Role of vagus nerve in postprandial antro-pyloric coordination in conscious dogs

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Short title: Vagus and antro-pyloric coordination

Grant support: This study was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases [RO1 DK55808 (Toku Takahashi)].

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Abstract

It is generally believed that gastric emptying of solids is regulated by a coordinated motor pattern between the antrum and pylorus. We studied the role of the vagus nerve in mediating postprandial coordination between the antrum and pylorus. Force transducers were implanted on the serosal surface of the body, antrum, pylorus and duodenum in seven dogs. Dogs were given either a solid or a liquid meal and gastro-duodenal motility was recorded over 10 hours. Gastric emptying was evaluated with radiopaque markers mixed with a solid meal. Dogs were treated with hexamethonium, L-NAME, or transient vagal nerve blockade by cooling. Postprandial motility pattern showed three distinct phases: early, intermediate, and late. In the late phase, profound pyloric relaxations predominantly synchronized with giant antral contractions, which were defined as postprandial antro-pyloric coordination. Gastric emptying study revealed that the time at which gastric contents entered into the duodenum occurred concomitantly with antro-pyloric coordination. Treatment by vagal blockade or hexamethonium significantly reduced postprandial antral contractions and pyloric relaxations of the late phase. L-NAME changed pyloric motor patterns from relaxation-dominant to contraction-dominant. Solid gastric emptying was significantly attenuated by the treatment with hexamethonium, L-NAME and vagal blockade. Postprandial antro-pyloric coordination was not seen after the feeding of a liquid meal. It is concluded that postprandial antro-pyloric coordination plays an important role to regulate gastric emptying of a solid food. Postprandial antro-pyloric coordination is regulated by the vagus nerve and nitrergic neurons in conscious dogs.

Key words: functional dyspepsia, gastric emptying, nicotinic receptor, nitrergic neuron.
Introduction

The mechanism of regulating gastric emptying is different between liquid and solid foods. The proximal stomach has a major role in emptying liquids by the gastroduodenal pressure gradient, while the distal stomach play a role in mixing, grinding, and emptying of solid foods (27). It is generally believed that gastric emptying of solids is regulated by coordinated motor pattern between the antrum and pylorus (16, 21, 25). In the digestive state, coordinated antro-pyloric motor pattern propels gastric content aborally, and resistance to flow from the stomach is provided by tonic and phasic pyloric motor activity (32).

The regulation of gastric emptying is thought to be an important physiological function of the pylorus (36). In conscious dogs, gastro-pyloro-duodenal contractions are correlated with the rate of solid gastric emptying. The rate of gastric emptying showed a significant positive correlation with the contractions of the body, antrum, pylorus and duodenum (13). In humans, the onset of solid emptying is associated with an increase in the rate of occurrence of antral pressure waves and the half-time for solid emptying is inversely correlated with the rate of coordinated contractions among the antrum, pylorus and duodenum (16).

We have previously reported that in conscious rats, synchronized contractions between the antrum and pylorus are observed in response to a solid food (17, 19). However, it remains unclear whether digested food is evacuated into the duodenum during the period of the synchronized contractions between the antrum and pylorus. Although the relaxation of the pylorus is believed to have an important role to empty gastric content, it is also uncertain whether the pylorus relaxes in the postprandial state. Furthermore, there has been no direct evidence reported that the gastric contents are emptied by the coordination between the antral contraction and the pyloric relaxation.
Insulin-induced hypoglycemia accelerates solid gastric emptying via stimulating vagal efferent activity. Cholinergic muscarinic blockade with atropine inhibits the hypoglycemia-induced acceleration of solid gastric emptying in humans (3). We have shown that vagotomy abolished the postprandial antro-pyloric coordination induced by solid food ingestion in rats (19). These indicate that the vagus nerve has a major role in regulating solid gastric emptying and postprandial motility. However, it remains to be investigated by what mechanism the vagus nerve regulates gastric emptying.

A certain subgroup of patients with functional dyspepsia (FD) show depressed activity of the vagus nerve (15). Quartero et al. (31) showed that gastric emptying of solids in patients with FD were 1.5 times slower than that of controls. A significant delay of emptying was present in almost 40% of patients with FD (31). In patients with FD, however, the influence of the impaired activity of the vagus nerve on delayed gastric emptying still remains unclear.

We hypothesize that the gastric contents are emptied by the coordination between the antral contraction and the pyloric relaxation in the postprandial state. We also hypothesize that postprandial antro-pyloric coordination is under the control of vagus nerve.

To prove these hypotheses, we studied the motor pattern of the antrum and pylorus in the digestive state of solid foods. We also studied the role of vagus nerve in mediating postprandial antro-pyloric coordination in conscious dogs.

**Materials and Methods**

*Preparation of animals*
All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All aspects of this research were approved by the Durham Veterans Affairs Medical Center, Durham, NC.

Seven mongrel dogs (five females and two males) weighing between 10 and 20 kg were used in this study. Food was withheld for twelve hours prior to surgery. Induction occurred with a single intravenous injection of 7 mg/kg of propofol. General anesthesia was maintained by intratracheal inhalation of isoflurane in oxygen. The abdomen was prepared aseptically for a 10 cm ventral midline laparotomy. Four strain gauge force transducers were implanted on the seromuscular layer to record a circular muscle motor activity. Three transducers (Model F12IS, 8x14 mm, Star Medical Inc., Tokyo, Japan) were sutured to the gastric body (at the level of the short gastric artery), the antrum (2-3 cm proximal to the pyloric ring), and the proximal duodenum (3-4 cm distal to the ring), respectively. One additional transducer (Model F12IS-P, 4x12 mm, Star Medical Inc., Tokyo, Japan) was applied to the pyloric sphincter. A bundle of lead wires was led out through a skin incision made between the scapulas via a subcutaneous tunnel in the costal flank. After the surgery, the lead wires were covered with a jacket protector. The dogs were allowed to recover from the surgery for two weeks. During the following experiments, the lead wires were connected to a recording system (Mac Lab; ADInstruments, Colorado Springs, CO).

Measurement of gastroduodenal motor activity after ingestion of solid meals and liquid meals

In order to clarify the postprandial gastroduodenal motor activity in response to a solid or a liquid meal, after a 12-hour fast dogs were given either 200 g of commercial based canned dog food (Beef Dinner, Pro-Pet L.L.C., St. Marys, Ohio) or a 200 ml of chicken soup (Tu Sabor Latino, Maggi). The basic motor activities were recorded over ten hours. The test meal was provided
fifteen to twenty minutes after the termination of phase III activity of the duodenum. The solid meal contained 200 kcal and consisted of 9.0% protein, 5.0% fat, and carbohydrate. The liquid meal contained 15 kcal.

**Effect of hexamethonium and L-NAME on postprandial motor activity**

The dogs were treated with hexamethonium or L-NAME to investigate the involvement of nicotinic or nitrergic regulation in postprandial coordination between the antrum and pylorus, respectively. The dogs were given 200 g of canned dog food. Drugs were administered through the cephalic vein when the apparent antro-pyloric coordination was observed. Hexamethonium was injected at 1 mg/kg bolus, followed by a continuous infusion at a rate of 1 mg/kg/hr for twenty minutes. L-NAME was administered 2.5 mg/kg bolus intravenously followed by infusion at a rate of 2.5 mg/kg/hr for fifteen minutes. A bolus of 40 mg/kg of L-arginine was injected to reverse the effect of L-NAME at the end of the study. Each study was performed in at least a three-day interval.

**Vagal blockade by cooling**

After finishing the baseline experiments, each dog underwent a second operation. Bilateral cervical vagal skin flaps were prepared for transient vagal nerve blockade. Under general anesthesia bilateral skin incisions were made on the ventral neck. Bilateral vago-sympathetic nerve trunks with surrounding connective and adipose tissues were isolated and then a 5 cm portion was wrapped by the skin flap, as previously described (8, 14). The dogs were allowed to recover for two weeks.
To clarify the involvement of the vagus nerve in postprandial coordination between the antrum and pylorus in the late phase, vagal blockade by cooling was applied to the dogs when postprandial coordination between the antrum and pylorus was observed. Transient vagal nerve blockade was initiated by circulating absolute ethanol at –20°C through U-shaped copper tubes hooked through the skin loops. The temperature of the skin loops was maintained at 4°C. Nerve blockade was achieved within fifteen to-twenty minutes after the start of cooling and was characterized by deep respirations and bilateral Horner’s syndrome, as previously reported (8, 14).

**Gastric emptying study**

Gastric emptying study was carried out with ring-shaped (4.5x4.5x1.0 mm, Sitzmarks, Konsyl Pharmaceuticals Inc., Fort Worth, Texas) or cubic (1.0x1.0x1.0 mm) radiopaque markers, which were made of polychlorinated vinyl with 33% barium sulfate and a density of 1.2 g/cm³. After a 12-hour fast, twenty radiopaque markers with 5 ml barium sulfate suspension (60% w/v) were ingested with the same regular canned dog food. The dogs were monitored fluoroscopically every sixty minutes for six hours to determine the location of the markers.

To further investigate the role of antro-pyloric coordination regulating solid gastric emptying, the dogs were treated with hexamethonium, L –NAME and vagal blockade. Three hours after the ingestion of canned dog food with twenty radiopaque markers (1 mm), treatment with hexamethonium, L –NAME and vagal blockade was started and lasted for 1 hour. The emptied markers from the stomach were counted during the treatment. In control experiments, dogs were treated with saline infusion for 1 hour. Sham vagal blockade was performed by circulating absolute ethanol at room temperature through the skin loops.
Data and Statistical analysis

A voluntary area under the curve during 10 minutes was calculated using a computer-assisted system and expressed as a motility index (MI). To investigate the effects of L-NAME, hexamethonium, and vagal blockade on the contractile components or relaxant components of the antrum and pylorus, area under the curve of each contraction or relaxation was calculated for 10 minutes. The summed area under the curve was compared before and after the various treatments. Values are shown as mean ± SE. Statistical analysis was performed by paired t test or analysis of variance (ANOVA). When significant differences were detected in ANOVA, differences between means were checked by Bonferroni’s method. P values <0.05 were considered significant.

Materials

Hexamethonium was obtained from Sigma (St. Louis, MO). L-NAME was obtained from Research Biochemical International (Natick, MA).

Results

Gastro-duodenal motor activity in response to solid meal

Representative postprandial gastro-duodenal motor activity after a solid meal ingestion is shown in Figure 1. Three different phases were recognized in the gastro-duodenal motor activity (Table 1). The early phase was characterized by receptive relaxation in the body followed by the phasic synchronized contractions in the body, antrum, and pylorus with a cycle of 4-5/min. In response to feeding, pyloric contractions with high amplitude were initially observed prior to these phasic contractions of the antrum and pylorus. Afterwards, the pylorus showed excitatory motor activity
dominantly in this phase (Figure 1A). The early phase was observed immediately after starting the feeding and lasted for 20-30 minutes.

In the intermediate phase, the phasic synchronized contractions remained in the body and antrum, while the pyloric phasic contractions were gradually disappearing. The antral phasic contractions were followed by pyloric excitatory or inhibitory motor activities (Figure 1B). The duodenum showed phasic contractions in this phase. The intermediate phase was observed 20-30 minutes after the feeding and lasted for 90-120 minutes.

Two to three hours after the feeding, the motor pattern of the antrum, pylorus and duodenum changed and showed its characteristic feature (Figure 1C). In the late phase, contractions of the body were propagated to the antrum with a 3:1 ratio and the antrum slowed contractions with low frequency (<3 cpm) and high amplitude (>20 g: giant contraction). The pylorus showed relaxations with large negative amplitude and wide duration, which occurred after 1-2 seconds in response to the contraction of the antrum. We defined “antro-pyloric coordination” as pyloric relaxations were predominantly associated with slow antral contractions (<3 cpm). In the late phase, the antro-pyloric coordination was frequently observed and lasted for three to four hours. The duodenum-exhibited contractions synchronized with the pyloric relaxations (Figure 1C). The late phase lasted for six-eight hours after the feeding.

Figure 2 shows the comparison of the MI in the antrum and pylorus in each phase. Significant differences were observed among the phases in the pylorus. Especially, the positive MI was observed in the early phase, while negative MI was observed in the late phase. This reflects that contractions are dominant in the early phase, while relaxations are dominant in the late phase of the pylorus.
Transition of motor pattern from postprandial state to interdigestive state ingestion is shown in Figure 3A. Approximately eight to ten hours after the feeding, the amplitude of antral contractions and the consequent pyloric relaxations gradually increased (Figure 3A-f). Phase III-like motor activity was resumed and was followed by an interdigestive pattern (Figure 3A-g).

**Gastro-duodenal motor activity in response to a liquid meal**

Representative postprandial gastroduodenal motor activity after a liquid intake is shown in Figure 3B. Phasic synchronized contractions were observed in the antrum, pylorus and duodenum immediately after the liquid meal ingestion (Figure 3B-b). Antral contractions and pyloric relaxations, which were seen in the late phase following solid meal ingestion, were not observed following liquid meal ingestion (Figure 3B-b, Figure 3B-c). Approximately two to three hours after the feeding, the amplitude of antral contractions and pyloric relaxations gradually increased (Figure 3B-d). These were followed by an interdigestive pattern (Figure 3B-e).

**Effects of hexamethonium and L-NAME on postprandial antro-pyloric coordination in the late phase**

Hexamethonium reduced antral contractions to 10.9±4.3% of pre-treatment level ($P<0.01$) and abolished pyloric relaxations (Figure 4A). L-NAME significantly increased the amplitude of phasic contractions of the duodenum. L-NAME changed the pyloric motor pattern from relaxation-dominant to contraction-dominant in the late phase. The contractile component of the pylorus is increased to 345.6 ± 56.3% ($P<0.01$ by paired $t$ test) of pre-treatment level, while the relaxant component of the pylorus is reduced to 45.6 ± 8.7% ($P<0.01$ by paired $t$ test) of pre-
treatment level of L-NAME (Figure 4B). Thus, antro-pyloric coordination was abolished by hexamethonium and L-NAME.

**Effects of vagal blockade on the postprandial coordination in the late phase**

When vagal cooling was started three hours after solid food ingestion, both frequency and the amplitude gradually diminished during the vagal blockade in the antrum, pylorus and duodenum. Components of the antral contractions and the pyloric relaxations in the late phase were significantly reduced to 11.2±2.3% and 26.9±11.2% of pre-treatment level ($P<0.05$ by paired $t$ test) by vagal blockade, respectively. After the removal of vagal cooling, the antro-pyloric coordination was restored (Figure 5A and 5B).

In one dog, phasic contractions were observed in the pylorus during the vagal blockade, although the contractions of the antrum and duodenum were significantly attenuated by vagal blockade. These isolated pyloric contractions disappeared after the removal of vagal cooling (Figure 5B).

**Gastric emptying study**

The time-course of gastric emptying of 4.5-mm and 1-mm non-digestive markers with 200 g of solid food is shown in Figure 6. The 1-mm markers were not emptied by 60 minutes, and then were emptied linearly from 60 to 360 minutes. By 360 minutes, 37.5±11.1% of 1-mm markers was evacuated. The 4.5-mm markers did not leave the stomach by 120 minutes after ingestion. By 360 minutes, 12.5 ±4.8% of 4.5-mm markers was emptied (Figure 6A).

The number of radiopaque markers emptied from the stomach was 13.7±3.5 % during 180-240 min after the feeding in saline-treated dogs. Treatment with hexamethonium, L–NAME (Figure
6B) and vagal blockade (Figure 6C) almost completely abolished the number of radiopaque markers emptied from the stomach.

Simultaneous observation of antro-pyloric coordination with the force transducers and the fluoroscope revealed that the time at which smaller markers entered into the duodenum occurred concomitantly with antro-pyloric coordination (Figure 7).

Discussion

Postprandial gastric motor patterns involve different phases and antro-pyloric coordination observed in the late phase contributes to solid gastric emptying

It has been reported that the postprandial motor patterns may be altered following a solid meal. We have previously shown that postprandial gastric motor patterns in response to a solid food have different phases in conscious rats (17, 19). Feeding of rat chow immediately caused phasic contractions of the antrum and pylorus. These contractions were sustained up to 7 to 10 minutes after feeding. Both the antrum and pylorus contracted randomly in this period. This period seems to reflect the grinding process of the gastric content.

Forty minutes after the feeding, contractions with low frequency (< 3 cycles/min) and high amplitude of the antrum were frequently observed. The peak contraction of the antrum occurred mostly 2-4 seconds before the peak contraction of the pylorus (19). Although this period seems to reflect the emptying process of the gastric content, it remains unclear whether these synchronized contractions are associated with the emptying process.

In humans, Brown et al. (5) recorded the changes in configuration of the distal stomach during digestion and expulsion of a solid meal using real-time ultrasonography. They divided postprandial time course into three periods: the early postprandial state (first 20 minutes of
observation), the intermediate state (20-60 minutes), and the late postprandial state (60-120 minutes). Liquids are decanted into the duodenum in the early phase. Large particles are blocked from entering the duodenum by closure of the pylorus in the intermediate phases. Large particles are ground and transformed into small particles by the gastric antrum in the late phase (5).

In the current study, we observed three separate postprandial gastro-duodenal motor patterns following a solid meal in conscious dogs. The early phase was characterized by receptive relaxation in the body followed by the phasic synchronized contractions in the body, antrum and pylorus. The pylorus dominantly showed excitatory motor activity in this phase.

In the intermediate phase, the phasic synchronized contractions remained in the body and antrum. The antral phasic contractions were followed by pyloric excitatory or inhibitory motor activities.

In the late phase (more than 2 hours after the feeding), the motor pattern of the antrum changed significantly showing contractions with low frequency (<3 cpm) and high amplitude (>20 g). The pylorus showed relaxations with large amplitude and wide duration, which well associated with antral contractions. The duodenum exhibited the contractions synchronized with the pyloric relaxations. The antro-pyloric coordination observed in the late phase seems to have a role to empty digested food into the duodenum. The synchronized motor pattern between the pyloric relaxations and duodenal contractions appears to prevent the reflux of duodenal contents to the stomach.

The mechanism of regulating gastric emptying is different between liquids and solids. The emptying of liquids from the stomach is thought to be primarily a function of the pressure gradient between the proximal stomach and the duodenum (27). Pulsatile transpyloric flow of
liquids into the duodenum occurs predominantly during the non-lumen occlusive stage of the contractions of the proximal stomach (2). Thus, antral contractions do not play a role in regulating gastric emptying of liquids. There is no significant relation observed between the antral motility and emptying of the liquid meal (6).

In contrast, there is a positive correlation between the antral motility and emptying of solids (6). Antral pump and pyloric opening are of paramount importance for emptying in solids. Solids are retained in the stomach until the antrum has ground them into particles small enough to become suspended in the antral fluid. Large solid particles retained in the stomach by the pyloric closure and retropelled and triturated in the antral mill. In the emptying state, the strong antral contractions were regularly associated with inhibition of the pylorus. Thus, antral contractions are related to pyloric opening as expressed “antral contraction followed by pyloric opening sequence” (25).

Here, we showed the differences in time course of motor pattern of the antrum and pylorus following solid meal ingestion. In contrast, the coordinated motor pattern between the antrum and pylorus is not observed following a liquid ingestion. We also showed that the pyloric relaxations in response to a solid food are highly synchronized with giant contractions of the antrum. This suggests that antro-pyloric coordination observed in the late phase contributes to solid gastric emptying. It seems that liquid emptying does not need the antro-pyloric coordination.

It is generally believed that gastric emptying of solids is regulated by coordinated motor pattern between the antral contractions and pyloric relaxations (16, 21, 25). To our knowledge, this is the first report demonstrating the importance of postprandial coordination between the antral contractions and pyloric relaxations.
Postprandial antro-pyloric coordination plays a role to empty undigested large particles.

The pyloric sphincter is involved in the regulation of solid gastric emptying primarily by its degree of opening (22). In contrast, the simultaneous contractions of the antrum and pylorus generate the force that solid particles get back into the gastric body (7, 27).

It is generally accepted that gastric emptying of undigested solids is mainly related to the antral phase III activity of the migrating motor complex (MMC) in the interdigestive state (29). Pyloric relaxation during the interdigestive state is thought to be the mechanism that allows the passing of particles that are too large to transverse the pylorus. Pyloric ring is open most widely in phase III and that large undigested particles are expelled by pyloric relaxation in phase III of MMC in conscious dogs (28).

Contrary to these opinions, it has recently been demonstrated that gastric emptying of undigested solids after a meal can occur unrelated to the antral phase III, at least up to a particle size of 3 mm and perhaps even 7 mm in humans (34). Others studied gastric emptying of simultaneously ingested solid food and various sizes of markers (1.0, 3.2, and 5.0 mm in diameter) in conscious dogs. Emptying rates of these markers (1.0, 3.2, and 5.0 mm) were nearly 100%, 70%, and 20%, respectively, in five hours after the feeding (26).

It remains unclear how undigested large particles are emptied from the stomach in the postprandial state. It has been demonstrated that no pyloric relaxation is observed in the postprandial state and that large undigested particles are emptied by pyloric relaxation in phase III of MMC (28). In contrast, others demonstrated that gastric emptying of undigested solids after a meal can occur unrelated to the antral phase III activity (34). As shown in Fig. 3A-a and 3B-a, the pyloric relaxation was most significantly observed in phase III of interdigestive phase. However, in the late postprandial state pylorus also
relaxes 40-60% compared to the phase III cycle (See Fig 3A-d and Fig. 3A-e). Our gastric emptying study with radiopaque markers revealed that large markers as well as small markers passed through the pylorus at the late postprandial phase. This finding suggests that the pyloric relaxations of the postprandial state may contribute to the emptying process of large undigested particles.

*Postprandial antro-pyloric coordination is regulated via the vagus nerve.*

We have previously demonstrated that postprandial synchronized motor pattern between the antrum and pylorus is not observed in rats that received truncal vagotomy (17, 19). We investigated the role of the vagus nerve on antro-pyloric coordination in conscious dogs.

Hexamethonium, a nicotinic receptor antagonist, significantly reduced antral contractions and abolished pyloric relaxations. There was no more antro-pyloric coordination observed during hexamethonium treatment. Furthermore, we showed that the transient vagal blockade by cooling significantly impaired antro-pyloric coordination. This indicates that the initiation of postprandial antro-pyloric coordination in the late phase is under the regulation of the vagus nerve. We also showed that gastric emptying was significantly attenuated by hexamethonium and vagal blockade.

We have previously showed that electrical vagal stimulation causes pyloric relaxations and that vagal stimulation-induced pyloric relaxations were abolished by L-NAME in rats (18). In the current study, L-NAME changed the pyloric motor pattern from relaxation-dominant to contraction-dominant. These suggest that the pyloric relaxations observed in the late phase are mainly mediated via the vagal efferent and NO neurons of the myenteric plexus.

L-NAME reduced the component of the pyloric relaxation to less than half in the late phase. In addition to NO, pituitary adenylate cyclase activating polypeptide (PACAP), vasoactive intestinal
polypeptide (VIP), and adenosine triphosphate (ATP) have been shown to mediate the pyloric relaxations (1, 20, 30, 33). L-NAME resistant relaxations observed in this study may be mediated by PACAP VIP, and/or ATP in conscious dogs.

Intraduodenal infusion of nutrients has been shown to suppress antral contractions and stimulate isolated pyloric pressure waves (IPPWs) in pigs (37) and dogs (10). In our current study, isolated pyloric contractions have never been observed following solid food ingestion in conscious dogs. Isolated pyloric contractions were observed only during the vagal blockade, as shown in Figure 7B. This suggests that the vagal inhibitory neural pathway innervating to the pyloric region has a dominant role to regulate pyloric motility.

Impaired vagal activity may delay solid gastric emptying in patients with FD

The incidence of delayed gastric emptying in patients with FD is reported to be 9-40% (4, 9, 24, 31). When radiopaque markers (4.5 mm in diameter) are applied with solid food, almost all markers were emptied in four hours after the feeding in healthy controls. In contrast, 80% of markers still remained in the stomach in patients with FD (23). Half of radiopaque capsules (5 mm in diameter) were emptied in healthy controls in two hours, while none of them emptied within two hours in every patient with FD (35). These results suggest that emptying mechanism of large particle in the postprandial state is impaired in patients with FD. The incidence of postprandial antro-pyloric coordination is significantly reduced in FD patients (12). The patients with FD sometimes show the reduced activity of the vagus nerve (11, 15). Our findings showed that the vagus nerve play a role in mediating postprandial antro-pyloric coordination. It is to be
investigated whether impaired vagal activity would generate delayed gastric emptying through the impairment of postprandial antro-pyloric coordination in patients with FD.

Our current study showed that postprandial motility was divided into three distinct phases: early, intermediate, and late. In the late phase, profound pyloric relaxations predominantly synchronized with antral contractions, which were defined as postprandial antro-pyloric coordination. Postprandial antro-pyloric coordination was significantly reduced by vagal blockade. Gastric emptying observations distinguished that the time at which gastric contents entered into the duodenum occurred concomitantly with postprandial antro-pyloric coordination.

In conclusion, we emphasize that postprandial antro-pyloric coordination is associated with gastric emptying of solid food that the vagus nerve mediates postprandial antro-pyloric coordination in conscious dogs.

Acknowledgment

The authors are indebted to Gerald Olson, DVM, MS, and Rick Leary, RVT, for their skilled technical assistance in Durham VA animal facility. This study was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases [RO1 DK55808 (Toku Takahashi)].
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**Table 1. Characteristics of postprandial gastro-duodenal motor activity**

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<th>Intermediate phase</th>
<th>Late phase</th>
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<td>Phasic contractions synchronized with corporeal contractions</td>
<td>Phasic contractions synchronized with corporeal contractions</td>
<td>Giant contractions synchronized with corporeal contractions</td>
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<td><strong>Pylorus</strong></td>
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<td>Contractions and relaxations</td>
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<td><strong>Duodenum</strong></td>
<td>Silent</td>
<td>Phasic contractions</td>
<td>Phasic contractions synchronized with pyloric relaxations</td>
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Figure Legends

Figure 1 Postprandial gastro-duodenal motor activity after a solid meal. Receptive relaxation was seen in the body. Note that initial pyloric contractions were observed prior to phasic contractions in the antrum and pylorus. In the early phase, phasic synchronized contractions were observed in the body, antrum and pylorus with cycle of 4-5/min. The pylorus showed excitatory activity dominantly in this period (A). In the intermediate phase, phasic synchronized contractions continued in the body and antrum. The antral phasic contractions were followed by pyloric excitatory or inhibitory motor activities (B). In the late phase, contractions of the body propagated to the antrum with a ratio of 3:1. The antrum slowed contractions with low frequency (<3 cpm) and high amplitude (>20 g). The pylorus showed relaxations with large negative amplitude and a wide duration, which occurred 1-2 seconds after the antral contractions. The duodenum exhibited contractions synchronized with the pyloric relaxations (C).

Figure 2 The comparison of the MI in the antrum and pylorus after a solid meal. The MI in the pylorus showed a significant difference among the phases. The MI index represents the area under the curve, taking into account both frequency and amplitude. The positive MI was observed in the early phase, while negative MI was observed in the late phase. This reflects that contractions are dominant in the early phase, while relaxations are dominant in the late phase of the pylorus (*P<0.05, **P<0.01 by Bonferroni).

Figure 3 Transition of motor pattern from postprandial state to interdigestive state after solid meal ingestion (A) and liquid meal ingestion (B). Approximately eight to ten hours after solid
meal ingestion, the amplitude of antral contractions and the consequent pyloric relaxations gradually increased (A-f). Phase III-like motor activity was resumed and was followed by an interdigestive pattern (A-g). The amplitude of pyloric relaxation observed at the late phase (A-d and A-e) was 40-60%, compared to that observed at the phase III cycle (A-a).

Immediately after the liquid meal ingestion, phasic synchronized contractions were observed in the antrum, pylorus and duodenum (B-b). No antro-pyloric coordination was observed following liquid meal ingestion (B-b and B-c). Approximately 2 to 3 hours after the feeding, the amplitude of antral contractions and pyloric relaxations gradually increased (B-d). These were followed by an interdigestive pattern (B-e).

Figure 4 Effect of hexamethonium (A) and L-NAME (B) on postprandial antro-pyloric coordination. Hexamethonium reduced antral contractions to 10.9±4.3% of controls and abolished pyloric relaxations (A). L-NAME changed the pyloric motor pattern from relaxation dominant to contraction dominant in the late phase. Thus, antro-pyloric coordination was abolished after the administration of hexamethonium and L-NAME.

Figure 5 Effect of transient vagal cooling on postprandial antro-pyloric coordination. Both the frequency and the amplitude gradually diminished during the vagal blockade in the antrum, pylorus and duodenum. Postprandial antral contractions and pyloric relaxations were significantly reduced by vagal blockade. Antro-pyloric coordination was also significantly attenuated by vagal blockade. After the removal of vagal cooling, coordinated motor pattern between the antrum and pylorus was restored (A and B).
In one dog, phasic contractions were observed of the pylorus during the vagal blockade, although the contractions of the antrum and duodenum were significantly attenuated by vagal blockade. These isolated pyloric contractions were disappeared after the removal of vagal cooling (B).

Figure 6 Gastric emptying study with radiopaque markers for 6 hours (A). Effects of hexamethonium, L-NAME (B) and vagal blockade (C) on gastric emptying during 180-240 minutes after the feeding. The 1-mm markers were not emptied by 60 minutes after the solid meal ingestion, and then were emptied linearly from 60 to 360 minutes. By 360 minutes, 37.5 ±11.1% of 1-mm markers was evacuated. The 4.5-mm markers remained in the stomach by 120 minutes after the ingestion. By 360 minutes 12.5 ±4.8% of 4.5-mm markers was emptied (A).

The number of radiopaque markers emptied from the stomach was 13.7±3.5% during 180-240 min after the feeding in saline-treated dogs. Treatment with hexamethonium, L-NAME (B) and vagal blockade (C) almost completely abolished the number of radiopaque markers emptied from the stomach.

Figure 7 Simultaneous observation of antro-pyloric coordination with the force transducers and radiopaque markers (4.5-mm). The time at which radiopaque markers (shown as closed triangles) entered into the duodenum occurred concomitantly with antro-pyloric coordination (shown as arrows).
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7

(A) Antrum and Pylorus recordings showing 40 g and 20 g forces with a 1 min time scale.

(B) Images of Duodenum and Antrum with a Pyloric transducer marked.