Vagus-Dependent and Vagus-Independent Mechanisms of Action of the Erythromycin Derivative EM574 and Motilin in Dogs

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ABSTRACT—The motor-stimulating action of de(N-methyl)-N-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574) on the upper gastrointestinal tract was studied in fasted conscious dogs using chronically implanted force transducers and compared with those of porcine motilin and cisapride. EM574 induced gastric phase III-like migrating contractions and increased the plasma motilin levels slightly. The gastric motility induced by low doses of EM574 and motilin was abolished by a $5HT_3$-receptor antagonist ondansetron and acute vagal blockade, whereas under these conditions, high doses of both agents induced contractions, which were abolished by atropine. Cisapride-induced gastric motility was inhibited by atropine and acute vagal blockade, but not by ondansetron. EM574 did not stimulate gastric secretion in the basal state. These results indicate that EM574- and motilin-induced gastrointestinal motility is attributable mainly to motor-stimulating vagal cholinergic neurons, and $5HT_3$-receptors are probably involved in the process. At high doses, EM574 and motilin also appear to stimulate cholinergic neurons in a non-vagal pathway, probably the enteric nervous system.

Keywords: EM574, Motilin, Cisapride, Cholinergic neuron, Enteric nervous system

Motilin, a 22-amino acid peptide, is considered to be involved in the occurrence of interdigestive migrating contractions (IMC) that originate in the stomach and migrate to the ileum in humans and dogs (1). Erythromycin has been reported to induce IMC-like contractions in humans and dogs when administered at low, microbially ineffective doses (2–4). An erythromycin derivative, de(N-methyl)-N-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574), has potent gastrointestinal motor-stimulating activity in the dog, but has no antibacterial activity (5). A binding study indicated that EM574 is a motilin receptor agonist in humans (6). It has been reported that erythromycin- or its derivative EM523-induced gastrointestinal motility is strongly inhibited by atropine and hexamethonium (7–11). Inatomi et al. (8) and Ohtawa et al. (9) found that EM523-induced gastric motility was strongly inhibited by $5HT_3$-receptor antagonists. These results suggested that the motor-stimulating actions of erythromycin or its derivatives are brought about through the cholinergic neural pathway and $5HT_3$ receptors, but the mechanisms of their actions on gut motility should be fully elucidated. In this study,

the effect of EM574 on plasma motilin level was investigated to determine whether endogenous motilin release is involved in its action. We then examined the effects of various receptor antagonists and acute vagal blockade on EM574- and motilin-induced gastrointestinal motility in order to determine their mechanisms of action. The effect of another prokinetic agent, cisapride (12), a $5HT_4$-receptor agonist, was also investigated to compare its action with those of EM574 and motilin.

MATERIALS AND METHODS

Preparation of animals

Thirteen male beagle dogs weighing 7.0–12.7 kg (Oriental Yeast Co., Tokyo) were used. The studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee of Animal Care at Takeda Chemical Industries. Under pentobarbital Na (30 mg/kg, intravenously (i.v.)) anesthesia, a Silastic® cannula (OD: 0.85″, ID: 0.40″; Dow Corning, Corning, NY, USA) was inserted into the superior vena cava through a branch vein of the right external jugular

vein, and the external end was sutured onto the adjacent skin. This cannula was filled with heparin-containing saline, capped with a plastic stopper and used as a route for intravenous injection of drugs and blood sampling. The abdomen was opened and force transducers (F-12 IS-60; Star Medical, Tokyo) were sutured to the serosa of the gastrointestinal tract to measure circular muscle contractions. The transducers were placed on the gastric antrum 3 cm proximal to the pyloric ring, mid-duodenum and on the small intestine 30 cm distal to the ligament of Treitz. In 3 dogs, force transducers were also sutured onto the greater curvature of the stomach 12 – 15 cm proximal to the pyloric ring and the small intestine 120 cm distal to the ligament of Treitz. Vagal nerve blockade experiments were done after the vagosympathetic nerves were isolated in skin loops on each side of the neck (13) in 3 dogs. For gastric secretion experiments, gastric fistulae were tied in the glandular stomachs through incisions in the stomachs in 3 dogs. A Silastic® cannula for administering drugs intraduodenally (i.d.) was inserted into the duodenal lumen through an incision 6 cm distal to the pylorus. The force transducer leads and Silastic® cannula were run subcutaneously (s.c.) along the costal flank to an incision between the scapulae and fixed to the adjacent skin with silk sutures. After the operation, a jacket was placed on each dog to protect and keep the lead wires and the Silastic® cannula in place. During the experiments, the dogs were housed in individual experimental cages, fed a dry-type dog meal (20 g/kg) once a day while water was given ad libitum.

Measurement of gastrointestinal motility

Gastrointestinal motility was recorded on a polygraph (EMR3701; Graphtech, Tokyo) by connecting the force transducer leads to the connecting cables from the amplifiers under the protective jacket. The test drugs were given i.d. or i.v. during the quiescent period (phase I) 15 min after the termination of spontaneous phase III contractions in the stomach. In order to measure the motility quantitatively, the antral signals were input into a signal processor (7T18; Nihon Denki-Sanei, Tokyo) every 100 msec, and the motor index of drug-induced contractions was calculated using the previously described procedures (7). Namely, the area of contractions induced by the drug administration was calculated, expressed as percent of the area assuming that maximum contraction of IMC lasted for 1 min, and used as the motor index.

In experiments in which the effects of receptor antagonists were examined, the antagonists were given i.v. 3 min before intraduodenal administration of 10 μg/kg EM574 or starting an intravenous infusion of 1 μg/kg/hr motilin for 30 min. The inhibition by each antagonist was calculated by comparing the motor index after saline

pretreatment with that after antagonist pretreatment and expressing it as a percentage. In the case of cisapride-induced contractions, receptor antagonists were given i.v. after gastric contractions were induced by intraduodenal administration of 1 mg/kg cisapride, because the motor index after cisapride fluctuated and definite comparisons, as described above, could not be made.

Measurement of plasma motilin levels

Five dogs with chronically implanted force transducers were used in this study. EM574 (30 μg/kg) was given i.d. 30 min after the termination of phase III contractions in the stomach. Blood samples were taken via the venous cannula into heparinized syringes during phase III, at 10-min intervals in the phase I state and at 5, 10, 20, 30, 40, 50 and 60 min after EM574 administration. These samples were put into chilled tubes containing aprotinin (Trasylol®, 400 kallikrein unit; Bayer, Tokyo), centrifuged, and the supernatants were stored at −80°C. The plasma motilin level was determined by a radioimmunoassay technique using rabbit anti-canine motilin antiserum (9).

Vagal cooling experiments

This experiment was performed on 3 dogs whose vagosympathetic nerves had been previously isolated in skin loops on each side of the neck. Transient nerve blockade was accomplished by circulating 50% v/v alcohol at −20°C (cooled with dry ice) through copper cooling jackets placed around the skin loops (13). The temperature of each skin loop was monitored with a thermometer stuck to the cooling jackets and maintained at 2 – 5°C for 20 – 40 min by controlling the flow rate.

Gastric secretion experiments

Three dogs with gastric fistulae and chronically implanted force transducers were used. EM574 (10 μg/kg) was given i.d. after observing the basal secretion for 30 min during phase I, and 1 hr later, tetragastrin (5 μg/kg) was given s.c. The gastric secretions were collected every 15 min, centrifuged, and the volume of the supernatant was measured. The acidity was determined by automatic titration (TTT85; Radiometer, Copenhagen, Denmark) to pH 7.0 with 0.1 N NaOH, and the total acid output was calculated by multiplying the supernatant volume by the acidity value.

Drugs

EM574 and CP-99994 ((2S,3S)-cis-3-(2-methoxybenzylamine)-2-phenyl piperidine) were synthesized at Takeda Chemical Industries, Ltd. The following drugs were used: porcine motilin (Peptide Institute, Inc., Minou), erythromycin A (EMA; Abbott Laboratories, North

Chicago, IL, USA), cisapride (extracted from Risamol®; Yoshitomi, Osaka), SR48968 ((S)-N-methyl-N[4-(4-acetyl-amino-4-phenyl piperidine)-2-(3,4-dichlorophenyl)butyl]-benzamide) (kindly supplied by Sanofi, Paris, France), phentolamine hydrochloride, propranolol hydrochloride, mepyramine maleate, famotidine, naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), methysergide (Sandoz Pharmaceuticals, Basel, Switzerland), ketanserin, ondansetron (Jyunsei Chemical, Osaka), tetragastrin (San-a Chemical, Tokyo), atropine sulfate and hexamethonium chloride (Wako Pure Chemical, Osaka). EM574 was dissolved in absolute ethanol, to which lactobionic acid (27 mg/ml) was added in a volume of less than 5% of the final volume, and then diluted with saline. Cisapride was dissolved in 0.5% w/v tartaric acid solution. Famotidine, ketanserin and ondansetron were dissolved in 0.1 N HCl or acetic acid and then neu-

tralized with NaOH. SR48968 was dissolved in dimethylsulfoxide and then diluted with 9 volumes of saline. The other agents used were dissolved in saline.

Statistics

The results are presented as means and the standard errors. The relative potencies of the drugs were calculated using a parallel line assay. Statistical analysis was carried out using the Dunnett multiple group comparison test or by Student's *t*-test. Differences at $P < 0.05$ were considered significant.

RESULTS

Effects of drugs administered intravenously and intraduodenally

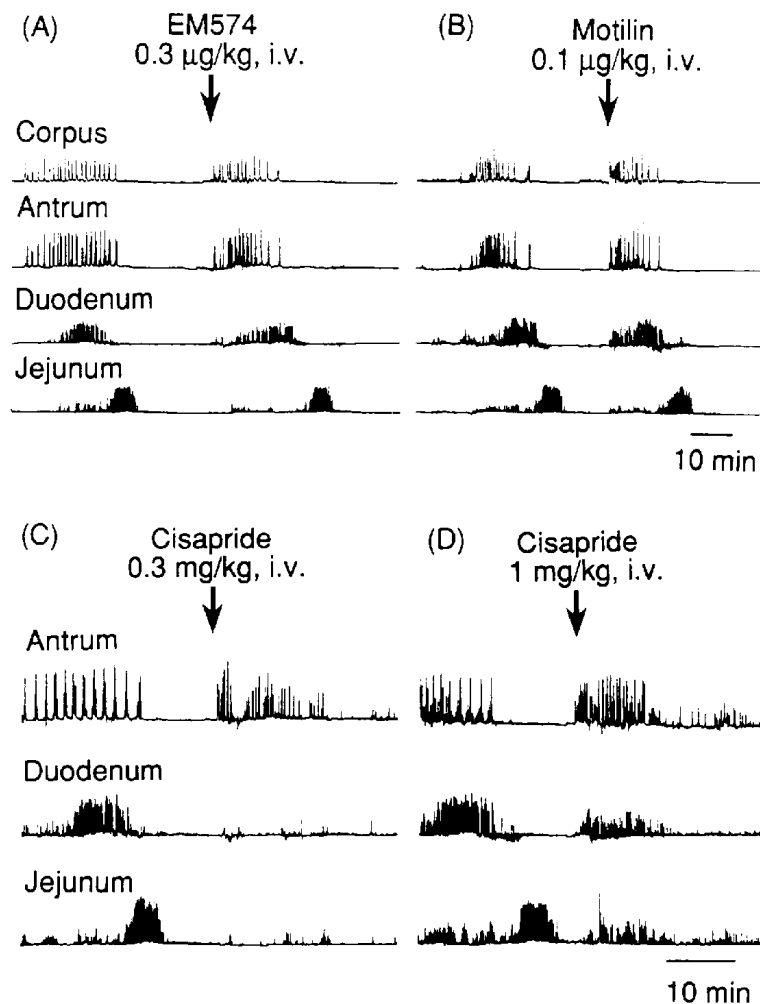The gastrointestinal motor-stimulating activity of

Fig. 1. Effects of i.v. EM574, 0.3 μg/kg (A); motilin, 0.1 μg/kg (B); cisapride, 0.3 mg/kg (C) and 1 mg/kg (D), on gastrointestinal contractile activity in the fasting conscious dog. EM574 and motilin induced IMC-like migrating contractions. The contractile pattern of cisapride-induced contractions was different from those induced by EM574 and motilin.
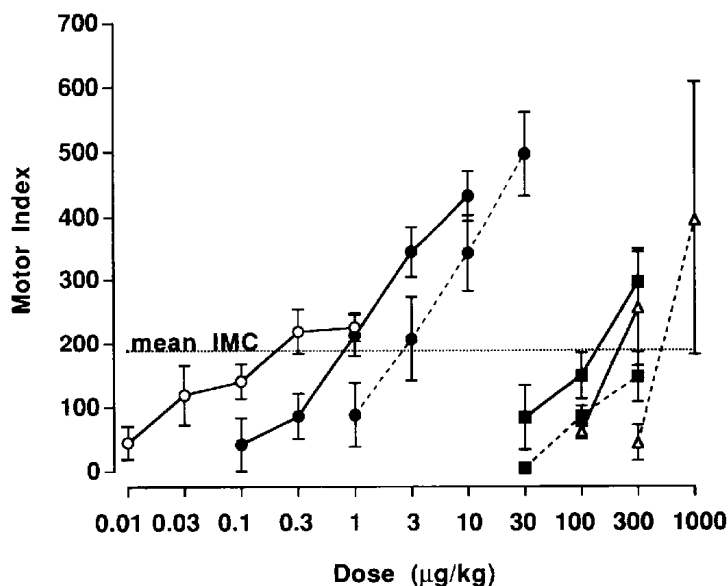
Fig. 2. Dose-response curves of the gastric motility induced by EM574, motilin, EMA and cisapride in conscious fasting dogs. The results are shown as means and the standard errors of data for 4–6 dogs. The mean IMC indicates the mean motor index of interdigestive migrating contractions in 6 dogs. —●— EM574, i.v.; ···●··· EM574, i.d.; —○— motilin, i.v.; —■— EMA, i.v.; ···■··· EMA, i.d.; —△— cisapride, i.v.; ···△··· cisapride, i.d.

EM574 was investigated in the fasted dog and compared with those of motilin, EMA and cisapride. The administration of vehicle given i.v. or i.d. did not have any effect on the gastrointestinal motility. EM574 (0.3 μg/kg and higher, i.v.) induced phase III-like contractions starting from the stomach (Fig. 1A), and the motor index increased in a dose-dependent manner (Fig. 2). Given i.d., EM574 (1 μg/kg and higher) induced IMC-like migrating contractions within 5 min after being administered. EMA, given i.v.and i.d., also induced phase III-like contractions. EM574 was 322 and 186 times more potent than EMA at inducing gastric contractions after intravenous and intraduodenal administration, respectively (Fig. 2). Motilin (0.01 μg/kg and higher, i.v.) induced phase III-like contractions (Figs. 1B and 2). The maximum response was obtained with 0.3 μg/kg, and the gastric motility did not increase further in response to higher doses. Given i.d., 10 μg/kg motilin did not induce gastrointesinal contractions. Cisapride (100 μg/kg and higher, i.v.) induced contractions of the stomach, but the contractile pattern differed from those induced by motilin and EM574 (Fig. 1, C and D). Cisapride (300 μg/kg and higher, i.d.) induced contractions within 6–25 min of administration. EM574 at 30 μg/kg, i.d. induced vomiting in 1 of 5 dogs. Motilin also caused vomiting at 0.3 and 1 μg/kg, i.v. in 2 and 3 of six dogs, respectively.

Effects on plasma motilin levels

As the action of EM574 on gastrointesinal motility

resembled that of motilin, the effect of EM574 on endogenous motilin release was investigated. EM574 (30 μg/kg, i.d.) increased antral motility markedly; the motor index exceeded the values during IMC 10 min after administration and then gradually decreased (Fig. 3). The mean plasma motilin concentration increased significantly in response to EM574, but its peak value was less than that during phase III.

Effects of receptor antagonists

The effects of pretreatment with various receptor antagonists on EM574 (10 μg/kg, i.d.) and motilin (1 μg/kg/hr, i.v., for 30 min)-induced gastrointestinal motility were examined. Pretreatment with atropine (0.1 mg/kg, i.v.) inhibited EM574- and motilin-induced gastric motility significantly by 95% and 94%, respectively (Table 1). Hexamethonium (10 mg/kg, i.v.) significantly inhibited the responses evoked by EM574 and motilin by 76% and 70%, respectively. Phentolamine (2 mg/kg, i.v.) and propranolol (1 mg/kg) did not affect EM574- and motilin-induced gastric motility significantly. Mepyramine (3 mg/kg), famotidine (1 mg/kg), methysergide (2 mg/kg) and ketanserine (1 mg/kg) inhibited neither EM574- nor motilin-induced gastric motility. The 5HT$_3$-receptor antagonist ondansetron (0.1 mg/kg, i.v.) inhibited EM574- and motilin-induced gastric motility by 91% and 94%, respectively. Naloxone, CP-99994 and SR48968 had no significant effect on EM574- or motilin-induced gastric motility.
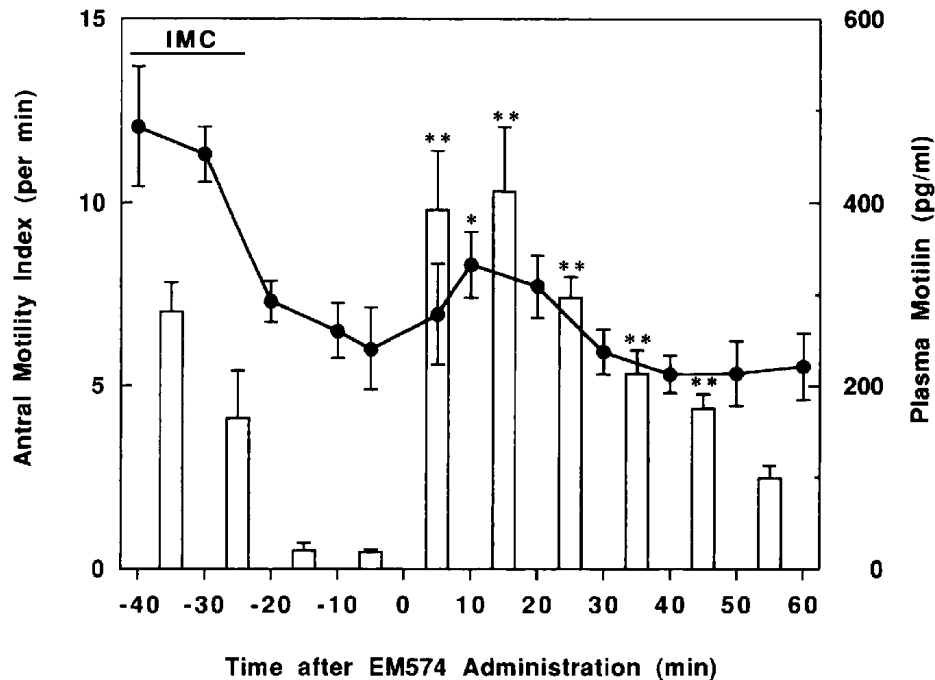
Fig. 3. Effect of i.d. EM574 (30 μg/kg) on gastric contractile activity and plasma motilin concentration. The results are shown as means and the standard errors of data for 5 dogs. *P<0.05, **P<0.01, compared with the value just before EM574 administration. □ motility, —●— motilin.

Table 1. Effects of receptor antagonists on EM574- and motilin-induced gastric motility in conscious fasting dogs

Antagonist	Receptor	Dose (mg/kg, i.v.)	Gastric motor index			
			EM574[a]		Motilin[b]	
Saline	—		266±36		250±22	
Atropine	Muscarinic	0.1	12± 5**	(95)	15±10**	(94)
Hexamethonium	Nicotinic	10	65±25**	(76)	75±17**	(70)
Phentolamine	α	2	221±51	(17)	182±41	(27)
Propranolol	β	1	247±29	(7)	198±28	(21)
Mepyramine	H_1	3	332±62	(−25)	367±41	(−47)
Famotidine	H_2	1	322±56	(−21)	246±32	(2)
Methysergide	$5HT_1$, $5HT_2$	2	262±34	(2)	253±46	(−1)
Ketanserin	$5HT_2$	1	358±61	(−35)	300±56	(−20)
Ondansetron	$5HT_3$	0.1	23± 6**	(91)	15± 4**	(94)
Naloxone	Opioid	1	240±23	(10)	213±26	(15)
Saline			313±41		361±22	
CP-99994	NK_1	0.3	278±78	(11)	320±49	(11)
SR48968	NK_2	0.3	292±25	(7)	391±42	(−8)

The values are means±S.E. of data for 3 or 5 dogs. The experiments with CP-99994 and SR48968 were conducted on 3 dogs, and the others were conducted on 5 dogs. [a]EM574 (10 μg/kg) was given i.d., [b]porcine motilin (1 μg/kg/hr) was given i.v. for 30 min. The numbers in parentheses are each a percentage inhibition relative to saline treatment. **P<0.01 vs saline treatment.

The gastric motility stimulated by cisapride (1 mg/kg, i.v.) was inhibited strongly by atropine (0.1 mg/kg, i.v.) and hexamethonium (10 mg/kg, i.v.) by $97 \pm 2\%$ and $98 \pm 1\%$ (n = 3), respectively, but no inhibition was observed after treatment with ondansetron (0.1 mg/kg, i.v.).

Intravenous infusion of EM574 (3 μg/kg/hr) and motilin (1 μg/kg/hr) induced phase III-like contractions, and the effect was sustained during the infusion for at least 1 hr. Ondansetron (0.1 mg/kg, i.v.) abolished the gastric motility induced by EM574 at a dose of 3 μg/kg/hr or less for more than 60 min. However, gastric contractions were induced when the dose of EM574 was increased to 10 μg/kg/hr and higher under ondansetron (0.1 mg/kg) treatment, and the contractions were not inhibited even by 3 mg/kg ondansetron, but were abolished by 0.1

mg/kg, i.v., atropine (Fig. 4A). The same responses to ondansetron were also observed with motilin-induced gastric contractions; the gastric motility induced by 1 μg/kg/hr, i.v., motilin was abolished by ondansetron at 0.1 mg/kg, i.v., but intravenous infusion of 3 μg/kg/hr motilin induced gastric contractions after this ondansetron pretreatment. The contractions were not inhibited by a higher dose of ondansetron, but were abolished by atropine at 0.1 mg/kg, i.v. (Fig. 4B).

Effects of acute vagal blockade

The role of the vagus nerve in EM574- and motilin-induced gastrointestinal contractions was investigated using a cooling technique for tentative blockade of the vagus nerve. Intravenous infusion of EM574 (3 μg/kg/hr) induced phase III-like contractions of the stomach, duo-
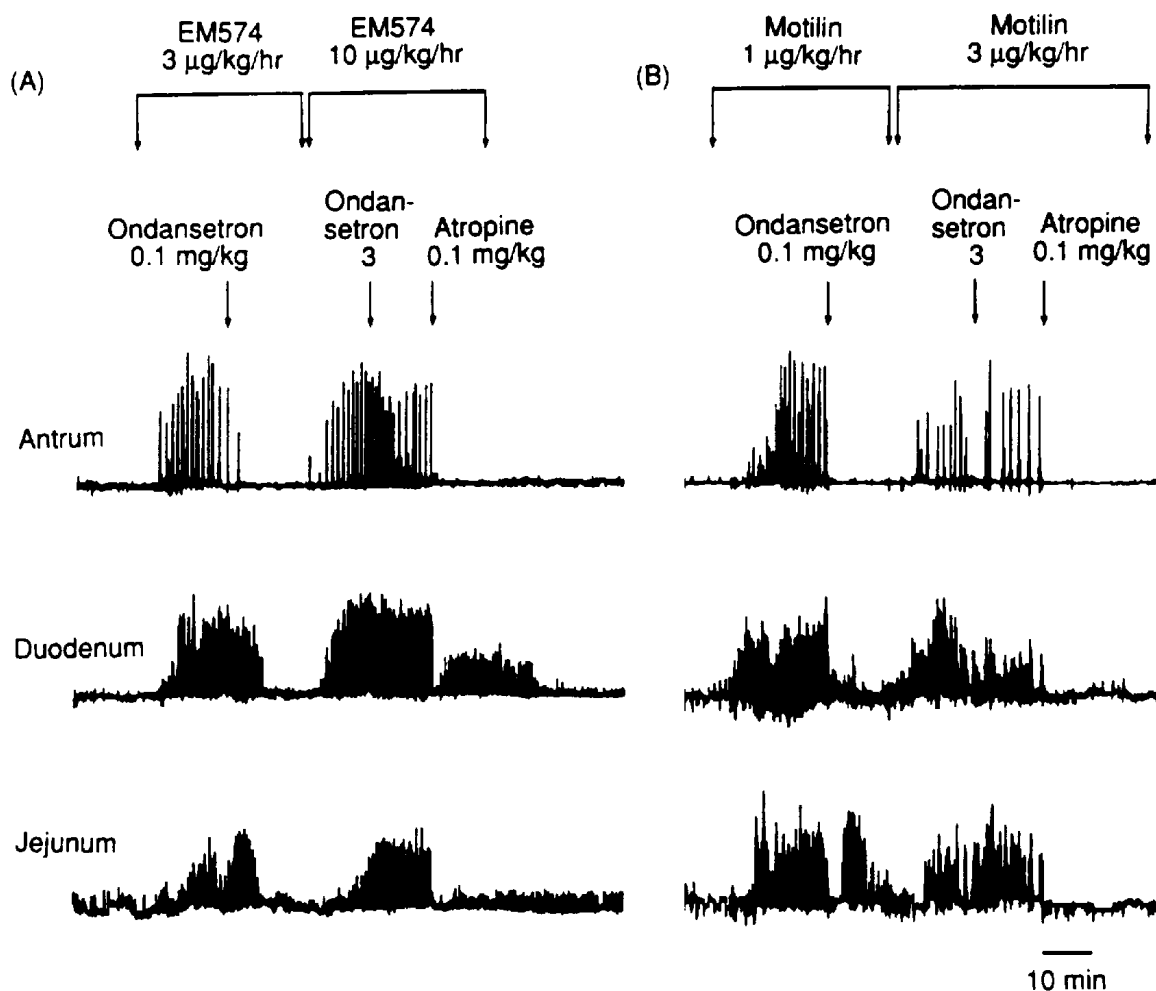

Fig. 4. Effects of ondansetron and atropine on gastrointestinal contractions induced by EM574 (A) and motilin (B). Ondansetron (0.1 mg/kg, i.v.) inhibited gastric contractions induced by i.v. EM574 (3 μg/kg/hr) and motilin (1 μg/kg/hr). These inhibitory effects lasted for over 1 hr (data not shown). Increasing the doses of EM574 and motilin to 10 and 3 μg/kg/hr, respectively, resulted in strong gastric contractions, which were not inhibited by ondansetron at by 3 mg/kg, i.v., but were abolished by atropine (0.1 mg/kg, i.v.).

(A) EM574 3 µg/kg/hr   EM574 10 µg/kg/hr

Vagal Blockade

Antrum

Duodenum

Jejunum

(B) Motilin 1 µg/kg/hr   Motilin 3 µg/kg/hr

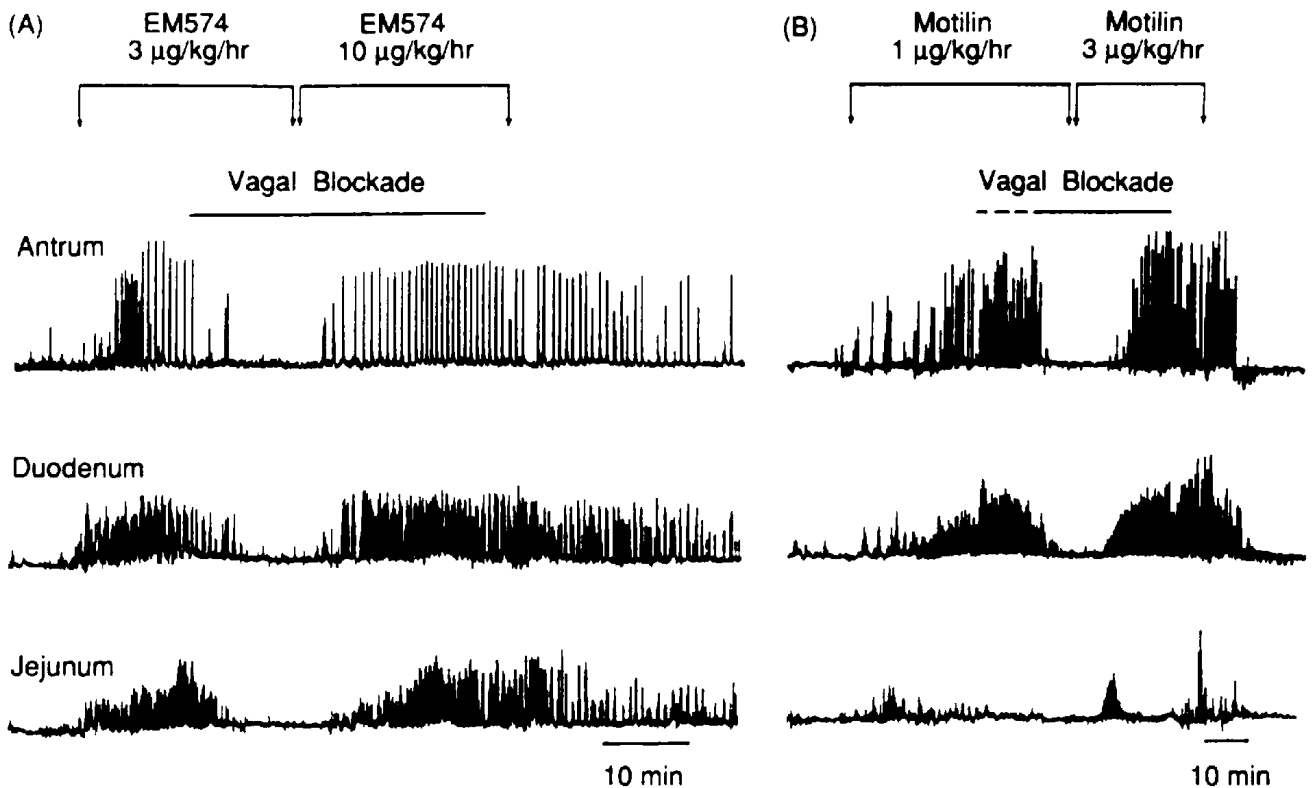Vagal Blockade

10 min   10 min

Fig. 5. Effects of vagal blockade on EM574- (A) and motilin- (B) induced gastrointestinal contractions in conscious dogs. The period of bilateral vagal blockade is indicated by an unbroken bar and that of unilateral vagal blockade by a dotted bar. Bilateral vagal blockade inhibited the gastrointestinal motility induced by EM574 (3 µg/kg/hr) and motilin (1 µg/kg/hr). After increasing the doses of EM574 and motilin, strong contractions were induced. Unilateral vagal blockade did not affect motilin-induced gastrointestinal motility (B).

denum and intestine. Unilateral vagal blockade did not inhibit EM574-induced contractions (data not shown). But bilateral vagal blockade almost completely inhibited the contractions in each region (Fig. 5). Under bilateral vagal blockade, increasing the dose of EM574 to 10 µg/kg/hr and higher induced strong contractions. The gastrointestinal contractions induced by 1 µg/kg/hr motilin, i.v. were not affected by unilateral vagal blockade, but were abolished by bilateral vagal blockade, under which, motilin, at doses of 3 µg/kg/hr and higher, induced contractions in each region (Fig. 5). Cisapride, 1 and 3 mg/kg, i.v., did not induce contractions under bilateral vagal blockade.

Effects on gastric secretion

In the phase I state, EM574 (10 µg/kg, i.d.) induced a marked increase in gastric motility; the motility peak was observed 15 min after administration, and then the motility gradually decreased. The gastric secretion volume and acid output were not affected by EM574 (Fig. 6), whereas tetragastrin (5 µg/kg, s.c.) increased both markedly.

DISCUSSION

In the present study, EM574 showed strong gastric motor-stimulating activity, it was 322 times more potent than EMA, and its mode of action resembled that of motilin. The 5HT$_4$-receptor agonist cisapride also induced gastric contractions, but its mode of action differed from those of EM574 and motilin. The finding that EM574 mimicked the motor-stimulating activity of motilin suggested that EM574 causes contractions by releasing endogenous motilin from motilin-containing cells in the duodenal and upper jejunal mucosa. Although EM574 (30 µg/kg, i.d.) induced marked gastric motility, the resulting plasma motilin level increase was slight, and the peak plasma motilin value was lower than that during phase III. There are several reports concerning the effects of erythromycin and its derivative EM523 on endogenous motilin release in human subjects: some investigators showed that erythromycin did not affect plasma motilin levels (14–17), whereas others found that administration of erythromycin and EM523 increased them significantly (11, 18, 19). Slight increases in serum motilin concentra-
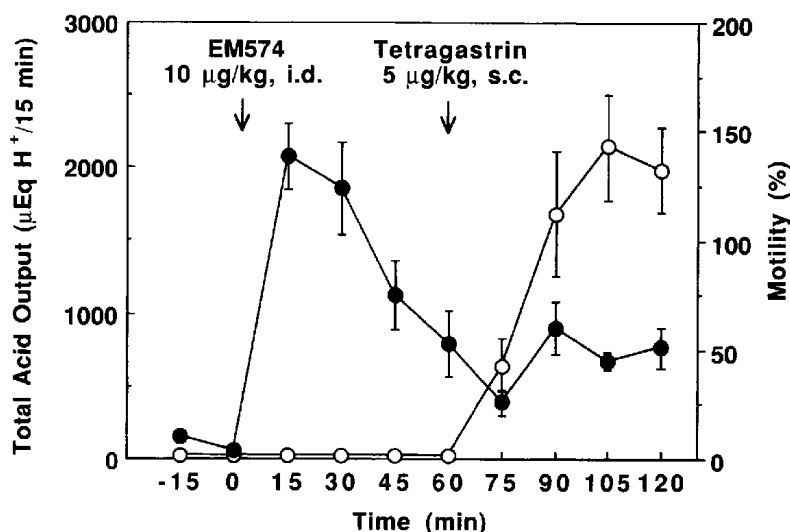
Fig. 6. Effects of EM574 (10 μg/kg, i.d.) and tetragastrin (5 μg/kg, s.c.) on gastric motility and gastric acid secretion in conscious dogs with gastric fistulae. The results are shown as the means and the standard errors of two experiments on 3 dogs. ○ total acid output, ● gastric motility.

tions evoked by erythromycin and EM523 in dogs have also been reported (3, 9). However, the increased plasma motilin levels reported in humans and dogs are not large enough to account for the total actions of erythromycin and EM523. Erythromycin and its derivatives have been reported to be motilin receptor agonists on rabbit and human gastrointestinal smooth muscle (20, 21), and we showed that EM574 acts as a motilin receptor agonist on human gastric antral muscle (6). These findings suggest that EM574 causes contractions mainly by acting on motilin receptors and that endogenous motilin released in association with EM574-induced contractions is involved, in part, in the motor-stimulating action.

The gastrointestinal contractions induced by EM574 were strongly inhibited by atropine and hexamethonium, and similar results have been obtained for erythromycin (10, 11) and EM523 (7–9). The effect of motilin on gastrointestinal motility was also inhibited by atropine and hexamethonium. These findings indicate that EM574 and motilin exert their actions by stimulating cholinergic neurons. In addition to cholinergic antagonists, EM574- and motilin-stimulated gastric motility was strongly inhibited by the 5HT$_3$-receptor antagonist ondansetron, indicating that 5HT$_3$ receptors are involved in their actions. 5HT$_3$ receptors have been identified in the area postrema (22–24) of the brain, on the enteric nervous system (25–27) and on afferent vagus nerves (24, 27, 28) of several animal species. As motilin-induced contractions of the extrinsically denervated stomach (Heidenhain pouch) were not inhibited by 5HT$_3$-antagonists (29), the 5HT$_3$ receptors involved in the action of motilin and

EM574 are probably not located in the enteric nervous system. It is possible that ondansetron acts at the area postrema, because the blood-brain barrier is incomplete in this region of the central nervous system and this area is highly vascularized. Vagus nerve homogenates were found to have high affinity binding sites for 5HT$_3$ ligands (24, 28), and it is well-known that vagal afferents can be stimulated by serotonin and convey gut motility signals (30, 31), suggesting that ondansetron inhibits motilin signals by acting on vagal afferents. Thus, we conclude that motilin and EM574 act through 5HT$_3$ receptors on the vagus nerve and/or in the area postrema and that the motilin signal is transmitted to the efferent pathway of the vagus nerve and thereby stimulates cholinergic neurons.

A particularly interesting observation in this study was that the gastric motility induced by low doses of EM574 and motilin was almost completely inhibited by 0.1 mg/kg ondansetron, but high doses of EM574 and motilin induced contractions after treatment with a very high dose (3 mg/kg) of ondansetron, and this activity was abolished by atropine. As the effects of high doses of EM574 and motilin were observed under treatment with ondansetron at doses as high as 30 times the dose that inhibited the effects of low doses of EM574 and motilin, ondansetron did not appear to competitively antagonize EM574 and motilin at motilin receptors. Therefore, 5HT$_3$ receptors appear to be present on the neuronal pathway stimulated by low doses, but not on that stimulated by high doses of EM574 and motilin.

It is well-known that the vagus plays an important role in the control of gastrointestinal motility. Hall et al. (13)
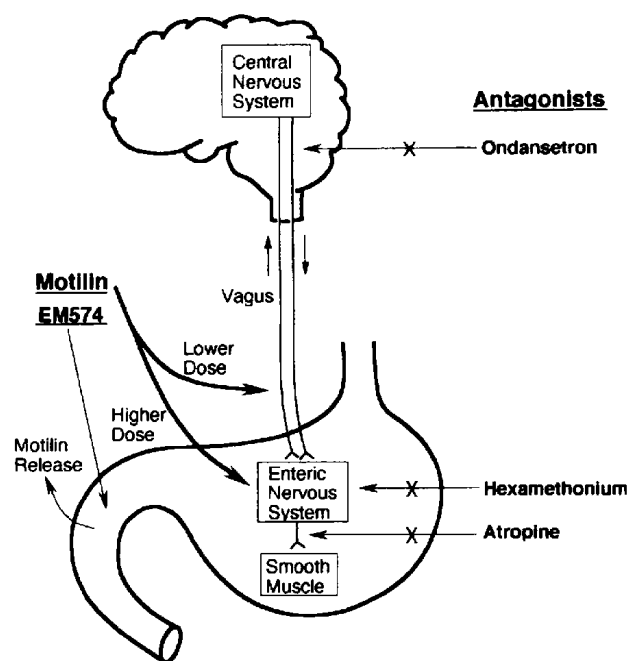
Fig. 7. Diagram illustrating the sites of action of EM574 and motilin. EM574 and motilin at lower doses seem to stimulate motilin receptors on vagal afferent neurons, and the signals are transmitted via vagal efferent cholinergic neurons. 5HT₃-receptor antagonists probably block the stimulation of this pathway. At high doses, EM574 and motilin also stimulate cholinergic neurons in the enteric nervous system. Hexamethonium and atropine inhibit the actions of EM574 and motilin by blocking the stimulation of both pathways.

reported that cooling-induced bilateral vagus nerve blockade abolished the occurrence of gastric contractions during IMC. Bilateral vagal blockade abolished the gastrointestinal motility induced by 3 μg/kg/hr or less EM574 and 1 μg/kg/hr or less motilin, but higher doses of both agents induced strong gastrointestinal contractions under vagal blockade. These results indicate that EM574 and motilin induce gastrointestinal contractions by stimulating vagal cholinergic nerves; and at higher doses, they evoke contractions via stimulation of non-vagal pathways. Although the sympathetic trunks in the neck are also blocked by cooling, they are involved exclusively with sympathetic innervation of the head and neck structures and play no role in the innervation of the gut below this level (32). A plausible explanation is that EM574 and motilin at high doses stimulate cholinergic nerves present in the enteric nervous system. The results of ondansetron treatment and acute vagal blockade suggest that the site of action of 5HT₃-receptor antagonists is on the vagal pathway. Figure 7 is a diagram showing the sites of action of EM574 and motilin: at relatively low doses, EM574 and motilin stimulate the vagal cholinergic neurons; and at higher doses, they also stimulate the

cholinergic neurons of the enteric nervous system, resulting in strong gastrointestinal contractions. As the action of cisapride was inhibited by atropine, hexamethonium and vagal blockade, we conclude that vagal cholinergic neurons are involved in its action. However, cisapride-induced gastric contractions were not affected by ondansetron, and high doses of cisapride did not induce contractions under vagal blockade, suggesting that the neuronal pathway involved in the action of cisapride differs from those involved in the actions of EM574 and motilin.

Insulin and 2-deoxy-D-glucose are potent stimulants of gastric acid secretion via vagal nerve activation (33, 34). As EM574 causes gastric motility by stimulating vagal cholinergic neurons, the possibility that EM574 stimulates gastric secretion was considered. However, EM574 did not stimulate basal gastric acid secretion in conscious dogs. Koch et al. (35) reported that infusing 13-norleucine-motilin into dogs with Heidenhain pouches did not stimulate acid secretion. Konturek et al. (36) demonstrated that infusing 13-norleucine-motilin into dogs with gastric fistulae increased gastric acid secretion slightly, but the amount of acid secreted was less than 3% of that stimulated by 8 μg/kg/hr pentagastrin. These results indicate that EM574 and motilin have little or no effect on basal gastric secretion, and that gastric motility and acid secretion are stimulated via different vagal cholinergic neurons. EM574 seems to stimulate the vagal neurons associated with gastrointestinal motility selectively.

In summary, the results of the present study indicate that EM574, like motilin, stimulates vagal cholinergic neurons and that, at higher doses, it also stimulates non-vagal cholinergic neurons in the enteric nervous system, resulting in the induction of strong gastrointestinal contractions. The involvement of endogenous motilin release in the gastrointestinal stimulant action of EM574 appears minor, and 5HT₃ receptors are likely to be associated with the transmission of signals in the vagal pathway.

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