Effect of Serotonin (5-HT\textsubscript{3})-Receptor Antagonists YM060, YM114 (KAE-393), Ondansetron and Granisetron on 5-HT\textsubscript{4} Receptors and Gastric Emptying in Rodents

Keiji Miyata, Mayumi Yamano, Takeshi Kamato and Shinobu Akuzawa

Neuroscience and Gastrointestinal Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd.,
21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan

Received June 5, 1995 Accepted August 9, 1995

ABSTRACT—We investigated the effects of YM060 ((R)-5-[(1-methyl-3-indolyl)carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazole hydrochloride) and YM114 (KAE-393) ((R)-5-[(2,3-dihydro-1-indolyl)-carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazole hydrochloride) on 5-HT\textsubscript{4} receptors and gastric emptying in normal and cisplatin-treated rats and compared results with those for ondansetron and granisetron. YM060, YM114, ondansetron and granisetron dose-dependently inhibited the specific binding of \[^{3}H\]GR113808 ([1-[(2-methylsulphonyl)amino]ethyl]-4-piperidin-yl)methyl 1-methyl-1H-indole-3-carboxylate} in guinea pig striatum, with pK\textsubscript{i} values of 5.53, 5.13, 5.21 and 5.63, respectively. According to the pK\textsubscript{i} values reported in 5-HT3-receptor binding of \[^{3}H\]GR65630 to rat cortical membranes, the affinity of YM060, YM114, ondansetron and granisetron for 5-HT\textsubscript{4} receptors was approximately 5, 5, 3.5 and 3.5 log units lower than that for 5-HT\textsubscript{3} receptors, respectively. In the guinea pig longitudinal muscle with myenteric plexus and rat esophageal tunica muscularis mucosae, YM060 and YM114 showed neither 5-HT\textsubscript{4}-agonistic nor antagonistic properties. Although ondansetron produced concentration-dependent increases in the magnitude of the twitch response in longitudinal muscle, it did not possess 5-HT\textsubscript{3}- and 5-HT\textsubscript{4}-agonistic activity. Granisetron antagonized 5-HT-induced relaxation of the rat esophagus with an apparent pA\textsubscript{2} value of 5.39. Intravenous YM060, YM114, ondansetron and granisetron significantly enhanced gastric emptying of glass beads and improved cisplatin-induced slowing of gastric emptying in rats. These results indicate that the selectivity of YM060 and YM114 for 5-HT\textsubscript{4} receptors is higher than that of ondansetron and granisetron and that these 5-HT\textsubscript{3} antagonists have gastroprokinetic activity in normal and cisplatin-treated rats without affecting 5-HT\textsubscript{4} receptors.

Keywords: YM060, YM114 (KAE-393), 5-HT\textsubscript{4} receptor, Gastric emptying

The use of cisplatin in the treatment of cancer is usually accompanied by the side effects of severe nausea and vomiting. Recently, a number of compounds that inhibit these side effects have been developed. These compounds all act via inhibition of 5-HT\textsubscript{3} receptors. YM060 and YM114 (KAE-393) are 4,5,6,7-tetrahydrobenzimidazole derivatives. These compounds have been reported to be potent and selective 5-HT\textsubscript{3}-receptor antagonists of 5-HT\textsubscript{3}-induced von Bezold-Jarisch reflex in rats (1, 2), contraction of the guinea pig colon (3, 4) and depolarization of the rabbit nodose ganglion (2, 5).

To date, a number of 5-HT\textsubscript{3}-receptor antagonists have been synthesized. Among these, tropisetron (6), cisapride (7), zacopride (8), ondansetron (9) and renzapride (10) have been reported to possess gastroprokinetic activities in rats or guinea pigs. Compounds that block the 5-HT\textsubscript{3} receptor are therefore suggested to increase the rate of gastric emptying.

Recent studies have revealed that the peripheral 5-HT\textsubscript{4}-receptor subtype is involved in the control of gastrointestinal motility through the release of acetylcholine (11 – 13) and that 5-HT\textsubscript{4}-receptor agonists facilitate gastric emptying (14, 15). Some of the prokinetic benzamides possess not only 5-HT\textsubscript{4}-receptor agonistic but also 5-HT\textsubscript{3}-receptor antagonistic properties (16 – 18). Concerning YM060 and YM114, however, the effects of these compounds on 5-HT\textsubscript{4} receptors have not yet been examined.

In the present study, we examined the effects of YM060 and YM114 on 5-HT\textsubscript{4} receptors and gastric emptying in normal and cisplatin-treated rats, and compared results with those for ondansetron and granisetron. We found that YM060 and YM114 dose-dependently inhibited the specific binding of \[^{3}H\]GR113808 in guinea pig striatum, with pK\textsubscript{i} values of 5.53, 5.13, 5.21 and 5.63, respectively. According to the pK\textsubscript{i} values reported in 5-HT3-receptor binding of \[^{3}H\]GR65630 to rat cortical membranes, the affinity of YM060, YM114, ondansetron and granisetron for 5-HT\textsubscript{4} receptors was approximately 5, 5, 3.5 and 3.5 log units lower than that for 5-HT\textsubscript{3} receptors, respectively. In the guinea pig longitudinal muscle with myenteric plexus and rat esophageal tunica muscularis mucosae, YM060 and YM114 showed neither 5-HT\textsubscript{4}-agonistic nor antagonistic properties. Although ondansetron produced concentration-dependent increases in the magnitude of the twitch response in longitudinal muscle, it did not possess 5-HT\textsubscript{3}- and 5-HT\textsubscript{4}-agonistic activity. Granisetron antagonized 5-HT-induced relaxation of the rat esophagus with an apparent pA\textsubscript{2} value of 5.39. Intravenous YM060, YM114, ondansetron and granisetron significantly enhanced gastric emptying of glass beads and improved cisplatin-induced slowing of gastric emptying in rats. These results indicate that the selectivity of YM060 and YM114 for 5-HT\textsubscript{4} receptors is higher than that of ondansetron and granisetron and that these 5-HT\textsubscript{3} antagonists have gastroprokinetic activity in normal and cisplatin-treated rats without affecting 5-HT\textsubscript{4} receptors.
with those of other 5-HT₄-receptor antagonists ondansetron and granisetron.

MATERIALS AND METHODS

Animals
Male Hartley guinea pigs weighing 550–925 g (Hamri Co., Sashima, Ibaraki) and male Wistar rats weighing 210–300 g (SLC, Hamamatsu) were used. The animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 12-hr light-dark cycle. In the in vivo experiments, rats were fasted overnight before the experiments but allowed free access to water.

Radioligand binding studies for 5-HT₄ receptor
Guinea pigs were killed by cervical dislocation, and the brain was removed and dissected. Pooled striatal brain tissue was placed in 15 volumes of 50 mM HEPES buffer (pH 7.4) at 4°C, homogenized (Polytron, setting 6) for 12 sec and centrifuged at 48,000 × g for 10 min. The resulting pellets were resuspended in 50 mM HEPES buffer to make a homogenate at 30 mg/ml.

The 5-HT₄-receptor binding studies were performed as described below using [³H]GR113808, a potent and selective 5-HT₄-receptor ligand (19). Assay tubes containing 50 μl of [³H]GR113808 (85 Ci/mmol) in HEPES buffer, 300 μl of buffer, 50 μl of buffer or a competing agent, and 100 μl of tissue preparation were incubated at 37°C for 30 min. Incubation was terminated by rapid vacuum filtration and washing with 3 ml of ice-cold buffer through GF/B glass-fiber filters using a Brandel Cell Harvester (Gaithersburg, MD, USA). Filters were presoaked with a solution of 0.1% polyethyleneimine to reduce filter binding. Bound radioactivity on the filter was measured by liquid scintillation spectroscopy. Non-specific binding was determined by the addition of 30 nM 5-HT. All determinations were performed in triplicate.

Isolated longitudinal muscle with myenteric plexus of the ileum of guinea pig
The ileum of guinea pigs was removed and cleaned in fresh Krebs-bicarbonate buffer (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 11.1 mM dextrose, 25.0 mM NaHCO₃ and 1.2 mM KH₂PO₄) at room temperature and then divided longitudinally into segments approximately 20 mm in length. Longitudinal muscle with myenteric plexus was prepared by the methods of Paton and Vizi (20). After the luminal contents were washed out, segments were stretched over a glass rod, and the longitudinal muscle was removed by gentle stroking with a cotton swab at an angle to the mesenteric attachment. The tissues were vertically suspended in 30-ml organ baths containing Krebs-bicarbonate solution warmed to 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. The tissues were attached to isometric force-displacement transducers (SB-11T; Nihon Kohden Co., Tokyo) connected to an ink oscillograph (EPR-241A; Toa Electronics, Tokyo) through a carrier amplifier (AP-621G, Nihon Kohden). Platinum electrodes were placed near the top and bottom of the tissue in a manner that avoided contact with the tissue. Transmural stimulation was carried out by rectangular pulses of 3-msec duration (40–50 V) at a frequency of 0.1 Hz delivered from an electrical stimulator (SEN-2201, Nihon Kohden). Twitch responses to electrical stimulation were isometrically recorded under a resting tension of 1 g.

After the preparations exhibited stable baseline tensions and consistent twitch heights (approximately 1 hr), they were subjected to supramaximal stimulation (100 V), and the maximal contractile responses were taken as 100%. The twitch response was then decreased by voltage reduction to about 50% of that at 100 V. After a stable submaximal response was obtained, a cumulative concentration-response curve to test drugs was constructed by increasing bath concentrations of test drugs approximately threefold. The responses to test drugs were measured in terms of their ability to enhance the twitch response to that obtained at supramaximal voltage (100%) and are expressed as the percentage enhancement of the twitch response.

In another series of experiments to evaluate the mechanism by which test drugs enhance the twitch responses, desensitization of the ileum was carried out by treatment with 5-methoxytryptamine and 2-methyl-5-HT, either alone or in combination (21). After cumulative concentration-response curves to the test drugs were constructed, the tissues were washed 3 times with Krebs-bicarbonate solution and exposed to 5-methoxytryptamine (10 μM), 2-methyl-5-HT (10 μM), or a combination of 5-methoxytryptamine and 2-methyl-5-HT (each at 10 μM) for 30 min before rechallenge with the test drug. To evaluate 5-HT₄-receptor agonistic activity, the percentage enhancement of the twitch response was calculated by subtracting the responses obtained under the 5-methoxytryptamine-desensitized condition from those obtained with the test drugs alone.

Isolated rat esophageal tunica muscularis mucosae
Rats were killed by cervical dislocation, and a 20-mm segment of intra-thoracic esophagus was excised. The outer longitudinal and circular muscle layers of the esophagus were removed, and the inner segment (tunica muscularis mucosa) was vertically suspended in a 10-ml organ bath containing Krebs-bicarbonate solution warmed to 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. Tissues were attached to an isometric
force-displacement transducer (SB-1T) connected to an ink oscillograph (SS-100F; Sekonic, Tokyo) through a carrier amplifier (AP-621G), placed under a resting tension of 0.5 g and left to equilibrate with Krebs-bicarbonate solution for about 1 hr prior to the start of the experiment. Tissues were attached to an isotonic force-displacement transducer (SB-1T) connected to an ink oscillograph (SS-100F; Sekonic) through a carrier amplifier (AP-621G).

After the preparations were induced to contract with $3 \times 10^{-6}$ M carbachol, a cumulative concentration-relaxation response to 5-HT was constructed by increasing bath concentrations of 5-HT approximately threefold. The tissues were washed 3 times with Krebs-bicarbonate solution and exposed to test drugs for 30 min before rechallenge with carbachol and 5-HT. As the cumulative concentration-response curves for 5-HT under these conditions could be constructed twice in the same preparations without significantly changing $E_{\text{max}}$ and $E_{C50}$ values (data not shown), each test drug was examined at one concentration in the same preparation.

Gastric emptying in rats

Rats were fasted overnight with water ad libitum. Gastric emptying was measured by following the passage of glass beads (1 mm in diameter) from the stomach. One hundred glass beads were orally given via a Nelaton's catheter with 1 ml of water. Fifteen minutes later, the rats were killed by cervical dislocation, the stomach was removed and opened and the number of beads remaining in the stomach was counted. Test drugs or vehicle were intravenously injected to the tail vein of rats 15 min before glass bead administration. Gastric emptying was evaluated as the number of glass beads expelled from the stomach.

In another series of experiments to evaluate the gastroprokinetic effect of test drugs under delayed conditions, the rats were treated with cisplatin at an intraperitoneal dose of 10 mg/kg 30 min before glass bead administration (22) and killed by cervical dislocation 30 min after the beads were given. Test drugs or vehicle were intravenously injected 15 min before cisplatin.

Statistical evaluation

All values represent the mean±S.E.M. or the mean with 95% confidence limits. In the receptor binding studies, $E_{C50}$ values, the concentration required to inhibit specific binding by 50%, were computed by logit-log analysis from the following equation:

$$\log\left(\frac{B_0 - B_t}{B_n - B_t}\right) = n \log(\text{test drug concentration}) - \log(E_{C50})$$

where $B_0$ and $B_t$ are binding in the absence and presence of test drugs, respectively; $B_n$ is nonspecific binding and $n$ is the slope factor identical to the Hill coefficient. $K_i$ values were calculated according to the following equation:

$$K_i = E_{C50}(1 - [L]/K_d)$$

where $[L]$ is the radioligand concentration and $K_d$ is the dissociation constant of the radioligand. $E_{C50}$ values for 5-HT$_4$-receptor agonistic activities of 5-HT and zacopride in isolated longitudinal muscle with myenteric plexus were calculated using the contractile responses by subtracting the responses obtained under 5-methoxytryptamine-pretreatment from those obtained with 5-HT or zacopride alone. $E_{C50}$ value of 5-HT for 5-HT$_4$-receptor agonistic activity in isolated esophageal tunica muscularis was calculated as the concentration causing 50% relaxation of the maximal response to 5-HT. $pA_2$ values for 5-HT$_4$-receptor antagonistic activity were calculated according to the method of Arunlakshana and Schild (23). The statistical significance of twitch response between mean values was determined with Students t-test for paired data. The statistical significance of glass bead values was determined by means of the Kruskal-Wallis H-test, and differences between treatment groups were compared by the Mann-Whitney U-test. Probabilities of <5% (P < 0.05) were considered significant.

Drugs

YM060 {[(R)-5-[[1-methyl-3-indolyl]carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazole hydrochloride}, YM114 (KAE-393) {[(R)-5-[[2,3-dihydro-1-indolyl]carbonyl]-4,5,6,7-tetrahydro-1H-benzimidazole hydrochloride}, granisetron, ondansetron, zacopride, tropisetron, GR113808 {[(1S)-2-(2-methylsulphonyl)aminoethyl]-4-piperidin-yl}methyl 1-methyl-1H-indole-3-carboxylate, FK1052 {{(+-)}-8,9-dihydro-10-methyl-7-[[5-methyl-4-imidazolyl] methyl]pyrido[1,2-a]-indole-6(7H)-one hydrochloride} and 2-methyl-5-HT were prepared by Yamanouchi Pharmaceutical Co. 5-HT creatinine sulfate, 5-methoxytryptamine hydrochloride and cisplatin (Briplatin) were purchased from E. Merck (Darmstadt, Germany), Fluka AG (Buchs, Switzerland) and Bristol-Myers Squibb (Wallingford, CT, USA), respectively. [3H]GR113808 was obtained from Amersham International plc (Little Chalfont, England). All drug doses are given as the free base.

RESULTS

$[^3H]GR113808$ binding

The specific binding of [3H]GR113808 in guinea pig striatum was saturable. Scatchard analysis revealed that the binding site was a single population of high affinity ($K_d = 0.16 \pm 0.01 \text{nM}$, $B_{\text{max}} = 78.7 \pm 2.2 \text{ fmol/mg protein}$ ($n=3$)). All the ligands inhibited the specific binding of
[\textsuperscript{3}H]GR113808 in a concentration-dependent manner (Fig. 1). Hill coefficients were not significantly different from unity. pKi values are listed in Table 1. On the basis of these, the ranking of affinities for 5-HT\textsubscript{4} receptors was GR113808 > tropisetron > zacopride > granisetron > YM060 > FK1052 > ondansetron > YM114.

Fig. 1. Inhibitory effect of YM060 (○), YM114 (●), ondansetron (△), granisetron (□), GR113808 (■), tropisetron (○), zacopride (♂) and FK1052 (△) on [\textsuperscript{3}H]GR113808 binding in homogenates of guinea pig striatum. Each point represents the mean ± S.E.M. for 3 to 9 experiments. Test compounds were incubated at 37°C for 30 min in the presence of [\textsuperscript{3}H]GR113808.

Fig. 2. Effects of 5-HT (○), YM060 (●), YM114 (△), ondansetron (♂), granisetron (□) and zacopride (▼) on electrically stimulated longitudinal muscle with myenteric plexus from guinea pig ileum. The maximal contractile response to supramaximal stimulation (100 V) was taken as 100%. Each point represents the mean ± S.E.M. for 5 to 11 animals.

Table 1. Affinities of various compounds that compete for 0.1 nM [\textsuperscript{3}H]GR113808 binding in homogenates of guinea pig striatum

| Compounds  | pKi (−log M) | Hill coefficient |
|------------|-------------|-----------------|
| YM060      | 5.53 (5.50−5.55) | 1.08 (0.85−1.30) |
| YM114      | 5.13 (5.08−5.19) | 0.86 (0.74−0.99) |
| Ondansetron| 5.21 (5.15−5.28) | 1.03 (0.87−1.20) |
| Granisetron| 5.63 (5.62−5.64) | 0.78 (0.71−0.84) |
| GR113808   | 9.72 (9.64−9.79) | 1.34 (1.07−1.61) |
| Tropisetron| 6.84 (6.77−6.91) | 0.96 (0.83−1.09) |
| Zacopride  | 6.30 (6.28−6.33) | 0.89 (0.74−1.04) |
| FK1052     | 5.41 (5.39−5.43) | 1.33 (1.15−1.50) |

Isolated longitudinal muscle with myenteric plexus

YM060 and YM114 (10\textsuperscript{−6}−10\textsuperscript{−4} M) did not enhance the twitch responses to transmural electrical stimulation of isolated longitudinal muscle with myenteric plexus of guinea pig ileum. Although granisetron (10\textsuperscript{−7}−10\textsuperscript{−6} M) enhanced transmural stimulation-induced contraction, the degree of maximal enhancement was only 20.9 ± 6.3% (n=5) at 10\textsuperscript{−6} M (Fig. 2). In contrast, 5-HT (10\textsuperscript{−9}−10\textsuperscript{−6} M), zacopride (3×10\textsuperscript{−8}−3×10\textsuperscript{−5} M) and ondansetron (10\textsuperscript{−7}−10\textsuperscript{−5} M) produced concentration-dependent increases in the magnitude of the submaximal electrically evoked twitch response in these preparations (Fig. 2).

The effect of low concentrations of 5-HT (10\textsuperscript{−9}−3×10\textsuperscript{−7} M) was significantly inhibited by pretreatment with 5-methoxytryptamine (10\textsuperscript{−5} M), and that of a high concentration of 5-HT (10\textsuperscript{−5} M) was inhibited by the pretreatment with 2-methyl-5-HT (10\textsuperscript{−5} M). Furthermore, in the presence of 5-methoxytryptamine and 2-methyl-5-HT, the effect of 5-HT (10\textsuperscript{−9}−10\textsuperscript{−5} M) was almost completely abolished (Fig. 3). The concentration-response curve to zacopride (3×10\textsuperscript{−7}−3×10\textsuperscript{−5} M) was also inhibited by pretreatment with 5-methoxytryptamine (Fig. 4). As a result, the 5-HT\textsubscript{4}-receptor agonistic activities of 5-HT and zacopride were maximal at concentrations of 10\textsuperscript{−7} and 10\textsuperscript{−5} M, with EC\textsubscript{50} values of 3.5 (2.1−5.7)×10\textsuperscript{−9} (n=4) and 4.6 (3.6−5.9)×10\textsuperscript{−7} M (n=7), respectively. On the other hand, the contractile effect of ondansetron on the transmural electrical stimulation-induced twitch response was not affected by either 5-methoxytryptamine or 2-methyl-5-HT (Fig. 5).

Isolated esophageal tunica muscularis mucosae

The cumulative administration of 5-HT (3×10\textsuperscript{−9}−10\textsuperscript{−6} M) caused a concentration-dependent relaxation of rat esophagus precontracted with carbachol (3×10\textsuperscript{−6} M), with an EC\textsubscript{50} value of 2.1 (1.9−2.3)×10\textsuperscript{−8} M (n=8). GR113808 (3×10\textsuperscript{−10}−10\textsuperscript{−8} M), a potent and specific 5-HT\textsubscript{4}-receptor antagonist, caused a concentration-dependent shift to the right of the 5-HT concentration-response
Fig. 3. 5-HT-induced enhancement of twitch in electrically stimulated longitudinal muscle with myenteric plexus from guinea pig ileum with (●) or without (■) pretreatment with 5-methoxytryptamine and 2-methyl-5-HT (10^{-5} M). A: 5-methoxytryptamine pretreatment, B: 2-methyl-5-HT pretreatment, C: both 5-methoxytryptamine and 2-methyl-5-HT pretreatment. The maximal contractile response to supramaximal stimulation (100 V) was taken as 100%. Each point represents the mean ± S.E.M. for 3 or 4 animals.

Fig. 4. Zacopride-induced enhancement of twitch in electrically stimulated longitudinal muscle with myenteric plexus from guinea pig ileum with (●) or without (■) pretreatment with 5-methoxytryptamine (10^{-5} M). The maximal contractile response to supramaximal stimulation (100 V) was taken as 100%. Each point represents the mean ± S.E.M. for 10 animals.

Fig. 5. Ondansetron-induced enhancement of twitch in electrically stimulated longitudinal muscle with myenteric plexus from guinea pig ileum with (●) or without (■) pretreatment with 5-methoxytryptamine (10^{-5} M) (A) or 2-methyl-5-HT (10^{-5} M) (B). The maximal contractile response to supramaximal stimulation (100 V) was taken as 100%. Each point represents the mean ± S.E.M. for 3 to 8 animals.
curves without a decrease in maximal response. The slope of the Schild plot of GR113808 was not different from unity (1.17 [0.49-1.84]). The pA2 value for GR113808 was 9.37 (9.00-10.28) (n=12), which is consistent with the data summarized by Grossman et al. (19). Under the conditions described above, YM060, YM 114, ondansetron and FK1052 (3 x 10^{-5} M) failed to antagonize the relaxation induced by 5-HT. Tropisetron (3 x 10^{-6} M) and granisetron (3 x 10^{-5} M) had low affinity for 5-HT4 receptors with apparent pA2 values of 6.65 ± 0.31 (n = 4) and 5.39 ± 0.31 (n = 4), respectively (Fig. 6).

Gastric emptying

Intravenous YM060, YM114, ondansetron and granisetron were examined for their effects on gastric emptying in conscious rats. The number of glass beads expelled over 15 min in vehicle-treated control rats in the YM060-, YM114-, ondansetron- and granisetron-treated groups were 48.5±6.0 (n = 10), 39.4±4.6 (n = 10), 28.4±4.9 (n = 9) and 26.1±2.4 (n = 8), respectively. YM060 (1-10 μg/kg), YM114 (3, 10 μg/kg), ondansetron (10-100 μg/kg) and granisetron (3-30 μg/kg) significantly enhanced the gastric emptying of glass beads in rats (Fig. 7).

Pretreatment of rats with cisplatin (10 mg/kg, i.p.) resulted in a significant decrease in gastric emptying of glass beads over 1 hr. Values in the vehicle-treated control rats for the YM060-, YM114-, ondansetron- and granisetron-treated groups fell from 56.2±7.9 (n = 10), 78.5±6.1 (n = 10), 59.9±6.3 (n = 9) and 50.2±6.2 (n = 10), respectively, to 18.9±5.9 (n = 8), 9.5±3.2 (n = 10), 4.0±2.0 (n = 10) and 14.8±1.7 (n = 10), respectively. This cisplatin-induced slowing of gastric emptying was also significantly reversed by YM060, YM114, ondansetron and granisetron in a dose-dependent manner at
Fig. 7. Effects of YM060, YM114, ondansetron and granisetron on gastric emptying in conscious rats. One hundred glass beads were orally given to rats, and gastric emptying was evaluated as the number of glass beads expelled from the stomach over 15 min. Test drugs or vehicle were intravenously injected to rats 15 min before glass bead administration. Each bar represents the mean ± S.E.M. for 7 to 10 animals. **P < 0.01, compared with the vehicle-treated control group (Mann-Whitney U-test).

Fig. 8. Effects of YM060, YM114, ondansetron and granisetron on cisplatin-induced delay of gastric emptying in conscious rats. Gastric emptying was evaluated as the number of glass beads expelled from the stomach over 30 min. Cisplatin (10 mg/kg) and test drugs (or vehicle) were intraperitoneally and intravenously injected to rats 30 and 15 min before glass bead administration, respectively. Each bar represents the mean ± S.E.M. for 8 to 10 animals. *P < 0.05, **P < 0.01, compared with the control group without cisplatin treatment. #P < 0.05, ##P < 0.01, compared with the cisplatin-treated control group (Mann-Whitney U-test).
longitudinal muscle with myenteric plexus of the ileum response to transmural electrical stimulation of isolated receptor. In the present study, we evaluated the effects of colliculi neurons, and they designated this the 5-HT4 receptor. In the present study, we evaluated the effects of 5-HT4-receptor antagonists YM060 and YM114 on 5-HT4 receptors by using the following three assay systems: [3H]GR113808 binding in guinea pig striatum, twitch response to transmural electrical stimulation of isolated longitudinal muscle with myenteric plexus of the ileum of guinea pig and 5-HT-induced relaxation of the rat esophagus precontracted with carbachol.

The present study revealed that YM060 and YM114 are more selective 5-HT4-receptor antagonists than ondansetron and granisetron. Among compounds that possess 5-HT4-receptor antagonistic activity, it has been reported that the substituted benzamide derivatives renzapride, cisapride, zacopride and metoclopramide have 5-HT4-receptor agonistic activity (17, 18), tropisetron and FK1052 have 5-HT4-receptor antagonistic activity (24 - 27), and that zacopride has 5-HT3-receptor agonistic activity in addition to its 5-HT4-receptor antagonistic and 5-HT3-receptor agonistic activities (28, 29). YM060 and YM114 are tetrahydrobenzimidazole derivatives that are structurally unrelated to substituted benzamide. In the present study, YM060 and YM114 as well as ondansetron and granisetron dose-dependently inhibited the specific binding of [3H]GR113808 in guinea pig striatum. Based on pKi values, their affinity for 5-HT4 receptors was approximately 4 log units lower than that of GR113808. Furthermore, pKi values of YM060, YM114, ondansetron and granisetron for the 5-HT3 receptor were reported to be 10.48 (10.41 - 10.56), 10.24 (10.18 - 10.29), 8.70 (8.64 - 8.77) and 9.15 (9.02 - 9.28), respectively, in [3H]-GR65630 binding to rat cortical membrane (30), indicating that the affinity of YM060, YM114, ondansetron and granisetron for 5-HT4 receptors was approximately 5, 5, 3.5 and 3.5 units lower than that for 5-HT3 receptors, respectively. Therefore, the selectivity of YM060 and YM114 for 5-HT3 receptors over 5-HT4 receptors is greater than that of ondansetron and granisetron. YM060 and YM114 did not show 5-HT2- and 5-HT2-receptor agonistic activity in isolated longitudinal muscle with myenteric plexus from guinea pig ileum, and they failed to antagonize the relaxation induced by 5-HT through 5-HT4 receptors in isolated rat esophageal tunica muscularis mucosae. Ondansetron produced increases in the magnitude of the electrically evoked twitch response by a mechanism unrelated to 5-HT3- and 5-HT4-receptor agonistic activity. Granisetron showed weak 5-HT4-receptor antagonistic activity (apparent pA2=5.39) in rat esophagus. Although FK1052, as mentioned above, has been reported to be a 5-HT3- and 5-HT4-receptor dual antagonist (26, 27), we could not detect 5-HT4-receptor antagonistic activity by this compound.

One of the most important findings of this study is that in rats at least, the gastroprokinetic effect of YM060 and YM114 is attributable to mechanisms other than 5-HT3-receptor antagonism, although a contribution by 5-HT3 receptors cannot be ruled out. Several compounds known to block 5-HT3 receptors, such as metoclopramide (31), tropisetron (6), cisapride (7), zacopride (8), renzapride (10) and ondansetron (9), have been shown to facilitate gastric emptying. In the present study, YM060 and YM114, like ondansetron and granisetron, enhanced gastric emptying under normal conditions, and they improved delayed gastric emptying induced by cisplatin in rats. However, the 5-HT4-receptor antagonists MDL-72222 and LY277359 do not facilitate gastric emptying in guinea pigs and rats (6, 32, 33). Although the ranking of potency in normal and cisplatin-pretreated rats (YM060 > YM114 > granisetron > ondansetron) in the present study is roughly consistent with that in inhibition of the von Bezold-Jarisch reflex induced by intravenous 5-HT in anesthetized rats, a difference was seen in the effective dose range between their enhancement of gastric emptying and inhibition of the von Bezold-Jarisch reflex (2, 3). Moreover, tropisetron and granisetron are reported to have little gastroprokinetic activity in dogs (34).

Among the various 5-HT-receptor subtypes, it has been suggested that both 5-HT4 and 5-HT3 receptors are involved in the control of gastrointestinal motility. 5-HT4-receptor agonists facilitate cholinergic neurotransmission and enhance gastrointestinal motility and gastric emptying (14, 15). As mentioned above, YM060, YM114, ondansetron and granisetron did not show 5-HT4-receptor agonistic activity in the present study, indicating that the stimulative activity of these compounds on gastric emptying does not involve a mechanism via 5-HT4 receptors.

The cytotoxic agent cisplatin induces emesis via the activation of 5-HT3 receptors in ferrets, dogs and man. Concerning the mechanism, it has been proposed that cytotoxic agents stimulate the release of 5-HT from enterochromaffin cells of the gastrointestinal mucosa. Released 5-HT, in turn, causes stimulation of 5-HT3 receptors located in the afferent vagal fibers, thereby eliciting the vomiting reflex (35 - 37). Cisplatin is also reported to delay gastric emptying in rats (22, 38). Although the mechanism by which cisplatin slows gastric emptying has not yet been established, a contribution by 5-HT3 receptor has been suggested (38). In the present study, YM060,
YM114, ondansetron and granisetron dose-dependently reversed the delay in gastric emptying induced by cisplatin at doses that enhance gastric emptying in normal rats. These results suggest that the 5-HT₃-receptor antagonist used here improve cisplatin-induced slowing of gastric emptying through, at least in part, 5-HT₃-receptor antagonistic activity.

In conclusion, the present data indicate that YM060 and YM114 are more selective 5-HT₃-receptor antagonists than ondansetron and granisetron without affinity for 5-HT₄ receptors. YM060 and YM114, like ondansetron and granisetron, enhanced gastric emptying and improved cisplatin-induced slowing of gastric emptying in rats. The effects of these agents in increasing gastric emptying may be partly related to 5-HT₃-receptor antagonism.

REFERENCES

1 Miyata K, Kamato T, Nishida A, Ito H, Katsuyama Y, Iwai A, Yuki H, Yamano M, Tsutsurni R, Ohta M, Takeda M and Honda K: Pharmacologic profile of (R)-5-[1-methyl-3-indoly]carbonyl-4,5,6,7-tetrahydro-1H-benzimidazole hydrochloride (YM060), a potent and selective 5-hydroxytryptamine₂ receptor antagonist, and its enantiomer in the isolated tissue. J Pharmacol Exp Ther 259, 15–21 (1991)

2 Miyata K, Ito H, Yamano M, Hidaka K, Kamato T, Nishida A and Yuki H: Comparison of the effects of trimetubine and YM114 (KAE-393), a novel 5-HT₃ receptor antagonist, on stress-induced defecation. Eur J Pharmacol 250, 303–310 (1993)

3 Miyata K, Kamato T, Yamano M, Nishida A, Ito H, Katsuyama Y, Yuki H, Tsutsurni R, Ohta M, Takeda M and Honda K: Serotonin (5-HT₃) receptor blocking activities of YM060, a novel 4,5,6,7-tetrahydrobenzimidazole derivative, and its enantiomer in anesthetized rats. J Pharmacol Exp Ther 259, 815–819 (1991)

4 Kamato T, Ito H, Suzuki K, Miyata K and Honda K: Studies on serotonin (5-HT₃) receptor antagonist effects of enantiomers of 4,5,6,7-tetrahydrobenzimidazole derivatives. Jpn J Pharmacol 67, 185–194 (1991)

5 Ito H, Hidaka K, Miyata K, Kamato T, Nishida A and Honda K: Characterization of YM060, a potent and selective 5-hydroxytryptamine₃ receptor antagonist, in rabbit nodose ganglion and N1E-115 neuroblastoma cells. J Pharmacol Exp Ther 263, 1127–1132 (1992)

6 Buchheit KH, Costall B, Engel G, Gunning SJ, Naylor RJ and Richardson BP: 5-Hydroxytryptamine receptor antagonism by metoclo-pramid and ICS205-930 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)

7 Schuurkes JAJ, Van Neuten JM, Van Daele PGH, Reymtjens AJ and Janssen PAJ: Motor stimulating properties of cisapride on isolated gastrointestinal preparations of the guinea pig. J Pharm Pharmacol Exp Ther 234, 775–783 (1985)

8 Alphin RS, Smith WL, Jackson CG, Droppleman DA and Sancilio LF: Zacopride (AHR-11190B): A unique and potent gastrointestinal prokinetic and antiemetic agent in laboratory animals. Dig Dis Sci 31, 4825 (1986)

9 Costall B, Gunning SJ, Naylor RJ and Tyers MB: The effect of GR38032F, novel 5-HT₃ receptor antagonist on gastric emptying in the guinea pig. Br J Pharmacol 91, 263–264 (1987)

10 Sanger GJ: Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL-24924: Potential clinical importance of BRL24924. Br J Pharmacol 91, 77–87 (1987)

11 Dumuis A, Bouhelal R, Sebben M, Copy R and Bockaert J: A non-classical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol Pharmacol 34, 880–887 (1988)

12 Eglen RM, Swank SR, Walsh LKM and Whiting RL: Characterization of 5-HT₃ and "atypical" 5-HT receptors mediating guinea pig ileal contractions in vitro. Br J Pharmacol 101, 513–520 (1990)

13 Kilbinger H and Wolf D: Effects of 5-HT₄ receptor stimulation on basal and electrically evoked release of acetylcholine from guinea pig myenteric plexus. Naunyn Schmiedebergs Arch Pharmacol 345, 270–275 (1992)

14 Linnik MD, Butler BT, Gaddis RR and Ahmed NK: Analysis of serotonergic mechanisms underlying benzamide-induced gastro-prokinesis. J Pharmacol Exp Ther 259, 501–507 (1991)

15 Rizzi CA, Coccini T, Onori L, Manzo L and Tonini M: Benzimidazolone derivatives: A new class of 5-hydroxytryptamine₄ receptor agonists with prokinetic and acetylcholine releasing properties in the guinea pig ileum. J Pharmacol Exp Ther 261, 412–419 (1992)

16 Dumuis A, Sebben M and Bockaert J: BRL24924: a potent agonist at a non-classical 5-HT receptor positively coupled with adenylate cyclase in colliculi neurons. Eur J Pharmacol 162, 381–384 (1989)

17 Dumuis A, Sebben M and Bockaert J: The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT₃ receptor (5-HT₃) positively coupled to adenylate cyclase in neurons. Naunyn Schmiedebergs Arch Pharmaco 340, 403–410 (1989)

18 Bockaert J, Sebben M and Dumuis A: Pharmacological characterization of 5-hydroxytryptamine (5-HT₃) receptors positively coupled to adenylate cyclase in adult guinea pig hippocampal membranes: Effect of substituted benzamide derivatives. Mol Pharmacol 37, 408–411 (1990)

19 Grossman CJ, Kilpatrick GJ and Bunce KT: Development of a radioligand binding assay for 5-HT₄ receptors in guinea pig and rat brain. Br J Pharmacol 109, 618–624 (1993)

20 Paton WDM and Vizi ES: The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea pig ileum longitudinal muscle strip. Br J Pharmacol 35, 10–29 (1969)

21 Craig DA, Eglen RM, Walsh LKM, Perkins LA, Whiting RL and Clarke DE: 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT₃ and putative 5-HT₄ receptors in guinea pig ileum. Naunyn Schmiedebergs Arch Pharmacol 342, 9–16 (1990)

22 Karasawa T, Yoshida N, Furukawa K, Omoya H and Ito T: Characterization of 5-hydroxytryptamine M-receptors by BRL-24924. Eur J Pharmacol 183, 2181 (1990)

23 Arunlakshana O and Schild HO: Some quantitative uses of drug antagonists. Br J Pharmacol 14, 48–58 (1959)

24 Clarke DE, Craig DA and Fozard JR: The 5-HT₃ receptor: Naughty, but nice. Trends Pharmacol Sci 10, 385–386 (1989)
25 Craig DA and Clarke DE: Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine_4 receptor. J Pharmacol Exp Ther 252, 1378–1386 (1990)

26 Kadowaki M, Nagakura Y, Tomoi M, Mori J and Kohsaka M: Effect of FK1052, a potent 5-hydroxytryptamine_3 and 5-hydroxytryptamine_4 receptor dual antagonist, on colonic function in vivo. J Pharmacol Exp Ther 266, 74–80 (1993)

27 Nagakura Y, Kadowaki M, Tokoro K, Tomoi M, Mori J and Kohsaka M: Pharmacological characterization of FK1052, a dihydropyrido-indole derivative, as a new serotonin 3 and 4 dual receptor antagonist. J Pharmacol Exp Ther 265, 752–758 (1993)

28 Middlefell VC and Price TL: 5-HT_3 receptor agonism may be responsible for the emetic effects of zacopride in the ferret. Br J Pharmacol 103, 1011–1012 (1991)

29 Sancilio LF, Pinkus LM, Jackson CB and Munson HR Jr: Studies on the emetic and antiemetic properties of zacopride and its enantiomers. Eur J Pharmacol 192, 365–369 (1991)

30 Ito H, Akuzawa S, Tsutsunri R, Kiso T, Kamato T, Nishida A, Yamano M and Miyata K: Comparative study of the affinities of 5-HT_3 receptor antagonists, YM060, YM114 (KAE-393), granisetron and ondansetron in rat vagus nerve and cerebral cortex. Neuropharmacology 34, 631–637 (1995)

31 Harrington RA, Hamilton CW, Brogden RN, Linkewich JA, Romankiewicz JA and Heel RC: Metoclopramide, an updated review of its pharmacological properties and clinical use. Drugs 25, 451–494 (1983)

32 Cohen ML, Bloomquist W, Gidda JS and Lacefield W: LY277359 maleate: A potent and selective 5-HT_3 receptor antagonist without gastroprokinetic activity. J Pharmacol Exp Ther 254, 350–355 (1990)

33 Costall B, Naylor RJ and Tyers MB: Recent advances in the neuropharmacology of 5-HT_3 agonists and antagonists. Rev Neurosci 2, 41–65 (1988)

34 Gullikson GW, Loeffler RF and Virina MA: Relationship of serotonin-3 receptor antagonist activity to gastric emptying and motor-stimulating actions of prokinetic drugs in dogs. J Pharmacol Exp Ther 258, 103–110 (1991)

35 Ireland SJ and Tyers MB: Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. Br J Pharmacol 90, 229–238 (1987)

36 Hawthorn J, Ostler KJ and Andrews PRL: The role of the abdominal visceral innervation of 5-hydroxytryptamine M-receptors in vomiting induced by cytotoxic drugs cyclophosphamide and cisplatin in the ferret. J Exp Physiol 73, 7–21 (1988)

37 Andrews PLR, Davis CJ and Maskell L: The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can J Physiol Pharmacol 68, 325–345 (1990)

38 Eeckhout C and Vedder A: 5-HT_3 antagonists reverse the cisplatin induced slowing of gastric emptying in fed rats. Gastroenterology 94, A111 (1988)