EFFECT OF 4-(o-BENZYLPHENOXY)-N-METHYLIBUTYLAMINE HYDROCHLORIDE (MCI-2016) ON THE SCOPOLAMINE-INDUCED DEFICIT OF SPONTANEOUS ALTERNATION BEHAVIOR IN RATS

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Abstract—To predict the possible activity on memory disorders, the effect of MCI-2016 was compared with those of physostigmine, choline chloride, methamphetamine, apomorphine, imipramine and calcium hopantenate by applying scopolamine-induced deficit of spontaneous alternation behavior (scopolamine-SA) as a proposed animal model for senile dementia. MCI-2016 was shown to improve the scopolamine-SA at doses of 25 to 100 mg/kg p.o. without producing any remarkable behavioral abnormalities. As for the effect of reference drugs, two types of cholinomimetic drugs (physostigmine and choline chloride) and methamphetamine were shown to be active. In the cases of physostigmine and methamphetamine, however, behavioral abnormalities were observed at those dose levels effective on scopolamine-SA. MCI-2016 potentiated the effect of physostigmine on scopolamine-SA at non active doses of 10 to 20 mg/kg p.o. In comparison with the deleterious effect of scopolamine on spontaneous alternation (SA) behavior itself, none of the test drugs except for imipramine were shown to disrupt the SA. Considering the disruptive or improving actions of various agents on SA or scopolamine-SA, it may be suggested that the present model is relatively sensitive to those drugs which affect the cholinergic mechanism either directly or indirectly. Mechanisms of the actions of MCI-2016 and methamphetamine were also discussed with reference to possible involvement of cholinergic mechanisms.

Spontaneous alternation (SA) is the characteristic behavior by which animals typically alternate their choices between the arms of a T-maze more often than they repeat their initial choice if allowed two consecutive unrewarded runs (1, 2). SA behavior is a learning task with no strong source of motivation, and the involvement of cholinergic mechanisms in the behavior has been suggested by Egger et al. (3). Indeed, scopolamine is suggested to reduce the SA behavior by impairment of recent memory (1, 4). Although no conclusive animal models are yet available, scopolamine-induced deficit of SA behavior has been proposed as one of the useful approaches for the evaluation of drugs for memory disorders such as senile dementia (2).

MCI-2016 (4-(o-benzylphenoxy)-N-methylbutylamine hydrochloride) is a new drug having anti-anoxic, EEG activating and antidepressive actions in laboratory evaluations (5-7). While the drug has a stimulative influence on central noradrenergic mechanisms, previous investigation suggests to us the possible involvement of central cholinergic mechanisms in the anti-anoxic and EEG activating effects. In addition to these pharmacological propensities of MCI-2016, the drug has been reported to be effective for the treatment of cerebrovascular diseases in recent clinical trials (personal com-
munication).

Considering that the symptoms of memory impairments are often observed in patients of senile dementia or cerebrovascular diseases, the present investigation was designed to predict the possible activity of MCI-2016 on memory impairments by applying scopolamine-induced deficit of SA behavior as a model.

Materials and Methods

Animals: Male rats of the Wistar strain were purchased from Japan Laboratory Animals Inc. They weighed 170 to 190 g upon arrival in the laboratory and were housed in groups of 5, with a 12 hr diurnal light cycle.

Drugs: MCI-2016 (4-(o-benzylphenoxy)-N-methylbutylamine hydrochloride) was synthesized in our laboratory. Other drugs used were physostigmine sulfate (Sigma), choline chloride (Sigma), methamphetamine hydrochloride (Dainippon), apomorphine hydrochloride (Sigma), imipramine hydrochloride (Fujisawa), calcium hopantenate (Ca hopantenate, Tanabe) and scopolamine hydrobromide (Sigma).

Spontaneous alternation behavior (SA): The apparatus was a T-maze made of black acrylic with independently hinged plastic tops on the start alley and goal arms. Both the stem and each arm of the maze were 50 cm long. The first 20 cm of the stem and last 20 cm of each arm were divided by guillotine doors into start and goal boxes, respectively. The alley of the maze were 9 cm wide, and the walls were 11 cm high. A 100 W bulb was situated 100 cm above the central part of the apparatus. Before experiments, animals (200–220 g, 7–8 week old) were adapted in the sound reducing room for at least 3 hr.

Each animal was placed in the start box of the maze. After 10 sec, the three guillotine doors were raised without delay, allowing the animals to locomote in the maze. Upon entering the goal box in one of the arms and having the guillotine door closed behind it, the animals were then removed to a holding bucket for 10 sec and was given a second trial in an identical fashion (3). The percentage of SA (SA%) was determined as: (number of animals that alternated their goals in two trials/number of animals used) ×100(%). Scopolamine was injected i.p. (1 mg/kg) 30 min before testing SA. The test drug was administered either p.o. or i.p. at each selected time. The antagonistic (improving) percentage of the drug for scopolamine-induced deficit of SA was determined as follows: (SA% in drug+ scopolamine treated group–SA% in scopolamine group)/(SA% in saline treated group–SA% in scopolamine treated group) ×100(%).

Exploratory behavior in rats (Open field test): The open field is a square box (100×100 cm, 40 cm high) with the bottom and wall painted black. The apparatus was placed in a dark room illuminated by a 100 W bulb situated 100 cm above the central part of the field which was divided into 16 blocks (25×25 cm). Five to six rats (240–260 g) were used for each dose level, and they were accustomed to the environment for at least 3 hr. The number of ambulations and rearings of each animal were calculated for 3 min.

Observation of the stereotyped behavior in rats: Each group of 5 rats (200–220 g) was given drugs at the highest doses that were used for the SA experiments. At least 30 min prior to the experiments, the animals were individually placed in the observation box and allowed to adapt to the environment. The box was made of clear plastic (five rooms, size: 25×20×16 cm) and open on top, with a wire mesh cover. The behavior was scored every 15 min for 1 hr according to the following scale (8): 0, normal; 1, intermittent sniffing and constant exploration; 2, continuous sniffing and/or repetitive head
and limb movements; 3, discontinuous gnawing, biting or licking; 4, continuous gnawing, biting or licking.

**Statistical analysis:** $x^2$-analysis was used for the evaluation of data in the SA experiments. In the case of exploratory and stereotyped behaviors, the student's $t$-test was applied.

**Results**

**General description of SA behavior:** Immediately after raising the door of the start box, most of the animals began to locomote slowly in the maze and entered one of the goal boxes within 5 to 10 sec. The animals which failed to enter the goal box within 30 sec were excluded from the study. The locomotion time in the 2nd trial was not very different from that in the 1st trial. Among the various groups (saline group, scopolamine alone group, scopolamine+drug treated group, alternated group and non-alternated group), however, scopolamine alone and scopolamine+methamphetamine groups exhibited a tendency to locomote sooner than the other groups.

**Effects on scopolamine-induced deficit of SA behavior:** Under our experimental con-

| Drugs                | Dose (mg/kg) | Scopolamine 1 mg/kg i.p. | Number of animals alternated | Percent alternation | Percent improvement |
|----------------------|-------------|--------------------------|------------------------------|---------------------|---------------------|
| Saline               |             |                          | 137/182                      | 75.3                | —                   |
| Scopolamine alone    | +           |                          | 56/180***                   | 31.1                | —                   |
| MCI-2016             | 25 p.o.     | +                        | 6/15                         | 40.0                | 25.1                |
|                      | 50          | +                        | 7/15                         | 46.6                | 35.1                |
|                      | 100         | +                        | 10/16####                   | 66.7                | 80.6                |
| Physostigmine        | 0.1 i.p.    | +                        | 5/10                         | 50.0                | 42.8                |
|                      | 0.3         | +                        | 13/20####                   | 65.0                | 76.7                |
|                      | 1.0         | +                        | 7/10##                      | 70.0                | 88.0                |
| Choline chloride     | 150 p.o.    | +                        | 16/30###                    | 53.3                | 50.2                |
|                      | 300         | +                        | 15/30                        | 50.0                | 42.8                |
|                      | 450         | +                        | 13/20###                    | 65.0                | 76.7                |
| Methamphetamine     | 0.1 i.p.    | +                        | 4/10                         | 40.0                | 25.1                |
|                      | 0.3         | +                        | 5/10                         | 50.0                | 42.8                |
|                      | 1.0         | +                        | 9/10######                  | 50.0                | >100                |
| Apomorphine          | 0.1 i.p.    | +                        | 4/10                         | 40.0                | 25.1                |
|                      | 0.3         | +                        | 5/10                         | 50.0                | 42.8                |
|                      | 1.0         | +                        | 3/10                         | 30.0                | 0                   |
| Imipramine           | 25 p.o.     | +                        | 3/10                         | 30.0                | 0                   |
|                      | 50          | +                        | 3/10                         | 30.0                | 0                   |
|                      | 100         | +                        | 4/10                         | 40.0                | 25.1                |
| Ca-hopantenate       | 250 p.o.    | +                        | 4/10                         | 40.0                | 25.1                |
|                      | 500         | +                        | 3/10                         | 30.0                | 0                   |
|                      | 1000        | +                        | 4/10                         | 40.0                | 25.1                |

<sup>a</sup>) Significantly different from the saline group at $P<0.01$, <sup>b</sup>) significantly different from the scopolamine alone group at $*P<0.05$, $**P<0.02$, $***P<0.01$. MCI-2016, choline chloride, imipramine and Ca-hopantenate were administered 30 min before scopolamine. Physostigmine, methamphetamine and apomorphine were simultaneously administered with scopolamine.
ditions, the saline treated group alternated at a greater than chance level (SA%: 75.3%, P<0.01), while the scopolamine alone group alternated at a less than chance level (SA%: 31.1%, P<0.01). Therefore, SA was impaired by 59% under scopolamine treatment (P<0.01 vs saline treated group). Table 1 summarizes the effects of MCI-2016 and other reference drugs on scopolamine-induced deficit of SA (scopolamine-SA). MCI-2016 exhibited a dose dependent improvement of the behavior at 25 to 100 mg/kg p.o., showing a significant difference from the scopolamine alone group at 100 mg/kg p.o. (80.6% improvement, P<0.02). Significant improvement of the behavior was also obtained by two cholinomimetic drugs: at 0.3 to 1 mg/kg i.p. for physostigmine and at 150 or 450 mg/kg p.o. for choline chloride.

In addition to the activities of cholinomimetic drugs, those of four drugs which are known as dopaminergic stimulants (methamphetamine and apomorphine), an antidepressant (imipramine) and a cerebral metabolic activator (Ca-hopantenate) were examined in a similar fashion (Table 1). Except for the case of methamphetamine, which showed marked improvement at 1 mg/kg i.p. (P<0.01), none of the tested drugs had significant effects.

**Combined effects of MCI-2016 and physostigmine on scopolamine-SA:** To examine further the combined effect, MCI-2016 and physostigmine were administered either orally (MCI-2016, 5–20 mg/kg p.o.) or intraperitoneally (physostigmine, 0.03 mg/kg i.p.) at those selected doses which had little influence on the scopolamine-SA by themselves. As described in Fig. 1, physostigmine itself showed a weak antagonistic effect (28% improvement). By combining with MCI-2016, the % improvement increased proportionally to the doses of MCI-2016. At 20 mg/kg p.o., the % improvement amounted to 71%.

**Effects on spontaneous alternation behavior (SA):** The drugs listed in Table 1 were examined to determine if they affected the SA behavior itself, and the results are summarized in Table 2. The saline treated group alternated at the SA ratio of 78.6%. MCI-2016 and physostigmine were demonstrated to have little influence on the SA. Choline chloride and methamphetamine exhibited a tendency to slightly increase the SA at higher doses. Although no dose response relationships were noticed, apomorphine, imipramine and Ca-hopantenate slightly decreased the SA. Among them, 50 mg/kg p.o. of imipramine was shown to significantly worsen the SA.

**Effects on exploratory and stereotyped behaviors:** Further experiments were conducted to obtain information on whether the drug effects shown in Table 1 were selective or accompanied by general behavioral changes. Among the drugs tested, physostigmine was shown to markedly suppress the exploratory behavior at 0.3 to 1 mg/kg i.p. without producing the stereotyped behavior.
Table 2. Effects of MCI-2016 and other reference drugs on spontaneous alternation behavior in rats

| Drugs       | Dose (mg/kg) | Number of animals alternated | Percent alternation | Percent difference from control^a |
|-------------|--------------|------------------------------|--------------------|-----------------------------------|
| Control (Saline) |              | 55/70                        | 78.6               |                                   |
| MCI-2016    | 25 p.o.      | 8/10                         | 80.0               | 1.8                               |
|             | 50           | 8/10                         | 80.0               | 1.8                               |
|             | 100          | 8/10                         | 80.0               | 1.8                               |
| Physostigmine | 0.1 i.p.     | 8/10                         | 80.0               | 1.8                               |
|             | 0.3          | 8/10                         | 80.0               | 1.8                               |
|             | 1.0          | 8/10                         | 80.0               | 1.8                               |
| Choline chloride | 150 p.o.   | 7/10                         | 70.0               | −10.9                             |
|             | 300          | 9/10                         | 90.0               | 14.5                              |
|             | 450          | 10/10                        | 100                | 27.2                              |
| Methamphetamine | 0.1 i.p.   | 7/10                         | 70.0               | −10.9                             |
|             | 0.3          | 9/10                         | 90.0               | 14.5                              |
|             | 1.0          | 10/10                        | 100                | 27.2                              |
| Apomorphine | 0.1 i.p.     | 8/10                         | 80.0               | 1.8                               |
|             | 0.3          | 6/10                         | 60.0               | −24.0                             |
|             | 1.0          | 7/10                         | 70.0               | −10.9                             |
| Imipramine  | 25 p.o.      | 8/10                         | 80.0               | 1.8                               |
|             | 50           | 5/10                         | 50.0**             | −36.4                             |
|             | 100          | 6/10                         | 60.0               | −24.0                             |
| Ca-hopantenate | 250 p.o.  | 7/10                         | 70.0               | −10.9                             |
|             | 500          | 7/10                         | 70.0               | −10.9                             |
|             | 1000         | 7/10                         | 70.0               | −10.9                             |

**P<0.02 vs control group. MCI-2016, choline chloride, imipramine and Ca-hopantenate: 60 min pretreated. Physostigmine, methamphetamine and apomorphine: 30 min pretreated. ^a) Percent difference from the control alternation.

In contrast, methamphetamine significantly increased the exploratory behavior in ambulatory response (0.1–0.3 mg/kg i.p.) and rearing response (0.3–1 mg/kg i.p.). Only the rearing response was increased significantly by 0.1 mg/kg i.p. of apomorphine (Table 3). Both methamphetamine and apomorphine produced a stereotyped behavior mainly characterized by continuous sniffing accompanied by occasional licking at 1 mg/kg i.p. (Fig. 2). Other drugs including MCI-2016 exhibited no stereotyped behavior at the highest doses employed, although moderate changes in exploratory behavior were obtained (Table 3).

Fig. 2. Induction of stereotyped behavior by methamphetamine and apomorphine in rats. Vertical bars represent the S.E.M. (●) methamphetamine, 1 mg/kg i.p.; (○) apomorphine, 1 mg/kg i.p.

Discussion
Among numerous findings, dysfunction
Table 3. Effects of MCI-2016 and reference drugs on exploratory behavior in rats

| Drugs              | Dose (mg/kg) | Number of ambulations (mean±S.E.M.) | Number of rearing (mean±S.E.M.) | N\(^a\) |
|--------------------|--------------|------------------------------------|---------------------------------|--------|
| Control            |              | 46.2±2.7                           | 16.3±1.0                        | 39     |
| MCI-2016 25 p.o.   | 57.5±7.0     | 20.0±2.6                           | 6                               |        |
| 50                 | 43.3±6.2     | 23.3±3.8                           | 6                               |        |
| 100                | 41.2±5.1     | 14.5±3.0                           | 6                               |        |
| Physostigmine 0.1 i.p. | 49.4±5.0 | 13.2±1.7                           | 5                               |        |
| 0.3                | 35.4±3.0*    | 10.2±1.3**                         | 5                               |        |
| 1.0                | 3.4±2.0**    | 0**                               | 5                               |        |
| Choline chloride 150 p.o. | 40.4±2.7 | 16.8±4.3                           | 5                               |        |
| 300                | 48.6±3.2     | 21.0±1.5*                          | 5                               |        |
| 450                | 51.6±8.1     | 13.4±3.2                           | 5                               |        |
| Methamphetamine 0.1 i.p. | 66.2±6.2** | 17.8±4.9                           | 6                               |        |
| 0.3                | 65.4±5.4**   | 23.6±2.3**                         | 6                               |        |
| 1.0                | 62.6±10.8    | 29.6±5.0**                         | 6                               |        |
| Apomorphine 0.1 i.p. | 50.2±3.3   | 22.7±2.1*                          | 6                               |        |
| 0.3                | 46.0±4.4     | 20.4±3.4                           | 6                               |        |
| 1.0                | 29.8±8.5     | 15.5±2.9                           | 6                               |        |
| Imipramine 25 p.o. | 43.5±1.4     | 12.2±2.1                           | 6                               |        |
| 50                 | 41.8±5.5     | 18.2±2.8                           | 6                               |        |
| 100                | 38.8±5.8     | 11.3±1.3**                         | 6                               |        |
| Ca-hpantenate 250  | 63.0±4.6**   | 23.3±4.0                           | 6                               |        |
| 500                | 45.5±5.8     | 18.0±2.2                           | 6                               |        |
| 1000               | 54.5±10.1    | 17.5±2.9                           | 6                               |        |

\(^a\) Number of animals used. \(^*P<0.05, **P<0.01\). MCI-2016, choline chloride, imipramine and Ca-hpantenate were administered 60 min before observation. Physostigmine, methamphetamine and apomorphine were administered 30 min before observation.

in specific cholinergic mechanisms has been suggested to be partially responsible for the deficits in recent memory observed with old age (9, 10). For example, comparison of the memory and cognitive failures induced by scopolamine with the performance of aged subjects showed a marked similarity in their patterns (11). In addition, clinical effects of several cholinomimetic drugs on memory disorders have also been reported (12-14).

From the point of pharmacological evaluation, however, the lack of satisfactory animal models retards the discovery of new drugs effective for memory and cognitive disorders. Under these circumstances, a new model for senile dementia has been proposed by Pepeu et al. (2). This model has been derived from the observation that scopolamine disrupts the spontaneous alternation (SA) behavior, which is considered as one of the cholinergically mediated learning behaviors (1-4). In the present study, the usefulness of this model was first examined. Using 7 to 8 week old rats, we have obtained values for the SA% of about 80% and 30% in the saline and scopolamine treated groups, respectively. The reproducibility of the SA% in these groups was good, and our results were found to be almost similar to those of Pepeu et al. and Egger et al. (2, 3). Although some controversies must be taken into consideration for the interpretation of SA behavior (1, 15, 16), the present model was judged to be basically applicable for drug evaluation, so
we then examined the effects of cholinomimetic drugs. Under our conditions, physostigmine and choline chloride were demonstrated to significantly antagonize the scopolamine-SA at 0.1–1 mg/kg i.p. and at 150 or 450 mg/kg p.o., respectively. The present results confirmed the report by Pepeu et al. (2) who showed the efficacy of choline and phosphatidylserine. Since these drugs have been reported to stimulate the cholinergic system (17–19), it may be suggested that cholinomimetic drugs are highly sensitive to scopolamine-SA.

Taking into consideration these observations and the possibilities for the efficacy of various drugs on scopolamine-SA, the effect of the new drug MCI-2016 was then investigated in line with those of various types of drugs. MCI-2016, when administered alone, dose-dependently antagonized the scopolamine-SA at 25 to 100 mg/kg p.o. and was found to be more active than choline chloride. Furthermore, MCI-2016 was demonstrated to have a combination effect with physostigmine (0.03 mg/kg i.p.) on scopolamine-SA at those doses which by themselves were inactive (10–20 mg/kg p.o.). Although direct evidence to neurochemically support the cholinomimetic action is not available yet, a number of results from previous (5, 6) and present investigations may suggest that the cholinergic mechanism is in part involved in the improving effect of MCI-2016. These results are: a) Potentiating effect of MCI-2016 on physostigmine-induced anti-hypoxic, EEG arousal and anti-scopolamine-SA actions and b) anti-hypoxic and EEG arousal effects of MCI-2016 which were antagonized by anti-cholinergic drugs (atropine or scopolamine). As for the involvement of other factors, MCI-2016 increases the turnover rate of norepinephrine (NE) and also inhibits NE uptake (unpublished data). Considering that NE uptake inhibition generally leads to feed back regulation of NE turnover, participation of other mechanisms such as acceleration of NE synthesis or increase of NE turnover via the cholinergic system may be postulated. In this respect, Morgan and Pfeil (20) reported that physostigmine increased the NE turnover which was blocked by atropine. If these results were applied to MCI-2016, the increase of NE turnover may also in part suggest the cholinomimetic action of MCI-2016. Comparable with the effect of MCI-2016 is that of methamphetamine which showed an improving effect on scopolamine-SA at 0.1–1 mg/kg i.p. Involvement of a dopaminergic mechanism in the action of methamphetamine is not plausible since apomorphine has been found to be inactive. It has been reported by Robinson et al. (21) that acetylcholine turnover in the hippocampus was increased by methamphetamine which was further blocked by phenoxybenzamine. Taking account of these facts, it may be possible that methamphetamine exhibits the effect by indirectly stimulating the cholinergic mechanism via the NE system as one of the causative mechanisms. Although the precise mechanism of action remains to be elucidated, these considerations with MCI-2016 and methamphetamine may indicate that NE-ACh interaction is important for the effects of these drugs. Yonkov and Roussinov (22, 23), while applying the scopolamine-induced memory deficit model, reported the memory facilitating effects of various central stimulants (strychnine, caffeine, methylphenidate and amphetamine). They suggested the complex interrelationships of various transmitter mechanisms for the incidence of the effect of these drugs. In addition, Mewaldt and Ghoneim (13) suggested two possible mechanisms for the improving effects of physostigmine and methamphetamine through experiments on human memory deficit with scopolamine: the first is stimulation of specific memory
process (either cholinergic or adrenergic), and the second is through a general arousal effect. If these hypotheses were applied to scopolamine-SA, then various drugs may have the possibilities to exhibit positive effects. Furthermore, since MCI-2016 and methamphetamine in line with physostigmine change the spontaneous EEG to an arousal pattern, these arousal effects may also affect the improving effects.

Other drugs examined were imipramine and Ca-hopantenate. Imipramine was selected because the drug resembles MCI-2016 in the sense that the drug has antidepressive (7) and NE uptake inhibitory actions. Although the in vivo antidepressive activity of imipramine was several times potent than that of MCI-2016, it failed to improve the scopolamine-SA. This may thus indicate that the antidepressive effect is not directly related to the improving effect of MCI-2016. In addition, Ca-hopantenate, which has been claimed to have some influence on psychological symptoms in patients of vascular or senile dementia (personal communication), failed to improve the scopolamine-SA even at higher doses (250–1000 mg/kg p.o.).

In accordance with the deleterious effect of scopolamine on SA, the influence of each of the tested drugs on SA behavior was also checked. Among the drugs, only imipramine caused a significant decrease of SA. This phenomenon may be considered to result from the anti-cholinergic action of imipramine. The reason why imipramine failed to worsen the scopolamine-SA is not clear at present. Perhaps scopolamine (1 mg/kg i.p.) itself caused enough disruption of SA (3) which did not allow imipramine to further worsen the behavior. Although not statistically significant, methamphetamine and choline chloride exhibited a tendency to slightly enhance the SA. The precise mechanism for the SA enhancing effect of these drug are not clear in the present experiments.

From the point of clinical application, the drug should be free from behavioral disorders. In the present study, physostigmine strongly decreased the exploratory behavior, and methamphetamine caused stereotyped behavior at effective dose levels. In the case of MCI-2016, however, stereotyped behavior was not observed at all, and the exploratory behavior was only slightly decreased at the highest dose. In consideration for clinical application, these properties of MCI-2016, therefore, may be advantageous over other drugs.

Memory impairments or learning deficits are produced by various treatments, and it is not clear as yet that only the cholinergic mechanism may be responsible for memory deficits (24–26). Although the present results with MCI-2016 may lead us to expect its possible potency for memory or cognitive disorders, further experiments on MCI-2016 are required to obtain more information from the point of neuropharmacological actions and of other animal models. While the scopolamine-SA model is found to be a simple and sensitive method for preliminarily predicting the potentials of drugs on memory or learning disorders, the results must be carefully interpreted, considering the promising but confusing mechanisms of memory disorders.

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