Effects of nitroglycerin on liquid gastric emptying and antropyloroduodenal motility

W.-M. Sun, S. Doran, K. L. Jones, E. Ooi, G. Boeckxstaens, G. S. Hebbard, T. Lingenfelsers, J. E. Morley, J. Dent, and M. Horowitz. Effects of nitroglycerin on liquid gastric emptying and antropyloroduodenal motility. Am. J. Physiol. 275 (Gastrinest. Liver Physiol. 38): G1173–G1178, 1998.—The effects of the nitric oxide donor nitroglycerin on gastric emptying and antropyloroduodenal motility were evaluated in nine healthy male subjects (ages 19–36 yr). Antropyloroduodenal pressures were recorded with a manometric assembly that had nine side holes spanning the antrum and proximal duodenum and a pyloric sleeve sensor; gastric emptying was quantified scintigraphically. In each subject, the emptying of 300 ml of 25% glucose labeled with 99mTc was assessed on two separate days during intravenous infusion of either nitroglycerin (5 µg/min in 5% dextrose) or 5% dextrose (control). Studies were performed with the subject in the supine position; blood pressure and heart rate were monitored. Nitroglycerin had no significant effect on blood pressure or heart rate. Nitroglycerin slowed gastric emptying (P < 0.02), and this was associated with greater retention of the drink in the proximal stomach (P < 0.05). In both nitroglycerin and control studies, ingestion of the drink was associated with an increase in the number of isolated pyloric pressure waves (P < 0.05) and antral pressure wave sequences (P < 0.05). Nitroglycerin reduced the number of isolated pyloric pressure waves (P < 0.05), basal pyloric pressure (P < 0.05), and the number of antral pressure wave sequences (P < 0.05), but not the total number of antral pressure waves. The rate of gastric emptying and the number of isolated pyloric pressure waves were inversely related during control (P = 0.03) and nitroglycerin (P < 0.05) infusions. We conclude that in normal subjects, 1) gastric emptying of 300 ml of 25% glucose is inversely related to the frequency of phasic pyloric pressure waves, and 2) nitroglycerin in a dose of 5 µg/min inhibits pyloric motility, alters the organization but not the number of antral pressure waves, and slows gastric emptying and intragastric distribution of 25% glucose.

nitric oxide; nutrient meal

GASTRIC EMPTYING is dependent on the organization of motor activity in the proximal stomach, antrum, pylorus, and duodenum, as well as on passive forces generated by intragastric volume and gravity (2, 16, 19). The delivery of nutrients from the stomach to the small intestine is closely regulated, largely as a result of feedback from chemo- and mechanoreceptors in the lumen of the small intestine (6, 19, 21). Infusion of nutrients into the small intestine is associated with inhibition of fundic and antral motility, stimulation of phasic and tonic pressure waves localized to the pylorus, and retardation of gastric emptying (4, 13, 14, 32). It has been suggested that stimulation of pyloric motility is the most important of these mechanisms, because the occurrence of phasic and tonic pyloric pressure waves is associated with cessation of transpyloric flow (32). There is, however, little information about the relationship between gastric emptying of nutrients and pyloric motility. The pathways mediating the effects of small intestinal nutrient exposure on gastric and pyloric motility are also poorly defined.

There is evidence that nitric oxide (NO) is an important inhibitory neurotransmitter in the gastrointestinal tract, including the stomach and pylorus (5, 10, 23–26, 33). In animal studies, inhibition of NO synthesis is associated with suppression of proximal gastric relaxation (10, 23), stimulation of antral, pyloric, and duodenal motility, and slowing of gastric emptying (24, 25). The latter effect may be more marked for nutrient compared with nonnutrient meals (25). The importance of NO mechanisms in the regulation of gastric emptying and gastric motility in humans is uncertain. Although one study (20) evaluated the effect of the NO donor nitroglycerin on gastric emptying, interpretation of these observations is difficult because nitroglycerin was given 40 min after ingestion of a drink of unstated caloric content. Two studies (11, 12) have provided evidence that nitroglycerin suppresses postprandial antral motility in humans, but antral motility was assessed using ultrasound, which is a less precise technique than manometry. There is also no information about the effects of NO on the organization of antral pressure waves; the latter appears to be a major determinant of transpyloric flow (16, 31). We have recently reported (28) that the stimulation of both tonic and phasic isolated pyloric pressure waves by intraduodenal triglyceride infusion is inhibited by nitroglycerin. This latter observation suggests that nitroglycerin may accelerate gastric emptying in humans and that the slowing of gastric emptying by small intestinal nutrients occurs through inhibition of an NO mechanism. However, the effect of nitroglycerin on postprandial pyloric motility has not been evaluated. Furthermore, the impact of nitroglycerin on gastric emptying is also likely to be dependent on its effects on proximal gastric and antral motor function (16).
The aims of this study were to evaluate in normal subjects 1) the relationship between gastric emptying and both phasic and tonic pyloric motility and 2) the effects of nitroglycerin on antropyloroduodenal motility and both gastric emptying and intragastric distribution after ingestion of a nutrient liquid meal.

**METHODS**

Subjects. Studies were carried out in nine male volunteers, ages 19–36 yr. All were nonsmokers, and none had a history of gastrointestinal disease or was taking medication. The experimental protocol was approved by the Human Ethics Committee of the Royal Adelaide Hospital in 1995, and each subject gave written informed consent.

Experimental protocol. All volunteers underwent paired studies on separate days, at least 7 days apart. The experimental protocol is summarized in Fig. 1. Each subject participated in 2 studies on separate days on which they received either intravenous nitroglycerin or dextrose (control).

Subjects remained supine throughout the study. Antropyloroduodenal pressures were recorded for 20 min during antral phase 1 interdigestive motor activity (30). Each subject was then given, in randomized single-blind order, an intravenous infusion of either nitroglycerin or 5% dextrose (Anginine, Wellcome Australia) at a rate of 5 µg/min or the same volume infusion of either nitroglycerin or dextrose (control).

Subjects gave written informed consent. Experimental protocol. All volunteers underwent paired studies on separate days, at least 7 days apart. The experimental protocol is summarized in Fig. 1. After subjects fasted overnight, at about 0900 a sleeve/side hole manometric catheter was passed through an anesthetized nostril and the sleeve sensor was positioned across the pylorus using feed-through IIci computer, controlled using proprietary software.

**Fig. 1.** Summary of experimental protocol. Each subject participated in 2 studies on separate days on which they received either intravenous nitroglycerin mixed in 5% glucose or control (5% glucose), BP, blood pressure.
(MAD 16, Synectics/Royal Adelaide Hospital/C. H. Malbert) developed from the LabView package (National Instruments, Austin, TX).

Recordings were analyzed only for periods in which the sleeve sensor was positioned correctly according to previously validated criteria: the antral TMPD was equal to or more positive than −20 mV; the duodenal TMPD was equal to or more positive than −15 mV; and the difference between the two readings was at least 15 mV (2, 13, 14, 28, 30). The antral and duodenal TMPD was recorded continuously, and the position of the manometric assembly was adjusted when necessary (14, 31). All phasic pressure waves (≥4 mmHg not attributable to respiration or straining) were evaluated. The latter were identified by their synchronous onset and identical amplitude in all side holes. Pressure waves were considered to be temporally related if their onset was from 5 s before to 10 s after the time of onset of the reference pressure wave in the most orad side hole. These related pressure waves were regarded as a pressure wave sequence. The number of antral pressure wave sequences was determined (14, 30, 31).

Distal antral pressure waves were defined as those recorded at the side hole 3 cm orad to the sleeve sensor. The number of duodenal pressure waves recorded by the duodenal TMPD side hole was counted. Isolated pyloric pressure waves (IPPW) were defined as phasic pressure waves detected by the sleeve sensor that were seen simultaneously in not more than one side hole over the length of the sleeve and that occurred in the absence (±5 s) of the onset of an associated pressure wave of any magnitude that was ascribable to either gastric or duodenal contraction (14, 30). Pyloric tone, defined as the difference between the basal pressures recorded by the sleeve sensor and that in the distal antrum (antral TMPD channel) (2, 14, 30), was measured 2 min before and every minute after commencement of the intravenous nitroglycerin infusion. Because pyloric tone was referenced to distal antral pressure, it was possible for the pressure to be negative (14).

The number of antral pressure wave sequences, IPPW, and mean pyloric tone were calculated for consecutive 20-min periods from 20 min before the commencement of the intravenous infusion (nitroglycerin or control) until 80 min after completion of the infusion.

Relationships between gastric emptying and antropyloroduodenal pressure events were evaluated in 40-min periods starting from the end of ingestion of the drink.

Statistical analysis. Differences between the two experiments were assessed using repeated-measures analysis via mixed-model ANOVA (a model with a mixture of fixed and random effects) after log transformation was performed to normalize the data (27). Paired comparisons were done using tests of simple effects (slices of interactions) (35), and the Tukey test was employed for multiple comparisons. Changes in blood pressure and heart rate were evaluated with ANOVA.

Relationships between gastric emptying and motility were assessed using linear regression with robust variance estimation via mixed-model analysis to allow for repeated values in each subject (34). Data are shown as means ± SE. A P value <0.05 was considered significant in all analyses.

RESULTS

One subject experienced mild headache during infusion of nitroglycerin, and another experienced headache during dextrose infusion. No other adverse effects were noted. There were no significant changes in blood pressure or heart rate attributable to either infusion (data not shown). The manometric catheter was positioned correctly across the pylorus for >95% of recording time in all studies.

Gastric emptying. Gastric emptying data are summarized in Fig. 3. There was no difference in the lag phase between nitroglycerin and control (2.6 ± 1.8 vs. 0.8 ± 0.2 min). Although the difference in t50 (148 ± 11 vs. 133 ± 11 min) between nitroglycerin and placebo was not significant, nitroglycerin slowed emptying from both the total (P < 0.02) and proximal (P < 0.05) stomach. The content of the distal stomach did not differ between nitroglycerin and placebo.

Antropyloroduodenal pressures. In both nitroglycerin and control studies there was an initial increase (P < 0.05) in the frequency of IPPW, with a subsequent decrease (P < 0.05) after ingestion of the glucose (Fig. 4A). The overall number of IPPW between 0 and 180 min was reduced by nitroglycerin (70 ± 15 vs. 40 ± 13, P < 0.05). After the drink was ingested, basal pyloric pressure increased (P < 0.05) during the control infusion but decreased (P < 0.05) during nitroglycerin infusion; pyloric tone was accordingly less during nitroglycerin infusion (P < 0.05) (Fig. 4B). There was no

1. D. A. Austin, J. E. Austin, and J. E. Button, The LabView package (National Instruments, Austin, TX). (MAD 16, Synectics/Royal Adelaide Hospital/C. H. Malbert) developed from the LabView package (National Instruments, Austin, TX).

2. Statistical analysis. Differences between the two experiments were assessed using repeated-measures analysis via mixed-model ANOVA (a model with a mixture of fixed and random effects) after log transformation was performed to normalize the data (27). Paired comparisons were done using tests of simple effects (slices of interactions) (35), and the Tukey test was employed for multiple comparisons. Changes in blood pressure and heart rate were evaluated with ANOVA.

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5. Relationships between gastric emptying and motility were assessed using linear regression with robust variance estimation via mixed-model analysis to allow for repeated values in each subject (34). Data are shown as means ± SE. A P value <0.05 was considered significant in all analyses.

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difference in the number of distal antral pressure waves (recorded at the side hole 3 cm orad to the sleeve) between 0 and 180 min in the two groups (30 ± 6 vs. 25 ± 8). However, the number of antral pressure wave sequences was lower (P < 0.05) during nitroglycerin administration than with placebo (Fig. 4C).

Relationship between gastric emptying and antropyloroduodenal pressures. In both experimental conditions the regressions between the rate of gastric emptying and the number of the IPPW were significant and inverse (control: P = 0.03; nitroglycerin: P < 0.05) (Fig. 5). The regressions between gastric emptying and pyloric tone (control: P = 0.22; nitroglycerin: P = 0.86) and between the rate of gastric emptying and the number of the antral pressure wave sequences (control: P = 0.23; nitroglycerin: P = 0.10) were not significant.

DISCUSSION

The major observations in this study are that after ingestion of 300 ml of 25% glucose in the supine position, 1) there is an inverse relationship between the rate of gastric emptying and the frequency of phasic pressure waves localized to the pylorus, and 2) the NO donor nitroglycerin, in a dose of 5 µg/min, slows gastric emptying and intragastric distribution, affects the organization but not the number of antral pressure waves, and inhibits pyloric motility.

Previous studies (13, 14, 31) have provided persuasive evidence that phasic and tonic pressure waves localized to the pylorus influence transpyloric flow by acting as a brake. Gastric emptying of nutrient-
containing liquids, such as 25% glucose, is regulated predominantly by feedback from receptors in the lumen of the small intestine (6, 19, 21), and the presence of nutrients in the small intestine (fat, glucose, amino acids, and acid) stimulates phasic (IPPW) and tonic pyloric motility (13, 14, 32). It has not been established whether IPPW or pyloric tone is the more important of these two mechanisms. Although the demonstration of a correlation does not establish causation, it is not surprising that there is an inverse relationship between gastric emptying of a nutrient liquid and the frequency of IPPW. In contrast, there was no significant relationship between gastric emptying and pyloric tone. Although the number of subjects that we studied was relatively small, this observation suggests that IPPW may play a greater role than pyloric tone in modulating transpyloric flow.

The observation that nitroglycerin inhibits both phasic and tonic pyloric motor responses to a nutrient liquid meal is consistent with previous observations in animals (1, 25) and humans (28) and suggests that NO mechanisms may be important in mediating the effects of small intestinal nutrients on gastric emptying and pyloric motility. Studies in animals using NO synthase inhibitors such as N\(^\text{G}\)-nitro-L-arginine-methyl-ester and N\(^\text{G}\)-nitro-L-arginine indicate that NO inhibits pyloric motility (1, 25). NO synthase activity has been reported to be absent in infantile hypertrophic pyloric stenosis (33). The importance of NO mechanisms in the control of gastric motility does not appear to be nutrient specific (20, 28). For example, we have demonstrated in normal volunteers that the stimulation of phasic and tonic pyloric motility by intraduodenal triglyceride infusion is attenuated by nitroglycerin (28). Despite this, it would certainly still be of interest to evaluate the effects of nitroglycerin on pyloric motility after ingestion of other nutrients and different concentrations of glucose.

Although the overall reductions in both IPPW and pyloric tone by nitroglycerin were substantial, there was a suggestion that suppression of basal pyloric pressure persisted throughout, whereas the effect on IPPW was biphasic. However, this is likely to be attributable to the greater variability in the number of IPPW compared with basal pyloric pressure in the control experiment; suppression of IPPW by nitroglycerin was most evident when the number of IPPW was greater in the control study. The magnitude of the observed reduction in pyloric tone is likely to be mechanically significant. An increase in basal pyloric pressure as low as 2 mmHg has the capacity to prevent transpyloric flow (32). There was a modest, albeit significant, overall slowing of gastric emptying induced by nitroglycerin, rather than the acceleration that might have been predicted from the effects of nitroglycerin on phasic and tonic pyloric pressures. This observation is consistent with a previous report (20) and suggests that nitroglycerin influences motor mechanisms, other than the pylorus, that are important in the regulation of gastric emptying. We noted that administration of nitroglycerin was associated with increased retention of the drink in the proximal stomach, consistent with a reduction in proximal gastric tone, which would favor slower gastric emptying (4, 16). The effects of nitroglycerin or NO synthase inhibitors on proximal gastric motility in humans have not been evaluated to our knowledge, but animal studies have established that proximal gastric relaxation in response to small intestinal nutrient infusion is attenuated by NO synthase inhibitors (10, 23). Antral pressure waves, particularly those temporally associated with duodenal pressure waves, are also likely to be important in the rate of gastric emptying (9, 31), and these were reduced by nitroglycerin. The magnitude of the effect of nitroglycerin on proximal stomach emptying was small, albeit statistically significant, and nitroglycerin had no effect on the content of the distal stomach. Accordingly, it is unlikely that the observed effect of nitroglycerin on antral (or pyloric) motility is secondary to changes in the intragastric distribution of the meal. Together, our observations strengthen the concept that the fundus, antrum, and pylorus all play a role in the regulation of gastric emptying of nutrient-containing liquids and that these mechanisms interact and have the capacity to compensate so that changes in the overall rate of emptying are minimized (3, 16, 22). The slowing of gastric emptying induced by NO synthase inhibitors observed in animal studies (24, 25) may primarily reflect the stimulation of proximal gastric motility, particularly because proximal gastric tone is increased by these agents (23). Ideally, the effect of NO mechanisms on proximal stomach, antral, and pyloric motility in humans would be evaluated by concurrent measurements, but such studies would pose substantial technical challenges. In the current study a dose of nitroglycerin known to have cardiovascular effects and be well tolerated (15) was evaluated. It would be of interest to evaluate the effects of different doses of nitroglycerin on gastric emptying (interpretation of our observations should be limited to the dose of nitroglycerin that was used) and also to determine the impact of intragastric volume and gravity, which have major effects on gastric emptying of low nutrient liquids (2, 3, 16, 18). Similarly, evaluation of the effects of nitroglycerin on gastric emptying in patients with gastroparesis would be of interest, particularly in diabetic gastroparesis, in which there is evidence that defective proximal gastric relaxation may reflect impaired expression of NO synthase in the gastric myenteric plexus (29).

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