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EM574, an erythromycin derivative, is a motilin receptor agonist in the rabbit

Fumihiko Sato [a], Masahiro Sekiguchi [b], Shogo Marui [c], Nobuhiro Inatomi [a,*], Akio Shino [b], Zen Itoh [d], Satoshi Ōmura [e]

[a] *Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan*
[b] *Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan*
[c] *Pharmaceutical Research Laboratories I, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan*
[d] *GI Laboratory, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371, Japan*
[e] *The Kitasato Institute, Tokyo 108, Japan*

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Abstract

This study was performed to examine whether an erythromycin derivative, de(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574) is a motilin receptor agonist in the rabbit gastrointestinal tract. EM574 and porcine motilin induced contractions in segments of isolated rabbit intestine with pEC_{50} values of 8.26 ± 0.04 and 8.69 ± 0.07, respectively, but not in rat or guinea pig preparations. The sensitivity and efficacy of the response to both compounds in rabbits decreased aborally and was insensitive to pretreatment with atropine or tetrodotoxin, but was markedly suppressed under Ca^{2+}-free conditions. EM574 and porcine motilin specifically displaced [^{125}I-Tyr^{23}]canine motilin bound to gastric antral smooth muscle homogenates with pIC_{50} values of 8.21 ± 0.13 and 9.20 ± 0.11, respectively. The pEC_{50} value for the contractile response and pIC_{50} value for the receptor binding for motilin, EM574, erythromycin A and three other derivatives correlated well ($r = 0.94$, $P < 0.01$). Tissue section autoradiography in the antrum revealed that specific labeled motilin binding sites were localized in the circular muscle layer and myenteric plexus, and could be reduced in the presence of an excess of EM574. These results indicate that EM574 is a potent motilin receptor agonist in the rabbit gastrointestinal tract.

Keywords: Erythromycin; EM574; Motilin; Receptor binding; (Rabbit)

1. Introduction

Motilin, a polypeptide of 22 amino-acid residues (Brown et al., 1973), is thought to be involved in the occurrence of the interdigestive migrating motor complex in the dog (Itoh et al., 1978) and the human (Peeters et al., 1980). We have previously shown that erythromycin A, a 14-membered macrolide, induces strong gastrointestinal contractions similar to naturally occurring interdigestive migrating motor complex and motilin-induced contractions in the dog (Itoh et al., 1984a,b, 1985). In order to obtain a compound with more potent motilin-like contractile activity than erythromycin without antibacterial activity, a large number of erythromycin derivatives were synthesized. De(*N*-methyl)-*N*-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM574) was selected as the best candidate (Ōmura et al., 1987; Tsuzuki et al., 1989). EM574 is 248 times as potent as erythromycin A for gastric motor-stimulating activity in conscious dogs, but has no antibacterial activity (Tsuzuki et al., 1989), and is currently under development as a prokinetic agent.

In vitro studies have shown that erythromycin and its derivatives bind to motilin receptors located on gastrointestinal smooth muscle tissue with the same regional and species specificities as motilin itself (Peeters et al., 1989; Depoortere et al., 1990, 1993) and that they induced contractions of muscle segments with a potency related to their receptor affinity (Depoortere et al., 1989). These findings suggest that erythromycin and its derivatives act as motilin receptor agonists.

In the present study we examined, using in vitro experiments, whether EM574 acts as a motilin receptor agonist in the rabbit. The experiments involved contraction studies

* Corresponding author. Tel.: (81-6) 300-6142; Fax: (81-6) 300-6306.

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using intestinal preparations, receptor binding studies and tissue section autoradiography in the gastric antrum. The effects of erythromycin A and three other erythromycin derivatives, *N*-methyl-9,9-dihydroerythromycin A 6,9-epoxide iodide (EM502), de(*N*-methyl)-*N*-ethyl-8,9-anhydroerythromycin A 6,9-hemiacetal (EM523) and *N*-propargyl-8,9-anhydroerythromycin A 6,9-hemiacetal bromide (EM536) (Sunazuka et al., 1989; Tsuzuki et al., 1989) on contractile activity and receptor binding were also investigated to confirm the relationship between these two variables.

2. Materials and methods

2.1. Animals

Male New Zealand White rabbits weighing 3–4 kg, female Hartley guinea pigs weighing 300–350 g and male Sprague-Dawley rats weighing 250–300 g were used in the experiments. The animals were killed with an overdose of pentobarbital.

2.2. Contractile activity

The contractile responses of rabbit, guinea pig and rat small intestinal segments were tested according to the method of Adachi et al. (1981). In brief, the duodenum, jejunum and ileum were isolated and 3-cm segments were mounted along their longitudinal axes in organ baths containing 20 ml of Krebs' solution, continuously gassed with 95% O_2-5% CO_2 and kept at 37°C. Intestinal segments from rabbit were preloaded with 1.0 g and guinea pig and rat segments were preloaded with 0.5 g. Isotonic contractions were recorded on a polygraph (Recti-Horiz-8K; NEC San-ei Instruments, Tokyo, Japan) by means of an isotonic transducer and amplifier (ME-4012; NEC San-ei). Each drug was added cumulatively to the organ bath, and the mean of the increase in tonus was expressed relative to that induced by 10^{-4} M acetylcholine chloride before the start of cumulative dosing.

Pharmacological analysis of the contractile responses to EM574 and motilin was performed by pretreating rabbit duodenal segments with atropine (10^{-6} M), tetrodotoxin (10^{-6} M), mepyramine (10^{-5} M) or (2*S*,3*S*)-*cis*-3-(2-methoxybenzylamine)-2-phenyl piperidine (CP-99994; 3×10^{-7} M) for 5 min, verapamil (10^{-5} M) for 30 min or Ca^{2+}-free media for 40 min. Effective concentrations of atropine, tetrodotoxin (Adachi et al., 1981), mepyramine (Chang et al., 1979), CP-99994 (Holzer et al., 1995) and verapamil (Matthijs et al., 1988) in isolated intestine as described in the literature were used.

2.3. Preparation of ^{125}I-motilin

Synthetic [Tyr^{23}]canine motilin was radioiodinated with $Na^{125}I$ by the lactoperoxidase method. It was purified by reverse-phase high-performance liquid chromatography on a TSK gel ODS 120 T column (4.6×250 mm; Tosoh, Tokyo, Japan) eluted with a 25–33% linear gradient of acetonitrile containing 0.1% trifluoroacetic acid for 50 min at 1 ml/min. A single major peak of radioactivity (specific activity 2000 cpm/fmol) was obtained.

2.4. Receptor binding

^{125}I-Motilin binding to the smooth muscle tissue of the rabbit gastric antrum was examined according to the method of Bormans et al. (1986) with minor modifications. All steps were carried out at 4°C, unless otherwise stated. The smooth muscle tissue was dissected free from mucosa and serosa, minced finely with scissors and then homogenized in 10 vols. of 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose, 10 mM $MgCl_2$ and 0.1 mM phenylmethylsulfonyl fluoride using a Potter S homogenizer at 1500 rpm for 15 s. The homogenate was centrifuged at $1000 \times g$ for 15 min, washed four times and resuspended in 150 mM NaCl. The protein concentration was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard. The antral smooth muscle homogenate (1 mg of protein) was incubated with 40 fmol of ^{125}I-motilin and a displacer for 60 min at 30°C in 0.8 ml of binding buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM phenylmethylsulfonyl fluoride and 1.5% bovine serum albumin (pH 8.0)). The reaction was terminated by adding 3.2 ml of ice-cold 10 mM Tris buffer (pH 8.0) containing 10 mM $MgCl_2$ and 1% bovine serum albumin, followed by centrifugation at $1000 \times g$ for 15 min. The pellet was washed with the binding buffer and counted in a gamma counter (ARC-1000; Aloka, Tokyo, Japan). Non-specific binding was measured in the presence of excess (10^{-6} M) unlabeled [Tyr^{23}]canine motilin. The K_d and B_{max} values were calculated from the self-displacement curve according to the method of Scatchard (1949).

2.5. Tissue section autoradiography

The distribution of motilin receptors in the rabbit gastric antrum was investigated by the method of Sheikh et al. (1991) with minor modifications. Frozen sections (7 μm) of rabbit antral tissue were mounted on gelatine-coated slides and incubated for 2 h at 25°C with 2×10^{-10} M ^{125}I-motilin in the presence or absence of 10^{-6} M unlabeled [Tyr^{23}]canine motilin or 3×10^{-6} M EM574 in 0.25 ml of the binding buffer (see Section 2.4). After incubation, the mounted sections were washed three times in the binding buffer, and then washed in the binding buffer without bovine serum albumin. The slides were then placed for 1 h in fixative containing 0.5% glutaraldehyde and 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4) at 4°C. They were then rinsed in chilled distilled water, dried, coated with NR-M2 photographic emulsion (Konica,

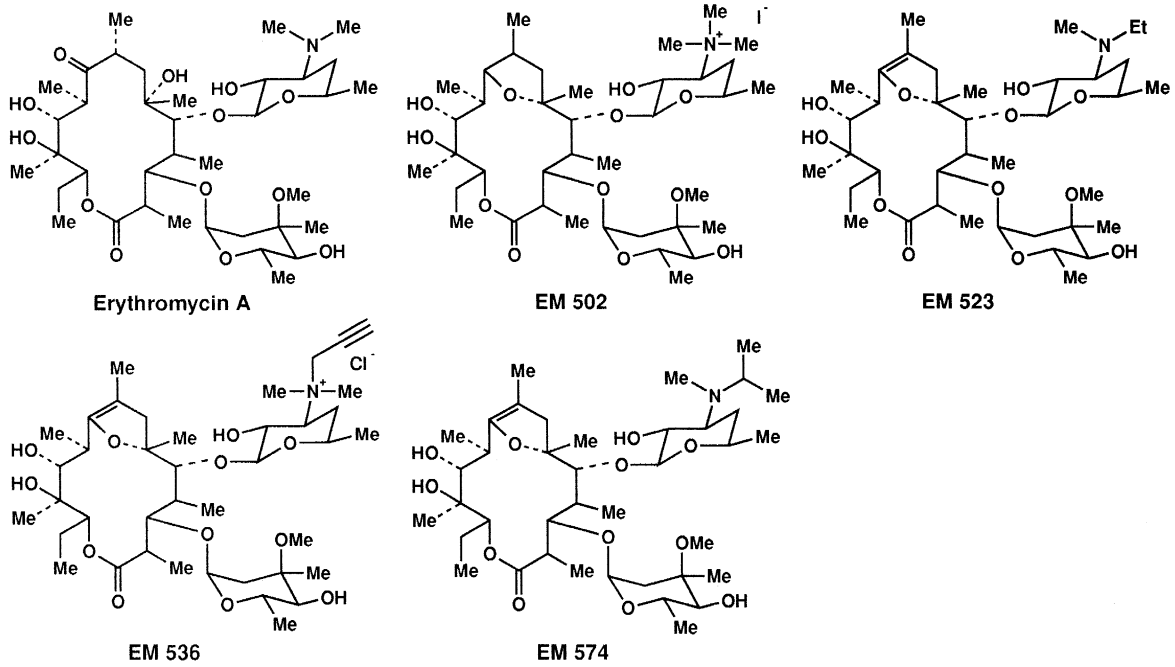


Fig. 1. Chemical structures of erythromycin A, EM502, EM523, EM536 and EM574.

Tokyo, Japan), and exposed for 20 days at 4°C. The slides were developed and counterstained with Kernechtrot. The distribution of autoradiographic grains at 10 sites each (1000 μm^2) in the mucosal, submucosal, longitudinal muscle and circular muscle layer and the myenteric plexus sections was counted using an image analyzer (Cosmozone 1 SA; Nikon, Tokyo, Japan).

2.6. Drugs and chemicals

CP-99994, EM502, EM523, EM536 and EM574 were synthesized at Takeda Chemical Industries. The chemical structures of these erythromycin derivatives and erythromycin A (Abbott Laboratories, North Chicago, IL, USA) are shown in Fig. 1. Erythromycin and its derivatives were solubilized in lactobionic acid as described previously (Satoh et al., 1990). The following drugs were used in this study: synthetic [Tyr^{23}]canine motilin, porcine motilin and substance P (Peptide Institute, Osaka, Japan), verapamil, bovine serum albumin (fraction V), phenylmethylsulfonyl fluoride and atropine sulfate (Wako, Osaka, Japan), $Na^{125}I$ (Amersham, Amersham, UK), tetrodotoxin and mepyramine maleate (Sigma, St. Louis, MO, USA), acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan), pentagastrin (Sumitomo Pharmaceutical, Osaka, Japan). All other reagents were of analytical grade from commercial sources.

3. Results

3.1. Contractile activity

The rabbit intestinal segments showed rhythmic contractions superimposed on the basal tonus. EM574 caused

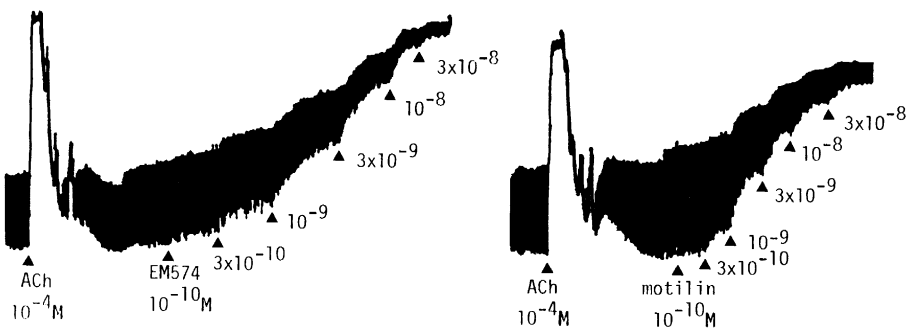


Fig. 2. Responses of isolated rabbit duodenum to acetylcholine, EM574 or porcine motilin. After supramaximal dosing of acetylcholine followed by washing-out, EM574 or motilin was applied in cumulative concentrations. EM574 as motilin produced an increase in the basal tonus of the segment in a dose-dependent manner.

an increase in the basal tonus in a dose-dependent manner without affecting the frequency of phasic contractions (Fig. 2). The sensitivity to EM574 differed according to the site in the intestine; the minimum effective concentrations for the duodenum, jejunum and ileum were 3×10^{-10}, 1×10^{-8} and 1×10^{-8} M, respectively (Fig. 3). The maximum contractile responses of the duodenum, jejunum and ileum were 90%, 47% and 10%, respectively, of the response induced by 10^{-4} M acetylcholine. EM574 did not induce contractions in the guinea pig or rat intestinal segments even at 10^{-5} M. Erythromycin A and the erythromycin derivatives EM502, EM523 and EM536 also induced contractions in the isolated rabbit duodenal segments. Their dose–response curves were almost parallel, with pEC_{50} values of 6.52 ± 0.26 ($n = 3$), 7.21 ± 0.05 ($n = 3$), 7.95 ± 0.09 ($n = 4$) and 7.84 ± 0.05 ($n = 3$), respectively; the pEC_{50} value for EM574 was 8.26 ± 0.04 ($n = 6$) (Fig. 4).

Porcine motilin did not induce contractions in the guinea pig or rat intestinal segments. Motilin induced dose-dependent contractions in the rabbit intestinal segments; the response was very similar to that induced by EM574 (Fig. 2). As with the results obtained with EM574, the sensitivity of the response to motilin decreased aborally; the minimum effective concentrations for the duodenum, jejunum and ileum were 1×10^{-10}, 3×10^{-9} and 1×10^{-8} M, respectively (Fig. 3). The maximum contractile responses of the duodenum, jejunum and ileum were 89%, 50% and 11%, respectively, of that induced by 10^{-4} M acetylcholine. The pEC_{50} value in the duodenum for porcine motilin was 8.69 ± 0.07 ($n = 6$); the pEC_{50} value for [Tyr^{23}]canine motilin was 8.58 ± 0.07 ($n = 3$) (Fig. 4).

The contractile responses of the duodenal segments to EM574 (3×10^{-9} or 10^{-8} M) and porcine motilin (3×10^{-9} M) were not sensitive to pretreatment with atropine (10^{-6} M, $n = 3$), tetrodotoxin (10^{-6} M, $n = 4$), mepyramine (10^{-5} M, $n = 3$) or CP-99994 (3×10^{-7} M, $n = 3$). Adding verapamil (10^{-5} M) to the organ bath markedly diminished the rhythmic contractions of the segments and decreased the basal tonus, and treatment of the segments for 30 min with 10^{-5} M verapamil suppressed the contractile response to EM574 and porcine motilin. The contractile responses to 10^{-8} M EM574 and 3×10^{-9} M motilin after verapamil treatment were $53 \pm 12\%$ ($n = 3$) and $61 \pm 8\%$ ($n = 3$), respectively, of those before treatment. After change of the media to a Ca^{2+}-free solution, the segments relaxed and spontaneous motion disappeared. Under these conditions, adding EM574 (10^{-8} M) and porcine motilin (3×10^{-9} M) induced only a slight contraction; the contractile responses to EM574 and motilin under Ca^{2+}-free conditions were $13 \pm 11\%$ ($n = 3$) and $17 \pm 7\%$ ($n = 3$), respectively, of those under normal conditions. Adding 2.5 mM Ca^{2+} to Ca^{2+}-free media restored the amplitude of the contractions to approximately the same level as seen in the control media.

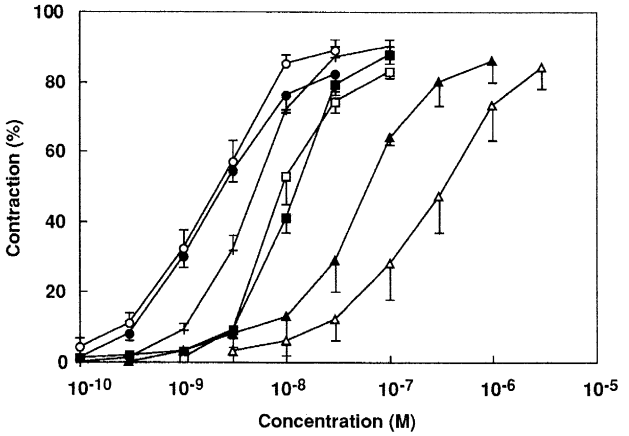


Fig. 4. Contractile effects of porcine motilin (○), [Tyr^{23}]canine motilin (●), erythromycin A (△) and its derivatives EM502 (▲), EM523 (□), EM536 (■) and EM574 (+) on isolated rabbit duodenum. Contractile responses elicited by each stimulant are expressed as percentages of the contraction elicited by 10^{-4} M acetylcholine in the same preparation. The dose-response curves are almost parallel. Each point represents the mean ± S.E.M. of 3–6 experiments.

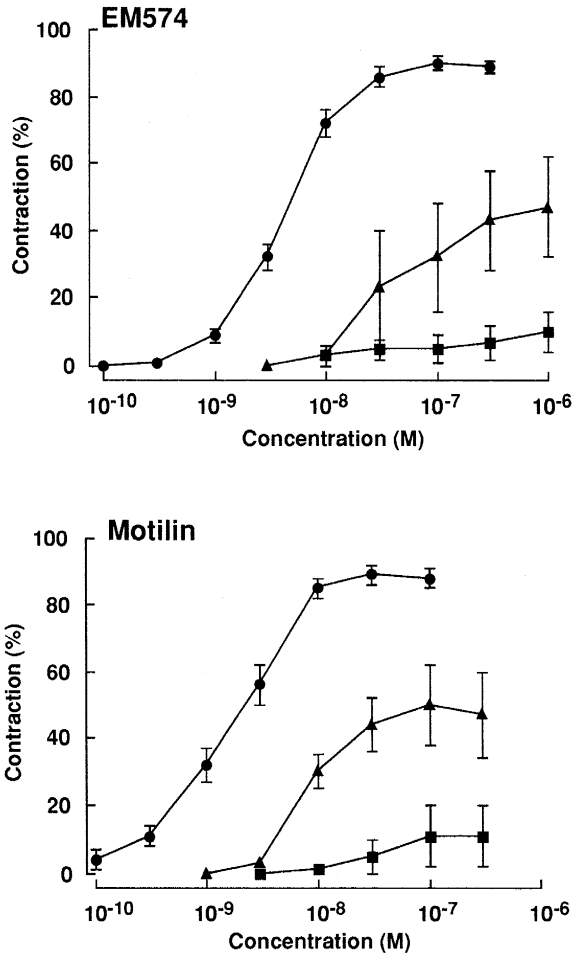


Fig. 3. Contractile responses of isolated rabbit duodenum (●), jejunum (▲) and ileum (■) to EM574 and porcine motilin. The responses elicited by each stimulant are expressed as percentages of the contraction elicited by 10^{-4} M acetylcholine in the same preparation. Each point represents the mean ± S.E.M. of 3–6 experiments.

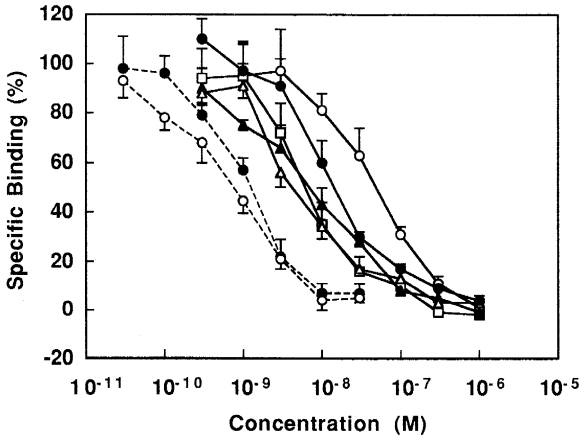


Fig. 5. Displacement studies with porcine motilin (- - -○- - -), [Tyr^{23}]canine motilin (- - -●- - -), erythromycin A (—○—) and its derivatives EM502 (—●—), EM523 (—△—), EM536 (—▲—) and EM574 (—□—). The antral smooth muscle tissue homogenates (1 mg of protein) were incubated with 5×10^{-11} M labeled motilin and various concentrations of the competitors. The binding displacement curves are almost parallel. Each point represents the mean ± S.E.M. of three to five experiments.

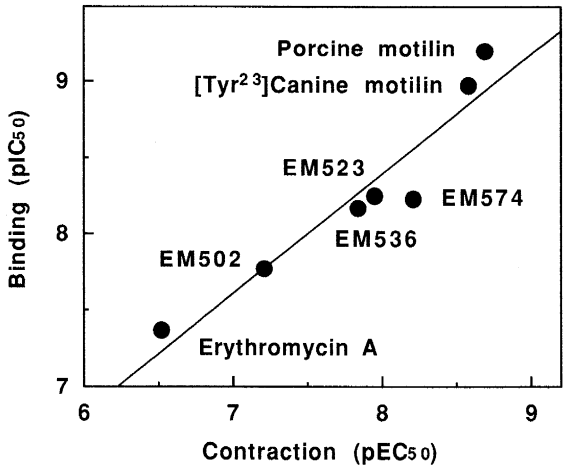


Fig. 6. Correlation ($r = 0.94$; $P < 0.01$) between contractile activity (pEC_{50}) and binding affinity (pIC_{50}) for porcine motilin, [Tyr^{23}]canine motilin and erythromycin derivatives.

3.2. Receptor binding

Non-specific binding to smooth muscle tissue homogenates of rabbit gastric antrum was less than 20% of the total binding as determined from the radioligand bound in the presence of excess unlabeled motilin. Porcine and [Tyr^{23}]canine motilin displaced labeled motilin bound to the antral homogenates with pIC_{50} values of 9.20 ± 0.11 ($n = 3$) and 8.97 ± 0.06 ($n = 5$), respectively (Fig. 5). Scatchard analysis of the self-displacement curves revealed linear relationships consistent with single binding sites with a pK_d value of 8.92 ± 0.06 and a maximal binding capacity of 24 ± 4 fmol/mg protein ($n = 5$, data not shown). Pentagastrin and substance P, at concentrations of up to 10^{-6} M, did not reduce labeled motilin binding (data not shown). EM574 displaced labeled motilin bound to the antral homogenates with a pIC_{50} value of 8.21 ± 0.13 ($n = 4$); erythromycin A and the erythromycin derivatives EM502, EM523 and EM536 also displaced labeled motilin with pIC_{50} values of 7.37 ± 0.10 ($n = 3$), 7.77 ± 0.06 ($n = 3$), 8.25 ± 0.16 ($n = 5$), 8.17 ± 0.15 ($n = 3$), respectively (Fig. 5). These displacement curves were almost parallel. A plot of binding affinity (pIC_{50} value) versus contractile activity (pEC_{50} value) demonstrated that changes in receptor binding were reflected by changes in contractile activity (Fig. 6). The pIC_{50} and pEC_{50} values for each motilin and erythromycin derivative were strongly correlated ($r = 0.94$, $P < 0.01$).

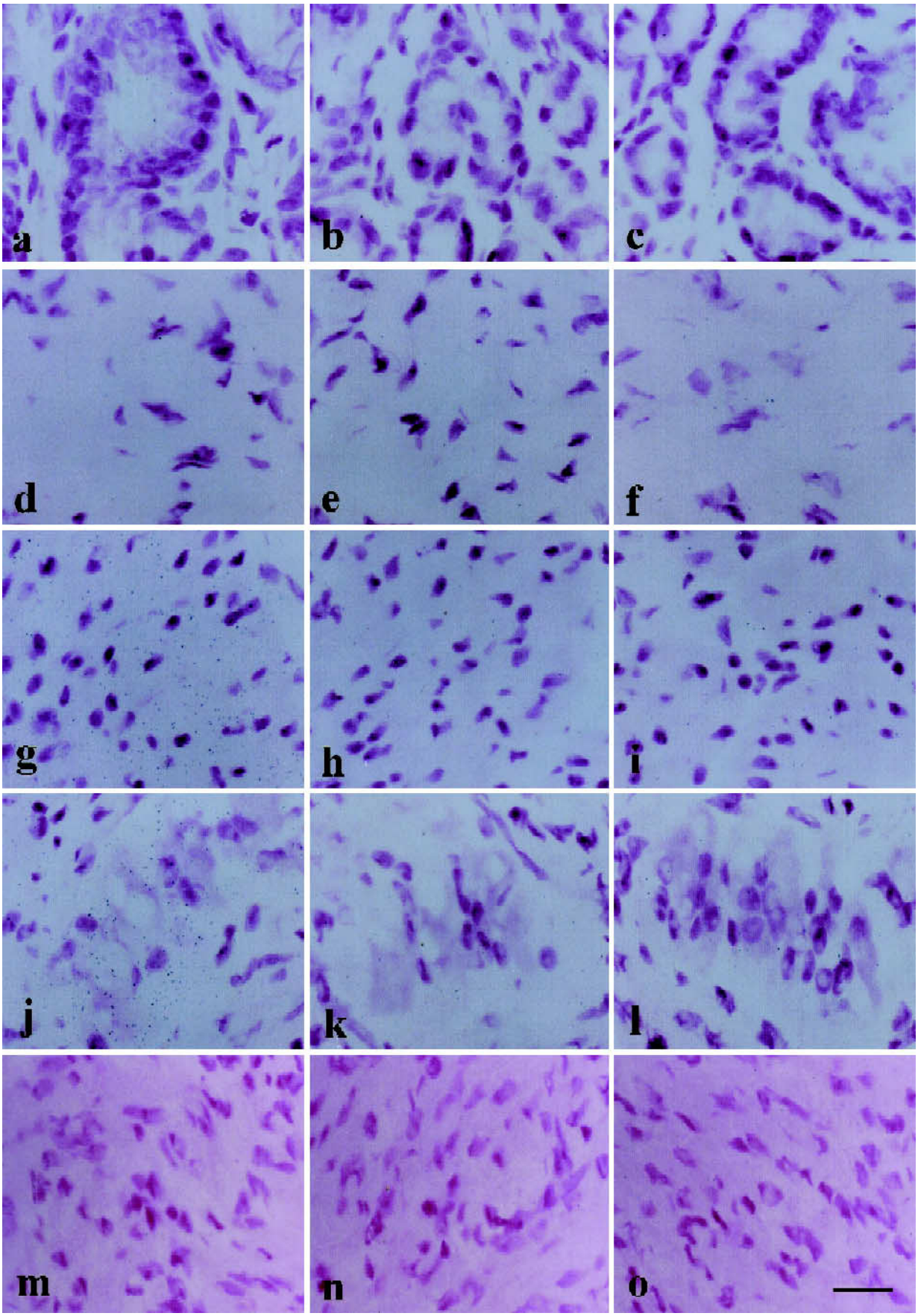
3.3. Autoradiographic analysis

The localization of motilin binding sites in the rabbit gastric antrum was determined by tissue section autoradiography. High densities of autoradiographic grains were observed on the circular muscle layer and myenteric plexus, and these were significantly reduced in the presence of 10^{-6} M unlabeled motilin or 3×10^{-6} M EM574 (Fig. 7, Table 1). The densities of grains in the mucosal, submucosal and longitudinal layers were less than those in the circular muscle layers and myenteric plexus, and were not affected by the addition of excess unlabeled motilin or EM574 (Fig. 7, Table 1).

Table 1
Distribution of autoradiographic grains in the rabbit antrum after incubation with ^{125}I-motilin

Competitor added to ^{125}I-motilin	Grains/1000 μm^2 [a]				
	Mucosal layer	Submucosal layer	Circular muscle layer	Myenteric plexus	Logitudinal muscle layer
Control	5.6 ± 0.7	4.5 ± 1.0	22.2 ± 5.2	19.4 ± 6.0	4.4 ± 0.4
Unlabeled motilin	5.3 ± 1.1	3.8 ± 1.0	6.2 ± 0.8 [b]	7.3 ± 1.5 [b]	3.9 ± 0.7
EM574	5.1 ± 1.0	3.8 ± 1.2	5.9 ± 1.2 [b]	8.8 ± 2.4 [b]	3.9 ± 0.7

Values were obtained by incubating tissue sections with 2×10^{-10} M [^{125}I-Tyr^{23}]canine motilin in the presence or absence of 1×10^{-6} M unlabeled [Tyr^{23}]canine motilin or 3×10^{-6} M EM574 for 2 h. Grain densities were calculated from light microscope autoradiographs using an image analyzer.
[a] Data are expressed as mean ± S.D. of 10 sites. [b] $P < 0.01$ compared to control (Dunnett's test).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4. Discussion

It has been reported that erythromycin improves impaired gastric emptying in patients with severe diabetic gastroparesis (Janssens et al., 1990), postvagotomy gastroparesis (Mozwecz et al., 1990; Xynos et al., 1992), idiopathic gastroparesis (Richards et al., 1990) and gastroparesis after cancer chemotherapy (Maliakkal et al., 1991). In addition, erythromycin accelerates gastric emptying in normal subjects (Annese et al., 1992). Thus erythromycin's prokinetic activity may be useful for the treatment of gastrointestinal disorders. However, erythromycin itself may disrupt the intestinal bacterial flora and induce resistance in bacterial strains due to its antibacterial activity. For this reason, erythromycin derivatives with potent gastrointestinal motor-stimulating activity and no antibacterial activity are promising candidates as prokinetic agents. In the present study we examined whether EM574, a highly potent stimulant of gastric motility without antibacterial activity (Tsuzuki et al., 1989), would act as a motilin receptor agonist in the rabbit gastrointestinal tract, which is usually used as an in vitro model system for studies on motilin (Adachi et al., 1981; Bormans et al., 1986; Peeters et al., 1989).

Previous pharmacological analysis has shown that erythromycin and its derivatives have the same regional and species specificities as motilin, and that the contractile response is not sensitive to neural blocking agents such as atropine and tetrodotoxin and is dependent on extracellular Ca^{2+} (Matthijs et al., 1988; Peeters et al., 1989, 1991; Satoh et al., 1990; Depoortere et al., 1990). Moreover, recent binding studies have indicated that erythromycin and its derivatives displace motilin bound to rabbit and human gastrointestinal smooth muscle tissue (Kondo et al., 1988; Depoortere et al., 1989, 1990). These data suggest that erythromycin and its derivatives are motilin receptor agonists despite the fact that there is no similarity between the structure of erythromycin derivatives and that of motilin.

In the present contraction experiments, EM574 induced contractions of isolated rabbit (but not rat or guinea pig) intestinal segments with the same regional and species specificity as motilin, duodenum being more sensitive than ileum. Atropine (cholinergic blockade), tetrodotoxin (blockade of axonal conduction), mepyramine (histamine H_1 blockade) or CP-99994 (tachykinin NK_1 blockade) did not affect the contractile activity of EM574 or motilin, and the contractile responses to both compounds were strongly dependent on extracellular Ca^{2+}. These results indicate that EM574, like motilin, exerts a direct effect on intestinal smooth muscle, which is in agreement with previous pharmacological data for other erythromycin derivatives as mentioned above.

In order to demonstrate that EM574 stimulates contractile activity via motilin receptors, receptor binding studies were performed. It has already been reported that there is a significant correlation between the binding affinity to rabbit antral smooth muscle homogenates and the contractile activity in rabbit duodenal segments of erythromycin derivatives (Depoortere et al., 1989) and those of several peptide fragments of [Leu^{13}]porcine motilin (Macielag et al., 1992). Therefore, we used the antrum for binding studies and the duodenum for contraction experiments to minimize the number of animals, and analyzed the relationship between contractile activity and binding affinity of erythromycin and its derivatives including EM574 and motilin. EM574 and other erythromycin derivatives displaced labeled motilin bound to gastric antral smooth muscle homogenates. A highly significant correlation was observed between the contractile activity (pEC_{50}) and binding affinity (pIC_{50}) of the erythromycin derivatives and motilin. These results suggest that EM574 stimulates contractile activity via motilin receptors on the surface of smooth muscle cells in the rabbit gastrointestinal tract. In the binding experiments EM523 was slightly more potent than EM574, while in the contraction studies the order of potency was reversed. The two experiments were carried out at different pH values (pH 8.0 for binding experiments; pH 7.4 for contraction studies). This may have accounted for the small difference in the pIC_{50} and pEC_{50} values. In addition, a difference between the tissue specimens used cannot be ruled out.

In order to confirm the localization of motilin receptors on smooth muscle cells and the effect of EM574 on the receptors, tissue section autoradiography was performed. This revealed that specific motilin binding sites were located on the circular muscle layer and myenteric plexus, and suggests that EM574 shares motilin binding sites in both areas. The predominant distribution of motilin binding sites on the circular muscle layer has already been reported by Sakai et al. (1994), but the present findings are the first direct morphological evidence of specific myenteric plexus binding sites. In accord with the report by Bormans et al. (1986), Scatchard plot analysis detected only a single class of binding sites in the smooth muscle tissue containing the myenteric plexus. The motilin binding sites detected are probably those located on the circular muscle layer, because the total number of the binding sites present throughout the circular muscle layer is apparently much greater than that of the binding sites on the myen-

Fig. 7. Autoradiographic distribution of motilin binding sites in rabbit gastric antrum. Sections were incubated with 2×10^{-10} M [^{125}I-Tyr^{23}]canine motilin in the presence or absence of 1×10^{-6} M unlabeled [Tyr^{23}]canine motilin or 3×10^{-6} M EM574 for 2 h. (a,b,c) mucosal layer, (d,e,f) submucosal layer, (g,h,i) circular muscle layer, (j,k,l) myenteric plexus, (m,n,o) longitudinal muscle layer; (a,d,g,j,m) without competitor, (b,e,h,k,n) + unlabeled [Tyr^{23}]canine motilin, (c,f,i,l,o) + EM574; bar = 20 μm.

teric plexus due to the difference in size of each area. Thus, motilin receptors located on the circular muscle layer, but not on the myenteric plexus, seem to be responsible for the pIC_{50} values obtained in the binding studies, and this is in line with the significant correlation between the binding affinity and contractile activity, which is insensitive to neural blocking agents.

Recently, Parkman et al. (1995) showed that in isolated rabbit antrum, erythromycin induced phasic and tonic contractions at a higher dose (5×10^{-5} M), and that at a subthreshold dose (10^{-7} M) it increased the frequency, but not the amplitude, of bethanechol- and substance P-induced phasic contractions. The authors demonstrated that, while the inotropic effect was not sensitive to anticholinergic agents, the chronotropic effect was inhibited with such agents, and that both effects were inhibited with motilin tachyphylaxis, suggesting that motilin receptors are located on both smooth muscle cells and enteric cholinergic nerves. Thus, it is probable that the motilin binding sites on the myenteric plexus in the antrum observed by autoradiographic analysis are involved in the release of acetylcholine by erythromycin or motilin. Moreover, Kitazawa et al. (1993) reported that anticholinergic agents inhibit the tonic response (slowly fading contractions), but not the phasic response (rapid, initial contractions) of rabbit duodenal segments to motilin. Therefore, motilin binding sites on enteric neurons may be present on the duodenum as on the antrum in the rabbit.

In isolated canine gastrointestinal preparations, motilin did not induce contractions (Jennewein et al., 1975; Fox et al., 1984) and specific motilin binding could not be demonstrated (Peeters et al., 1988). Since the motor-stimulating actions of motilin (Green et al., 1976; Fox et al., 1983) and erythromycin derivatives (Itoh et al., 1984a; Inatomi et al., 1989) are inhibited by anticholinergic agents in dogs in vivo, it seems that motilin receptors in dogs must be located at neural sites. Intravenous administration of EM502, EM523, EM536 and EM574 increases canine gastric motility with potencies of 65, 18, 2890 and 248 times that of erythromycin A, respectively (Sunazuka et al., 1989; Tsuzuki et al., 1989), whereas the contractile activities of these erythromycin derivatives on isolated rabbit duodenal segments are 8, 62, 38 and 87 times more potent, respectively. This poor correlation suggests that the structure of motilin receptors on canine nerves is slightly different from that of the receptors on rabbit smooth muscle cells. Further studies using gene cloning will be needed to elucidate the diversity of motilin receptors in different species and/or tissues.

In conclusion, we have demonstrated that EM574 is a highly potent motilin receptor agonist in the rabbit, as reported recently for the human (Satoh et al., 1994). Since, unlike motilin, EM574 is active when it is administered by the oral or intraduodenal route (Inatomi et al., 1996), it would seem to be promising as a prokinetic agent.

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