Effect of 5-{3-[(*(2S)*-1,4-Benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole HCl (MKC-242), a Novel 5-HT_{1A}-Receptor Agonist, on Aggressive Behavior and Marble Burying Behavior in Mice

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ABSTRACT—Behavioral effects of 5-{3-[(*(2S)*-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole HCl (MKC-242), a novel 5-HT_{1A}-receptor agonist, were evaluated using animal models of anxiety and obsessive compulsive disorder and compared against reference compounds. MKC-242 suppressed foot shock-induced fighting behavior without loss of motor coordination in mice as the reference compounds did. The ED₅₀ values of MKC-242, buspirone, tandospirone and diazepam were 1.7, 42, 80 and 2.0 mg/kg, p.o., respectively. The duration of the suppression of fighting by MKC-242 was longer than those of buspirone and tandospirone and comparable to that of diazepam. Similar results were also obtained with the water-lick conflict test in rats. The plasma concentration of MKC-242 in rats was much higher than the reported value of buspirone during 0.25–6 hr after oral administration. In addition, MKC-242 reduced marble burying behavior without reduction of motor activity. Fluoxetine, tandospirone and diazepam also reduced the behavior at non-sedative doses. These findings indicate that MKC-242 possesses a longer-lasting anxiolytic effect than azapirones. This might be due to the high concentration of the compound in plasma. In addition, it is also suggested that MKC-242 possesses an antiobsessional effect.

Keywords: MKC-242, 5-HT_{1A} receptor, Anxiolytic, Aggressive behavior, Marble burying behavior

5-HT has been implicated in anxiety for this quarter century. The transmitter binds to receptor subtypes 5-HT₁ to 5-HT₇ (1). For the last years, attention has been focussed on the 5-HT_{1A} receptor, a subtype of the 5-HT₁-receptor family. Although clinical efficacies of buspirone and tandospirone, which act as agonists for 5-HT_{1A} receptors, has been established in the treatment of general anxiety disorder (GAD) (2, 3), their effects on the other types of anxiety are yet not established. Obsessive compulsive disorder (OCD) is one of them. Since 5-HT uptake inhibitors were effective in the treatment of this disorder (4), several 5-HT receptor subtypes were assessed to explain the mechanism. Lesch and Osterheider (5) suggested the involvement of 5-HT_{1A} receptors in the mechanism of action of antiobsessional agents because attenuation of the hypothermic effect of ipsapirone was observed after three weeks of fluoxetine treatment in OCD patients. However, the clinical efficacy of buspirone has been controversial (6, 7).

There are some difficulties in performing studies on the involvement of 5-HT_{1A} receptors on anxiety using azapirones, buspirone and its related compounds for two reasons. One problem is that their common metabolite 1-(2-pyrimidinyl)-piperazine (1-PP) has low affinity for 5-HT_{1A} receptors but moderate affinity for α_2 -adrenoceptors and acts as an antagonist (8). Although there have been several negative reports of 1-PP in animal models of anxiety (9, 10), Gower and Tricklebank (11) reported the anxiolytic effect of the metabolite in the water-lick conflict test. In contrast, the anxiogenic effect of yohimbine, another α_2 -adrenoceptor antagonist, was reported in the elevated plus maze test in rats (12). These possible interaction of α_2 -adrenoceptor and anxiety makes it difficult to explain the involvement of 5-HT_{1A} receptors in anxiety using azapirones.

The other reason is rapid metabolism of azapirones. Indeed, Caccica and his co-workers (13) reported that the serum concentration of buspirone was under the detect-

able level 1 hr after oral administration (10 mg/kg) to rats. The rapid metabolism may cause the short-lasting and insufficient 5-HT_{1A} agonistic action of azapirones.

Recently, we reported that a novel benzodioxan derivative, MKC-242 (5-{3-[(2*S*)-1,4-benzodioxan-2-ylmethyl]-amino}propoxy}-1,3-benzodioxole HCl), increased punished drinking and social interaction after oral administration in rats (14). The compound bound to 5-HT_{1A} receptors with very high affinity and acted as an agonist for the receptor (15). MKC-242 is considered to be more beneficial in studying the involvement of 5-HT_{1A} receptors for various types of anxiety, since the compound does not possess the 1-PP structure and also selectively bound to 5-HT_{1A} receptors (15).

In the present study, we compared the duration of anxiolytic and taming effects of MKC-242 to those of azapirones and diazepam in the water-lick conflict test in rats and foot shock-induced fighting test in mice. Plasma concentration of MKC-242 in rats was also measured. In addition, to investigate the possible involvement of 5-HT_{1A} receptors in OCD, the effect of MKC-242 was evaluated in the marble burying test, which has been considered as an animal model of OCD (16).

MATERIALS AND METHODS

Animals

Male Wistar rats (Japan Laboratory Animals, Inc., Tokyo) weighing 150–250 g and male Sprague-Dawley rats (Charles River Japan, Inc., Atsugi) weighing 250–300 g were used for the water-lick conflict test and plasma concentration, respectively. Male ICR mice (Japan SLC, Inc., Shizuoka) weighing 25–35 g were used in marble burying and spontaneous motor activity (SMA). Male ddY mice (Japan SLC, Inc.) weighing 17–28 g were used in the other experiments. All animals were group housed (rats: 5–6/cage, mice: 10/cage) in an air-conditioned room on a 12-hr light-dark cycle (dark period: 19:00–07:00 hours). They received food and water ad libitum except when indicated. Each animal was used only once, except the isolation induced fighting test.

Foot shock-induced fighting behavior

We used the method of Tedeschi et al. (17), with modifications. A pair of mice was placed on the stainless grid floor and covered with a glass beaker (500 ml). A continuous electric shock (240 V AC, internal impedance 110 k Ω) was administered to them for 1 min. Only pairs showing more than 5 fighting episodes within 1 min were used. The fighting episode was determined as a standing face to face posture with boxing or biting one another. Groups of pairs ($n=8$) were treated with test compounds. One hour after the drug administration, the mice were

replaced on the grid floor and administered the electric shock. The number of fighting episodes induced by the foot-shock was counted for 2 min. The ED₅₀ values were obtained from the mean values of number of fighting episodes using the Litchfield-Wilcoxon method.

Isolation-induced fighting behavior

A modified method based on the one described by Yen et al. (18) was used. At the age of 3 weeks, each mouse was separated to an individual cage and housed for at least 11 weeks. After the isolation, a pair of mice was placed into a circular open field (20-cm diameter, 30-cm height). Only pairs that started fighting within 3 min and had more than 5 fighting episodes during the 5-min observation were used. Groups of pairs ($n=8$) were treated with test compounds. One hour after the drug administration, the pair was replaced into the open field again, and their behavior was observed for 5 min. If the pair showed at least one fighting episode, it was recorded as positive. The mice were repeatedly used after at least 5 days rest. The ED₅₀ values were obtained from the data at 3 or 4 doses of test compounds by Probit analysis.

Water-lick conflict test

A modification of the one described by Vogel et al. (19) was used. Details were described previously (14). Briefly, the water deprived rat was placed in a conflict test apparatus (MVC-005; Muromachi Kikai Co., Ltd., Tokyo) after the pre-test sessions. The pre-test sessions consisted of the free-licking session and the punished-licking session. Only rats whose licking were sufficiently suppressed were selected. Groups of rats ($n=12-15$) were treated with test compounds. They were replaced in the conflict test boxes 1–4 hr after the administration. Each rat was allowed to drink water through the spout, and the rat received an electric shock (240 V AC, internal impedance 230 k Ω , 2 sec) after every 20 licks or every 2 sec of continuous licking. The number of received shocks was counted for 3 min. The doses of test compounds were determined to cause equipotent increase of punished drinking 1 hr after their administration in the previous study (14). In the study, MKC-242, diazepam, buspirone and tandospirone were active at doses of 0.0625–0.25, 10–40, 10–40 and 10–40 mg/kg, p.o., respectively.

Rota-rod test

Mice were trained to stay on a rod (MK-600; Muromachi Kikai Co., Ltd., Tokyo) rotating at 6 rpm before the test day. Then, they were put on the rod (6 rpm) again (pre-test session). Only mice that stayed on the rod over 3 min were used for the test. A total of 212 mice were trained, and 179 mice could stay over 3 min in the pre-test session. Groups of mice ($n=8$) were treated with

test compounds. They were put on the rota-rod again 1 hr after the treatment. The mice that dropped within 1 min were determined as positive. The ED_{50} values were obtained from the data at 4 or 5 doses of test compounds using Probit analysis.

Marble burying test

A modified method based on the one described by Ichimaru et al. (16) was used. Groups of mice ($n=8-9$) were treated with test compounds. One hour after the treatment, the mouse was placed in a stainless steel mesh-topped transparent plastic box ($30 \times 20 \times 13$ cm) with 20 clean glass marbles (15-mm-diameter) evenly spaced 5 cm apart on sawdust (5-cm-deep) in a sound-proof box. The number of marbles buried with sawdust 30 min after the placement was counted.

Spontaneous motor activity (SMA)

A group of 5 mice was placed in a Plexiglas cage on the Animex Auto (MK-110; Muromachi Kikai Co., Ltd.) 1 hr after oral administration of a dose of the test compounds or vehicle. SMA was automatically counted for 30 min by the static electricity derived from spontaneous movement of animals. The value for each dose of a compound was estimated as the average count from 5 experiments.

Measurement of plasma concentration of ^{14}C -MKC-242

^{14}C -MKC-242 was administered to male rats orally at a dose of 1 mg/kg. Blood was collected from the abdominal posterior vena cava under ether anesthesia using a heparinized syringe. Then the blood was centrifuged (15,000 rpm, 1 min) to obtain plasma. The plasma was stored at $-20^{\circ}C$ until measurement of the concentration. To 100 μ l of the plasma, 100 μ l of purified water, 100 μ l of methanol solution of internal standard (I.S.) 5-{3-[(2*S*)-1,4-benzodioxan-2-ylmethyl]amino}butoxy}-1,3-benzodioxole HCl (1 μ g/ml) and 700 μ l of diethylether were added. The mixture was stirred for 30 sec and centrifuged at 15000 rpm for 1 min to separate the ether layer. The ether extraction was repeated again, and the obtained ether layers were combined and evaporated to dryness under a nitrogen stream. The residue was dissolved in 400 μ l of HPLC eluent, 50 μ l of which were injected into HPLC apparatus for the measurement of unchanged compound by the fluorometric analysis. Recovery of the unchanged compound and I.S. by this method were 92.3–93.5% and 96.6–99.4%, respectively. The half-life ($t_{1/2}$) was calculated from the actual observed values within a specified duration by the least squares method. The areas under the curve (AUC) was calculated by the trapezoidal rule to the last measured concentration.

HPLC

The apparatus was composed of a Hitachi pump, L-6200 (Hitachi Factory, Co., Ltd., Tokyo), 7161 injector (Rheodyne, Cotati, CA, USA), TCM column oven (Waters, Milford, MA, USA) and 650-10LC detector or 650-10S fluorescence detector (Hitachi Factory, Co., Ltd.). Conditions were as follows: column: Inertsil ODS-2 (4.6 mm i.d. $\times$ 150 mm; G.L. Science, Co., Ltd., Tokyo); column temperature: $50^{\circ}C$; eluent: acetonitrile/water/trifluoroacetic acid (TFA) = 22:78:0.02 (v/v/v); flow rate: 1 ml/min; detection: fluorescence (Ex 283 nm, Em 338 nm).

Drugs

MKC-242, tandospirone citrate, fluoxetine and 5-{3-[(2*S*)-1,4-benzodioxan-2-ylmethyl]amino}butoxy}-1,3-benzodioxole HCl were synthesized in our laboratory. ^{14}C -MKC-242 was supplied by Mitsubishi Chemical Safety Institute, Ltd., Yokohama. Other compounds were obtained from standard commercial sources. MKC-242, tandospirone and ^{14}C -MKC-242 were suspended in 0.5% carboxymethyl cellulose solution. Fluoxetine and diazepam (Soparochim, Milano, Italy) was suspended in 0.5% Tween 80 solution. Buspirone (Sigma, St. Louis, MO, USA) and chlorpromazine (Sigma) were dissolved in distilled water. All drugs were given either in a volume of 1 ml/kg to rats or 10 ml/kg to mice.

Statistics

Statistical differences between behavioral data for the differently treated groups were located using the one way analysis of variance test (ANOVA). After a positive ANOVA result, individual differences between groups were tested by the Dunnett two-tailed test. In the case of comparing 2 groups, their statistical differences were tested by Student's *t*-test. Differences between groups were considered significant if $P < 0.05$.

RESULTS

Fighting behavior

The paired mice who received electric shocks through the floor grids exhibited fighting behavior. MKC-242 dose-dependently suppressed the number of fighting episodes as buspirone, tandospirone and diazepam did (Fig. 1). The ED_{50} values in the test were summarized at Table 1. The ED_{50} values of MKC-242, diazepam, buspirone and tandospirone at 2 hr were 1.5, 2.9, 3.8 and 4.0-fold higher than the values at 1 hr, respectively (Table 1). In the rota-rod test, all of vehicle-treated mice could stay on the rota-rod in the test session ($n=32$). Test compounds increased the number of mice unable to stay on rota-rod in a dose dependent manner. The ED_{50} values of tested

Fig. 1. Effects of MKC-242, buspirone, tandospirone and diazepam on the shock-induced fighting behavior in mice. Test compounds were given orally 1 hr before testing. The data are each a mean $\pm$ S.E. ($n=8$). * $P<0.05$, compared with the control group by the Dunnett two-tailed test after ANOVA. The mean values of the number of fighting episodes during the pre-test session were 8.9–10.6, 8.3–8.4, 7.9–10.3, 10.8–12.5 and 9.1–10.5 in the vehicle-, MKC-242-, buspirone-, tandospirone- and diazepam-treated mice, respectively.

Table 1. The ED_{50} values of MKC-242, buspirone, tandospirone and diazepam in inhibition of the foot shock-induced fighting behavior in mice

	ED_{50} (mg/kg, p.o.)		
	1 hr	2 hr	3 hr
MKC-242	1.7	2.6	4.5
Buspirone	42	160	N.T.
Tandospirone	80	320	N.T.
Diazepam	2.0	5.7	8.4

All compounds were given at the indicated time before the test. All values are ED_{50} values (mg/kg, p.o.) calculated by the Litchfield-Wilcoxon method. N.T.: Not tested.

Table 2. The ED_{50} values of MKC-242, buspirone, tandospirone and diazepam in the rota-rod test in mice

Compound	ED_{50} (mg/kg, p.o.)
MKC-242	7.8
Buspirone	132
Tandospirone	322
Diazepam	4.2

All compounds were given 1 hr before testing. The data are ED_{50} values (mg/kg, p.o.) calculated by the Probit analysis.

Fig. 2. The duration of anti-conflict effects of MKC-242, buspirone, tandospirone (A) and diazepam (A and B) in the water-lick conflict test in rats. Vehicle ($\circ$); MKC-242, 0.125 mg/kg, p.o. ($\bullet$); buspirone, 20 mg/kg, p.o. ($\blacksquare$); tandospirone, 20 mg/kg, p.o. ($\blacktriangle$); or diazepam, 10 mg/kg, p.o. ($\blacktriangledown$) (A) or 20 mg/kg, p.o. ($\blacklozenge$) (B) was given at the indicated times before testing. The data are each a mean value $\pm$ S.E. ($n=12-15$). * $P<0.05$, compared with the vehicle control group at each time by the Dunnett two-tailed test after ANOVA or by Student's unpaired t -test. The mean values of the number of shocks during the pre-test session were 8.2–12.7, 9.5–12.0, 10.0–11.4, 9.8–12.4 and 8.2–12.6 in the vehicle-, MKC-242-, buspirone-, tandospirone- and diazepam-treated rats, respectively.

compounds are summarized in Table 2. The ED₅₀ values of MKC-242, buspirone, tandospirone and diazepam in the rota-rod test were 4.6, 3.1, 4.0 and 2.1-fold higher than their ED₅₀ values in the foot shock-induced fighting test, respectively.

The number of fighting episodes in isolated mice was 6.1 ± 0.2 in the pre-test session ($n=72$). All of the vehicle-treated mice showed fighting episodes in the test session, 4.5 ± 0.6 times ($n=16$). MKC-242 and buspirone decreased the incidence of fighting behavior induced by isolation in a dose-dependent manner. The ED₅₀ values of MKC-242 and buspirone in the isolation-induced fighting in mice were 0.84 and 20 mg/kg, respectively.

Duration of action in water-lick conflict test

MKC-242 (0.125 mg/kg) and a high dose of diazepam (20 mg/kg) significantly increased the received shocks 1 and 2 hr after oral administration (Fig. 2), while buspirone (20 mg/kg), tandospirone (20 mg/kg) and a low dose of diazepam (10 mg/kg) significantly increased the number of received shocks only 1 hr after oral administration (Fig. 2).

Table 3. Effects of MKC-242 and the other 5-HT_{1A} receptor ligands on burying behavior and SMA in mice

Compounds	Dose (mg/kg, p.o.)	No. of buried marbles	SMA (counts)
MKC-242	cont	13.4 ± 1.5	2321 ± 297
	0.1	15.1 ± 0.7	N.T.
	0.2	11.6 ± 2.3	N.T.
	0.4	13.1 ± 1.5	N.T.
	0.8	12.7 ± 1.3	N.T.
	1.6	9.0 ± 1.5	2406 ± 205
	3.2	$5.8 \pm 1.6^*$	2170 ± 210
	6.4	$1.0 \pm 0.6^*$	$1244 \pm 142^*$
	12.8	N.T.	$454 \pm 145^*$
Buspirone	cont	12.9 ± 2.2	2710 ± 149
	10	13.2 ± 1.7	2790 ± 252
	20	7.7 ± 2.4	2356 ± 202
	40	8.0 ± 2.5	2204 ± 200
	80	$5.3 \pm 1.9^*$	$1845 \pm 171^*$
	160	$0.6 \pm 0.4^*$	$871 \pm 173^*$
Tandospirone	cont	16.0 ± 0.9	2469 ± 218
	20	15.0 ± 1.2	N.T.
	40	12.6 ± 1.9	1990 ± 172
	80	$10.1 \pm 1.3^*$	1787 ± 201
	160	$6.7 \pm 1.9^*$	$1266 \pm 180^*$
	320	$4.4 \pm 1.9^*$	$824 \pm 188^*$

All values are reported as the mean $\pm$ S.E. ($n=9$ for marble burying and 5 for reduction of SMA). N.T.: Not tested. * $P < 0.05$, compared with the control group by the Dunnett test after ANOVA.

Marble burying behavior

The effects of MKC-242 and reference compounds on burying behavior and SMA are summarized at Tables 3 and 4. MKC-242, fluoxetine, tandospirone and diazepam significantly suppressed the number of buried marbles without significant reduction of SMA at 3.2, 40, 80 and 4 mg/kg, respectively. Buspirone reduced both the number of buried marbles and SMA at the same doses, while chlorpromazine significantly reduced SMA without any effect on the number of buried marbles at 5 mg/kg.

Plasma concentration of MKC-242

Plasma concentrations of MKC-242 after oral administration (1 mg/kg) to rats are shown in Fig. 3. MKC-242 in plasma reached the maximal concentration (C_{max}) (1712 ng/ml) 15 min after the administration. None of this compound was detected in the plasma at 24 hr after the administration. The AUC value was 2943 ng·hr/ml. The $t_{1/2}$ value calculated from the data measured up to 6 hr was 1.3 hr.

Table 4. Effects of reference compounds on burying behavior and SMA in mice

Compounds	Dose (mg/kg, p.o.)	No. of buried marbles	SMA (counts)
Fluoxetine	cont	14.7 ± 1.2	2725 ± 167
	5	14.2 ± 1.2	N.T.
	10	9.2 ± 2.3	N.T.
	20	10.0 ± 1.8	N.T.
	40	$7.3 \pm 2.1^*$	2591 ± 315
	80	$3.3 \pm 1.9^*$	2323 ± 318
	160	$1.7 \pm 1.3^*$	$1755 \pm 205^*$
	320	N.T.	$1086 \pm 149^*$
Diazepam	cont	14.9 ± 1.4	2406 ± 137
	0.25	15.3 ± 0.9	N.T.
	0.5	12.6 ± 0.6	N.T.
	1	11.4 ± 1.2	N.T.
	2	11.9 ± 1.4	N.T.
	4	$7.6 \pm 1.9^*$	1912 ± 294
	8	$1.3 \pm 0.8^*$	$1514 \pm 282^*$
	16	N.T.	$1004 \pm 156^*$
	32	N.T.	$983 \pm 136^*$
Chlorpromazine	cont	13.9 ± 1.4	2710 ± 149
	1.25	13.2 ± 2.0	N.T.
	2.5	13.8 ± 1.3	2754 ± 130
	5	14.2 ± 2.1	$1700 \pm 166^*$
	10	$4.1 \pm 2.1^*$	$1081 \pm 232^*$

All values are reported as the mean $\pm$ S.E. ($n=9$ for marble burying and 5 for reduction of SMA). N.T.: Not tested. * $P < 0.05$, compared with the control group by the Dunnett test after ANOVA.

Fig. 3. Plasma concentration-time curve of MKC-242 after oral administration of MKC-242 (1 mg/kg, p.o.) to rats. The data are each a mean value $\pm$ S.E. ($n=4$).

DISCUSSION

Our study showed that MKC-242 reduced aggressive behavior in mice as the reference compounds did. In addition, the duration of the taming and anti-conflict effect of MKC-242 was longer than that of azapirones and comparable to that of diazepam.

Since foot shock-induced fighting behavior was attenuated by benzodiazepines, it has been used as a screening model for anxiolytics (20). The taming effects of azapirones have also been reported in foot shock-induced fighting (21) and the other types of aggressive behavior (22, 23). In the present study, buspirone and tandospirone dose-dependently reduced the fighting behavior without impairment of motor coordination. The results were consistent with the previous report (21). In the present study, MKC-242 also reduced shock-induced fighting behavior without loss of motor coordination at lower doses than those of buspirone and tandospirone. The result supports the concept that stimulation of 5-HT_{1A} receptors causes a taming effect, since the compound selectively bound to 5-HT_{1A} receptors. Indeed, the potency of the taming effects of MKC-242, buspirone and tandospirone obtained in this experiment were reflected in their K_i values for 5-HT_{1A} receptors, which were 0.39 nM, 24 nM and 45 nM, respectively (15).

The present results from the time course experiments of shock-induced fighting and water-lick conflict tests indicated that MKC-242 possesses a longer-lasting pharmacological effect than those of buspirone or tandospirone and comparable to that of diazepam. This difference in the duration of pharmacological action could be ex-

plained by the pharmacokinetic experiments. Indeed, Caccica and his co-workers (13) reported that unchanged buspirone in plasma was under the detectable level even 1 hr after oral administration of buspirone (10 mg/kg) in rats. The $t_{1/2}$ value of MKC-242 calculated from the present result was 2.6 times longer than the value of buspirone previously reported (13). In addition, the plasma concentration of MKC-242 obtained in our study was much higher than the value reported for buspirone. The C_{max} and AUC values of buspirone after 10 mg/kg oral administration in rats were 0.35 nmol/ml (148 ng/ml) and 0.27 nmol·hr/ml (114 ng·hr/ml), respectively (13). Compared with these values, the C_{max} and AUC values of MKC-242 obtained here were 11 and 26-fold higher even after one-tenth less administration (1 mg/kg, p.o.). These pharmacokinetic properties may explain the potent and long-lasting anxiolytic effect of MKC-242.

Although the pharmacokinetic parameters obtained in the present study was only at one dose (1 mg/kg, p.o.), plasma concentration of radioactivity was measured after administration of ¹⁴C-MKC-242 at the doses of 0.3, 1 and 3 mg/kg, p.o. (H. Nakai et al., unpublished data). At these doses, $t_{1/2}$ values of radioactivity in plasma were almost the same as the value of unchanged MKC-242 obtained in the present study, and AUC values increased in a dose-dependent manner. In addition, the AUC value of unchanged MKC-242 was approximately 70% of that of radioactivity after oral administration (1 mg/kg). Thus, it is suggested that a sufficient concentration of unchanged MKC-242 exists 1 or 2 hr after administration of MKC-242 (0.125 mg/kg, p.o.) in the water-lick conflict test.

MKC-242 reduced aggressive behavior at 10 to 25 times higher doses than those at which the compound increased punished drinking or social interaction time (14). Buspirone and tandospirone also required 5 to 8-fold higher doses for their taming effects than those required for anti-conflict effects (14). Since the taming effect of a 5-HT_{1A}-receptor agonist in isolated mice was augmented by co-administration of small doses of methiosepin, the effect is considered to be mediated via presynaptic 5-HT_{1A}-receptor agonist action (24). On the other hand, it is considered that postsynaptic or both pre- and postsynaptic 5-HT_{1A} receptors are responsible for the anti-conflict effect (25, 26). If MKC-242 and azapirones act as agonists more preferentially at postsynaptic 5-HT_{1A} receptors than presynaptic ones, the discrepancy of effective doses of these compounds in different models may be explained by the difference of acting points. It is also possible that the discrepancy may be due to differences in experimental conditions, i.e., animal species and the intensities of stress used.

MKC-242 also reduced marble burying behavior at doses without reduction of SMA. The same result was

obtained for tandospirone. These results suggest the contribution of 5-HT_{1A} receptors to the burying behavior. Compared to these 5-HT_{1A} receptor agonists, buspirone reduced burying behavior only at sedative doses. The difference may be explained by their selectivity for 5-HT_{1A} and D₂ receptors (15). MKC-242 and tandospirone bound to D₂ receptors at 240- and 15-fold higher concentration than those required for 5-HT_{1A} receptors. While buspirone has equipotent affinity for those two receptors. This lack of selectivity may cause the reduction of SMA by buspirone. The contribution of 5-HT_{1A} receptors is also supported by the previous finding that the decrease of burying behavior by fluvoxamine, a 5-HT uptake inhibitor, was antagonized by NAN-190, a 5-HT_{1A}-receptor antagonist (16).

Although it is possible that the marble burying behavior may reflect both compulsiveness and fear of novelty, the latter is not considered to be a major factor because of the lack of habituation in this behavior (27, 28). In addition, fluoxetine more specifically reduced marble burying behavior compared to diazepam in the present study. The pharmacological profile indicates that the behavior is more likely a model of OCD than anxiety, even though the model has been originally established as a screening method to evaluate anxiolytics (28). Positive results for other serotonin re-uptake inhibitors were also reported in previous studies (16, 29). Since MKC-242 reduced burying behavior at non-sedative doses, it may be effective for the treatment of OCD.

The following clinical results suggested that patients with OCD exhibited increased 5-HT metabolism and turnover: First, 5-hydroxyindole-3-acetic acid (5-HIAA) levels in cerebrospinal fluid were higher in OCD patients compared to controls (30). Second, the high levels of 5-HIAA were reduced by chronic treatment with clomipramine (31). Third, the untreated patients with OCD became markedly more anxious and obsessional after oral administration of m-CPP, a 5-HT-receptor agonist (4). These clinical findings suggest an increase in central serotonergic function in OCD patients, while it is known that 5-HT_{1A}-receptor agonists reduce 5-HT neuronal activity via presynaptic 5-HT_{1A} receptors (15, 24). Thus, it can be considered that the anti-obsessional effects of 5-HT_{1A}-receptor agonists are mediated by the reduction of 5-HT neuronal activity via stimulation of 5-HT_{1A} receptors. Although the clinical efficacy of buspirone has been controversial (6, 7), a clinical study using more specific and longer lasting 5-HT_{1A} receptor agonist should be performed to clarify the involvement of the receptor in OCD.

In summary, MKC-242 reduced aggressive behavior without loss of motor coordination. The duration of taming and anti-conflict effects of MKC-242 was longer

than that of buspirone and tandospirone. It can be explained by the pharmacokinetic difference between MKC-242 and azapirones. MKC-242 also reduced marble burying behavior without sedation. These findings suggest that MKC-242 has a longer-lasting anxiolytic effect and antiobsessional effect.

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