Rapid Antidepressant Effect of Ketamine Anesthesia During Electroconvulsive Therapy of Treatment-Resistant Depression

Comparing Ketamine and Propofol Anesthesia

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Background: Reports of the superiority of the antidepressant effect of ketamine during the conduct of electroconvulsive therapy (ECT) have been limited. We conducted an open-label trial of ketamine to determine whether ketamine as the anesthetic during ECT would provide a greater antidepressant effect than the antidepressant effect obtained with propofol.

Methods: Between April 2006 and April 2007, 31 inpatients with treatment-resistant depression gave written consent for ECT and to participate in this study. An anesthesiologist who was unaware of the mental symptoms of the subjects assigned them to receive propofol or ketamine anesthetic according to the preferences of the patients, and the patients underwent 8 ECT sessions for 4 weeks. The Hamilton Depression Rating Scale (HDRS) was valuated before ECT and after the completion of the second, fourth, sixth, and eighth ECT sessions.

Results: The HDRS scores improved earlier in the ketamine group, with decreases in HDRS scores that were significantly greater in the ketamine group.

Conclusions: The results suggested that it is possible to improve symptoms of depression earlier by using ketamine anesthesia.

Key Words: treatment-resistant depression, antidepressant response, ketamine, electroconvulsive therapy (ECT)

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The Sequenced Treatment Alternatives to Relieve Depression study demonstrated positive outcomes in no more than 30% of the patients even after 4 adequate treatment options had been performed.[1] There are limits to the therapeutic efficacy of the treatment of depression with the current antidepressant agents that enhance serotonin, noradrenaline, or dopamine nerve function. As a result, attention is being focused on glutamate pathway dysfunction as a novel pathophysiology of depression and on the antidepressant effect of the *N*-methyl-D-aspartate (NMDA) receptor antagonists.[2] The results of animal experiments in learned helplessness rats, a traditional model of depression, have suggested that NMDA receptor antagonists may have anxiolytic and antidepressant actions.[3–6] In recent years, it has been reported that lamotrigine[7] and riluzole,[8] which inhibit glutamate release, exhibit an antidepressant effect in humans as well and studies have been reported in which the NMDA receptor antagonist ketamine also exerted an antidepressant effect in humans when a single dose was infused intravenously.[9–10]

The principal action of ketamine is its NMDA receptor antagonist action, and it blocks the calcium-ion influx that occurs when glutamate binds to the NMDA receptor. Ketamine suppresses the cerebral cortex and thalamus and converts the brain waves in the electroencephalogram (EEG) to slow waves, but it is a dissociative anesthetic that exhibits a stimulant action on the cerebral limbic system. Ketamine is covered by the national health insurance system as a general anesthetic in Japan, and there are reports that no significant adverse events have been observed even when used as an anesthetic during electroconvulsive therapy (ECT).[11,12]

There have been few studies on the superiority of the antidepressant effect of ketamine anesthesia during ECT in earlier research, and there have been only a few case reports in which a rapid antidepressant effect of ketamine anesthesia has been inferred when used during ECT. Ostroff et al[13] reported the case of a 47-year-old woman with a depressive state in schizoaffective disorder. The patient was resistant to treatment with numerous antidepressant drugs and mood-stabilizing drugs, and bilateral ECT was pursued by inducing anesthesia with ketamine at 0.5 mg/kg. Electroconvulsive therapy was performed 6 times, and a rapid improvement in her symptoms of depression was observed after completion of the first ECT session. In addition, Goforth et al[14] reported a case in which they observed rapid improvement in symptoms starting with completion of the first ECT session when they performed ECT using ketamine at 1.5 mg/kg intramuscularly in a 54-year-old male patient with psychotic major depression.

However, ECT itself often has a rapid antidepressant effect beginning with the completion of the first session, and it remained unknown whether the rapid antidepressant effect in these case reports was actually an effect of ketamine.

We therefore conducted an open-label trial to determine whether a greater antidepressant effect would be obtained by using ketamine as the anesthetic during the performance of ECT than by using propofol.

MATERIALS AND METHODS

Subjects

We screened 52 patients with treatment-resistant depression who had consented to ECT while inpatients in the Department of

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Psychiatry of the National Center Hospital of Neurology and Psychiatry during the period between April 2006 and April 2007. All patients fulfilled the diagnostic criteria for major depression according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, per the Structured Clinical Interview for DSM-IV Axis I Disorders, and they had failed to respond to at least 2 adequate drug therapies for their current depression episode with a total score of 20 or higher on the 17-item Hamilton Depression Rating Scale (HDRS).

The exclusion criteria for this study were (1) complication by any serious physical disease, such as cardiovascular disease, cerebrovascular disorder, intracranial hypertension, respiratory tract disease, and severe fracture; (2) hypertension, glaucoma, arterial aneurysm, or cerebrovascular malformation; (3) presence of a foreign body, such as a pacemaker, intracranial electrode, and clips; (4) history of seizures; (5) history of substance abuse or dependence, including alcohol abuse; (6) status 4 or 5 evaluated according to the criteria of the American Society of Anesthesiologists; (7) history of serious adverse effects related to anesthetics, for example, allergy; (8) concomitant presence of a mental disorder other than major depression, such as dementia and bipolar disorder; (9) pregnancy; (10) being a minor; and (11) any other reason that the attending physician judged to make performing ECT inappropriate from a therapeutic standpoint.

All of screened patients underwent detailed pre-ECT examinations (psychiatric interview and physical examination, blood examination, chest x-ray, electrocardiography, and brain computed tomography, and if the results of the tests fulfilled any of the exclusion criteria, the patient was excluded from participation in the study).

Fifty-two patients were screened, and 31 of them (16 men and 15 women, aged 32–78 years old) who did not meet any of the exclusion criteria and from whom written consent for the study was obtained participated in this study (Fig. 1).

Electroconvulsive Therapy Administration

An anesthesiologist with no knowledge of the mental symptoms of the subjects assigned them to a propofol anesthesia group (N = 20, 10 men and 10 women) or a ketamine anesthesia group (N = 11, 5 men and 6 women) according to the preference of the patients. The anesthetic consisted of intravenous atropine sulfate (0.25 mg) with either ketamine (0.8 mg/kg at the first ECT session) or propofol (0.8 mg/kg at the first ECT session) by intravenous bolus. The quantity of the anesthetic agent was revised in the subsequent sessions in consideration of the initial anesthetic effect. Succinylcholine (1 mg/kg) was given intravenously as a muscle relaxant after induction of anesthesia. Thymatron System IV (Somatics Inc, Lake Bluff, Ill) was used, and brief pulse ECT was performed twice a week for a total of 8 times. The seizure threshold was determined by the half-age method during the first ECT session. The dose of anesthetic, the stimulation intensity, and the seizure duration on the EEG were recorded for each ECT session.

No changes in oral medication, including antidepressant drugs, were made between before the start of ECT and the completion of the ECT sessions.

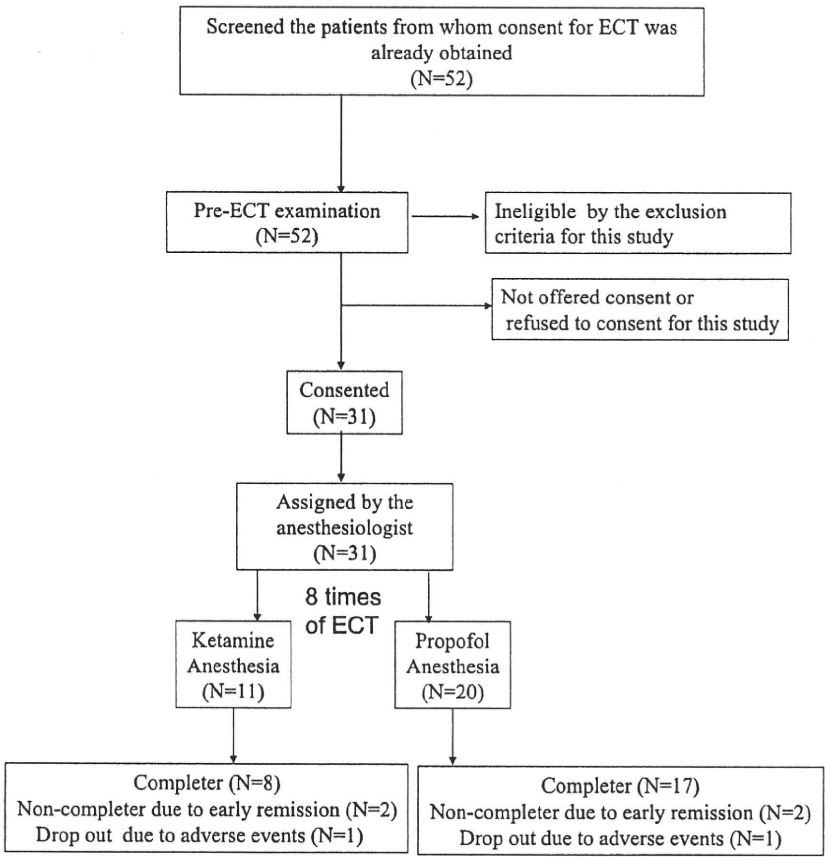

FIGURE 1. Participant flow of this study.

Measures

The 17-item HDRS was used as the primary end point to evaluate improvement in the symptoms of depression, administered by a psychiatrist before the start of ECT and on the day of completion of the second (1 week later), fourth (2 weeks later), sixth (3 weeks later), and eighth sessions (4 weeks later). Adverse events were collected by spontaneous report at every ECT session and during hospitalization.

Statistical Analysis

The statistical analysis was performed with the SPSS 16.0 software (SPSS Inc, Chicago, Ill), and the nonpaired t test and Pearson χ^2 test were used in the baseline analysis. Differences between the 2 groups in mean values for stimulus intensity and seizure duration on the EEG were analyzed for each session by the nonpaired t test. To analyze the differences in antidepressant effect between the 2 groups, we calculated the decrease in total score on the 17-item HDRS (δ HDRS-17) between the baseline and the completion of each ECT session. Then, the differences were analyzed between the mean δ HDRS-17 values in the propofol and ketamine groups after the completion of each ECT session by the nonpaired t test. Adverse events were analyzed by the Pearson χ^2 test.

RESULTS

Baseline Analysis

As shown in Table 1, in the baseline analysis, the mean (SD) age was 59.3 (13.5) years in the ketamine group and 55.1 (15.4) years in the propofol group; the duration of the current depression phase was 2.8 (2.1) years in the ketamine group and 2.7 (2.0) years in the propofol group; the number of previous adequate antidepressant trials was 6.5 (2.7) in the ketamine group and 6.7 (2.2) in the propofol group; there was a history of ECT in 2 patients in the ketamine group and in 3 patients in the propofol group; the δ HDRS-17 was 31.9 (4.5) in the ketamine group and 30.3 (5.4) in the propofol group; and none of the differences between the 2 groups were significant.

The mean anesthetic dose was 0.86 mg/kg (40–60 mg) in the ketamine group and 0.94 mg/kg (34–84 mg) in the propofol group.

TABLE 1. Baseline Characteristics of the Ketamine and Propofol Groups

	Ketamine Group (n = 11)	Propofol Group (n = 20)	P
Male	5 (45%)	10 (50%)	0.893
Age, yr	59.3 (13.5)	55.1 (15.4)	0.469
Duration of current depressive episode, yr	2.8 (2.1)	2.7 (2.0)	0.982
No. failures of adequate therapy	6.5 (2.7)	6.7 (2.2)	0.843
Positive history of ECT in past episodes	2 (18%)	3 (15%)	0.187
δ HDRS-17 before the ECT session	31.9 (4.5)	30.3 (5.4)	0.436
Medication			
SSRI	6 (55%)	10 (50%)	0.809
TCA	5 (45%)	10 (50%)	0.809
AAP	3 (27%)	6 (30%)	0.873
BZP	4 (36%)	4 (20%)	0.319

AAP indicates atypical antipsychotic; BZP, benzodiazepine; SSRI, serotonin-selective reuptake inhibitor; TCA, tricyclic antidepressant.

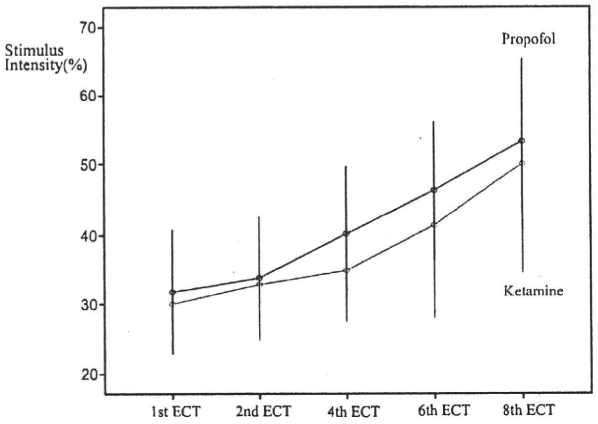

FIGURE 2. Stimulus intensity (in percent) in each ECT session (sessions 1, 2, 4, 6, and 8).

Noncompleters and Dropouts

Because depressive symptoms remitted completely with the second or third ECT session, ECT was completed in 6 sessions in 2 patients in the propofol group and 2 patients in the ketamine group. These patients were included in the statistical analysis of antidepressant effect. Two patients dropped out, 1 in the propofol group because ECT was discontinued after 2 sessions because of strong delirium and another in the ketamine group because the patient strongly complained of sense of fears with hallucinations upon awakening from anesthesia, and the anesthetic was switched to thiopental. Both dropouts were included in the analysis of adverse events, but they were excluded from the analysis of antidepressant effect (Fig. 1).

Stimulus Intensity and Seizure Duration on the EEG

Gradual increases in stimulus intensity were necessary in both groups as the number of ECT sessions increased. Stimulus intensity tended to be lower in the ketamine group in the series of sessions as a whole, but no significant differences were observed according to the nonpaired t test at any of the points (Fig. 2).

Seizure duration on the EEG tended to be longer in the ketamine group in the series of sessions as a whole, but the results of the analyses by the nonpaired t test showed significant differences only in the first (P = 0.015) and sixth sessions

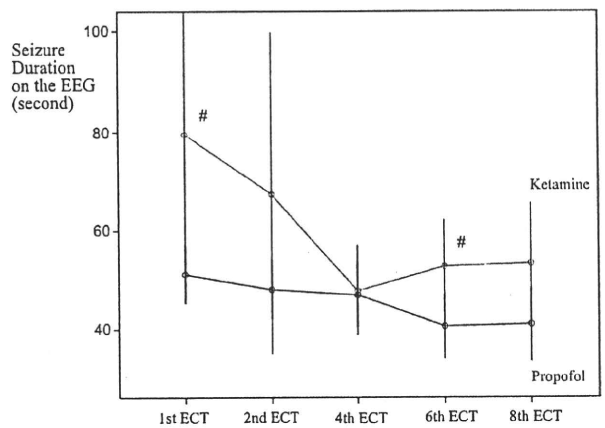

FIGURE 3. Seizure duration on the EEG (second) during each ECT session (sessions 1, 2, 4, 6, and 8).

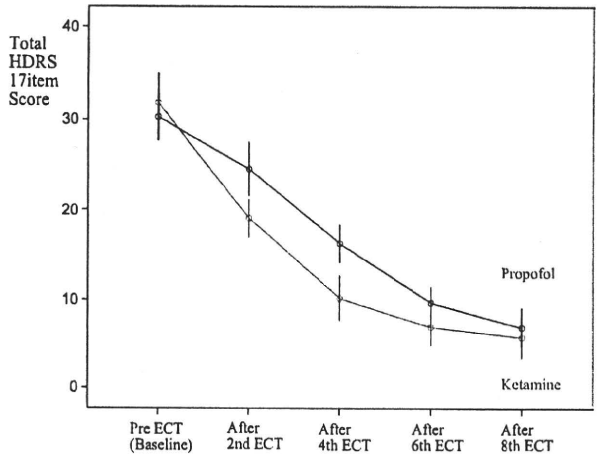

FIGURE 4. Total score on the 17-item HDRS before the start of ECT (pre-ECT) and after sessions 2, 4, 6, and 8.

($P = 0.027$; # in Fig. 3). The differences in the second, fourth, and eighth sessions were not significant (Fig. 3).

Antidepressant Effect

Decreases in the δ HDRS-17 were observed in both groups while the number of ECT sessions increased (Fig. 4).

The decrease in the δ HDRS-17 was significantly greater in the ketamine group than in the propofol group after the completion of the second ($P = 0.000$) and fourth sessions ($P = 0.000$; # in Fig. 5), but the differences after the completion of the sixth ($P = 0.086$) and eighth sessions ($P = 0.360$) were not significant (Fig. 5).

Adverse Events

The adverse events in the ketamine and propofol groups were headache (36% vs 40%), nausea (9% vs 15%), angialgia at the site of injection of the anesthetic (0% vs 45%), hypertension during the ECT session (55% vs 20%), sense of fears with hallucinations upon awakening from anesthesia (27% vs 0%), brief delirium within 1 hour after awakening (9% vs 15%), and prolonged delirium longer than 1 hour (0% vs 5%; Table 2).

Angialgia was significantly more common in the propofol group ($\chi^2_1 = 6.975$, $P = 0.008$), and intrainterventional hypertension ($\chi^2_1 = 3.876$, $P = 0.049$) and sense of fears with hallucinations upon awakening from anesthesia ($\chi^2_1 = 6.039$, $P = 0.014$) were more common in the ketamine group, but there were no serious adverse effects in either group.

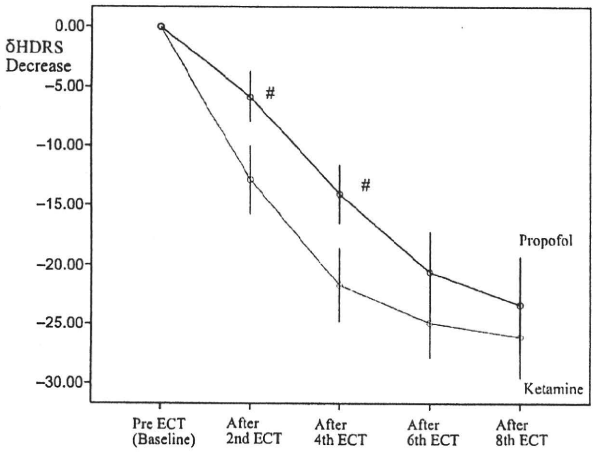

FIGURE 5. Change in δ HDRS-17 between the baseline (pre-ECT) and after sessions 2, 4, 6, and 8.

TABLE 2. Adverse Events

	Ketamine Group (n = 11), n (%)	Propofol Group (n = 20), n (%)	*P*
Headache	4 (36)	8 (40)	0.842
Nausea	1 (9)	3 (15)	0.639
Angialgia	0 (0)	9 (45)	0.008
Hypertension during ECT	6 (55)	4 (20)	0.049
Sense of fear with hallucinations upon awakening from anesthesia	3 (27)	0 (0)	0.014
Brief delirium (within 1 h)	1 (9)	3 (15)	0.639
Prolonged delirium (longer than 1 h)	0 (0)	1 (5)	0.451

DISCUSSION

According to the report of Berman et al[9] of a ketamine-versus-saline, placebo-controlled, double-blind, single-dose study in depressed patients, a significant decrease in the depression scale score was observed within 3 hours in the group of 9 treatment-resistant patients who were infused with ketamine at 0.5 mg/kg intravenously, and the decrease persisted for 72 hours. In 2006, a placebo-controlled double-blind crossover study was conducted in 18 nonpsychotic recurrent–major depression inpatients who were resistant to 2 adequate antidepressant drugs.[10] A significant improvement in depression was observed in the ketamine group within 2 hours, and the difference was maintained for an entire week. Moreover, 71% of the 17 subjects who received a single dose of ketamine responded, and 29% fulfilled the remission criteria. The adverse events in this study, that is, perceptual disturbance, confusion, elevation of blood pressure, euphoria, dizziness, and increased libido, were found to be more common in the ketamine group than in the placebo group, but no serious adverse effects were observed. These studies showed that ketamine has a clinical antidepressant effect when administered in a single dose, but until now, there has been no clinical research showing its superiority from the standpoint of an antidepressant action during ECT anesthesia.

On the other hand, ketamine is a general anesthetic that has been routinely used, and it has long been used as an anesthetic during the conduct of ECT.[12]

In addition, possessing a seizure-inducing action and increasing seizure duration are known as distinctive properties of ketamine as an ECT anesthetic. Nonbarbiturate anesthetics, including propofol, and barbiturate anesthetics, including thiopental, which are commonly used as ECT anesthetics, have an anticonvulsant action, and sometimes seizure induction is inadequate. According to a report on 471 patients who underwent ECT with methohexital, 72 (15%) required the maximal stimulation intensity, but in 24 (33%) of the 72 patients, seizure duration was insufficient or no seizure occurred at all even at the maximal stimulation intensity.[15] Switching to ketamine anesthesia has been found to be a useful method as a seizure induction technique when seizure induction at the maximal stimulation intensity is inadequate during ECT for which an

anesthetic that has such an anticonvulsant action has been used for anesthesia. There is a report of a switch to ketamine in 36 patients in whom seizure induction was inadequate or who were intolerant at the maximal stimulation intensity with methohexital, and seizure duration increased in 83% without any significant adverse effects.[16]

Moreover, in recent years, attention has been focused on the possibility of a cognitive function–preserving action by ketamine anesthesia during the conduct of ECT. The possibility that it suppresses excitotoxicity and has a neuroprotective action in relation to hippocampal synaptic plasticity and so on has been shown in an ECT rat model of ketamine anesthesia.[17] A cognitive function–preserving action during ECT in humans has also been reported in a small group of subjects,[18] and it has been suggested that ketamine anesthesia may reduce the cognitive impairment caused by ECT.[19]

Thus, despite the advantage of the antidepressant action that ketamine itself possesses and the advantages of its seizure-inducing action and cognitive function–preserving action during ECT, little attention has ever been paid to the superiority of the antidepressant effect of ketamine in ECT.

Our results showed that until the completion of the fourth ECT session, the δ HDRS-17 was statistically significantly higher in the ketamine group than in the propofol group. This finding suggests that ketamine anesthesia has an early antidepressant effect during ECT that was superior to propofol anesthesia and the effect may come from the antidepressant effect of ketamine itself.

However, the results of the statistical analysis showed that from the sixth ECT session onward, the antidepressant effect in the propofol group had caught up to the antidepressant effect in the ketamine group. That seems to have been due to the influence of the antidepressant effect of ECT itself being sufficiently expressed in both groups while the number of ECT session increased and the differences between the δ HDRS-17 in the 2 groups become smaller.

Because of this, ketamine may be useful when an early antidepressant effect is needed clinically in severe cases in which, for example, a suicide attempt is imminent clinically.

No serious adverse events were observed in either group in this study, but hypertension and sense of fears with hallucinations upon awakening from anesthesia were significantly more common in the ketamine group. Especially because of hypertension, some sort of arrangement, such as using an appropriate antihypertensive agent, seems necessary during the delicate anesthesia management of ECT.

Furthermore, in this study, there was not the case that showed a dependence of ketamine clinically after ECT session and serious psychedelic effect induced by ketamine, but we should pay enough attention to the dependence and the psychedelic properties of ketamine when we use it as an anesthetic agent of ECT.

The limitations of this study were that it was an open trial and the number of subjects was small. A large-scale double-blind trial is anticipated in the future to verify the superiority of the antidepressant effect of ketamine anesthesia in ECT.

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Pramipexole for stage 2 treatment-resistant major depression: An open study

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ABSTRACT

Objective: To examine the effectiveness and safety of adjunctive pramipexole in the treatment of stage 2 treatment-resistant major depressive disorder.

Methods: This study included patients with moderate or non-psychotic severe major depressive disorder according to DSM-IV-TR criteria despite at least two adequate treatment trials with antidepressants from different pharmacological classes. Pramipexole 0.25 to 2 mg daily was added to antidepressant therapy. Previous treatments were continued unchanged, but no new treatments were allowed. We conducted assessments at baseline and at weeks 2, 4, 6, and 8. We defined response as a 50% or greater reduction on the Montgomery–Åsberg Depression Rating Scale (MADRS).

Results: Ten patients (4 men, 6 women) aged 43.7 ± 11.4 years received pramipexole at mean dose of 1.3 ± 0.6 mg/d. Mean MADRS scores improved significantly from baseline to endpoint (mean differences = 11.4, 95% CI [4.1, 18.7], $P = 0.0064$). At the endpoint, six of 10 (60%) were responders on MADRS (≥50% reduction). Two patients (20%) terminated early due to mild somatic and psychiatric adverse effects.

Conclusion: These preliminary data suggest that the addition of pramipexole to antidepressant treatment may be effective and well tolerated in patients with stage 2 treatment-resistant major depressive disorder.

1. Introduction

Treatment-resistant major depression is a major issue in clinical practice, and the search for new, more effective treatments is ongoing. In general, treatment-resistant major depression is defined as the persistence of significant or moderate depressive symptoms despite at least two treatment trials with antidepressants from different pharmacological classes [stage 2 major depression according to the staging of depression based on prior treatment response proposed by Thase and Rush (1995)]. Each prior treatment must have been used in an adequate dose for an adequate period (i.e., a minimum of the equivalent of 150 mg of imipramine for 4 weeks) (Thase and Rush, 1995). The prevalence of treatment-resistant major depression is estimated to be 5–10% among all patients with major depression (Inoue et al., 2002). Nevertheless, most studies have investigated non-responders to single antidepressant trials [stage 1 major depression by Thase and Rush (1995)] and defined these patients as having treatment-resistant major depression (Thase and Rush, 1995). Furthermore, because of short treatment periods and small doses of antidepressants in several studies, there has been little evidence regarding effective therapy for stage 2 treatment-resistant major depression (Stimpson et al., 2002). Electroconvulsive therapy, lithium augmentation, and thyroid augmentation are recommended as treatment options in the World Federation of Societies of Biological Psychiatry Guidelines for Biological Treatment of Unipolar Depressive Disorders (Bauer et al., 2002), and have some evidence for stage 1 depression, but unexpectedly, little evidence for stage 2 treatment-resistant major depression has been noted (Stimpson et al., 2002). It is noteworthy that growing evidence for the treatment of stage 2 treatment-resistant major depression has shown clinical efficacy for atypical antipsychotic drugs (olanzapine and aripiprazole) as adjunctive therapy in large-scale randomized clinical trials (Thase et al., 2007; Marcus et al., 2008). Moreover, adjunctive repetitive transcranial magnetic stimulation was shown to be effective for stage 2 major depression in a small randomized clinical trial (Fitzgerald et al., 2006), whereas monotherapy with repetitive transcranial magnetic stimulation has been proven effective only for stage 1 major depression (O'Reardon et al., 2007).

A growing number of studies report abnormalities in the dopaminergic system in major depression, and the efficacy of pro-dopaminergic drugs, including dopamine receptor agonists, for major depression have been reported (Papakostas, 2006). The idea that major depression resistant to treatment with multiple serotonergic- and noradrenergic-based antidepressants may be responsive to pro-dopaminergic drugs is rational in terms of the mechanism of action of

Abbreviations: MADRS, Montgomery-Åsberg Depression Rating Scale; GAF, Global Assessment of Functioning; HDRS, Hamilton Depression Rating Scale; LOCF, last-observation-carried forward; CI, confidence interval; SSRI, selective serotonin reuptake inhibitor; DSM-IV-TR, Diagnostic and statistical manual of mental disorders, 4th edition, text revision.

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these drugs. Several studies have reported that dopamine receptor agonists (bromocriptine, pergolide, pramipexole, and ropinirole) are effective for stage 1 major depression that fails to respond to at least a single adequate conventional antidepressant treatment trial (Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). As mentioned above, the clinical efficacy of dopamine receptor agonists for stage 2 treatment-resistant major depression has not been assessed.

This pilot prospective, open study was undertaken to investigate the efficacy and safety of pramipexole in patients with stage 2 treatment-resistant major depression.

2. Methods

2.1. Subjects

We conducted an 8-week, open-label trial of pramipexole for patients with stage 2 treatment-resistant major depression at the Department of Psychiatry, Hokkaido University Hospital, Sapporo, Japan. The inclusion period started in June 2005 and ended in October 2008.

We included patients of both sexes, aged 20 to 70 years, with a diagnosis of moderate or non-psychotic severe major depressive disorder according to DSM-IV-TR criteria despite at least two treatment trials with antidepressants from different pharmacological classes, each used in an adequate dose for an adequate time period (i.e., a minimum of the equivalent of 150 mg of imipramine for 4 weeks) (Thase and Rush, 1995). Patients with scores of 20 or greater on the Montgomery–Åsberg Depression Rating Scale (MADRS, 10 items) (Montgomery and Åsberg, 1979) or scores of 60 or less on the Global Assessment of Functioning (GAF) Scale (even if MADRS scores were less than 20) were included. Patients with organic brain syndrome, schizophrenia, bipolar or schizoaffective disorder, severe physical illness, a history of substance use, or marked suicidality were excluded. All subjects provided written informed consent, and the trial was approved by the institutional review board of Hokkaido University Graduate School of Medicine.

2.2. Intervention and measurements

Subjects entering this study were prescribed pramipexole. All took one or two antidepressants, and doses of these drugs were held constant throughout the study. Pramipexole administration was started at 0.125 mg twice daily and increased 0.25–0.5 mg/day every 7 days to a target range of 0.5–2 mg/day. Higher doses (up to 3 mg/day) were permitted as needed. Dose escalations continued until 1) achievement of the primary endpoint (defined as a reduction of 50% or more from baseline in MADRS score), 2) drug intolerance, or 3) the 8-week protocol completion. Dosages were adjusted individually for patients.

Clinical assessments of adverse events and drug compliance were performed at each visit (every day for three inpatients) by trained psychiatrists at baseline and weeks 2, 4, 6, and 8. Outcomes were assessed using the MADRS score, the 17-item Hamilton Depression Rating Scale (HDRS) (Williams, 1988), and the GAF scale. The primary efficacy measure was the MADRS score. Secondary efficacy measures were the 17-item HDRS and GAF scores. Spontaneously reported adverse events were recorded at each visit.

2.3. Data analysis

All analyses were carried out on an intent-to-treat basis. Longitudinal efficacy outcomes (MADRS, HDRS, and GAF) were analyzed using paired *t*-tests comparing baseline and last-observation-carried forward (LOCF) results, with α set at 0.05; all tests were 2-tailed.

The primary outcome was defined as treatment response based on a ≥50% reduction in MADRS score over 8 weeks using LOCF methodology. Remission was defined as MADRS score<10 at the last visit (LOCF).

Secondary outcomes were determined using HDRS and GAF scores. Secondary treatment response was defined as a ≥50% reduction in HDRS score or a 10-point improvement (increase) in GAF score. Functional recovery was defined as GAF score>70 (Haykal and Akiskal, 1999; Furukawa et al., 2001). Changes in scores from baseline to final study visit were calculated for the MADRS, HDRS, and GAF. Pearson's correlation coefficients between GAF changes and MADRS or HDRS changes were calculated to assess potential predictors of functional improvement.

All continuous data are presented as means with standard deviations or 95% confidence intervals (CIs).

3. Results

Clinical and demographic characteristics of subjects are shown in Tables 1 and 2. All patients were diagnosed with non-psychotic major depressive disorder, moderate ($n=9$) or severe ($n=1$) with melancholic features. The mean peak dose of pramipexole was 1.3 mg/day (SD = 0.6). Eight of 10 patients (80%) completed the 8-week trial. Two patients discontinued the trial due to lack of efficacy and adverse events. As shown in Table 2, all 10 patients took one or two concurrent antidepressants.

Six of 10 patients (60%) were judged to be treatment responders based on the MADRS (≥50% reduction). Among the 10 patients, MADRS scores improved statistically significantly from baseline to the primary endpoint (mean difference = 11.4, 95% CI [4.1, 18.7], $P=0.0064$). Six patients achieved a MADRS score<10 at last visit (LOCF), yielding a 60% remission rate. As seen in Fig. 1A, this improvement was seen in week 2 and remained statistically significant throughout the study and at endpoint (LOCF). Eight items on the MADRS showed significant mean changes from baseline to endpoint (LOCF): apparent sadness, 1.90 (95% CI 0.92, 2.88), $P=0.0018$; reported sadness, 1.30 (95% CI 0.47, 2.13), $P=0.0063$; inner tension, 1.30 (95% CI 0.40, 2.20), $P=0.0095$; reduced sleep, 1.40

Table 1
Baseline characteristics of 10 patients with major depressive disorder.

Characteristic	Value
Diagnosis	
Major depressive disorder, single episode, *n* (%)	6 (60)
Major depressive disorder, recurrent, *n* (%)	4 (40)
Sex	
Female, *n* (%); male, *n* (%)	4 (40); 6 (60)
Age at entry, mean ± SD (yr)	43.7 ± 11.4
Range	29 – 64
Marital status	
Married, *n* (%); single, *n* (%)	7 (70); 3 (30)
Employment status	
Employed, *n* (%); unemployed, *n* (%)	7 (70) ; 3 (30)
Education, mean ± SD (yr)	13.7 ± 2.4
Length of current major depressive episode, mean ± SD (yr)	2.3 ± 1.3
Age at onset of first episode, mean ± SD (yr)	39.6 ± 11.5
Depression episodes, life time, *n* (%)	
1 episode	6 (60)
2 episodes	4 (40)
Patients with failed adequate antidepressant trials, *n* (%)	
2 trials	5 (50)
3 trials	3 (30)
4 trials	1 (10)
5 trials	1 (10)
Baseline MADRS score, mean ± SD	23.9 ± 7.0
Baseline HDRS score, mean ± SD	16.4 ± 4.4
Baseline GAF score, mean ± SD	46.2 ± 8.8

MADRS = Montgomery–Åsberg Depression Rating Scale, HDRS = 17-item Hamilton Depression Rating Scale, GAF = Global Assessment of Functioning.

Table 2
Clinical data of 10 patients with treatment-resistant depression treated with pramipexole.

Subjects	Age	Sex	MADRS Response	Previous antidepressant treatment for current episode (maximal dose and duration, weeks [W])
1	52	M	Responder	Milnacipran (150 mg, 8 W), amitriptyline[a] (150 mg, 4 W), clomipramine (150 mg, >8 W), imipramine (150 mg, 4 W)
2	37	M	Responder	Amoxapine[a] (300 mg, >8 W), clomipramine[a] (150 mg, >8 W), milnacipran (150 mg, 6 W), imipramine (200 mg, 8 W), paroxetine (40 mg, >8 W)
3	41	M	Non-responder	Mianserin[a] (60 mg, >8 W), paroxetine (40 mg, 7 W), milnacipran (200 mg, 8 W), amitriptyline[a] (150 mg, >8 W)
4	56	F	Responder	Milnacipran (100 mg, 8 W), clomipramine[a] (150 mg, 7 W), imipramine[a] (200 mg, >8 W), mianserin (60 mg, 8 W)
5	33	M	Non-responder	Nortriptyline[a] (150 mg, 8 W), sertraline[a] (100 mg, 8 W), paroxetine (40 mg, 8 W)
6	51	M	Responder	Amoxapine[a] (150 mg, >8 W), imipramine[a] (300 mg, 6 W), paroxetine (40 mg, 8 W)
7	39	F	Responder	Imipramine[a] (150 mg, >8 W), amitriptyline (150 mg, 8 W), paroxetine[a] (40 mg, >8 W)
8	64	F	Non-responder	Fluvoxamine (150 mg, 8 W), milnacipran (100 mg, 8 W), mianserin[a] (30 mg, 6 W)
9	29	F	Responder	Amitriptyline[a] (150 mg, 8 W), mianserin (60 mg, 8 W)
10	35	M	Non-responder	Maprotiline[a] (150 mg, 6 W), sertraline[a] (100 mg, 6 W)

MADRS = Montgomery–Åsberg Depression Rating Scale, M = male, F = female.
[a] Concurrent antidepressant.

(95% CI 0.04, 2.76), $P = 0.0445$; concentration difficulties, 1.70 (95% CI 0.74, 2.66), $P = 0.0030$; lassitude, 1.50 (95% CI 0.37, 2.63), $P = 0.0150$; inability to feel, 1.60 (95% CI 0.63, 2.57), $P = 0.0046$; and pessimistic thoughts, 1.10 (95% CI 0.18, 2.02), $P = 0.0243$). Our subjects took tricyclic or tetracyclic antidepressants with pramipexole, and three subjects took SSRIs with tricyclic or tetracyclic antidepressants

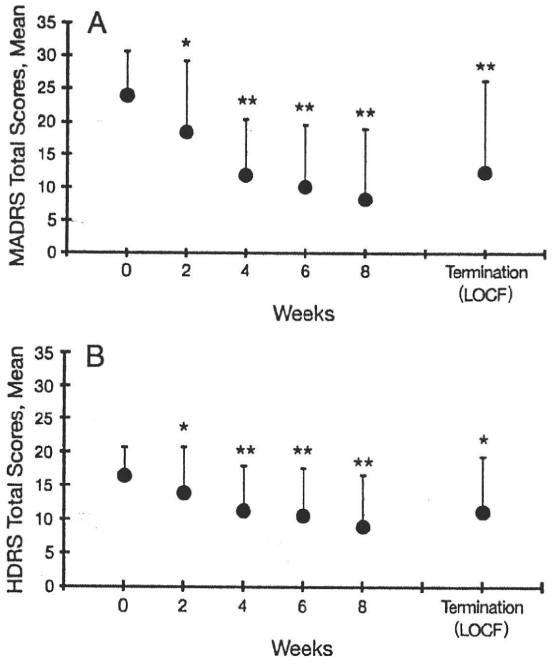

Fig. 1. MADRS (A) and HDRS (B) scores with pramipexole treatment ($n = 10$). Error bars represent SD. *$P<0.05$ and **$P<0.01$ indicate statistical significance from baseline to given point, using paired t test. Termination represents LOCF. MADRS = Montgomery–Åsberg Depression Rating Scale, HDRS = 17-item Hamilton Depression Rating Scale, LOCF = last observation carried forward.

(Table 2). Accordingly, most of them had received pharmacological treatments producing enough inhibition of both noradrenaline and serotonin reuptake. It is difficult to say what kinds of antidepressants are appropriate for adjunctive pramipexole therapy.

HDRS scores also decreased at week 2, and this improvement remained statistically significant throughout the study and at endpoint (LOCF) [mean differences = 5.2, 95% CI 1.4, 9.0, $P = 0.0135$] (Fig. 1B). Five of 10 patients (50%) were judged to be treatment responders based on the HDRS (≥50% reduction). Five patients achieved a HDRS score ≤7 at last visit (LOCF), yielding a 50% remission rate. One patient, who was a responder and remitter on the MADRS (subject 1 in Table 2) was only a partial responder (27% reduction) on the HDRS, because physical symptoms remained and kept the HDRS score high. Five items of the HDRS showed statistically significant mean differences from baseline to endpoint (LOCF): depressed mood, 1.00 (95% CI 0.11, 1.89), $P = 0.0319$; work and activities, 1.30 (95% CI 0.54, 2.06), $P = 0.0037$; psychomotor retardation, 0.4 (95% CI 0.03, 0.76), $P = 0.0368$; anxiety psychic, 0.90 (95% CI 0.37, 1.43), $P = 0.0039$; and somatic symptoms general, 0.60 (95% CI 0.10, 1.10), $P = 0.0239$.

GAF scores improved significantly from baseline to endpoint [mean difference = 13.5, 95% CI (5.1, 21.9), P = 0.0055], with six of 10 patients (60%) showing a GAF-based treatment response. Two patients were judged as achieving functional recovery defined as a GAF score > 70. GAF score changes increased as a function of the improvements of depressive symptoms, HDRS scores, and MADRS scores [HDRS, $r = -0.73$, 95% CI (−0.93, −0.18), $P = 0.0174$; MADRS, $r = -0.80$, 95% CI (−0.95, −0.35), $P = 0.0050$].

Two patients (20%) discontinued treatment because of lack of efficacy and side effects ($n = 1$ at week 6, nausea and appetite loss; subject 8 in Table 2) and psychiatric side effects ($n = 1$ at week 2, irritability and buying sprees, which did not fulfill the criteria of hypomanic or manic episode of DSM-IV-TR; subject 5 in Table 2). Reported side effects were nausea, appetite loss, sea sickness-like symptoms, and consciousness of something non-existent [Leibhaftige Bewusstheit; Jaspers (1973)] ($n = 1$ each) in four patients. All side effects were mild and improved by pramipexole termination, dose reduction, or use of an antiemetic agent.

4. Discussion

In this open-label, nonrandomized, prospective study, we found that pramipexole added to antidepressants seemed to be effective for improving stage 2 treatment-resistant major depression in six of 10 patients (60%) based on the primary outcome (MADRS score). Statistically significant improvements in depressive symptoms were seen overall in the entire sample. Using secondary outcome measures (HDRS and GAF), similarly high response rates were observed (50–60%), indicating that pramipexole improved not only depressive symptoms, but also psychosocial function. No serious side effects were seen, which is consistent with previous studies (Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005), and the discontinuation rate was relatively low (2 of 10 patients [20%]). Two of the 10 patients were not able to complete the open-label trial due to intolerable side effects, but among the remaining eight patients, pramipexole was reasonably well tolerated.

Pramipexole improved total MADRS scores. We analyzed whether improvements differed among the sub-items of this scale. Eight of 10 items of the MADRS showed significant improvements. Accordingly, it does not appear that pramipexole improves only a certain subset of depressive symptoms. Improvements in the 17-item HDRS ($HDRS_{17}$) were seen in five sub-items, all of which were symptoms of so-called "Bech's $HDRS_6$", which is more clearly unidimensional and more sensitive to changes than the $HDRS_{17}$ (Carmody et al., 2006). Both the MADRS and the $HDRS_6$ are related highly to the core concept of depression and had acceptable effect sizes in two clinical trials

including highly treatment-resistant and non-treatment-resistant patients with major depression, respectively (Carmody et al., 2006). Hence, our data on changes in sub-items over time are in good agreement with a previous study (Carmody et al., 2006).

The antidepressant effects of pramipexole were first observed in several animal models and, later, in a series of controlled and uncontrolled clinical studies in which pramipexole was used as monotherapy or augmentation therapy in patients with bipolar and unipolar depression (Aiken, 2007). A randomized controlled trial of pramipexole compared three doses of pramipexole to fluoxetine and placebo in 174 subjects with non-refractory unipolar major depression (Corrigan et al., 2000). At 8 weeks, pramipexole performed comparably to fluoxetine and significantly better than placebo; pramipexole 1.0 mg per day resulted in significant improvement over baseline compared with the placebo group. In addition, six previous studies have reported that dopamine receptor agonists, including pramipexole, in addition to antidepressants improved major depressive disorder refractory to at least one standard antidepressant trial (Bouckoms and Mangini, 1993; Inoue et al., 1996; Izumi et al., 2000; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). However, two studies did not define treatment-resistant depression clearly (Bouckoms and Mangini, 1993; Sporn et al., 2000). Four studies included not only major depressive disorder, but also major depressive episode associated with bipolar disorder (Bouckoms and Mangini, 1993; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005). Although these six studies might include stage 2 treatment-resistant major depressive disorder as defined by Thase and Rush (1995), the efficacy of dopamine agonists for stage 2 major depressive disorder remains unclear. Our study suggests that a dopamine receptor agonist may be an effective adjunctive therapy for stage 2 treatment-resistant major depressive disorder.

The clinical efficacy of pramipexole for treatment-resistant bipolar depression is clearer than that for treatment-resistant unipolar depression (Goldberg et al., 2004; Zarate et al., 2004). In addition to the open trials with dopamine receptor agonists described above (Bouckoms and Mangini, 1993; Sporn et al., 2000; Lattanzi et al., 2002; Cassano et al., 2005), two preliminary randomized, double-blind, placebo-controlled trials were undertaken for treatment-resistant bipolar depression among patients with non-response to at least two adequate trials of standard antidepressants with concomitant mood stabilizers during the current depressive episode (Goldberg et al., 2004) or non-response to at least one adequate antidepressant trial regardless of concomitant mood stabilizer (Zarate et al., 2004). Both studies showed significant antidepressant effects of pramipexole. Because bipolar disorder is one of the most common reasons for treatment-resistant major depression (Sharma et al., 2005; Inoue et al., 2006), the clinical efficacy of pramipexole suggests that pramipexole may be effective for unrecognized bipolar depression that has been treated as treatment-resistant major depression.

This study does not prove efficacy or safety of this agent because it was nonrandomized and uncontrolled, but it does apply a prospective outcomes assessment, with no changes allowed in any other treatments. Thus, it provides useful pilot data that tend to support performing randomized studies of pramipexole in stage 2 treatment-resistant major depressive disorder. However, two limitations should be noted. First, open-label case series tend to over-estimate the effectiveness of novel interventions. Second, this study examined the short-term efficacy of pramipexole. Future studies examining long-term efficacy are needed because a high proportion of patients with more advanced levels of treatment-resistant major depression relapsed after responding to later stage therapies in STAR*D (Rush et al., 2006).

5. Conclusion

Pramipexole may have benefit for stage 2 treatment-resistant major depressive disorder. Further studies to confirm the clinical efficacy of pramipexole are warranted.

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Neuropharmacology and Analgesia

Combined treatment with MAO-A inhibitor and MAO-B inhibitor increases extracellular noradrenaline levels more than MAO-A inhibitor alone through increases in β-phenylethylamine

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ABSTRACT

Monoamine oxidase inhibitors (MAO inhibitors) have been widely used as antidepressants. However, it remains unclear whether a difference exists between non-selective MAO inhibitors and selective MAO-A inhibitors in terms of their antidepressant effects. Using in vivo microdialysis methods, we measured extracellular noradrenaline and serotonin levels following administration of Ro 41-1049, a reversible MAO-A inhibitor and/or lazabemide, a reversible MAO-B inhibitor in the medial prefrontal cortex (mPFC) of rats. We examined the effect of local infusion of β-phenylethylamine to the mPFC of rats on extracellular noradrenaline and serotonin levels. Furthermore, the concentrations of β-phenylethylamine in the tissue of the mPFC after combined treatment with Ro 41-1049 and lazabemide were measured. The Ro 41-1049 alone and the combined treatment significantly increased extracellular noradrenaline levels compared with vehicle and lazabemide alone. Furthermore, the combined treatment increased noradrenaline levels significantly more than Ro 41-1049 alone did. The Ro 41-1049 alone and the combined treatment significantly increased extracellular serotonin levels compared with vehicle and lazabemide alone, but no difference in serotonin levels was found between the combined treatment group and the Ro 41-1049 group. Local infusion of low-dose β-phenylethylamine increased extracellular noradrenaline levels, but not that of serotonin. Only the combined treatment significantly increased β-phenylethylamine levels in tissues of the mPFC. Our results suggest that the combined treatment with a MAO-A inhibitor and a MAO-B inhibitor strengthens antidepressant effects because the combined treatment increases extracellular noradrenaline levels more than a MAO-A inhibitor alone through increases in β-phenylethylamine.

1. Introduction

Non-selective MAO inhibitors were first developed as antidepressants. Subsequently, selective MAO inhibitors such as selective monoamine oxidase A (MAO-A) inhibitor (clorgyline) and selective monoamine oxidase B (MAO-B) inhibitor (selegiline) were introduced. However, irreversible MAO inhibitors entail risks of causing hypertensive attacks after consumption of tyramine-rich food (Blackwell et al., 1967) and of causing serotonin syndrome in cases of co-administration of non-selective MAO inhibitors and tricyclic antidepressants (TCA) or selective serotonin reuptake inhibitors (SSRIs) (Schuckit et al., 1971; Ananth and Luchins, 1977; Sternbach, 1991). Consequently, MAO inhibitors have been used only infrequently as the first-line antidepressant of the depression treatment for the reasons described above (Lam et al., 2009).

Reversible monoamine oxidase A inhibitors (RIMAs) were developed later. Moclobemide, an RIMA, has an antidepressant effect that is equal to that of SSRIs and different side effect profiles from SSRIs (Papakostas and Fava, 2006). In several countries, RIMAs are used for the treatment of depression. Now, RIMAs are recognized as important antidepressants. They are used as first-line antidepressants for the treatment of depression (Lam et al., 2009).

Several reports have described that the MAO-A inhibition contributes to the mechanism of antidepressant effects of MAO inhibitors more than MAO-B inhibition (Lipper et al., 1979; Mann et al., 1989). Moreover, Larsen et al. (1991) reported that RIMA has equal antidepressant effects to those of irreversible MAO inhibitors. However, Lotufo-Neto et al. (1999) examined antidepressant effects of MAO inhibitors in a meta-analysis and described the possibility that non-selective MAO inhibitors are more effective than RIMA. Consequently, it is likely that MAO-B inhibition also contributes to an

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antidepressant effect. Nevertheless, no consensus has been reached on the matter.

For this study, to examine the pharmacological mechanism of antidepressant effects of MAO-A and MAO-B inhibitors, we measured extracellular noradrenaline and serotonin levels after administration of Ro 41-1049, an RIMA, and/or lazabemide, a reversible MAO-B inhibitor in the medial prefrontal cortex (mPFC) of rats using the in vivo microdialysis method. A main substance of MAO-B, β-phenylethylamine, exists in the brain; it is related to catecholamine release (Mesfioui et al., 1998; Nakamura et al., 1998; Burchett and Hicks, 2006). Accordingly, we also measured extracellular noradrenaline and serotonin levels after local infusion of β-phenylethylamine to the mPFC of rats. In addition, the concentrations of β-phenylethylamine in the tissues of the mPFC after administration of Ro 41-1049 and lazabemide were measured.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 180–280 g were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were housed in groups of four and maintained on a 12 h light–dark cycle (light phase: 06:30–18:30) in a temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after a 10-day period of acclimatization. All procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee. They complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.2. Drugs

After dissolution in saline, Ro 41-1049 (N-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxamide hydrochloride) (Research Biochemical Inc., Natick, U.S.A.) and lazabemide (N-(2-aminoethyl)-5-chloro-2-pyridinecarboxamide hydrochloride) (F. Hoffman-La Roche Ltd., Switzerland) were injected intraperitoneally (i.p.) at a volume of 1 ml/kg. Then β-phenylethylamine (Sigma Chemical Co., St. Louis, U.S.A.) was dissolved in artificial cerebrospinal fluid (CSF) and was thereafter administered from microdialysis probes (reverse-dialysis). The doses of the selective MAO-A and MAO-B inhibitors were chosen, respectively, to inhibit MAO-A and MAO-B fully and selectively (Da Prada et al., 1990).

2.3. Microdialysis procedures

2.3.1. Surgery and perfusion

Experiments were performed according to a procedure described in a previous report (Kitaichi et al., 2004). Briefly, rats were implanted stereotaxically under pentobarbital anesthesia (30 mg/kg i.p.) using an AG-4 guide cannulae (Eicom Corp., Kyoto, Japan) leading to the surface of the mPFC at the following coordinates relative to the bregma: A + 3.2, ML + 0.8, DV + 1.0 mm. Dialysis probes with 0.22 mm outer diameter (A-I-4-03; Eicom Corp.) were then inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the mPFC. Rats were housed individually after these operations.

Experiments were performed using freely moving rats. On the following day, 24 h after surgery, perfusion was started using artificial CSF (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow rate of 1 μl/min. Following initial perfusion for 2 h, dialysate samples were collected in sample vials containing 50 μl of 0.05 M acetic acid every 40 min for 440 min.

2.3.1.1. Experiment 1: Acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on extracellular noradrenaline and serotonin concentrations. Rats received a single injection (i.p.) of vehicle, Ro 41-1049 (30 mg/kg), lazabemide (10 mg/kg), or the combination of Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg), 200 min after the first dialysate samples were collected. Extracellular noradrenaline and serotonin levels were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (Eicom Corp.).

2.3.1.2. Experiment 2: Local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) into the mPFC on extracellular noradrenaline and serotonin concentrations. Rats received local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) via reverse microdialysis into the mPFC (local reverse-dialysis) during 0–240 min, 200 min after the first dialysate samples were collected. Extracellular noradrenaline and serotonin levels were determined using HPLC-ECD (Eicom Corp.).

2.3.2. Analytical procedures for noradrenaline

The HPLC system consisted of a liquid chromatograph pump (EP-300; Eicom Corp.), a degasser (DG-300; Eicom Corp.), a reverse phase ODS column (Eicompak CA-5ODS 150 2.1 mm; Eicom Corp.), an ECD-300 electrochemical detector (Eicom Corp.), and a data acquisition system (PowerChrom; AD Instruments Pty. Ltd., Sydney, Australia). For the noradrenaline analysis, 30 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 5% (v/v) methanol, 50 mg/l Na_2EDTA and 500 mg/l L-octanesulfonic acid. Separations were conducted at 25 °C with a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. Noradrenaline standard solutions were injected every working day and the peak heights for the standard were used for comparison to determine the amount of noradrenaline in the samples.

2.3.3. Analytical procedures for serotonin

To determine serotonin concentrations, the same equipment as that used for the noradrenaline analysis with the exception of a different reverse phase ODS column, an Eicompak PP-ODS 30 4.6 mm (Eicom Corp.) was used. For serotonin analysis, 20 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 1% (v/v) methanol, 50 mg/l Na_2-EDTA and 500 mg/l sodium L-decanesulfonate. Separations were conducted at 25 °C with a flow rate of 0.5 ml/min. The electrochemical detector was set at an oxidation potential of 400 mV. Standard solutions for serotonin were injected every working day, and the peak heights for the standards were used for comparison to determine the amount of serotonin in the samples.

2.4. Experiment 3: Effect of acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on β-phenylethylamine concentrations in the mPFC

Rats were administered vehicle, Ro 41-1049 (30 mg/kg), lazabemide (10 mg/kg) or the combination of Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg). All rats were killed by decapitation 4 h after drug administration. Brains were quickly removed and frozen at −80 °C. We entrusted the measurement of β-phenylethylamine concentrations of the mPFC to S-Medical Service Inc. (Tokyo, Japan). β-Phenylethylamine was measured using gas chromatography–mass spectrometry.

2.5. Statistical analysis

All data are given as the mean values ± S.E.M. of individual rats from each group. The noradrenaline and serotonin contents of dialysate samples were expressed as absolute values (pg/fraction).

In experiment 1, to investigate the combined effect of Ro 41-1049 and lazabemide (2×2 design) on extracellular noradrenaline and serotonin concentrations, repeated measures analysis of variance (ANOVA) for absolute values was used during the 0–240 min interval after MAO inhibitors administration. The respective areas under the curve for the 0–240 min periods were compared among the four groups using one-way ANOVA, followed by Duncan's test. Differences

in absolute values measured at each time point of collection among the four groups were analyzed using a one-way ANOVA followed by Duncan's test. Differences were considered significant at $P<0.05$.

In experiment 2, to investigate the effect of local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) into the mPFC on extracellular noradrenaline and serotonin concentrations, repeated measures ANOVA for absolute values was used during the 0–240 min interval during local reverse-dialysis of β-phenylethylamine. The areas under the curve for the 0–240 min periods were compared among the three groups (β-phenylethylamine 0, 10, and 100 μmol/l) using a one-way ANOVA, followed by Duncan's test. Differences in absolute values measured at each time point of collection between three groups were analyzed using a one-way ANOVA followed by Duncan's test. Differences were considered significant at $P<0.05$.

In experiment 3, differences in brain β-phenylethylamine concentrations among the four groups were analyzed using a one-way ANOVA, followed by Duncan's test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Effect of the combined treatment with acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on extracellular noradrenaline and serotonin concentrations in the mPFC (Fig. 1A and B)

Acute administration of Ro 41-1049 alone and the combination of Ro 41-1049 and lazabemide increased extracellular noradrenaline concentrations (Fig. 1A). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of MAO inhibitors treatment [$F(3,47)=13.416$, $P<0.0001$] and time [$F(6,282)=38.237$, $P<0.0001$] on extracellular noradrenaline concentrations. In addition, the interaction between MAO inhibitors and time was significant [$F(18,282)=17.899$, $P<0.0001$]. The combined treatment (Ro 41-1049 and lazabemide) group showed significantly higher concentrations of extracellular noradrenaline compared with the vehicle, the Ro 41-1049 and the lazabemide groups (Duncan's test, vs. vehicle, 80–240 min, $P<0.01$, 40 min, $P<0.05$; vs. Ro 41-1049 group, 120–240 min, $P<0.01$, 80 min, $P<0.05$; vs. lazabemide group, 80–240 min, $P<0.01$). Significantly higher concentrations of extracellular noradrenaline were found for the Ro 41-1049 group than for the vehicle and the lazabemide groups (Duncan's test, vs. vehicle group, 160 and 200 min, $P<0.01$, 80, 120 and 240 min, $P<0.05$; vs. lazabemide group, 80–240 min, $P<0.05$).

Acute administration of Ro 41-1049 and the combination with Ro 41-1049 and lazabemide increased extracellular serotonin concentrations (Fig. 1B). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of MAO inhibitors treatment [$F(3,44)=3.992$, $P=0.0134$] and time [$F(6,264)=5.420$, $P<0.0001$] on extracellular serotonin concentrations. In addition, the interaction between MAO inhibitors and time was significant [$F(18,264)=2.229$, $P=0.0034$]. Significantly higher concentrations of extracellular serotonin were found for the combined treatment (Ro 41-1049 and lazabemide) group than for the vehicle and the lazabemide groups (Duncan's test, vs. vehicle group, 160 and 200, $P<0.01$, 80, 120, and 240 min, $P<0.05$; vs. lazabemide group, 160 and 200, $P<0.01$, 120, and 240 min, $P<0.05$). No significant difference was found between the combined treatment and Ro 41-1049 groups at any time point. Significantly higher concentrations of extracellular serotonin were found for the Ro 41-1049 (30 mg/kg) group than for the vehicle or the lazabemide group (Duncan's test, vs. vehicle group, 80–200 min, $P<0.05$; vs. lazabemide group, 120–200 min, $P<0.05$).

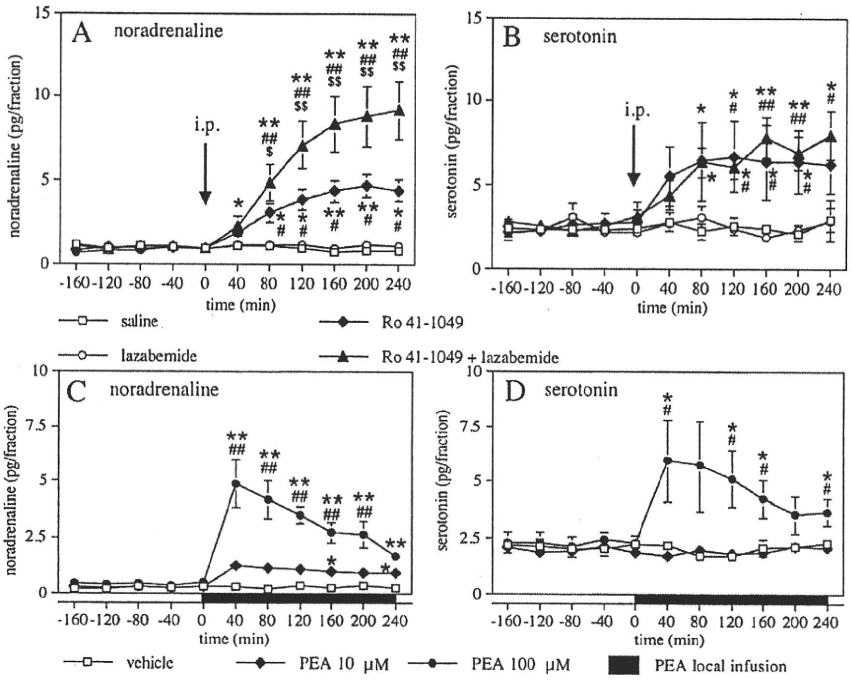

Fig. 1. (A and B) Effect of the combined treatment with acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on extracellular noradrenaline and serotonin concentrations during 0–240 min in the mPFC. Values represent the mean ± S.E.M. (pg/40 min fraction): Noradrenaline, $N=14$ (vehicle group), $N=12$ (Ro 41-1049 and lazabemide groups), $N=13$ (combined treatment group); Serotonin, $N=11$ (vehicle and lazabemide groups), $N=12$ (Ro 41-1049 group), $N=14$ (combined treatment group). $^{**}P<0.01$, $^{*}P<0.05$ vs. vehicle group, $^{\#\#}P<0.01$, $^{\#}P<0.05$ vs. lazabemide group, $^{\$\$}P<0.01$, $^{\$}P<0.05$ vs. Ro 41-1049 group. (C and D) Effect of local infusion of β-phenylethylamine (PEA) (0, 10, and 100 μmol/l) on extracellular noradrenaline and serotonin concentrations during 0–240 min in the mPFC. Values represent the mean ± S.E.M. (pg/40 min fraction): Noradrenaline, $N=4$ (vehicle group), $N=6$ (β-phenylethylamine 10 μmol/l group), $N=5$ (β-phenylethylamine 100 μmol/l group); Serotonin, $N=4$ (vehicle group), $N=5$ (β-phenylethylamine 10 and 100 μmol/l groups). $^{**}P<0.01$, $^{*}P<0.05$ vs. vehicle group, $^{\#\#}P<0.01$, $^{\#}P<0.05$ vs. β-phenylethylamine 10 μmol/l group.

Table 1
Acute effect of the combined treatment with Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on β-phenylethylamine concentrations in the mPFC.

	Vehicle	Ro 41-1049	Lazabemide	Combined treatment with Ro 41-1049 and lazabemide
β-phenylethylamine	3.87 ± 2.74	2.42 ± 0.79	2.10 ± 0.41	38.83 ± 3.90[a, b, c]

Values represent the mean ± S.E.M. (ng/g tissue). $N=6$ (vehicle group), $N=4$ (Ro 41-1049 group), $N=8$ (lazabemide and combined treatment groups).
[a] $P<0.01$ vs. vehicle group.
[b] $P<0.01$ vs. lazabemide group.
[c] $P<0.01$ vs. Ro 41-1049 group.

3.2. Effect of acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on the area under the curve during 0–240 min for extracellular noradrenaline and serotonin concentrations in the mPFC

The areas under the curve of each of the four groups (vehicle, Ro 41-1049, lazabemide and combined treatment with Ro 41-1049 and lazabemide groups) during 0–240 min for extracellular noradrenaline in the mPFC were, respectively, 228 ± 30 ($N=14$), 832 ± 122 ($N=12$), 271 ± 56 ($N=12$) and 1459 ± 288 ($N=13$) pg min. One-way ANOVA indicated significant main effects of MAO inhibitors on the area under the curve (0–240 min) for extracellular noradrenaline levels [$F(3,47)=13.183$, $P=0.0001$]. The area under the curve (0–240 min) for extracellular noradrenaline of the combined treatment (Ro 41-1049 and lazabemide) group was significantly higher than those of the vehicle, Ro 41-1049, or lazabemide group (Duncan's test; $P<0.01$, respectively). The area under the curve (0–240 min) for extracellular noradrenaline of the Ro 41-1049 group was significantly greater than that of either the vehicle or lazabemide group (Duncan's test; $P<0.05$, respectively).

The areas under the curve of each of the four groups (vehicle, Ro 41-1049, lazabemide and combined treatment with Ro 41-1049 and lazabemide groups) during 0–240 min for extracellular serotonin in the mPFC were, respectively, 609 ± 105 ($N=11$), 1440 ± 410 ($N=12$), 616 ± 86 ($N=11$), and 1487 ± 195 ($N=14$) pg min. One-way ANOVA indicated significant main effects of MAO inhibitors on the area under the curve (0–240 min) for extracellular serotonin levels [$F(3,44)=4.087$, $P=0.0121$]. The areas under the curve (0–240 min) for extracellular serotonin of the combined treatment (Ro 41-1049 and lazabemide) group and the Ro 41-1049 group were significantly higher than that of either the vehicle or lazabemide group (Duncan's test; $P<0.05$). No difference was found between the area under the curve (0–240 min) for extracellular serotonin of the combined treatment (Ro 41-1049 and lazabemide) group and the Ro 41-1049 group.

3.3. Effect of local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) on extracellular noradrenaline and serotonin concentrations during 0–240 min in the mPFC (Fig. 1C and D)

Local infusion of β-phenylethylamine (10 and 100 μmol/l) into the mPFC increased extracellular noradrenaline concentrations (Fig. 1C). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of β-phenylethylamine treatment [$F(2,12)=35.844$, $P<0.0001$] and time [$F(6,72)=9.377$, $P<0.0001$] on extracellular noradrenaline concentrations. In addition, the interaction between β-phenylethylamine and time was found to be significant [$F(21,72)=5.305$, $P<0.0001$]. Significantly higher concentrations of extracellular noradrenaline were found for the β-phenylethylamine (100 μmol/l) group than for the vehicle and β-phenylethylamine (10 μmol/l) groups (Duncan's test; vs. vehicle group, 40–240 min, $P<0.01$; vs. β-phenylethylamine (10 μmol/l) group, 40–200 min, $P<0.01$). In addition, significantly higher concentrations of extracellular noradrenaline were found for the β-phenylethylamine (10 μmol/l) group than for the vehicle group (Duncan's test, 160, 240 min, $P<0.05$).

Local infusion of β-phenylethylamine (100 μmol/l) into the mPFC increased extracellular serotonin concentrations (Fig. 1D). Two-way ANOVA with repeated measures (0–240 min) indicated significant main effects of β-phenylethylamine treatment [$F(2,11)=4.854$, $P=0.0308$] and the interaction between β-phenylethylamine and time [$F(12,66)=2.332$, $P=0.0148$] on extracellular serotonin concentrations. The time effect was not significant [$F(6,66)=1.348$, $P=0.2489$]. Significantly higher concentrations of extracellular serotonin were found for the β-phenylethylamine (100 μmol/l) group than for the vehicle and β-phenylethylamine (10 μmol/l) groups (Duncan's test, 40, 120, 160, 240 min, $P<0.05$, respectively). However, local infusion of β-phenylethylamine (10 μmol/l) did not increase extracellular serotonin concentrations compared with the vehicle group.

3.4. Effect of local infusion of β-phenylethylamine (0, 10, and 100 μmol/l) on the area under the curve during 0–240 min for extracellular noradrenaline and serotonin concentrations in the mPFC

The areas under the curve of three groups (vehicle, the β-phenylethylamine 10 μmol/l and the β-phenylethylamine 100 μmol/l groups) during 0–240 min for extracellular noradrenaline and serotonin in the mPFC were the following: noradrenaline, 74 ± 20 ($N=4$), 245 ± 49 ($N=6$) and 650 ± 53 ($N=5$) pg min, respectively; serotonin, 402 ± 35 ($N=4$), 382 ± 34 ($N=5$) and 944 ± 239 ($N=5$) pg min, respectively. One-way ANOVA indicated significant main effects of β-phenylethylamine on the area under the curve (0–240 min) for the extracellular noradrenaline and serotonin levels [$F(2,12)=37.066$, $P=0.0001$; $F(2,11)=4.609$, $P=0.0352$, respectively]. The area under the curve (0–240 min) for extracellular noradrenaline and serotonin of the β-phenylethylamine (100 μmol/l) group was significantly higher than that of the vehicle or the β-phenylethylamine (10 μmol/l) group (Duncan's test; $P<0.01$). In addition, the area under the curve (0–240 min) for extracellular noradrenaline of the β-phenylethylamine (10 μmol/l) group was significantly higher than that of the vehicle group (Duncan's test; $P<0.05$). The area under the curve (0–240 min) for extracellular serotonin concentrations of the β-phenylethylamine (10 μmol/l) group was not different from that of the vehicle group.

3.5. Effect of acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on β-phenylethylamine concentrations in the mPFC (Table 1)

One-way ANOVA indicated significant main effects of MAO inhibitors on the tissue concentrations of β-phenylethylamine [$F(3,22)=50.031$, $P=0.0001$]. Significantly higher concentrations of β-phenylethylamine was found for the combined treatment with Ro 41-1049 and lazabemide than for the vehicle, Ro 41-1049, or lazabemide group (Duncan's test, $P<0.01$). However, Ro 41-1049 alone or lazabemide alone did not affect β-phenylethylamine concentrations.

4. Discussion

Acute administration of Ro 41-1049 increased extracellular noradrenaline levels significantly compared with vehicle and lazabemide. Increased extracellular noradrenaline levels by MAO-A inhibition have been described in several reports (Curet et al., 1998; Kitaichi et al., 2006). On the other hand, combined treatment with Ro 41-1049 and lazabemide increased extracellular noradrenaline levels more than not only vehicle and lazabemide alone did, but also more than Ro 41-1049

alone did. As Table 1 shows, the concentrations of β-phenylethylamine in the mPFC tissue were significantly higher in the combined treatment (Ro 41-1049 and lazabemide) group than in any of the other three groups. Furthermore, local infusion of β-phenylethylamine increased extracellular noradrenaline levels significantly in the mPFC. In short, it is thought that through the increases in β-phenylethylamine, combined treatment with a MAO-A inhibitor and a MAO-B inhibitor induced more increases in extracellular noradrenaline levels than a MAO-A inhibitor alone did. It is possible that MAO-A inhibition together with MAO-B inhibition strengthens antidepressant effects more than MAO-A inhibition alone did.

We previously reported that the selective MAO-A inhibitor clorgyline increased extracellular serotonin concentrations in the mPFC of rats (Kitaichi et al., 2006). In the present study, Ro 41-1049 administration alone and combined treatment with Ro 41-1049 and lazabemide significantly increased extracellular serotonin levels, although no significant difference between these two groups was found. Celada and Artigas (1993) reported that the irreversible MAO-A inhibitor clorgyline, together with the irreversible MAO-B inhibitor selegiline, increased extracellular serotonin levels more than clorgyline alone did. Selegiline is partly metabolized to L-amphetamine (Karoum et al., 1982). Therefore, another mechanism other than MAO inhibition might explain the enhancement of serotonin increase by selegiline in their study. In our study, local infusion of high-dose β-phenylethylamine (100 μmol/l) increased extracellular serotonin levels significantly, but low-dose β-phenylethylamine (10 μmol/l) did not. As described above, local infusion of low-dose β-phenylethylamine (10 μmol/l) increased extracellular noradrenaline levels. Consistent with our finding, a previous study also reported that extracellular dopamine levels in the nucleus accumbens were increased by local infusion of β-phenylethylamine at 1 μmol/l, but extracellular serotonin levels were increased by that of β-phenylethylamine at 100 μmol/l or more (Nakamura et al., 1998). Therefore, the increase in β-phenylethylamine by combined treatment with a MAO-A inhibitor and a MAO-B inhibitor in this study might not be sufficient to strengthen serotonin levels more than MAO-A inhibition alone because only high-dose infusion of β-phenylethylamine increased extracellular serotonin levels.

A few studies have been undertaken to investigate β-phenylethylamine concentrations by administration of selective MAO-B inhibitors in the mPFC of rats, although previous reports show that selegiline, which is a selective MAO-B inhibitor but which inhibits MAO-A at high dosage, increases striatal β-phenylethylamine concentrations in the brain (Paterson et al., 1991). In fact, β-phenylethylamine is a main substrate of MAO-B. However, no change was found in β-phenylethylamine concentrations in the mPFC tissue by single administration of the selective MAO-B inhibitor lazabemide. Only when a MAO-A inhibitor and a MAO-B inhibitor were administered together, significant increases in β-phenylethylamine were observed. High concentrations of β-phenylethylamine are reportedly metabolized also by MAO-A, although β-phenylethylamine is mainly metabolized by MAO-B (Schoepp and Azzaro, 1981). This finding might account for the lack of increase in β-phenylethylamine levels of the mPFC by the highly selective MAO-B inhibitor lazabemide.

It is well known that β-phenylethylamine is related to catecholamine release (Mesfioui et al., 1998; Nakamura et al., 1998; Burchett and Hicks, 2006). Recently, Xie and Miller (2008) reported that β-phenylethylamine inhibited noradrenaline uptake and induced efflux of noradrenaline through trace amine-associated receptor 1, which exists in the brainstem and other brain regions, including the prefrontal cortex (Bunzow et al., 2001). In their study, 1 μmol/l β-phenylethylamine affected not only noradrenaline, but also serotonin and dopamine. In this study, low-dose β-phenylethylamine increased extracellular noradrenaline levels, but high-dose β-phenylethylamine did both extracellular noradrenaline and serotonin levels (Fig. 1C and D). A study undertaken by Xie and Miller (2008) examined a single dose of β-phenylethylamine and did not investigate differences in potencies of β-phenylethylamine for noradrenaline and serotonin uptake inhibition. On the other hand, differences have been found in the in vivo potencies of β-phenylethylamine for uptake inhibition of monoamines (Nakamura et al., 1998). Taken together, uptake inhibition or increased efflux of noradrenaline induced by increased β-phenylethylamine by combination of a MAO-A inhibitor and a MAO-B inhibitor in our study might engender a further increase in extracellular noradrenaline, which was increased by a MAO-A inhibitor. The effect of β-phenylethylamine on uptake inhibition and efflux of noradrenaline and serotonin must be elucidated more precisely in the future.

Results of this study suggest that the greater increase in extracellular noradrenaline levels in the mPFC through increased β-phenylethylamine levels after combined treatment with a MAO-A inhibitor and a MAO-B inhibitor might strengthen antidepressant effects of MAO-A inhibitors. As described in the Introduction, Lotufo-Neto et al. (1999) reported a meta-analysis of antidepressant effects of MAO inhibitors and pointed out the possibility that non-selective MAO inhibitors are more effective than RIMA. Our results are consistent with their meta-analysis results. There are more MAO-B than MAO-A in the human brain, but more MAO-A than MAO-B in the rat brain (Riederer et al., 1987). Moreover, the distribution of MAO-A and MAO-B is different between the human brain and the rat brain. The role of MAO-B in antidepressant effects might be greater in humans than in rats; stronger antidepressant effects of combined treatment with a MAO-A inhibitor and a MAO-B inhibitor might be likely to be induced in humans.

Irreversible MAO inhibitors present a risk of causing hypertensive attacks when consuming tyramine-rich food (Blackwell et al., 1967). Tyramine taken orally is metabolized by MAO-A and MAO-B (Youdim and Weinstock, 2004). Therefore, a combination of a MAO-A inhibitor and a MAO-B inhibitor might inhibit tyramine metabolism more than a MAO-A inhibitor alone would; such a combination would increase the risk of a tyramine reaction. Tyramine restriction might be necessary in combined treatment with a MAO-A inhibitor and a MAO-B inhibitor, although such a combination of reversible inhibitors might produce less tyramine potentiation clinically (Youdim and Weinstock, 2004).

In conclusion, combined treatment with the reversible MAO-A inhibitor Ro 41-1049 and reversible MAO-B inhibitor lazabemide significantly increased extracellular noradrenaline levels more than Ro 41-1049 alone did. The increase in β-phenylethylamine levels might be the mechanism of action. On the other hand, no difference was found between combined treatment with Ro 41-1049 and lazabemide and treatment with Ro 41-1049 alone in the effects on extracellular serotonin levels in the mPFC. The possibility exists that antidepressant effects of combined treatment with a MAO-A inhibitor and a MAO-B inhibitor or non-selective MAO inhibitors are stronger than that of a MAO-A inhibitor alone.

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Neuropharmacology and Analgesia

Sertraline increases extracellular levels not only of serotonin, but also of dopamine in the nucleus accumbens and striatum of rats

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are a first-line treatment for depression. Recent reports in the literature describe differences in antidepressant effects among SSRIs. Although each SSRI apparently has different pharmacological actions aside from serotonin reuptake inhibition, the relations between antidepressant effects and unique pharmacological properties in respective SSRIs remain unclear. This study was designed to compare abilities of three systemically administered SSRIs to increase the extracellular levels of serotonin, dopamine, and noradrenaline acutely in three brain regions of male Sprague–Dawley rats. We examined effects of sertraline, fluvoxamine, and paroxetine on extracellular serotonin, dopamine, and noradrenaline levels in the medial prefrontal cortex, nucleus accumbens and striatum of rats using *in vivo* microdialysis. Dialysate samples were collected in sample vials every 20 min for 460 min. Extracellular serotonin, dopamine, and noradrenaline levels were determined using high-performance liquid chromatography with electrochemical detection. All SSRI administrations increased extracellular serotonin levels in all regions. Only sertraline administration increased extracellular dopamine concentrations in the nucleus accumbens and striatum. All SSRI administrations increased extracellular noradrenaline levels in the nucleus accumbens, although fluvoxamine was less effective. These results suggest that neurochemical differences account for the differences in clinical antidepressant effects among SSRIs.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) have antidepressant effects equal to those of tricyclic antidepressants. However, they have better tolerability and lower rates of treatment discontinuation than tricyclic antidepressants do (Montgomery and Kasper, 1995; Anderson, 2000). Most treatment guidelines for depression recommend SSRIs as a first-line treatment (Rush et al., 1998; Janicak et al., 2001; Kennedy et al., 2001). Several SSRIs have been developed and put on the market. Various SSRIs have common pharmacological characteristic of inhibiting serotonin reuptake on serotonin transporters. However, clinical reports describe that switching to another SSRI is effective for the treatment of depression, for which one SSRI is ineffective (Joffe et al., 1996; Thase et al., 1997, 2001). In STAR*D trial—which elucidated clinical efficacies of switching to sertraline, bupropion-SR, and venlafaxine-XR in nonresponders or patients intolerant to citalopram, an SSRI—the remission rate of switching to sertraline, an SSRI, was shown to be 17.6% by the 17-item Hamilton Depression Rating Scale. It was not different from those of other treatments (Rush et al., 2006). Recently, Ruhé et al. (2006) reviewed that response rates of switching to a second SSRI were about 50–70% after the first SSRI treatment had failed. In a meta-analysis study, Cipriani et al. (2009) reported that mirtazapine, escitalopram, venlafaxine, and sertraline exhibited greater effectiveness in unipolar depressive patients than duloxetine, fluoxetine, fluvoxamine, and paroxetine, which suggests that antidepressant effects of various SSRIs might differ despite their shared classification as antidepressants.

The respective SSRIs have different pharmacological actions aside from their serotonin reuptake inhibition (Carrasco and Sandner, 2005). These different actions might be related to differences in antidepressant effects of SSRIs. In receptor binding studies, some SSRIs display marked affinity for noradrenaline transporters, dopamine transporters, muscarinic receptors, and sigma receptors (Sánchez and Hyttel, 1999; Owens et al., 2001; Narita et al., 1996). Notably, sertraline is capable of dopamine reuptake inhibition; paroxetine shows the capability of noradrenaline reuptake inhibition in rats

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(Goodnick and Goldstein, 1998). However, the relations between antidepressant effects and unique pharmacological properties in respective SSRIs remain unclear.

The biological basis of depression is hypothesized as a lack of neurotransmitters such as serotonin and noradrenaline. The increases in extracellular serotonin and noradrenaline levels in the brain are thought to be related closely to the antidepressant effect. However, recent evidence suggests that dopamine might be related also to the pathogenesis and treatment of depression (Kapur and Mann, 1992; Papakostas, 2006). Several drugs that stimulate dopamine have antidepressant effects (Papakostas, 2006). Several lines of evidence suggest that the medial prefrontal cortex, nucleus accumbens and striatum—the projection regions of three main dopamine systems (Björklund and Dunnett, 2007)—are closely related to depression (Konarski et al., 2008; Nestler and Carlezon, 2006). Particularly, some reports have described that dopaminergic neurons in the nucleus accumbens are related to depression-like behaviors in animal models (Nestler and Carlezon, 2006) and anhedonia, which is a core symptom of major depressive disorder (Gorwood, 2008). The relations between dopaminergic neurons in the nucleus accumbens and depression have been receiving increasing attention.

To clarify differences in the antidepressant effects among SSRIs, we examined the effects of three SSRIs (sertraline, fluvoxamine, and paroxetine) not only on extracellular serotonin and noradrenaline levels, but also on extracellular dopamine levels in the medial prefrontal cortex, nucleus accumbens, and striatum of rats using *in vivo* microdialysis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 270–400 g, obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), were housed in groups of four and maintained on a 12 h light–dark cycle (light phase: 06:30–18:30) in a temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after a 10-day period of acclimatization. The Hokkaido University School of Medicine Animal Care and Use Committee approved all procedures, which complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.2. Drugs

Sertraline hydrochloride (a gift from Pfizer Inc., USA), fluvoxamine maleate (a gift from Solvay Pharmaceuticals S.A., The Netherlands), and paroxetine hydrochloride (a gift from GlaxoSmithKline plc., UK) were used. Sertraline hydrochloride, fluvoxamine maleate and paroxetine hydrochloride were dissolved, respectively, in distilled water adding 2 drops of Tween 80 to achieve a final concentration of 10 mg/ml, 15 mg/ml and 5 mg/ml. Those were injected intraperitoneally (i.p.) as a volume of 2 ml/kg. The dosages of sertraline hydrochloride (20 mg/kg), fluvoxamine maleate (30 mg/kg), and paroxetine hydrochloride (10 mg/kg) were chosen based on earlier *in vivo* and *ex vivo* studies explained below. From the inhibitory potency (ED_{50}) of serotonin reuptake of sertraline, fluvoxamine, and paroxetine in human (9.1, 18.6, and 5.0 mg/day *in vivo*, respectively) (Suhara et al., 2003; Meyer et al., 2004) and rats (3.1, 10.4, and 1.9 mg/kg *ex vivo*, respectively) (Koe et al., 1983; Thomas et al., 1987), sertraline 20 mg/kg, fluvoxamine 30 mg/kg and paroxetine 10 mg/kg in rats are estimated, respectively, as equivalent to 59 mg/day, 54 mg/day, and 26 mg/day in human (clinical daily doses of sertraline, fluvoxamine, and paroxetine are, respectively, 50–200 mg/day, 50–250 mg/kg, and 20–60 mg/kg) (Bauer et al., 2002).

2.3. Experimental procedures

All experiments were performed with nonrestrained, freely moving rats. Sertraline (20 mg/kg), fluvoxamine (30 mg/kg), paroxetine (10 mg/kg), and distilled water with Tween 80 were administered i.p. 120 min after the first dialysate samples were collected. In every experiment, eight rats (two rats for each treatment group) were examined, with all experiments subsequently repeated. Extracellular serotonin, dopamine, and noradrenaline levels were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (Eicom Corp., Kyoto, Japan).

2.4. Microdialysis procedures

Experiments were performed according to a procedure described in a previous report (Kitaichi et al., 2008). Stereotaxically and under pentobarbital anesthesia (30 mg/kg i.p.), AG-4, AG-8, and AG-8 guide cannulae (Eicom Corp.) were implanted respectively into rats, leading to the surface of the medial prefrontal cortex, nucleus accumbens, and striatum at the following coordinates relative to the bregma from the stereotaxic atlas of Paxinos and Watson (1997): A + 3.2, ML + 0.8, DV + 1.0 mm; A + 1.7, ML + 0.8, + DV 6.0 mm; and A + 0.5, ML + 3.0, DV + 3.5 mm. Dialysis probes with 0.22 mm outer diameter (A-I-4-03, A-I-8-02, A-I-8-03; Eicom Corp.) were then inserted into the guide cannulae so that 3.0, 2.0, and 3.0 mm of the probes were exposed, respectively, to the medial prefrontal cortex, nucleus accumbens, and striatum tissues. Only one probe was implanted in each rat. Rats were housed individually after these operations. Experiments were performed in freely moving rats. On the next day, 24 h after surgery, perfusion was started using artificial cerebrospinal fluid (CSF) (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, and 1.0 mM $MgCl_2$) at a flow rate of 2 μl/min. Following initial perfusion for 2 h, dialysate samples were collected in sample vials containing 50 μl of 0.05 M acetic acid every 20 min for 460 min.

2.5. Analytical procedures for serotonin and dopamine

The HPLC system consisted of a liquid chromatograph pump (EP-300; Eicom Corp.), a degasser (DG-300; Eicom Corp.), a reverse-phase ODS column (Eicompak PP-ODS 30 4.6 mm; Eicom Corp.), an electrochemical detector (ECD-300; Eicom Corp.), and a data acquisition system (PowerChrom; AD Instruments Pty. Ltd., Sydney, Australia). For serotonin and dopamine analysis, 20 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 1% (v/v) methanol, 50 mg/l Na_2EDTA, and 500 mg/l sodium L-decanesulfonate. Separations were conducted at 25 °C with a flow rate of 0.5 ml/min. In the electrochemical detector, an oxidation potential was set at 400 mV. Standard solutions for serotonin and dopamine were injected every working day, and the peak heights for the standards were used for comparison to determine the amounts of serotonin and dopamine in the samples.

2.6. Analytical procedures for noradrenaline

To determine noradrenaline concentrations, we used identical equipment to that used for the serotonin and dopamine analysis, with the exception that a different reverse-phase ODS column (Eicompak CA-5ODS 150 2.1 mm; Eicom Corp.) was used. For noradrenaline analysis, 30 μl of dialysate was injected into the HPLC system that used a 0.1 M phosphate buffer (pH 6.0) mobile phase containing 5% (v/v) methanol, 50 mg/l Na_2EDTA and 500 mg/l L-octanesulfonic acid. Separations were conducted at 25 °C with a flow rate of 0.23 ml/min. The electrochemical detector was set at an oxidation potential of 550 mV. Noradrenaline standard solutions were injected every working day and the peak heights for the standard were used for comparison to determine the amount of noradrenaline in the samples.

2.7. Statistical analysis

All data are given as the mean values ± S.E.M. of individual rats from each group. The serotonin, dopamine, and noradrenaline contents of dialysate samples were expressed as absolute values (pg/fraction). To investigate the effects of sertraline, fluvoxamine, and paroxetine on extracellular serotonin, dopamine, and noradrenaline concentrations, repeated measures analysis of variance (ANOVA) for absolute values was used during the 0–360 min interval after SSRI administration. When the main and/or interaction effects were found to be significant, subsequent post-hoc comparisons (differences in absolute values measured at each time point of collection among four groups) were made using Duncan's test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg), and paroxetine (10 mg/kg) on extracellular serotonin, dopamine, and noradrenaline concentrations in the medial prefrontal cortex (Fig. 1)

Acute administration of sertraline, fluvoxamine, and paroxetine increased extracellular serotonin concentrations in the medial prefrontal cortex (Fig. 1A). Two-way ANOVA with repeated measures (0–360 min) indicated significant interaction between treatment and time [$F(54,540)=3.520$, $P<0.0001$] and a significant main effect of time [$F(18,540)=13.970$, $P<0.0001$] on extracellular serotonin concentrations. The main effect of treatment was nearly significant [$F(3,30)=2.875$, $P=0.0526$]. The sertraline, fluvoxamine, and paroxetine groups showed significantly higher concentrations of extracellular serotonin than the vehicle group (Duncan's test: sertraline group vs. vehicle group, 80–140 min, $P<0.05$; fluvoxamine group vs. vehicle group, 40–100 min, $P<0.01$; 120–180 min, $P<0.05$; paroxetine group vs. vehicle group, 40 min, $P<0.01$; 100–140 min, $P<0.05$). The administration of fluvoxamine and paroxetine increased extracellular serotonin levels significantly compared with that of sertraline (Duncan's test, 40 min $P<0.01$, respectively).

Acute administration of sertraline, fluvoxamine, and paroxetine had no effect on extracellular dopamine concentrations in the medial prefrontal cortex (Fig. 1B). Two-way ANOVA with repeated measures (0–360 min) indicated significant interaction between treatment and time [$F(54,540)=1.445$, $P=0.0243$] and a significant main effect of time [$F(18,540)=4.737$, $P<0.0001$]. The main effect of treatment was not significant [$F(3,30)=0.272$, $P=0.8448$]. No significant difference was found among groups for extracellular dopamine concentrations at any time point (Duncan's test).

As for extracellular noradrenaline concentrations (Fig. 1C), two-way ANOVA with repeated measures (0–360 min) indicated significant interaction between treatment and time [$F(54,540)=1.664$, $P=0.0029$] and a significant main effect of time [$F(18,540)=10.211$, $P<0.0001$]. The main effect of treatment was not significant [$F(3,30)=0.604$, $P=0.6173$]. No significant difference was found between any SSRI groups and the vehicle group on extracellular noradrenaline concentrations at any time point (Duncan's test). The paroxetine group showed significantly higher concentrations of extracellular noradrenaline than the sertraline group did (Duncan's test, 320 min, $P<0.05$).

3.2. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg), and paroxetine (10 mg/kg) on extracellular serotonin, dopamine, and noradrenaline concentrations in the nucleus accumbens (Fig. 2)

Acute administration of sertraline, fluvoxamine, and paroxetine increased extracellular serotonin concentrations in the nucleus accumbens (Fig. 2A). Two-way ANOVA with repeated measures (0–360 min) revealed significant main effects of treatment [$F(3,26)=19.159$, $P<0.0001$] and time [$F(18,468)=30.275$, $P<0.0001$] on extracellular

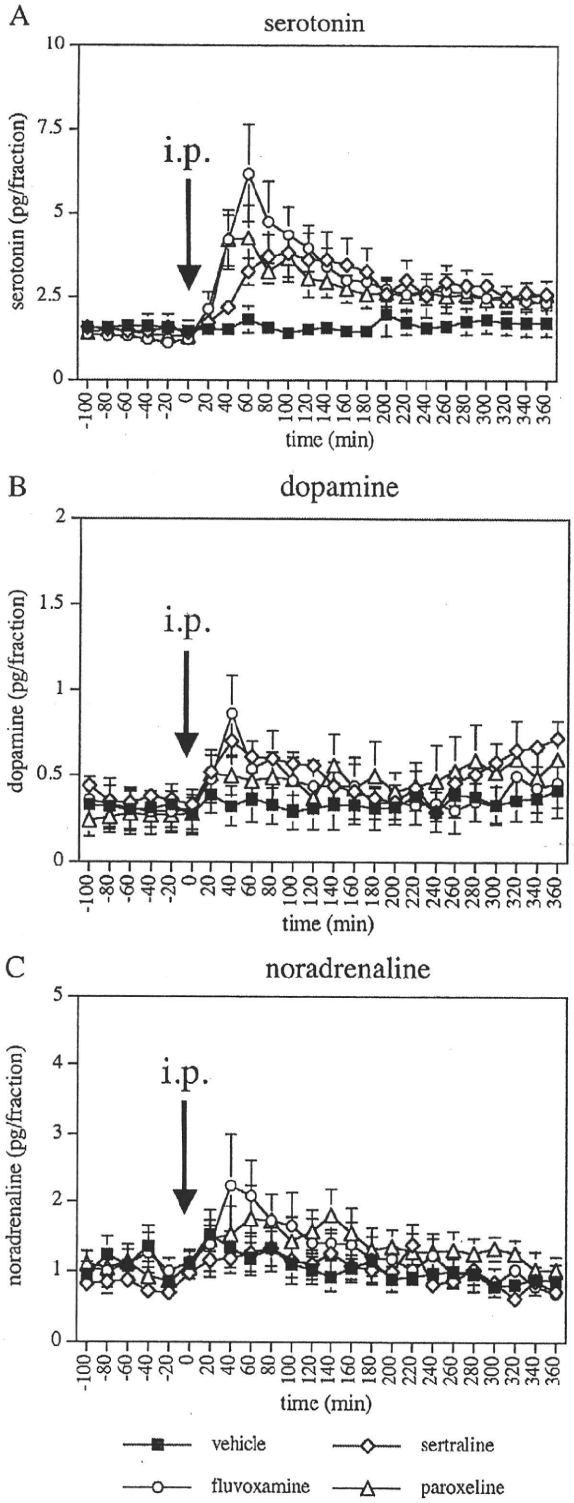
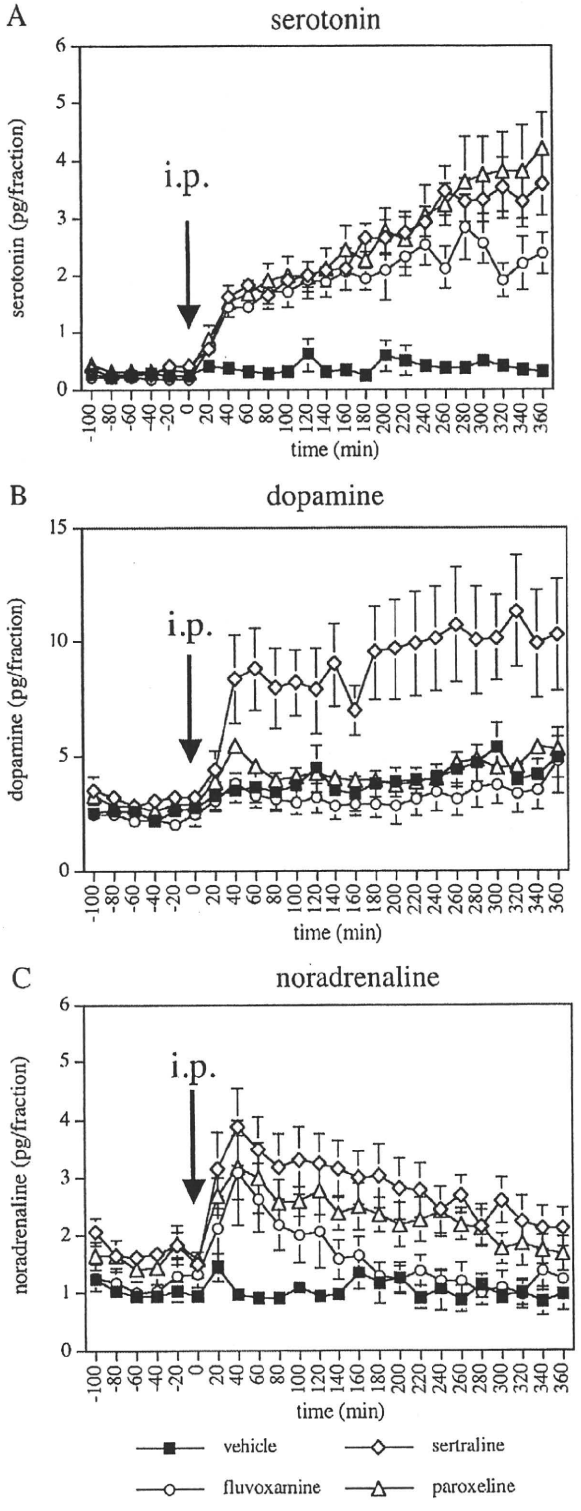


Fig. 1. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg) and paroxetine (10 mg/kg) on extracellular serotonin (A), dopamine (B), and noradrenaline (C) concentrations in the medial prefrontal cortex. Values represent the mean ± S.E.M. (pg/20 min fraction). $N=10$ (vehicle group), $N=9$ (sertraline group), $N=7$ (fluvoxamine group), $N=8$ (paroxetine group).Serotonin (A): the sertraline group vs. the vehicle group, 80–140 min, $P<0.05$; fluvoxamine group vs. vehicle group, 40–100 min, $P<0.01$; 120–180 min, $P<0.05$; paroxetine group vs. vehicle group, 40 min, $P<0.01$; 100–140 min, $P<0.05$; fluvoxamine group vs. sertraline group, 40 min $P<0.01$; paroxetine group vs. sertraline group, 40 min $P<0.01$.Dopamine (B): no significant difference between groups.Noradrenaline (C): paroxetine group vs. sertraline group, 320 min, $P<0.05$.

serotonin concentrations. Furthermore, the interaction of treatment and time was significant [$F(54,468)=4.611$, $P<0.0001$]. The sertraline, fluvoxamine, and paroxetine groups showed significantly higher concentrations of extracellular serotonin than the vehicle group did (Duncan's test: sertraline group vs. vehicle group, 80–360 min, $P<0.01$; fluvoxamine group vs. vehicle group, 40–300 min and 360 min, $P<0.01$, 320 and 340 min, $P<0.05$; paroxetine group vs. vehicle group, 40–360 min, $P<0.01$; 20 min, $P<0.05$). The administration of sertraline and paroxetine increased extracellular serotonin levels significantly more than fluvoxamine did (Duncan's test: sertraline group vs. fluvoxamine group, 320 min, $P<0.05$; paroxetine group vs. fluvoxamine group, 320 min, $P<0.01$, 340, and 360 min, $P<0.05$).



Acute administration of sertraline increased extracellular dopamine concentrations in the nucleus accumbens (Fig. 2B). Two-way ANOVA with repeated measures (0–360 min) indicated significant main effects of treatment [$F(3,27)=5.355$, $P=0.0050$] and time [$F(18,486)=9.120$, $P<0.0001$] on extracellular dopamine concentrations. In addition, the interaction of treatment and time was significant [$F(54,486)=2.911$, $P<0.0001$]. The administration of sertraline increased extracellular dopamine levels significantly more than vehicle, fluvoxamine, or paroxetine did (Duncan's test: sertraline group vs. vehicle group, 40–100 min, 140–260 min, 320 and 340 min, $P<0.01$; 280, 300, and 360 min, $P<0.05$; sertraline group vs. fluvoxamine group, 60–100 min and 140–340 min, $P<0.01$; 40, 120, and 360 min, $P<0.05$; sertraline group vs. paroxetine group, 100, 140, 180–260 min, 300, and 320 min, $P<0.01$, 60, 80, 160, 280, 340, and 360 min, $P<0.05$).

Acute administration of sertraline and paroxetine increased extracellular noradrenaline concentrations in the nucleus accumbens (Fig. 2C). Two-way ANOVA with repeated measures (0–360 min) indicated significant main effects of treatment [$F(3,27)=5.530$, $P=0.0043$] and time [$F(18,486)=14.878$, $P<0.0001$] on extracellular noradrenaline concentrations. In addition, the interaction of treatment and time was significant [$F(54,486)=2.568$, $P<0.0001$]. The administration of sertraline, fluvoxamine, and paroxetine increased extracellular noradrenaline levels significantly more than the vehicle did (Duncan's test: sertraline group vs. vehicle group, 40–220 min, 260, and 300 min, $P<0.01$, 20, 240, 280, and 320–360 min, $P<0.05$; fluvoxamine group vs. vehicle group, 40–80 min, $P<0.05$; paroxetine group vs. vehicle group, 60, 140, and 260 min, $P<0.01$, 40, 80–120 min, 160, 180, 220, 240, and 280 min, $P<0.05$). The administration of sertraline and paroxetine increased extracellular noradrenaline levels significantly more than fluvoxamine did (Duncan's test: sertraline group vs. fluvoxamine group, 180–220 min, 260, and 300 min, $P<0.01$, 100, 140, 160, 240, 280, and 320 min, $P<0.05$; paroxetine group vs. fluvoxamine group, 240–280 min, $P<0.05$).

3.3. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg), and paroxetine (10 mg/kg) on extracellular serotonin, dopamine, and noradrenaline concentrations in the striatum (Fig. 3)

Acute administration of sertraline, fluvoxamine, and paroxetine increased extracellular serotonin concentrations in the striatum (Fig. 3A). Two-way ANOVA with repeated measures (0–360 min) indicated significant main effects of treatment [$F(3,16)=5.336$, $P=0.0097$] and time [$F(18,288)=27.312$, $P<0.0001$] on extracellular

Fig. 2. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg), and paroxetine (10 mg/kg) on extracellular serotonin (A), dopamine (B), and noradrenaline (C) concentrations in the nucleus accumbens. Values represent the mean ± S.E.M. (pg/20 min fraction). $N=8$ (vehicle, sertraline, and fluvoxamine groups), $N=7$ (paroxetine group).Serotonin (A): sertraline group vs. vehicle group, 80–360 min, $P<0.01$; fluvoxamine group vs. vehicle group, 40–300 min and 360 min, $P<0.01$; 320 and 340 min, $P<0.05$; paroxetine group vs. vehicle group, 40–360 min, $P<0.01$; 20 min, $P<0.05$; sertraline group vs. fluvoxamine group, 320 min, $P<0.05$; paroxetine group vs. fluvoxamine group, 320 min, $P<0.01$; 340 and 360 min, $P<0.05$.Dopamine (B): sertraline group vs. vehicle group, 40–100 min, 140–260 min, 320, and 340 min, $P<0.01$; 280, 300, and 360 min, $P<0.05$; sertraline group vs. fluvoxamine group, 60–100 min and 140–340 min, $P<0.01$; 40, 120, and 360 min, $P<0.05$; sertraline group vs. paroxetine group, 100, 140, 180–260 min, 300, and 320 min, $P<0.01$; 60,80, 160, 280, 340, and 360 min, $P<0.05$.Noradrenaline (C): sertraline group vs. vehicle group, 40–220 min, 260, and 300 min, $P<0.01$; 20, 240, 280, and 320–360 min, $P<0.05$; fluvoxamine group vs. vehicle group, 40–80 min, $P<0.05$; paroxetine group vs. vehicle group, 60, 140, and 260 min, $P<0.01$; 40, 80–120 min, 160, 180, 220, 240, and 280 min, $P<0.05$; sertraline group vs. fluvoxamine group, 180–220 min, 260 and 300 min, $P<0.01$; 100, 140, 160, 240, 280, and 320 min, $P<0.05$; paroxetine group vs. fluvoxamine group, 240–280 min, $P<0.05$.

serotonin concentrations. In addition, the interaction of treatment and time was significant [$F(54,288)=5.184$, $P<0.0001$]. The sertraline, fluvoxamine, and paroxetine groups showed significantly higher concentrations of extracellular serotonin than the vehicle group did (Duncan's test: sertraline group vs. vehicle group, 260, 280, and 320–360 min, $P<0.01$; 40–80 min, 180–240 min, and 300 min, $P<0.05$; fluvoxamine group vs. vehicle group, 60–360 min, $P<0.01$; 40 min, $P<0.05$; paroxetine group vs. vehicle group, 320–360 min, $P<0.01$, 60, 80, 200, 260, and 280 min, $P<0.05$). The fluvoxamine group showed significantly higher concentrations of extracellular serotonin than the paroxetine group did (Duncan's test, 180 min, $P<0.05$).

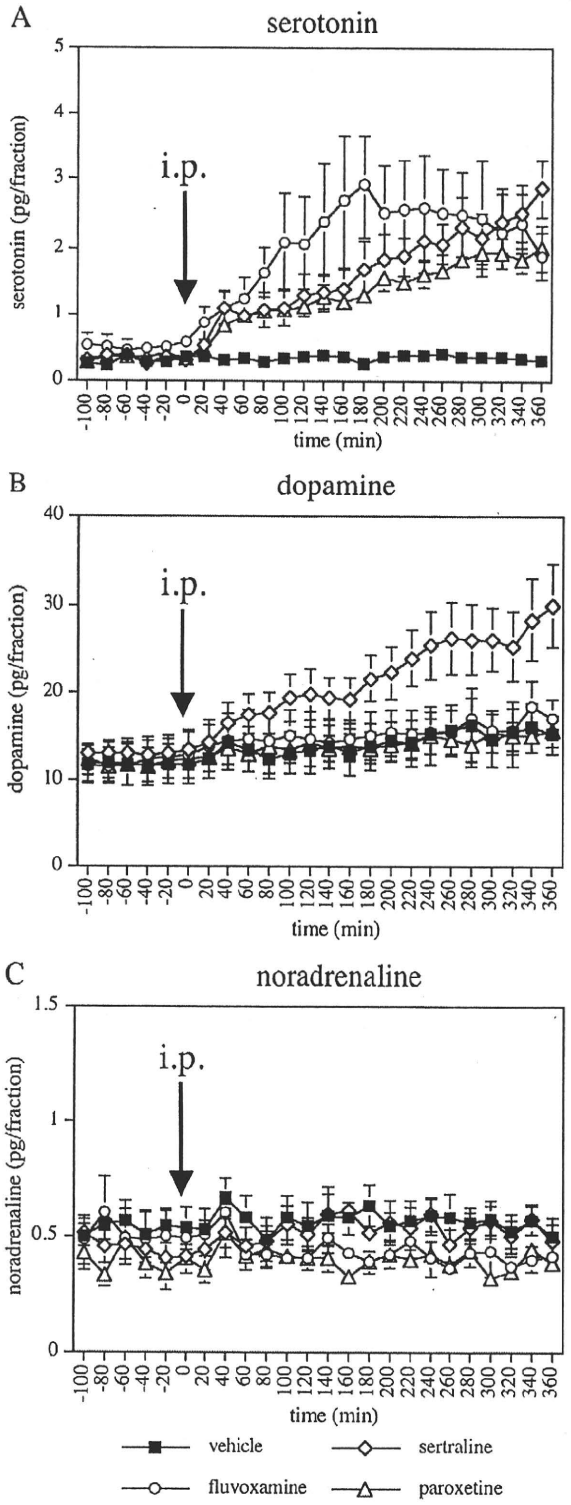


Fig. 3. Effects of sertraline (20 mg/kg), fluvoxamine (30 mg/kg), and paroxetine (10 mg/kg) on extracellular serotonin (A), dopamine (B), and noradrenaline (C) concentrations in the striatum. Values represent the mean ± S.E.M. (pg/20 min fraction). (A) and (B), $N=5$ (vehicle, sertraline, fluvoxamine, and paroxetine groups); (C), $N=9$ (vehicle, sertraline and paroxetine groups), $N=8$ (fluvoxamine group). Serotonin (A): sertraline group vs. vehicle group, 260, 280, and 320–360 min, $P<0.01$; 40–80 min, 180–240 min, and 300 min, $P<0.05$; fluvoxamine group vs. vehicle group, 60–360 min, $P<0.01$; 40 min, $P<0.05$; paroxetine group vs. vehicle group, 320–360 min, $P<0.01$; 60, 80, 200, 260, and 280 min, $P<0.05$; fluvoxamine group vs. paroxetine group, 180 min, $P<0.05$.Dopamine (B): sertraline group vs. vehicle group, 360 min, $P<0.01$; 180–260 min and 300–340 min, $P<0.05$; sertraline group vs. fluvoxamine group, 360 min, $P<0.01$; 220–260 min and 300–340 min, $P<0.05$; sertraline group vs. paroxetine group, 360 min, $P<0.01$; 180–340 min, $P<0.05$.Noradrenaline (C): no significant difference between groups.

Acute administration of sertraline increased extracellular dopamine concentrations in the striatum (Fig. 3B). Two-way ANOVA with repeated measures (0–360 min) indicated a significant interaction between treatment and time [$F(54,288)=4.877$, $P<0.0001$] and a significant main effect of time [$F(18,288)=19.813$, $P<0.0001$] on extracellular dopamine concentrations. The main effect of treatment was not significant [$F(3,16)=2.132$, $P=0.1363$]. The sertraline group showed significantly higher concentrations of extracellular dopamine than the vehicle, fluvoxamine, or paroxetine group did (Duncan's test: sertraline group vs. vehicle group, 360 min, $P<0.01$, 180–260 min and 300–340 min, $P<0.05$; sertraline group vs. fluvoxamine group, 360 min, $P<0.01$; 220–260 min and 300–340 min, $P<0.05$; sertraline group vs. paroxetine group, 360 min, $P<0.01$; 180–340 min, $P<0.05$).

Regarding extracellular noradrenaline levels in the striatum, no difference was found among groups (Fig. 3C). Two-way ANOVA with repeated measures (0–360 min) revealed neither a main effect of treatment [$F(3,31)=1.952$, $P=0.1418$] nor an interaction between treatment and time [$F(54,558)=1.005$, $P=0.4686$] on extracellular noradrenaline concentrations. Only a significant main effect of time was shown [$F(18,558)=1.911$, $P=0.0131$].

4. Discussion

All SSRIs (sertraline, fluvoxamine, and paroxetine) increased extracellular serotonin levels in all areas (medial prefrontal cortex, nucleus accumbens, and striatum) as results of serotonin reuptake inhibition. The inhibition of serotonin reuptake by these SSRIs seems approximately equal and sufficient *in vivo*. These results suggest that the dosages of three SSRIs derived from the *in vivo* and ex vivo experiments of human and rats are appropriate.

Only sertraline administration increased extracellular dopamine levels compared with vehicle, fluvoxamine, and paroxetine administration in the nucleus accumbens and striatum. This result is the first report of *in vivo* experiments, although it had been anticipated from the *in vitro* experiments. Fluvoxamine and paroxetine administrations had no effect on extracellular dopamine levels.

Previous reports described that sertraline has the moderate affinity for dopamine transporters (Sánchez and Hyttel, 1999; Owens et al., 2001) and has the ability of dopamine reuptake inhibition (Goodnick and Goldstein, 1998). It is likely that this dopamine reuptake inhibition by sertraline is one mechanism by which dopamine increases in the nucleus accumbens and striatum. Muneoka et al. (2009) reported a tendency for acute administration of sertraline to increase dopamine in the nucleus accumbens tissue, but that finding was not statistically significant. Two possibilities can be considered: administration of sertraline 5 mg/kg was too low when we considered a clinical dosage; and dopamine levels in tissues did not sharply reflect the pharmacological effect of dopamine reuptake inhibition. On the other hand, sertraline did not increase extracellular