ELSEVIER

European Journal of Pharmacology 280 (1995) 235–238

ejp

Short communication

Antidepressant-like effect by postsynaptic 5-HT_{1A} receptor activation in mice

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Received 8 March 1995; revised 25 April 1995; accepted 28 April 1995

Abstract

The antidepressant-like effect of 5-{3-[((2*S*)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole (MKC-242), a novel 5-HT_{1A} receptor agonist, was studied in the forced swimming test in mice injected i.c.v. with 5,7-dihydroxytryptamine to destroy 5-HT neurons or treated with *p*-chlorophenylalanine to inhibit 5-HT synthesis. MKC-242 reduced immobility time of mice pretreated with vehicle and these drugs, although it did not affect their locomotor activity. The anti-immobility effect was antagonized by 5-HT_{1A} receptor antagonists such as propranolol and *N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropanamide. These findings support the hypothesis that postsynaptic 5-HT_{1A} receptors play an important role in the antidepressant-like effect of 5-HT_{1A} receptor agonists.

Keywords: 5-HT_{1A} receptor; MKC-242 (5-{3-[((2*S*)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole); Forced swimming test; Antidepressant activity; (Mouse)

1. Introduction

5-HT_{1A} receptors are present on the soma and dendrites of 5-HT neurons and on postsynaptic neurons in various regions of the limbic system (Pazos et al., 1988). The receptor agonists such as azapirones and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) have an antidepressant-like effect (Lucki et al., 1994): they reduce the immobility of rats in the forced swimming test, a test that is sensitive to a wide variety of antidepressant agents (Porsolt et al., 1977). Evidence is accumulating supporting postsynaptic 5-HT_{1A} receptors for the antidepressant-like effect (Luscombe et al., 1993; Wieland and Lucki, 1990), while the importance of presynaptic 5-HT_{1A} receptors is also reported (Cervo et al., 1988; Cervo and Samanin, 1987, 1991). The study using potent and selective 5-HT_{1A} receptor agonists on pre- and postsynaptic 5-HT_{1A} receptor mechanisms should contribute to a better understanding of the pharmacological effects of 5-HT_{1A} receptor agonists.

We have recently developed a potent and selective 5-HT_{1A} receptor agonist, 5-{3-[((2*S*)-1,4-benzodioxan-2-ylmethyl)amino]propoxy}-1,3-benzodioxole (MKC-242), with anxiolytic and antidepressant-like effects in rats (water-lick conflict test; social interaction test; behavioral deficit induced by restraint stress; forced swimming test) (Egawa et al., 1993). MKC-242 has high affinity for 5-HT_{1A} receptors ($K_i = 0.35$ nM), lower affinity for α_1-adrenoceptors ($K_i = 21$ nM) and dopamine D_2 receptors ($K_i = 83$ nM), and little activity at other 5-HT receptor subtypes and neurotransmitter receptors including 5-HT_{1B}, 5-HT_{1C}, 5-HT_2, 5-HT_3 receptors, α_2-adrenoceptors, β-adrenoceptors, dopamine D_1, benzodiazepine and $GABA_A$ receptors (Yoshikawa et al., 1994). In this paper, we studied the antidepressant-like effect of MKC-242 in the forced swimming test in mice treated with 5,7-dihydroxytryptamine or *p*-chlorophenylalanine. We report here that the effect of MKC-242 may be mediated by postsynaptic 5-HT_{1A} receptors.

2. Materials and methods

Male *ddY* mice (4 weeks old) were housed 5–7 per cage under standard conditions: room temperature (21

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SSDI 0014-2999(95)00254-5

± 1° C), light/dark cycle (lights on 08:00 h, off 20:00 h). They had free access to food and water and were allowed to adapt to the laboratory for at least 1 week before the experiments. The animals were subjected to the forced swimming test according to the method of Porsolt et al. (1977) with a modification. The test for 6 min was carried out on the 6th day after a 5 min swim once a day for 5 days, as repeated exposure to the swim test increased immobility time of mice. The behavior was videorecorded and immobility time during the total 6 min was measured by an observer who was unaware of the drug treatment. A mouse was judged to be immobile when it remained floating in the water, making only the necessary movements to keep its head above water. The pilot experiment showed that the effect of 8-OH-DPAT on immobility time was more pronounced during the total 6 min than during the last 4 min under the conditions used here. After each swim test, the wet fur of the mice was immediately wiped with a towel and dried with a heater. Locomotor activity of the mouse was determined using an Animex activity meter (type S, Farad Electronics, Sweden). Brain amine contents were determined as previously reported (Matsuda et al., 1989). 8-OH-DPAT in saline and MKC-242 in 0.5% carboxymethylcellulose were administered 30 min before the Porsolt test, and (−)-propranolol at 10 mg/kg in saline and *N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropanamide (WAY100135) at 10 mg/kg in saline were injected 1 h before the test. *p*-Chlorophenylalanine was i.p. injected 1, 3, and 5 days prior to the Porsolt test at 400 mg/kg. For lesioning of 5-HT neurons, the mice were anesthetized with pentobarbital (40 mg/kg i.p.), and injected i.c.v. with 4 μl 5,7-dihydroxytryptamine (75 μg as free base) dissolved in saline containing 0.04% ascorbic acid. Desipramine at 5 mg/kg was i.p. injected 30 min before 5,7-dihydroxytryptamine to protect noradrenergic neurons. The animals were allowed to recover for 2 weeks after surgery before the experiments.

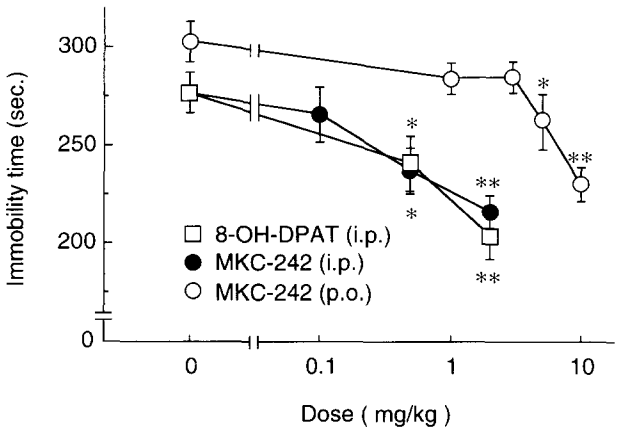

Fig. 1. Effects of MKC-242 and 8-OH-DPAT on immobility time of mice in the forced swimming test. Points are means ± S.E. of 8 mice. MKC-242 and 8-OH-DPAT were administrated 30 min (i.p.) or 60 min (oral) before the test. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. vehicle (Duncan multiple test).

The data were analyzed with the Duncan test following a positive one-way analysis of variance (ANOVA) or Mann-Whitney test using statistical data analysis software (SPSS, Chicago, IL, USA).

3. Results

I.p. injection of MKC-242 and 8-OH-DPAT reduced the immobility time of mice in a dose-dependent manner (Fig. 1). MKC-242 was an orally active drug. MKC-242 at 0.5–2.0 mg/kg (i.p.) did not cause any behavioral change or motor dysfunction that could have interfered with the measurement of immobility, although, at doses more than 5.0 mg/kg, it produced sedation: the locomotor activities (counts/10 min,

Table 1
Effects of 5,7-dihydroxytryptamine and *p*-chlorophenylalanine on the reduction of immobility time caused by MKC-242 and on brain noradrenaline, dopamine, 5-HT and 5-hydroxyindoleacetic acid contents

Treatment	Contents (ng/g wet weight)				Immobility time (s)	
	Noradrenaline	Dopamine	5-HT	5-Hydroxyindoleacetic acid	Vehicle	MKC-242
Experiment A						
Control	503 ± 41	1533 ± 89	667 ± 18	231 ± 16	279 ± 5	233 ± 12 [b]
5,7-Dihydroxytryptamine	434 ± 25	1582 ± 49	105 ± 7 [a]	33 ± 4 [a]	288 ± 5	232 ± 9 [b]
Experiment B						
Control	570 ± 20	1442 ± 41	777 ± 17	304 ± 10	272 ± 10	220 ± 8 [b]
p-Chlorophenylalanine	558 ± 12	1414 ± 18	259 ± 24 [a]	40 ± 4 [a]	251 ± 12	194 ± 14 [b]

Values are means ± S.E. of 8–10 mice. Brain amine and the metabolite contents were determined 24 h after the last injection of *p*-chlorophenylalanine and 2 weeks after 5,7-dihydroxytryptamine. [a] $P < 0.001$ vs. control, [b] $P < 0.01$ vs. vehicle (Mann-Whitney test).

means ± S.E. of 10 mice) 30 min after injection were 337 ± 49 (vehicle), 376 ± 50 (0.5 mg/kg), 272 ± 42 (2.0 mg/kg), 121 ± 33 (5.0 mg/kg, $P < 0.01$ vs. vehicle). The anti-immobility effect of MKC-242 (0.5 mg/kg i.p.) was completely blocked by (−)-propranolol, a β-adrenoceptor and 5-HT_{1A} receptor antagonist (Martin et al., 1992), and WAY100135, a specific 5-HT_{1A} receptor antagonist (Fletcher et al., 1993): immobility times (s, means ± S.E. of 8 mice) were: control, 295 ± 11; MKC-242, 223 ± 6 ($P < 0.01$ vs. control); (−)-propranolol, 302 ± 9; WAY100135, 290 ± 4; (−)-propranolol + MKC-242, 288 ± 8; WAY100135 + MKC-242, 287 ± 5.

Table 1 shows the effects of lesions of 5-HT neurons with 5,7-dihydroxytryptamine and the treatment with *p*-chlorophenylalanine on MKC-242-induced reduction of immobility time in the forced swimming test. The anti-immobility effect of MKC-242 was not altered either by 5,7-dihydroxytryptamine or *p*-chlorophenylalanine which decreased 5-HT and 5-hydroxyindoleacetic acid content without changing noradrenaline and dopamine content (ANOVA (2 × 2) as follows: 5,7-dihydroxytryptamine, $F(1,24) = 0.398$, $P = 0.534$ and *p*-chlorophenylalanine, $F(1,22) = 0.245$, $P = 0.625$).

4. Discussion

The present study showed that MKC-242 had an anti-immobility effect in the forced swimming test in mice, in agreement with the previous finding using rats (Egawa et al., 1993). MKC-242 is a potent and selective 5-HT_{1A} receptor agonist (Yoshikawa et al., 1994). Thus, it is conceivable that the antidepressant-like effect of MKC-242 is mediated by an activation of 5-HT_{1A} receptors. Indeed, the anti-immobility effect of MKC-242 was antagonized by (−)-propranolol and WAY100135, 5-HT_{1A} receptor antagonists.

There are controversial reports concerning the sites of action for 5-HT_{1A} receptor agonists such as 8-OH-DPAT and azapirones in the forced swimming test. Cervo's group supports a presynaptic site for the action (Cervo et al., 1988). They showed that the destruction of 5-HT neurons using 5,7-dihydroxytryptamine and the inhibition of 5-HT synthesis with *p*-chlorophenylalanine prevented the antidepressant-like behavioral effect of 8-OH-DPAT in the test (Cervo and Samanin, 1987, 1991). This view has been challenged, however, by evidence supporting a postsynaptic site of action for 5-HT_{1A} receptor agonists in the forced swimming test: 5,7-dihydroxytryptamine and *p*-chlorophenylalanine failed to alter the reduction of immobility time produced by 8-OH-DPAT and azapirones (Luscombe et al., 1993; Wieland and Lucki, 1990).

The present study demonstrated that the anti-immobility effect of MKC-242 was not affected by 5,7-dihydroxytryptamine and *p*-chlorophenylalanine which decreased markedly 5-HT and its metabolite levels in the brain. Furthermore, the dysfunction of 5-HT neurons induced by 5,7-dihydroxytryptamine or *p*-chlorophenylalanine did not alter the behavior of mice in the forced swimming test. These findings suggest that 5-HT neurons are not involved in the anti-immobility effect of MKC-242, in agreement with the previous view supporting a postsynaptic site for the antidepressant-like effect of 5-HT_{1A} receptor agonists. However, the whereabouts of the postsynaptic 5-HT_{1A} receptors responsible for the antidepressant-like effect remain to be determined. Further studies on modulation of target neurons by 5-HT_{1A} receptor agonists are required to clarify the exact mechanism for the antidepressant-like effect of 5-HT_{1A} receptor agonists.

Acknowledgements

We thank Mitsubishi Chemical Co. (Yokohama) and Wyeth Research (U.K.) Ltd. for their generous gifts of MKC-242 and WAY100135, respectively. This research was supported by grants from the Ministry of Education, Science and Culture of Japan and from Mitsubishi Chemical Co.

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