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Short communication

The 5-HT$_{1A}$ receptor agonist MKC-242 increases the exploratory activity of mice in the elevated plus-maze

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Abstract

The effect of (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole HCl (MKC-242), a 5-HT$_{1A}$ receptor agonist, on mouse behavior was examined in the elevated plus-maze. MKC-242 significantly increased the percentage of open-arm entries and the percentage of open-arm time, indices of anxiety reduction, while it did not increase the enclosed-arm entries and time spent in enclosed arms. The effect of MKC-242 was antagonized by a low dose of the 5-HT$_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide which alone did not affect the behavior. These findings suggest that MKC-242 increases the exploratory activity of mice in the elevated plus-maze via activation of 5-HT$_{1A}$ receptors, probably the presynaptic autoreceptors.

Keywords: MKC-242; 5-HT$_{1A}$ receptor; Plus-maze test; (Mouse)

1. Introduction

5-HT$_{1A}$ receptors are considered target molecules for the treatment of anxiety (De Vry, 1995). Although the elevated plus-maze is a well-established setup for studies of anxiety (Lister, 1987; Hoggs, 1996), this test has yielded inconsistent effects with 5-HT$_{1A}$ receptor agonists (Setem et al., 1999). This may be due to a lack of selectivity for 5-HT$_{1A}$ receptors of the ligands used in the previous studies including 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Hamon et al., 1988). We previously reported that (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole HCl (MKC-242) is a potent and selective 5-HT$_{1A}$ receptor agonist (Matsuda et al., 1995). This compound has an anxiolytic-like effect in the water-lick conflict and social interaction tests (Abe et al., 1996), but this effect has not been studied in the elevated plus-maze test. We now examined the effect of MKC-242 on mouse behavior in the elevated plus-maze and whether this effect is blocked by the specific 5-HT$_{1A}$ receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100635).

2. Materials and methods

2.1. Animals and drugs

Four-week-old male *dd*Y mice were maintained under controlled environmental conditions (22 ± 1 °C; 12–12 h light/dark cycle, lights on at 08:00 h; food and water ad libitum) for at least 1 week before being used in the experiments. All mice were experimentally naive. Procedures involving animals and their care were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. MKC-242 and WAY100635 were gifts from Mitsubishi Pharma (Yokohama, Japan). MKC-242 and diazepam were suspended (10 ml/kg) in 0.5% w/v carboxymethylcellulose (CMC), and WAY100635 was dissolved in saline. All drugs were freshly prepared.

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2.2. Elevated plus-maze test

The plus-maze was made of white-colored wood and consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 20 cm). The open arms did not have a lip around the edge. The arms extended from a central platform (5 × 5 cm). The open arms, the central platform, and the floor of the closed arms were made of white wood. The apparatus was mounted on a wood base, raising it 40 cm above the floor. The maze was placed in a dark room, and the open arms were illuminated using dim red light (2 × 40 W). A mouse (5–6 weeks old) was placed in the center of the plus-maze facing the open arm. Entry of the mouse into open and enclosed arms of the maze and time spent in the open arms, enclosed arms and platform were assessed for 5 min using a video tracking system (AXIS-90, Neuroscience, Osaka, Japan). Arm entries were defined as entry of all four paws into an arm.

2.3. Locomotor activity

The locomotor activity of the mouse was measured with a Supermex (Muromachi Kikai, Tokyo). A mouse was placed in a clear plastic cage (24 × 17 × 12 cm) for a habituation period and then injected with CMC or MKC-242. The locomotor activity in each 5-min period was measured for 90 min after administration of the drug.

2.4. Statistics

Data from the elevated plus-maze test were analyzed using two-way analysis of variance (ANOVA) followed by the Tukey–Kramer test, or one-way ANOVA followed by the Dunnett test. Data from locomotor activity testing were analyzed using repeated two-way ANOVA for treatment as between-subjects factor and repeated measures with time as within-subject factor. Statistical analyses were performed using a software package (Stat View 5.0) for the Apple Macintosh computer. P values of 5% or less were considered statistically significant.

3. Results

We first examined the effect of diazepam on mouse behavior in the elevated plus-maze (Table 1A). Although diazepam at 0.5–1.0 mg/kg increased the open-arm entries, it did not affect the percentage of open-arm entries. Diazepam at 2.0 mg/kg increased the two indices of anxiety reduction, that is, the percentage of open-arm entries, and

Table 1
Effects of diazepam and MKC-242 on the behavior of mice in the elevated plus-maze test

(A) Diazepam

	Vehicle	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	F-values	P-values
Arm entries						
Open arms	2.9 ± 0.8	4.4 ± 0.8	6.2 ± 0.7[a]	7.2 ± 1.1[b]	$F(3,63)=4.627$	$P=0.0056$
Enclosed arms	12.8 ± 1.2	19.9 ± 1.9[a]	22.8 ± 1.5[b]	16.3 ± 2.6	$F(3,63)=5.304$	$P=0.0026$
Total	15.7 ± 1.7	24.3 ± 2.4[a]	28.9 ± 1.8[b]	23.4 ± 3.0	$F(3,63)=5.663$	$P=0.0017$
% Open arm entries	16.8 ± 3.3	17.0 ± 2.1	21.8 ± 2.5	32.4 ± 5.2[b]	$F(3,63)=4.401$	$P=0.0073$
Time spent						
Open arms	19.3 ± 6.5	26.4 ± 5.0	33.3 ± 5.0	51.2 ± 12.1[a]	$F(3,63)=3.159$	$P=0.0311$
Enclosed arms	194.9 ± 11.4	186.7 ± 6.9	173.8 ± 7.7	174.7 ± 17.6	$F(3,63)=0.750$	NS
Platform	85.8 ± 7.6	86.9 ± 3.2	92.8 ± 7.6	74.1 ± 13.7	$F(3,63)=0.783$	NS
% Time spent in open arms	9.6 ± 3.3	12.6 ± 2.5	16.0 ± 2.3	25.6 ± 5.9[a]	$F(3,63)=3.410$	$P=0.0231$

(B) MKC-242

	Vehicle	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg	F-values	P-values
Arm entries						
Open arms	1.4 ± 0.3	3.1 ± 0.3[a]	4.6 ± 0.6[b]	4.4 ± 0.5[b]	$F(3,65)=11.077$	$P<0.0001$
Enclosed arms	11.0 ± 1.1	13.2 ± 0.6	12.2 ± 1.0	12.1 ± 1.2	$F(3,65)=0.847$	NS
Total	12.4 ± 1.3	16.4 ± 0.7[a]	16.8 ± 1.1[a]	16.5 ± 1.5[a]	$F(3,65)=3.288$	$P=0.0264$
% Open arm entries	10.3 ± 2.1	18.9 ± 1.7	27.3 ± 3.1[b]	27.8 ± 2.9[b]	$F(3,65)=11.188$	$P<0.0001$
Time spent						
Open arms	9.6 ± 3.3	16.9 ± 2.7	35.7 ± 6.8[b]	37.1 ± 6.0[b]	$F(3,65)=7.760$	$P=0.0002$
Enclosed arms	229.4 ± 7.7	203.1 ± 7.4	186.6 ± 9.5[b]	189.3 ± 8.6[b]	$F(3,65)=5.647$	$P=0.0017$
Platform	61.0 ± 6.6	80.0 ± 6.1	77.7 ± 4.9	73.7 ± 6.1	$F(3,65)=2.072$	NS
% Time spent in open arms	4.1 ± 1.3	8.0 ± 1.4	16.5 ± 3.4[b]	16.6 ± 2.7[b]	$F(3,65)=7.183$	$P=0.0003$

Results are means ± S.E.M. for 16–17 mice. Diazepam (i.p.) and MKC-242 (p.o.) were administered 30 and 60 min before the experiment, respectively.
[a] $P<0.05$, compared with vehicle-treated group.
[b] $P<0.01$, compared with vehicle-treated group.

Table 2
Effect of WAY100635 on the activity of MKC-242 in the elevated plus-maze test

	Saline/CMC	Saline/MKC-242	WAY100635/CMC	WAY100635/MKC-242	*F*-values	*P*-values
Arm entries						
Open arms	1.4 ± 0.3	4.6 ± 0.6[a]	2.5 ± 0.6	1.9 ± 0.5[c]	$F(1,65)=12.748$	$P=0.0007$
Enclosed arms	10.2 ± 1.4	10.5 ± 1.0	11.4 ± 1.3	11.2 ± 1.4	$F(1,65)=0.027$	NS
Total	11.6 ± 1.7	15.1 ± 1.3	13.9 ± 1.5	13.1 ± 1.6	$F(1,65)=1.914$	NS
% Open arm entries	12.0 ± 3.3	30.9 ± 3.3[a]	16.3 ± 4.8	11.4 ± 3.0[c]	$F(1,65)=10.628$	$P=0.0018$
Time spent						
Open arms	9.6 ± 3.7	54.2 ± 8.7[a]	17.4 ± 5.4	20.6 ± 9.0[b]	$F(1,65)=8.292$	$P=0.0055$
Enclosed arms	236.7 ± 7.9	171.6 ± 14.6[a]	218.1 ± 10.4	211.3 ± 11.1[b]	$F(1,65)=6.531$	$P=0.0131$
Platform	53.7 ± 6.3	74.2 ± 8.7	64.4 ± 7.2	68.0 ± 7.4	$F(1,65)=1.285$	NS
% Time spent in open arms	4.0 ± 1.5	26.1 ± 5.2[a]	7.5 ± 2.5	8.8 ± 3.7[b]	$F(1,65)=8.684$	$P=0.0045$

Results are means ± S.E.M. for 16–17 mice. WAY100635 (i.p.) and MKC-242 (p.o.) were administered 90 and 60 min before the experiment, respectively.
[a] $P<0.01$, compared with saline/CMC-treated group.
[b] $P<0.05$.
[c] $P<0.01$, compared with saline/MKC-242-treated group.

the time spent in the open arms expressed as a percentage of the total time spent in either type of arm, while it did not affect the enclosed-arm entries and enclosed arm time. This result suggests that this model is sensitive to diazepam as previously reported (Moser, 1989). Then, we examined the effect of MKC-242 at different doses on mouse behavior in the elevated plus-maze (Table 1B). MKC-242 at 0.3 and 1.0 mg/kg significantly increased open-arm entries and the total number of entries, but not enclosed-arm entries. MKC-242 also increased the time spent in open arms, but instead decreased the time spent in enclosed arms. MKC-242 increased the two indices of anxiety-reduction. MKC-242 (0.1, 0.3 and 1.0 mg/kg) did not affect spontaneous locomotor activity ($n=7–8$; $F(51,539)=1.199$, $P=0.1732$) (data not shown). The effects of MKC-242 on the percentage of open-arm entries and the percentage of open time were not observed in mice pretreated with WAY100635 (Table 2).

4. Discussion

The percentage of open-arm entries and the time spent in the open arms expressed as a percentage of the total time spent in either type of arm are generally used as indices of anxiety reduction in the elevated plus-maze test. The present study showed that MKC-242 at 0.3–1.0 mg/kg significantly increased both the number of open-arm entries and the time spent in the open arms. However, it did not affect the number of enclosed-arm entries and it decreased the time spent in the enclosed arms. In addition, we observed that MKC-242 did not have any effect on spontaneous activity. These findings suggest that the effect of MKC-242 to increase the number of open-arm entries and the time spent in the open arms is not secondary to non-specific stimulatory actions. A number of previous studies have shown that 5-HT_{1A} receptor agonists have inconsistent effects in the elevated plus-maze. In this connection, File et al. (1996) provided evidence that stimulation of presynaptic receptors results in anxiolysis, whereas the postsynaptic receptors are anxiogenic. The opposition of pre- and postsynaptic receptors may explain the relatively weak anxiolytic profile seen with systemic administration of 5-HT_{1A} receptor agonists. MKC-242 is a full agonist for presynaptic 5-HT_{1A} receptors and a partial agonist for postsynaptic 5-HT_{1A} receptors (Matsuda et al., 1995). These properties of MKC-242 may be responsible for the anxiolytic-like effect in the plus-maze. In contrast to MKC-242, buspirone, a partial agonist for 5-HT_{1A} receptors, has inconsistent effects in the elevated plus-maze (Handley and McBlane, 1993). This may be due not only to a difference in the experimental conditions, but also to a lack of specificity of buspirone for 5-HT_{1A} receptors (Cole and Rodgers, 1994; Collinson and Dawson, 1997). It should be noted that MKC-242 and 8-OH-DPAT affect cholinergic neurons in the rat cerebral cortex via different mechanisms (Somboonthum et al., 1997).

We also observed that the effects of MKC-242 in the maze were fully reversed by a 0.1 mg/kg dose of the 5-HT_{1A} receptor antagonist, WAY100635. The finding suggests that the anxiolytic-like effect of MKC-242 is mediated by activation of 5-HT_{1A} receptors. 5-HT_{1A} receptors are not only localized on serotonergic neurons as presynaptic autoreceptors but also on other neurons as postsynaptic receptors. Previous studies in rats have shown that a low dose of WAY100635 blocked the presynaptic, but not the postsynaptic, 5-HT_{1A} receptor-mediated responses (Romero et al., 1996; Dawson et al., 1999; Hajós-Korcsok et al., 1999). In a separate experiment, we also found that, in mice, presynaptic and postsynaptic 5-HT_{1A} receptors differ in sensitivity to WAY100635 (unpublished): a low dose of WAY100635 blocked the MKC-242-induced decrease in cortical 5-HT release, a presynaptic 5-HT_{1A} receptor-mediated response, but not the MKC-242-induced increase in the dopamine release, a postsynaptic 5-HT_{1A} receptor-mediated response (Sakaue et al., 2000). Taken together, the present findings suggest that presynaptic 5-HT_{1A} receptors play a key role in the anxiolytic-like effect of MKC-242.

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