Reduction of Wrap Restraint Stress-Induced Defecation by MKC-242, a Novel Benzodioxan Derivative, via 5-HT_{1A}-Receptor Agonist Action in Rats

Michikazu Abe and Ken-Ichi Saito

Pharmaceuticals Laboratory I, Yokohama Research Center, Mitsubishi Chemical Corporation, 1000, Kamoshida, Aoba-ku, Yokohama 227-8502, Japan

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ABSTRACT—Effects of MKC-242 (5-{3-[(*2S*)-1,4-benzodioxan-2-ylmethyl]amino]propoxy}-1,3-benzodioxole HCl), a novel 5-HT_{1A}-receptor agonist, and reference compounds on wrap restraint stress-induced defecation were evaluated in rats. Wrapping restraint stress increased defecation in rats. The increase was attenuated by putative 5-HT_{1A}-receptor agonists, MKC-242 and 8-hydroxy-2-(*di-n*-propylamino)tetralin (8-OH-DPAT). The suppressive effect of MKC-242 on wrap stress-induced defecation was antagonized by prior administration of a 5-HT_{1A}-receptor antagonist, WAY100135. MKC-242 did not affect spontaneous defecation and 5-HT-induced defecation. Diazepam and amitriptyline also significantly reduced the stress-induced defecation. However, amitriptyline showed a potent anti-cholinergic effect in the oxotremorine-induced tremor test and reduced spontaneous defecation. In contrast to MKC-242 and 8-OH-DPAT, buspirone and tandospirone tended to suppress the increase at high doses. A major metabolite of buspirone and tandospirone, 1-(2-pyrimidinyl)piperazine, antagonized the suppressive effect of MKC-242. These findings suggest that stimulation of 5-HT_{1A} receptors reduces stress-induced defecation but not spontaneous and 5-HT-induced defecation and that MKC-242 may be useful for the treatment of irritable bowel syndrome.

Keywords: MKC-242, 5-HT_{1A} receptor, Stress, Irritable bowel syndrome, Defecation

Irritable bowel syndrome (IBS) has been considered to be associated with psychological stress (1). Indeed, anxiolytics and antidepressants have been used for the treatment of this disorder (1, 2). However, another opinion that such a medication is insufficient for the treatment of this disorder has been also suggested (3). In addition, the possible involvement of the anticholinergic property of tricyclics was presented (1).

On the other hand, azapirones such as buspirone and tandospirone have been used as a novel class of anxiolytics that are less sedative and more anxi-selective than benzodiazepines (4, 5). The mechanism of their anxiolytic action is considered to be an agonist action for one of the 5-HT receptor subtypes, 5-HT_{1A} receptors (5, 6). In clinical trial, although there were no statistically significant differences, the improvement rating of tandospirone was higher than that of the placebo in patients with irritable colon syndrome (7). This indicates the possible involvement of 5-HT_{1A} receptors for irritable bowel syndrome.

In preclinical studies, various stresses including forced

swimming, conditioned emotional response (CER), restraint stress and cold stress have been reported to increase fecal output (8, 9). Among them, restraint stress has been commonly used to screen for compounds that are effective for the treatment of IBS (10–12). Williams et al. (10) reported that wrap restraint stress causes intestinal symptoms in rats similar to IBS in humans without formation of ulcers. This indicates the model may be relevant as an animal model of IBS. However, pharmacological effects of 5-HT_{1A}-receptor agonists in the wrap stress model have not been studied yet. Effects of 5-HT_{1A}-receptor agonists on stress-induced defecation seem to depend upon the stress used. Buspirone reduced the CER-induced cecal motor response (13). Ipsapirone reduced CER-induced defecation but not forced swimming-induced nor restraint stress-induced defecation (8). Their effects on the CER stress-induced cecal responses have been considered to reflect their anxiolytic effect.

Recently, we reported that a novel benzodioxan derivative, 5-{3-[(*2S*)-1,4-benzodioxan-2-ylmethyl]amino]pro-

poxy}-1,3-benzodioxole HCl (MKC-242), selectively bound to 5-HT_{1A} receptors and acted as an agonist for the receptor (14). Unlike azapirones, the compound does not possess the 1-(2-pyrimidinyl)piperazine (1-PP) structure. MKC-242 showed an anxiolytic-like effect in the punished drinking and social interaction test and an antidepressant-like effect in the forced swimming test and restraint stress-induced behavioral deficit models (15).

In the present study, we investigated the effect of MKC-242 in wrap stress-induced defecation as an animal model of IBS and compared it with the effects of reference compounds including other 5-HT_{1A}-receptor agonists.

MATERIALS AND METHODS

Animals

Male Wistar rats (Japan Laboratory Animals, Inc., Tokyo) weighing 250–350 g and male ddY mice (Japan SLC, Inc., Shizuoka) weighing 14–24 g were used. All animals were group housed (rats, 5–6/cage; mice, 8–11/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. Each animal was used only once.

Wrap restraint stress-induced defecation

The method described by Williams et al. (10) was used with slight modifications. Rats were acclimated to the observation metal mesh cage placed on a tray for 5 hr (9:00–14:00). After the acclimation, they were lightly anesthetized with ether. Then, their forelimbs were restrained with adhesive tape (12.5-mm width). The restrained limbs and thoracic trunk were wrapped with the other adhesive tape (50-mm width). Immediately after that, rats were returned to the observation cage. The number of feces dropped on the tray was counted 1 hr after the wrapping. Test compounds were administered 1 hr or 30 min before the restraint.

Spontaneous defecation

Rats were individually housed in the metal mesh cage placed on a tray with free access to food and water for 3 days. They were treated with test compounds twice daily (9:00 and 17:00). The number of feces was counted before each treatment. The test was started at 9:00 on the first day.

5-HT-induced defecation

Rats were subcutaneously administered 5-HT (10 mg/kg) or saline. The number of feces dropped was counted 1 hr after the administration. Test compound was given 1 hr before the 5-HT administration.

Oxotremorine-induced tremor

Mice were orally treated with test compounds. One hour after the treatment, they were intraperitoneally administered oxotremorine (0.2 mg/kg). The intensity of tremor was observed 10 min after the oxotremorine injection and scored on a scale of 0 to 2 (0, absent; 1, moderate; 2, intense), as described by Ögren et al. (16).

Drugs

MKC-242, tandospirone citrate, fluoxetine and *N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropanamide (WAY100135) were synthesized in our laboratory. Other compounds were obtained from standard commercial sources. MKC-242, tandospirone, WAY100135 and loperamide (Sigma, St. Louis, MO, USA) were each suspended in 0.5% carboxymethylcellulose (CMC) solution. Fluoxetine and diazepam (Koa Seiyaku, Yokohama) were each suspended in 0.5% Tween 80 solution. Buspirone (Sigma) was dissolved in distilled water. Oxotremorine (Sigma), 5-HT (Sigma), 1-PP (Aldrich, Milwaukee, WI, USA) and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; RBI, Natick, MA, USA) were each dissolved in saline. Amitriptyline HCl (Nihon Shinyaku, Kyoto) was dissolved in distilled water for the wrap-stress experiment and in 0.5% CMC for the spontaneous defecation experiment. All drugs were given either in a volume of 1 ml/kg to rats or 10 ml/kg to mice.

Statistics

The statistical differences in parametric data between the differently treated groups were determined by the one way analysis of variance test (ANOVA). After a positive ANOVA result, individual differences between groups were tested by the Dunnett's two-tailed test or the Tukey-Kramer test. The statistical differences in non-parametric data were determined by the Kruskal-Wallis test. After a positive result in the Kruskal-Wallis test, individual differences between the control group and drug-treated groups were tested by Dunnett's two-tailed test with rank data. When 2 group were compared, their statistical differences were tested by Student's *t*-test for parametric data or the Mann-Whitney *U*-test for non-parametric data. Differences between groups were considered to be significant if $P < 0.05$.

RESULTS

Wrap-restraint stress significantly increased defecation (Fig. 1). The stress-induced defecation was reduced by prior administration of MKC-242 in a dose-dependent manner (Fig. 1A). Prior treatment with 8-OH-DPAT (0.1 and 0.3 mg/kg, s.c.) also significantly reduced the stress-induced defecation (Fig. 1B). To clarify the contribution

Fig. 1. Effects of 5-HT_{1A} receptor ligands on wrap restraint stress-induced defecation in rats. All values are reported as the mean ± S.E. (n=6 for A and B, n=8 for C) of the number of feces during 1-hr wrapping. *P<0.05, by Dunnett's test (A and B) or the Tukey-Kramer test (C) after ANOVA. #P<0.05, by Student's *t*-test. †P<0.05, by the Mann-Whitney *U*-test. A: MKC-242 and loperamide (L) were orally administered 1 hr before the restraint. Normal (nor) and control (cont) indicate the stress-free-control and stressed-control, respectively. B: 8-OH-DPAT was subcutaneously administered 1 hr before the restraint. C: WAY100135 (30 mg/kg) or 0.5% CMC (CMC) was subcutaneously administered 1 hr before the restraint. MKC-242 (1 mg/kg) or CMC was orally administered 30 min before restraint.

of 5-HT_{1A} receptors for the reduction of stress-induced defecation by MKC-242, the effect of WAY100135, a 5-HT_{1A}-receptor antagonist, was investigated. Although WAY100135 (30 mg/kg, s.c.) did not affect the stress-induced defecation by itself, it antagonized the reducing effect of MKC-242 on stress-induced defecation (Fig. 1C). In contrast to MKC-242 and 8-OH-DPAT, buspirone and tandospirone only tended to decrease the stress-induced defecation at relative higher doses (Table 1). Diazepam and amitriptyline significantly decreased the stress-in-

Table 1. Effects of anxiolytics and antidepressants on the wrap stress-induced defecation in rats

Treatment	Dose (mg/kg, p.o.)	Fecal pellets
Normal (stress free)	0	0.00 ± 0.00 [†]
Control	0	7.40 ± 0.87
Loperamide	10	3.67 ± 1.12 [#]
Buspirone	10	8.33 ± 1.38
	30	6.50 ± 1.48
	100	4.17 ± 1.25
Normal (stress free)	0	0.00 ± 0.00 [†]
Control	0	8.00 ± 1.13
Loperamide	10	2.83 ± 0.87 [#]
Tandospirone	30	8.67 ± 0.42
	100	7.67 ± 1.05
	300	4.50 ± 1.29
Normal (stress free)	0	0.00 ± 0.00 [†]
Control	0	8.83 ± 0.54
Loperamide	10	5.17 ± 0.98 [#]
Diazepam	3	6.50 ± 1.34
	10	5.67 ± 1.36
	30	2.00 ± 1.13 [*]
Normal (stress free)	0	0.00 ± 0.00 [†]
Control	0	8.00 ± 0.78
Loperamide	10	4.50 ± 0.50 [#]
Amitriptyline	10	6.67 ± 1.50
	30	8.50 ± 1.03
	100	2.50 ± 1.20 [*]
Normal (stress free)	0	0.00 ± 0.00 [†]
Control	0	9.00 ± 0.93
Loperamide	10	3.00 ± 1.41 [#]
Fluoxetine	10	6.83 ± 1.78
	30	7.67 ± 1.23
	100	6.67 ± 1.41

Test compounds were administered 1 hr before the restraint. All data are reported as the mean ± S.E. (n=5–6) of the number of feces during 1-hr wrapping. *P<0.05, compared with the stress control group by Dunnett's test after ANOVA. #P<0.05, compared with the stress control group by Student's *t*-test. †P<0.05, compared with the stress control group by the Mann-Whitney *U*-test.

duced defecation at 30 and 100 mg/kg, p.o., respectively (Table 1). Fluoxetine did not have any effect on the defe-

Fig. 2. Effect of 1-PP on the reduction of stress-induced defecation by MKC-242 in rats. MKC-242 (1 mg/kg, p.o.) and 1-PP (30 mg/kg, s.c.) were administered 30 min and 1 hr before the restraint, respectively. All values are reported as the mean ± S.E. (n=12) of the number of feces during 1-hr wrapping. *P<0.05, by the Tukey-Kramer test after ANOVA.

Fig. 3. Effects of MKC-242 and amitriptyline on spontaneous defecation of rats under the stress-free condition. Vehicle (○), MKC-242 (●, 3 mg/kg; ■, 10 mg/kg) and amitriptyline (▲, 30 mg/kg) were orally administered twice daily. All values are reported as the mean ± S.E. (n=5) of the total number of feces during 24 hr. *P<0.05, compared with the vehicle control group by Dunnett's test after ANOVA.

cation at the tested doses (Table 1).

To evaluate the involvement of a metabolite of buspirone, 1-PP, on the defecation, the effect of 1-PP was investigated. Although 1-PP (30 mg/kg, s.c.) did not affect the stress-induced defecation by itself, it antagonized the reducing effect of MKC-242 on stress-induced defecation (Fig. 2).

Twice daily treatment with amitriptyline (30 mg/kg, p.o.) reduced spontaneous defecation under the stress-free condition (Fig. 3). The reduction was statistically significant on days 2 and 3. In addition, amitriptyline (30 and 100 mg/kg, p.o.) significantly reduced the incidence of oxotremorine-induced tremor (Table 2). On the other hand, MKC-242 did not affect spontaneous defecation and oxotremorine-induced tremor at 3 and 10 mg/kg,

Table 2. Effects of MKC-242 and reference compounds on the oxotremorine-induced tremor in mice

Treatment	Dose (mg/kg, p.o.)	Intensity of tremor
Control	0	1.88±0.13
MKC-242	1	1.88±0.13
	3	2.00±0.00
	10	2.00±0.00
	30	1.00±0.38
Control	0	2.00±0.00
Amitriptyline	3	1.63±0.18
	10	1.13±0.23
	30	0.25±0.16*
	100	0.00±0.00*
Control	0	1.75±0.16
Buspirone	10	1.88±0.13
	30	1.88±0.13
	100	1.63±0.18
	300	0.13±0.13*
Control	0	1.75±0.16
Tandospirone	10	2.00±0.00
	30	2.00±0.00
	100	1.75±0.16
	300	1.50±0.19
Control	0	2.00±0.00
Fluoxetine	3	1.63±0.18
	10	1.88±0.13
	30	2.00±0.00
	100	1.88±0.13

Test compounds were administered 1 hr before oxotremorine (0.2 mg/kg, i.p.) injection. All data are reported as the mean ± S.E. (n=8) of the score obtained 10 min after the oxotremorine injection. *P<0.05, compared with the control group by Dunnett's test with rank data after the Kruskal-Wallis test.

Fig. 4. Effect of MKC-242 on 5-HT-induced defecation in rats. The number of feces was counted 1 hr after 5-HT- or saline-injection. MKC-242 and CMC was orally administered 1 hr before the injection. All values are reported as the mean $\pm$ S.E. ($n=8$). $^{\dagger}P<0.05$, by the Mann-Whitney U -test.

p.o. (Fig. 3, Table 2). These doses were higher than the doses active in the wrap-restraint stress-induced defecation test.

Subcutaneous administration of 5-HT significantly increased defecation under the stress-free condition (Fig. 4). The 5-HT-induced defecation was not affected by prior administration of MKC-242 at the doses effective in stress-induced defecation (Fig. 4).

DISCUSSION

In the present study, MKC-242 reduced restraint stress-induced defecation as 8-OH-DPAT did. Moreover, the effect of MKC-242 was antagonized by a 5-HT_{1A}-receptor antagonist, WAY100135. These results indicate that the stimulation of 5-HT_{1A} receptors reduces stress-induced defecation.

In contrast to MKC-242 and 8-OH-DPAT, two azapirones including buspirone and tandospirone only tended to decrease the stress-induced defecation. The relatively weak effects of these azapirones might be due to their major metabolite, 1-PP, because the reducing effect of MKC-242 on stress-induced defecation was antagonized by the prior administration of 1-PP. Buspirone was rapidly metabolized to 1-PP (17). Since 1-PP has low affinity for 5-HT_{1A} receptors but high affinity for α_2 -adrenoceptors (18), it is unlikely that 1-PP antagonized the reducing effect of MKC-242 on the stress-induced defecation via 5-HT_{1A}-receptor agonist action. It has been reported that 1-PP and other α_2 -adrenoceptor antagonists increased fecal excretion in rats (19). Such an in-

creasing effect of 1-PP on fecal excretion may abolish the reducing effects of 5-HT_{1A}-receptor agonists on stress-induced defecation.

Stress-induced 5-HT release in the colon is considered to be responsible for the stress-induced defecation (20). It is suggested that 5-HT_{1A}-receptor agonists may reduce stress-induced defecation via its action on a point upstream of 5-HT release, because MKC-242 did not decrease the 5-HT-induced defecation. Miyata et al. (20) reported that diazepam reduced stress-induced defecation but not 5-HT- and thyrotropin-releasing hormone-induced defecation and suggested the central action of the compound. In behavioral studies, 5-HT_{1A}-receptor agonists have shown an anxiolytic effect like benzodiazepines (see the Introduction). The anxiolytic effects of 5-HT_{1A}-receptor agonists and benzodiazepines may reduce stress-induced physiological changes including 5-HT release in the colon. Indeed, gastric ulcer, another stress-induced change, was also inhibited by treatment with buspirone in the communication box method in mice (21).

From another point of view, it is also possible that 5-HT_{1A} receptors in the myenteric plexus may contribute to the reduction of stress-induced defecation. It has been reported that 5-HT_{1A} receptor stimulation in enteric neurons inhibited excitatory postsynaptic potentials and acetylcholine release in the myenteric plexus (22–24) and smooth muscle contraction (24), while another 5-HT receptor subtype, 5-HT₃ receptors, are distributed widely in the enteric nervous system (25). 5-HT₃-receptor antagonists including YM114 (26), KF18259 (11) and GK-128 (12) reduced stress-induced defecation in the same model used in the present study. Thus, stimulation of 5-HT₃ receptors are considered to enhance intestinal motility. In the central nervous systems (CNS), 5-HT_{1A} receptor stimulation reduces serotonergic neuronal activity via somatodendritic autoreceptors (5, 14). If 5-HT_{1A} receptors negatively modulate 5-HT neuronal activity in the myenteric plexus as seen in the CNS, 5-HT₃ receptor stimulation may be reduced by 5-HT_{1A}-receptor agonists. In wrap restraint model, both 5-HT_{1A}-receptor agonists and 5-HT₃ antagonists reduce defecation. It is noteworthy that these two types of compounds also reduce emesis in animal models with different profile (27–29).

Although MKC-242 binds to 5-HT_{1A} receptors with higher affinity than 8-OH-DPAT (14, 30), MKC-242 reduced stress-induced defecation at approximately tenfold higher doses than 8-OH-DPAT. Since 8-OH-DPAT has poor oral bioavailability (30), the compound was subcutaneously administered in the present study, while MKC-242 and the other reference compounds were orally administered. The difference of administration route may cause the difference of effective doses.

In the present study, amitriptyline reduced not only stress-induced defecation but also spontaneous defecation. The compound binds to M₁-muscarinic receptor and acts as an antagonist (31). Such an anti-cholinergic effect of amitriptyline was detected in the oxotremorine test at relative lower dose than the dose required to decrease stress-induced defecation. These results suggest that the anti-cholinergic effect of amitriptyline may be responsible for the reducing effect on stress-induced defecation. Another antidepressant, fluoxetine, did not reduce the stress-induced defecation. The compound selectively inhibits 5-HT uptake, but has little effect on catecholamine uptake and muscarinic receptors (32). The negative result on fluoxetine may be due to its very low anti-cholinergic effect. In contrast to amitriptyline, MKC-242 did not reduce spontaneous defecation and oxotremorine-induced tremor at tenfold higher doses than the dose at which it decreased stress-induced defecation. This indicates that MKC-242 has little anti-cholinergic effect and that its reducing effect on defecation is specific for the stressed condition but not the stress-free condition.

In conclusion, MKC-242 reduced wrap restraint stress-induced defecation at doses having no effect on spontaneous defecation. The reduction might be mediated via 5-HT_{1A} receptor activation. This suggests that MKC-242 may be a new candidate for the treatment of IBS.

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