

Pharmacological Properties of KF18259, a Novel 5-HT₃-Receptor Antagonist, in Rats: Inhibition of the Distal Colonic Function

Nobuyuki Kishibayashi, Shunji Ichikawa, Toshihide Yokoyama, Akio Ishii and Akira Karasawa

Department of Pharmacology, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

Received July 28, 1993 Accepted September 28, 1993

ABSTRACT—We investigated the effects of KF18259 (*endo*-(8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-1-isobutyl-2-oxo-1,2-dihydro-4-quinolinecarboxylate hydrochloride), a novel 5-HT₃-receptor antagonist, in a variety of rat models, which are assumed to be mediated via 5-HT₃ receptors, in comparison with those of YM060 ((*R*)-5-[(1-methyl-3-indolyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole hydrochloride), granisetron and ondansetron. KF18259 inhibited wrap-restraint stress-induced defecation. The doses of KF18259 to inhibit wrap-restraint stress-induced defecation were lower than those to inhibit the 5-HT-induced von Bezold-Jarisch reflex and the cisplatin-induced slowing of gastric emptying. In contrast, the doses of YM060, granisetron and ondansetron to inhibit these three responses were similar. Moreover, KF18259 inhibited the wrap-restraint stress-induced propulsive motility of the proximal and distal colon. The effect of KF18259 on the distal colon was as potent as that on defecation and was more potent than that on the proximal colon. These results indicate that KF18259 potently inhibits the distal colonic function. KF18259 may be a useful tool for the discrimination of the 5-HT₃-receptors located on the distal colon and other tissues.

Keywords: 5-HT₃-receptor antagonist, Defecation, Colonic propulsion

5-Hydroxytryptamine (5-HT) is present in the gastrointestinal tract, where it is mainly localized in the enterochromaffin cells of the mucosa (1). 5-HT mediates various physiological and pharmacological actions of the gastrointestinal system through the activation of four types of 5-HT receptors, including 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ receptors (2–4). Among them, 5-HT₃ receptors are widely distributed in the enteric nervous system (5) and play important roles in the regulation of gastrointestinal motility (2, 6–8). In fact, the potent and highly selective 5-HT₃-receptor antagonist ondansetron (GR 38032F) accelerates gastric emptying (9, 10) and inhibits cisplatin-induced emesis in animals and humans (10–14). It also reverses the cisplatin-induced slowing of gastric emptying in fed rats (15).

Some studies (16, 17) have revealed an association between the occurrence of stressful experience and the appearance of disturbances in bowel function, indicating that the gut function is affected by various stresses. In humans, stress commonly results in gastrointestinal disorders like irritable bowel syndrome (IBS) (18, 19), in association with changes in gastrointestinal motility (16) and digestive transit (17). Recently, it was reported (20) that

the selective 5-HT₃-receptor antagonists YM060 and granisetron (BRL43694) inhibit the restraint stress-induced defecation in rats, suggesting that endogenous 5-HT mediates stress-induced changes in bowel function through the 5-HT₃ receptor.

KF18259, *endo*-(8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-1-isobutyl-2-oxo-1,2-dihydro-4-quinolinecarboxylate hydrochloride (Fig. 1), is a potent and selective 5-HT₃-receptor antagonist (21). KF18259 inhibits the [³H]quipazine binding to 5-HT₃-receptor sites in NG108-15 cells

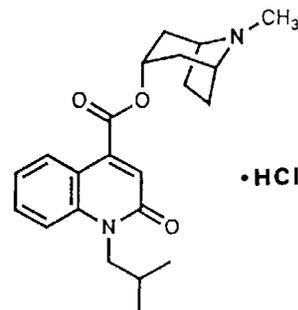


Fig. 1. Chemical structure of KF18259.

and the 5-HT-induced transient bradycardia in rats (21). In the present study, we determined the effects of KF18259 on wrap-restraint stress-induced defecation, which is an appropriate animal model for IBS (17), the 5-HT-induced von Bezold-Jarisch reflex and the cisplatin-induced slowing of gastric emptying in rats. We also determined the effects of KF18259 on the wrap-restraint stress-induced propulsive motility of the proximal and the distal colon in rats. The purposes of the present study were 1) to compare the activity of KF18259 among a variety of animal models, which are supposed to involve 5-HT₃ receptors, and 2) to compare the effect of KF18259 with those of the other 5-HT₃-receptor antagonists.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 150 to 250 g were purchased from Japan SLC, Inc. (Hamamatsu). The animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 12-hr light-dark cycle.

Drugs

KF18259 (*endo*-(8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-1-isobutyl-2-oxo-1,2-dihydro-4-quinolinecarboxylate hydrochloride), YM060 ((*R*)-5-[(1-methyl-3-indolyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole hydrochloride), granisetron (BRL43694: *N-endo*-9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-indazole-3-carboxamide hydrochloride), and ondansetron (GR38032F: (±)-1,2,3,9-tetrahydro-3-[(methylimidazol-1-yl)methyl]-9-methyl-4*H*-carbazol-4-one hydrochloride) were synthesized in our laboratories. 5-HT creatinine sulfate was purchased from E. Merck (Darmstadt, Germany). Cisplatin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phenol red, charcoal and trichloroacetic acid were purchased from Wako Pure Chemical Industries (Osaka). Test drugs were dissolved in distilled water. All drugs were orally administered to rats at a volume of 5 ml/kg.

Wrap-restraint stress-induced defecation

The wrap-restraint stress was induced in rats according to the reported procedure (17). The rats were lightly anesthetized with ether, and the foreshoulders, upper forelimbs and the thoracic trunk were wrapped in paper tape to restrict, but not prevent, movement. The animals recovered from anesthesia within 2–5 min and immediately moved around in cages and ate and drank, but had been restricted from mobility of forelimbs, which prevented them from grooming the face, upper head and the neck. Fecal pellet output induced by wrap-restraint stress was

weighted during the 1st hr after stress. The test drugs were given p.o. 1 hr before stress. The ID₅₀ values were calculated as the doses that reduced stress-induced defecation by 50%.

5-HT-induced von Bezold-Jarisch reflex

The 5-HT-induced von Bezold-Jarisch reflex assay was carried out with a modification of the reported procedure (22). Each animal was anesthetized with urethane (1.25 g/kg, i.p.) and then the trachea was cannulated. Blood pressure was recorded from the left carotid artery via a saline/heparin-filled pressure transducer, from which the heart rate was continuously monitored. The Bezold-Jarisch reflex was induced by rapid bolus i.v. injection of 5-HT (30 µg/kg). The test drug was given p.o. 1 hr before the injection of 5-HT. Each animal was anesthetized 30 min after the test drug was given. The ID₅₀ values were calculated as the doses that reduced the 5-HT-induced bradycardia by 50%.

Cisplatin-induced slowing of gastric emptying

The cisplatin-induced slowing of gastric emptying was elicited with a modification of the reported procedure (23). The animals were deprived of food 24 hr prior to the experiment but allowed free access to water until 3 hr before the experiment. A solution of 50 mg phenol red in 100 ml aqueous sodium carboxymethyl cellulose (1.5% w/v) was used as a test meal. Slowing of gastric emptying was induced by i.p. injection of cisplatin (3 mg/kg) 30 min before the test meal was given. Fifteen minutes after the test meal was given, the animals were sacrificed by cervical dislocation. In each experiment, a group of 4 animals was sacrificed immediately after administration of the meal and considered as a standard (100% phenol red in the stomach) to avoid the errors associated with terminal convulsions of the animal. The stomach was then exposed by laparotomy and removed.

The stomach was incised in 40 ml of NaOH solution (0.1 N) and its content was dissolved. One ml of the supernatant was added to 2 ml of trichloroacetic acid solution (7.5% w/v) to precipitate the proteins. After centrifugation (2500 × g for 15 min), 1 ml of the supernatant was added to 1 ml of NaOH (1 N) to develop the maximum intensity of the color. The absorbance at 560 nm of the solution was then measured with a spectrophotometer (model U-1080; Hitachi, Tokyo).

The test drug was given p.o. 1 hr before the test meal was given. The gastric emptying (G.E.) for each rat was calculated according to the following formula:

$$\text{G.E. (\%)} = \{1 - (\text{Amount of phenol red recovered from the test stomach}) / (\text{Average amount of phenol red recovered from the standard stomach})\} \times 100$$

The ID₅₀ values were calculated as the doses that

reduced the cisplatin-induced slowing of gastric emptying by 50%.

Wrap-restraint stress-induced propulsive motility of the proximal colon

The wrap-restraint stress-induced propulsive motility of the proximal colon was determined with a modification of the reported method (24). Each animal was anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the cecum was exposed by laparotomy. A vinyl tube of about 1 mm in diameter was inserted into the cecum at the beginning of the colon. The other end of the tube was taken out of the back. The animals were kept one by one for 4 or 5 days and deprived of food 24 hr prior to the experiment.

In the experiment examining the wrap-restraint stress-induced propulsive motility of the proximal colon, a suspension of 5 g charcoal in 100 ml aqueous Arabic gum (10% w/v) was used as a test meal. Each animal was lightly anesthetized with ether and administered 0.5 ml of the test meal into the colonic tubing. At the same time, the foreshoulder, upper forelimbs and the thoracic trunk were wrapped in paper tape to restrict, but not prevent, the movement. One hour after the test meal was given, the animals were sacrificed by cervical dislocation. The colon was then exposed by laparotomy, and the percentage traverse of the charcoal meal in the colon was determined. The test drug was given p.o. 1 hr before the test meal was given. The ID₅₀ values were calculated as the doses that reduced the wrap-restraint stress-induced propulsive motility of the proximal colon by 50%.

Wrap-restraint stress-induced propulsive motility of the distal colon

The wrap-restraint stress-induced propulsive motility of the distal colon was elicited with a modification of the reported procedure (25). Each animal was lightly anesthetized with ether, and a teflon ball of 3 mm in diameter was inserted into the colon 4 cm proximal to the anus. At the same time, the foreshoulder, upper forelimbs and the thoracic trunk were wrapped in paper tape to restrict, but not prevent, movement. The time required to evacuate the teflon ball was determined. The test drug was given p.o. 1 hr before the teflon ball was inserted. The ID₅₀ values were calculated as the doses that reduced the wrap-restraint stress-induced propulsive motility of the distal colon by 50%.

Statistical analyses

The result was expressed as the mean \pm S.E.M. Differences between the mean values in the normal group and control group were analyzed by the Wilcoxon rank sum test. Differences between the mean values in groups of

each drug treatment and control group were analyzed by the Steel multiple comparison test following the Kruskal-Wallis test. A difference at $P < 0.05$ was considered statistically significant.

RESULTS

Inhibition of wrap-restraint stress-induced defecation

The wrap-restraint stress significantly increased fecal pellet output in fed rats. Figure 2 shows the time course of changes in fecal pellet output produced during the wrap-restraint stress. Stool weight was increased by the stress only during 0–1 hr. After p.o. administration, all the drugs used in the present study dose-dependently inhibited wrap-restraint stress-induced increases in stool weight (Fig. 3). The ID₅₀ values for the drugs are shown in Table 1.

Inhibition of the 5-HT-induced von Bezold-Jarisch reflex

The Bezold-Jarisch reflex was induced by rapid bolus i.v. injection of 5-HT (30 μ g/kg) in fed rats. As shown in Fig. 4, all the drugs in the present study dose-dependently inhibited the 5-HT-induced von Bezold-Jarisch reflex. The ID₅₀ values for the drugs are shown in Table 1.

Inhibition of cisplatin-induced slowing of gastric emptying

The i.p. injection of cisplatin at doses ranging from 3 to 10 mg/kg slowed the gastric emptying in a dose-dependent manner (Fig. 5). As shown in Fig. 6, all the drugs in the present study dose-dependently inhibited the cisplatin (3 mg/kg i.p.)-induced slowing of gastric emptying in rats. The ID₅₀ values for the drugs are shown in Table 1.

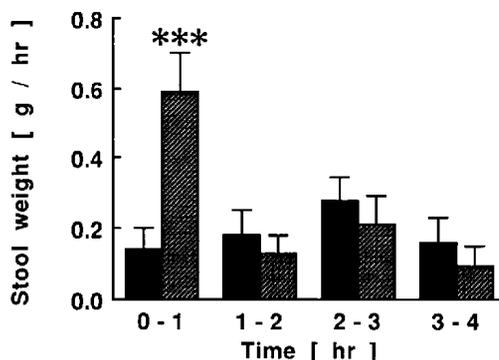


Fig. 2. Effect of the wrap-restraint stress on defecation in fed rats. Each bar represents the mean \pm S.E.M. of 12 rats. Symbols indicate the nonstress normal group (■) or the stress group (▨). ***; $P < 0.001$, compared with the value in the nonstress normal group (Wilcoxon rank sum test).

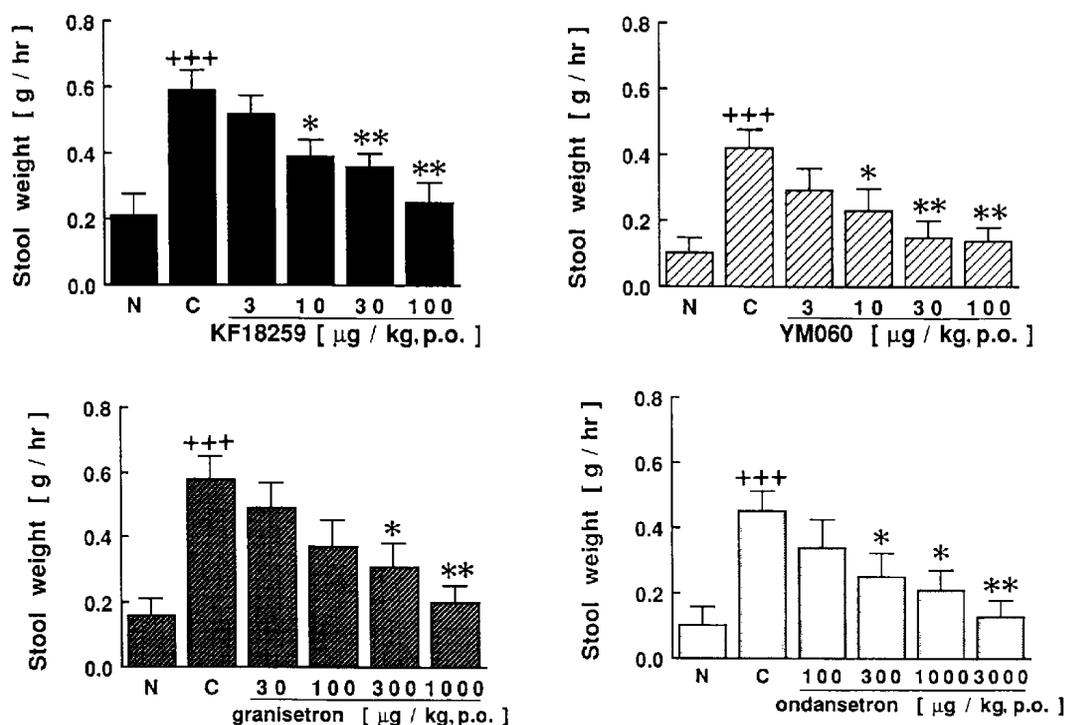


Fig. 3. Effects of KF18259, YM060, granisetron and ondansetron on wrap-restraint stress-induced defecation in fed rats. Each bar represents the mean \pm S.E.M. of 12 rats. +++: $P < 0.001$, compared with the value in the normal (N) group (Wilcoxon rank sum test). *: $P < 0.05$, **: $P < 0.01$, compared with the value in the control (C) group (Steel multiple comparison test).

Table 1. Effects of KF18259, YM060, granisetron and ondansetron on the wrap-restraint stress-induced defecation, the 5-HT-induced von Bezold-Jarisch reflex, the cisplatin-induced slowing of gastric emptying and the wrap-restraint stress-induced propulsive motility of the proximal and the distal colon in rats

Drugs	ID ₅₀ (µg/kg, p.o.)				
	Wrap-restraint stress-induced defecation	5-HT-induced von Bezold-Jarisch effect	Cisplatin-induced slowing of gastric emptying	Wrap-restraint stress-induced propulsive motility of the proximal colon	Wrap-restraint stress-induced propulsive motility of the distal colon
KF18259	13	170	50	50	10
YM060	5.2	1.6	5.4	21	69
Granisetron	120	140	250	620	740
Ondansetron	270	250	230	3400	1600

The ID₅₀ values were calculated as the doses which reduced stress-induced defecation, the 5-HT-induced bradycardia, the cisplatin-induced slowing of emptying, or the wrap-restraint stress-induced propulsive motility of the proximal or the distal colon, respectively, by 50%. The ID₅₀ values are presented as the mean of 5–12 animals.

Inhibition of the wrap-restraint stress-induced propulsive motility of the proximal colon

The wrap-restraint stress significantly increased the propulsive motility of the proximal colon in rats. Figure 7 shows that all the drugs in the present study dose-dependently inhibited the wrap-restraint stress-induced propulsive motility of the proximal colon. The ID₅₀ values for the drugs are shown in Table 1.

Inhibition of the wrap-restraint stress-induced propulsive motility of the distal colon

The wrap-restraint stress significantly increased the propulsive motility of the distal colon in rats. Figure 8 shows that all the drugs in the present study dose-dependently inhibited the wrap-restraint stress-induced propulsive motility of the distal colon. The ID₅₀ values for the drugs are shown in Table 1.

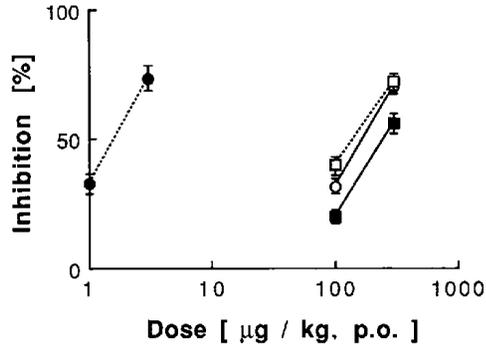


Fig. 4. Effects of KF18259 (○), YM060 (●), granisetron (□) and ondansetron (■) on the Bezold-Jarisch effect in fed rats. Each bar represents the mean \pm S.E.M. of 5 rats.

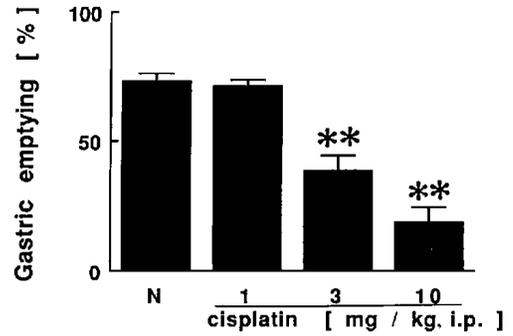


Fig. 5. Effect of cisplatin on the gastric emptying in rats. Each bar represents the mean \pm S.E.M. of 10 rats. **: $P < 0.01$, compared with the value in the normal (N) group (Steel multiple comparison test).

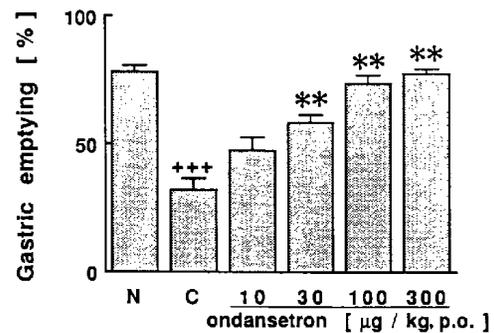
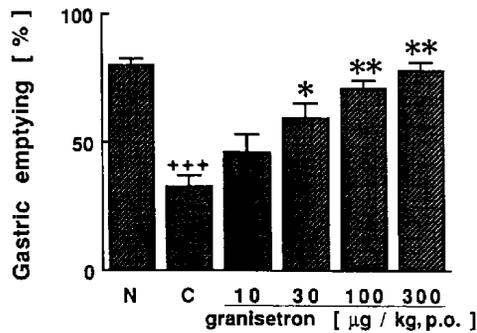
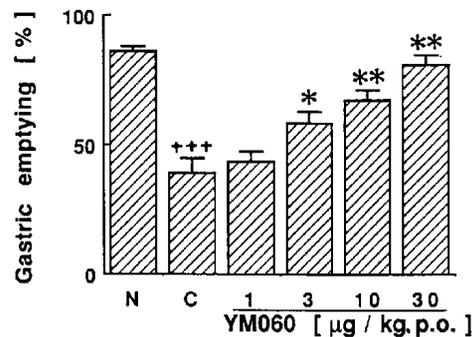
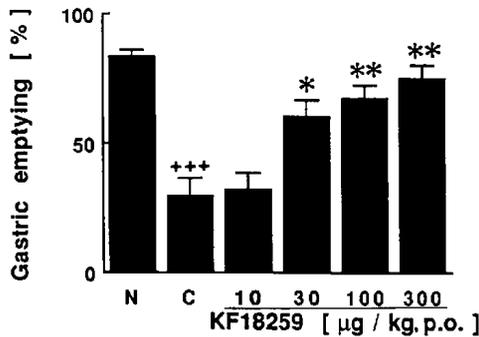


Fig. 6. Effects of KF18259, YM060, granisetron and ondansetron on the cisplatin-induced slowing of gastric emptying in rats. Each bar represents the mean \pm S.E.M. of 10 rats. +++: $P < 0.001$ compared with the value in the normal (N) group (Wilcoxon rank sum test). *: $P < 0.05$, **: $P < 0.01$, compared with the value in the control (C) group (Steel multiple comparison test).

DISCUSSION

The wrap-restraint stress model is an appropriate animal model for IBS (17). It has already been reported (26) that wrap-restraint stress-induced defecation is inhibited by granisetron and ondansetron. However, the effects of 5-HT₃-receptor antagonists on the wrap-restraint stress-induced propulsive motility of the proximal and the distal colon have never been reported prior to the

present study. The present study demonstrates that the 5-HT₃-receptor antagonists, including KF18259, granisetron and ondansetron, attenuate the wrap-restraint stress-induced propulsive motility of the proximal and the distal colon. These results suggest that the inhibition by the 5-HT₃-receptor antagonist of wrap-restraint stress-induced defecation is due to the inhibitory effect on the propulsive motility of the colon. Moreover, these results support the notion that the 5-HT₃-receptor antagonist is effective in

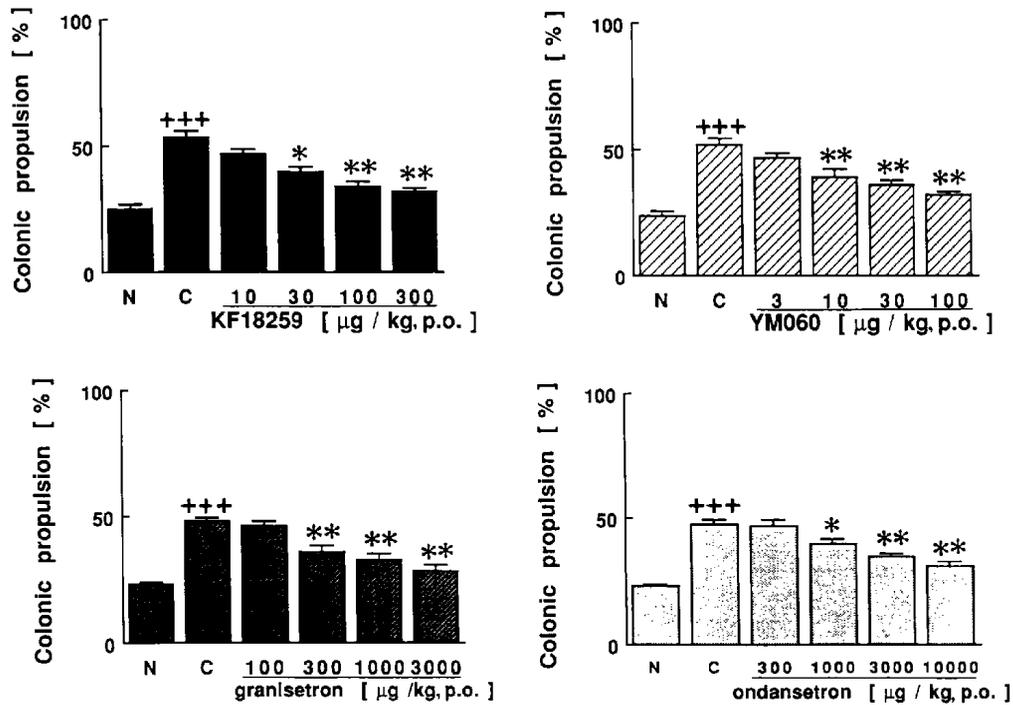


Fig. 7. Effects of KF18259, YM060, granisetron and ondansetron on the wrap-restraint stress-induced propulsive motility of the proximal colon in rats. Each bar represents the mean \pm S.E.M. of 8 rats. $+++$: $P < 0.001$, compared with the value in the normal (N) group (Wilcoxon rank sum test). *: $P < 0.05$, **: $P < 0.01$, compared with the value in the control (C) group (Steel multiple comparison test).

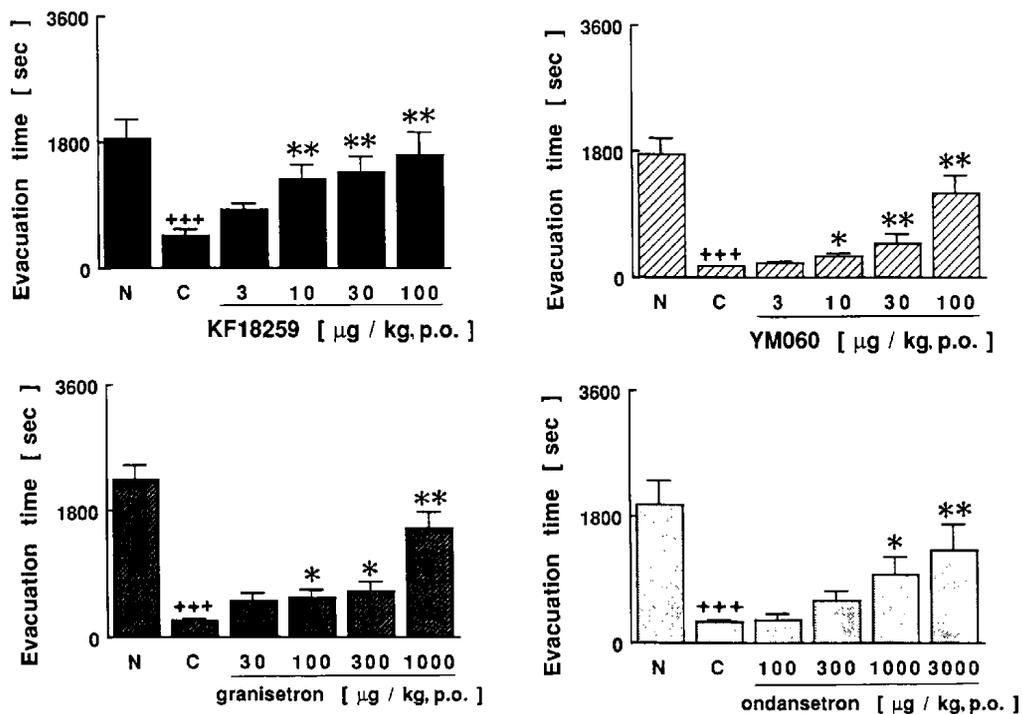


Fig. 8. Effects of KF18259, YM060, granisetron and ondansetron on the wrap-restraint stress-induced propulsive motility of the distal colon in rats. Each bar represents the mean \pm S.E.M. of 10 rats. $+++$: $P < 0.001$, compared with the value in the normal (N) group (Wilcoxon rank sum test). *: $P < 0.05$, **: $P < 0.01$, compared with the value in the control (C) group (Steel multiple comparison test).

the diarrhea associated with IBS (27, 28).

The present study indicated that the doses of KF18259 to inhibit wrap-restraint stress-induced defecation were lower than those to inhibit the 5-HT-induced von Bezold-Jarisch reflex and the cisplatin-induced slowing of gastric emptying. In contrast, YM060, granisetron and ondansetron almost equipotently inhibited these three responses, respectively. The inhibitory effect of KF18259 on the wrap-restraint stress-induced propulsive motility of the distal colon was more potent than that of the proximal colon and was as potent as the inhibitory effect of KF18259 on wrap-restraint stress-induced defecation. These results indicate that KF18259 potently inhibits the distal colonic function.

There are several possible mechanisms for the potent inhibitory effect of KF18259 on the distal colon. Whereas KF18259 exhibits a high affinity for 5-HT₃ receptors ($K_i = 0.47$ nM) (21), it has no affinity for any other receptors, including 5-HT₁, 5-HT₂ and 5-HT₄ receptors (T. Yokoyama et al., unpublished observations). Therefore, it is unlikely that KF18259 inhibited the distal colonic function via a mechanism independent of 5-HT₃ receptors. It has been proposed that 5-HT₃ receptors are divided into three subtypes, 5-HT_{3A}, 5-HT_{3B} and 5-HT_{3C} (3). In addition, it is known that ICS 205-930 and MDL 72222, selective 5-HT₃-receptor antagonists, are more potent in the rabbit heart than in the guinea pig ileum (29, 30). Recently, Maricq et al. reported that the messenger RNAs encoding the 5-HT₃ receptors in the intestine are different from those in the brain and the heart (31). Under these circumstances, it is suggested that the potent effect of KF18259 on the distal colon may be due to the difference in the 5-HT₃-receptor subtype.

In conclusion, the present results demonstrate that the 5-HT₃-receptor antagonist inhibit the propulsive motility of the colon associated with wrap-restraint stress, presumably resulting in the inhibition of the defecation. Moreover, the present results demonstrate that KF18259 potently inhibits the distal colonic function stimulated by wrap-restraint stress. Thus, KF18259 may be a useful tool for the discrimination of 5-HT₃ receptors located on the distal colon and other tissues.

Acknowledgments

We wish to thank C. Takashima and M. Watanabe for their skilful technical assistance, H. Hayashi and Y. Miwa for preparation of several compounds, and Dr. F. Suzuki and Dr. T. Hirata for encouragement and support.

REFERENCES

- Pentilla, A.: Histochemical reactions of enterochromaffin cells and the 5-hydroxytryptamine content of the mammalian duodenum. *Acta Physiol. Scand.* **281**, Supp. 1-77 (1966)
- Buchheit, K.-H., Engel, G., Muschler, E. and Richardson, B.P.: Study of the contractile effect of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum. *Naunyn Schmiedebergs Arch. Pharmacol.* **32**, 36-41 (1985)
- Richardson, B.P. and Engel, G.: The pharmacology and function of 5-HT₃ receptors. *Trends Neurosci.* **9**, 424-428 (1986)
- Dumuis, A., Sebben, M. and Bockaert, J.: The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT₄) positively coupled to adenylate cyclase in neurons. *Naunyn Schmiedebergs Arch. Pharmacol.* **340**, 403-410 (1989)
- Hendriks, R., Bornstein, J.C. and Furnness, J.B.: Evidence for two types of 5-hydroxytryptamine receptor on secretomotor neurons of the guinea-pig ileum. *Naunyn Schmiedebergs Arch. Pharmacol.* **339**, 409-414 (1989)
- Buchheit, K.-H., Costall, B., Engel, G., Gunning, S.J., Naylor, R.J. and Richardson, B.P.: 5-Hydroxytryptamine receptor antagonism by metoclopramide and ICS 205-930 in the guinea-pig leads to enhancement of contractions of stomach muscle strips induced by electrical field stimulation and facilitation of gastric emptying in vivo. *J. Pharm. Pharmacol.* **37**, 664-667 (1985)
- Gidda, J.S., Evans, D.C., Prime, P., Shenck, K. and Cohen, M.L.: Role of 5-HT₃ receptor antagonists in gastrointestinal motility. *Gastroenterology* **95**, 867 (1988)
- Gullikson, G.W., Loeffler, R.F., Bianchi, R.G., Perkins, W.E. and Bauer, R.F.: Relationship of 5-HT₃ receptor antagonist activity to gastrointestinal prokinetic activity in conscious dogs. *Gastroenterology* **95**, 869 (1988)
- Costall, B., Gunning, S.J., Naylor, R.J. and Tyers, M.B.: The effect of GR38032F, a novel 5-HT₃ receptor antagonist on gastric emptying in the guinea-pig. *Br. J. Pharmacol.* **91**, 263-264 (1987)
- Fozard, J.R.: The development and early clinical evaluation of selective 5-HT₃ receptor antagonists. *In* The Peripheral Actions of 5-Hydroxytryptamine, Edited by Fozard, J.R., pp. 374-376, Oxford University Press, Oxford (1989)
- Andrews, P.L.R., Rapeport, W.G. and Sanger, G.J.: Neuropharmacology of emesis induced by anti-cancer therapy. *Trends Pharmacol. Sci.* **9**, 334-341 (1988)
- Bermudez, J., Boyle, E.A., Miner, W.D. and Sanger, G.J.: The anti-emetic potential of the 5-hydroxytryptamine₃ receptor antagonist BRL 43694. *Br. J. Cancer* **58**, 644-650 (1988)
- King, F.D. and Sanger, G.J.: 5-HT₃ receptor antagonists. *Drugs Future* **14**, 875-889 (1989)
- Kilpatrick, G.J., Bunce, K.T. and Tyers, M.B.: 5-HT₃ receptors. *Med. Res. Rev.* **10**, 441-475 (1990)
- Eeckhout, C. and Vedder, A.: 5-HT₃ antagonists reverse the cisplatin induced slowing of gastric emptying in fed rats. *Gastroenterology* **94**, A111 (1988)
- Yano, S., Akahane, M. and Harada, M.: Role of gastric motility in development of stress-induced gastric lesions of rats. *Japan. J. Pharmacol.* **28**, 607-615 (1978)
- Williams, C.L., Villar, R.G., Peterson, J.M. and Burks, T.F.: Stress-induced changes in intestinal transit in the rat: A model for irritable bowel syndrome. *Gastroenterology* **94**, 611-621 (1988)
- Thompson, W.C.: Progress report: The irritable bowel. *Gut* **25**, 305-320 (1984)
- Narducci, F., Snape, W.J., Battle, W.M., London, R.L. and

- Cohen, S.: Increased colonic motility during exposure to a stressful situation. *Dig. Dis. Sci.* **30**, 40–44 (1985)
- 20 Miyata, K., Kamata, T., Nishida, A., Ito, H., Yuki, H., Yamamo, M., Tsutsumi, R., Katsuyama, Y. and Honda, K.: Role of the serotonin₁ receptor in stress-induced defecation. *J. Pharmacol. Exp. Ther.* **261**, 297–303 (1992)
- 21 Hayashi, H., Miwa, Y., Miki, I., Ichikawa, S., Yoda, N., Ishii, A., Kono, M. and Suzuki, F.: 5-HT₃ receptor antagonists. 1. New quinoline derivatives. *J. Med. Chem.* **35**, 4893–4902 (1992)
- 22 Dunber, A.W., McClelland, C.W. and Sanger, G.J.: BRL 24924: a stimulant of gut motility which is also a potent antagonist of the Bezold-Jarisch reflex in anaesthetised rats. *Br. J. Pharmacol.* **88**, Supp. 319P (1986)
- 23 Scarpignato, C., Capovilla, T. and Bertaccini, G.: Action of caerulein on gastric emptying of the conscious rat. *Arch. Int. Pharmacodyn. Ther.* **246**, 286–294 (1980)
- 24 Ueda, M., Matsuda, S., Kawakami, S., Mineshita, T. and Takeda, H.: On the propulsive motility of large intestine of mice and effects of pharmacological agents on it. *Oyo-Yakuri/Pharmacometrics* **3**, 265–294 (1969) (Abs. in English)
- 25 Yamada, K. and Onoda, Y.: Effects of trimebutine on colonic propulsion in mice. *J. Smooth Muscle Res.* **28**, 87–93 (1992)
- (Abs. in English)
- 26 Kadowaki, M., Nagakura, Y., Tokoro, K., Tomoi, M., Kohsaka, M. and Kuwahara, A.: Effect of FK1052, a potent 5-HT₃ and 5-HT₄ receptor antagonist, on gastrointestinal function. *Gastroenterology* **102**, A739 (1992)
- 27 Steadman, C.J., Talley, N.J., Phillips, S.F. and Mulvihill, C.: Trial of a selective serotonin type 3 (5-HT₃) receptor antagonist ondansetron (GR38032F) in diarrhea predominant irritable bowel syndrome (IBS). *Gastroenterology* **96**, A394 (1990)
- 28 Maxton, D.G., Haigh, C.G. and Whorwell, P.J.: Clinical trial of ondansetron, a selective 5-HT₃ antagonist, in irritable bowel syndrome (IBS). *Gastroenterology* **100**, A468 (1991)
- 29 Donatsch, P., Engel, G., Richardson, B.P. and Stadler, P.: ICS 205-930: a highly selective and potent antagonist at peripheral neuronal 5-hydroxytryptamine (5-HT) receptors. *Br. J. Pharmacol.* **81**, Supp. 34P (1984)
- 30 Fozard, J.R.: MDL 72222: a potent and highly selective antagonist at neural 5-hydroxytryptamine receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **326**, 36–44 (1984)
- 31 Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M. and Julius, D.: Primary structure and functional expression of the 5-HT₃ receptor, a serotonin-gated ion channel. *Science* **254**, 432–437 (1991)