Effect of Motilin on Colonic Motor Activity in the Interdigestive State in Conscious Dogs

CHIKASHI SHIBATA, IWAO SASAKI, HIROO NAITO, MICHINAGA TAKAHASHI, TAKASHI DOI, NORIYA OHTANI, KAORI KOYAMA and SEIKI MATSUNO

The First Department of Surgery, Tohoku University School of Medicine, Sendai 980-77


The aim of the present study was to investigate the effect of motilin at various doses on colonic motility in the interdigestive state. Colonic motility was investigated in five dogs equipped with strain gauge force transducers on the gastric antrum, and on the proximal, middle, and distal colon. Exogenously infused motilin (0.2, 0.5, 1.0 and 2.0 μg/kg-hr) dose-dependently increased colonic motility, but the doses of motilin that significantly enhanced colonic motility were 1.0 and 2.0 μg/kg-hr in all areas of the colon. Motilin at 0.2 μg/kg-hr increased the plasma motilin concentration to almost equal to the physiological peak values. The excitatory effect of motilin (2.0 μg/kg-hr) was abolished by atropine but was not affected by hexamethonium. These results indicate that exogenously infused motilin, which increased plasma motilin concentration to above the physiological peak level, stimulated colonic motility by affecting postsynaptic cholinergic neurons.

It is well known that plasma motilin concentrations in the interdigestive state fluctuate cyclically; their peak coinciding with the end of interdigestive phase III contractions in the stomach (Itoh et al. 1978; Lee et al. 1978). Exogenously infused motilin at a physiological dose level evokes interdigestive phase III contractions in the stomach (Itoh et al. 1975, 1977).

However, relationship between plasma motilin concentrations and occurrence of colonic motor complexes in the interdigestive state remains obscure; it is not known whether or not the physiological increase in the plasma motilin concentration actually is a physiological stimulus inducing colonic motor complexes in the interdigestive state. Considering the fact that the cycle of fluctuation in the plasma motilin concentration (90–120 min) (Itoh et al. 1978) is longer than the intervals of the occurrence of the colonic motor complexes in the fasted state (25–

Received October 18, 1994; revision accepted for publication February 23, 1995.
Address for reprints: Chikashi Shibata, The First Department of Surgery, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-77, Japan.
40 min) (Shibata et al. 1991, 1993), motilin is not considered to be the only factor inducing colonic motor complexes in the interdigestive state. There have been few reports describing the effect of motilin on colonic motility; in an in vitro study, motilin elicited phasic contractions in the whole colon (Strunz et al. 1975). Bickel and Belz (1988) reported that motilin administered intravenously evoked colonic motor complexes in the right half of the colon. Furthermore, the mechanism of the excitatory effect of motilin on colonic motility has not yet been elucidated in conscious dogs.

The objectives of this study were to investigate 1) effect of motilin of various doses on motility of the entire colon; 2) the involvement of the cholinergic pathways in the excitatory effect of motilin on colonic motor activity.

**Materials and Methods**

*Preparation of animals.* Five mongrel dogs weighing 14–18 kg of both sexes were used. The dogs were anesthetized with an intravenous injection of Nembutal (Abbott, North Chicago, IL, USA) at a dose of 25 mg/kg body weight, and laparotomized by a midline incision. Three strain gauge force transducers (F-121S; Star Medical Inc., Tokyo) were sutured onto the serosal surface of the colon, and one more transducer was placed on the gastric antrum in order to measure the contractile activity of the circular muscle layer. The transducer on the gastric antrum was placed 4 cm proximal to the pylorus in order to aid in distinguishing between the interdigestive and digestive states. The transducers on the proximal colon were fixed 5 cm distal to the ileocecal sphincter, and transducers on the distal colon 8 cm proximal to the anal verge. Transducers on the middle colon were sutured at the mid-point between the other two transducers on the colon; the distance between neighboring transducers was 12–16 cm. Lead wires from the transducers were pulled out from a stab wound made between the scapulae through a subcutaneous tunnel and connected to a small connector. A silicone tube (01-102; Create Medic Co., Ltd., Yokohama) was positioned in the superior vena cava from the right external jugular vein in order to infuse the test materials. The lead wires, small connector and tube were covered with a jacket to protect them against the dog's scratching. The dogs were housed in individual cages and fed solid meal (ED-1; Oriental Yeast Co., Tokyo) at the ratio of 20 g/kg body weight once a day. Water was given freely. Ten days were allowed as a recovery period after surgery.

*Recording of gastrointestinal motility.* Gastrointestinal motor activity was monitored continuously by connecting the small connector to an amplifier (MS-088; Star Medical, Inc.), and was recorded on a computer (Eight Star; Star Medical Inc.) and a multi-channel recorder (MS-0863; Graphtec Co., Tokyo) simultaneously.

*Experimental protocol.* All experiments were performed during the interdigestive state. The interdigestive state was defined as the time between the first
Motilin and Colonic Motility

Motilin and Colonic Motility

appearance of the quiescent period (phase I in the interdigestive migrating contractions) and feeding in the gastric antrum.

In the interdigestive state, the periodic occurrence of bursts of colonic contractions was observed. These colonic contractions consisted of tonic contractions imposed upon phasic contractions were defined as colonic motor complexes, as reported previously (Sarna et al. 1984).

Synthetic canine motilin (0.2, 0.5, 1.0, 2.0 µg/kg-hr) (Sigma Chemical Company, St. Louis, MO, USA) was infused through the silicone tube in the superior vena cava for exactly 30 min by means of an infusion pump (PSK-51; Nikkiso Co., Tokyo) in the interdigestive state. Infusion was begun 20-40 min after the end of interdigestive phase III contractions in the gastric antrum. Motor indexes during motilin-infusion were totaled and compared to the motor indexes obtained by the infusion of the same volume of normal saline. Blood samples were drawn from a peripheral vein in an anterior limb every 10 min during motilin infusion. On the other day, in order to investigate the physiological peak value of plasma motilin concentration, blood samples were taken from the silicone tube every 10 min until interdigestive phase III contractions appear. Infusion of motilin was repeated three times per dose per dog, and blood samples were taken one time per dose per dog in five dogs. Atropine sulfate (0.1 mg/kg) or hexamethonium (5 mg/kg) was administered intravenously 3 min prior to the start of the infusion of the 2.0 µg/kg-hr dose of motilin. Colonic motor indexes during motilin- and saline-infusion were totaled and compared.

Measurement of immunoreactive motilin. Blood samples were collected in ice-chilled glass tubes containing ethylenediaminetetraacetic acid and aprotinin (NT-EA0205; Nipro Co., Osaka), and the plasma was separated by centrifuging for 10 min. Blood samples were stored at -30°C until assayed. Immunoreactive motilin was measured with a commercial motilin (canine) RIA kit (RIK 7160; Peninsula Co., Belmont, CA, USA). The antibody in this kit reacts to both canine and porcine motilin 100%, and does not crossreact with secretin, gastrin, or substance P. The intraassay variability coefficient was 5%. All sera were measured in a duplicate assay.

Data analysis. Motor indexes were calculated by summing the areas between the base line and the contraction waves at all colonic transducer sites, with a commercial computer program (Eight Star; Star Medical Inc.). All motor indexes were expressed as percentages with 100 as the control values. All the values are expressed as the mean ± s.e. One way analysis of variance (ANOVA) and paired t-test were used in investigating dose-dependency and the effect of antagonists, respectively. P values less than 0.05 were regarded as significant.

Drugs and peptides. Canine motilin was purchased from Sigma Chemical Co., St. Louis, MO, USA. It was dissolved in distilled water and diluted to the required concentrations in a saline containing 0.2% albumin. Atropine sulfate and hexamethonium bromide were kindly supplied by Tanabe Seiyaku Co., Ltd.,
Osaka, and Yamanouchi Seiyaku Co., Ltd., Osaka, respectively.

RESULTS

Motilin increased motor indexes in all areas of the colon in a dose-dependent manner. However, the doses of motilin that significantly increased colonic motility in all areas of the colon compared to the control were 1.0 and 2.0 μg/kg-hr (Fig. 1). Motilin at 0.2 μg/kg-hr showed no effect on colonic motility anywhere in the colon in most of cases (Fig. 2A). Motilin at 2.0 μg/kg-hr always induced colonic motor complexes at all transducer sites (Fig. 2B). The peak in the level of the plasma motilin concentrations was obtained at the end of the 30 min-infusion of exogenous motilin. Administration of 0.2 μg/kg-hr of motilin elicited an increase in the serum concentration of motilin almost equivalent to the natural physiological peak value (Table 1). Other doses of motilin evoked a plasma motilin concentration to above the physiological peak level (Table 1). The numbers of defecations were 0, 1, 2, 1 and 2 during motilin-infusion at doses of 0, 0.2, 0.5, 1.0 and 2.0 μg/kg-hr, respectively. Pretreatment with atropine abolished the occurrence of colonic motor complexes during motilin-infusion (2.0 μg/kg-hr) and significantly depressed the colonic motor index compared to the control in all areas of the colon (Fig. 3 and 4A). Hexamethonium did not significantly affect colonic motor activity during motilin-infusion at the dose of 2.0 μg/kg-hr; p values were 0.056, 0.417, and 0.650, in the proximal, middle, and distal colon, respectively (Fig. 3 and 4B).

![Fig. 1. Effect of motilin on interdigestive colonic contractile activity. Motilin increased the colonic motor indexes in a dose-dependent manner. However, effect of 0.2 and 0.5 μg/kg-hr of motilin in increasing colonic motility was not significant along the entire colon. 1.0 and 2.0 μg/kg-hr of motilin had a significant effect on motility and this was demonstrated throughout the colon. *signifies values significantly different from the control. □, proximal colon; ■, middle colon; ▲, distal colon.](image-url)
Motilin and Colonic Motility

Fig. 2. Effect of 0.2 μg/kg-hr (A) and 2.0 μg/kg-hr (B) of motilin on colonic motor activity in dog No. 1. 0.2 μg/kg-hr of motilin did not affect colonic motility at any of the transducer sites in most of cases. The motilin dosages of 2.0 μg/kg-hr always induced colonic motor complexes along the entire colon.

Table 1. Comparison of the normal physiological peak plasma motilin concentrations with those induced by exogenous motilin infusion

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Physiological peak values</th>
<th>Doses of exogenous motilin (μg/kg-hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>1</td>
<td>696</td>
<td>681</td>
</tr>
<tr>
<td>2</td>
<td>788</td>
<td>747</td>
</tr>
<tr>
<td>3</td>
<td>713</td>
<td>649</td>
</tr>
<tr>
<td>4</td>
<td>673</td>
<td>556</td>
</tr>
<tr>
<td>5</td>
<td>487</td>
<td>534</td>
</tr>
<tr>
<td>Mean ± s.e.</td>
<td>671 ± 50</td>
<td>633 ± 40</td>
</tr>
</tbody>
</table>

Values are the plasma motilin concentrations measured with specific radioimmunoassay (pg/ml).
Fig. 3. Effect of atropine and hexamethonium on colonic motor indexes during motilin-infusion (2.0 μg/kg-hr). Atropine significantly inhibited the colonic motor index compared to the control in all areas of the colon. Hexamethonium had no significant effect on the colonic motor index during motilin infusion in any of the areas of the colon. Control values signify the colonic motor index induced by the 2.0 μg/kg-hr dose of motilin alone. *denotes significantly different values from those of the control. □, control; ■, atropine; ■■, hexamethonium.

Fig. 4. Effect of atropine (A) and hexamethonium (B) on motilin-induced colonic motor complexes in dog No. 5. Atropine (0.1 mg/kg) abolished the occurrence of the colonic motor complexes induced by 2.0 μg/kg-hr of motilin in all areas of the colon. The occurrence of motor complexes during motilin infusion was not inhibited by pretreatment with hexamethonium (5 mg/kg) along entire colon.
DISCUSSION

The present study showed that exogenously infused motilin, which increased the plasma motilin concentration to above the physiological peak value, stimulated motility of entire colon by affecting postsynaptic cholinergic neurons. Because 0.2 \( \mu \text{g/kg-hr} \) of motilin, which increased the plasma motilin concentration to almost equal to the physiological peak values, had no effect on motility anywhere of the colon, motilin was not considered to be a physiological stimulus for colonic motility in the interdigestive state.

In a previous study, we have shown that intervals between colonic motor complexes in the fasted state in dogs with either innervated or extrinsically denervated middle colonic loops, were not significantly different from dogs with intact colons (Shibata et al. 1991). Based on such findings, we have speculated about the possible role of the intrinsic nerves and/or humoral factors as a regulator of the occurrence of the colonic motor complexes in the interdigestive state. Motilin was thought as a possible physiological hormonal factor to induce colonic motor complexes in the interdigestive state, since this peptide plays an important role in the control of gastric motility in the interdigestive state (Itoh et al. 1978). However, in the present study, we were not able to pinpoint the role of motilin in the physiological regulation of colonic motility in the interdigestive state. Therefore the role played by the intrinsic nerves should also be investigated.

Intravenous injection of 3 \( \mu \text{g/kg} \) of motilin was reported to induce colonic motor complexes (Bickel and Belz 1988). This is not in conflict with our present results, because intravenous volus injection of 3 \( \mu \text{g/kg} \) of motilin must elicit a plasma motilin concentration to above the physiological peak value. Their study was referred only to the right half of the colon. We were able to show that there was no regional differences in the effect of motilin on colonic motility.

Atropine significantly depressed the increase in the colonic motor index induced by motilin at the 2.0 \( \mu \text{g/kg-hr} \) dose level. Atropine is known to inhibit basal colonic motility (Basilisco and Phillips 1994). In the canine stomach, the effect of motilin inducing interdigestive phase III contractions is inhibited by atropine or hexamethonium (Itoh 1990). Although hexamethonium tended to inhibit motilin-induced motor response in the proximal colon, the effect was not significant (\( p=0.056 \)). It is noteworthy that motilin may induce motor complexes in the colon by predominantly affecting the postsynaptic excitatory cholinergic neurons.

References


