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

Yuji Kitaichi[a,b,] Takeshi Inoue[b]*, Shin Nakagawa[b], Shuken Boku[b], Takeshi Izumi[c] and Tsukasa Koyama[b]

[a] Department of Molecular Stressphysiology, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, Munich 80804, Germany

[b] Department of Psychiatry, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan

[c] Department of Neuropharmacology, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan

*Correspondence address: Takeshi Inoue, M.D., Ph.D.

Phone: +81-11-706-5160, FAX:+81-11-706-5081, E-mail: tinoue@med.hokudai.ac.jp

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.

Keywords: MAO-A inhibitor, MAO-B inhibitor, β-phenylethylamine, noradrenaline, serotonin, in vivo microdialysis

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 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.) as 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_2EDTA 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 hr 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 (Figs. 1A and 1B)

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, $P<0.05$).

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 (Figs. 1C and 1D)

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 Ro41-1049 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 (2004) 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 (Figs. 1C and 1D). A study undertaken by Xie and Miller (2004) 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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Figure legends

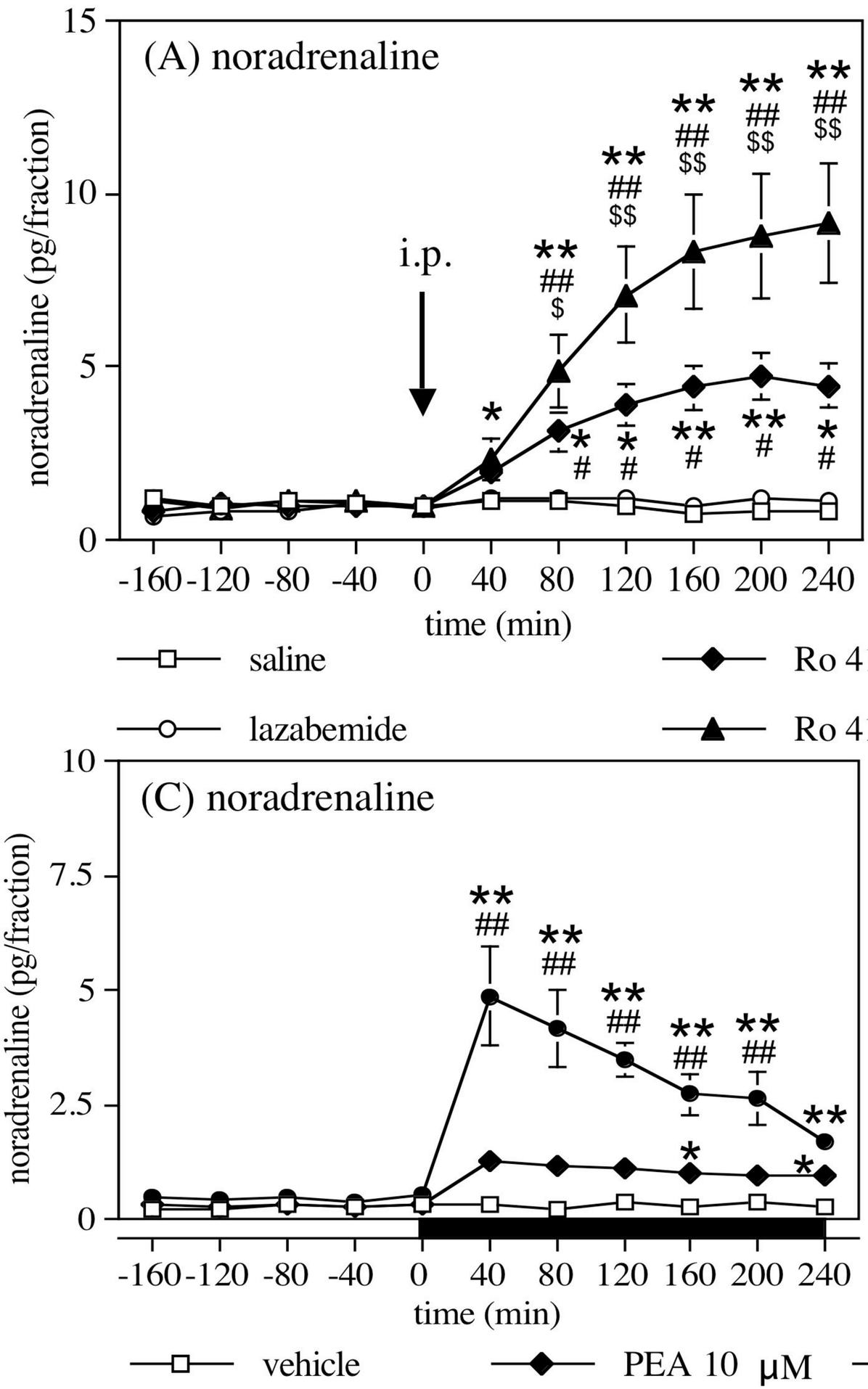
Fig. 1. (A and B) Effect of the combined treatment with acute Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg) on extracellular noradrenaline and serotonin concentrations during 0–240 min in the mPFC. Values represent the mean ± S.E.M. (pg/40 min fraction): Noradrenaline, *N*=14 (vehicle group), *N*=12 (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.

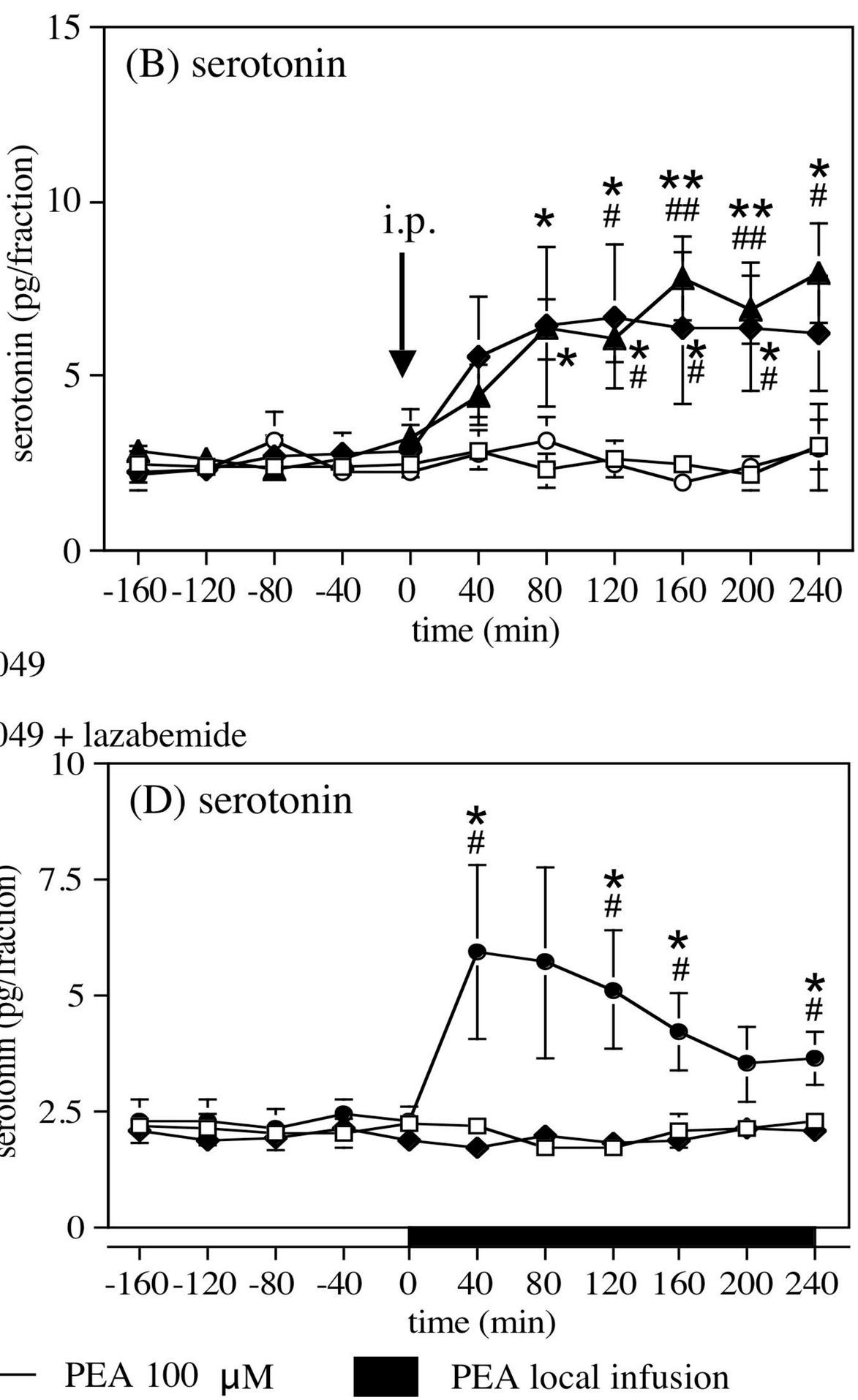
(C and D) Effect of local infusion of β-phenylethylamine (PEA) (0, 10, and 100 μmol/l) on extracellular noradrenaline and serotonin concentrations during 0–240 min in the mPFC. Values represent the mean ± S.E.M. (pg/40 min fraction): Noradrenaline, *N*=4 (vehicle group), *N*=6 (β-phenylethylamine 10 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[abc]

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.





saline — Ro 41-1049

lazabemide — Ro 41-1049 + lazabemide

vehicle — PEA 10 μM — PEA 100 μM — PEA local infusion