Stimulating action of KW-5139 (Leu¹³-motilin) on gastrointestinal motility in the rabbit

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1 The gastrointestinal motor stimulating action of the motilin analogue, KW-5139 (Leu¹³-motilin), was investigated both in the anaesthetized rabbit and in rabbit isolated smooth muscle tissues.

2 KW-5139 (0.3–10 µg kg⁻¹, i.v.) produced motor stimulating actions in the gastric antrum, ileum and descending colon, the excitatory responses of which were initiated at the same time but declined with different time courses. The rank order of the excitatory response was: descending colon ≥ gastric antrum >> ileum.

3 Atropine (1–3 mg kg⁻¹, i.v.) or naloxone (1 mg kg⁻¹, i.v.) completely suppressed the excitatory response to KW-5139 in the gastric antrum, but only partially attenuated that in the descending colon. This suggests that the mechanism of the excitatory response is different in the gastric antrum and the descending colon, and that cholinergic neural pathway is involved in the response of the gastric antrum.

4 KW-5139 (0.1 nM–1 µM) caused concentration-dependent contractions of the gastric antrum, duodenum, jejunum, ileum and the descending colon *in vitro*. In the rabbit intestine, the contractile response to KW-5139 was strongest in the duodenum and weakest in the ileum.

5 The contractile response to KW-5139 in the intestinal segments were not affected by tetrodotoxin, but were decreased by verapamil, or pretreatment with a high concentration of porcine motilin, confirming the involvement of motilin receptors in the response to KW-5139.

6 The present results suggest that the rabbit is a suitable species for the investigation of motilin on gut motility, because of the high responsiveness of the descending colon as well as the upper gastrointestinal tract.

Keywords: Motilin; rabbit gastrointestinal tract *in vivo*; motor stimulating action

Introduction

Motilin, a 22 amino acid polypeptide isolated from the mucosae of upper small intestine of the hog (Brown *et al.*, 1971; 1973), stimulates mammalian gastrointestinal (GI) motility in *in vivo* and *in vitro* experiments, although the mechanism of the stimulating action seems to differ among species. Motilin appears to stimulate motility of the stomach and small intestine of the dog by excitation of the cholinergic neurones (Inatomi *et al.*, 1989; Itoh, 1990; Itoh *et al.*, 1991; Kitazawa *et al.*, 1992), whereas this peptide produces contraction of the stomach, pylorus and the duodenum of the rabbit and man by direct action on the smooth muscle cells (Segawa *et al.*, 1976; Adachi *et al.*, 1981; Poitras *et al.*, 1987; Lüdtke *et al.*, 1989; Satoh *et al.*, 1990).

Motilin given intravenously to the dog initiates a contractile response, the period of which is similar to that of the interdigestive migrating motor complex (Itoh *et al.*, 1976; Lee *et al.*, 1983; Itoh, 1990), while the peptide does not affect isolated strips of dog GI tissues (Segawa *et al.*, 1976; Poitras *et al.*, 1987). Therefore, it was impossible to compare the response of motilin between *in vivo* and *in vitro* experiments. Isolated rabbit and human GI smooth muscle have been shown to exhibit high contractile responsiveness (Segawa *et al.*, 1976; Adachi *et al.*, 1981; Lüdtke *et al.*, 1989; Satoh *et al.*, 1990) and high affinity binding sites (Bormans *et al.*, 1986; Depoortere *et al.*, 1991) to motilin, and, thus, the duodenal strip of the rabbit was used for a biological assay of motilin-like peptides (Vogel & Brown, 1990). Recently, the amino acid sequence of rabbit motilin has been established

and the physiological significance of motilin in GI motility has been proposed (Banfield *et al.*, 1992). Our previous study demonstrated that KW-5139 (Leu¹³-motilin), an analogue of porcine motilin, produces a contraction of rabbit isolated duodenum by stimulation of both the smooth muscle and cholinergic neurones (Kitazawa *et al.*, 1993).

In spite of the high responsiveness and frequent usage of rabbit tissue in motilin studies, few *in vivo* studies concerning the effect of motilin on rabbit GI motility have been published (Fox, 1990). The present study was undertaken to determine the effect of KW-5139 on the rabbit GI motility in both *in vivo* and *in vitro* experiments. Preliminary results of this study have been presented in abstract form (Ishii *et al.*, 1992).

Methods

In vivo study

Male rabbits (Japan White strain), weighing 2.3–3.3 kg, were anaesthetized with urethane (1.3 g kg⁻¹, i.p.). After anaesthesia had been induced, the trachea was cannulated to facilitate respiration. Polyethylene cannulae were inserted into the left carotid artery for measurement of systemic blood pressure through a pressure transducer (MPU-0.5, Nihon Kohden), and into the right ear vein for systemic application of KW-5139 and the other drugs. The abdominal cavity was opened by midline incision, and the gastric antrum, ileum and the descending colon were exposed. To measure GI motility, intraluminal pressure changes were detected by rubber balloons, inserted into the gastric antrum, ileum, or the descending colon through a small incision in the GI wall. The measurement of the motility was performed usually in

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two different GI regions (gastric antrum and ileum, or gastric antrum and descending colon) of each animal at the same time. The balloons were filled with distilled water (23–24°C, pressure applied to the balloon was usually set at 10 cmH₂O) and connected to a pressure transducer equipped with an ink-writing polygraph (RM-6200, Nihon Kohden). The animals were allowed to equilibrate for 60 min at which time steady contractile activity and blood pressure were established. Mean blood pressure of the urethane-anaesthetized rabbit was 92 ± 5.5 mmHg ($n = 6$). Each dose of KW-5139 (0.1 – $10 \mu\text{g kg}^{-1}$), atropine, pyrilamine or naloxone was dissolved in saline, and administered into the right ear vein. The actions evoked on the GI motility and blood pressure were recorded. When KW-5139 was applied at 60 min intervals, we could obtain the reproducible mechanical response over 8–9 h. For quantitative analysis of GI motility, the motor index was calculated by the measurement of the area surrounded by the contraction waves and baseline during the 10 min period, before and after administration of KW-5139, and used for comparison of regional responsiveness. The increase in motility induced by KW-5139 was calculated by the following equation: Increase in motility (%) = $100 \times$ motor index after KW-5139 stimulation/motor index before KW-5139 stimulation.

In vitro study

White male rabbits were anaesthetized with Na pentobarbitone (30 mg kg^{-1} , i.v.) and exsanguinated. After a mid-line incision, the antrum of the stomach, duodenum, jejunum, ileum and the descending colon were surgically isolated. Both longitudinal and circular muscle layers ($20 \times 2 \text{ mm}$) were prepared from the gastric antrum. These smooth muscle layers and intestinal segments (20 – 30 mm) of duodenum, jejunum, ileum and descending colon were suspended separately, each in an organ bath (40 ml), containing Tyrode solution continuously bubbled with a gas mixture of 95% O₂–5% CO₂. Bathing Tyrode solution was kept at $28 \pm 1^\circ\text{C}$ in accordance with the study of Adachi *et al.* (1981)

to prevent excessive spontaneous contractions. The mechanical activity of the preparation was measured with an isotonic transducer (TD-112S, Nihon Kohden) and recorded on an ink-writing recorder. The initial load was set at 1 g for gastric longitudinal and circular muscles or 4 g for intestinal segments. These preparations were rinsed with a fresh nutrient solution every 15 min, and were allowed to equilibrate for 60 min to establish a steady baseline and spontaneous contractile activity. Prior to the addition of KW-5139, each smooth muscle preparation was subjected to 3 or 4 stimulations with acetylcholine (ACh, $100 \mu\text{M}$) for 30 s to stabilize the contractility. The concentration-dependent contractions were determined after cumulative addition of KW-5139 (0.1 , 0.3 , 1 , 3 , 10 , 30 , 100 , 300 nM , $1 \mu\text{M}$) in the bath at 2 min cycles. The amplitude of the maximum contractile response during each 2 min cycle was determined and expressed as a percentage of the response to $100 \mu\text{M}$ ACh, which was sufficient to produce the maximum contraction in each preparation. EC₅₀ values were calculated from the concentration-response curves and expressed as geometric means with 95% confidence limits in parentheses.

Drugs

Substances used in the present experiments were as follows, acetylcholine chloride (Ovisot, Daiichi Seiyaku), atropine sulphate (Nakarai), pyrilamine maleate (Sigma), naloxone hydrochloride (Sigma), tetrodotoxin (Sigma), verapamil hydrochloride (Sigma), porcine-motilin (Peninsula Laboratories Inc.), KW-5139 (Leu¹³-motilin, Kyowa Hakko Kogyo Co. Ltd.) was produced by recombinant DNA techniques (Miyashita *et al.*, 1988). The drugs and motilin peptides were dissolved in saline or distilled water.

Statistics

All data, except for EC₅₀ values, are expressed as means $\pm$ s.e.mean. The significance of difference was determined by

Figure 1 Typical effects of three increasing doses of KW-5139 on the motility of gastric antrum, ileum and descending colon of the anaesthetized rabbit. Each dose of KW-5139 (1 – $10 \mu\text{g kg}^{-1}$) was administered intravenously at the point indicated by (●).

Figure 2 Dose-related gastrointestinal motor stimulating action induced by KW-5139 in the anaesthetized rabbit. Effects of KW-5139, given i.v., on the motility of the gastric antrum (open columns), ileum (hatched columns) and the descending colon (stippled columns) are illustrated. Ordinate indicates an index of motility (%) for 10 min after administration of KW-5139 (control = 100%, see Methods). Columns represent the means with s.e.mean of 4–8 animals. *Significantly different from the motor index before stimulation with KW-5139 (paired *t* test).

a paired *t* test or Sign-Wilcoxon test, and *P* values less than 0.05 were considered significant.

Results

Effect on gastrointestinal motility in vivo

The lowest dose ($0.1 \mu\text{g kg}^{-1}$) of KW-5139 given intravenously did not produce any GI motility changes; however, doses of $0.3 \mu\text{g kg}^{-1}$ or higher produced a motor stimulating action in the gastric antrum, ileum and the descending colon which occurred almost at the same time (Figures 1 and 2). The systemic blood pressure did not change significantly at any dose (data not shown). Excitatory responses produced in the gastric antrum and the descending colon appeared rapidly after the application of KW-5139 (within 10–20 s), and lasted for about 15–20 min, but the response in the ileum was less notable and short-living compared with that in other two regions. The excitatory responses in the gastric antrum were, somewhat different from those in the descending colon. KW-5139 induced mainly a phasic contraction in the antrum, while, in the descending colon, the peptide induced both a tonic and phasic response, the latter being superimposed on the former (Figure 1). Figure 2 shows the dose-response relationships of the GI motor stimulating action of KW-5139 in the three GI regions. The rank order of the excitatory response was: descending colon $\gg$ gastric antrum $\gg$ ileum. The doses of KW-5139 required to increase spontaneous motility by 50% were $3.2 \pm 1.4 \mu\text{g kg}^{-1}$, i.v. ($n = 4$) for the descending colon and $4.6 \pm 1.4 \mu\text{g kg}^{-1}$, i.v. ($n = 5$) for the gastric antrum, respectively. On the other hand, the action on the ileum induced by KW-5139 ($10 \mu\text{g kg}^{-1}$, i.v.) was only $17 \pm 8\%$ ($n = 4$) of the spontaneous motility.

To examine the mechanisms underlying the excitatory response to KW-5139, the effects of some autonomic drugs were determined. Atropine (1 or 3 mg kg^{-1} , i.v.) almost completely suppressed the gastric contraction but only partly suppressed the colonic contraction induced by KW-5139 ($10 \mu\text{g kg}^{-1}$) (Figure 3). The inhibition by atropine (3 mg kg^{-1}) of the motility in the gastric antrum ($90 \pm 5.7\%$,

Figure 3 Effects of atropine on the excitatory response of gastric antrum and descending colon to KW-5139 in the anaesthetized rabbit. (a) Control mechanical response induced by KW-5139 ($10 \mu\text{g kg}^{-1}$, i.v.). After treatment with atropine (b, 1 mg kg^{-1} , i.v.; c, 3 mg kg^{-1} , i.v.) for 7 min, a reduction in motor stimulating action induced by KW-5139 was observed. KW-5139 was administered intravenously at the point indicated by (●).

$n = 4$) was significantly ($P < 0.05$) greater than that in descending colon ($55 \pm 6.6\%$, $n = 4$). Naloxone (1 mg kg^{-1} , i.v.) significantly ($P < 0.05$) suppressed the gastric response to KW-5139 ($10 \mu\text{g kg}^{-1}$) (inhibition; $62 \pm 6.8\%$, $n = 5$) without markedly decreasing the colonic response (inhibition; $15 \pm 8.5\%$, $n = 5$) (Figure 4a). Since histamine is known to contract the rabbit GI smooth muscle, the effect of pyrilamine (1 mg kg^{-1} , i.v.) on the mechanical response to KW-5139 was tested. As shown in Figure 4b, pyrilamine did not markedly decrease the gastric and colonic excitatory responses induced by KW-5139 ($10 \mu\text{g kg}^{-1}$). Relative excitatory responses after treatment with pyrilamine were $105 \pm 15\%$ ($n = 3$) in the gastric antrum, and $84 \pm 2.4\%$ ($n = 3$) in the descending colon, respectively.

Effect on isolated smooth muscle preparations

KW-5139, applied in the organ bath, caused contraction of the gastric antrum (both longitudinal and circular muscle), duodenum, jejunum, ileum and the descending colon in a concentration-dependent manner with different sensitivity from region to region (Figure 5). The minimum effective concentrations were 1 nM for duodenum, 3 nM for jejunum and descending colon, and 10 nM for gastric circular and longitudinal muscle, and ileum, respectively. The EC_{50} values (95% confidence limits) and maximum amplitudes of the contraction (expressed as a % of the $100 \mu\text{M}$ ACh-induced response) were 100 (44 – 242) nM and $21 \pm 6.8\%$ ($n = 9$) (longitudinal muscle of the gastric antrum), 28 (21 – 36) nM and $64 \pm 14.8\%$ ($n = 7$) (circular muscle of the gastric antrum), 4.2 (3.0 – 5.4) nM and $88 \pm 7.1\%$ ($n = 6$) (duodenum), 5.5 (4.4 – 6.9) nM and $77 \pm 7.5\%$ ($n = 5$) (jejunum), 12 (3.7 – 39) nM and $13 \pm 1.8\%$ ($n = 8$) (ileum), 20 (13 – 28) nM and $42 \pm 6.6\%$ ($n = 7$) (descending colon), respectively. These results indicate that the circular muscle is more sensitive to

Figure 4 Effects of naloxone and pyrilamine on the excitatory response of gastric antrum and descending colon induced by KW-5139 in the anaesthetized rabbit. (i) Control mechanical responses induced by KW-5139 ($10 \mu\text{g kg}^{-1}$, i.v.). After pretreatment with naloxone (a (ii), 1 mg kg^{-1} , i.v.) or pyrilamine (b (ii), 1 mg kg^{-1} , i.v.) for 10 min, motor stimulating action induced by KW-5139 was observed. KW-5139 was applied intravenously at the point indicated by (●).

KW-5139 than the longitudinal muscle in the gastric antrum, that the duodenum is the most sensitive and that the ileum is the least sensitive of the intestinal smooth muscle preparations tested.

Figure 6 represents a comparison of the reproducibility of the contractile response to KW-5139 in rabbit GI smooth muscles. After cumulative application of KW-5139 at 2 min cycles to obtain the first concentration-response curve, the preparations were rinsed 4–5 times with fresh Tyrode solution, equilibrated for 1 h, and then subjected to another cumulative application of KW-5139 for the second concentration-response curve. We could obtain reproducible concentration-related contractions in the duodenum, ileum and the descending colon at 1 h interval applications; however, in the gastric circular muscle, the amplitude of the contractile response was significantly suppressed at the second cumulative application without causing a notable change of EC_{50} . The EC_{50} value (95% confidence limits) and the maximum contractions were $26 (19\text{--}34) \text{ nM}$ and $79 \pm 19.2\%$ ($n=3$) in the first application, and $44 (12\text{--}77) \text{ nM}$ and $15 \pm 12.6\%$ ($n=3$) in the second, respectively. In the gastric longitudinal muscle, the maximum contraction was also suppressed at the second application (data not shown).

Pharmacological properties of the response to KW-5139 were examined in the duodenum (upper intestine) and the descending colon (lower intestine) segments, which showed

Figure 5 Concentration-contraction relationships of KW-5139 in isolated smooth muscle preparations of the rabbit gastrointestinal tract. (a) Concentration-response curves for longitudinal (●) and circular (○) muscle of the gastric antrum. (b) Concentration-response curves for duodenum (○), jejunum (●), ileum (Δ) and descending colon (▲). Relative contraction was expressed as a % of the amplitude of the acetylcholine ($100 \mu\text{M}$)-induced response in each region. Points represent the means $\pm$ s.e.mean of 5–6 experiments.

relatively high responsiveness to KW-5139 and good reproducibility of the response. Tetrodotoxin ($1 \mu\text{M}$) did not markedly decrease the initial phasic contractile response to KW-5139 ($10\text{--}100 \text{ nM}$), in agreement with previous studies (Depoortere *et al.*, 1991; Kitazawa *et al.*, 1993). In contrast, pretreatment with verapamil ($1 \mu\text{M}$) for 30 min diminished the spontaneous contractility and significantly ($P<0.05$) decreased the KW-5139-induced contraction. Relative amplitudes after verapamil were $15.4 \pm 5.9\%$ ($n=5$) for the duodenum (10 nM KW-5139) and $36.0 \pm 6.7\%$ ($n=6$) for the descending colon (100 nM KW-5139), respectively. These results indicate that KW-5139 acts directly on the intestinal smooth muscle to produce the phasic response, and that external Ca^{2+} plays an important role in the contractile response.

Effects of long-lasting exposure to motilin peptide (motilin peptide-induced desensitization) on the response to KW-5139 were examined in the duodenum and the descending colon to clarify whether or not KW-5139 acts on the motilin receptor. After observing the control contractile responses to KW-5139, porcine motilin and ACh, the preparation was markedly contracted by exposure to a high concentration ($10 \mu\text{M}$) of KW-5139 or porcine motilin for 10 min. The evoked contraction, sustained during the presence of KW-5139 in the bath, returned to the basal level in 20–40 min after repeated rinses with fresh Tyrode solution. Thereafter, the contractions induced by the test stimulants were

Figure 6 Reproducibility of the contractile response to KW-5139 in four different regions of the gastrointestinal tract of the rabbit. KW-5139 was cumulatively applied during 2 min cycles and the first concentration-response curve was obtained (O) in circular muscle of gastric antrum (a), and segments of duodenum (b), ileum (c) and descending colon (d). After 3–4 rinses with fresh Tyrode solution and equilibrium for 1 h, KW-5139 was cumulatively applied again and the second concentration-response relationship was established (●). Relative contraction was expressed as a % of the amplitude of acetylcholine (100 μ M)-induced contraction. *Significantly different from the correspondent control value (O) (paired *t* test). Points represent the means $\pm$ s.e.mean of 3–7 experiments.

examined. As shown in Figure 7a and Table 1, pretreatment with KW-5139 (10 μ M for 10 min) in the duodenum significantly decreased the contractile responses to KW-5139 (10 nM) and porcine motilin (10 nM) without inhibiting the contraction induced by ACh (100 μ M). In addition, pretreatment with porcine motilin (10 μ M for 10 min) also significantly decreased the contraction induced by KW-5139 and porcine motilin but did not change the response to 100 μ M ACh (Figure 7b, Table 1). Similarly, effects of pretreatment with motilin peptides on the response to KW-5139, porcine motilin and ACh were examined in the descending colon. Continuous exposure to motilin peptides caused marked transient contractions, which were slightly different from that in the duodenum, and the elevated tonus easily returned to the baseline level by repeated rinses. Pretreatment with KW-5139 or porcine motilin (10 μ M for 10 min) significantly decreased the responsiveness to KW-5139 and porcine motilin. However, in both cases, ACh (100 μ M)-induced contractions were not affected (Figure 7c, d, Table 1).

Discussion

From the present results of the *in vitro* mechanical study, it is assumed that KW-5139 acted on the motilin receptor and caused the contraction of the rabbit GI tissue. Evidence for this assumption is as follows: first, the difference in sensitivity to KW-5139 among the regions of the small intestine was similar to that to porcine motilin (Sato *et al.*, 1990) and second, the pharmacological properties of the contraction induced by KW-5139 (tetrodotoxin-resistance and verapamil-sensitiveness) were consistent with those of porcine motilin

Figure 7 Typical effects of pretreatment with a high concentration of KW-5139 or porcine motilin on the contractile response to KW-5139, porcine motilin, and acetylcholine in the rabbit duodenum and the descending colon. (a, b) The contractile responses to KW-5139 (10 nM, KW, ●) and porcine motilin (10 nM, MT, ○) in the duodenum were significantly decreased after the pretreatment with KW-5139 (10 μ M for 10 min, a) or porcine motilin (10 μ M for 10 min, b). (c, d) The contractile responses to KW-5139 (100 nM, KW, ●) and porcine motilin (100 nM, MT, ○) in the descending colon were also selectively decreased after pretreatment with KW-5139 (10 μ M for 10 min, c) or porcine motilin (10 μ M for 10 min, d). On the other hand, the contractile response to acetylcholine (ACh, 100 μ M, ▲) was not decreased in any conditions.

Table 1 Contractile responses to KW-5139, porcine motilin (MT) and acetylcholine (ACh) before and after treatment with a high concentration of KW-5139 or porcine motilin in the rabbit isolated duodenum and descending colon

		Amplitude of contractile response					
		Before treatment			After treatment		
<i>Duodenum</i>		ACh (100 μ M)	KW-5139 (10 nM)	MT (10 nM)	ACh (100 μ M)	KW-5139 (10 nM)	MT (10 nM)
KW-5139	10 μ M	100	92.0 $\pm$ 10.2	90.7 $\pm$ 10.5	110.4 $\pm$ 9.3	16.4 $\pm$ 4.2*	19.2 $\pm$ 4.7*
MT	10 μ M	100	86.5 $\pm$ 17.7	74.0 $\pm$ 10.6	95.7 $\pm$ 6.7	7.2 $\pm$ 1.9*	10.8 $\pm$ 3.2*
<i>Colon</i>		ACh (100 μ M)	KW-5139 (100 nM)	MT (100 nM)	ACh (100 μ M)	KW-5139 (100 nM)	MT (100 nM)
KW-5139	10 μ M	100	22.5 $\pm$ 5.4	15.0 $\pm$ 3.4	116.8 $\pm$ 7.0	1.1 $\pm$ 1.1*	0.8 $\pm$ 0.6*
MT	10 μ M	100	33.2 $\pm$ 14.6	40.2 $\pm$ 12.9	114.4 $\pm$ 8.5	0.0 $\pm$ 0.0*	1.0 $\pm$ 1.0*

Each preparation was treated with 10 μ M KW-5139 or porcine motilin for 10 min as shown in Figure 7. The amplitude of the contractile response is expressed as a percentage of the contractile response to 100 μ M ACh before the treatment. Values are means $\pm$ s.e. means of 5–6 experiments. * P < 0.05, significantly decreased from the response to the agent before treatment (Sign-Wilcoxon test).

(Adachi *et al.*, 1981; Satoh *et al.*, 1990). Third, cross-desensitization between KW-5139- and porcine motilin-induced contraction occurred, as was the case with the ACh release induced by both peptides (Kitazawa *et al.*, 1993).

The dog has been used as a good experimental species for the study of the action of motilin *in vivo* (Itoh, 1990). It is well known that motilin administered intravenously in dogs causes a series of strong contractions or electrical activity changes corresponding to phase III of the interdigestive migrating motor complex in the gastric antrum and the duodenum without causing simultaneous motility changes in the small intestine, and that the contractions produced migrate through the small intestine towards the terminal ileum (Itoh *et al.*, 1976; Wingate *et al.*, 1976; Matsumoto *et al.*, 1986; Inatomi *et al.*, 1989; Itoh, 1990). The observations in man with motilin are in agreement with the results in dogs (Janssens *et al.*, 1983).

In the present study, KW-5139 produced simultaneous excitatory responses of the gastric antrum, ileum and the descending colon of the anaesthetized rabbit. The dose of KW-5139 required to produce the response in this species (0.3–10 μ g kg⁻¹, i.v.) was slightly higher than that in the dog (Kitazawa *et al.*, 1992). There was considerable difference in the sensitivity of the intestinal regions to KW-5139 between the rabbit and the dog. The order of the excitatory response in the *in vivo* study (descending colon $\geq$ gastric antrum $\gg$ ileum) was similar to that of the amplitude of the maximum contraction in the *in vitro* study (gastric antrum (circular muscle) $>$ descending colon $\gg$ ileum). The higher responsiveness of the gastric antrum than the ileum might be due to the regional difference in the density of motilin receptors, which is high in the stomach and decreases aborally toward the terminal ileum in the rabbit (Bormans *et al.*, 1986).

There have been controversial results concerning the effect of motilin on colonic motility in the dog. Motilin did not evoke an excitatory response of the colon (Itoh *et al.*, 1976; Matsumoto *et al.*, 1986; Itoh, 1990), while motilin produced the colonic motor complex (Bickel & Belz, 1988). In contrast, the present results as well as the previous mechanical studies on the rabbit colon *in vitro* (Satoh *et al.*, 1990; Depoortere *et al.*, 1991; Hasler *et al.*, 1992), have demonstrated that motilin obviously stimulates motor activity of the rabbit colon. Moreover, radioligand binding studies in the rabbit have demonstrated that the density of motilin receptors in the colon was comparable to that in the upper GI tract (Depoortere *et al.*, 1991; Hasler *et al.*, 1992). From these results, motilin is considered to act not only on the upper GI tract but also on the colon of the rabbit, and thus, this species seems to be useful to examine the effect of motilin on colonic motility. The physiological significance of motilin in the rabbit GI function has not yet been clarified. Recently, however, the presence of rabbit motilin, different from porcine motilin in amino-acid positions 8-16-20-22, was demonstrated in the duodenal mucosa (Banfield *et al.*, 1992).

Therefore, it is likely that motilin regulates the motility of both upper and lower GI tract of the rabbit.

Fox *et al.* (1983) and Inatomi *et al.* (1989) found that the contractile responses of the canine stomach to motilin were almost completely blocked by treatment with atropine or tetrodotoxin, and suggested that the action of motilin depends on the activation of cholinergic neurones. In contrast, naloxone was ineffective on the motilin-induced contractile response of the canine stomach (Inatomi *et al.*, 1989). We found that the contractile response of gastric antrum in rabbits induced by KW-5139 was markedly decreased by atropine and naloxone. These findings suggest that a neural pathway involving acetylcholine and opiate peptide is required for the action of KW-5139 in the rabbit gastric antrum. However, it was impossible to evaluate further the pharmacological properties of the KW-5139-induced contraction of the gastric antrum *in vitro*, because the gastric contraction was not reproducible even with application at intervals of 60 min, which is quite different from those of the other regions. This phenomenon might reflect the difference of contractile mechanisms (e.g., receptor or receptor-coupled signal transduction) between gastric and intestinal tissues; however, clarification of the difference was beyond the scope of the present experiments.

In contrast to the case with the gastric antrum, the colonic motor stimulating action of KW-5139 *in vivo* was partly (about 50%) decreased by atropine and not affected by naloxone. In the descending colon, a non-cholinergic mechanism in addition to the cholinergic pathway thus seems to play a role in the excitatory action of KW-5139. One possible mechanism of the non-cholinergic response is the direct action on the smooth muscle cells, as was demonstrated in isolated smooth muscle segments (this study, Adachi *et al.*, 1981; Satoh *et al.*, 1990; Depoortere *et al.*, 1991; Kitazawa *et al.*, 1993) and isolated smooth muscle cells (Hasler *et al.*, 1992). On the other hand, electrical field stimulation of the rabbit colon elicits an atropine-resistant contraction (Koelbel *et al.*, 1989). Moreover, Fox *et al.* (1984, 1991) showed that motilin stimulated the motility of the canine small intestine through non-cholinergic neural mechanisms including the decrease of vasoactive intestinal polypeptide release. Therefore, the possibility that motilin stimulates the non-cholinergic neural pathway in the rabbit colon cannot be excluded completely.

In summary, our present study *in vivo* demonstrates the stimulation of GI motility by motilin in the rabbit. Because of the high sensitivity to motilin, especially of the colon, the rabbit is considered to be a useful species to evaluate the response of the GI tract to motilin.

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