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Effects of 5-HT_2 Receptor Antagonist on Cycloheximide-Induced Amnesia in Mice

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NABESHIMA, T., K. ITOH, K. KAWASHIMA AND T. KAMEYAMA. *Effects of 5-HT_2 receptor antagonist on cycloheximide-induced amnesia in mice.* PHARMACOL BIOCHEM BEHAV **32**(3) 787–790, 1989.—A role played by serotonergic neuronal system in cycloheximide (CXM)-induced amnesia was studied in mice using a step-down passive avoidance task. CXM (30 mg/kg SC) given immediately after training caused impairment of memory. Nonselective serotonin (5-HT) antagonist methysergide and selective 5-HT_2 antagonist ritanserin significantly attenuated impairment of memory caused by CXM. 5-HT_1 antagonist (±)-pindolol had no effect on CXM-induced amnesia. The antiamnesic effect of ritanserin on CXM-induced amnesia was antagonized by 5-HT (ICV), but not by nonselective 5-HT agonist 5-methoxy-N,N-dimethyltryptamine and 5-HT_{1A} selective agonist 8-hydroxy-2-(di-n-propylamino)tetralin at the dose level which did not cause the memory disruption. Scopolamine antagonized the antiamnesic effects of methysergide and ritanserin on CXM-induced amnesia. These results suggest that 5-HT_2 receptors and cholinergic neuronal system may play an important role in memory formation.

Cycloheximide Serotonergic system Cholinergic system Ritanserin Methysergide Amnesia Mice

IT is well-known that a protein synthesis inhibitor cycloheximide (CXM) impairs learning and memory in rodents (5, 6, 8). Several investigators have suggested that the amnesic action of CXM might be due to disturbances in catecholaminergic neurotransmission (9,10), and in cholinergic neuronal system (20, 22, 28). Kameyama *et al.* (14) and Nabeshima *et al.* (20) have also reported that the opioidergic and GABAergic neuronal systems might participate in the amnesic action induced by CXM. It is therefore possible to attribute the amnesic action of CXM to effects on numerous neurotransmitter system.

It has been shown that posttrial intracerebral administration of serotonin (5-HT) impairs retention of avoidance learning in rats (27). 5-Hydroxytryptophan (5-HT precursor), p-chloroamphetamine (5-HT releaser), and 5-methoxy-N,N-dimethyltryptamine (5-HT agonist) also impair avoidance learning in rats (3, 13, 16, 21, 25). On the contrary, serotonergic antagonists produce the enhancement of the memory of previously learned aversive habit in mice (1). These results suggest that 5-HT plays an important role in the processes underlying learning and memory in animals. Recently, neurochemical investigations of Alzheimer-type dementia have demonstrated a marked change of serotonergic neuronal system (2, 4, 22). Therefore, it aroused an interest in the study of relation between serotonergic neuronal system and memory.

The present study was undertaken to investigate an involvement of serotonergic neuronal system in CXM-induced amnesia in mice.

METHOD

Animals

Male mice of the ddY strain (Shizuoka Laboratory Animal Center) weighing 30–35 g were used. Animals were kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity, light-dark cycle; light on between 8:00 and 20:00) and were provided with commercial food and water ad lib.

Drugs

Cycloheximide (CXM; Sigma), serotonin (5-HT; Sigma) and scopolamine (Katayama Chemical) were dissolved in 0.9% saline. 5-Methoxy-N,N-dimethyltryptamine (5-MeODMT; Sigma) and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; Research Biochemicals, Inc.) were dissolved in 0.9% saline containing 0.5% ascorbic acid. Ritanserin (Janssen-Kyowa) was dissolved in distilled water with a small amount of lactic acid, and sodium bicarbonate was used to bring the pH of the solution up to about 4.

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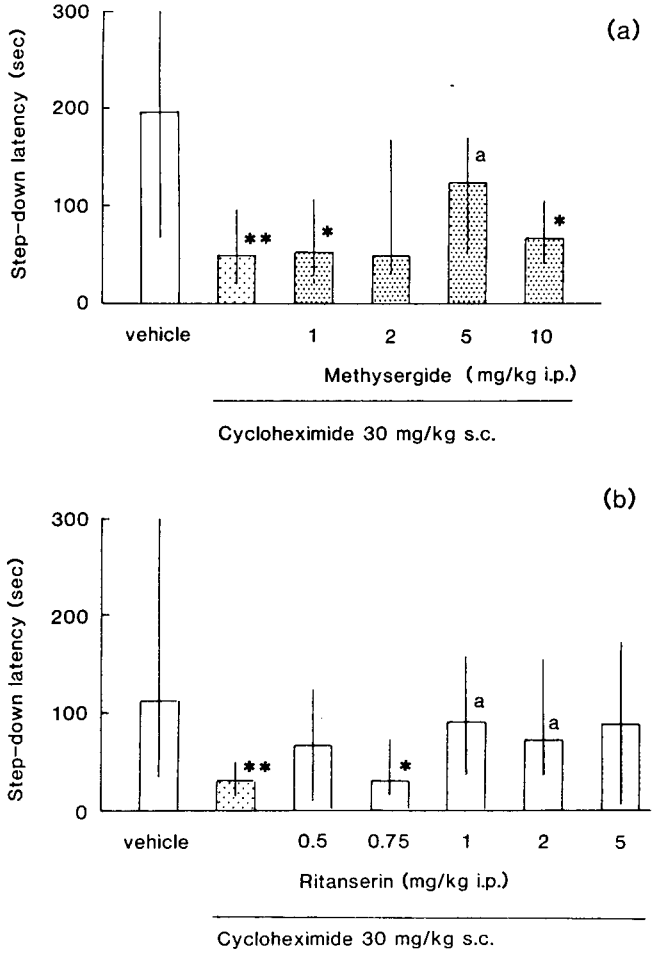


FIG. 1. Effects of methysergide and ritanserin on CXM-induced amnesia in mice. Mice were given CXM in combination with methysergide (a) or ritanserin (b) immediately after training. The retention test was performed 24 hr after training. Each value is the median of 15–20 animals. $*p<0.05$, $**p<0.01$ compared with vehicle group. $^{a}p<0.05$ compared with CXM group.

Other drugs were as follows: methysergide (Sandoz), (±)-pindolol (Sigma). The doses of drugs which did not affect on learning and memory by themselves were used for the experiments of antagonism against CXM-induced amnesia.

Apparatus

The passive avoidance apparatus consisted of a Plexiglas rectangular inner box (21 × 21 × 40 cm high) with a grid floor and semi-soundproof wooden outer box (35 × 41 × 91 cm high) with a 15-W illumination lamp (14). The grid floor consisted of 26 parallel steel rods (0.3 cm in a diameter) set 0.8 cm apart. In the center of the grid floor, a wooden platform (4 × 4 × 4 cm) was set. The intermittent electric shocks (1 Hz, 0.5 sec, 60 V DC) were delivered to the grid floor by an isolated stimulator (Nihon Koden SEN 3201).

Passive Avoidance Procedures

Training. Each mouse was placed gently on the wooden platform set in the center of the grid floor. When the mouse stepped down from the platform and placed all its paws on the grid floor, the intermittent electric shocks were delivered. The step-down latency was measured, and the animals of the step-down latency 3–15 sec were used for the retention test.

Retention test. Twenty-four hr after training, each mouse was placed on the platform again, and the step-down latency was recorded up to a maximum cut-off time of 5 min.

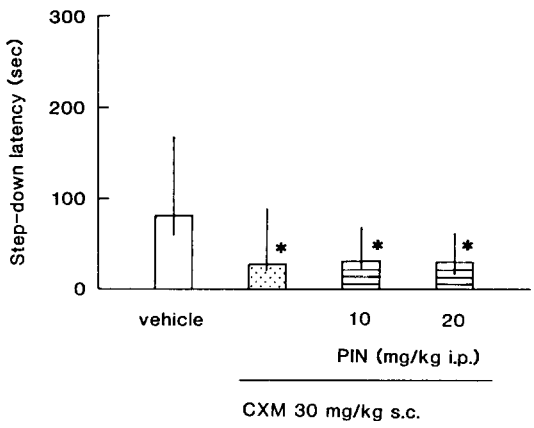


FIG. 2. Effect of (±)-pindolol (PIN) on CXM-induced amnesia in mice. Mice were given CXM in combination with (±)-pindolol immediately after training. The retention test was performed 24 hr after training. Each value is the median of 15 animals. $*p<0.05$ compared with vehicle group.

Experimental Procedures

CXM-induced amnesia. Mice were given CXM (30 mg/kg SC) immediately after training, since CXM induced amnesia in a dose-dependent fashion, and the effect reached a plateau at the dose of 30 mg/kg (20).

Effects of methysergide, ritanserin and (±)-pindolol on CXM-induced amnesia in mice. Mice were given CXM (30 mg/kg SC) in combination with vehicle, methysergide (1, 2, 5 and 10 mg/kg IP), ritanserin (0.5, 0.75, 1, 2, and 5 mg/kg IP) or (±)-pindolol (10 and 20 mg/kg IP) immediately after training. The retention test was performed 24 hr after training.

Effects of 5-HT, 5-MeODMT and 8-OH-DPAT on ritanserin-induced recovery from CXM-induced amnesia in mice. Mice were given CXM (30 mg/kg SC) in combination with ritanserin (1 mg/kg IP) immediately after training. 5-HT (1 and 2.5 μg/mouse ICV), 5-MeODMT (4 and 8 mg/kg SC) or 8-OH-DPAT (0.5 and 0.75 mg/kg IP) was also given simultaneously. The ICV injection of 5-HT was carried out according to the method of Haley and McCormick (12). The retention test was performed 24 hr after training.

Effects of scopolamine on methysergide- and ritanserin-induced recovery from CXM-induced amnesia in mice. Mice were given CXM (30 mg/kg SC) in combination with ritanserin (1 mg/kg IP) or methysergide (5 mg/kg IP) immediately after training. Scopolamine (0.5 mg/kg SC) was also given simultaneously. The retention test was performed 24 hr after training.

Statistical Analysis

Data were analyzed by Mann-Whitney U-test. All data were expressed as medians and interquartile ranges.

RESULTS

Effects of Methysergide, Ritanserin and (±)-Pindolol on CXM-Induced Amnesia in Mice

Vehicle-treated mice received electric shocks in training showed

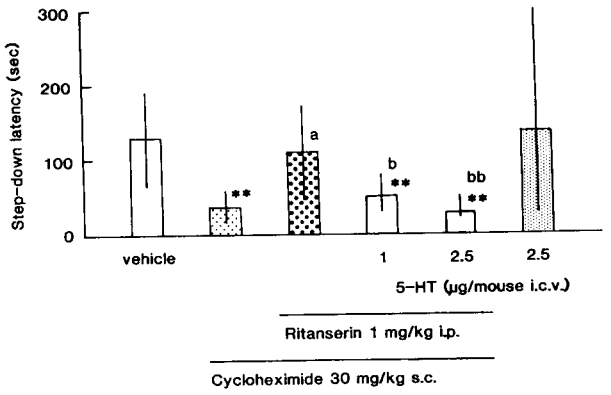


FIG. 3. Effect of 5-HT on ritanserin-induced recovery from CXM-induced amnesia in mice. Mice were given CXM in combination with ritanserin immediately after training. 5-HT was also given simultaneously. The retention test was performed 24 hr after training. Each value is the median of 15–23 animals. $^{**}p<0.01$ compared with vehicle group. $^{a}p<0.05$ compared with CXM group. $^{b}p<0.05$, $^{bb}p<0.01$ compared with CXM + ritanserin group.

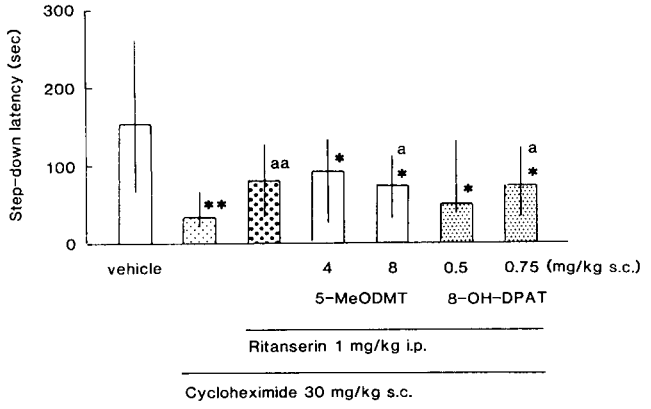
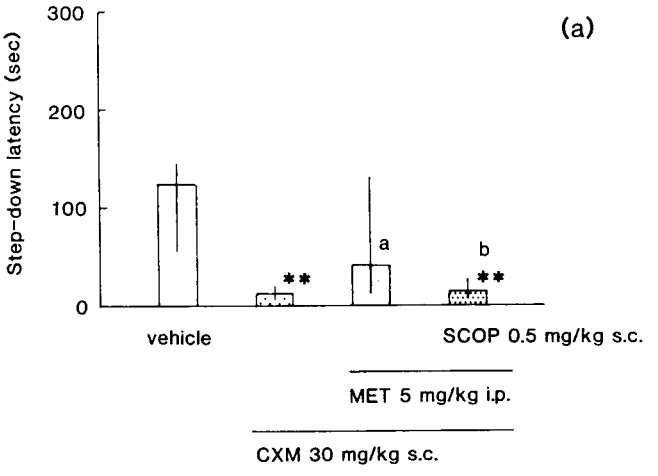


FIG. 4. Effects of 5-MeODMT and 8-OH-DPAT on ritanserin-induced recovery from CXM-induced amnesia in mice. Mice were given CXM in combination with ritanserin immediately after training. 5-MeODMT or 8-OH-DPAT was also given simultaneously. The retention test was performed 24 hr after training. Each value is the median of 15–30 animals. $^{*}p<0.05$, $^{**}p<0.01$ compared with vehicle group. $^{a}p<0.05$, $^{aa}p<0.01$ compared with CXM group.



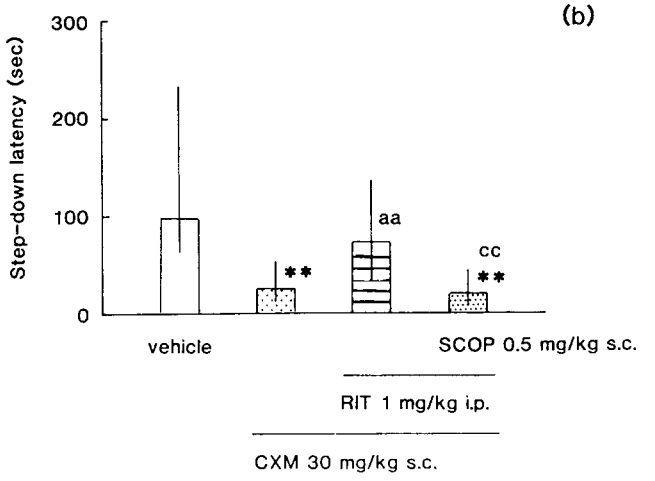


FIG. 5. Effects of scopolamine (SCOP) on methysergide (MET)- and ritanserin (RIT)-induced recovery from CXM-induced amnesia in mice. Mice were given CXM in combination with methysergide (a) or ritanserin (b) immediately after training. Scopolamine was also given simultaneously. The retention test was performed 24 hr after training. Each value is the median of 15–30 animals. $^{**}p<0.01$ compared with vehicle group. $^{a}p<0.05$, $^{aa}p<0.01$ compared with CXM group. $^{b}p<0.05$ compared with CXM + methysergide group. $^{cc}p<0.01$ compared with CXM + ritanserin group.

prolonged step-down latencies in the retention test (Fig. 1 a,b), while the step-down latencies in nonshocked animals did not change between training and the retention test (data not shown). CXM (30 mg/kg SC) significantly decreased the step-down latencies in the retention test (Fig. 1 a,b). Methysergide (5 mg/kg) and ritanserin (1 and 2 mg/kg) significantly improved CXM (30 mg/kg SC)-induced disruption of the memory (Fig. 1 a,b). These effects were a bell-shaped manner. (±)-Pindolol (10 and 20 mg/kg) had no antiamnesic effect (Fig. 2), although (±)-pindolol (10 mg/kg) significantly suppressed the 5-MeODMT- and PCP-induced head weaving by interacting with 5-HT receptors (19).

Effects of 5-HT, 5-MeODMT and 8-OH-DPAT on ritanserin-induced recovery from CXM-induced amnesia in mice.

5-HT (1 and 2.5 μg/mouse, ICV) significantly antagonized the antiamnesic effect of ritanserin on cycloheximide-induced amnesia (Fig. 3). 5-MeODMT (4 and 8 mg/kg) and 8-OH-DPAT (0.5 and 0.75 mg/kg) failed to attenuate ritanserin-induced recovery from CXM-induced amnesia (Fig. 4). The dose ranges of 5-MeODMT and 8-OH-DPAT at which they failed to cause amnesia were used, were administered, since 5-MeODMT (15 mg/kg) and 8-OH-DPAT (1 mg/kg) themselves caused the memory deficit (data not shown).

Effects of Scopolamine on Methysergide- and Ritanserin-Induced Recovery From CXM-Induced Amnesia in Mice.

The antiamnesic effects of methysergide (5 mg/kg) and ritanserin (1 mg/kg) on CXM-induced memory impairment were significantly attenuated by scopolamine (0.5 mg/kg) (Fig. 5, a,b). This dose level of scopolamine itself did not cause the memory disruption when it was administered immediately after training (data not shown).

DISCUSSION

The serotonergic neuronal system on learning and memory may

be inhibitory in nature. This hypothesis is supported by numerous studies that have shown that enhancement of serotonergic activity impairs learning (3, 13, 21, 25, 27). In the present study, methysergide (nonselective 5-HT antagonist) and ritanserin (selective 5-HT_2 antagonist) (17) significantly attenuated impairment of memory in mice caused by CXM, but (±)-pindolol (5-HT_1 antagonist) (11) was ineffective. Moreover, the antiamnesic effect of ritanserin on CXM-induced amnesia was antagonized by 5-HT, but was not attenuated by 5-MeODMT (nonselective 5-HT agonist) and 8-OH-DPAT (5-HT_{1A} selective agonist) (18) at the dose level which did not cause memory disruption. These results suggest that the activation of 5-HT_2 receptors may be involved in impairment of memory in mice. Day *et al.* have also suggested that serotonergic system is related to CXM-induced amnesia in rats (7). In addition, 5-HT_2 antagonists (pirenperone, ketanserin, methysergide, metergoline and mianserin) produce the enhancement of the memory of a previously learned aversive habit (1). Therefore, 5-HT_2 receptors may play an important role in learning and memory.

Serotonergic neurons from the median raphe nucleus appear to tonically inhibit cholinergic neurons in the cortex, and serotonergic neurons from the dosal raphe nucleus appear to tonically inhibit cholinergic neurons in the hippocampus and cortex (24). [^{3}H]Ketanserin binding to 5-HT receptors increased in the rat cortex following basal forebrain lesions with ibotenic acid (26). Lesions of the nucleus basalis magnocellularis (nBM) markedly decrease [^{3}H]ketanserin binding in rat cortex (23). These results indicate that there are cholinergic-serotonergic interactions in the brain sites which are important to memory process. We found that scopolamine antagonized the antiamnesic effects of methysergide and ritanserin on CXM-induced amnesia in the present study. In addition, physostigmine (0.125 and 0.25 mg/kg) significantly attenuates CXM-induced amnesia (20). Thus, it is possible that cholinergic and serotonergic systems are closely linked together in memory process.

We conclude that 5-HT_2 receptors and cholinergic neuronal system are linked to amnesia in mice.

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