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Short communication

# Involvement of dopamine receptors in the anti-immobility effects of dopamine re-uptake inhibitors in the forced swimming test

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**Abstract**

The effects of dopamine re-uptake inhibitors, bupropion and nomifensine on immobility in the forced swimming test were studied in mice. Bupropion and nomifensine reduced immobility time dose-dependently. Both drugs significantly displayed anti-immobility effects at doses without altering locomotor activity. Anti-immobility effects of bupropion and nomifensine were inhibited by the dopamine D1 receptor antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-HCl (SCH 23390) and the dopamine D2 receptor antagonist sulpiride. These findings suggest that dopamine may be related to depression and dopamine D1 and dopamine D2 receptors play a role in the effects of dopamine re-uptake inhibitors.
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*Keywords:* Bupropion; Nomifensine; Dopamine D1 receptor; Dopamine D2 receptor; Antidepressant; Forced swimming test

## 1. Introduction

It is well recognized that depression is related to noradrenaline and serotonin (5-HT) and that both noradrenaline and/or 5-HT re-uptake inhibitors are available to improve depression (Blier and de Montigny, 1994; Briley and Moret, 1993). Selective serotonin re-uptake inhibitors (SSRIs) such as fluvoxamine, fluoxetine or paroxetine are useful to avoid affinity for muscarinic or histamine receptors, although classical tricyclic antidepressants (i.e., imipramine) have an affinity for these neurotransmitter receptors (Hyttel, 1994; Montgomery, 1990).

It was suggested that dopamine also participates in depression (Brown et al., 1993; Kapur and Mann, 1992). Dopamine is implicated in regulation of mood (Brown et al., 1993) and it was shown that, in the animal model of depression, brain extracellular dopamine levels are decreased (Rossetti et al., 1993). Recently, it has been considered that dopamine may be related to the effects of antidepressants (Joca et al., 2000; Maj and Rogoz, 1999). The forced swimming test established by Porsolt et al. (1977) is widely used to evaluate of antidepressants. It was reported that dopamine receptor agonists enhance antidepressant effects of animals in the forced swimming test (Joca et al., 2000; Maj and Rogoz, 1999). Moreover, dopamine D2 receptor agonists display antidepressant effects in humans (Shopsin and Gershon, 1978; Waehrens and Gerlach, 1981). It was reported that antisense dopamine D2 receptor administration inhibits antidepressant effects of imipramine (Dziedzicka-Wasylewska et al., 2000).

Bupropion and nomifensine are selective dopamine re-uptake inhibitors. Bupropion is clinically used in humans as an antidepressant or in therapy for withdraw of nicotine (Ascher et al., 1995; Martin et al., 1990; Richmond and Zwar, 2003). It was reported that bupropion reduces immobility time in the forced swimming test, while relatively high doses have been used to induce apparent anti-immobility effects in mice (Martin et al., 1990).

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However, the mechanisms for antidepressant effects of bupropion have not yet been fully clarified. Nomifensine is also a dopamine re-uptake inhibitor and shortens immobility time in rats (Hernando et al., 1994), although these effects of nomifensine on immobility have not been examined in detail. In the present paper, to clarify the involvement of dopamine on depression, we studied the effects of bupropion and nomifensine on the immobility time of mice in the forced swimming test and the involvement of dopamine receptors in the effects of bupropion and nomifensine.

## 2. Materials and methods

### 2.1. Animals

Male ddY mice weighing 25–30 g were purchased from SLC Japan (Japan). Mice were housed in the clear polycarbonate cages (22.5×33.8×14.0 cm) and in groups of five mice under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with room temperature at 23±1 °C and humidity at 55±5%. The mice were given free access to water and food pellets for mice and rats (F-2, Funahashi Farm, Japan). The experimental procedure was approved by the Kobe Pharmaceutical University Animal Care and Use Committee.

### 2.2. Drug treatment

Bupropion HCl (ICN, USA) and nomifensine HCl (Sigma, USA), *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-HCl (SCH 23390, Sigma) and sulpiride (Sigma) were used. Bupropion, nomifensine and SCH 23390 were, respectively, dissolved in saline. Sulpiride was dissolved in a few drops of 0.1 N HCl and diluted with saline. Bupropion and nomifensine were injected i.p. SCH 23390 and sulpiride were administered s.c. and i.p., respectively. SCH 23390 and sulpiride were injected 30 min before bupropion or nomifensine. Mice of control group received saline.

### 2.3. Forced swimming test

The forced swimming test was performed according to the methods described by Porsolt et al. (1977) and our previous report (Yamada and Sugimoto, 2001). Thirty minutes after the injections of bupropion and nomifensine, each mouse was placed in a 25-cm glass cylinder (10 cm diameter) containing 10 cm of water at 23±1 °C. Immobility was recorded during a 6-min swimming test. A mouse was judged as being in an immobile state, when the mouse floated and its hindlimbs appeared immobile with only small movements of the forepaws being made to keep the head above the water level. Observers of the experiments were not informed which drugs had been administered to each mouse.

### 2.4. Measurement of locomotor activity

Locomotor activity of mice was measured by a digital counter with an infrared sensor (NS-AS01, Neuroscience, Japan) following the method described in previous reports (Sugimoto et al., 2000; Narita et al., 2003). An infrared sensor was set over an open-top clear polycarbonate cage (22.5×33.8×14.0 cm) where each mouse was placed. The locomotor activity for 10 min was determined 30 min after injection of bupropion or nomifensine. The apparatus detects the movement of animals on the basis of released infrared rays associated with their temperature, and records a digital count.

### 2.5. Statistics

Dose-related effects of bupropion and nomifensine on immobility were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Other results were analyzed by two-way ANOVA followed by Tukey's test.

## 3. Results

### 3.1. Effects of bupropion and nomifensine on immobility time in the forced swimming test

Fig. 1A and B shows the effects of bupropion and nomifensine on immobility time. Both bupropion and nomifensine reduced immobility time dose-dependently. Bupropion at 10 mg/kg and nomifensine 2.5 mg/kg, which showed anti-immobility effects, did not affect locomotor activity (Fig. 1C).

### 3.2. Effects of SCH 23390 and sulpiride on bupropion- and nomifensine-induced anti-immobility effects

Fig. 2 shows the effects of SCH 23390 and sulpiride on bupropion 10 mg/kg- and nomifensine 2.5 mg/kg-induced anti-immobility effects. SCH 23390 significantly inhibited the anti-immobility effects of both bupropion and nomifensine. Sulpride dose-dependently antagonized the anti-immobility effects of bupropion and nomifensine. SCH 23390 and sulpiride did not affect the locomotor activity of mice treated with dopamine re-uptake inhibitors or saline (data is not shown).

## 4. Discussion

The present results demonstrate that dopamine re-uptake inhibitors bupropion and nomifensine reduced the

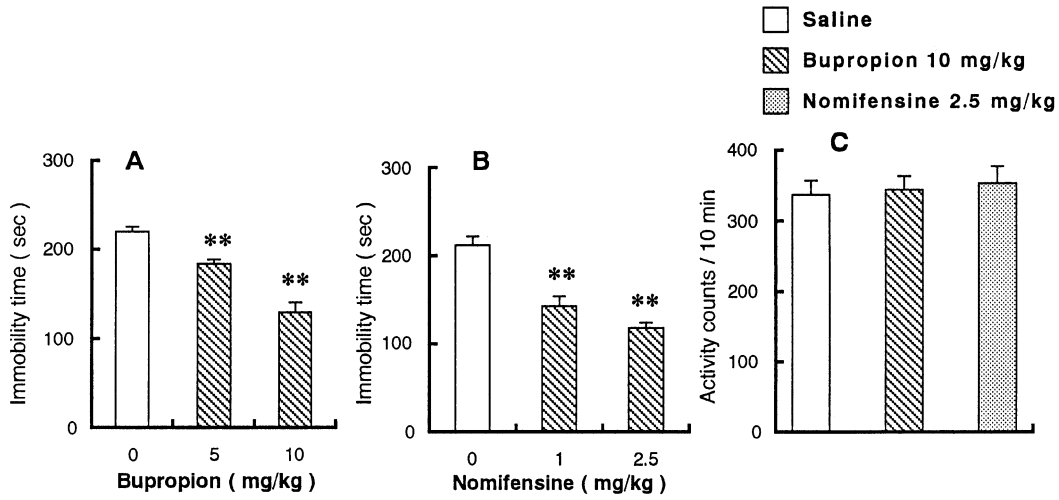


Fig. 1. Effects of bupropion and nomifensine on immobility in forced swimming test and locomotor activity in mice. (A) Effects of bupropion on immobility in forced swimming test. Results are shown as means±S.E. ($N$=5–7). Bupropion was given i.p. **$P$<0.01. (B) Effects of nomifensine on immobility in forced swimming test. Results are shown as means±S.E. ($N$=5–7). Nomifensine was given i.p. **$P$<0.01. (C) Effects of bupropion and nomifensine on locomotor activity in mice. Results are shown as means±S.E. ($N$=5). Bupropion at 10 mg/kg and nomifensine at 2.5 mg/kg were given i.p., respectively. Locomotor activity was measured 30 min after the injection of bupropion and nomifensine.

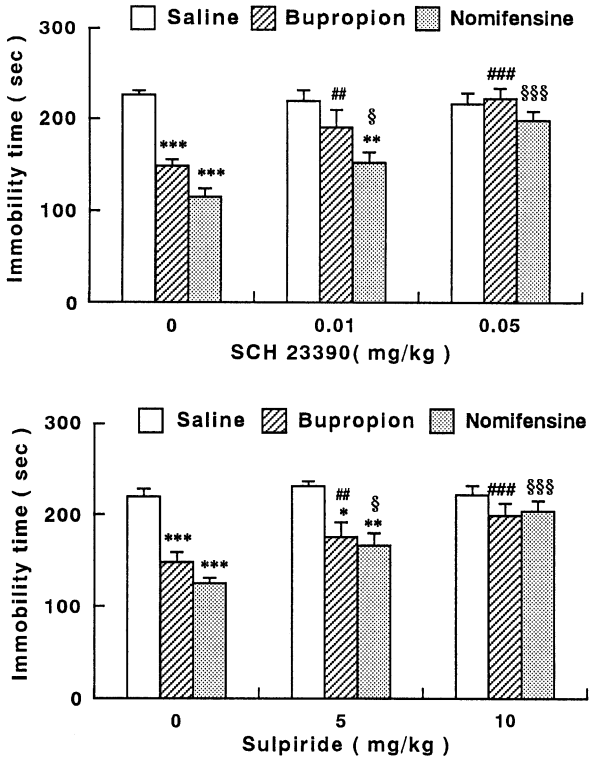


Fig. 2. Effects of SCH23390 and sulpiride on anti-immobility effects of bupropion and nomifensine. Results are shown as means±S.E. ($N$=5–7). Bupropion at 10 mg/kg and nomifensine at 2.5 mg/kg were administered i.p. SCH23390 and sulpiride were administered s.c. and i.p., respectively. SCH23390 and sulpiride were injected 30 min before bupropion or nomifensine. **$P$<0.01, ***$P$<0.0001 vs. respective saline-pretreated group. ##$P$<0.01, ###$P$<0.001 vs. saline+bupropion-treated group. §§$P$<0.01, §§§$P$<0.001 vs. saline+nomifensine-treated group.

immobility time of mice in the forced swimming test. It was previously reported that bupropion reduced the immobility time in mice (Martin et al., 1990), which agrees with the present findings. Inasmuch as the previous study by Martin et al. (1990) used higher doses of bupropion (32 mg/kg) than those in the present study, our findings demonstrated that a smaller dose of 10 mg/kg also induced anti-immobility effects. Inasmuch as they used a different strain of mice, the NMRI mouse, for their experiments on the anti-immobility effects of bupropion, strain differences may be involved in the effects of differing bupropion doses. Another dopamine re-uptake inhibitor, nomifensine also showed anti-immobility effects at a dose of 2.5 mg/kg, which is consistent with a previous study (Hernando et al., 1994). These results demonstrated that dopamine re-uptake inhibitors demonstrate antidepressant-like effects in the forced swimming test similar to that of other antidepressants (Porsolt et al., 1977; Yamada and Sugimoto, 2001, 2002) and that dopamine may participate in depression.

Bupropion has been reported to increase locomotor activity at higher doses (Ascher et al., 1995; Martin et al., 1990). However, in the present study, bupropion at a dose of 10 mg/kg, showed anti-immobility effects, but did not affect locomotion. Nomifensine at 2.5 mg/kg did not affect locomotor activity either, although this dose displayed apparent anti-immobility effects. Therefore, locomotion was not related to the anti-immobility effects of bupropion and nomifensine in our study.

The involvement of dopamine receptors in the anti-immobility effects of bupropion and nomifensine was

examined. As shown in the results, the selective dopamine D1 receptor antagonist SCH 23390 significantly antagonized the anti-immobility effects of bupropion. Anti-immobility effects of nomifensine were also antagonized by SCH 23390. It was reported that the dopamine D1 receptor agonist (±)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol (SKF 38393) enhances anti-immobility effects of SSRIs, including paroxetine or fluvoxamine, suggesting that the dopamine D1 receptor may participate in the antidepressant effects of SSRIs (Renard et al., 2001). The dopamine D1 receptor antagonist SCH 23390 inhibited both bupropion- and nomifensine-induced anti-immobility effects. It suggests that dopamine D1 receptor is involved in the anti-immobility effects of bupropion and nomifensine. Our results indicate that dopamine D1 receptor may play a role in depression.

Both the anti-immobility effects of bupropion and nomifensine were reduced by the dopamine D2 receptor antagonist sulpiride. Previous findings indicate that the anti-immobility effects of bupropion were antagonized by another dopamine D2 receptor antagonist, pimozide (Martin et al., 1990), which is consistent with the present findings using sulpiride. These findings suggest that the dopamine D2 receptor is involved in the anti-immobility effects of bupropion and nomifensine. It was also reported that the anti-immobility effects of imipramine, which inhibits both 5-HT and noradrenaline, were reduced by antisense dopamine D2 receptor (Dziedzicka-Wasylewska et al., 2000). In clinical studies, it was reported that the dopamine D2 receptor agonist is effective for treating depressive patients (Waehrens and Gerlach, 1981). Our results support the hypothesis that activation of dopamine D2 receptors may potentially relieve depression.

Co-administration of dopamine receptor antagonists SCH23390 and/or sulpiride with dopamine re-uptake inhibitors did not affect locomotor activity. The absence of changes in locomotion following administration of these drugs, suggests that the antagonistic effects of dopamine receptor antagonists on the anti-immobility effects of bupropion and nomifensine are not related to locomotor activity.

The present findings suggest that the anti-immobility effects of dopamine re-uptake inhibitors bupropion and nomifensine are mediated by dopamine D1 and D2 receptors. It was reported that bupropion and nomifensine can inhibit re-uptake noradrenaline in addition to dopamine (Ascher et al., 1995; Kunstmann et al., 1987). However, our results demonstrate that inhibitory effects of SCH 23390 and sulpiride on anti-immobility effects of bupropion and nomifensine were significant and reversed immobility to the control level. Therefore, it is suggested that dopamine more than noradrenaline contributes to the anti-immobility effects of bupropion and nomifensine.

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