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Buspirone Enhances Immobility in the Forced Swim Test in Mice

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KITAMURA, Y. AND T. NAGATANI. *Buspirone enhances immobility in the forced swim test in mice.* PHARMACOL BIOCHEM BEHAV **55**(3) 445–451, 1996.—We studied the effects of buspirone and 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) on duration of immobility in mice in the forced swim test. Buspirone [3–10 mg/kg, intraperitoneally (IP)] potently and dose dependently increased the duration of immobility in mice. In contrast, following a single dose of 8-OH-DPAT (1–3 mg/kg, IP), there was a dose-dependent decrease in the duration of immobility. Pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (200 mg/kg, IP, 3 days before further drug treatment) did not alter the effects of buspirone or 8-OH-DPAT. The increase in the duration of immobility induced by buspirone (3 mg/kg, IP) was blocked by NAN-190 [1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)-piperazine hydrobromide, 1 mg/kg, IP], a postsynaptic 5-HT_{1A} receptor antagonist. However, the effect of 8-OH-DPAT (1 mg/kg, IP) was not blocked by NAN-190 (1 mg/kg, IP). The effect of buspirone (3 mg/kg, IP) was blocked by apomorphine (0.3 mg/kg, IP), a dopamine receptor agonist. Based on the results of this study, it is suggested that the effects of buspirone and of 8-OH-DPAT on immobility in the forced swim test may occur through different mechanisms. **Copyright © 1996 Elsevier Science Inc.**

Buspirone 8-OH-DPAT Forced swim test 5-hydroxytryptamine (5-HT)$_{1A}$ receptor

IT has been suggested that buspirone [8-(4-[4-(2-pyrimidinyl)-1-piperaziyl]butyl)-8-azaspiro(4,5)decane-7,9-dion], a non-benzodiazepine anxiolytic (20) with high affinity for the 5-HT_{1A} receptor and dopamine 2 receptor (28), possesses the therapeutic properties of an antidepressant (21). Cervo and co-workers (5) reported that buspirone had no effect on immobility in rats in the forced swim test, either when injected systemically as a single dose or when given as a three-injection course over a 24-h period. In contrast, direct injection of buspirone into the dorsal raphe significantly reduced the duration of immobility. The anti-immobility effect of buspirone in the dorsal raphe was completely blocked by the injection of (−)-propranolol and (−)-pindolol in the same area, indicating that the effect of intraraphe buspirone on immobility may be due to activation of presynaptic 5-HT_{1A} receptors. Wieland and Lucki (27) found that subchronic treatment with buspirone significantly reduced the duration of immobility in rats. They also found that pretreatment with *p*-chlorophenylalanine did not alter the duration of immobility induced by tandospirone (an azapirone derivative similar to buspirone). This latter result indicates that the effect of buspirone may be exerted via postsynaptic 5-HT_{1A} receptors.

8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), a compound that has high affinity for 5-HT_{1A} and 5-HT_7 receptor (22,28), reduced immobility in mice in the forced swim test. Several studies have linked this effect of 8-OH-DPAT in the forced swim test to actions at both presynaptic and postsynaptic 5-HT_{1A} receptors (4,5,7,14,27). As stated above, Cervo and co-workers (5) reported that pretreatment with intraraphe (−)-propranolol or (−)-pindolol, 5-HT_{1A} receptor antagonists, before 8-OH-DPAT, completely antagonized the reduction of immobility induced by 8-OH-DPAT. The reduction in the duration of immobility produced by 8-OH-DPAT treatment was blocked by the administration of the 5-HT neurotoxin 5,7-dihydroxytryptamine (7). These effects of 8-OH-DPAT on immobility seem to be due to the activation of presynaptic 5-HT_{1A} receptors in the dorsal raphe. In contrast, Wieland and Lucki (27) reported that pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine, did not alter the duration of immobility, and that *p*-chlorophenylalanine did not attenuate the 8-OH-DPAT–induced reduction in immobility in rats. Luscombe and colleagues (14) found that selective destruction of 5-HT neurons with 5,7-dihydroxytryptamine or *p*-chlorophenylalanine did not change the effect of 8-OH-

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DPAT in the forced swim test in mice. These findings suggest that 8-OH-DPAT reduces the duration of immobility through its action at postsynaptic 5-HT_{1A} receptors.

Although these reports suggest that the effects on immobility in the forced swim test exerted by buspirone and by 8-OH-DPAT are mediated by pre- and/or postsynaptic 5-HT_{1A} receptors, there remain some discrepancies.

We performed the present study to examine the effects of buspirone and 8-OH-DPAT on immobility in mice in the forced swim test, and to determine whether 5-HT_{1A} receptors were involved in these effects.

METHODS

Animals

Male ICR mice (Charles River, Yokohama, Japan) weighing 25–35 g were used. They were housed in groups of 20 under controlled conditions of light (from 0700 to 1900 h), temperature (23 ± 2°C), and humidity (55 ± 15%). The animals were allowed free access to standard laboratory food and tapwater.

Drugs

The following drugs were used: buspirone hydrochloride (Sigma Chemical Co., St. Louis, MO), apomorphine hydrochloride (Sigma), *p*-chlorophenylalanine ethylester (Sigma), prazosin hydrochloride (Sigma), 8-OH-DPAT hydrobromide (Research Biochemicals Inc., South Natick, MA), NAN-190 [1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)-piperazine hydrobromide; Research Biochemicals Inc.], proadifen (SKF-525A hydrochloride; Research Biochemicals Inc.), (−)-sulpiride (Research Biochemicals Inc.), spiperone hydrochloride (Research Biochemicals Inc.), spiroxatrine (Research Biochemicals Inc.), 1-(2-pyrimidinyl)-piperazine dihydrochloride (1-PP; Aldrich Chemical Company, Inc., Milwaukee, WI), and haloperidol (Serenace injection; Dainippon Pharm., Tokyo, Japan). NAN-190 (−)-sulpiride, prazosin, spiperone, and spiroxatrine were suspended in 0.5% Tween 80 solution, and the other drugs were dissolved in saline on the day of testing. All agents were injected intraperitoneally (IP) in a volume of 10 ml/kg body wt.

Measurement of Immobility

To measure immobility, we essentially followed the method of Porsolt and colleagues (16). Mice were placed individually in plastic cylinders (height 24 cm, diameter 10 cm) containing 19 cm of water at 25°C. The total period of immobility was recorded with analysis program TARGET series/7M (Neuroscience Inc., Tokyo, Japan) and mice were assessed for immobility for 6 min. Buspirone, 8-OH-DPAT, and 1-PP were given 30 min before observation. NAN-190, apomorphine, (−)-sulpiride, spiperone, spiroxatrine, and prazosin were given 5 min before the injection of buspirone or 8-OH-DPAT. Proadifen was given 30 min before the injection of buspirone. To inhibit 5-HT neurotransmission, mice received three injections of the tryptophan hydroxylase inhibitor, *p*-chlorophenylalanine (200 mg/kg, IP), 72, 48, and 24 h before the test.

Locomotor Activity

Locomotor activity was measured in a drum-shaped cage (diameter 23 cm, width 6 cm) made with thin stainless-steel bars. A mouse was placed in the turning cage, which turned when the mouse moved. Drug-treated mice (30 min after the administration of buspirone and 8-OH-DPAT) were placed into the turning cage and the number of turns they made in 6 min was counted.

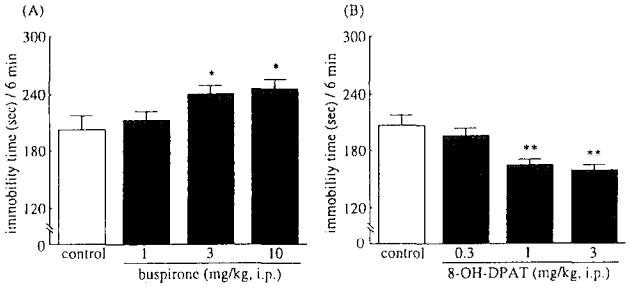

FIG. 1. Effects of buspirone (A) and 8-OH-DPAT (B) on the duration of immobility of mice in the forced swim test. Buspirone and 8-OH-DPAT were given 30 min before the test. Values are expressed as the means ± SEM of eight to 16 animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test. $*p < 0.05$; $**p < 0.01$, significant difference from the control value.

Statistics

Values are expressed as group means and SEM. The data were analyzed by one-way analysis of variance (ANOVA), and posthoc comparison of means was carried out with Dunnett's test or Tukey's test for multiple comparisons.

RESULTS

Figure 1 shows the effects of treatment with buspirone and 8-OH-DPAT on the duration of immobility of mice in the forced swim test. Buspirone (3–10 mg/kg, IP) potently and dose-dependently increased the duration of immobility [$F(3, 51) = 3.53, p < 0.05$] (Fig. 1A), while 8-OH-DPAT (administered 1–3 mg/kg, IP) significantly decreased the duration of immobility [$F(3, 52) = 7.18, p < 0.05$] (Fig. 1B). The effects of buspirone and 8-OH-DPAT on the ambulation of mice as measured in the drum-shaped cage are shown in Table 1. Buspirone, at a dose of 10 mg/kg, IP, significantly reduced locomotor activity. 8-OH-DPAT (1 mg/kg, IP) did not change locomotor activity, but at the dose of 3 mg/kg, it decreased ambulation [$F(4, 45) = 6.29, p < 0.01$]. Figure 2 shows that the effects of buspirone (3 mg/kg, IP) [$F(3, 28) = 3.56, p <$

TABLE 1

EFFECTS OF BUSPIRONE AND 8-OH-DPAT ON THE NUMBER OF TURNS IN THE TURNING CAGE IN MICE

Drug	Dose (mg/kg)	No. of Turns
Control		88.5 ± 4.1
Buspirone	3	97.4 ± 3.5
	10	60.4 ± 7.0*
8-OH-DPAT	1	80.4 ± 8.5
	3	52.1 ± 9.6*

Buspirone and 8-OH-DPAT were given 30 min before the test. Values are expressed as the means ± SEM of 10 animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test.

*$p < 0.05$, significant difference from the control value.

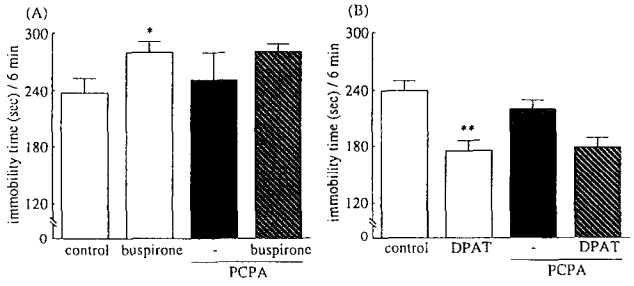

FIG. 2. Effects of buspirone (A) and 8-OH-DPAT (DPAT) (B) on the duration of immobility in the forced swim test in *p*-chlorophenylalanine-treated mice. *p*-Chlorophenylalanine (200 mg/kg, IP, 3 days) was given 72, 48, and 24 h before the test. Buspirone (3 mg/kg, IP) and 8-OH-DPAT (1 mg/kg, IP) were given 30 min before the test. Values are expressed as the means ± SEM of eight to 16 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. $*p < 0.05$; $**p < 0.01$, significant difference from the control value.

TABLE 3

INFLUENCE OF PROADIFEN ON THE IMMOBILITY-ENHANCING EFFECT OF BUSPIRONE IN MICE IN THE FORCED SWIM TEST

Drug	Immobility Time (s)
Control	231.1 ± 11.2
Buspirone	266.5 ± 10.5*
Proadifen	213.2 ± 15.8
Buspirone + proadifen	271.2 ± 11.9

Proadifen (50 mgkg, IP) was given 30 min before the injection of buspirone. Buspirone (3 mg/kg, IP) was given 30 min before the test. Values are expressed as the means ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Tukey's test.

$*p < 0.05$, significant difference from the control value.

0.05] and 8-OH-DPAT (1 mg/kg, IP) [$F(3, 52) = 9.53$, $p < 0.01$] on the duration of immobility in the forced swim test were not blocked by pretreatment with *p*-chlorophenylalanine (200 mg/kg, IP, 3 days). Further, *p*-chlorophenylalanine alone did not modify the duration of immobility.

Table 2 shows that 1-PP (1–10 mg/kg, IP), the major metabolite of buspirone, had no effect on duration of immobility in the forced swim test [$F(3, 28) = 1.43$, $p < 0.05$]. Proadifen, which inhibits the formation of 1-PP from buspirone, did not affect the buspirone-induced increase in the duration of immobility in the forced swim test [$F(3, 28) = 4.96$, $p < 0.01$] (Table 3). Figure 3A illustrates that NAN-190 (1 mg/kg, IP), the postsynaptic 5-HT_{1A}-receptor antagonist, blocked the effect of buspirone (3 mg/kg, IP) [$F(3, 28) = 5.40$, $p < 0.01$] but did not inhibit the effect of 8-OH-DPAT (1 mg/kg, IP) [$F(3, 28) = 7.38$, $p < 0.01$] (Fig. 3B).

Table 4 shows that the 5-HT_{1A}-receptor antagonists spiperone (0.03 mg/kg, IP) [$F(3, 28) = 11.54$, $p < 0.01$] and spiroxatrine (0.03 mg/kg, IP) [$F(3, 51) = 4.71$, $p < 0.01$] antagonized the effect of 8-OH-DPAT (1 mg/kg, IP). Figure 4 illustrates the effect of apomorphine, a dopamine receptor agonist, on the immobility-enhancing effect of buspirone in the forced swim test. Apomorphine (0.3 mg/kg, IP) antagonized the effect of buspirone (3 mg/kg, IP) [$F(3, 39) = 3.13$, $p < 0.05$]. The effect of 8-OH-DPAT (1 mg/kg, IP) was blocked by the dopamine D_2-receptor antagonist (−)-sulpiride (3 mg/kg, IP) [$F(3, 28) = 7.77$, $p < 0.01$] (Fig. 5).

TABLE 2

EFFECTS OF 1-(2-PYRIMIDINYL)-PIPERAZINE (1-PP) ON THE DURATION OF IMMOBILITY OF MICE IN THE FORCED SWIM TEST

Drug	Dose (mg/kg)	Immobility Time (s)
Control		226.4 ± 15.8
1-PP	1	233.7 ± 6.6
	3	235.9 ± 5.8
	10	231.5 ± 13.9

1-PP was given 30 min before the test. Values are expressed as the mean ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test.

Table 5 shows that the α_1-adrenoceptor antagonist, prazosin (0.3 mg/kg, IP) did not antagonize the effect of buspirone (3 mg/kg, IP) [$F(3, 28) = 4.49$, $p < 0.05$].

Table 6 shows the effects of treatment with the 5-HT_{1A}-receptor antagonists and other drugs on duration of immobility in mice. NAN-190 (0.3–3 mg/kg, IP) [$F(3, 28) = 0.33$, $p < 0.1$] did not affect the duration of immobility. Spiperone (0.01–0.03 mg/kg, IP) [$F(3, 36) = 4.60$, $p < 0.01$] and spiroxatrine (0.03 mg/kg, IP) [$F(2, 21) = 3.49$, $p < 0.05$] also did not affect the duration of immobility. However, spiperone and spiroxatrine increased the duration of immobility at 0.1 mg/kg, IP. Although 0.3 mg/kg of apomorphine [$F(3, 31) = 35.76$, $p < 0.01$] did not affect the duration of immobility, it potently decreased the duration at doses > 1 mg/kg, IP. (−)-Sulpiride (3 mg/kg, IP) [$F(2, 21) = 3.97$, $p < 0.05$] and haloperidol (0.3 mg/kg, IP) increased the duration of immobility [$F(3, 28) = 34.04$, $p < 0.05$]. Prazosin (0.3 mg/kg, IP) [$F(2, 21) = 4.30$, $p < 0.05$] did not affect the duration of immobility, but it increased the duration at 1 mg/kg (Table 6).

Table 7 shows that the immobility-decreasing effect of 1 mg/kg 8-OH-DPAT was blocked by buspirone (1 mg/kg, IP) [$F(3, 28) = 3.09$, $p < 0.05$]. This dose (1 mg/kg, IP) of buspirone

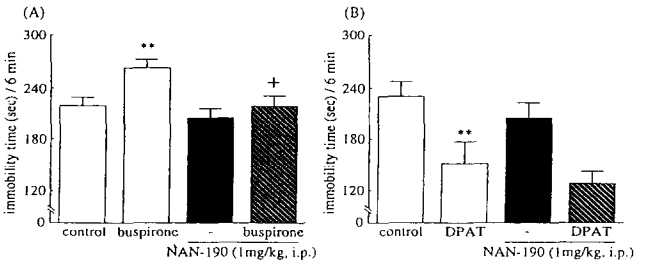

FIG. 3. Effect of NAN-190 on the effects of buspirone (A) and 8-OH-DPAT (DPAT) (B) on the duration of immobility of mice in the forced swim test. NAN-190 (1 mg/kg, IP) was given 5 min before the injection of buspirone (3 mg/kg, IP) and 8-OH-DPAT (1 mg/kg, IP). Buspirone (3 mg/kg, IP) and 8-OH-DPAT (1 mg/kg, IP) were given 30 min before the test. Values are expressed as the means ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. $**p < 0.01$, significant difference from the control value. $^+p < 0.05$, significant difference from the value for buspirone-treated mice.

TABLE 4

EFFECTS OF SPIPERONE AND SPIROXATRINE ON THE IMMOBILITY-DECREASING EFFECT OF 8-OH-DPAT IN MICE IN THE FORCED SWIM TEST

Drug	Immobility Time (s)
Control	224.2 ± 6.2
8-OH-DPAT	195.8 ± 12.2*
Spiperone	237.8 ± 10.9
8-OH-DPAT + spiperone	233.3 ± 11.7†
Control	224.3 ± 12.7
8-OH-DPAT	176.1 ± 11.2*
Spiroxatrine	248.0 ± 8.7
8-OH-DPAT + spiroxatrine	254.44 ± 8.8†

Spiperone (0.03 mg/kg, IP) and spiroxatrine (0.03 mg/kg, IP) were given 5 min before the injection of 8-OH-DPAT. 8-OH-DPAT (1 mg/kg, IP) was given 30 min before the test. Values are expressed as the means ± SEM of eight to 16 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test.

*$p < 0.05$, significant difference from the control value.

†$p < 0.05$, significant difference from the value for 8-OH-DPAT–treated mice.

did not affect the duration of immobility in the forced swim test when administered alone.

DISCUSSION

In this study, we examined the influence of buspirone and 8-OH-DPAT on the immobility of mice in the forced swim test. Buspirone (3–30 mg/kg, IP) dose-dependently increased the duration of immobility, and 8-OH-DPAT (1–3 mg/kg, IP) decreased the duration of immobility. The effect of 8-OH-DPAT on immobility is in accord with results in earlier reports (4,5,7,14,27). However, buspirone clearly increased the duration of immobility, and this finding was different from results in earlier studies. For example, Wieland and Lucki (27) reported that buspirone [10 mg/kg, subcutaneously (SC)] administered 0.5, 19, and 23 h after the pretest in rats decreased the duration of immobility. Further, Cervo and co-workers (5) reported that a single injection of buspirone, at doses ranging from 0.1 to 10 mg/kg (SC), had no effect on duration of immobility in rats. Przegaliński and colleagues (18) also reported that buspirone administered as a single dose (5–20 mg/kg, IP), or repeatedly three times, did not affect the duration of immobility in rats. It is difficult to explain why buspirone enhanced immobility in the forced swim test in mice in our present study. Differences in the number of injections, the route of injection, the animal species (mouse or rat), or differences in the experimental devices used could be reflected in the experimental results.

To investigate the possibility that the changes in immobility were associated with changes in locomotor activity, we examined the effect of buspirone and 8-OH-DPAT on locomotor activity in mice in a drum-shaped cage. The high dose of buspirone (10 mg/kg) decreased locomotor activity. This suggests that a decrease in locomotor activity may have contributed to the buspirone-induced increase in the duration of immobility in the forced swim test. However, buspirone did not affect spontaneous activity doses < 3 mg/kg, IP. Accordingly, at doses < 3 mg/kg, buspirone seemed to exert its effect on immobility without affecting spontaneuos motor activity. In contrast, 8-OH-DPAT (3 mg/kg, IP) decreased locomotor activity. It is unlikely that the tendency for 8-OH-DPAT to reduce immobility in the forced swim test relates to the drug's effect on locomotor activity. One would expect that a drug which decreases immobility would enhance locomotor activity.

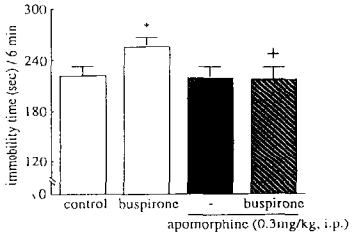

FIG. 4. Effect of apomorphine on the immobility-enhancing effect of buspirone in mice in the forced swim test. Apomorphine (0.3 mg/kg, IP) was given 5 min before the injection of buspirone (3 mg/kg, IP). Buspirone (3 mg/kg, IP) was given 30 min before the test. Values are expressed as the means ± SEM of eight to 16 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. *$p < 0.05$, significant difference from the control value. †$p < 0.05$, significant difference from the value for buspirone-treated mice.

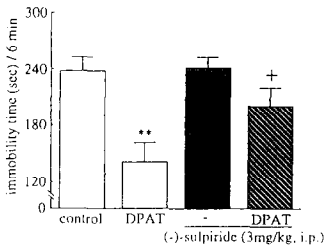

FIG. 5. Effect of (−)-sulpiride on the immobility-decreasing effect of 8-OH-DPAT (DPAT) in mice in the forced swim test. (−)-Sulpiride (3 mg/kg, IP) was given 5 min before the injection of 8-OH-DPAT (1 mg/kg, IP). 8-OH-DPAT (1 mg/kg, IP) was given 30 min before the test. Values are expressed as the means ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. **$p < 0.01$, significant difference from the control value. †$p < 0.05$, significant difference from the value for 8-OH-DPAT-treated mice.

TABLE 5

EFFECT OF PRAZOSIN ON THE IMMOBILITY-ENHANCING EFFECT OF BUSPIRONE IN MICE IN THE FORCED SWIM TEST

Drug	Immobility Time (s)
Control	227.0 ± 12.5
Buspirone	273.4 ± 6.4*
Prazosin	208.0 ± 21.4
Buspirone + prazosin	254.7 ± 9.6

Prazosin (0.3 mg/kg, IP) was given 30 min before the injection of buspirone. Buspirone (3 mg/kg, IP) was given 30 min before the test. Values are expressed as the means ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Tukey's test.

*$p < 0.05$, significant difference from the control value.

TABLE 6

EFFECTS OF 5-HT$_{1A}$ RECEPTOR ANTAGONISTS AND OTHER DRUGS ON THE DURATION OF IMMOBILITY OF MICE IN THE FORCED SWIM TEST

Drug	Dose (mg/kg, IP)	Immobility time (s)
Control		198.9 ± 13.8
NAN-190	0.3	207.5 ± 11.0
	1	186.5 ± 19.4
	3	195.7 ± 13.5
Control		242.2 ± 6.2
Spiperone	0.01	245.2 ± 14.4
	0.03	233.8 ± 10.9
	0.1	281.1 ± 4.5*
Control		224.0 ± 11.5
Spiroxatrine	0.03	241.3 ± 8.4
	0.1	261.1 ± 9.4†
Control		227.0 ± 14.3
Apomorphine	0.3	225.4 ± 12.1
	1	159.7 ± 16.7†
	3	26.3 ± 18.3*
Control		207.5 ± 16.0
(−)-sulpiride	3	222.4 ± 9.2
	10	252.6 ± 7.7†
Control		227.0 ± 12.5
Prazosin	0.3	208.0 ± 21.4
	1	163.6 ± 11.1†
Control		236.2 ± 8.2
Haloperidol	0.03	236.2 ± 13.8
	0.1	264.6 ± 8.2
	0.3	279.4 ± 11.6†

All drugs was given 35 min before the test. Values are expressed as the means ± SEM of eight to 16 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test.

*$p < 0.01$, significant difference from the control value, †$p < 0.05$.

However, 8-OH-DPAT was found to decrease spontaneous locomotor activity.

It is well known that buspirone is rapidly metabolized to 1-PP, and that the brain levels of 1-PP are higher than those of buspirone (3) following buspirone administration. In the present study, 1-PP (1–10 mg/kg, IP) did not affect the duration of immobility. Similar results were reported by Cervo and colleagues (6), who found that the administration of 1-PP (0.3–3 mg/kg, SC) did not affect immobility in the forced swim test in rats. In addition, pretreatment with proadifen (50 mg/kg, IP), a nonselective inhibitor of drug metabolism (1), which inhibits the formation of 1-PP from buspirone, did not change the effect of buspirone. Thus, the immobility-enhancing effect of buspirone (3 mg/kg, IP) in our study seemed to be exerted by buspirone itself, rather than by 1-PP.

It is well known that both buspirone and 8-OH-DPAT display a high affinity for the 5-HT$_{1A}$ receptor (28). Buspirone and 8-OH-DPAT produce various effects in the rat, mediated via 5-HT$_{1A}$ receptor, such as the induction of a behavioral syndrome characteristic of 5-HT-receptor stimulation (25), hypothermia (12), hyperphagia in rats not deprived of food (9), anxiolytic effects (13,23), and antidepressant effects (4,5, 7,14,27). 5-HT$_{1A}$ receptors are located presynaptically on 5-HT cell bodies and postsynaptically on 5-HT nerve terminals (26).

TABLE 7

EFFECT OF BUSPIRONE ON THE IMMOBILITY-DECREASING EFFECT OF 8-OH-DPAT IN MICE IN THE FORCED SWIM TEST

Drug	Immobility Time (s)
Control	232.4 ± 8.2
8-OH-DPAT	175.5 ± 11.0*
Buspirone	212.4 ± 9.2
8-OH-DPAT + Buspirone	222.6 ± 9.0†

Buspirone (1 mg/kg, IP) was given 5 min before the injection of 8-OH-DPAT. 8-OH-DPAT (1 mg/kg, IP) was given 30 min before the test. Values are expressed as means ± SEM of eight animals. Data were analyzed by one-way ANOVA, followed by Tukey's test.

*$p < 0.01$, significant difference from the control value.

†$p < 0.05$, significant difference from the value for 8-OH-DPAT–treated mice.

We investigated the influence of *p*-chlorophenylalanine, which inhibits 5-HT synthesis, on the effects of buspirone and 8-OH-DPAT. *p*-Chlorophenylalanine (200 mg/kg, IP, 3 days) alone did not affect the duration of immobility and did not alter the effect of buspirone or 8-OH-DPAT. Accordingly, it seems that buspirone (3 mg/kg, IP) and 8-OH-DPAT (1 mg/kg, IP) may exert their effects mainly via the postsynaptic 5-HT$_{1A}$ receptors. These findings agree with earlier reports (14,27). We confirmed that 5-HT and 5-hydroxyindole acetic acid levels in mouse whole brain decreased to 65% and 45% of control levels, respectively, after *p*-chlorophenylalanine treatment (data not shown). It is reasonable to assume that the effects of these drugs in the forced swim test in mice are not mediated by presynaptic 5-HT$_{1A}$ receptors, even if 5-HT$_{1A}$ receptors play some role in mediating the effects of these drugs in the forced swim test.

We examined the effect of receptor antagonists on the effects of buspirone and 8-OH-DPAT. The immobility-enhancing effect of buspirone (3 mg/kg, IP) was blocked by NAN-190 (1 mg/kg, IP), which is a well-known antagonist of postsynaptic 5-HT$_{1A}$ receptors. It is likely that the effect of buspirone involves 5-HT$_{1A}$ receptors in some way. NAN-190 also shows a high affinity for α_1-adrenoceptors (10) and acts as an antagonist of these receptors in vivo (8). However, the effect of buspirone was not affected by the selective α_1-adrenoceptor antagonist prazosin (0.3 mg/kg, IP). Accordingly, the α_1-adrenoceptor-blocking activity of NAN-190 did seem sufficient to account for its blockade of buspirone's effects on immobility.

NAN-190 (1 mg/kg, IP) did not block the immobility-decreasing effect of 8-OH-DPAT (1 mg/kg, IP). Thus, the effect of 8-OH-DPAT on immobility seems not to relate directly to postsynaptic 5-HT$_{1A}$-receptor function. However, the immobility-decreasing effect of 8-OH-DPAT (1 mg/kg, IP) was blocked by the 5-HT$_{1A}$-receptor antagonists spiperone (0.03 mg/kg, IP) and spiroxatrine (0.03 mg/kg, IP) when they were injected 5 min before the administration of 8-OH-DPAT. This result agrees with a previous study, in which spiroxatrine (1–30 mg/kg, orally) attenuated the 8-OH-DPAT (3 mg/kg, SC)–induced decrease in immobility in the forced swim test in mice (14). In addition, it is well established that 8-OH-DPAT is a specific ligand for the 5-HT$_{1A}$ receptor. It is well known that NAN-190 antagonizes the behavioral syndrome

(flat body posture, forepaw treading) induced by 8-OH-DPAT, but that it does not affect the hypothermic, hormonal, or hyperglycemic response to 8-OH-DPAT (17,30). These studies thus suggest that NAN-190 is not always an effective antagonist of 8-OH-DPAT's effects. 8-OH-DPAT may decrease immobility via a NAN-190–insensitive 5-HT_{1A} receptor. Alternately, buspirone may recognize a NAN-190–sensitive 5-HT_{1A} receptor. Further studies are in progress to clarify these points.

Numerous investigations have demonstrated interactions between brain 5-HT_{1A}-receptor function and dopamine neurons. Haloperidol-induced catalepsy in rats can be antagonized by treatment with 8-OH-DPAT (11,15). Tatarczyńska and colleagues (24) showed that the antireserpine action (reserpine-induced hypothermia) of 8-OH-DPAT (1 mg/kg, IP) in mice was reduced by haloperidol (1 mg/kg, IP). 8-OH-DPAT may activate the dopaminergic system via 5-HT_{1A} receptors. In addition, the dopaminergic system has been shown to be involved in the immobility in the forced swim test (2). Thus, enhancement of the dopaminergic system produces a decrease in the duration of immobility in the forced swim test (2). We confirmed here that apomorphine, a dopamine-receptor agonist, decreased the duration of immobility in the forced swim test in mice. Thus, the possibility of involvement of the dopaminergic system in the effects observed in the present study should not be ignored. The effect of 8-OH-DPAT was actually antagonized by the selective dopamine D_2-receptor antagonist (−)-sulpiride (3 mg/kg, IP). Similar results were obtained in an earlier study by Cervo and colleagues (4). Thus, it seems reasonable to assume that the effect of 8-OH-DPAT on the duration of immobility was due to activation of the dopaminergic system through postsynaptic NAN-insensitive 5-HT_{1A} receptors.

Buspirone also has affinity for dopamine D_2 receptors and appears to work as an antagonist (19,29). We confirmed here that haloperidol and (−)-sulpiride, dopamine D_2-receptor antagonists, increased the duration of immobility in the forced swim test in mice. In addition, the immobility-enhancing effect of buspirone was blocked by the dopamine-receptor agonist apomorphine. Accordingly, it is likely that the effect of buspirone was exerted by antagonism of dopamine D_2 receptors. Furthermore, buspirone's antagonism of the effect of 8-OH-DPAT also may occur via the dopamine D_2-receptor system. Nevertheless, there remains the possibility that the antagonism by buspirone may occur at the 5-HT_{1A} receptor, with buspirone acting as a partial agonist. However, as we assume that buspirone and 8-OH-DPAT bind to different recognition sites on the 5-HT_{1A} receptor (NAN-190–sensitive site and NAN-190–insensitive site), that possibility seem to be very low. Further studies are in progress to clarify these issues.

In summary, buspirone clearly increased the duration of immobility in mice in the forced swim test, contrary to previous reports. 8-OH-DPAT clearly decreased immobility. The effect of buspirone did not appear to be associated with its effect on spontaneous activity. Further, the effect of buspirone seemed to be exerted by buspirone itself, rather than by its metabolite, 1-PP. 8-OH-DPAT seemed to reduce the duration of immobility by activating the dopamine system via 5-HT_{1A} receptors. Buspirone seemed to increase the duration of immobility owing to its dopamine D_2-receptor antagonist-like effect, rather than to its effect on 5-HT_{1A} receptors.

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