Role of vagus nerve in postprandial antropyloric coordination in conscious dogs

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Ueno, Tomio, Kenichiro Uemura, Mary B. Harris, Theodore N. Pappas, and Toku Takahashi. Role of vagus nerve in postprandial antropyloric coordination in conscious dogs. Am J Physiol Gastrointest Liver Physiol 288: G487–G495, 2005. First published October 14, 2004; doi:10.1152/ajpgi.00195.2004.—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 gastroduodenal motility was recorded over 10 h. Gastric emptying was evaluated with radiopaque markers mixed with a solid meal. Dogs were treated with hexamethonium, N^2-nitro-L-arginine methyl ester (l-NAME), or transient vagal nerve blockade by cooling. A postprandial motility pattern showed three distinct phases: early, intermediate, and late. In the late phase, profound pyloric relaxations predominantly synchronized with giant antral contractions that were defined as postprandial antropyloric coordination. A gastric emptying study revealed that the time at which gastric contents entered into the duodenum occurred concomitantly coordination was not seen after feeding a liquid meal. It is concluded that gastric emptying of solids is regulated by a coordinated antropyloric coordination between the antrum, pylorus, and duodenum. Functional dyspepsia; gastric emptying; nicotinic receptor; nitrergic neuron.

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, whereas the distal stomach plays a role in mixing, grinding, and emptying solid foods (27). It is generally believed that gastric emptying of solids is regulated by a coordinated motor pattern between the antrum and pylorus (16, 21, 25). In the digestive state, a coordinated antropyloric motor pattern propels gastric content aborally, and resistance to flow from the stomach is provided by tonic and phasic pyloric motor activity (32).

Regulation of gastric emptying is thought to be an important physiological function of the pylorus (36). In conscious dogs, gastropyloroduodenal 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 (17, 19) that in conscious rats, synchronized contractions between the antrum and pylorus are observed in response to a solid food. 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 relaxation of the pylorus is believed to have an important role in emptying gastric content, it is also uncertain whether the pylorus relaxes in the postprandial state. Furthermore, there has been no direct evidence reported that gastric contents are emptied by coordination between antral contraction and 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 antropyloric coordination induced by solid food ingestion in rats (19). These studies 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) showed 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 is 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 antropyloric coordination is under the control of the 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 the vagus nerve in mediating postprandial antropyloric coordination in conscious dogs.

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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 (5 females and 2 males) weighing between 10 and 20 kg were used in this study. Food was withheld for 12 h before surgery. Induction occurred with a single intravenous injection of 7 mg/kg 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 F121S, 8 × 14 mm; Star Medical, 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 F121S-P, 4 × 12 mm; Star Medical) 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 2 wk. 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 and liquid meals. To clarify the postprandial gastroduodenal motor activity in response to a solid or a liquid meal after a 12-h fast, dogs were given either 200 g of commercial canned dog food (Beef Dinner; Pro-Pet, St. Marys, OH) or 200 ml of chicken soup (Tu Sabor Latino, Maggi, Venezuela). The basic motor activities were recorded over 10 h. The test meal was provided 15–20 min after the termination of the fasting period. After the meal was ingested, each dog underwent a second operation. Bilateral cervical skin incisions were made on the dogs. Skin was incised to the underlying connective and adipose tissues were isolated, and a 5-cm portion was then wrapped by the skin flap as previously described (8, 14). The dogs were allowed to recover for 2 wk.

Vagal blockade by cooling. After the baseline experiments were finished, each dog underwent a second operation. Bilateral cervical skin incisions were made on the dogs. Skin was incised to the underlying connective and adipose tissues were isolated, and a 5-cm portion was then wrapped by the skin flap as previously described (8, 14). The dogs were allowed to recover for 2 wk.

To clarify 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 15–20 min after the start of cooling and was characterized by deep respirations and bilateral Horner's syndrome, as previously reported (8, 14).

Gastric emptying study. A gastric emptying study was carried out with ring-shaped (4.5 × 4.5 × 1.0 mm, Sitzmarks; Konsyl Pharmaceuticals, Fort Worth, TX) or cubic (1.0 × 1.0 × 1.0 mm) radiopaque markers made of polychlorinated vinyl with 33% barium sulfate and a density of 1.2 g/cm³. After a 12-h fast, 20 radiopaque markers with 5 ml of barium sulfate suspension (60% wt/vol) were ingested with the same regular canned dog food. The dogs were monitored fluoroscopically every 60 min for 6 h to determine the location of the markers.

To further investigate the role of antropyloric 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 20 radiopaque markers (1 mm), treatment with hexamethonium, L-NAME, and vagal blockade was started and lasted for 1 h. The emptied markers from the stomach were counted during the treatment. In control experiments, dogs were treated with saline infusion for 1 h. 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 min was calculated using a computer-assisted system and expressed as a motility index (MI). To investigate 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 min. The summed area under the curve was compared before and after the various treatments. Values are shown as means ± SE. Statistical analysis was performed by Student’s paired t-test or ANOVA. When significant differences were detected in ANOVA, differences between means were checked by the Bonferroni 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

Gastroduodenal motor activity in response to solid meal. Representative postprandial gastroduodenal motor activity after ingestion of a solid meal is shown in Fig. 1. Three different phases were recognized in the gastroduodenal 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 before these phasic contractions of the antrum and pylorus. Afterward, the pylorus showed excitatory motor activity dominantly in this phase (Fig. 1A). The early phase was observed immediately after starting the feeding and lasted for 20–30 min.

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 (Fig. 1B). The duodenum showed phasic contractions in this phase. The intermediate phase was observed 20–30 min after feeding and lasted 90–120 min. Two to three hours after the feeding, the motor pattern of the antrum, pylorus, and duodenum changed and showed its characteristic feature (Fig. 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 counts/min (cpm)) and high amplitude (>20 g, giant contraction). The pylorus showed relaxations with large negative
amplitude and wide duration, which occurred after 1–2 s in response to the contraction of the antrum. We defined “antro-pyloric coordination” as pyloric relaxations that were predominantly associated with slow antral contractions (<3 cpm). In the late phase, the antropyloric coordination was frequently observed and lasted for 3 to 4 h. The duodenum exhibited contractions synchronized with the pyloric relaxations (Fig. 1C). The late phase lasted for 6–8 h 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, whereas negative MI was observed in the late phase. This reflects that contractions are dominant in the early phase, whereas relaxations are dominant in the late phase of the pylorus.

Transition of motor pattern from postprandial state to interdigestive state ingestion is shown in Fig. 3A. Approximately 8–10 h after the feeding, the amplitude of antral contractions and the consequent pyloric relaxations gradually increased (Fig. 3Af). Phase III-like motor activity was resumed and was followed by an interdigestive pattern (Fig. 3Ag).

Gastroduodenal motor activity in response to a liquid meal. Representative postprandial gastroduodenal motor activity after a liquid intake is shown in Fig. 3B. Phasic synchronized contractions were observed in the antrum, pylorus, and duodenum immediately after the liquid meal ingestion (Fig. 3Bb). Antral contractions and pyloric relaxations, which were seen in the late phase after solid meal ingestion, were not observed after liquid meal ingestion (Fig. 3B, b and c). Approximately 2 to 3 h after the feeding, the amplitude of antral contractions and pyloric relaxations gradually increased (Fig. 3Bd). These were followed by an interdigestive pattern (Fig. 3Be).

Effects of hexamethonium and L-NAME on postprandial antropyloric coordination in the late phase. Hexamethonium reduced antral contractions to 10.9 ± 4.3% of pretreatment level (P < 0.01) and abolished pyloric relaxations (Fig. 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 pretreatment level, whereas the relaxant component of the...
Pylorus relaxations in the late phase were significantly reduced in the pylorus, and duodenum. Components of antral contractions and relaxations remained in the body and antrum. The antral phasic synchronized contractions were followed by pyloric excitatory or inhibitory relaxations.

Pylorus contractions and relaxations in the pylorus were significantly attenuated by vagal blockade, although the contractions of the antrum in the late phase (5).

In the present study, we observed three separate postprandial gastroduodenal motor patterns after 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 pyloric synchronized contractions remained in the body and antrum. The antral pyloric contractions were followed by pyloric excitatory or inhibitory motor activities.

**DISCUSSION**

Postprandial gastric motor patterns involve different phases, and antropyloric coordination observed in the late phase contributes to solid gastric emptying. It has been reported that the postprandial motor patterns may be altered after 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 7–10 min 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 feeding, contractions with low frequency (<3 cycles/min) and high amplitude of the antrum were frequently observed. Peak contraction of the antrum occurred mostly 2–4 s 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 the postprandial time course into three periods: the early postprandial state (first 20 min of observation), the intermediate state (20–60 min), and the late postprandial state (60–120 min). 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 phase. Large particles are ground and transformed into small particles by the gastric antrum in the late phase (5).

In the present study, we observed three separate postprandial gastroduodenal motor patterns after 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 pyloric synchronized contractions remained in the body and antrum. The antral pyloric contractions were followed by pyloric excitatory or inhibitory motor activities.
In the late phase (>2 h 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 was well associated with antral contractions. The duodenum exhibited the contractions synchronized with the pyloric relaxations. The antropyloric coordination observed in the late phase seems to have a role in emptying 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 nonlumen 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 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 were 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, expressed as “antral contraction followed by pyloric opening sequence” (25).

Here, we showed the differences in time course of motor pattern of the antrum and pylorus after solid meal ingestion. In contrast, the coordinated motor pattern between the antrum and pylorus is not observed after liquid meal ingestion. Solid particles were retained in the stomach by the pyloric closure and retropelled and triturated in the antral mill. In the emptying state, pyloric relaxations in response to a solid food are highly synchronized with giant contractions of the antrum. This suggests that antral activity observed in the late phase contributes to solid gastric emptying. It seems that liquid emptying does not need antropyloric 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 antropyloric coordination plays a role in emptying 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 need to 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. The pyloric ring is open most widely in phase III and 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 (26) 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, 5 h after the feeding.

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. 3, Aa and Bz, the pyloric relaxation was most significantly observed in phase III of the interdigestive phase. However, in the late postprandial state, pylorus also relaxes 40–60% compared with the phase III cycle (See Fig. 3, Ad and Ae). 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 antropyloric coordination is regulated via the vagus nerve. We have previously demonstrated (17, 19) that postprandial synchronized motor pattern between the antrum and pylorus is not observed in rats that received truncal vagotomy. We investigated the role of the vagus nerve on antropyloric coordination in conscious dogs.

Hexamethonium, a nicotinic receptor antagonist, significantly reduced antral contractions and abolished pyloric relaxations. There was no more antropyloric coordination observed by vagal blockade. Antropyloric coordination was also significantly attenuated by vagal blockade. After the removal of vagal cooling, a coordinated motor pattern between the antrum and pylorus was restored (A and B). In 1 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 disappeared after the removal of vagal cooling (B).

Fig. 5. Effect of transient vagal cooling on postprandial antropyloric 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. Antropyloric coordination was also significantly attenuated by vagal blockade. After the removal of vagal cooling, a coordinated motor pattern between the antrum and pylorus was restored (A and B). In 1 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 disappeared after the removal of vagal cooling (B).
ation of postprandial antropyloric 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 present 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), VIP, and 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 in pigs (37) and dogs (10). In our present study, isolated pyloric contractions have never been observed after solid food ingestion in conscious dogs.

Isolated pyloric contractions were observed only during the vagal blockade, as shown in Fig. 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 4 h after the feeding in healthy controls. In contrast, 80% of markers still remained in the stomach in patients with FD (23). One-half of radiopaque capsules (5 mm in diameter) were emptied in healthy controls in 2 h, whereas none of them were emptied within 2 h in every patient with FD (35). These results suggest that the emptying mechanism of large particles in the postprandial state is impaired in patients with FD. The incidence of postprandial antropyloric coordination is significantly reduced in FD patients (12). Patients with FD sometimes show the reduced activity of the vagus nerve (11, 15). Our findings showed that the vagus nerve plays a role in mediating postprandial antropyloric coordination. It is to be investigated whether impaired vagal activity would generate delayed gastric emptying through the impairment of postprandial antropyloric coordination in patients with FD.

Our present 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 antropyloric coordination. Postprandial antropyloric 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 antropyloric coordination.
In conclusion, we emphasize that postprandial antropyloric coordination is associated with gastric emptying of solid food and that the vagus nerve mediates postprandial antropyloric coordination in conscious dogs.

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