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European Journal of Pharmacology 406 (2000) 411–418

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Monoamine oxidase inhibitors reduce conditioned fear stress-induced freezing behavior in rats

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Received 31 August 2000; accepted 7 September 2000

Abstract

The present study examined the acute anxiolytic effects of monoamine oxidase inhibitors on freezing behavior, a putative index of anxiety induced by conditioned fear stress. The selective serotonin 1A receptor agonist tandospirone (0.1–10 mg/kg) inhibited freezing dose dependently. The irreversible, non-selective monoamine oxidase inhibitors tranylcypromine (3 and 15 mg/kg) and phenelzine (30 and 80 mg/kg) reduced freezing significantly. Clorgyline (10 mg/kg, irreversible selective monoamine oxidase A inhibitor), *N*-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazole carboxamide (Ro 41-1049) (30 mg/kg, reversible selective monoamine oxidase A inhibitor), selegiline (3 mg/kg, irreversible selective monoamine oxidase B inhibitor) and lazabemide (10 mg/kg, reversible selective monoamine oxidase B inhibitor) had no effect on freezing behavior. However, combined administration of clorgyline (10 mg/kg) and selegiline (3 mg/kg) reduced freezing significantly, as well as combined administration of clorgyline (10 mg/kg) and lazabemide (10 mg/kg), Ro 41-1049 (30 mg/kg) and selegiline (3 mg/kg), or Ro 41-1049 (30 mg/kg) and lazabemide (10 mg/kg). These effects of monoamine oxidase inhibitors on freezing were not due to non-specific motor effects. These results suggest that acute inhibition of both monoamine oxidase A and B reduced anxiety or fear, while inhibition of monoamine oxidase A or B alone failed to reduce anxiety or fear.

Keywords: Anxiety; Conditioned fear stress; Freezing behavior; Monoamine oxidase inhibitor

1. Introduction

Several lines of evidence have shown that monoamine oxidase inhibitors, a class of antidepressants, are effective in the treatment of various anxiety disorders. The clinical efficacy of monoamine oxidase inhibitors for social phobia has been investigated by several studies and has been confirmed by placebo-controlled double-blind studies (Liebowitz et al., 1992; Versiani et al., 1992). The clinical effects of monoamine oxidase inhibitors on other anxiety disorders are not established well compared with social phobia, but the anxiolytic effect of monoamine oxidase inhibitors has been reported for panic disorder, agoraphobia with panic attack and post-traumatic stress disorder (Sheehan et al., 1980; Frank et al., 1988; van Vliet et al., 1993).

In spite of the clinical effects of monoamine oxidase inhibitors on anxiety disorders, anxiolytic effects of monoamine oxidase inhibitors in animal models have not been proven. The conflict test, a classic animal model of anxiety, and the elevated plus-maze test are generally not useful to screen the anxiolytic effects of monoamine oxidase inhibitors (Chopin and Briley, 1987). Only one report showed that chronic, but not acute, treatment with monoamine oxidase inhibitors produces anxiolytic effects in a conflict test (Fontana et al., 1989). The conflict test and elevated plus-maze test were originally developed to screen the anxiolytic effect of benzodiazepines. Newer serotonergic anxiolytics, such as selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors, are also ineffective for these animal models of anxiety (Chopin and Briley, 1987). Because of this lack of suitable animal

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00706-8

models for the anxiolytic effects of monoamine oxidase inhibitors, examining the mechanism of the action of monoamine oxidase inhibitors as anxiolytics is difficult.

We reported that various serotonergic drugs, such as 5-hydroxytryptamine 1A (5-HT_{1A}) receptor agonists, selective 5-HT reuptake inhibitors and L-5-hydroxytryptophan (L-5-HTP), inhibit freezing behavior induced by conditioned fear (Hashimoto et al., 1996, 1997; Inoue et al., 1996a; Muraki et al., 1999). Our studies suggest that facilitation of 5-HT neurotransmission decreases anxiety because selective 5-HT reuptake inhibitors and L-5-HTP are assumed to increase the output from the 5-HT synapse. However, dopamine reuptake inhibitor or noradrenaline reuptake inhibitor was not anxiolytic in conditioned fear (Hashimoto et al., 1996). Accordingly, the conditioned fear model is useful to detect the anxiolytic potential of newer serotonergic drugs.

The mechanism of action of monoamine oxidase inhibitors as anxiolytics is not known. The effect of each monoamine oxidase inhibitor on the monoamines 5-HT, dopamine or noradrenaline might be associated with the anxiolytic effects as monoamine oxidase inhibitors inhibit the metabolism of these monoamines and increase extracellular concentrations of these monoamines (Butcher et al., 1990; Celada and Artigas, 1993; Finberg et al., 1993). Among monoamines, central 5-HT may have a key role and mediate anxiolytic effects of monoamine oxidase inhibitors because recent clinical and experimental evidence strongly suggests the 5-HT hypothesis of anxiety (Eriksson and Humble, 1990; Inoue et al., 1995, 1996a; Hashimoto et al., 1996). Thus, the anxiolytic effects of monoamine oxidase inhibitors may be detectable in conditioned fear.

This study examined the acute anxiolytic effects of monoamine oxidase inhibitors on freezing behavior, a putative index of anxiety, induced by conditioned fear stress (re-exposure to an environment paired previously with inescapable electric footshock). Monoamine oxidase (E.C. 1.4.3.4) has two forms, A-form and B-form, that deaminate different monoamines as substrates. Monoamine oxidase A deaminates dopamine, noradrenaline and 5-HT, and monoamine oxidase B deaminates dopamine (Green and Costain, 1981). In this study, the effects of selective monoamine oxidase A and B inhibitors, and non-selective monoamine oxidase inhibitors, were examined and compared in the conditioned fear model. Reversible monoamine oxidase inhibitors have been developed recently, and their pharmacological characteristics are well known (Da Prada et al., 1990). The effects of the reversible monoamine oxidase A inhibitor *N*-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazole carboxamide (Ro 41-1049) and the reversible monoamine oxidase B inhibitor lazabemide were compared with those of the irreversible monoamine oxidase A inhibitor clorgyline and the irreversible monoamine oxidase B inhibitor selegiline (Da Prada et al., 1990; Youdim and Finberg, 1991).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 230–250 g, were housed in groups of four and were maintained in a 12 h light–dark cycle (light phase: 06:30–18:30 h), temperature-controlled environment (22 ± 1°C) with free access to food and water. All experiments were made between 08:00 and 13:00 h. Experiments began after a 14-day period of acclimatization.

2.2. Drugs

Tandospirone (3aα, 4β, 7β, 7aα-hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4, 7-methano-1*H*-isoindole-1, 3(2*H*)-dione dihydrogen citrate) (Sumitomo Seiyaku, Japan) was dissolved in saline and injected subcutaneously (s.c.) as a volume of 1 ml/kg. Tranylcypromine hydrochloride (trans-2-phenyl-cyclopropylamine hydrochloride) (RBI, Natick, USA), phenelzine sulphate ((2-phenethyl)hydrazine sulphate) (Sigma, St. Louis, MO, USA), clorgyline hydrochloride (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine hydrochloride) (RBI), selegiline (*R*(−)-*N*, α-dimethyl-*N*-2-propynyl-benzeneethanamine HCl) (former L-deprenyl; RBI), Ro 41-1049 (RBI) and lazabemide (*N*-(2-aminoethyl)-5-chloro-2-pyridinecarboxamide hydrochloride) (F. Hoffman-La Roche, Switzerland) were dissolved in saline and were injected intraperitoneally (i.p.) as a volume of 1 ml/kg. The doses of the selective monoamine oxidase A and B inhibitors were chosen to fully and selectively inhibit monoamine oxidase A and B, respectively (Waldmeier and Felner, 1978; Da Prada et al., 1989, 1990; Celada and Artigas, 1993). The doses of the non-selective monoamine oxidase inhibitors were chosen to fully inhibit both monoamine oxidase A and B (Da Prada et al., 1989; Celada and Artigas, 1993).

2.3. Procedure of conditioned fear

As described previously (Inoue et al., 1996b), rats were individually subjected to inescapable electric footshock for a total of 2.5 min [five footshocks (2.5 mA scrambled shock, 30 s duration) that were delivered at intershock intervals of 35–85 s (mean 60 s)] in a shock chamber with a grid floor ($19 \times 22 \times 20$ cm, Medical Agent, Japan). Electric shocks were administered using a Model SGS-02D Shock Generator (Medical Agent) that produces a high-voltage, high-resistance circuit with the resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator gave a shock intensity of 0.2 mA to rats. Twenty-four hours after a single footshock session, rats were treated

with drugs or saline. Four hours (30 min for tandospirone) after the injection, the rats were placed in a shock chamber without shocks and were observed for 5 min (conditioned fear). Conditioned fear, as measured by freezing, develops from the contextual stimuli of the conditioned chamber with these procedures (Fanselow, 1980). During the observation period, the duration of freezing behavior was recorded using a modification of a time-sampling procedure (Fanselow, 1980) previously described by Inoue et al. (1996b). Freezing was defined as the absence of all observable movement of the skeleton and the vibrissae, except those related to respiration. All other behavior was scored as activity. The animal was classified as showing either freezing or active behavior according to its behavior throughout the entire 10-s period. The percentage score (% freezing) represented the number of 10-s periods the animal froze for the entire 10 s. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.4. Motor activity

Motor activity was measured at the representative doses of the drugs that significantly inhibited freezing. Rats were housed individually for 3 days before testing, and their motor activity in home cages was recorded as described by Ohmori et al. (1994) automatically for 10 min by electronic digital counters with infrared cell sensors. Horizontal movement was digitized and fed into a computer. Locomotion contributed predominantly to the count, but other body movements also contributed to the count when those movements contained substantial horizontal components. Drugs were administered i.p. 4 h before testing.

2.5. Data analysis

All the data are presented as the means ± S.E.M. of the individual values of the rats from each group. The statistical analysis of the data was performed using a one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. The combined treatment with two drugs was analyzed by two-way ANOVA.

3. Results

3.1. Effect of non-selective monoamine oxidase inhibitors on conditioned freezing

The selective 5-HT_{1A} receptor agonist tandospirone (0.1–10 mg/kg), a new anxiolytic, inhibited freezing dose dependently [$F(3,28) = 23.143$, $P = 0.0001$] (Fig. 1). Tandospirone at the doses of 1 and 10 mg/kg reduced conditioned freezing significantly. Irreversible, non-selective monoamine oxidase inhibitors, tranylcypromine (3–15 mg/kg) and phenelzine (10–80 mg/kg) inhibited freezing dose dependently [tranylcypromine, $F(2,21) = 30.461$, $P = 0.0001$; phenelzine, $F(3,28) = 31.556$, $P = 0.0001$] (Figs. 2 and 3). Tranylcypromine at doses 3–15 mg/kg and phenelzine at doses 30–80 mg/kg significantly reduced conditioned freezing.

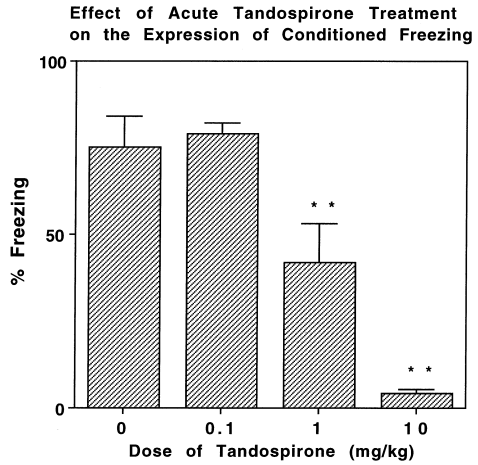

Fig. 1. Effect of the selective 5-HT_{1A} receptor agonist tandospirone on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with tandospirone or saline (s.c.). Thirty minutes after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. One-way ANOVA, $F(3,28) = 23.143$, $P = 0.0001$. $N = 8$. $^{**}P < 0.01$ vs. control.

3.2. Effect of combined treatment with monoamine oxidase A and B inhibitors

Combined administration of clorgyline (10 mg/kg), an irreversible selective monoamine oxidase A inhibitor, and selegiline (3 mg/kg), an irreversible selective monoamine oxidase B inhibitor, reduced freezing significantly, whereas either drug alone failed to change freezing (Fig. 4). Two-way ANOVA showed significant main effects of clorgyline and selegiline, and a significant interaction effect [effect of clorgyline, $F(1,28) = 14.091$, $P = 0.0008$; effect of selegiline, $F(1,28) = 14.88$, $P = 0.0006$; effect of interaction, $F(1,28) = 13.706$, $P = 0.0009$]. Post-hoc analysis showed that the combined treatment group significantly decreased freezing compared with clorgyline, selegiline and vehicle groups ($P < 0.01$), while clorgyline and selegiline groups did not decrease freezing compared with the controls.

Combined administration of Ro 41-1049 (30 mg/kg), a reversible selective monoamine oxidase A inhibitor, and lazabemide (10 mg/kg), a reversible selective monoamine oxidase B inhibitor, reduced freezing significantly, whereas

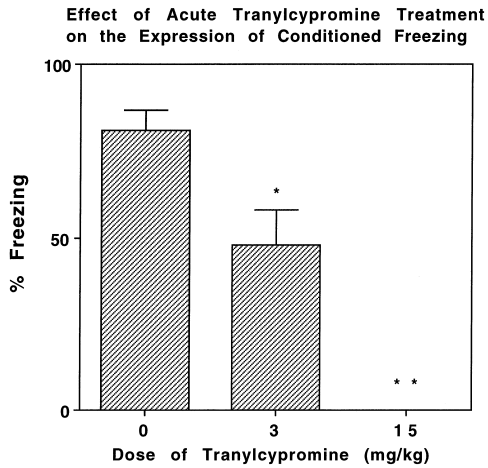

Fig. 2. Effect of the irreversible, non-selective monoamine oxidase inhibitor tranylcypromine on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with tranylcypromine or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. One-way ANOVA, $F(2,21) = 30.461$, $P = 0.0001$. $N = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

either drug alone failed to change freezing (Fig. 5). Two-way ANOVA showed no significant main effect of Ro 41-1049, but a significant main effect of lazabemide, and a significant interaction effect [effect of Ro 41-1049, $F(1,44) = 3.767$, $P = 0.0587$; effect of lazabemide, $F(1,44) = 11.759$, $P = 0.0013$; effect of interaction, $F(1,44) = 16.093$, $P = 0.0002$]. Post-hoc analysis showed that the combined treatment group significantly decreased

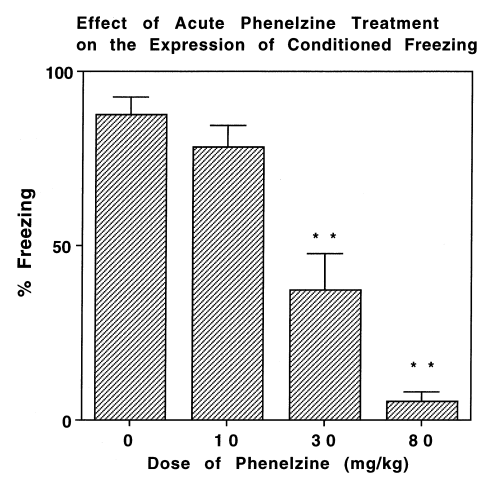

Fig. 3. Effect of the irreversible, non-selective monoamine oxidase inhibitor phenelzine on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with phenelzine or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. One-way ANOVA, $F(3,28) = 31.556$, $P = 0.0001$. $N = 8$. $^{**}P < 0.01$ vs. control.

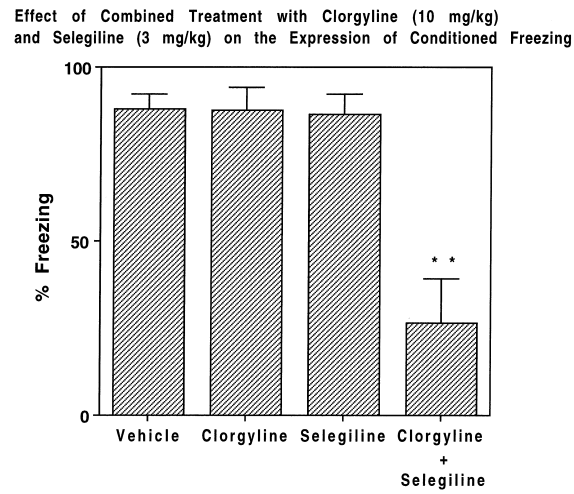

Fig. 4. Effect of combined treatment with the irreversible, selective monoamine oxidase A inhibitor clorgyline (10 mg/kg) and the irreversible, selective monoamine oxidase B inhibitor selegiline (3 mg/kg) on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with drugs or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. Two-way ANOVA, effect of clorgyline, $F(1,28) = 14.091$, $P = 0.0008$; effect of selegiline, $F(1,28) = 14.88$, $P = 0.0006$; effect of interaction, $F(1,28) = 13.706$, $P = 0.0009$. $N = 8$. $^{**}P < 0.01$ vs. vehicle, clorgyline and selegiline groups.

freezing compared with Ro 41-1049, lazabemide and vehicle groups ($P < 0.01$), while Ro 41-1049 and lazabemide

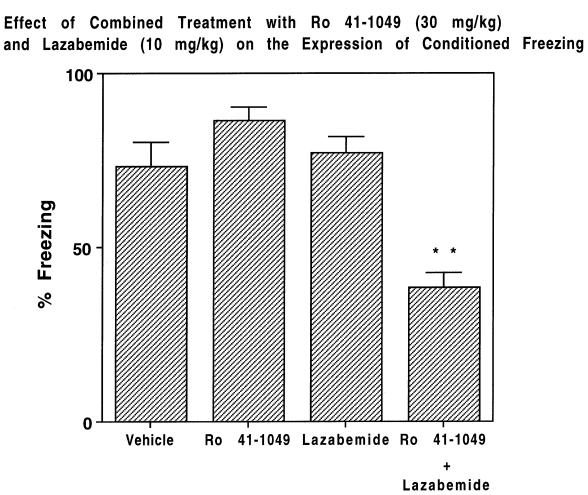

Fig. 5. Effect of combined treatment with the reversible, selective monoamine oxidase A inhibitor Ro 41-1049 (30 mg/kg) and the reversible, selective monoamine oxidase B inhibitor lazabemide (10 mg/kg) on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with drugs or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. Two-way ANOVA, effect of Ro 41-1049, $F(1,44) = 3.767$, $P = 0.0587$; effect of lazabemide, $F(1,44) = 11.759$, $P = 0.0013$; effect of interaction, $F(1,44) = 16.093$, $P = 0.0002$. $N = 8$ except for vehicle and lazabemide groups ($N = 16$). $^{**}P < 0.01$ vs. vehicle, Ro 41-1049 and lazabemide groups.

groups did not decrease freezing compared with the controls.

Combined administration of clorgyline (10 mg/kg) and lazabemide (10 mg/kg) reduced freezing significantly, whereas either drug alone failed to change freezing (Fig. 6). Two-way ANOVA showed significant main effects of clorgyline and lazabemide, and a significant interaction effect [effect of clorgyline, $F(1,28) = 17.195$, $P = 0.0003$; effect of lazabemide, $F(1,28) = 6.392$, $P = 0.0174$; effect of interaction, $F(1,28) = 16.777$, $P = 0.0003$]. Post-hoc analysis showed that the combined treatment group significantly decreased freezing compared with clorgyline, lazabemide and vehicle groups ($P < 0.01$), while clorgyline and lazabemide groups did not decrease freezing compared with the controls.

Combined administration of Ro 41-1049 (30 mg/kg) and selegiline (3 mg/kg) reduced freezing significantly, whereas either drug alone failed to change freezing (Fig. 7). Two-way ANOVA showed significant main effects of Ro 41-1049 and selegiline, and a significant interaction effect [effect of Ro 41-1049, $F(1,44) = 6.451$, $P = 0.0147$; effect of selegiline, $F(1,44) = 13.324$, $P = 0.0007$; effect of interaction, $F(1,44) = 10.819$, $P = 0.002$]. Post-hoc analysis showed that the combined treatment group significantly decreased freezing compared with Ro 41-1049, selegiline and vehicle groups ($P < 0.01$), while Ro 41-1049 and selegiline groups did not decrease freezing compared with the controls.

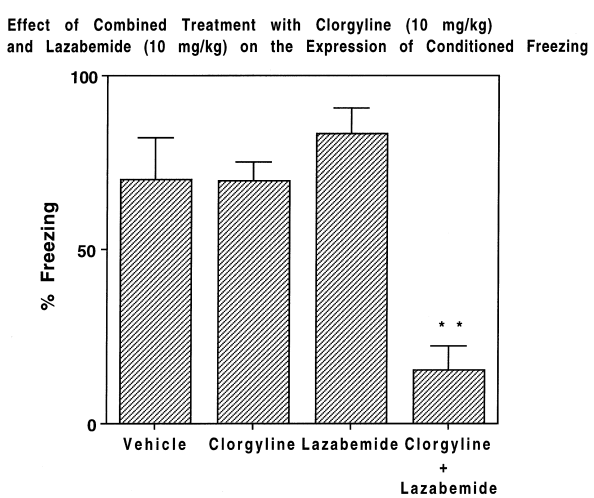

Fig. 6. Effect of combined treatment with the irreversible, selective monoamine oxidase A inhibitor clorgyline (10 mg/kg) and the reversible, selective monoamine oxidase B inhibitor lazabemide (10 mg/kg) on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with drugs or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. Two-way ANOVA, effect of clorgyline, $F(1,28) = 17.195$, $P = 0.0003$; effect of lazabemide, $F(1,28) = 6.392$, $P = 0.0174$; effect of interaction, $F(1,28) = 16.777$, $P = 0.0003$. $N = 8$. $^{**}P < 0.01$ vs. vehicle, clorgyline and lazabemide groups.

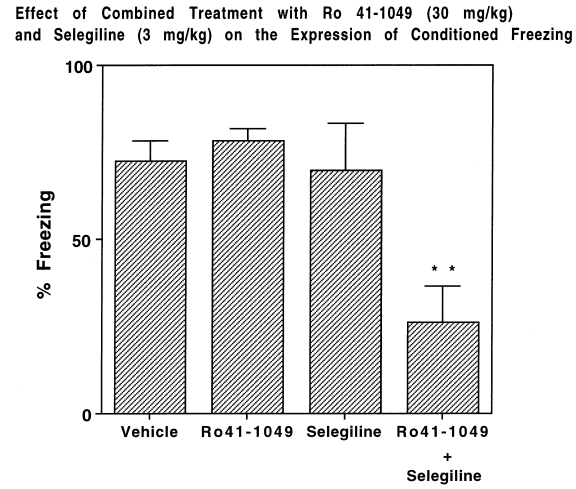

Fig. 7. Effect of combined treatment with the reversible, selective monoamine oxidase A inhibitor Ro 41-1049 (30 mg/kg) and the irreversible, selective monoamine oxidase B inhibitor selegiline (3 mg/kg) on the expression of conditioned freezing. Twenty-four hours after a single footshock session (2.5 mA for 5 min), rats were treated with drugs or saline (i.p.). Four hours after injection, the rats were placed in the shock chamber without shock and were observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. Two-way ANOVA, effect of Ro 41-1049, $F(1,44) = 6.451$, $P = 0.0147$; effect of selegiline, $F(1,44) = 13.324$, $P = 0.0007$; effect of interaction, $F(1,44) = 10.819$, $P = 0.002$. $N = 8$ except for vehicle and Ro 41-1049 groups ($N = 16$). $^{**}P < 0.01$ vs. vehicle, Ro 41-1049 and selegiline groups.

3.3. Effect of monoamine oxidase inhibitors on motor activity

Representative doses of drugs or any combination of drugs, which significantly inhibited freezing, did not increase or decrease motor activity [one-way ANOVA, $F(6,65) = 0.981$, $P = 0.4454$, $N = 8$ except for vehicle ($N = 24$)] (Fig. 8).

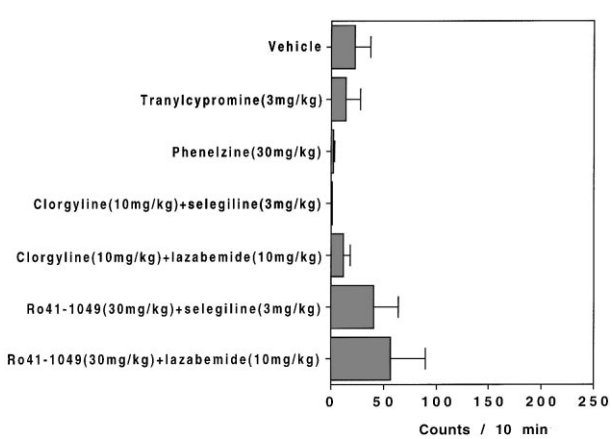

Fig. 8. Effect of monoamine oxidase inhibitors on motor activity (counts) for 10 min. Motor activity was counted 4 h after the injection of drugs. Represented are the mean counts ± S.E.M. of motor activity. One-way ANOVA, $F(6,65) = 0.981$, $P = 0.4454$. $N = 8$ except for vehicle ($N = 24$).

4. Discussion

In this study, non-selective monoamine oxidase inhibitors tranylcypromine and phenelzine, which inhibit both monoamine oxidase A and monoamine oxidase B, reduced conditioned freezing, a putative index of anxiety or fear, dose dependently. Effective doses of tranylcypromine (3 mg/kg) and phenelzine (30 mg/kg) for inhibiting conditioned freezing failed to affect motor activity in the home cages. Therefore, the reduction in freezing observed with these drugs appeared to be independent of any non-specific effect on motor activity at doses required to significantly reduce freezing. In the conditioned fear model, classic anxiolytic benzodiazepines and other newer serotonergic anxiolytics, such as selective 5-HT reuptake inhibitors and 5-HT_{1A} receptor agonists, significantly reduce the expression of conditioned freezing, i.e., anxiety or fear (Fanselow and Helmstetter, 1988; Rittenhouse et al., 1992; Hashimoto et al., 1996, 1997; Inoue et al., 1996a; Muraki et al., 1999). Consistent with our previous results (Inoue et al., 1996a), this study showed an anxiolytic effect of the selective 5-$HT1_A$ receptor agonist tandospirone (Hamik et al., 1990). Accordingly, the results of this study suggest anxiolytic effects of non-selective monoamine oxidase inhibitors in an animal model of anxiety. As described in Section 1, only few studies exist showing anxiolytic effects of monoamine oxidase inhibitors in animal models, while anxiolytic effects of monoamine oxidase inhibitors have been proven clinically. To our knowledge, this is the first report of non-selective monoamine oxidase inhibitors reducing conditioned freezing.

The question may arise as to whether monoamine oxidase A inhibition or monoamine oxidase B inhibition (or both) by non-selective monoamine oxidase inhibitors contributes to reducing conditioned freezing. In this study, selective monoamine oxidase A inhibitors or selective monoamine oxidase B inhibitors alone did not affect conditioned freezing at the doses used, which largely inhibit monoamine oxidase A or monoamine oxidase B, respectively (Waldmeier and Felner, 1978; Da Prada et al., 1989, 1990). Interestingly, the combined treatment with monoamine oxidase A and B inhibitors significantly decreased freezing, whether these monoamine oxidase inhibitors are reversible or irreversible. In this study, four combinations of monoamine oxidase inhibitors (clorgyline with selegiline, clorgyline with lazabemide, Ro 41-1049 with selegiline, and Ro 41-1049 with lazabemide) showed similar results reliably, suggesting that the anxiolytic effects of non-selective monoamine oxidase inhibitors in the conditioned fear model are mediated by the simultaneous inhibition of monoamine oxidase A and B.

In vivo microdialysis studies have showed that the irreversible non-selective monoamine oxidase inhibitor tranylcypromine increases extracellular 5-HT dramatically in the frontal cortex, whereas the irreversible monoamine oxidase A inhibitor clorgyline and the irreversible monoamine oxidase B inhibitor selegiline induces a slight increase and no increase, respectively (Celada and Artigas, 1993). Interestingly, the combined treatment with clorgyline and selegiline results in much larger increases in extracellular 5-HT in the frontal cortex than does either monoamine oxidase inhibitor alone (Celada and Artigas, 1993). Similar results were obtained with the combined administration of selegiline and the reversible monoamine oxidase A inhibitor brofaromine (Bel and Artigas, 1995). Our previous studies have indicated that facilitation of 5-HT neurotransmission decreases conditioned freezing, i.e., anxiety or fear (Hashimoto et al., 1996, 1997, 1999; Inoue et al., 1996a; Muraki et al., 1999). The results of these in vivo microdialysis studies may account for the results of this study of the simultaneous blockade of both monoamine oxidase A and B reducing conditioned freezing, whereas blockade of either monoamine oxidase alone failed.

Monoamine oxidase inhibitors increase extracellular concentrations of not only 5-HT, but also dopamine and noradrenaline in the brain (Butcher et al., 1990; Celada and Artigas, 1993; Finberg et al., 1993). Although previous studies suggested an important role of 5-HT for the anxiolytic effect of monoamine oxidase inhibitors on conditioned freezing (Hashimoto et al., 1996, 1999; Inoue et al., 1996a), other monoamines are also related to conditioned fear (Inoue et al., 1995). Accordingly, extracellular increases in noradrenaline or dopamine by monoamine oxidase inhibitors, or a combination of all three monoamines, may contribute to reducing conditioned freezing.

Among monoamine oxidase inhibitors used in this study, selegiline is metabolized in vivo to L-amphetamine and L-methamphetamine, weaker isomers than D-isomers (Reynolds et al., 1978). Increased brain levels of L-amphetamine and L-methamphetamine following selegiline administration may increase motor activity by increasing dopamine output, or may affect 5-HT release leading to decreased freezing (Kuczenski et al., 1995). The former possibility is excluded because selegiline with monoamine oxidase A inhibitors did not increase motor activity in this study. The latter possibility cannot be excluded, especially when selegiline is administered with clorgyline, which increases tissue 5-HT concentrations. However, the new monoamine oxidase B inhibitor lazabemide with monoamine oxidase A inhibitors also decreased conditioned freezing in this study. As lazabemide is not metabolized to amphetamine derivatives (Dingemanse et al., 1997), we conclude that monoamine oxidase B inhibition by selegiline and lazabemide contributes to the marked effect of combined treatment with monoamine oxidase A and B inhibitors on freezing.

Non-selective monoamine oxidase inhibitors (phenelzine) were first introduced to treat anxiety disorders (Sheehan et al., 1980; Frank et al., 1988; Liebowitz et al., 1992). Recently, reversible monoamine oxidase A in-

hibitors (RIMA; moclobemide and brofaromine) have been developed and used clinically, and have proven to be effective to treat various anxiety disorders (Versiani et al., 1992; van Vliet et al., 1993; Schneier et al., 1998). These clinical findings suggest that blockade of monoamine oxidase A is the main mechanism of the anxiolytic action of monoamine oxidase inhibitors. However, reversible monoamine oxidase A inhibitors examined in clinical trials were not completely selective for monoamine oxidase A inhibition, as moclobemide has active metabolites that block monoamine oxidase B (Da Prada et al., 1989), and brofaromine inhibits 5-HT reuptake (Waldmeier and Stocklin, 1989). Therefore, monoamine oxidase A inhibition alone producing anxiolytic effects has not been proven clinically. The results of this study using an animal model suggest that simultaneous blockade of both monoamine oxidase A and B produces an anxiolytic action, whereas selective monoamine oxidase A inhibitors are not anxiolytic. Further clinical and experimental research is necessary to clarify the mechanism of the anxiolytic action of monoamine oxidase inhibitors.

In conclusion, non-selective monoamine oxidase inhibitors reduced conditioned freezing significantly. Selective monoamine oxidase A or B inhibitors alone had no effect on freezing behavior. Combined administration of monoamine oxidase A and B inhibitors reduced freezing, whether these monoamine oxidase inhibitors were reversible or irreversible. These results suggest that acute inhibition of both monoamine oxidase A and monoamine oxidase B reduces anxiety or fear, while the inhibition of monoamine oxidase A or B alone fails to reduce anxiety or fear.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research No. 09470205 (T.K.) and No. 11670924 (T.I.) from the Japanese Ministry of Education, Science and Culture. The publishing cost of this article was supported by the memorial fund of the deceased psychiatrist Dr. Kozo Ito.

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