number of animals used. *P<0.05 and **P<0.01 vs. control group. #P<0.05 and ##P<0.01 vs. scopolamine control group.
### Table 5. Effects of MCI-2016 and aniracetam on cycloheximide-induced failure of passive avoidance response (PAR) in mice

| Drugs                  | Dose (mg/kg, p.o.) | N  | Latency to step down from the platform (sec±S.E.) |
|------------------------|--------------------|----|-----------------------------------------------|
| Control                | —                  | 39 | 156.8± 5.6                                    |
| Cycloheximide control  | 39                 |    | 120.3± 7.2**                                 |
| Cycloheximide-MCI-2016 | 200                | 20 | 150.6± 9.4*                                  |
|                        | 300                | 20 | 120.9±10.7**                                 |
| Cycloheximide-Aniracetam | 100              | 18 | 140.5±10.4                                   |
|                        | 200                | 17 | 146.9± 8.0*                                  |

Cycloheximide was administered just after PAR training. **P<0.01 vs. control group. *P<0.05 vs. cycloheximide control.

### Table 6. Effects of MCI-2016 and other reference drugs on the latency to enter the small compartment in normal rats

| Drugs                  | Dose (mg/kg, i.p.) | Pretreated time (hr) | N  | Latency to enter small compartment (sec±S.E.) |
|------------------------|--------------------|----------------------|----|-----------------------------------------------|
| Control-1              | saline             | 0.5                  | 6  | 19.4±0.38                                     |
| MCI-2016               | 10                 | 0.5                  | 6  | 24.2±7.1                                      |
|                        | 30                 | 0.5                  | 6  | 36.5±8.2                                      |
| Piracetam              | 100                | 0.5                  | 6  | 23.3±2.7                                      |
| Physostigmine          | 0.3                | 0.5                  | 6  | 20.0±2.6                                      |
| Scopolamine            | 1.5                | 0.5                  | 6  | 22.5±5.1                                      |
| Control-2              | saline             | 3                    | 6  | 23.3±4.6                                      |
| MCI-2016               | 30                 | 3                    | 6  | 23.3±4.9                                      |
| Physostigmine          | 0.3                | 3                    | 6  | 28.7±6.8                                      |
| Ca-hopantenate         | 500                | 3                    | 6  | 40.8±8.2                                      |

Drugs were administered to normal rats (no FS, no ECS) at 0.5 or 3 hr before testing the latency to enter the small compartment.

### Table 7. Effects of MCI-2016 and other reference drugs on the latency to step down from the platform in normal mice

| Drugs                  | Dose (mg/kg, p.o.) | Pretreated time (hr) | N  | Latency to enter small compartment (sec±S.E.) |
|------------------------|--------------------|----------------------|----|-----------------------------------------------|
| Control-1              | 1                  | 6                    | 6  | 7.8±2.4                                       |
| MCI-2016               | 100                | 1                    | 6  | 5.7±0.9                                       |
| Physostigmine          | 0.1                | 1                    | 6  | 9.2±1.5                                       |
| Choline chloride       | 400                | 1                    | 6  | 8.7±3.3                                       |
|                        | 200 (i.p.)         | 1                    | 6  | 9.0±3.2                                       |
| Piracetam              | 1000               | 1                    | 6  | 8.0±2.8                                       |
| Aniracetam             | 200                | 1                    | 6  | 11.5±4.5                                      |
| Scopolamine            | 1 (i.p.)           | 1                    | 6  | 20.3±7.6                                      |
| Control-2              | 24                 | 6                    |    | 7.8±1.1                                       |
| MCI-2016               | 300                | 24                   | 6  | 7.8±3.6                                       |
| Aniracetam             | 200                | 24                   | 6  | 4.5±0.9                                       |
| Cycloheximide          | 140 (s.c.)         | 24                   | 6  | 15.0±7.5                                      |

Drugs were administered to normal mice (no FS) at 1 or 24 hr before testing the latency to step down from the platform.
None of the test drugs showed significant changes on the latency to step down from the platform. Scopolamine 1 mg/kg, i.p., was shown to prolong the latency (control latency: 7.8±2.4 sec; scopolamine, 1 mg/kg, i.p., latency: 20.3±7.6 sec) (Table 7).

Discussion

In the present investigation, we examined the effects of MCI-2016 on passive avoidance response (PAR)-failure induced by post trial (training) treatments of electroconvulsive shock (ECS), scopolamine and cycloheximide. Although some differences exist for training to test (retention test) intervals among the three PAR-failure models except for scopolamine PAR-failure in rats, these procedures may be considered to cause retrograde amnesia by impairing the memory consolidation process (8). Under these conditions, MCI-2016 significantly prolonged the latency and improved the PAR at 10 to 30 mg/kg, i.p. (rats) and 25 to 200 mg/kg, p.o. (mice). For comparison, Ca-hopantenate which has been claimed to be effective for senile mental disorders (9), cholinomimetic agents (physostigmine and choline chloride) and nootropic agents (piracetam and aniracetam), which have also been reported to have beneficial effects on memory or cerebral impairment disorders (10-13), were also examined. In the ECS-PAR failure test, the retention test was performed at either 3 or 24 hr after the training. Since test drugs were administered just after ECS (retention test: 3 hr after drug administration) or 0.5 hr prior to the retention test (retention test: 24 hr after ECS), the interpretation of drug effects may be different. Essentially, if the drug was effective in the 3 hr schedule, it might be postulated to improve the impaired consolidation process, and if the drug was effective in the 24 hr schedule, it might be postulated to improve retrieval deficit. Considering that MCI-2016 improved the PAR-failure in both conditions at 10 to 30 mg/kg, i.p., the drug may facilitate consolidation and retrieval processes. Ca-hopantenate was more effective in the 3 hr schedule than in the 24 hr one at 250 to 500 mg/kg, i.p. In the case of piracetam, it was rather effective in the 24 hr schedule (60 mg/kg, i.p.). Considering the doses of drugs used, MCI-2016 may be superior to Ca-hopantenate and piracetam in its efficacy. Subsequently, the effect of physostigmine was also examined. The drug exhibited a tendency to improve the PAR failure at 0.1 to 0.3 mg/kg, i.p., in 24 hr experiments, but it did not reach a significant level in our experiments.

Anti-cholinergic agents are known to disrupt memory-cognitive function in humans as well as animals (14, 15). In the scopolamine-induced PAR failure in mice, physostigmine (0.1 mg/kg, i.p.) and choline chloride (200 mg/kg, i.p. and p.o.) showed a definite improvement for prolonging the latency. In the same model, MCI-2016 was also shown to be effective at 25 to 100 mg/kg, p.o. The latency time was increased to around 3 times that of the scopolamine-control by these drugs. Nootropic agents, piracetam and aniracetam, also showed beneficial effects at 1000 mg/kg, p.o., and 100 to 200 mg/kg, p.o., respectively. Partial involvement of the cholinergic mechanism has been presented with aniracetam by Kubota et al. (16), but little has been reported about such mechanisms with piracetam. Recent investigation indicated that MCI-2016 antagonized the decreased acetylcholine level produced by scopolamine injection (6). These considerations lead us to expect the possibility that the drugs tested (except for piracetam) counteract the PAR failure by scopolamine through partial modification of decreased cholinergic function.

We have also examined the influence of drugs on scopolamine-induced PAR failure in rats. Because scopolamine was administered 0.5 hr before the retention test, PAR-failure may be caused through its influence on the retrieval and/or retention process. Although scopolamine is known to increase locomotor activity, the drug exhibited no significant effect on the latency to enter the small compartment when administered in no FS and no ECS experienced rats. Similar data was also obtained in mice. Thus the motor activity change by scopolamine may be considered not to affect PAR directly. Deutsh and Rocklin (17) and Pazzagli and Pepeu (18) reported the memory failure when
scopolamine was injected 24 hr after the initial training. Although the clear explanation for the present model must await further study, MCI-2016 was also shown to improve the deficit at 30 mg/kg, i.p. Physostigmine also showed a moderate improvement at 0.3 mg/kg, i.p.

Cumin et al. (8) reported an improving effect of aniracetam on cycloheximide-induced PAR failure in mice. These effects were also confirmed in the present experiments. In this model, MCI-2016 also showed beneficial effects at 200 mg/kg, p.o. Judging from the effects of MCI-2016 on PAR failures in mice, it may be suggested that the drug is more effective on scopolamine-induced PAR failures. In the interpretation of the activity of drugs on PAR failure models, influence of drugs on motor function may be important. MCI-2016 showed no significant change in the latency to enter the small compartment (10–30 mg/kg, i.p., 0.5 and 3 hr pretreatments in rats) or to step down from the platform (100–300 mg/kg, p.o. 1 hr and 24 hr pretreatments in mice) when examined in no FS experienced animals. Although slight prolongation of latency was noticed after 30 mg/kg, i.p. (0.5 hr) of MCI-2016, it may be suggested that the effect of MCI-2016 on PAR failure is not produced through its influence on motor function.

Among various hypotheses for memory disorders, dysfunction theories of cholinergic mechanism have been presented repeatedly (19, 20). MCI-2016 potentiates the effects of physostigmine on anoxia, EEG and scopolamine-induced deficit of spontaneous alternation behavior (4, 5). In addition, the drug counteracts the decrease of acetylcholine following scopolamine, hypoxia and ischemia (6) and is also suggested to modify the release of acetylcholine (Saito et al., unpublished data). Coupled with the improving effect of MCI-2016 on scopolamine-induced PAR failure, the drug may influence the memory deficit partly through activation of cholinergic mechanisms. Involvement of other mechanisms must also be considered because MCI-2016 also showed a positive effect on other amnestic models. Meyer and Beattie (21) and Waugh et al. (22) suggested that memory difficulties or deficit in the cerebrovascular accidents or elderly are often due to memory retrieval failure. Although it is not clear at present what types of cognitive disorders are more sensitive to MCI-2016, the drug may be expected to have some beneficial effects.

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