Load-sensitive rat gastric vagal afferents encode volume but not gastric nutrients

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Mathis, Carole, Timothy H. Moran, and Gary J. Schwartz. Load-sensitive rat gastric vagal afferents encode volume but not gastric nutrients. Am. J. Physiol. Regul. Integrative Comp. Physiol. 43: R280–R286, 1998.—To assess nutrient sensitivity in a population of gastric load-sensitive vagal afferents, their neurophysiological activity was examined in anesthetized rats with inflated pyloric cuffs after gastric infusion of a range of volumes of nutrient and equiosmotic saline solutions. Responses to physiological saline loads (1, 2, 4, and 8 ml) were compared with responses elicited by the same volume range of carbohydrate (12.5% glucose), protein (12.5% peptone), and equiosmotic hypertonic (750 mosM) saline. The threshold load volume of physiological saline required to increase gastric vagal afferent activity was 1 ml. Thereafter, there was a dose-dependent relationship between increasing gastric volume and firing rate and between gastric volume and pressure. The dose-response relationships elicited by glucose, peptone, and equiosmotic hypertonic saline loads did not differ from those elicited by physiological saline loads. These data identify a population of gastric load-sensitive vagal afferents unresponsive to the chemical composition of gastric contents and are consistent with a role for gastric vagal volume signals but not gastric nutrient content in the negative feedback control of ingestion.

visceral afferents; food intake; vagus; brain-gut communication

VOLUME AND CHEMICAL characteristics of ingested food in the upper gut have been proposed to generate signals that mediate the negative feedback control of ingestion. The sensory component of vagus nerve is the major neuroanatomical link from the gastrointestinal sites that handle ingested nutrients to the central nervous system sites that mediate the control of food intake. An important role for meal-elicited vagal afferent signals in ingestion has been suggested by results from studies demonstrating that transection of or damage to the afferent vagus blocks the ability of duodenal nutrients to suppress sham feeding (21, 22, 24) and attenuates the ability of the brain-gut peptide cholecystokinin to suppress food intake (19, 20). Consistent with this role, electrophysiological studies have identified upper gut vagal afferent fibers sensitive to load volume (2, 10, 12) and load chemical characteristics, including macronutrient composition, pH, and osmolarity (10). Specifically, volume sensitivity has been identified in both gastric and duodenal vagal afferents, whereas studies using nutrient loads have identified duodenal, but not gastric, vagal afferent nutrient sensitivity. Whether gastric load-sensitive vagal afferents are also sensitive to the nutrient content of meals in the stomach is not known.

Results from behavioral studies of the ability of gastric nutrients to inhibit ingestion have led to contradictory conclusions concerning the existence and role of gastric nutrient sensitivity. The ability of gastric nutrient loads to inhibit feeding in rats has been investigated using an inflated pyloric cuff to confine loads to the stomach. With this technique, Deutsch and colleagues (3–7) have demonstrated that food intake is a function of both the load nutrient composition and gastric volume and have proposed that gastric nutrient content signals satiety. Recent studies employing more precise stimulus control (11) have demonstrated that gastric load volume, and not the chemical composition of a range of nutrient loads, dose-dependently determined the degree of inhibition of food intake. These data, in contrast to previous results, support the notion that gastric load volume, and not gastric nutrient content, signals satiety.

To evaluate nutrient sensitivity in a population of gastric load-sensitive vagal afferents, the present study examined vagal neurophysiological responses to a range of gastric volumes varying in nutrient content and osmolarity in rats with inflated pyloric cuffs. In addition, the change in gastric pressure produced by each load volume and nutrient type was assessed.

METHODS

Male Charles River Sprague-Dawley rats (n = 16, 300–400 g), food deprived overnight, served as subjects in experiments. Rats were anesthetized with a mixture of 4:3 ketamine HCl/xylocaine [100 mg/ml ketamine HCl (Aveco)/20 mg/ml Rompun (Mobay)]; this mixture was administered at a dose of 0.1 ml/kg im, and supplemental injections were administered as necessary to maintain a surgical level of anesthesia. Body temperature was monitored and maintained at 36–37°C with a warm water heating pad (Baxter, K Module).

Surgical procedures. A tracheostomy was performed to facilitate respiration, and a 5-Fr feeding tube was inserted into an incision in the cervical esophagus and advanced distally such that the tip of the tube terminated in the gastric corpus, <1 cm distal to the lower esophageal sphincter. This cannula permitted infusion of liquid gastric loads. A laparotomy was performed, and the following devices were implanted. At the fundic level, a saline-filled length of PE-10 manometry tubing (Intramed) was inserted into a fundic incision and advanced into the stomach such that the open tip of the catheter rested in the distal antrum, ~1 cm from the pylorus. This manometry tube was attached to a blood pressure transducer for the measurement of gastric pressure (mmHg). Gastric pressure signals were fed to amplifiers (WPI Transbridge), and output was recorded onto computer hard disk and videotape for subsequent analysis using computerized hardware and software (GW Instruments SuperScope). A second polyethylene tube (ID 1.2 mm, OD 1.7 mm) was
inserted through the same fundic incision and served as a gastric drainage catheter. During gastric infusions, this catheter was clamped to prevent the drainage of gastric contents.

A pyloric cuff fashioned from a Silastic inflatable bag (4 mm wide × 5 cm long) attached to Silastic tubing (width = 1 cm; Bourne Laboratory, White Plains, NY) was threaded around the pyloric sphincter region in two steps. First, the end of the cuff bag was threaded through a 5-mm space made at the top of the pylorus in between the antpyloric ligament and the distal antrum. The end of the cuff bag was then drawn through a 5-mm space made at the bottom of the pylorus between the pancreatic tissue and adjoining mesenteric blood supply. The beginning and end of the pyloric cuff bag were sutured to one another to form the cuff as a loop, and this loop spanned the antpyloro-duodenal junction. When inflated with 0.5–0.7 ml of distilled water, the pyloric cuff would completely occlude this junction and prevent gastric contents from reaching the duodenum. The combination of the feeding catheter, pyloric cuff, and gastric drain permitted the stomach to be distended reversibly by infusion of gastric loads, followed by drainage through the gastric drain catheter. The pyloric cuff inflation tube exited through the laparotomy incision. The abdominal muscle wall was closed with a purse string suture, and the outer skin was sealed with acrylic cement.

Neurophysiological recordings. The vagus nerve was exposed in the neck, and small bundles of nerve fibers from the left cervical vagal trunk were peeled off and the distal cut ends were placed on tungsten wire electrodes (A-M Instruments). Vagal afferent discharges were amplified using standard techniques and were monitored by oscilloscope and audio monitor. The activity of individual fibers was discerned by finer dissection of the nerve trunk and use of a dual time/amplitude window discrimination of spike activity (BAK Instruments). The dual time/amplitude window discriminator permitted identification of individual units with unique time-amplitude signatures. Vagal impulses were recorded on videotape for on-line and subsequent analysis of spike frequency and number using computerized hardware and software (SuperScope, GW Instruments).

Experimental protocol. To identify a unit for investigation, each fiber isolated as described above was screened for its spontaneous discharge activity. Only fibers with spontaneous activity were screened for their response to gastric load stimuli. Identification of a gastric afferent fiber employed a 2-ml gastric load as a probe stimulus. Only fibers with spontaneous activity were screened for their response to gastric load stimuli. Identification of a gastric afferent fiber employed a 2-ml gastric load as a probe stimulus. Only fibers with spontaneous activity were screened for their response to gastric load stimuli. Identification of a gastric afferent fiber employed a 2-ml gastric load as a probe stimulus.

Once a gastric load-sensitive vagal afferent was isolated, dose-response relationships between gastric load volume (ranging from 1 to 8 ml) and vagal afferent firing rate were performed with the following solutions: physiological saline (0.9%; 300 mosM, pH 7), hypertonic saline (2.25%; 750 mosM, pH 7), and one nutrient-containing solution consisting of either glucose (n = 8 units) (12.5%; 0.5 kcal/ml, 750 mosM, pH 7) or peptone (n = 8 units) (12.5%; 0.5 kcal/ml, 750 mosM, pH 7). All solutions were warmed to a temperature of 36–37°C before gastric infusion. The nutrients, osmotic concentrations, and volumes tested were chosen because they were within the range of those used to inhibit food intake when infused and confined in the stomach with a pyloric cuff (11).

Solutions were tested in a random order, and within one given solution, volumes were administered in increasing order (1, 2, 4, and 8 ml). Each gastric load volume was administered after a 2-min recording of baseline activity. Loads were infused manually at a rate of 0.5 ml/s and were maintained in the stomach for 30 s after the end of the infusion. Once a volume was tested, all gastric contents were removed by opening the gastric drain, allowing the stomach to empty by gravity. A 2-min delay was interposed between the drainage of one gastric load and the administration of the subsequent load. This pattern of load and drainage was repeated until the completion of the dose-response curve for each solution. Once a dose-response range for one solution was completed, the stomach was carefully rinsed by slowly (<0.2 ml/s) infusing 3–5 ml of warm isotonic saline while maintaining the gastric drain open.

To investigate the stationarity of unit activity during the course of the experiments, the response to 2-ml physiological saline loads was checked twice; once after completion of the dose-response relationship with physiological osmotic saline and once after the nutrient-containing solution.

The gastric receptive fields of the fibers under study were confirmed at the end of experiments by gently but firmly probing the external gastric wall with a blunt-tipped glass rod (tip diameter 1.2 mm) in the nondistended stomach as previously described (12, 13). A receptive field was defined as a portion of the stomach wall where probing a 2-mm-diameter region with a glass rod would elicit a rate of discharge at least two standard deviations above the spontaneous baseline rate. All the units tested except two had their receptive field along the great curvature in the antral part of the ventral stomach. The receptive field of one unit was located at the border of the ventral corpus and the fundus, and the receptive field of the remaining unit was located in the upper part of the ventral fundus.

Data analysis. Analyses were performed with the use of computerized hardware and software (SuperScope, GW Instruments). Neural activity was automatically analyzed for spike frequency. For each unit and each dose-response relationship, an increase in spike frequency was expressed in terms of a percentage of the maximal firing rate obtained during the collection of dose-response data for that fiber.

Average gastric pressure before and during the course of the same 30-s periods that were used for spike analysis were determined from the gastric pressure records with SuperScope software. Changes in intragastric pressure above baseline levels induced by gastric loads were calculated, and all pressure data were expressed as a percent of the maximal change induced by physiological saline gastric loads for subsequent statistical analysis.

For each group, the stationarity of responses to 2-ml physiological saline loads were tested by repeated one-way analysis of variance (ANOVA), with trial as the main factor. Statistically significant changes in vagal afferent spike frequency and gastric pressure were tested by separate two-way repeated-measures ANOVAs, with volume and composition of solution as factors. Statistical comparisons of the dose-response relationships for glucose and equimolar peptone were tested by two-way ANOVA, with nutrient (peptone or glucose) and volume as factors. Data are expressed as means ± SE, and differences between individual volume/solution pairs were determined using planned t-comparisons (P < 0.05).
RESULTS

Vagal afferent responses to gastric loads. Baseline firing rate among the 16 identified vagal afferent units with gastric receptive fields ranged from 0.1 to 16 spikes/s. During the course of all experiments, there were no significant variations in the increase in firing rate induced by 2-ml physiological saline gastric load tests for stationarity [glucose group, $F(1,7) = 0.256, P > 0.5$; peptone group $F(1,7) = 0.375, P > 0.6$].

Responses to glucose. An example of the neurophysiological responses to increasing volumes of physiological saline, 750 mosM saline, and equiosmotic hypertonic glucose in a single gastric vagal afferent fiber is shown in Fig. 1. Prestimulus baseline activity for units in this group ($n = 8$) was constant throughout the course of each study and was not affected by the composition of the gastric load [$F(2,11) = 1.157, P > 0.3$] (Figs. 1 and 2). Increasing volumes of gastric physiological saline loads dose-dependently increased the firing rate in these vagal afferents [$F(7,4) = 9.57, P < 0.01$] (Fig. 2, top). This significant dose-dependent increase in firing rate was also found for 750 mosM saline [$F(7,4) = 10.1, P < 0.01$] and equiosmotic glucose loads [$F(7,4) = 8.63, P < 0.01$] (Figs. 1 and 2). For all three solutions tested (physiological saline, 750 mosM saline, and 750 mosM glucose), the threshold load volume that elicited a significant increase in firing rate compared with baseline was 1 ml, the lowest volume tested, with maximal stimulation induced by a 4-ml load ($P$ values < 0.05) (Figs. 1 and 2). Comparisons of the dose-response curves generated for the vagal afferent responses to the range of gastric load volumes of physiological saline, 750 mosM saline, and 750 mosM glucose revealed no significant differences between these functions [$F(2,14) = 0.608, P > 0.5$]. Within each of the physiological saline, hypertonic saline, and equiosmotic glucose solutions, post hoc t-tests revealed that the gastric vagal afferent response to 2-ml loads was significantly greater than that produced by 1 ml and that the response to 4 ml was significantly greater than the 2-ml response ($P$ values < 0.05), indicating a graded volume-dependent response to gastric load size.

The change in intragastric pressure above baseline levels increased significantly with increasing gastric load volume for all three solutions tested in this group [physiological saline $F(7,3) = 9.31$, 750 mosM saline

![Electrophysiological recordings from a single gastric vagal afferent showing prestimulus baseline activity and response induced by increasing volumes of gastric loads composed of physiological saline, 750 mosM saline, and equiosmotic glucose. Neural spike under study is represented at top right.](image)
Fig. 2. Effects of gastric load volumes of physiological saline, 750 mosM saline, and equiosmotic glucose on gastric vagal afferent firing rate (top, n = 8) and gastric pressure (bottom). Increasing gastric load volume dose-dependently increased 1) firing rate in vagal afferents and 2) gastric pressure. The threshold volume of peptone tested that elicited a significant increase in vagal firing rate compared with baseline was 1 ml, with maximal stimulation induced by a 4-ml load. Dose-response relationships between gastric load volume and vagal afferent discharge (top) and gastric load volume and gastric pressure (bottom) were identical among these 3 solutions, irrespective of the nutrient content or osmolarity of the gastric load. Results are expressed as means ± SE.

F(7,3) = 9.01, 750 mosM glucose F(7,3) = 8.45; P values < 0.05) (Fig. 2, bottom). Furthermore, the pressure-volume relationship was not significantly different for physiological or 750 mosM saline solutions compared with 750 mosM glucose [F(2,14) = 2.1, P > 0.1]. Post hoc tests within each infusate solution revealed that the 2-ml load produced significantly greater changes in gastric pressure than the 1-ml load and that the pressure change generated by 4-ml loads was significantly greater than that produced by 2-ml volumes.

Responses to peptone. An example of the neurophysiological responses to increasing volumes of physiological saline, 750 mosM saline, and equiosmotic hypertonic peptone in a single gastric vagal afferent fiber is shown in Fig. 3. Prestimulus baseline activity for units in this group (n = 8) was constant throughout the course of each study and was not affected by composition of the gastric load [F(2,11) = 0.978, P > 0.5] (Figs. 3 and 4). Increasing volumes of gastric physiological saline loads dose-dependently increased the firing rate in these vagal afferents as well [F(7,4) = 8.37, P < 0.01] (Fig. 4, top). This significant dose-dependent increase in firing rate was also found for 750 mosM saline [F(7,4) = 9.35, P < 0.01] and equiosmotic peptone loads [F(7,4) = 7.27, P < 0.01]. For all three solutions tested (physiological saline, 750 mosM saline, and 750 mosM peptone), the threshold load volume that elicited a significant increase in firing rate compared with baseline was 1 ml, the lowest volume tested, with maximal stimulation induced by a 4-ml load (P values < 0.05) (Figs. 3 and 4). Comparisons of the dose-response curves generated for the vagal afferent responses to the range of gastric load volumes of physiological saline, 750 mosM saline, and 750 mosM peptone revealed no significant differences between these functions [F(2,14) = 0.454, P > 0.6]. Within each of the physiological saline, hypertonic saline, and equiosmotic peptone solutions, post hoc t-tests revealed that the gastric vagal afferent response to 2-ml loads was significantly greater than that produced by 1 ml and that the response to 4 ml was significantly greater than the 2-ml response (P values < 0.05), indicating a graded volumedependent response to gastric load size.

The change in intragastric pressure above baseline levels increased significantly with increasing gastric load volume for all three solutions tested in this group (physiological saline F(7,3) = 7.21, 750 mosM saline F(7,3) = 8.23, 750 mosM peptone F(7,3) = 9.21; P values < 0.05) (Fig. 4, bottom). Furthermore, the pressure-volume relationship was not significantly different for physiological or 750 mosM saline solutions compared with 750 mosM peptone [F(2,14) = 0.81, P > 0.6]. Post hoc tests within each infusate solution revealed that the 2-ml load produced significantly greater changes in gastric pressure than the 1-ml load and that the pressure change generated by 4-ml loads was significantly greater than that produced by 2-ml volumes.

Finally, comparisons between the dose-response curves generated for the vagal afferent responses to the range of load volumes of equicaloric 750 mosM glucose and 750 mosM peptone revealed no significant differences between these functions [F(1,15) = 0.002, P > 0.9] (Fig. 5).

**DISCUSSION**

The present study demonstrates that gastric load-sensitive vagal fibers signal gastric volume but not the chemical composition of the gastric contents. These results are consistent with previous data demonstrating a dose-dependent relationship between the neurophysiological response of gastric vagal afferent fibers and intragastric volume (14, 15). Although hypertonic
caloric and equiosmotic saline loads produced a volume-dependent increase in firing rate of gastric vagal afferents and in gastric pressure, none of the chemicals tested in the present study produced shifts in the dose-response relationships compared with that obtained with physiological saline loads. They also failed to induce significant changes in baseline vagal afferent activity.

Our protocols identified load-sensitive units with relatively low volume thresholds for an increase in firing rate above spontaneous baseline levels (1 ml) and low maximally effective volumes, where many individual fibers responded maximally at 4- or 8-ml volumes. There is clearly heterogeneity in the dynamic response range of gastric load-sensitive vagal afferents. Previous neurophysiological studies have identified populations of rat gastric vagal afferents whose thresholds ranged from 1 to 10 ml and whose maximal response rates are elicited by loads ranging from 8 to 15 ml (2, 15). Such heterogeneity has also been demonstrated in other vagally innervated gut tissues, including the esophagus (18) and the colon (8). In the present study, all but 2 of the 16 units tested had receptive fields in the distal antrum, whereas data from studies involving units with larger volumes required for maximal firing tend to represent a greater mixture of both fundus, antrum, and corpus receptive fields (2, 15).

The present results reveal that increasing volumes of nutrients and nonnutrients elicit equivalent volumedependent increases in gastric pressure. In contrast, duodenal carbohydrate and protein exposure have been demonstrated to stimulate both duodenal and gastric motility in the rat (16). Taken together, these data suggest that gastric nutrient content fails to alter gastric motility.

The present increases in both gastric pressure and vagal afferent firing rate with increasing gastric load volume support the idea that gastric pressure plays an important role in determining firing rate in these fibers, independent of the gastric load content.

Our screening criteria, requiring spontaneous baseline activity and an increase in firing rate in response to inflation, may exclude other potentially nutrient-sensitive populations of gastric vagal afferents. For example, some duodenal afferents have been shown to respond to chemical stimulation alone, whereas others...
respond to both mechanical and chemical stimulation (10). Typically, fibers sensitive to the gastrointestinal presence of chemicals only have been described as spontaneously silent, with a distinguishing brief on-off response when the stimulus is applied and withdrawn (10). In addition, the possibility of chemosensitivity in gastric splanchnic afferent fibers remains unexplored.

The response range of the present fibers suggests that they may be sufficient to carry volume signals important in the negative feedback control of ingestion. The characteristics of our dose-response relationship between gastric load volume and vagal afferent responses are in agreement with behavioral experiments in rats in which gastric loads induced volume-dependent reductions in subsequent food intake over a dose range of 1–10 ml (11). Support for the suggestion that gastric afferent innervation detects critical volume signals in the negative feedback control of ingestion comes from results of studies demonstrating that removal of food from the gastric cavity results in compensatory eating (1, 4, 9, 23), whereas the opposite maneuver reduces subsequent meal size (3, 9, 11). The inability of nutrient loads confined to the stomach to modulate gastric vagal afferent activity relative to saline is consistent with a role for volume but not nutrient sensitivity in the inhibition of food intake produced by gastric contents. Phillips and Powley (11) have shown that nutrient loads confined to the stomach with a pyloric cuff are no more potent than physiological saline in reducing food intake. Finally, studies of Deutsch and colleagues (5–7) have proposed that gastric loads of liquid-mixed nutrient meals, as well as fat, signal satiety. Although fat would therefore appear to be a reasonable stimulus to use in the present study, this work required rinsing the stomach in between successive trials to completely clear any residual gastric nutrient or nonnutrient solutions. Several trials with fat diets in pilot studies revealed that this was not reliably possible for these loads, because the fat tended to adhere to the gastric lining.

Perspectives

Although the present results demonstrate that nutrient content does not modify the gastric vagal afferent response to gastric loads, gastric load-sensitive fibers have been shown to be activated by the presence of nutrients in the duodenal lumen. Specifically, both carbohydrate (glucose) and protein (peptone) solutions have been demonstrated to stimulate antral contractions and bursts of antral vagal load-sensitive afferent activity (16). The presence of calories in the duodenal

![Fig. 4. Effects of gastric load volumes of physiological saline, 750 mosM saline, and equiosmotic peptone on gastric vagal afferent firing rate (top) and gastric pressure (bottom). Again, increasing gastric load volume dose dependently increased 1) firing rate in vagal afferents (n = 8) and 2) gastric pressure. The threshold volume of peptone tested that elicited a significant increase in vagal firing rate compared with baseline was 1 ml, with maximal stimulation induced by a 4-ml load. Dose-response relationships between gastric load volume and vagal afferent discharge (top) and gastric load volume and gastric pressure (bottom) were identical among these 3 solutions, irrespective of the nutrient content or osmolarity of the gastric load. Results are expressed as means ± SE.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00241.2016)

![Fig. 5. Comparison of the effects of gastric loads of 750 mosM glucose (n = 8) and peptone (n = 8) in 2 groups of gastric vagal load-sensitive afferents. Dose-response relationships between gastric load volume and vagal afferent discharge were identical for these 2 macronutrients.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00241.2016)
infusate elicited significant increases in afferent neural activity beyond that attributable to osmolarity alone. In addition, duodenal peptone was more effective than equicaloric glucose in eliciting this gastric vagal afferent activity. These data demonstrate that antral load-sensitive vagal afferents are differentially responsive to 1) the caloric content of nutrients in the duodenum and 2) the type of macronutrient infused into the duodenum. Thus activity in these antral load-sensitive vagal afferents, although apparently not modulated by the nutrient content of the gastric lumen, still provides nutrient- and calorie-specific signals that may be important in the negative feedback control of gastrointestinal function and ingestion.

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