Modulation by 5-HT$_{2A}$ Receptors of Aggressive Behavior in Isolated Mice

Masaki Sakaue$^1$, Yukio Ago$^2$, Chikako Sowa$^2$, Yayoi Sakamoto$^2$, Beni Nishihara$^2$, Yutaka Koyama$^2$, Akemichi Baba$^1$ and Toshio Matsuda$^2$*

$^1$Laboratory of Molecular Neuropharmacology and $^2$Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan

ABSTRACT—The present study examines whether isolation-rearing affects sensitivity of 5-hydroxytryptamine (5-HT)$_{2A}$ receptors and the functional interaction between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in mice. The 5-HT$_{2A}$-receptor agonist ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI)-induced head twitch response was significantly greater in isolated mice than in grouped mice. DOI increased isolation-induced aggressive behavior, and the 5-HT$_{2A}$-receptor antagonist ritanserin decreased it. The 5-HT$_{1A}$-receptor agonist ($S$)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole HCl (MKC-242) inhibited the DOI-enhanced aggressive behavior. MKC-242 inhibited DOI-induced head twitch response. These findings suggest that 5-HT$_{2A}$ receptors play a role in aggressive behavior in isolated mice and imply that the antiaggressive effect of MKC-242 may be mediated partly by the inhibition of 5-HT$_{2A}$-receptor function.

Keywords: 5-Hydroxytryptamine (5-HT)$_{2A}$ receptor, Aggression, 5-HT$_{1A}$ receptor

5-Hydroxytryptamine (5-HT)$_{1A}$-receptor agonists, which have an anxiolytic-like effect, reduce aggressive behavior in isolated male mice (1 – 4), but the exact mechanism remains unknown. We have shown that cortical dopaminergic activity is enhanced and the sensitivity of 5-HT$_{1A}$ receptors that stimulate cortical dopamine release is reduced in isolated mice (5). In addition, we showed that the antiaggressive effect of the selective 5-HT$_{1A}$-receptor agonist ($S$)-5-[3-[(1,4-benzodioxan-2-ylmethyl)amino]propoxy]-1,3-benzodioxole HCl (MKC-242) (6) was antagonized by flumazenil, a benzodiazepine-receptor antagonist (7). These findings suggest that dopaminergic neurons play a role in mediating the aggressive behaviors in isolated mice and that $\gamma$-aminobutyric acid neurons are involved in the antiaggressive effect of MKC-242. By contrast, previous studies have shown that the downregulation of 5-HT$_{2A}$ receptors is involved in the antidepressant-like and anxiolytic-like effects of 5-HT$_{1A}$-receptor agonists (8). However, it is not known whether 5-HT$_{2A}$ receptors are involved in isolation-induced aggressive behavior. In the present study, we examine the possible involvement of the 5-HT$_{2A}$ receptors in isolation-induced aggressive behavior and the effect of MKC-242 on 5-HT$_{2A}$-receptor-mediated response in mice.

Male ddY mice (4-week-old) were either housed in groups of 5 – 6/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 0800, food and water ad libitum). Procedures involving animals and their care were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. The isolated mice were prescreened for aggressive behavior once a day for one or two days prior to the experiment. An intruder mouse was introduced into the isolated mouse’s home cage for 3 min, and the isolated mice exhibiting bite marks were used for the drug test experiments 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 (5, 7), and a videotape recording of their behavior was made for 20 min. An assessment of the aggressive behavior (biting attacks, wrestling, lateral threats and tail switching) of two isolated mice was conducted for 20 min as the total fighting time according as previously reported (5, 7). The non-aggressive behavior (walking, rearing, self-grooming and social sniffing) of two isolated mice was counted for frequency for 20 min.

*Corresponding author. FAX: +81-6-6879-8159
E-mail: matsuda@phs.osaka-u.ac.jp

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In this study, isolated mice were used as opponents instead of the intruder mouse in the drug test session, because the procedure using two isolated mice indicated a higher aggression score. For analysis of the 5-HT_{2A}-receptor function, an examination of DOI-induced head twitch response was conducted: the total number of head twitches in a 10-min period was counted after DOI administration.

The following drugs were used: MKC-242 and N-2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY100635) (Mitsubishi Pharma Co., Yokohama); (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) (R.B.I., Natick, MA, USA); ritanserin (Sigma-Aldrich Co., Tokyo). All other chemicals used were of the highest purity available commercially and all drugs were freshly prepared. MKC-242 for oral administration (10 ml/kg) was suspended in 0.5% w/v carboxymethylcellulose (CMC). DOI and ritanserin were dissolved in sterile water and 0.1% Tween-80, respectively.

Statistical analyses were conducted by Student’s t-test, one-way analysis of variance followed by the Dunnett test, and Kruskal-Wallis followed by the Mann Whitney U test. P values of 5% or less were considered statistically significant.

The administration of DOI (2.5 – 5.0 mg/kg, i.p.) significantly induced the head twitch response, which was antagonized by the 5-HT_{2A}-receptor antagonist, ritanserin (data not shown). The head twitch responses induced by DOI (2.5 mg/kg, i.p.) (counts/10 min) in grouped and isolated mice were 13 ± 1 and 24 ± 1, respectively (means ± S.E.M. of 5 mice, P<0.001, Student’s t-test). The difference in DOI-induced head twitch response between the two groups was also observed in mice pretreated with proadifen, an inhibitor of the drug-metabolizing enzyme (9, 10): the head twitch responses induced by DOI (5 mg/kg, i.p.) (counts/10 min) in mice pretreated with proadifen (50 mg/kg, i.p., 30 min before DOI) were 23 ± 1 (grouped mice) and 33 ± 1 (isolated mice) (means ± S.E.M. of 6 to 9 mice, P<0.001, Student’s t-test). These observations indicate that the isolation enhances the sensitivity of the 5-HT_{2A} receptors. The enhanced responsiveness of 5-HT_{2A} receptors was also reported in isolated rats (11).

To study the involvement of 5-HT_{2A} receptors in mediating aggressive behavior, the effects of 5-HT_{2A}-receptor agonist and antagonist on aggressive behavior were examined in isolated mice (Fig. 1). DOI at 1 mg/kg increased the total fighting time of the isolated mice, but it at 5 mg/kg did not affect it. DOI at 1 and 5 mg/kg increased waking behavior. MKC-242 in the presence of DOI inhibited not only the aggressive behavior but also the non-aggressive behavior, although it alone inhibited the aggressive behavior selectively (5, 7). In view of the non-specific effect on aggressive behavior, the effects of DOI and DOI plus MKC-242 on aggressive behavior appear to be complex. Alternatively, the bell-shaped dose-response for the effect of DOI reported here suggests that a limited activation of 5-HT_{2A} receptors may enhance the aggressive behavior, while an excess activation may reduce the aggressive behavior, although the exact mechanism is not known. In contrast, the 5-HT_{2A} receptor antagonist ritanserin at 3 mg/kg significantly inhibited the aggressive behavior of isolated mice without having any effect on the non-aggressive behavior. This observation is similar to the previous observation by White et al. (2). They reported that 10 mg/kg of ritanserin inhibited aggressive behavior in isolated mice, although they did not examine the effect of ritanserin at the high dose on non-aggressive behavior. Thus, it is likely that an inhibition of 5-HT_{2A}-receptor function decreases the aggressive behavior in isolated mice.

With respect to the functional coupling between 5-HT_{1A} and 5-HT_{2A} receptors, the previous observations are controversial: acute administration of 8-hydroxy-2-(di-n-propylaminotetralin, a 5-HT_{1A}-receptor agonist, inhibits some 5-HT_{2A}-receptor-mediated behavioral responses (12, 13), although Kitamura et al. (14) reported that buspirone, a 5-HT_{1A}-receptor agonist, enhanced head twitch behavior induced by 5-hydroxy-L-tryptophan in mice. In these studies, it is not known whether the effect is indeed due to an activation of 5-HT_{1A} receptors. We re-examined the interaction between 5-HT_{1A} and 5-HT_{2A} receptors using the selective 5-HT_{1A}-receptor agonist MKC-242 and the selective 5-HT_{1A}-receptor antagonist WAY100635 (Fig. 2). MKC-242 inhibited the DOI-induced head twitch response in a dose-dependent manner, and the effect of MKC-242 was blocked by WAY100635. This suggests that an activation of 5-HT_{1A} receptors inhibits the 5-HT_{2A}-receptor function, although the exact synaptic relationship between 5-HT_{1A} and 5-HT_{2A} receptors remains unknown. It should be noted that the effective doses of MKC-242 reported here were the same as those of the drug for the antiaggressive effect reported previously (5). Taken together, it is possible that the antiaggressive effect of MKC-242 is partly mediated by the inhibition of 5-HT_{2A}-receptor function. In this connection, we observed that MKC-242 inhibited the aggressive behavior in isolated mice treated with DOI (Fig. 1). However, it is not known whether there is an antagonism between 5-HT_{1A} and 5-HT_{2A} receptors in aggressive behavior, since MKC-242 alone inhibits isolation-induced aggressive behavior (5, 7).

In conclusion, the present study demonstrates that 1) the 5-HT_{2A}-receptor function is enhanced by isolation-rearing, 2) the aggressive behavior of isolated mice is increased and decreased by DOI and ritanserin, respectively, and 3) MKC-242 inhibits the DOI-enhanced aggressive behavior. Furthermore, we observed that the 5-HT_{2A}-receptor-mediated head twitch response is inhibited by an activation of 5-
Fig. 1. Effects of DOI, ritanserin and MKC-242 on aggressive and non-aggressive behavior in isolated mice. Vehicle, DOI and ritanserin were p.o. administered 1 h before the experiments. MKC-242 was i.p. injected 30 min before DOI. Results are means ± S.E.M. of 15 to 18 pairs of isolated mice. *P<0.05, **P<0.01, compared with the vehicle (one-way analysis of variance followed by Dunnett test for the effects of DOI and ritanserin or Student’s t-test for the effect of MKC-242). †P<0.05, ††P<0.01, †††P<0.001, compared with DOI (Student’s t-test).

Fig. 2. Effects of MKC-242 and WAY100635 on DOI-induced head twitch response in mice. Vehicle (open) and MKC-242 (0.3 mg/kg, p.o.) (hatched) were administered 1 h before the experiment. WAY100635 (1 mg/kg, i.p.) was administered 30 min before vehicle and MKC-242. Head twitch response was analyzed for 10 min after the administration of DOI (5 mg/kg, i.p.). Results are means ± S.E.M. of 9 to 10 mice. A: **P<0.01, compared with CMC (one-way analysis of variance followed by the Dunnett test). B: **P<0.01, compared with saline/CMC, ††P<0.01, compared with saline/MKC-242 (Kruskal-Wallis followed by the Mann Whitney U test).
HT$_{1A}$ receptors. These findings suggest that 5-HT$_{2A}$ receptors have a role in the aggressive behavior. They also imply that the inhibition of 5-HT$_{2A}$-receptor function may contribute to the antiaggressive effect of MKC-242.

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REFERENCES

1. Olivier B, Mos J, Van del Heyden J and Hartog J: Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology (Berl) 97, 154 – 156 (1989)
2. White SM, Kucharik RF and Moyer JA: Effects of serotonergic agents on isolation-induced aggression. Pharmacol Biochem Behav 39, 729 – 736 (1991)
3. Sánchez C, Arnt J, Hyttel J and Moltzen EK: The role of serotonergic mechanisms in inhibition of isolation-induced aggression in male mice. Psychopharmacology (Berl) 110, 53 – 59 (1993)
4. Mendoza DL, Bravo HA and Swanson HH: Antiaggressive and anxiolytic effects of gepirone in mice, and their attenuation by WAY 100635. Pharmacol Biochem Behav 62, 499 – 509 (1999)
5. Matsuda T, Sakaue M, Ago Y, Sakamoto Y, Koyama Y and Baba A: Functional alteration of brain dopaminergic system in isolated aggressive mice. Jpn J Neuropsychopharmacol 21, 71 – 76 (2001)
6. Matsuda T, Yoshikawa T, Suzuki M, Asano S, Somboonthum P, Takuma K, Nakano Y, Morita T, Nakasu Y, Kim HS, Egawa M, Tobe A and Baba A: Novel benzodioxanderivative, 5-(3-[[25]-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 (1995)
7. Sakaue M, Ago Y, Murakami C, Sowa C, Sakamoto Y, Koyama Y, Baba A and Matsuda T: Involvement of benzodiazepine binding sites in an antiaggressive effect by 5-HT$_{1A}$ receptor activation in isolated mice. Eur J Pharmacol 463, 163 – 166 (2001)
8. Schreiber R and De Vry J: 5-HT$_{1A}$ receptor ligands in animal models of anxiety, impulsivity and depression: multiple mechanisms of action? Prog Neuro-psychopharmacol Biol Psychiat 17, 87 – 104 (1993)
9. Anders M: Enhancement and inhibition of drug metabolism. Annu Rev Pharmacol 11, 37 – 56 (1971)
10. Matsuda T, Nakano Y, Kanda T, Iwata H and Baba A: Gonadectomy changes the pituitary-adrenocortical response in mice to 5-HT$_{1A}$ receptor agonists. Eur J Pharmacol 200, 299 – 304 (1991)
11. Wright IK, Ismail H, Upton N and Marsden CA: Effect of isolation rearing on 5-HT agonist-induced responses in rat. Psychopharmacology (Berl) 105, 259 – 263 (1991)
12. Glennon RA, Darmani NA and Martin BR: Multiple populations of serotonin receptors may modulate the behavioral effects of serotonergic agents. Life Sci 48, 2493 – 2498 (1991)
13. Krebs Thomson K and Geyer MA: Evidence for a functional interaction between 5-HT$_{1A}$ and 5-HT$_{2}$ receptors in rats. Psychopharmacology (Berl) 140, 69 – 74 (1998)
14. Kitamura Y, Nagatani T and Watanabe T: Buspirone enhances head twitch behavior in mice. Eur J Pharmacol 253, 297 – 301 (1994)