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Low dose citalopram reverses memory impairment and electroconvulsive shock-induced immobilization

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Abstract

Citalopram, a selective serotonin reuptake inhibitor (SSRI), is one of the most widely used antidepressants. Recently, citalopram has been reported to improve working memory in patients with depression, and psychotic symptoms and behavioral disturbances in patients with dementia. However, the possibility of using citalopram in the treatment of cognitive disorders has not received much attention. The present study investigated the effects of citalopram on scopolamine- and Δ^9-tetrahydrocannabinol (THC)-induced impairment of spatial memory using an eight-arm radial maze and electroconvulsive shock (ECS)-induced immobilization (a behavioral model for the disturbance of consciousness). Low dose citalopram reversed both scopolamine- and THC-induced impairment of spatial memory, suppressed ECS-induced immobilization reversed the THC-induced decrease of acetylcholine (ACh) release in the dorsal hippocampus in vivo microdialysis, and enhanced tremors induced by oxotremorine, a muscarinic M_1 receptor agonist. Taken together these findings suggest that low dose citalopram is useful for the treatment of memory deficits and consciousness disturbance.

Keywords: Acetylcholine; Citalopram; Cannabinoids; Hippocampus; Maze; Scopolamine; Spatial memory; Electroconvulsive shock

1. Introduction

Citalopram, a selective serotonin reuptake inhibitor (SSRI), has been widely used in several disorders related to serotonergic dysfunction, including depression, anxiety, panic disorders, obsessive–compulsive disorder and premenstrual dysphoria (Masand and Gupta, 1999; Pollock, 2001). Citalopram exerts its clinical effects by inhibiting the uptake of serotonin (5-HT) from the synaptic cleft. It has been demonstrated by microdialysis that acute administration of citalopram raises the extracellular concentration of 5-HT in the rat dorsal and ventral hippocampus (Hjorth, 1993; Hjorth et al., 1997; Invernizzi et al., 1995). Also, local perfusion of citalopram increases acetylcholine (ACh) release in the rat frontal cortex and dorsal hippocampus (Consolo et al., 1994; Yamaguchi et al., 1997). Recently, citalopram has been reported to improve working memory in patients with depression (Zobel et al., 2004) and psychotic symptoms and behavioral disturbances in patients with dementia (Pollock et al., 2002). Furthermore, acute administration of citalopram facilitates memory consolidation in healthy volunteers (Harmer et al., 2002). Notwithstanding these results, the possibility of using citalopram in the treatment of cognitive disorders has not received much attention.

Memory impairment is a cardinal symptom of Alzheimer's disease (AD), and thought to be secondary, at least to some degree, to central cholinergic neuropathology (Bartus et al., 1982; Coyle et al., 1983). The cholinesterase inhibitors donepezil and tetrahydroaminoacridine have been shown to improve memory disorders in AD patients (Rogers et al., 1998;

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Summers et al., 1986). Scopolamine, a non-selective muscarinic receptor antagonist, has been used to induce memory disturbance in experimental animal models (Egashira et al., 2001; Hatip-Al-Khatib et al., 2004; Smith, 1988).

Delta^9-tetrahydrocannabinol (THC), a principal psychoactive component of marijuana, is known to impair learning and memory in humans (Ameri, 1999; D'Souza et al., 2004; Heishman et al., 1997; Hollister, 1986; Miller and Branconnier, 1983;). THC also impairs spatial memory in rats (Lichtman and Martin, 1996; Mallet and Beninger, 1998). We previously reported that THC-induced impairment of spatial memory is likely to be associated with dysfunction of the cholinergic and serotonergic systems (Egashira et al., 2002b; Mishima et al., 2002).

Disturbance of consciousness is an important symptom of cerebrovascular disorders and brain injury. We previously reported that electroconvulsive shock (ECS)-induced immobilization is suppressed by amantadine (Egashira et al., 2001), which has been used clinically to treat disturbance of consciousness (Zafonte et al., 1998). The immobilized state is induced by light ECS-treatment. Such ECS-induced immobilization is easily inhibited by air blowing, sound and tactile stimuli. Thus, ECS-induced immobilization is markedly different from catalepsy, which is induced by large doses of major tranquilizers. This immobilized state may be thought to represent a decreased level of consciousness. Therefore, ECS-induced immobilization is considered to be a model of consciousness disturbance.

In a recent study, we found that a very low dose of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, reversed impairment of spatial memory induced by THC in an eight-arm radial maze (Inui et al., 2004). 8-OH-DPAT at the same dose also reversed the THC-induced decrease of ACh release in the dorsal hippocampus in vivo microdialysis. Therefore, low dose 8-OH-DPAT is thought to reverse THC-induced impairment of spatial memory by enhancing ACh release in the dorsal hippocampus. Moreover, low doses of 8-OH-DPAT have been reported to prevent the impairment of spatial learning caused by intrahippocampal scopolamine through 5-HT$_{1A}$ receptors in the dorsal raphe (Carli et al., 2000). However, the effect of citalopram on impairment of spatial memory remains unexplored. Accordingly, the present study was conducted to investigate the effects of citalopram on scopolamine- and THC-induced impairment of spatial memory in the eight-arm radial maze in rats. We also examined the effect of citalopram on ECS-induced immobilization in rats. Furthermore, we examined the effect of citalopram on THC-induced decrease of ACh release in the dorsal hippocampus in rats. In addition, we examined the effect of citalopram on oxotremorine-induced tremors in mice, to investigate the involvement of cholinergic neurons.

2. Materials and methods

2.1. Animals

Male Wistar rats, aged 7 weeks and weighing 200–250 g, and male ddY mice, aged 4 weeks and weighing 20–25 g, were obtained from Kyudo (Saga, Japan). They were housed in groups of four to five per cage for rats, and eight to 10 per cage for mice, in a room with a controlled temperature of 23±2 °C, relative humidity of 60±10% and the lights on from 07.00 to 19.00 h. The animals scheduled to undergo the eight-arm radial maze task were placed under a restricted food intake (10–12 g daily, CE-2; Clea Japan, Tokyo, Japan) and maintained at approximately 80% of the body weight they had under free-feeding conditions during the experimental period. All animals had free access to drinking water in their home cages. All procedures regarding animal care and use were carried out at the Facilities for Experimental Animals, based on the regulations established by the Experimental Animal Care and Use Committee at Fukuoka University, Japan.

2.2. Drugs

THC was isolated from cannabis by Professor Y. Shoyama (Department of Medicinal Resources Regulation, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan). Citalopram hydrobromide was a generous gift from Zeria Pharmaceutical (Tokyo, Japan). Scopolamine hydrobromide and oxotremorine sesquifumarate were purchased from Sigma-Aldrich (St. Louis, MO, USA). THC was emulsified in 1% Tween 80 solution. Scopolamine and oxotremorine were dissolved in 0.9% saline solution. Citalopram was dissolved in distilled water.

2.3. Eight-arm radial maze test

We investigated the effects of citalopram on scopolamine- and THC-induced impairment of spatial memory in the eight-arm radial maze in rats. Behavioral testing was conducted as previously reported (Egashira et al., 2002b), using an eight-arm radial maze (Neuroscience Co., Tokyo, Japan) that was a modified version of the original maze developed by Olton and Samuelson (1976). The maze was elevated 50 cm from the floor. It consisted of a central platform 24 cm in diameter, with eight arms extending radially. Each arm was 50 cm long, 10 cm wide and 50 cm high with transparent plastic sides. Food cups for the reinforcers were placed near the end of each arm. The maze was located in a room containing many visual cues external to the maze. For behavioral analysis, an image motion analyzer, AXIS-30 (Neuroscience, Tokyo, Japan), was used to quantify the task performance of rats in the eight-arm radial maze. The high-speed analyzer had an automatic tracking system which allowed the movement of each rat to be tracked in the maze with a CCD camera equipped with a personal computer. A group of animals was trained, so that they would become habituated to the apparatus and food pellets, for 3 days before each test. A 10-min period of habituation was repeated three times a day, at intervals of more than 1 h. In each training session, the animal was placed within a circular plastic cage on the platform in the middle of the eight-arm radial maze. After 1 min, the cage was lifted and the animal was allowed to move freely in the maze. The trial continued until the animal had either entered all eight arms or 10 min had elapsed. Animals that proceeded through the

maze using non-spatial strategies, that is, repeatedly choosing the arm adjacent to (45°) or three arms away from (135°) the one currently visited, were excluded from the present experiment because they were thought not to have acquired spatial memory. The performance of the animal in each trial was assessed using three parameters: (1) the number of correct choices in the initial eight chosen arms, (2) the number of errors, which were defined as choosing arms which had already been visited, and (3) the time elapsed before the animal ate all eight pellets. The acquisition of spatial memory was defined as the ability to reach at least seven different arms in the first eight choices and all eight within the first nine choices for three consecutive trials. All animals met the above criterion within 21 days.

If a test animal met the above criteria, scopolamine (0.5 mg/kg) or THC (6 mg/kg) was administered intraperitoneally (i.p.) 30 or 60 min prior to the test. And, we selected the rats, which are impaired spatial memory by scopolamine or THC. After three days, all rats were retrained until they met the above criteria since we check whether the rats recovered or not. When the rats met the criterion, they were injected with scopolamine or THC and citalopram the following day. Citalopram was administered orally (p.o.) 60 min before the test.

2.4. ECS-induced immobilization test

We examined the effect of citalopram on ECS-induced immobilization in rats. The ECS-induced immobilization test was performed as previously described (Egashira et al., 2001). ECS (90 mA, 60 Hz, 0.2 s) was administered via saline-moistened earclip electrodes using an E.C. Stimulator; model MK-80 (Neuroscience, Tokyo, Japan). A mild clonic convulsion was observed in each rat receiving ECS. After 15 min they were removed and allowed to put up both forelimbs over a cork (height, 12 cm; diameter, 2.5 cm), which was placed in the middle of a Hall's open-field apparatus (bottom diameter, 30 cm). This unnatural stretched posture was considered to be ECS-induced immobilization. The duration of immobilization was measured for a 60-s observation period. Citalopram was administered p.o. 60 min prior to ECS-treatment.

2.5. Measurement of oxotremorine-induced tremors

We examined the effect of citalopram on tremors induced by oxotremorine, a muscarinic M_1 receptor agonist. Tremors were induced by oxotremorine as previously described (Egashira et al., 2003). Mice were used for the investigation of the effects of citalopram on oxotremorine-induced tremors. Immediately after an intraperitoneal injection of oxotremorine 0.3 mg/kg, each mouse was placed into a plastic container (10 × 30 × 30 cm), and the intensity of the tremors was recorded according to the following scores: 0, no abnormal behavior observed; 1, intermittent slight tremors; 2, occasional moderate tremors as well as intermittent slight tremors; 3, persistent moderate tremors; and 4, persistent severe tremors. Citalopram was administered orally 50 min before the injection of oxotremorine. Observation of the tremors was made at 5-min intervals starting 30 min after the oxotremorine treatment, and the intensity of the tremors was determined as the total score for 10–20 min.

2.6. Brain microdialysis for ACh release

We examined the effect of citalopram on THC-induced decrease of ACh release in the dorsal hippocampus in rats. Brain microdialysis was performed as previously described (Mishima et al., 2002). Only animals that completed the eight-arm radial maze were stereotaxically implanted with a guide cannula (AG-8; Eicom, Kyoto, Japan) under pentobarbital anesthesia (40 mg/kg, i.p.; Tokyo Kasei, Tokyo, Japan). The guide cannula was placed in the dorsal hippocampus (A: −3.8, L: 2.0, V: 2.0 mm from the bregma) according to the atlas of Paxinos and Watson (1998). The implanted cannula was then secured with dummy cannula kept in place by a cap. After surgery, each rat was injected with penicillin in hindquarter muscle (100,000 U) and housed singly after operation. The extracellular of ACh was measured in the dorsal hippocampus by microdialysis technique, three days after surgery, in unanesthetized freely moving rats. A microdialysis probe (A-UI-8-02; 2 mm dialysis membrane, Eicom, Kyoto, Japan) was perfused with Ringer's solution containing 0.1 mM eserine sulfate (Sigma-Aldrich, St Louis, MO, USA) at a flow rate of 1 μl/min by means of a syringe pump (CMA/100; Carnegie Medicine, Stockholm, Sweden). Samples (20 μl) were collected at 20-min intervals over a 40-min period (baseline) before THC (6 mg/kg, i.p.) and citalopram (0.01 mg/kg, p.o.) administration and over a 60–100-min period (time of behavioral test) after drug treatment. To achieve stable baseline readings, microdialysis was allowed to proceed for 120 min before the collection of fractions. The sampled ACh concentrations were then measured by a high-performance liquid chromatography-electrochemical detector system (Waters, Milford, MA, USA), utilizing an EicomPak AC column and enzyme column (EICOMPAK AC-GEL, Eicom, Kyoto, Japan). Samples were quantified by calculating the area under the curves using an integrator (Waters Model 730, Waters, Milford, MA, USA) and ACh concentration was then determined using an internal standard. The data were expressed as percentages of the baseline concentration.

2.7. Histology

After completion of the microdialysis experiment, the animals were anesthetized with ether and decapitated. Brains were removed, frozen and cut into 40-μm slices. The position of the guide cannula in the dorsal hippocampus site was confirmed by microscopic examination. Only data from animals in which the implantation was made at the desired site were analyzed.

2.8. Statistical analysis

Findings for the eight-arm radial maze task and ECS-induced immobilization test were evaluated for statistical significance using a one-way analysis of variance (ANOVA) followed by the Bonferroni test. ACh release and oxotremorine-induced tremors

were analyzed using one-way ANOVA followed by the Student–Newman–Keuls post hoc test. The criterion for statistical significance was considered to be $P<0.05$. Values were expressed as the mean±S.E.M.

3. Results

3.1. Effect of citalopram on scopolamine-induced impairment of spatial memory

Fig. 1 shows the effect of citalopram on scopolamine-induced impairment of spatial memory in the eight-arm radial maze. Scopolamine (0.5 mg/kg, i.p.) significantly reduced the number of correct choices and also increased the number of errors ($P<0.01$ by the Bonferroni test), indicating impairment of spatial memory. Citalopram at low doses significantly reversed scopolamine-induced impairment of spatial memory (correct choices: ($F(4,62)=22.413$, $P<0.001$ and errors: ($F(4,62)=18.230$, $P<0.001$ by one-way ANOVA). The number of correct choices was significantly increased at doses of 0.1 and 0.2 mg/kg (0.1 mg/kg, $P<0.05$; 0.2 mg/kg, $P<0.01$ by the Bonferroni test), while the number of errors decreased at doses of 0.05 and 0.2 mg/kg (0.05 mg/kg, $P<0.05$; 0.2 mg/kg, $P<0.01$ by the Bonferroni test). Furthermore, scopolamine significantly increased the running time in the eight-arm radial maze (vehicle, 65.7±4.7 s; scopolamine, 180.3±25.1 s, $P<0.01$ by the Bonferroni test), and citalopram 0.05 mg/kg significantly decreased this effect (0.05 mg/kg, 83.6±5.6 s, $P<0.01$ by the Bonferroni test). In addition, citalopram (0.2 mg/kg) alone had no effect on spatial memory in non-scopolamine treated rats (data not shown).

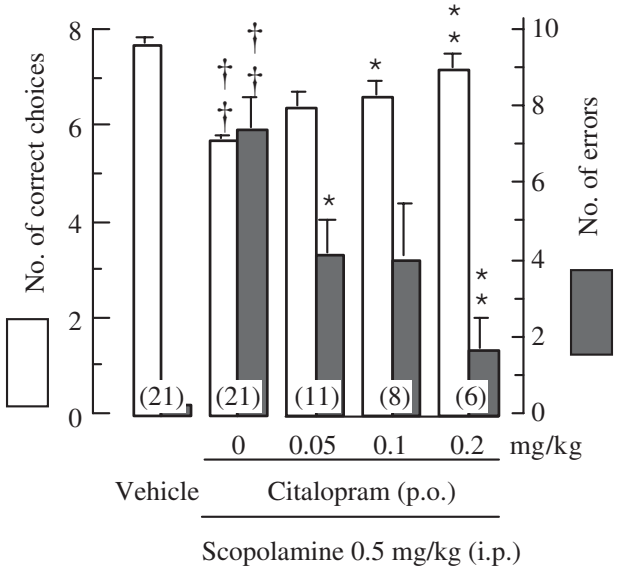

Fig. 1. Effect of citalopram on scopolamine-induced impairment of spatial memory in rats. Scopolamine was injected i.p. 30 min prior to the test. Citalopram was injected p.o. 60 min prior to the test. Values are expressed as the mean±S.E.M. $^{\dagger\dagger}P<0.01$ compared with vehicle, $^{*}P<0.05$, $^{**}P<0.01$ compared with scopolamine alone. The number of animals is shown at the bottom of each column.

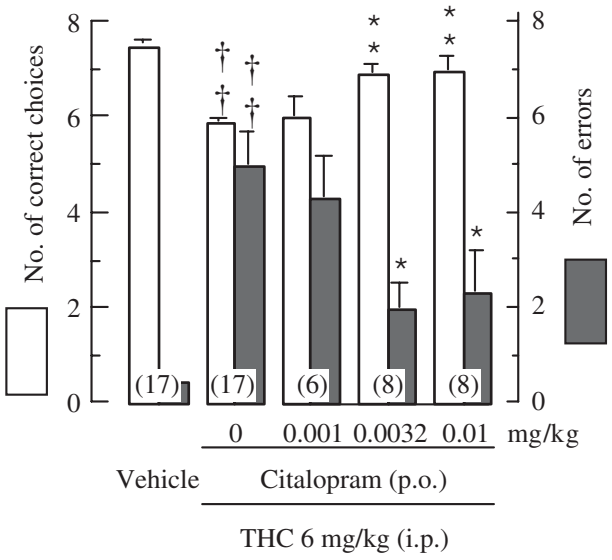

Fig. 2. Effect of citalopram on Δ^9-tetrahydrocannabinol-induced impairment of spatial memory in rats. Delta^9-tetrahydrocannabinol was injected i.p. 60 min prior to the test. Citalopram was injected p.o. 60 min prior to the test. Values are expressed as the mean±S.E.M. $^{\dagger\dagger}P<0.01$ compared with vehicle, $^{*}P<0.05$, $^{**}P<0.01$ compared with Δ^9-tetrahydrocannabinol alone. The number of animals is shown at the bottom of each column.

3.2. Effect of citalopram on THC-induced impairment of spatial memory

THC (6 mg/kg, i.p.) produced a marked impairment of spatial memory and therefore significantly reduced the number of correct choices and increased the number of errors ($P<0.01$ by the Bonferroni test, Fig. 2). Citalopram at very low doses significantly reversed THC-induced impairment of spatial memory (correct choices: ($F(4,51)=18.214$, $P<0.001$ and errors: ($F(4,51)=11.402$, $P<0.001$ by one-way ANOVA). The number of correct choices was significantly increased at doses of 0.0032 and 0.01 mg/kg ($P<0.01$ by the Bonferroni test), while the number of errors decreased at same doses ($P<0.05$ by the Bonferroni test). THC also significantly increased the running time in the eight-arm radial maze (vehicle, 60.2±6 s; THC, 311.5±50.1 s, $P<0.001$ by the Bonferroni test), but citalopram did not significantly decrease this effect (0.0032 mg/kg, 253.9±60.4 s; 0.01 mg/kg, 396.1±99.6 s). In addition, citalopram (0.01 mg/kg) alone had no effect on spatial memory in non-THC treated rats (data not shown).

3.3. Effect of citalopram on ECS-induced immobilization

The duration of immobilization was approximately 70% of the time during the 60-s observation period in vehicle-treated rats. Citalopram 0.05 and 0.1 mg/kg significantly suppressed ECS-induced immobilization ($F(2,46)=7.263$, $P<0.01$ by one-way ANOVA; 0.05 mg/kg, $P<0.05$; 0.1 mg/kg, $P<0.01$ by the Bonferroni test, Fig. 3). In addition, the immobilization was not appeared in non-ECS treated rats (data not shown).

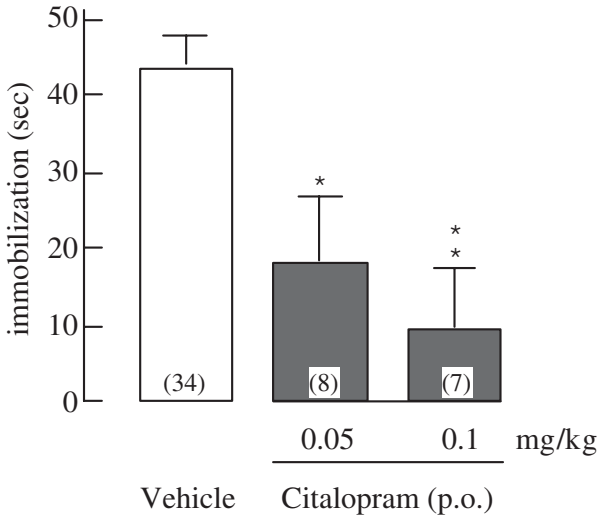

Fig. 3. Effect of citalopram on ECS-induced immobilization in rats. Citalopram was administered p.o. 60 min prior to ECS-treatment. Values are expressed as the mean±S.E.M. *$P<0.05$, **$P<0.01$ compared with vehicle. The number of animals is shown at the bottom of each column.

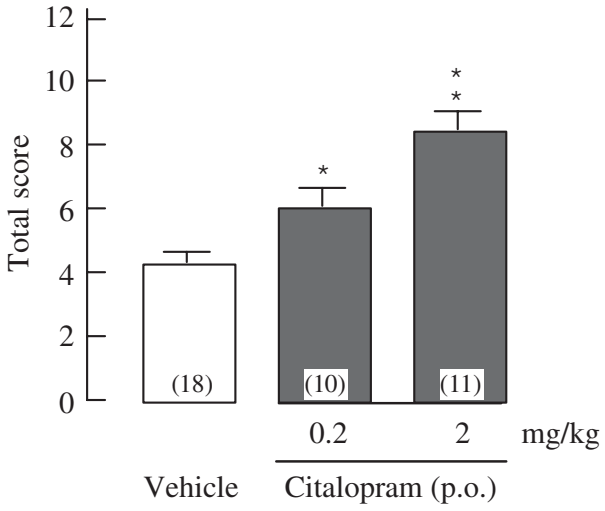

Fig. 5. Effect of citalopram on oxotremorine-induced tremors in mice. Citalopram was administered p.o. 50 min prior to oxotremorine injection. Values are expressed as the mean±S.E.M. *$P<0.05$, **$P<0.01$ compared with vehicle. The number of animals is shown at the bottom of each column.

3.4. Effect of citalopram on THC-induced decrease in ACh release in the dorsal hippocampus

Citalopram at a dose of 0.01 mg/kg, which reversed impairment of spatial memory, reversed the decrease in ACh release induced by THC in the dorsal hippocampus after 60–100 min compared with THC alone ($F(3,34)=4.4$, $P<0.05$ by one-way ANOVA; 0.01 mg/kg, $P<0.05$ by the Student–Newman–Keuls post hoc test, Fig. 4). In addition, the same dose of citalopram alone significantly did not increase ACh release compared with vehicle.

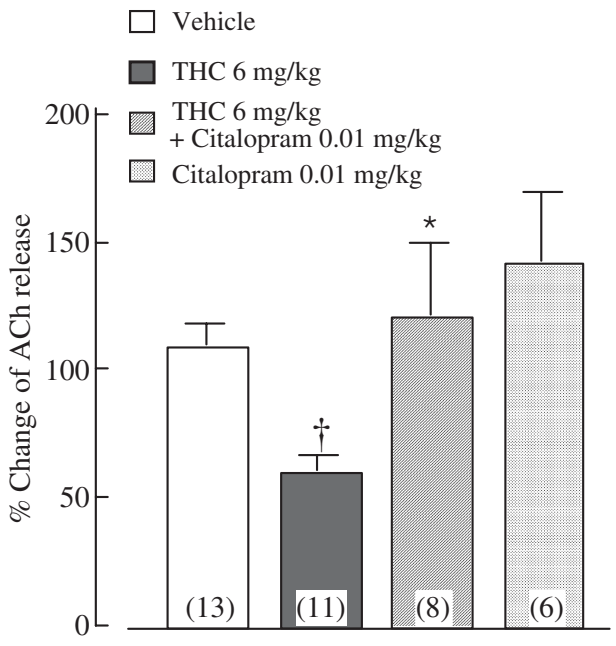

Fig. 4. Effect of citalopram on Δ^9-tetrahydrocannabinol-induced decrease in ACh release in the dorsal hippocampus in rats. Delta^9-tetrahydrocannabinol (6 mg/kg, i.p.) and citalopram (0.01 mg/kg, p.o.) were administered immediately after sampling. Values are expressed as percentages (mean±S.E.M.) of the baseline concentration. $^{\dagger}P<0.05$ compared with vehicle, *$P<0.05$ compared with Δ^9-tetrahydrocannabinol alone. The number of animals is shown at the bottom of each column.

3.5. Effect of citalopram on oxotremorine-induced tremors

Citalopram significantly enhanced oxotremorine-induced tremors ($F(2,36)=15.9$, $P<0.001$ by one-way ANOVA; 0.2 g/kg, $P<0.05$; 2 mg/kg, $P<0.01$ by the Student–Newman–Keuls post hoc test, Fig. 5). In addition, the tremors did not appear in non-oxotremorine treated mice, and citalopram (2 mg/kg) did not cause the tremors in non-oxotremorine treated mice (data not shown).

4. Discussion

In the present study, low dose citalopram reversed scopolamine-induced impairment of spatial memory in the eight-arm radial maze. The cholinesterase inhibitors donepezil and THA have been shown to improve memory disorders in AD patients (Rogers et al., 1998; Summers et al., 1986). These drugs also have beneficial effects on scopolamine-induced memory deficits in rats (Higgins et al., 2002; Murray et al., 1991). We also reported that THA reversed the scopolamine-induced impairment of spatial memory (Egashira et al., 2001). Recently, citalopram has been reported to improve the psychotic symptoms and behavioral disturbances in patients with dementia (Pollock et al., 2002). Furthermore, acute administration of citalopram facilitates memory consolidation in healthy volunteers (Harmer et al., 2002). These findings suggest that citalopram has potential as an enhancer of cognitive function in memory disorders.

We found that citalopram at very low doses reversed THC-induced impairment of spatial memory. Therefore, citalopram may be effective in the treatment of THC-induced memory

deficits. We previously reported that THC-induced impairment of spatial memory was reversed by physostigmine and THA (Mishima et al., 2002). We also found that THC (6 mg/kg, i.p.), which impairs spatial memory, produced a decrease in ACh release in the dorsal hippocampus, and these decreases were reversed by THA. We previously confirmed that microinjection of THC into the dorsal hippocampus impairs spatial memory (Egashira et al., 2002a). More recently, we reported that a very low dose of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, reversed the THC-induced impairment of spatial memory (Inui et al., 2004). Also, 8-OH-DPAT at the same dose reversed the THC-induced decrease of ACh release in the dorsal hippocampus. Thus, THC-induced inhibition of ACh release in the dorsal hippocampus is thought to be involved in impairment of spatial memory, and the enhancement of ACh release could ameliorate this impairment. Citalopram (0.01 mg/kg, p.o.), which reverses THC-induced impairment of spatial memory, reversed the THC-induced decrease of ACh release in the dorsal hippocampus in the present study.

These findings suggest that low dose citalopram reverses THC-induced impairment of spatial memory by enhancing ACh release in the dorsal hippocampus. Consolo et al. (1994) reported that citalopram facilitates in vivo release of ACh from the dorsal hippocampus by increasing synaptic 5-HT levels, and this facilitation is mediated by 5-HT_3 receptors located in this area. However, we found that citalopram had no effect on the 5-HT content of the dorsal hippocampus in THC-treated rats (N. Egashira et al., unpublished data). Therefore, it is unlikely that citalopram enhances ACh release in the dorsal hippocampus by increasing 5-HT levels in the 5-HT synaptic cleft. We have previously reported that the 5-HT content of the ventral hippocampus was significantly increased in THC-treated rats (Egashira et al., 2002b). Moreover, THC markedly decreased the extracellular 5-HT concentration in the ventral hippocampus. Therefore, the increase in 5-HT content in the ventral hippocampus in THC-treated rats is thought to result from inhibition of 5-HT release in this area. We also found that citalopram significantly decreased the 5-HT content of the ventral hippocampus in THC-treated rats (N. Egashira et al., unpublished data). These finding suggest that the 5-HT neuronal system is involved in the ameliorative effect of citalopram. Therefore, citalopram may reverse THC-induced impairment of spatial memory by enhancing not only ACh but also 5-HT neuronal system.

A brain-related feature of depression is cognitive dysfunction (Austin et al., 2001). Several studies have found impairments of memory and attention in depression (Hemmeter et al., 2000). Working memory impairment is consistently reported in severe or moderate depression (Austin et al., 2001; Pelosi et al., 2000). Citalopram has been reported to improve working memory in patients with depression (Zobel et al., 2004). A consistent feature of depression is dysfunction of the hypothalamus–pituitary–adrenocortical (HPA) system. An insufficient feedback mechanism of the central corticosteroid receptor is thought to induce hyperactivity of the HPA system (Holsboer, 2000). It has been reported that improvement of memory impairment by citalopram is correlated with HPA system normalization (Zobel et al., 2004). In this study, we found that citalopram reversed memory impairment by enhancing ACh release. Hence, the possibility exists that citalopram improves memory impairment in patients with depression by enhancing ACh release.

In the present study, we found that citalopram suppressed ECS-induced immobilization in a behavioral model of consciousness disturbance (Egashira et al., 2001). Therefore, citalopram may be useful for treating disturbed consciousness. Spingnol and Pepeu (1986) reported a decrease in the hippocampal ACh level, which was still statistically significant at 30 min after ECS application. Furthermore, in a microdialysis study, Zis et al. (1991) showed that a single ECS application increased ACh release in the rat striatum within 10 min, followed by a decrease below baseline value for approximately 30 min. In addition, we have found that THA suppresses ECS-induced immobilization (Egashira et al., 2001). Based on these findings, ECS-induced immobilization was considered to be associated with cholinergic neurons.

In the present study, citalopram significantly potentiated the tremors induced by oxotremorine, a muscarinic M_1 receptor agonist. These tremors are completely antagonized by scopolamine hydrobromide but not by scopolamine methyl bromide, a peripheral muscarinic receptor antagonist (Egashira et al., 2003), indicating that tremor enhancement is induced by increasing central cholinergic activity. Citalopram has been reported to be inactive on the muscarinic M_1 receptors in [^{3}H]-QNB binding studies in rats (Pietra et al., 1990). Therefore, high dose citalopram may potentiate the postsynaptic cholinergic activity by enhancing ACh release. Hence, citalopram may reverse, not only impairment of spatial memory, but also ECS-induced immobilization by enhancing activity of cholinergic and serotonergic neurons. In addition, oxotremorine-induced tremors have also been used as a model of Parkinson's disease (Oliver et al., 1991). In the present study, citalopram dose-dependently enhanced the oxotremorine-induced tremors. Consequently, citalopram, especially at high doses, should not be used in patients with Parkinson's disease.

In conclusion, the present results reveal that low dose citalopram reverses both scopolamine- and THC-induced impairment of spatial memory. Moreover, the present results show that low dose citalopram suppresses ECS-induced immobilization. Therefore, low dose citalopram may be useful for the treatment of memory deficit and disturbance of consciousness.

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