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Effects of a Novel Compound MCI-225 on Impaired Learning and Memory in Rats

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EGUCHI, J., T. YUASA, M. EGAWA AND A. TOBE. *Effects of a novel compound MCI-225 on impaired learning and memory in rats.* PHARMACOL BIOCHEM BEHAV 48(2) 345–349, 1994.—Effects of MCI-225, [4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride] on experimental amnesia were studied in rats and compared with those of THA [9-amino-1,2,3,4-tetrahydroacridine]. In the Morris-type water maze task, MCI-225 (1–10 mg/kg, PO) reduced the spatial learning impairment induced by scopolamine (0.5 mg/kg, IP). In a passive avoidance (PA) task, administration of MCI-225 prior to training (1–30 mg/kg, PO) lessened the carbon dioxide (CO_2)-induced amnesia in a dose-dependent manner. MCI-225 (1–100 mg/kg) did not affect gross behavior. THA (0.1–3 mg/kg, PO) reduced scopolamine-induced learning deficits in the water maze task, but the effect was not significant. THA (0.3–3 mg/kg, PO) also ameliorated the CO_2-induced amnesia, although slightly, in the PA task. THA (10 mg/kg, PO) increased locomotor activity and higher dose of THA (30 mg/kg, PO) induced tremor, hypersalivation, and muscle relaxation. These results suggest that MCI-225 lessens impairments in learning and memory without causing serious behavioral abnormalities.

MCI-225 Water maze PA Scopolamine CO_2 THA

IN order to evaluate the effects of new compounds on learning and memory, many experimental amnesic models have been used. It is known that the cholinergic neuron in the central nervous system (CNS) plays an important role in learning and memory (1), and scopolamine, an acetylcholine (ACh) receptor antagonist, is used to cause amnesia in animal models (17). Memory impairments are also induced by hypoxia or hypercapnia, which affect the metabolism of neurotransmitters including ACh and monoamines in the CNS (2,11).

MCI-225 (Fig. 1) is a newly synthesized thienopyrimidine analog. Previous studies suggest that MCI-225 has an interesting profile of action on the CNS (7,16).

In the present study, the antiamnesic effects of MCI-225 were evaluated in rats using scopolamine-induced learning impairment in the water maze task and CO_2-induced amnesia in a PA task. To clarify the profile of action, the effects of MCI-225 on gross behavior were also observed.

For comparison, the effects of THA, an acetylcholinesterase (AChE) inhibitor (9), were also examined. THA is reported to be effective in the treatment of senile dementia of the Alzheimer type (15,19), but produces signs of liver damage in humans (12) and adverse cholinergic effects in experimental animals (3).

METHOD

Animals

Male Wistar rats (160–320 g, 7–10 weeks old, purchased from Japan Laboratory Animals, Inc.) were used in all experiments except the PA task. Female Sprague-Dawley rats (160–180 g, purchased from Charles River, Ltd.) were used for the PA task. All rats were housed in groups of five, with a 12 h diurnal light cycle.

Water Maze (Place Navigation and Cued Navigation Experiments)

The procedure used was a modification of that described by Morris (14). A circular pool (150 cm in diameter, 45 cm high) was filled to a depth of 30 cm with 21.0°C water. The pool surface was divided into four quadrants of equal area, NE, NW, SE, and SW. A transparent plastic platform (12 cm diameter) was placed midway between the center and the rim of the pool in the NE quadrant. The platform was located 1 cm below the surface of the water in the place navigation experiment and 1 cm above the water in the cued navigation experiment.

Each rat received five trials, for which the starting loca-

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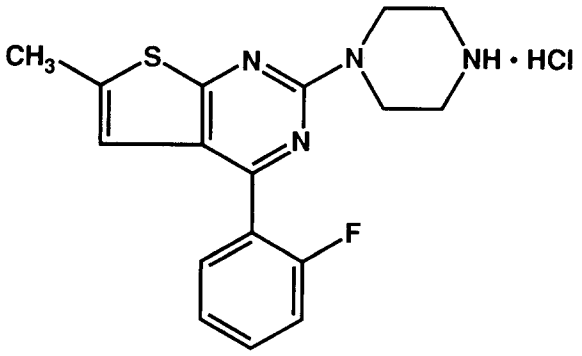

FIG. 1. Chemical formula of MCI-225.

tions were N-W-S-E-N, with 10 min intertrial intervals. A trial started when the rat, held facing the pool wall, was immersed in the water. The rat was then allowed 120 s to search for the platform; if the rat failed to escape within this time, it was placed on the platform. Regardless of whether the rat found the platform or not, it remained there for 30 s. Escape latency was recorded in each trial. Scopolamine (0.5 mg/kg, diluted in saline) or saline was injected IP 15 min before the first trial for both the place and cued navigation experiments. MCI-225 (suspended in 0.5% Tween 80), THA (diluted in distilled water), or vehicles were administered PO 1 h before testing for the place navigation experiment.

Passive Avoidance

A two compartment step-through PA apparatus consisting of an illuminated runway (6 × 25 cm) and a dark chamber (32 × 32 × 32 cm) with a grid floor, through which a scrambled footshock was delivered, was used. The rat could enter the dark chamber through an opening (6 × 6 cm).

On the first day, the rats received familiarization in place of training three times for which each animal was placed on the runway and allowed to enter the dark compartment. The next day, an acquisition trial was performed. This was similar to a training trial, except that the rats received a footshock (FS, 1.0 mA for 10 s) commencing 10 s after they entered the chamber. The latency to enter the dark compartment was recorded. Immediately after FS administration, the rats were placed in a box filled with CO_2 until respiratory arrest occurred. The rats were then revived by artificial respiration. Sham-treated rats were placed in an identical but air-filled box. Drugs or vehicles were administered orally 60 min before the acquisition trial.

Twenty-four hours later, a retention test was administered and the escape latency was measured during a 180 s observation.

Behavioral Study

Gross behavior was observed using a modification of the method of Denoble et al. (3). In brief, rats received orally drug or vehicle and were scored by a trained observer at 0.5, 1, 2, 4, 6, and 24 h after administration. Each rat was scored for hypersalivation, tremor, chromodacryorrhea, and muscle relaxation. Each dose was evaluated in six rats with each rat used only once.

Locomotor activity was recorded using an electromagnetic activity meter (MK ANIMEX SE, Muromachi Kikai, Tokyo, Japan) at 60 min intervals for a total of 240 min immediately after a pair of rats was placed in the activity cage (32 × 42 × 30 cm). Drugs or vehicles were administered orally just before the start of measurements.

Drugs

MCI-225 was synthesized in our laboratory. Scopolamine hydrobromide (Sigma) and THA (Janssen) were purchased commercially.

Data Analysis

Data from the water maze experiments were analyzed using repeated analysis of variance (ANOVA). Data from PA were analyzed using the Kruskal-Wallis test followed by Mann-Whitney's *U*-test. Data on locomotor activity were analyzed by two-tailed Student's *t*-test. All results were expressed as means ± SE. A 0.05 level of probability was accepted as significant.

RESULTS

Place Navigation in the Water Maze

Effects of MCI-225. In preliminary studies, the dose of scopolamine (0.5 mg/kg, IP) was carefully selected as the lowest dose capable of increasing the swimming latency. The escape latencies of each group in five consecutive trials are shown in Fig. 2. An analysis of the latencies in training trials revealed a significant overall group effect, $F(5, 168) = 12.0$, $p < 0.01$, with scopolamine (0.5 mg/kg, IP) increasing escape latencies in swimming compared with saline treated group, $F(1, 56) = 78.8$, $p < 0.01$. MCI-225 significantly shortened the escape latencies prolonged by scopolamine, [1 mg/kg: $F(1, 56) = 6.5$, $p < 0.05$; 3 mg/kg: $F(1, 56) = 16.0$, $p < 0.01$; 10 mg/kg: $F(1, 56) = 15.1$, $p < 0.01$]. A higher dose of MCI-225 (30 mg/kg) produced a slight reduction in scopolamine-induced learning impairment, but the effect was not significant.

Effects of THA. Figure 3 shows the escape latency of each

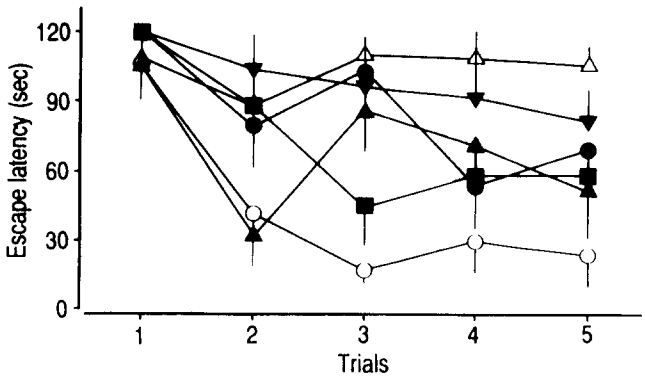

FIG. 2. Effects of MCI-225 on place navigation using a water maze task in scopolamine-treated rats. Saline IP + 0.5% Tween 80 PO (○), scopolamine 0.5 mg/kg, IP + 0.5% Tween 80 PO (△), scopolamine IP + MCI-225, 1 (●), 3 (▲), 10 (■), 30 (▼) mg/kg, PO. MCI-225 or Tween 80 were administered 60 min before the first trial (45 min prior to scopolamine or saline injection). $n = 8$ per group.

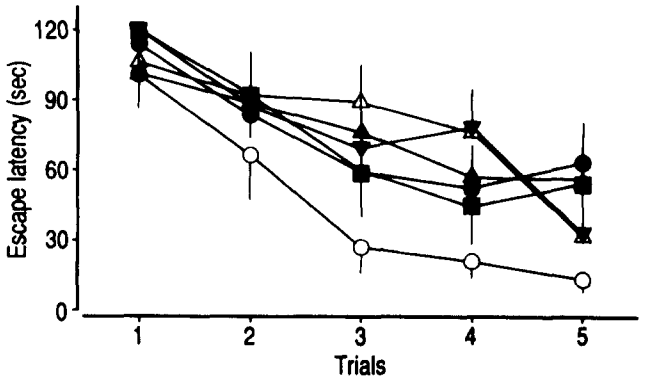

FIG. 3. Effects of THA on place navigation using a water maze task in scopolamine treated rats. Saline IP + D.W. PO (○), scopolamine 0.5 mg/kg, IP + D.W. PO (△), scopolamine IP + THA, 0.1 (●), 0.3 (▲), 1 (■), 3 (▼) mg/kg, PO. THA or D.W. were administered 60 min before the first trial (45 min prior to scopolamine or saline injection). n = 8 per group.

group in another experiment. Analysis of latencies revealed a significant overall group effect, $F(5, 168) = 3.2$, $p < 0.01$, with scopolamine significantly increasing the escape latency, $F(1, 56) = 15.5$, $p < 0.01$. THA (0.1–3 mg/kg) reduced scopolamine-induced learning impairment, but the effect was not significant in the range of doses used.

Cued Navigation in the Water Maze

When performance was assessed in the water maze using a visible platform (Fig. 4), an analysis of escape latencies revealed that scopolamine (0.5 mg/kg, IP) did not affect the latencies for finding the visible escape platform.

Passive Avoidance

Table 1 shows step-through latencies in the PA acquisition trial and retention test following CO_2 treatment. In the retention test, the load of CO_2 produced amnesia, with the CO_2 control group having significantly shorter latencies compared to the non-CO_2 control group. CO_2-induced amnesia was ameliorated in a dose-dependent manner by treatment with

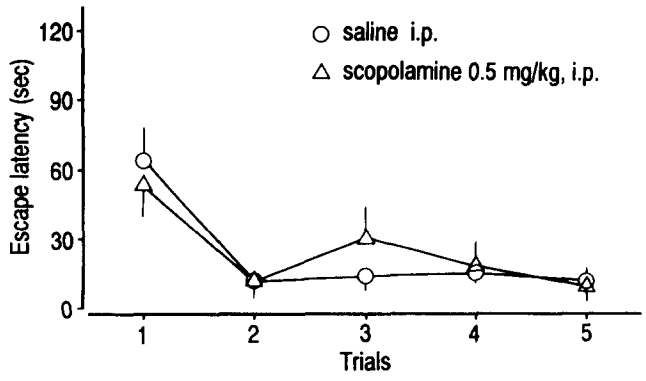

FIG. 4. Effects of scopolamine on cued navigation using a water maze task in rats. Saline or scopolamine were injected 15 min before the first trial. n = 8 per group.

MCI-225 at 1–30 mg/kg. In the acquisition trial, MCI-225 did not induce any changes at any dose used.

THA (0.3–3 mg/kg) caused a slight but significant prolongation in the escape latencies in the retention test. The highest dose of THA (10 mg/kg) did not influence latency. THA also increased the latencies in the acquisition trial compared to the CO_2 control group at doses of 0.3 and 10 mg/kg.

Behavioral Study

The influence of MCI-225 (1–100 mg/kg) and THA (1–30 mg/kg) on gross behavior were examined. MCI-225 did not induce behavioral changes within the range of doses used. THA produced tremor, hypersalivation, and muscle relaxation at 30 mg/kg. Neither chromodacryorrhea nor mortality were found at any dose tested.

Figures 5 and 6 represent the time course changes in locomotor activity during 4 h after the administration of a compound. MCI-225 (1–30 mg/kg) did not change the locomotor activity. THA produced a dose-dependent increase in locomotor activity, which was significant at 10 mg/kg compared to the vehicle control group ($p < 0.05$).

DISCUSSION

It is well known that scopolamine, an ACh receptor antagonist, reduces the memory-cognitive function in animals (20) and humans (4,5). The present results in which scopolamine impaired the acquisition of spatial learning in rats are in accordance with earlier observations in rodents (10,18). Latencies of animals treated with both scopolamine and MCI-225 (1–10 mg/kg) were shorter than scopolamine-treated subjects but longer than saline controls, thus implying that the memory impairment created by scopolamine was partially blocked with MCI-225. The scopolamine-induced learning deficit in the place navigation experiment is separate from the effects on motor function or motivational processes, because the same dose of scopolamine does not influence escape latencies in the cued navigation experiment. On the other hand, it has been shown that hypercapnia or hypoxia produce amnesia via the alteration of not only cholinergic but also noradrenergic and serotonergic neuronal functions in the CNS (2,11). MCI-225 (1–30 mg/kg) dose dependently ameliorated the CO_2-induced amnesia in PA task, as reflected by increasing latencies to enter the aversive dark chamber, in contrast to animals that only hypocapnic treatment. When using water maze or PA tasks, the influence of a test compound on gross behavior should also be considered, because behavioral changes induced by a compound affect the latencies obtained in those amnesic models. Up to 100 mg/kg, MCI-225 produced no behavioral changes. In addition, neither locomotor activity nor latencies on acquisition test for PA were influenced by MCI-225 (1–30 mg/kg). Consequently, it is concluded that MCI-225 has antiamnesic effects in the scopolamine and hypercapnia models.

Concerning the mechanism of action, MCI-225 may ameliorate the learning deficit at least partly via the activation of the cholinergic neuron. It has been reported that scopolamine-induced spatial learning impairment is antagonized by cholinomimetic agents, physostigmine and oxotremorine (10). MCI-225 clearly attenuated the impairment in this model. THA (0.1–3 mg/kg), an AChE inhibitor (9), appears to reduce escape latencies in the spatial task. THA is reported to partially normalize scopolamine-induced learning deficits in the water maze task in rats (8) and to be inactive in mice (10). The reason why the ameliorative effects of THA in the water

TABLE 1

EFFECTS OF MCI-225 AND THA ON CO_2 INDUCED AMNESIA IN RATS IN STEP-THROUGH PA RESPONSE

Treatment	(mg/kg, PO)	Number of Rats	FS	CO_2	Latency (s) Acquisition	Latency (s) Retention
Tween 80		20	+	−	0.7 ± 0.04	130.0 ± 13.67
		20	+	+	0.6 ± 0.04	0.9 ± 0.08*
MCI-225	0.3	10	+	+	0.6 ± 0.04	1.1 ± 0.10
	1	10	+	+	0.5 ± 0.04	3.9 ± 0.66†
	3	10	+	+	0.7 ± 0.08	13.1 ± 1.50†
	10	10	+	+	0.5 ± 0.04	21.8 ± 5.72†
	30	10	+	+	0.5 ± 0.04	41.0 ± 18.17†
Distilled water		10	+	−	1.0 ± 0.05	88.7 ± 20.24
		10	+	+	1.0 ± 0.05	1.0 ± 0.06*
THA	0.3	10	+	+	1.2 ± 0.03†	1.2 ± 0.03‡
	1	10	+	+	1.0 ± 0.06	1.5 ± 0.19‡
	3	10	+	+	1.0 ± 0.04	1.5 ± 0.16‡
	10	10	+	+	2.5 ± 0.39‡	1.0 ± 0.12

Drugs or vehicles were administered 1 h before the acquisition trial (25 h prior to the retention test). Overall $H(6) = 76.85, p < 0.01$ (MCI-225- and vehicle-treated groups), $H(5) = 37.26, p < 0.01$ (THA- and distilled water-treated groups).

*$p < 0.01$ vs. control group.

†$p < 0.05$.

‡$p < 0.01$ vs. CO_2-control group (Mann–Whitney's U-test).

maze task are weak may be that the effects of THA on motor function mask its antiamnesic action in this task. In the present study, THA significantly prolonged escape latencies in the acquisition trial for PA response at doses of 0.3 and 10 mg/kg.

Coupled with the improvements provided by MCI-225 on CO_2-induced PA failure, MCI-225 may also attenuate hypofunction in some neurotransmitter systems including noradrenaline (NA), serotonin (5HT), and ACh. In fact, it has been reported that MCI-225 (30 mg/kg) changes turnover of both NA and 5HT in rat brain (16). Because scopolamine (0.5 mg/kg, IP) decreases NA contents in rat brain (13), it is possible that MCI-225 also acts on the learning impairment induced by scopolamine through the noradrenergic neuron. The possibility that MCI-225 acts on noradrenergic neurons is supported by the fact that MCI-225 (10 and 30 mg/kg) reduces the resistance to the extinction of the food-rewarded runway response in dorsal noradrenergic bundle lesioned rats (7). Even at the highest dose tested (100 mg/kg), MCI-225 did not induce any behavioral changes or sedation. These results suggest that the pharmacological profile of MCI-225 might be different from that of AChE inhibitors like THA (3) and that the antiamnesic action of MCI-225 may be mediated via some neurotransmitter systems. On the other hand, THA (0.3–3 mg/kg) showed a slight effect on escape latencies in CO_2-induced PA failure. In this model, amnesia is accompanied

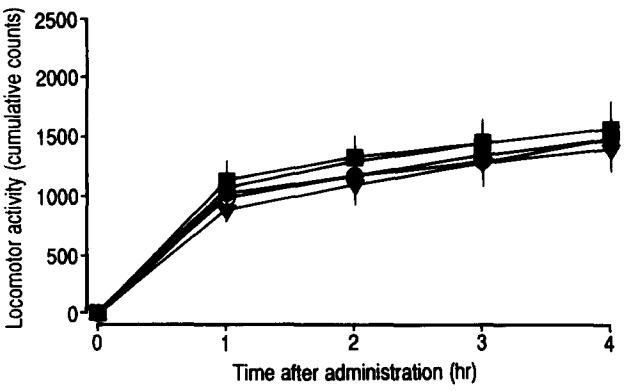

FIG. 5. Effects of MCI-225 on locomotor activity in rats. Locomotor activity was measured continuously for 4 h just after drug administration. Tween 80 (0.5%) PO (○), MCI-225, 1 (●), 3 (▲), 10 (■), 30 (▼) mg/kg, PO. n = 5–14 per group.

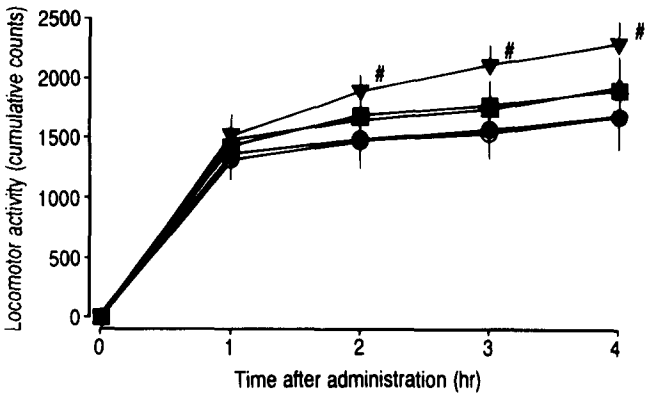

FIG. 6. Effects of THA on locomotor activity in rats. Locomotor activity was measured continuously for 4 h just after drug administration. D.W. PO (○), THA, 0.3 (●), 1 (▲), 3 (■), 10 (▼) mg/kg, PO. n = 8–9 per group. #: $p < 0.05$, vs. control group (Student's t-test).

by dysfunction in both the monoaminergic and cholinergic neuronal systems. Because THA does not substantially affect monoamine systems (6), it may be difficult to observe the sufficient effect of THA on CO_2-induced amnesia.

In contrast to MCI-225, THA (30 mg/kg) produced marked cholinomimetic effects such as tremor and salivation. Also, THA (10 mg/kg) increased locomotor activity and prolonged escape latencies in the acquisition trial for PA response. Denoble et al. (3) have reported that THA produces tremor at 6 mg/kg, SC, although THA protects against hypoxia-induced PA failure in rats at 0.3, 5 and 7 mg/kg, SC.

In conclusion, MCI-225 reduces the spatial learning impairment induced by scopolamine and reversed the memory deficit induced by CO_2 treatment without causing serious behavioral impairment. It is suggested that MCI-225 may have ameliorative effects on cognitive deficits.

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