Interdigestive migrating contractions are coregulated by ghrelin and motilin in conscious dogs

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Running title: Ghrelin and motilin coregulate IMCs

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Abstract

During fasting, gastrointestinal motility is characterized by cyclical motor contractions. These contractions have been referred to as interdigestive migrating contractions (IMCs). In dogs and humans, IMCs are known to be regulated by motilin. However, in rats and mice, IMCs are regulated by ghrelin. It is not clear how these peptides influence each other in vivo. The aim of the present study was to investigate the relationship between ghrelin and motilin in conscious dogs.

Twenty healthy beagles were used in this study. Force transducers were implanted in the stomach, duodenum, and jejunum to monitor gastrointestinal motility. Subsequent gastrointestinal motility was recorded and quantified by calculating the motility index. In examination 1, blood samples were collected in the interdigestive state, and levels of plasma ghrelin and motilin were measured. Plasma motilin peaks were observed during every gastric phase III, and plasma ghrelin peaks occurred in nearly every early phase I. Plasma motilin and ghrelin levels increased and decreased cyclically with the interdigestive states. In examination 2, saline or canine ghrelin was administered intravenously during phase II and phase III. After injection of ghrelin, plasma motilin levels were measured. Ghrelin injection during phases II and III inhibited phase III contractions and decreased plasma motilin levels. In examination 3, ghrelin was infused in the presence of the GHS-R antagonist [D-Lys3]-GHRP-6.
Continuous ghrelin infusion suppressed motilin release, an effect abrogated by the infusion of [D-Lys3]-GHRP-6. Examination 4 was performed to evaluate the plasma ghrelin response to motilin administration. Motilin infusion immediately decreased ghrelin levels.

In this study, we demonstrated that motilin and ghrelin cooperatively control the function of gastric IMCs in conscious dogs. Our findings suggest that ghrelin regulates the function and release of motilin and that motilin may also regulate ghrelin.

**Keywords:**

Ghrelin; Motilin; Interdigestive migrating contractions
Introduction

Ghrelin was first identified in rat stomach as an endogenous ligand for growth hormone secretagogue receptors (GHS-R).(17) Ghrelin molecules exist in the stomach and hypothalamus in 2 major endogenous forms: one form acylated at serine 3 (ghrelin) and in a desacylated form (des-acyl ghrelin).(13) Ghrelin stimulates GH secretion by binding to type 1a GHS-R, and acylation is essential for this binding. Des-acyl ghrelin is unable to initiate GH release. Hence, acylated ghrelin has been known as the active form and des-acyl ghrelin as the inactive form of the peptide.(7, 13, 18) Ghrelin is involved in various functions, including GH release, control of food intake and energy balance,(2, 40) glucose metabolism and insulin release,(6, 31) and cardiovascular actions.(4, 23)

Ghrelin has also been reported to stimulate gastrointestinal (GI) motility in rats and humans.(12, 20, 36) We have previously shown that ghrelin stimulates GH release in a dose-dependent manner. However, it does not stimulate digestive tract motor activity in either the fasted or the fed state in dogs.(25) Moreover, an intravenous (i.v.) injection of high-dose canine ghrelin significantly reduces the motility index (MI) in the gastric body (GB) in the fasted state.(25) In the interdigestive fasting period, the stomach and small intestine, including the duodenum, show cyclically remarkable motor contractions. These contractions have been called interdigestive migrating
contractions (IMCs).(14) IMC consists of 3 phases in the dog. Each phase is visually determined according to the following criteria: phase I is defined at the quiescent period; phase II consists of clusters of irregular contractions that follow phase I and precede phase III; and phase III is characterized by strong contractions lasting more than 15 min. IMCs are observed every 90 to 120 min in dogs and humans.(15, 42) The physiological importance of gastric IMCs pertains to the mechanical and chemical cleansing of the empty stomach in preparation for the next meal.(38) Stores of motilin in the duodenum play an important role in initiating gastric IMCs. IMCs are widely known to be regulated by motilin in dogs and humans. Intravenous infusion of motilin induces premature gastric phase III in dogs and humans.(16, 39) The plasma levels of motilin cyclically increase every 90 to 120 min in dogs and humans. In contrast, rodent IMC cycles are approximately 20 min.(12) Ghrelin regulates spontaneous phase III-like contractions in rats, and its administration induces phase III-like contractions in the rat stomach.(1) However, motilin administration fails to affect gastric emptying and GI transit in rats. Moreover, neither motilin nor motilin receptors have been found in rats.(9) Thus, it seems that the function of ghrelin and motilin in mediating interdigestive gastric contractions differs to some extent among humans, dogs, and rodents.

Ghrelin structurally resembles motilin, and they share an almost 50% similarity in
their amino acid sequences. The receptors of both peptides belong to the same family of G protein-coupled receptors and share 53% overall amino acid sequence identity.

On the basis of this structural similarity, these peptides are considered to be members of a new motilin-ghrelin peptide family. A recent study showed that peaks in plasma ghrelin levels were frequently observed 20 to 25 min after peaks in plasma motilin levels in dogs. However, how these 2 peptides influence each other in vivo has not been clarified. The aim of our study was to investigate the relationship between ghrelin and motilin in conscious dogs.

Materials and methods

Animal preparation

Twenty healthy beagle dogs of both sexes weighing 10 to 12 kg were used. The procedures were approved by the Review Committee on Animal Use of Gunma University, Maebashi, Japan. The dogs were anesthetized with a single i.v. injection of thiopental sodium (Ravonal; Tanabe Pharmaceutical Co. Ltd., Osaka, Japan) at a dose of 20 mg/kg/body weight, and general anesthesia was maintained by intratracheal inhalation of halothane (Fluothane; Takeda Chemical Industries Ltd., Osaka, Japan) and oxygen.

A silastic tube (Silastic 602-205; Dow Corning, Midland, MI, USA) was inserted
into the superior vena cava through a branch of the right external jugular vein (jugular cannula) and used for withdrawal of blood samples and administration of injections.

The jugular cannula was exteriorized through a skin incision on the neck and its outer end was fixed to the adjacent skin with silk sutures. After the abdominal cavity was opened, force transducers were implanted onto the serosal surfaces of the gastric body (GB), gastric antrum (GA), mid-duodenum (D), and jejunum (J) (20 and 40 cm distal to Treitz’s fascia). The lead wires of the force transducers were tunneled through the subcutaneous tissue and exteriorized through a skin incision made between the scapulae. After closure of the abdominal cavity, a jacket-type protector was placed on each dog to prevent the lead wires and tubes from being damaged by the dog scratching itself.

Dogs were housed in individual experimental cages, maintained on i.v. drip infusions of Lactec G (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) for 3 postoperative days, and gradually returned to a normal chow diet (15 g/kg body weight per day; Funabashi Farm Inc., Funabashi, Japan).

Monitoring of GI contractions

The wires from the transducer were connected to a telemeter, and the data were transmitted to the recording system (Eight Star system, Star Medical, Tokyo, Japan). The recorded signals were used to determine the MI. The MI was the integrated area
between the baseline (zero level) and contractile wave expressed as motor units measured by the Eight Star system. In addition, the data were used to identify each phase of the contractile activity.

*Measurement of active ghrelin and plasma motilin*

Whole blood samples were transferred into chilled tubes containing ethylenediaminetetraacetic acid-2Na and 500 U apoprotin, and centrifuged at 4°C at 3000 × g. Two plasma samples were immediately collected; one was used for ghrelin measurement, and the other was used for motilin measurement. For measuring the acylated ghrelin by ELISA, 0.1 mL of 1-N hydrochloric acid per mL of plasma sample was added to the sample. All plasma samples were stored at -80°C until hormone analyses were performed.

The plasma acylated ghrelin concentration was measured using an ELISA kit (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan). This kit employs a polyclonal antibody against N-terminal fragments containing n-octanoylated serine at position 3. These assay kits were designed for rats, mice, and humans. However, according to a recent study, canine ghrelin was able to be measured accurately with this kit.(41)

The plasma concentration of motilin was measured by radioimmunoassay. Each tube containing 100 μL of a standard or unknown sample, 100 μL of rabbit anticanine
motilin serum (RC91T), 200 μL of assay buffer for the unknown sample, or 100 μL of assay buffer and 100 μL of hormone-free serum was prepared by charcoal extraction. The RC91T antiserum was diluted to a final concentration of 1:150 000. After incubation of the tubes for 24 h at 4°C, 10 μL of $^{125}$I-(try$^{23}$) canine motilin (10 000 counts/min), which was prepared as previously described previously,(21, 32) was added to the tubes, followed by further incubation for 24 h at 4°C. The second antiserum (200 μL) was added to the tubes and incubated at room temperature for 2 h. Bound and free $^{125}$I-motilin were separated by centrifugation at $3000 \times g$ for 15 min. The supernatant was discarded, and the precipitate was counted in a Packard COBLA gamma counter (Downers Grove, IL).

*Plasma levels of ghrelin and motilin during the interdigestive state (Examination 1)*

Nine dogs were used in Examination 1. Experiments were initiated after confirming that gastric phase III contractions were occurring regularly; it typically took approximately 2 weeks after the procedure for this activity to be restored. After the first or second phase III contractions of the day were confirmed to have occurred, the IMC cycle was recorded at least 2 times in each dog, and blood samples were collected at 15 min intervals. Results of ghrelin and motilin concentrations were averaged and assigned to each phase and compared. Phase I was divided at its midpoint into early
phase I and late phase I (Fig. 1). In addition, ghrelin and motilin levels were compared at 30 min preceding and 30 min following the end of phase III on GA, and quantitative analysis was performed, as previously described. (28)

Effect of ghrelin on motilin release (Examination 2)

Six dogs were used in Examination 2. Saline or canine ghrelin (10 μg/kg) was administered i.v. as a slow, single-bolus injection during phase II or III contractions. The subsequent GI motility was recorded for at least 2 h. After randomly receiving ghrelin or saline, each animal was examined on 3 different days. The MI was calculated for the 30 min period after injection. Blood samples were withdrawn from the jugular catheter at –15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min after the injection, and the ghrelin and motilin plasma concentrations were measured.

Effect of ghrelin on IMC (Examination 3)

Four dogs were used in Examination 3 to observe that ghrelin inhibited phase III. Canine ghrelin was continuously infused for two hours during phase I (1μg/kg/hr) with data being recorded in the next phase III. Next, [D-Lys3]-GHRP-6 (Sigma-Aldrich Japan Co. Tokyo), an antagonist of GHS-R, was administrated before the continuous infusion of ghrelin. A dose of 70 nmol/kg of [D-Lys3]-GHRP-6 was administered
three times at 1hr intervals. The method and dose of [D-Lys3]-GHRP-6 injection was
determined according to recent reports.(10-12, 37) The same experiments were
performed at least twice on different days in each dog. Blood samples were withdrawn
from the jugular catheter at –20, 0, 20, 40, 60, 80, 100, 120, 140, 160 and 180 min after
the injection, and the ghrelin and motilin plasma concentrations were measured.

**Effect of motilin on ghrelin release (Examination 4)**

Five dogs were use in Examination 4. To clarify whether motilin regulates
ghrelin release in dogs, the reaction of the plasma ghrelin level was evaluated when
motilin was administered during phase I. Blood samples were withdrawn from the
jugular catheter at –15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min after the injection of
motilin, and ghrelin and motilin plasma concentrations were measured.

**Analysis of the MI**

GI motility was quantified by calculating the MI that was equivalent to the area under
the curve. The MI was calculated using a computer-assisted system (Eight Star system,
Version 6.0, Star Medical, Tokyo, Japan). The levels of fasted GI motility were
analyzed for 30 min following injection of either saline or canine ghrelin. MI analysis
was conducted blind to the saline and ghrelin doses. The MI of canine ghrelin was
compared with that of the saline level. The mean of three studies for each dog was calculated, and all data were expressed as means ± SE. The data were subjected to detailed statistical using the ANOVA followed by Fisher’s protected least significant difference method. Differences at $P$-values of <0.05 were considered to be significant.

The statistical calculations were carried out using JMP 5.0 software (SAS Institute, Japan).

**Materials**

Canine ghrelin [GSS (n-octanoyl) FLSPEHQKLQQRKESKKPPAKLQPR] was synthesized at Shimadzu Co. (Kyoto, Japan). Canine motilin was purchased from ScyTek Laboratories (Logan, UT).

**Statistical analysis**

All results were expressed as mean values (SEM) for each time point and for each treatment. The data were subjected to further statistical analysis (ANOVA). Time dependent changes in plasma hormone levels were compared by two way ANOVA analysis with repeated measurements on two factors and univariate testing of significance for planned comparisons. The paired data were compared using Student’s t-test. $P$-values of <0.05 were considered statistically significant. The statistical calculations were carried out using JMP 5.0 software (SAS Institute, Japan.).
Results

Plasma concentration of ghrelin in the fasted and fed states (Examination 1)

In the fasted state, IMCs were observed cyclically every 100 to 120 min. The peaks of plasma motilin levels were observed at every gastric phase III (18/18). (Fig.2) Basal motilin levels were 1103 ± 55.4, 1288 ± 40.16, 1615 ± 70.15, and 1154 ± 55.64 pg/mL for phases late I, II, III, and early I, respectively. In contrast, peaks in plasma ghrelin were observed nearly universally in gastric early phase I (17/18). Only dog No.7 showed ghrelin peaks in phase III, simultaneously with the motilin peak (Fig.2g). Ghrelin peaks were observed within 30 min in most cases (16/18) (except No.6 and No.7). An exception was dog No.6 in which the ghrelin peak did not follow the motilin peak. Basal ghrelin levels were 114.8 ± 24.92, 101.76 ± 19.48, 137.96 ± 29.53, and 188.13 ± 31.35 pg/mL for phases late I, II, III, and early I, respectively. In addition, ghrelin levels of early phase I were significantly greater than those in phase II (P = 0.0326). Motilin and ghrelin levels changed cyclically among gastric IMCs in conscious dogs (Fig.3). Motilin levels were compared between pre-phase III and post-phase III. Motilin levels of post-phase III were significantly decreased in comparison to pre-phase III (Fig.4a). In contrast, ghrelin levels of post-phase III were significantly higher than those in pre-phase III (Fig.4b).
Influence of ghrelin administration on motilin levels and IMC (Examination 2)

When saline injections were administered during gastric phase III contractions in the interdigestive state in conscious dogs, the phase III contractions were unchanged. When canine ghrelin (10 μg/kg) was injected in phase II of IMCs, phase III was inhibited. Administration of canine ghrelin significantly decreased the MI of the GA (data not shown). When canine ghrelin (10 μg/kg) was injected in phase III of the IMCs, phase III was inhibited, and the MIs of the GB and GA were significantly decreased (Figs.5 and 6). Generally, the plasma concentration of motilin gradually increased during phase II, leading to a maximum level in phase III (Fig.7a). However, administration of canine ghrelin during phase II and III contractions immediately decreased the plasma concentration of motilin (Fig.7b).

Effect of ghrelin on IMC (Examination 3)

Examination 3 was performed to prove that ghrelin inhibited phase III. During infusion of canine ghrelin (1μg/kg/hr for 2 hours), phase III was not observed in any dog (Fig.8a). In contrast, when [D-Lys3]-GHRP-6 (total dose of 70 nmol/kg) was preloaded, conventional phase III was observed in the presence of a canine ghrelin infusion (Fig.8b). In both experiments, ghrelin levels were increased by the infusion
of canine ghrelin. In the setting of ghrelin infusion, motilin levels were suppressed by 
the high concentration of ghrelin. In the presence of an antagonist, however, motilin 
levels increased regardless of elevated ghrelin levels and permitted the initiation in 
phase III. In other words, the rise of motilin levels was inhibited by ghrelin and, as a 
result, phase III was inhibited.

Influence of motilin administration on ghrelin release (Examination 4)

Canine motilin infusion (0.6 μg/kg/h for 20 min) immediately stimulated phase III 
contractions of the stomach and duodenum. The plasma concentration of motilin 
increased, and upon finishing the infusion, gradually decreased. The motilin infusion, 
in contrast, immediately decreased the plasma concentration of ghrelin, the level of 
which gradually increased again from the middle stage of the infusion (Fig.9).

Discussion

In this study, we demonstrated that ghrelin and motilin influenced each other in vivo 
in conscious dogs. This is the first study demonstrating that ghrelin administration 
decreases the plasma concentration of motilin and inhibits phase III of IMCs. A recent 
study has shown that peak plasma ghrelin levels are frequently observed 20 to 25 min 
after peak plasma motilin levels in the interdigestive state in conscious dogs. Ghrelin
peaks are observed in early phase I (43). In this prior study, however, ghrelin peaks followed motilin peaks only 72.2% of the time (13/18). In the present study ghrelin peaks followed motilin peaks in 83.3% (16/18) of cases. The shorter time intervals for blood sampling may explain this discrepancy. In a recent study, blood was collected at 20-25 min intervals. In the current study, blood samples were collected at 15 min intervals. The peaks of ghrelin may only persist for a short length of time and be missed by longer intervals of blood sampling.

The results of the present study indicate that ghrelin regulates the function and release of motilin, and that motilin might be the regulator of ghrelin. The negative results of canine ghrelin in our previous study suggest that ghrelin does not stimulate fasted GI motility in dogs. In the previous study, however, ghrelin was administered in phase I of IMCs. An i.v. injection of canine ghrelin (10 μL/kg) has been shown to significantly reduce the MI in the GB during phase I contractions.(25) This result may suggest that ghrelin competitively inhibits motilin because ghrelin has a structural resemblance to motilin and the ghrelin receptor exhibits a 50% identity with the motilin receptor.(3) As indicated by Peeters, these results suggest that both peptides may cross-react with their receptors.(26) In the present study, ghrelin decreased plasma motilin levels and inhibited phase III. As shown in Figure 8a and 8b, increase of motilin levels was not observed during ghrelin infusion, and IMCs were suppressed.
After two hours, the release of motilin began again. Conversely, when the ghrelin antagonist was preloaded, motilin release was not inhibited and conventional phase III was observed. These results suggest that ghrelin inhibits the release of motilin.

The present study shows that ghrelin inhibits gastric, but not duodenal, phase III contractions. Bormans et al. reported that motilin is only involved in the regulation of the MMCs originating from the stomach in humans. (5) Peeters et al. showed that somatostatin depresses motilin and pancreatic polypeptide, but facilitates the progression of the activity front towards the small intestine. (27) These studies suggest separate control mechanisms for gastric and duodenal IMCs in humans. Although plasma motilin levels are associated with the appearance of gastric phase III in dogs, (16) phase III in the small intestine sometimes occurs without a concomitant increase in plasma motilin concentration. (30) As shown in our recent study, after duodenectomy, an obvious phase III is no longer seen in the GA; however, a migrating phase III is seen in the upper J. (35) These results suggest that motilin regulates gastric, but not intestinal IMCs in dogs. The present study suggests that ghrelin regulates motilin release and gastric IMCs. Ghrelin does not inhibit duodenal phase III contractions, and for this reason, gastric and intestinal IMCs are thought to be controlled by different mechanisms.

Tack et al. showed that in humans, ghrelin infusion induces a premature gastric phase
III of the IMC, which is not mediated by motilin release. This conflicts with what has been observed in dogs. Unlike phase III of dogs, humans phase III by ghrelin infusion is not accompanied by an increase in the levels of motilin. In other words, IMCs, which are induced by ghrelin infusion in humans, are not attributable to motilin. One reason for this may be that the reactivity of ghrelin may be different in humans and dogs. For example, ghrelin acts to stimulate the motilin receptor in humans. Conversely, ghrelin acts to inhibit the release of motilin in dogs.

In humans, preprandial ghrelin peaks are observed, while conversely motilin peaks are observed late phase II and phase III of IMC. Because IMCs are observed in the interdigestive states, preprandial motilin peaks may also occur. Furthermore, motilin peaks might be observed before the peaks of ghrelin. In humans, whether a similar cooperative effect by ghrelin and motilin exists is unclear; however it is plausible that the two peptides are associated.

Our previous study has shown that exogenous motilin stimulates endogenous motilin release in dogs. The effect is mediated through muscarinic receptors on motilin-producing cells via preganglionic pathways involving 5-hydroxytryptamine 3 receptors. If exogenous motilin stimulates endogenous release of motilin, a positive feedback mechanism is likely to operate when the plasma motilin concentration increases in the interdigestive state. In another study, Nakajima et al. have shown
that the IMC cycle is mediated by a positive feedback mechanism via the interaction between motilin and 5-HT. (24) Because of these positive feedback systems, the plasma concentration of motilin increases gradually during phases II and III. Accordingly, an inhibitory mechanism is necessary to break the positive feedback system; otherwise, endogenous release of motilin will continue. However, how gastric phase III of IMCs is terminated has remained unclear since it was first reported that motilin regulates gastric phase III in 1976. (15) Our current study showed that a ghrelin injection decreased plasma motilin levels, and a peak in the plasma concentration of ghrelin was replaced by a peak in the plasma concentration of motilin. These results indicate that the increased plasma concentration of ghrelin terminates phase III of IMCs. In other words, ghrelin may regulate the release and function of motilin. Some reports have indicated that GI hormones regulate each other. In our previous study, we confirmed a concomitant increase in the plasma concentrations of motilin and pancreatic polypeptide (PP), and found that exogenous motilin at doses lower than the physiological level (0.01 μg/kg) significantly stimulates endogenous release of PP. (21) Our other previous studies showed that significant insulin release is observed in close association with the endogenous release of motilin, and physiological doses of motilin stimulate a dose-dependent release of insulin in the postprandial state in conscious dogs. (33-34)
In conclusion, the results of the present study suggest that gastric phase III and motilin release are terminated by increases in plasma ghrelin. We revealed the presence of cooperative regulation of motilin and ghrelin in the function of gastric IMCs in conscious dogs.

**Perspectives and Significance**

Understanding the mechanisms of IMCs may provide important insights in developing treatments for a variety of functional gastrointestinal diseases. The present study suggests that IMCs are regulated by interactions between ghrelin and motilin.

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**Disclosures**

The authors have no conflicts of interest to disclose.

**References**


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Figure legends

(Fig.1)

The IMC is composed of three phases. Fig.1 shows each phase. Phase I is divided into early and late sub-phases at the midpoint.

(Fig.2)

Data of nine dogs for the individual association between ghrelin(●) and motilin (○) peaks in the IMCs. Motilin peaks(⁎) uniformly occurred in phase III (18/18). Eighteen ghrelin peaks(#) were observed, 16 of which were in early phase I (16/18). With the exceptions of No.6 and No.7, all ghrelin peaks were observed subsequent to peaks of motilin in early phase I.

(Fig.3)

Correlation between plasma motilin (○) and ghrelin (●) concentrations from late phase I to the next late phase I in the interdigestive state in conscious dogs. Ghrelin and motilin concentrations were averaged and assigned to each phase. The motilin peak occurred during gastric phase III contractions, and ghrelin peaks occurred during early phase I. Values in all figures are means ± SE of mean values obtained in 9 dogs.
Motilin (a) and ghrelin (b) concentrations were compared 30 minutes before and 30 minutes after the end of phase III on GA. Motilin levels were significantly decreased post-30 (post-phase III) in comparison to pre-30 (pre-phase III) \( (P<0.0001) \). Ghrelin levels were significantly greater post-30 than pre-30 \( (P = 0.0001) \).

Simultaneous recordings of IMCs. IMCs were observed cyclically every 100 to 120 min. Phase III contractions were not altered after saline injection (data not shown).

Effect of ghrelin \( (10 \, \mu g/kg) \) (solid arrow) on gastric phase III contractions in the interdigestive state in conscious dogs (Fig.5). Phase III contractions were inhibited after ghrelin injection.

Comparison of MI between the ghrelin injection and control groups. Ghrelin injection group (open bars) and control group (filled bars). In the ghrelin injection group, the MI of the GA was significantly decreased during phase II contractions \( (P < 0.05, N = 6) \) (data not shown), and the MIs of the GB and GA were significantly decreased during phase III contractions \( (P < 0.05, N = 6) \). However, MIs of D and J did not change...
significantly.

(Figs.7a and 7b)

The association between plasma ghrelin and motilin levels and GI contractile activity.

The effect of saline injection on phase III contractions (Fig.7a). Identical to the results shown in Fig.3, peak plasma motilin levels (○) were observed during phase III contractions, and peak plasma ghrelin levels (●) were observed 15 min after peak motilin levels (221.8 ± 33.42 pg/mL, N = 6). The effect of ghrelin injection on phase III contractions (Fig.7b). Plasma ghrelin levels (●) were remarkably increased by the administration of ghrelin (2216 ± 56.92 pg/mL, N = 6), and plasma motilin levels (○) were decreased by ghrelin injection in conscious dogs. Values in all figures are means ± SE of mean values obtained from three observations in each of 6 dogs.

(Figs.8a and 8b)

Examination 3 was performed in two phases. For the first, canine ghrelin was continuously infused for 2 hours at phase I (solid arrow) (Fig.8a). In the second phase, [D-Lys3]-GHRP-6, an antagonist of GHS-R was added. A dose of 70 nmol/kg of [D-Lys3]-GHRP-6 was injected three times at 1 hr intervals (open arrow), before and during the continuous infusion of ghrelin (Fig.8b). In the first step, motilin release was
inhibited by ghrelin infusion, and IMCs were not observed (Fig. 8a). In the second step, the effects of ghrelin were inhibited by [D-Lys3]-GHRP-6, motilin was released as in the control, and phase III was observed (Fig. 8b).

(Fig. 9)

Plasma concentration of ghrelin and motilin in response to motilin infusion (0.6 μg/kg/h for 20 min) (solid arrow) in phase I contractions. Motilin infusion caused phase II and III contractions in the GB and GA. Plasma motilin levels (○) were increased by motilin infusion, and ghrelin levels (●) were decreased by motilin infusion in conscious dogs. Values in all figures are means ± SE of mean values obtained from 3 observations in each of 5 dogs.
Figure 1

IMC
(Interdigestive migrating contractions)
Figure 2

(a) Dog No.1

(b) Dog No.2

(c) Dog No.3

(d) Dog No.4

(e) Dog No.5

(f) Dog No.6

(g) Dog No.7

(h) Dog No.8

(i) Dog No.9
Phase III
Ghrelin 10 μg/kg iv

Gastric Body
Antrum
Duodenum
Jejunum 1
Jejunum 2

30min
Figure 6

Motility index (Phase III)

n=6

Control

Ghrelin

* p<0.05
Figure 7b

Ghrelin 10 µg/kg iv

Gastric Body

Antrum

Motilin – ○ ○ ○
Ghrelin – – –
Figure 8a

Ghrelin 1μg/kg/hr div

Ghrelin 1μg/kg/hr div

Gastric Body

Antrum

Duodenum

Jejunum

Motilin

Ghrelin
Figure 8b  
[D-Lys3]-GHRP-6 + Ghrelin 1μg/kg/hr div

[D-Lys3]-GHRP-6 (Total 70 nmol/kg)

Ghrelin 1μg/kg/hr div

Gastric Body
Antrum
Duodenum
Jejunum

Motilin
Ghrelin

(pg/ml)
(pg/ml)
(min)