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Short communication

Involvement of benzodiazepine binding sites in an antiaggressive effect by 5-HT_{1A} receptor activation in isolated mice

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Abstract

The effect of the benzodiazepine receptor antagonist flumazenil was examined on an antiaggressive effect of (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3- benzodioxole HCl (MKC-242), a 5-HT_{1A} receptor agonist. MKC-242 (0.1–1.0 mg/kg, p.o.) selectively reduced isolation-induced aggressive behavior in a dose-dependent manner. Flumazenil (10 mg/kg, i.p.) antagonized the antiaggressive effects of MKC-242 and diazepam, although it alone did not affect the behaviors of isolated mice. These findings suggest that a γ-aminobutyric $acid_A$ ($GABA_A$) receptor system is involved in the antiaggressive effect by 5-HT_{1A} receptor activation.

Keywords: 5-HT_{1A} receptor; MKC-242; Aggression; Flumazenil; Isolation; (Mouse)

1. Introduction

Since 5-HT_{1A} receptor agonists reduce aggressive behavior in isolated male mice (Olivier et al., 1989; White et al., 1991; Sánchez et al., 1993; Schreiber and De Vry, 1993), isolation-induced aggression is proposed to be useful as an animal model for assessing nonbenzodiazepine anxiolytic activity. It was also shown that (*S*)-5-[3-[(1,4-benzodioxan-2-ylmethyl) amino] propoxy]-1,3- benzodioxole HCl (MKC-242), a potent and selective 5-HT_{1A} receptor agonist (Matsuda et al., 1995b), has anxiolytic-like and antidepressant-like effects (Matsuda et al., 1995a; Abe et al., 1996, 1998). This compound also inhibited mouse aggressive behavior induced by foot shock and isolation (Abe et al., 1998). The exact mechanism of the antiaggressive effect of 5-HT_{1A} receptor agonists is not known. On the other hand, there is much evidence suggesting that there is a functional relationship between the γ-aminobutyric acid (GABA)/benzodiazepine and the 5-HT systems in the brain (Fernandez Guasti and Lopez Rubalcava, 1998; Lista et al., 1990; Lopez Rubalcava et al., 1992; Söderpalm et al., 1997). The present study aims to examine the possible involvement of the GABA system in the antiaggressive effect of 5-HT_{1A} receptor activation in isolated mice. The selective 5-HT_{1A} receptor agonist MKC-242 is shown to be a highly potent inhibitor of isolation-induced aggressive behavior, and we provide evidence to show that benzodiazepine receptor binding sites are involved in the mechanisms underlying the antiaggressive effect of MKC-242.

2. Materials and methods

2.1. Animals

Male ddY mice (4 weeks old) were either housed in groups of five to six/cage (24 × 17 × 12 cm) or isolated in the same size cage for more than 6 weeks before experiments under controlled environmental conditions (22 ± 1 °C; 12–12 light–dark cycle, lights on at 08:00 h, food and water ad libitum). Procedures involving animals and their care were conducted according to the Guiding Principles for

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the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Measurement of aggressive and non-aggressive behavior

The isolated mice were prescreened for aggressive behavior 1 day before the experiment. An intruder mouse was introduced into the isolated mouse's home cage for 3 min, and the isolated mice showing biting attacks were used for the experiments of drug test on the following day. Two isolated mice that were pretreated with drugs were placed in a neutral cage, which was the same size as their home cages as previously reported (Ojima et al., 1995), and their behaviors were videotaped for 20 min. Aggressive behaviors (biting attacks, wrestling, lateral threat and tail rattle) of two isolated mice for 20 min were assessed as total fighting time according to the method of Ojima et al. (1995). Non-aggressive behaviors (walking, rearing, self-grooming and social sniffing) of two isolated mice for 20 min were counted for frequency. In this study, we used isolated mice as opponents instead of an intruder mouse in the drug test session, because two isolated mice showed higher aggressive score. We confirmed using this method that serotonergic drugs were effective in reducing aggressive behavior: fluoxetine (30 mg/kg, p.o.), buspirone (10, 30 mg/kg, p.o.) and tandospirone (10, 30 mg/kg, p.o.) significantly reduced total fighting time (data not shown).

2.3. Drugs

The following drugs were used: MKC-242 and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100635) (Mitsubishi-Tokyo Pharmaceuticals, Yokohama, Japan); flumazenil (Nippon Roche, Kamakura, Japan). All other chemicals used were of the highest commercially available purity. All drugs were freshly prepared. MKC-242 and diazepam for oral administration (10 ml/kg) were suspended in 0.5% w/v carboxymethylcellulose (CMC). Flumazenil was dissolved in 0.1% Tween-80.

2.4. Statistics

Statistical analyses were conducted by one-way analysis of variance followed by Dunnett test, and Kruskal–Wallis followed by Mann–Whitney *U* test. *P* values of 5% or less were considered statistically significant.

3. Results

Fig. 1 shows the effect of MKC-242 on behaviors of two isolated mice. MKC-242 significantly reduced total fighting time in a dose-dependent manner (0.1–1.0 mg/kg, p.o.). On the other hand, MKC-242 did not affect walking, rearing, and grooming behaviors. MKC-242 inhibited sniffing, but the effect was not dose dependent. In addition, MKC-242 at the doses used here did not affect spontaneous locomotor activity (data not shown), which is in agreement with the previous report that it did not have a sedative effect (Matsuda et al., 1995a; Abe et al., 1998). The antiaggressive effect of MKC-242 was observed to be antagonized by pretreatment with the 5-HT_{1A} receptor antagonist WAY 100635 (1.0 mg/kg, i.p.) (data not shown).

Fig. 2 shows the effect of flumazenil on the antiaggressive effects of MKC-242 and diazepam. In contrast to MKC-242, diazepam at a dose that has a sedative effect (data not shown) has an antiaggressive effect. Flumazenil at a dose that completely blocked the effect of diazepam

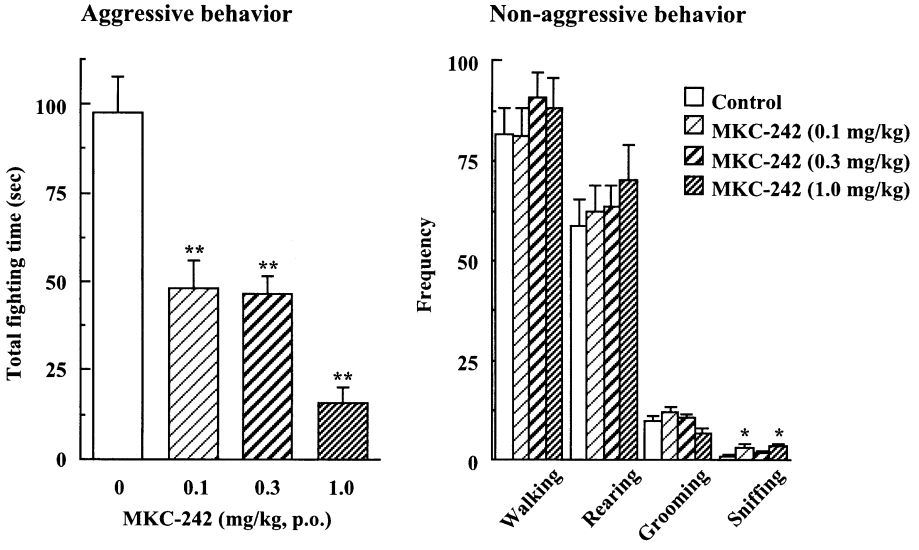

Fig. 1. Effect of MKC-242 on the aggressive and non-aggressive behaviors in isolated mice. Drugs were p.o. administered to the paired mice 1 h before the experiments. Results are means ± S.E.M. of 15–28 pairs of isolated mice. * $P < 0.05$, * * $P < 0.01$, compared with the control (Dunnett test).

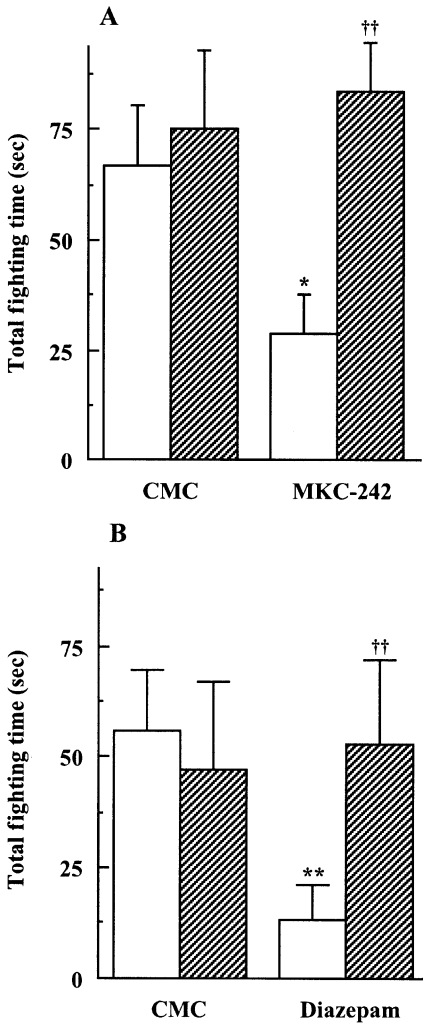

Fig. 2. Effect of flumazenil on the inhibition of isolation-induced aggressive behavior by MKC-2424 (A) and diazepam (B). MKC-242 (0.3 mg/kg) and diazepam (5.0 mg/kg) were p.o. administered 1 h before the experiment. Flumazenil (10 mg/kg, i.p., hatched) and vehicle (open) were injected 30 min before MKC-242 and diazepam. Aggression was analyzed 1 h after these drugs. Results are means ± S.E.M. of 6–12 pairs of isolated mice. * $P < 0.05$, * * $P < 0.01$, compared with control/CMC; †† $P < 0.01$, compared with vehicle/MKC-242 (A) or vehicle/diazepam (B) (Kruskal–Wallis followed by Mann–Whitney U test).

prevented the antiaggressive effect of MKC-242. Flumazenil alone had no effect on the aggressive (Fig. 2) and non-aggressive behaviors of the isolated mice (data not shown).

4. Discussion

The present study demonstrates that MKC-242 selectively reduces isolation-induced aggressive behavior in mice via an activation of 5-HT_{1A} receptors. The importance of 5-HT_{1A} receptors for the antiaggressive effect of 5-HT_{1A} receptor agonists is also reported (Millan et al., 1997; De Boer et al., 1999; Mendoza et al., 1999). The minimum effective dose (0.1 mg/kg, p.o.) for the antiaggressive effect of MKC-242 reported here was the same as that for the anxiolytic effect reported previously (Abe et al., 1996). It should be noted that MKC-242 was more potent in reducing isolation-induced aggression than other 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), decahydro-3-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-1,5-methano-6,7,9-metheno-2*H*-pentaleno [1,2-*d*]azepine-2,4(3*H*)-dione dihydrochloride (Wy-487-23),8-[2-[4-[2-methoxyphenyl]-1-piperazinyl]ethyl]-8-azaspiron[4,5]decane-7,9-dione dihydrochloride (BMY-7378), azapirones and 4-(benzodioxan-5-yl)1-(indan-2-yl) piperazine (S 15535) (White et al., 1991; Millan et al., 1997).

Lopez Rubalcava et al. (1992) reported that the anxiolytic-like effects of some 5-HT_{1A} receptor agonists might be counteracted by the benzodiazepine receptor antagonist flumazenil. The finding suggests that the anxiolytic-like effect of 5-HT_{1A} receptor agonists may involve an activation of $GABA_A$–benzodiazepine receptor complexes. The present study showed that the antiaggressive effect of MKC-242 was blocked by flumazenil. This finding suggests that benzodiazepine binding sites are involved in the antiaggressive effect of MKC-242. We also found that WAY100635 (1.0 mg/kg, i.p.) did not influence the effect of diazepam on spontaneous locomotor activity (data not shown). This implies that the GABA system is downstream of the 5-HT system in the expression of the behavioral effect of MKC-242. In this line, Söderpalm et al. (1997) have recently shown that in vivo administration of 8-OH-DPAT enhances the function of corticohippocampal $GABA_A$–benzodiazepine receptor complexes. They suggest that the anxiolytic-like action of 8-OH-DPAT may be explained by modulation of $GABA_A$–benzodiazepine receptor complexes. Furthermore, Stutzmann and LeDoux (1999) reported that 5-HT inhibits glutamatergic excitation via activation of GABAergic interneurons. The exact synaptic relationship between 5-HT_{1A} and $GABA_A$ receptors is not known.

In conclusion, MKC-242 selectively inhibited isolation-induced aggressive behavior in isolated mice via an activation of 5-HT_{1A} receptors. This effect is mediated by a functional interaction with the $GABA_A$–benzodiazepine receptor complexes.

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References

Abe, M., Tabata, R., Saito, K., Matsuda, T., Baba, A., Egawa, M., 1996. Novel benzodioxan derivative, 5-[3-[((2*S*)-1,4-benzodioxan-2-ylmeth-

yl) amino]propoxy]-1,3-benzodioxole HCl (MKC-242), with anxiolytic-like and antidepressant-like effects in animal models. J. Pharmacol. Exp. Ther. 278, 898–905.

Abe, M., Nakai, H., Tabata, R., Saito, K., Egawa, M., 1998. Effect of 5-[3-[((2*S*)-1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole HCl (MKC-242), a novel 5-HT1A-receptor agonist, on aggressive behavior and marble burying behavior in mice. Jpn. J. Pharmacol. 76, 297–304.

De Boer, S.F., Lesourd, M., Mocaer, E., Koolhaas, J.M., 1999. Selective antiaggressive effects of alnespirone in resident–intruder test are mediated via 5-hydroxytryptamine$_{1A}$ receptors: a comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. J. Pharmacol. Exp. Ther. 288, 1125–1133.

Fernandez Guasti, A., Lopez Rubalcava, C., 1998. Modification of the anxiolytic action of 5-HT$_{1A}$ compounds by GABA–benzodiazepine agents in rats. Pharmacol. Biochem. Behav. 60, 27–32.

Lista, A., Blier, P., De Montigny, C., 1990. Benzodiazepine receptors modulate 5-hydroxytryptamine neurotransmission in the rat hippocampus: in vivo electrophysiological evidence. J. Pharmacol. Exp. Ther. 254, 318–323.

Lopez Rubalcava, C., Saldivar, A., Fernandez Guasti, A., 1992. Interaction of GABA and serotonin in the anxiolytic action of diazepam and serotonergic anxiolytics. Pharmacol. Biochem. Behav. 43, 433–440.

Matsuda, T., Somboonthum, P., Suzuki, M., Asano, S., Baba, A., 1995a. Antidepressant-like effect by postsynaptic 5-HT$_{1A}$ receptor activation in mice. Eur. J. Pharmacol. 280, 235–238.

Matsuda, T., Yoshikawa, T., Suzuki, M., Asano, S., Somboonthum, P., Takuma, K., Nakano, Y., Morita, T., Nakasu, Y., Kim, H.S., Egawa, A., Tobe, A., Baba, A., 1995b. Novel benzodioxan derivative, 5-(3-[((2*S*)-1,4-benzodioxan-2-ylmethyl)amino]propoxy)-1,3-benzodioxole HCl (MKC-242), with a highly potent and selective agonist activity at rat central serotonin$_{1A}$ receptors. Jpn. J. Pharmacol. 69, 357–366.

Mendoza, D.L., Bravo, H.A., Swanson, H.H., 1999. Antiaggressive and anxiolytic effects of gepirone in mice, and their attenuation by WAY 100635. Pharmacol. Biochem. Behav. 62, 499–509.

Millan, M.J., Hjorth, S., Samanin, R., Schreiber, R., Jaffard, R., De Ladonchamps, B., Veiga, S., Goument, B., Peglion, J.L., Spedding, M., Brocco, M., 1997. S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: II. Modulation of hippocampal serotonin release in relation to potential anxiolytic properties. J. Pharmacol. Exp. Ther. 282, 148–161.

Ojima, K., Matsumoto, K., Tohda, M., Watanabe, H., 1995. Hyperactivity of central noradrenergic and CRF systems is involved in social isolation-induced decrease in pentobarbital sleep. Brain Res. 684, 87–94.

Olivier, B., Mos, J., Van del Heyden, J., Hartog, J., 1989. Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology 97, 154–156.

Sánchez, C., Arnt, J., Hyttel, J., Moltzen, E.K., 1993. The role of serotonergic mechanisms in inhibition of isolation-induced aggression in male mice. Psychopharmacology 110, 53–59.

Schreiber, R., De Vry, J., 1993. 5-HT$_{1A}$ receptor ligands in animal models of anxiety, impulsivity and depression: multiple mechanisms of action? Prog. Neuro-Psychopharmacol. Biol. Psychiatry 17, 87–104.

Söderpalm, B., Andersson, G., Enerbäck, C., Engel, J.A., 1997. In vivo administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT interferes with brain GABA$_A$/benzodiazepine receptor complexes. Neuropharmacology 8, 1071–1077.

Stutzmann, G.E., LeDoux, J.E., 1999. GABAergic antagonists block the inhibitory effects of serotonin in the lateral amygdala: a mechanism for modulation of sensory inputs related to fear conditioning. J. Neurosci. 19 (RC8), 1–4.

White, S.M., Kucharik, R.F., Moyer, J.A., 1991. Effects of serotonergic agents on isolation-induced aggression. Pharmacol. Biochem. Behav. 39, 729–736.