Gastric volume detection after selective vagotomies in rats

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Phillips, Robert J., and Terry L. Powley. Gastric volume detection after selective vagotomies in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1626–R1638, 1998.—Rats receiving intragastric infusions of 2.5, 5.0, 7.5, or 10.0 ml of normal saline while their pylori are reversibly occluded suppress meal size to the smallest infusion and display a dose-dependent reduction across volumes [Phillips, R. J., and T. L. Powley. Am. J. Physiol. 271 (Regulatory Integrative Comp. Physiol. 40): R766–R779, 1996]. To evaluate the contributions of the vagus to this detection of gastric volume, groups prepared with different selective vagotomies and equipped with pyloric cuffs and gastric catheters were tested. Liquid diet consumption during a 30-min feeding bout was measured after infusions of 5.0 and 10.0 ml of normal saline on cuff-open and cuff-closed trials. Consistent with earlier observations, sham animals with cuffs closed exhibited volume-dependent suppression of food intake to the infusions, and completely vagotomized animals did not inhibit feeding in response to the loads. In cuff-closed trials, the suppression function slopes of the selective vagotomy groups were intermediate to those of the shams and the completely vagotomized animals. Furthermore, for the different groups, the extent of suppression after vagotomy was proportional to the density of the afferent innervation and the different vagotomized animals. Furthermore, for the different groups, the extent of suppression after vagotomy was proportional to the density of the afferent innervation and the different vagotomized animals. Specifically, the group with the gastric branches spared (nonsignificantly attenuated in comparison to shams) and the group with the hepatic branch spared (significantly attenuated with respect to shams) both still exhibited significant dose-dependent suppression slopes (compared with completes), whereas the group with only celiac branches spared was not significantly different from completely vagotomized animals. In sum, the vagus nerve mediates the detection of the gastric volumes tested, and the different branches of the vagus make distinctive contributions to this afferent feedback.

duodenum; mechanoreceptors; stomach; vagus; visceral afferents

In a recent series of experiments using the pyloric cuff to separate gastric function from that of the more distal gastrointestinal (GI) tract, we demonstrated that rats were able to detect small changes in gastric volume and that such animals were also able to differentiate between different volumes of infusates (25). In addition, for a variety of test meals restricted to the gastric compartment, we observed that the only salient cue of the test stimuli was volume (or distension). These results reinforce previous findings that animals are able to detect subtle changes in gastric distension and to alter meal size in response to stomach fill (7, 39). However, the observations do not address the question of possible pathway(s) by which meal-related distension cues are transmitted from the periphery to central targets.

Although a role for spinal visceral afferents cannot be excluded, the prominent candidate pathway is the vagus nerve. Electrophysiological studies have shown that distension of the stomach evoked afferent impulses in cervical vagal fibers (13, 22). Furthermore, when neural activity in the hypothalamic feeding areas of dogs (35) and cats (1) was recorded during gastric distension by a balloon, neurons showed a significant increase in firing rate, and cutting the vagi eliminated this response. In support of these findings, behavioral studies have shown that rats are generally unable to detect distension of the stomach after subdiaphragmatic vagotomy (6, 10). Although it should be noted that even though Gonzalez and Deutsch (10) found that rats with only their hepatic branch of the vagus left intact were unresponsive to gastric distension, the animals were still able to respond to withdrawal of gastric contents, indicating some sparing of mechanosensory sensitivity.

Anatomically, the recently characterized pattern of GI innervation by the vagus nerve and the morphology of its afferent endings also implies a prominent role for the vagus in the detection of gastric volume (or distension). The nerve supplies an extensive network of afferent endings to the stomach muscle wall (4, 29), myenteric plexus (4, 29), and mucosa (3, 12). Vagal afferent endings in the GI tract smooth muscle and myenteric plexus consist of at least two morphologically distinct terminal types: intramuscular arrays (IMAs) and intraganglionic laminar endings (IGLEs). IGLEs are more extensively distributed throughout the GI tract and are located in association with the myenteric ganglia (20, 21, 29, 32), whereas IMAs appear to be more localized, particularly in sphincters and in the muscle wall of the forestomach (4, 17, 29).

Thus electrophysiological, behavioral, and anatomic findings point to the vagus as an important pathway for the transmission of information about gastric distension. This previous work does not, however, indicate which specific branch(es) of the vagus may carry information to the central nervous system (CNS). After passing through the diaphragm, the nerve separates successively into a hepatic branch, paired celiac branches, and paired gastric branches (30). The hepatic branch and gastric branches innervate both the stomach and first part of the small intestine, whereas the celiac branches appear to innervate the intestines and not the stomach (2, 24; see also results of present study). Thus one goal of the present study was to reaffirm the role of the vagus in the detection of physiological levels of stomach distension and then, more particularly, to employ different selective vagotomies, clarifying which subdiaphragmatic branch(es) of the vagus detect gastric distension. It is most likely, of course, that the gastric branches are involved in relaying distension information centrally, but it is also possible that the hepatic branch is involved. Predicting the role of the hepatic branch is problematic, however, because 1) its afferent supply to the stomach has only
recently been described (24), 2) its distribution is limited to circumscribed areas of the fundus and distal antrum (24), and 3) its stomach projection has not yet been functionally characterized. Thus a second goal was to characterize the afferent projections associated with the different branches of the vagus, to determine whether a relationship between the regional distributions of vagal afferents and an animal’s ability to detect gastric distension exists.

Finally, the experiment was designed to address two additional issues concerning vagal mechanoreceptor function. First, because previous results (e.g., Ref. 16) have suggested the possibility that sudden surgical removal of the negative feedback provided by GI mechanoreceptors might under some conditions lead to transient overeating, the first meals after vagotomy were analyzed. Second, because we have recently observed (23, 26, 27) that vagal mechanoreceptors exhibit dramatic reorganization and regeneration after axotomy, the present experiments included observations to evaluate possible plasticity of vagal projections to the stomach and intestines occurring during the present test period.

METHODS

Subjects. Male Sprague-Dawley rats (Harlan) were housed individually and maintained on a 12:12-h light-dark schedule (lights on at 0600, lights off at 1800) at 23°C. Before any surgery, animals had ad libitum access to tap water and pelleted rat chow (Lab Diet no. 5001, PMI Feeds). Five days before surgery, all animals were switched to Isocal, a nutritionally complete liquid diet (1.05 kcal/ml; Mead Johnson, Evansville, IN), to permit accurate measurement of consumption without spillage and to minimize problems associated with the vagotomy procedure; animals were maintained on Isocal for the duration of the experiment.

Surgical procedures. To evaluate the contributions of the various subdiaphragmatic branches of the vagus to the detection of gastric volume, five experimental groups equipped with pyloric cuffs and indwelling gastric catheters were tested. The groups consisted of rats with 1) all branches intact (shams), 2) only the ventral and dorsal gastric branches intact (gastrocs-spared group), 3) only the ventral and dorsal celiac branches intact (celiacs-spared group), 4) only the hepatic branch intact (hepatics-spared group), and 5) both the anterior and posterior vagal trunks transected above all abdominal branch points (completes).

Animals were anesthetized with pentobarbital sodium (60 mg/kg ip) and then treated with atropine (1.0 mg/kg sc). Each animal was laparotomized and selectively vagotomized as previously described (28). Briefly, the appropriate branch (or branches) was isolated by dissection, supported by a mic dissecting hook, and cauterized with a high-temperature ophthalmic cautery. For the sham-vagotomized group, the branches were exposed and manipulated, but left intact. Immediately after vagotomy or the sham procedure, in the same surgery, each rat was implanted with a gastric catheter and pyloric cuff as previously described (25). The gastric catheter consisted of Silastic tubing (ID = 0.025 in., OD = 0.047 in.; Specialty Manufacturing) located in the greater curvature of the nonglandular stomach and exteriorized at a modified Luer-Lok needle connector mounted on the dorsal surface of the skull with stainless steel screws and dental acrylic.

For implantation of the pyloric cuff, the body of the stomach was brought into view and the greater curvature was followed until the pylorus and duodenum could be visualized. A curved hemostat was then passed along the dorsal wall of the pylorus, carefully avoiding the gastroduodenal and gastroepiploic arteries. One end of the cuff (40 mm in length and 6 mm in width) was then drawn back through the opening, and the two ends of the cuff were stitched together. The cuff was then inflated with 0.5 ml of water to verify occlusion of the pylorus. Finally, the Silastic tubing attached to the cuff was tunneled subcutaneously to the back of the head, where it was anchored along with the gastric catheter.

Apparatus. After surgery, animals were housed individually in cylindrical acrylic infusion cages (OD = 12 in.) for the remainder of the experiments. Twenty-four hours after surgery, each animal’s catheter was connected to a flow-through swivel (Instech Laboratories) by polyethylene tubing protected by a steel spring, and then the cuff line was securely anchored to the outside of the spring. These connections were maintained chronically to permit inflation and deflation of the cuff as well as gastric infusions of various substances without handling or disturbing the animal. Infusions were delivered using a syringe pump (Harvard Apparatus).

Protocol. Each day, all animals were allowed ad libitum intake from 1700 until 1000 the next day. Short-term food intake (i.e., first 30-min intake each evening, equivalent to the first meal) was measured daily, and the total daily food intake (i.e., 17-h consumption) was also recorded. At 1000, the Isocal was removed. The pyloric cuffs were then inflated and deflated 30 min later. This habituated the animals to the procedure and prevented association of cuff inflation with the presentation of food, and the complete recovery of the 0.5 ml of water used to inflate them provided verification that the cuffs were patent. All swivel systems were tested daily (1030) for patency with a 1-ml saline infusion. Animals were weighed daily at 1620–1630.

Cuff inflation and infusions. On cuff-closed test days, animals had their cuffs inflated and they were infused intragastrically with either 0, 5, or 10 ml of normal saline immediately before 1700, at which time they were presented with Isocal and short-term (30 min) intake was subsequently evaluated. Cuff-open days were identical to cuff-closed days except the cuff was left deflated. The order of cuff-open and cuff-closed testing was counterbalanced over subjects within a given experimental condition and counterbalanced within subjects over the series of experimental conditions. Animals received infusions according to a schedule that was randomized for each subject, and testing occurred 2 out of every 4 days, with the same infusion volume being given on the first 2 days and no infusions on the following 2 days. Baseline (0 ml) and the first infusion volume tested were repeated, and the means of the two scores were used for statistical analysis. Infusions were administered at a rate of 2 ml/min; this rate was chosen and used throughout because it is well within the normal rate of consumption of a liquid diet by a rat (14). Different volumes of gastric loads were controlled by varying the timing of initiating the infusion. Onsets were phased so that all loads were completed at 1700, just as food was made available (e.g., 10-ml infusions were begun at 1655). If the cuff had been inflated during a trial, then it was be deflated at 1730.

Tracer protocols. To verify the selective vagotomies, a Fluorogold tracer strategy was used (28). Before death, each animal received an intraperitoneal injection of 1 mg of Fluorogold (Fluorochrome) in 1 ml of normal saline. Five days later, each animal was given a lethal dose of pentobarbital sodium and then perfused transcardially with 800 ml of 0.9% saline at 40°C followed by 800 ml of 10% phosphate-buffered...
with repeated measures. Post hoc analyses were performed with Tukey’s honestly significant difference test. Comparisons of baseline (0 ml) cuff-open and cuff-closed scores were performed with paired t-tests.

RESULTS

Assessment of vagotomies by the retrograde transport of Fluorogold. Examination of the pattern of labeled cells in the dorsal motor nucleus (DMNX) after retrograde transport of Fluorogold by vagal efferents made it possible to evaluate various vagal cuts for the five experimental groups (28), and all animals included in the respective groups used in the infusion tests displayed appropriate Fluorogold profiles. The columns of preganglionic somata representing the paired gastric- and paired celiac-spared branches were organized as paired, symmetrical, longitudinal distributions forming the DMNX. Figure 1 illustrates these columns at one of the coronal levels analyzed. Sham animals were determined as such by the presence of all columns in both the left and right DMNX columns (see Fig. 1A). Sparing of the ventral and dorsal gastric branches was denoted by a medial column of cells on each side, constituting approximately two-thirds of the frontal view for both of the DMNX columns (see Fig. 1C). The accessory celiac and celiac branches were formed by cells in the lateral poles of both sides of the DMNX (see Fig. 1E). The hepatic branch-spared animals were represented by the fewest preganglionic neurons, and these cells formed a more diffuse column essentially coextensive with that of the anterior gastric branch (see Fig. 1E). Finally, completely vagotomized animals were devoid of any labeled cell bodies in the DMNX (see Fig. 1B).

Gastric volume detection after selective vagotomies. All animals whose data were used for statistical analyses were verified as having had successful sham or selective vagotomy surgery by analyses of their Fluorogold pattern. In addition, they also had to have passed the cuff-verification tests, which consisted of visual determination of proper cuff placement on the pyloric sphincter and the prevention of the leakage of dye into the duodenum by the inflated cuff.

Cuff open vs. cuff closed. Baseline (0 ml gastric infusions) food intakes for the cuff-open and cuff-closed trials did not differ significantly (all P values > 0.05) for shams, gastrics-spared animals, hepatic-spared rats, and completes (see Figs. 2, A-C, and 3B). In contrast, the celiacs-spared group ate significantly more Isolec when their cuffs were closed (P < 0.05) and their small intestines were not stimulated (see Fig. 3A).

Gastric infusions. With the cuff open, increasing volumes of saline significantly reduced the food intake of shams (P < 0.05), but had no effect on the gastrics-spared animals, hepatic-spared group, celiacs-spared animals, or completes (see Figs. 2 and 3). Post hoc analyses of the shams’ cuff-open intake data showed that this group significantly suppressed to 10-ml infusions of saline (P < 0.05) but not to 5-ml infusions (P > 0.05).
When the pyloric cuff was closed, shams, gastrics-spared animals, and the hepatic-spared group showed similar response patterns to infusions (see Fig. 2). Infusions of increasing volumes produced dose-dependent suppressions (all $P$ values < 0.01), with both 5- and 10-ml infusions being significantly different from baseline (all $P$ values < 0.05). In addition, during cuff-closed infusions the three groups were able to differentiate between 5- and 10-ml infusion volumes (all $P$ values < 0.05).

Celiacs-spared animals also significantly suppressed food intake ($P < 0.05$), but only to the largest infusion volume of 10 ml ($P < 0.05$), and they were unable to differentiate between 5- and 10-ml infusions ($P > 0.05$); see Fig. 3A. In contrast to shams and the different selectively vagotomized animals that had some vagal innervation of the GI tract spared, completely vagotomized rats exhibited no indication that they detected either of the volumes infused ($P$ values > 0.05); see Fig. 3B.

Analyses of slopes. A linear regression analysis for the sham animals' baseline and saline infusion conditions indicated a significant effect on intake for volume infused in cuff-closed trials ($P < 0.01$), with a slope of 0.47 ml suppression for each milliliter infused. Comparison of the sham animals' suppression slope with the slopes of the selectively vagotomized groups revealed that the suppression scores of the gastrics-spared animals were only nonsignificantly attenuated ($P > 0.05$), whereas scores of the hepatic-spared, celiacs-spared, and complete animals were significantly attenuated (all $P$ values < 0.01). Further analyses of suppression slopes divulged that, whereas completely vagotomized animals and celiacs-spared animals were not significantly different ($P > 0.05$) from each other, hepatic-spared, gastrics-spared, and sham groups were significantly more sensitive to increasing infusion volumes than were completes (all $P$ values < 0.05).

Postsurgery food intake and body weight recovery for the five experimental groups: general pattern. Four days before surgery, the five groups did not differ significantly in their short-term Isocal intakes, daily Isocal intakes, or body weights (all $P$ values < 0.05; one-way ANOVA).
ANOVA), but by 12 days postsurgery this was not the case (all P values < 0.01; one-way ANOVA). To attenuate the possible effects of residual postsurgery anesthetic on first meal size, only the data from animals that had completed surgery 5 h before the presentation of the first meal were used for short-term intake analysis. Similar findings were found for both short-term or 30-min test and daily intake. Hepatic-spared and completely vagotomized rats ate significantly more than shams in the first 30 min of food presentation and the preceding 16.5 h (all P values < 0.05; Dunnett's t-test), see Fig. 5, A and B. In contrast, the short-term and daily intakes of gastrics-spared and celiacs-spared animals were no different from the values of sham-vagotomized animals (all P values > 0.05; Dunnett's t-test), see Fig. 5, A and B.

Histological determination of GI innervation by the five experimental groups using WGA-HRP. At least three rats from each of the five groups were used to determine the afferent projection pattern. The density and regional distribution of the sensory innervation of the GI tract were distinct for each of the five groups. Shams were the most heavily and completely innervated, with bundles and individual axons located throughout the stomach and small intestine, and crossing from the stomach into the proximal duodenum (see Fig. 2).

Fig. 2. Mean (±SE) milliliters of Isocal consumed in the cuff-closed (●) and cuff-open (○) conditions for shams (A; n = 9), gastrics-spared animals (B; n = 6), and the hepatic-spared group (C; n = 9). Although the response was attenuated in the hepatic-spared group, all 3 of these experimental groups had innervation of the gastric compartment by vagal afferents and showed a dose-response suppression to increasing volume of gastric infusions during cuff-closed trials. (Compare with results in Fig. 3.)

Postscopy food intake for the five experimental groups: first meals after denervation. Additional analysis of short-term and daily intake on the day of surgery revealed a significant effect for Isocal intake (all P values < 0.01; one-way ANOVA). To attenuate the possible effects of residual postsurgery anesthetic on first meal size, only the data from animals that had completed surgery 5 h before the presentation of the first meal were used for short-term intake analysis. Similar findings were found for both short-term or 30-min test and daily intake. Hepatic-spared and completely vagotomized rats ate significantly more than shams in the first 30 min of food presentation and the preceding 16.5 h (all P values < 0.05; Dunnett's t-test), see Fig. 5, A and B. In contrast, the short-term and daily intakes of gastrics-spared and celiacs-spared animals were no different from the values of sham-vagotomized animals (all P values > 0.05; Dunnett's t-test), see Fig. 5, A and B.

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Fig. 3. Mean (±SE) milliliters of Isocal consumed in the cuff-open (○) and cuff-closed (●) conditions for the celiacs-spared animals (A; n = 9) and the completely vagotomized animals (B; n = 9). Gastric compartment for both groups was devoid of vagal afferent innervation. Completes did not suppress to any infusion conditions, and celiacs-spared group only suppressed to the largest volume tested in cuff-closed condition.
Vagal sensory terminals were also found to be located in a manner consistent with previous findings (38). This typical pattern consisted of IGLEs distributed throughout the GI tract (see Fig. 6B) and IMAs focused primarily within the forestomach region of the stomach (see Fig. 6C). The innervation of the stomach in gastrics-spared animals was similar to shams, but the duodenum was only sparsely innervated, with most of the innervation occurring within the first centimeter. A large proportion of the duodenal innervation by the paired gastric branches was due to fibers that crossed over the pyloric sphincter to innervate the proximal duodenum. The innervation of the GI tract by the hepatic branch was similar to that previously reported (24). In this group, IMAs were located exclusively along the greater curvature of the fundic region of the ventral stomach wall, and IGLEs were densely distributed within the first 3–4 cm of the small intestine. The celiac branches appeared to provide no innervation to the stomach, and only started to densely innervate the small intestine 5–6 cm distal to the pyloric sphincter. Although most of the celiac innervation traveled in the anal direction, some reflected back to innervate the oral portion of the intestine. Afferent terminals provided by the celiac branches appeared to consist exclusively of IGLEs. Finally, the entire GI tract was in essence thoroughly denervated in the complete vagotomy cases. The only innervation consisted of a few
individual axons in the small intestine that most likely represented stray fascicles that were missed during the initial vagotomy procedure.

Plasticity of vagal afferents after selective vagotomy. The above description of vagal sensory innervation was based on bundles, axons, and terminals that were morphologically normal in appearance, but, for each of the four vagotomized groups, signs of vagal afferent regeneration and possible reorganization were found to occur in some but not all of the cases. Regenerating and reorganizing vagal structures were determined to be such, based on their abnormal morphological appearance and the presence of growth cone profiles. Presumably due to the brevity of the study (5–6 wk postvagotomy), however, the changes that were found were not as dramatic as those previously observed for longer postsurgery survival times (23, 26, 27). For all of the shams and two of the three gastrics-spared cases examined, no signs of vagal plasticity were found. In one of the gastrics-spared animals, however, distorted IMAs or abnormally long axons were found in the antrum of the dorsal stomach (see Fig. 7A). Vagal plasticity in the stomachs of the celiacs-spared and complete groups was similar in appearance and consisted of regenerating vagal bundles and individual axons entering near the lower esophageal sphincter and radiating toward the greater curvature. These small bundles had only begun to innervate the stomach and gave off individual axons that terminated primarily in growth cone profiles (see Fig. 7B). For all of the hepatic-spared and completely vagotomized animals surveyed, there was no sign of vagal plasticity occurring in the small intestine, although, for one of the three celiacs-spared animals, abnormal vagal profiles were found to occur at ~5 cm distal to the pylorus. These consisted of long, straight IMAs or axons that traveled in the longitudinal direction (see Fig. 7C) and abnormal innervation of myenteric ganglia (see Fig. 7D).

**DISCUSSION**

In addition to confirming previous reports that intact animals with their pylori occluded exhibit dose-dependent suppression of food intake to gastric infusions and that completely vagotomized animals do not inhibit feeding in response to gastric loads (e.g., Refs. 6, 10), the present results demonstrate for the first time that animals with selective vagotomies exhibit different responses to increasing gastric loads, depending on the density and distribution of terminals supplied to
the GI tract by the spared vagal afferents. The present observations also establish that signals associated with stomach distension are relayed to the CNS by multiple branches, namely the unpaired hepatic as well as the paired gastric branches, of the vagus. Furthermore, surgical interruption of some of the patterns of vagal afferent feedback, i.e., specific vagal branches, yielded transient overeating.

Verification tests. The interpretations of the present behavioral findings hinge on the accuracy of the vagotomy verification and the afferent labeling strategy. (They also depend on the ability to differentiate between normal and abnormal, i.e., regenerating or reorganizing, vagal innervation patterns, as discussed below.) To determine the accuracy of the selective vagotomy procedure, we used the retrograde tracer Fluorogold, which is taken up by spared vagal efferents and transported back to the DMNX (28), where the different columnar patterns of cell bodies indicate which vagal branch or branches are intact. This technique has been shown to be a reliable tool for verification of the selective vagotomy procedure (e.g., Refs. 2, 24, 28, 36). Concerning the second methodological point above, namely the issue of afferent labeling, it is important to note that WGA-HRP injected into the nodose ganglion in accordance with the present protocol produces relatively complete labeling of vagal afferent projections to the GI tract (24, 38) without labeling.

Fig. 7. Dark-field images of abnormal vagal innervation found in selectively vagotomized animals 5–6 wk postvagotomy. A: almost the entire figure consists of unusually long axons or IMAs (running from top to bottom) within the circular muscle of the antral region (dorsal stomach) of a gastrics-spared animal. Typically, IMAs are short arrays found mostly in the longitudinal muscle of the forestomach, and individual axons are never found to travel for such long distances in a straight trajectory; scale bar = 500 µm. B: growth cone profiles (arrows) originating from 1 or more axons that have reinnervated the dorsal stomach of a hepatic-spared animal. This was characteristic of the vagal plasticity found in ventral and dorsal stomachs of celiacs-spared, hepatic-spared, and completes groups. C: elongated IMAs or axons (arrows) found ~5 cm distal to the pyloric sphincter of a celiacs-spared animal. IMAs occur relatively rarely in the small intestine, but when they are found they are short processes close to the mesenteric border. D: atypical innervation of myenteric ganglia (arrowheads) by vagal afferents. Photograph was taken from ~5 cm distal to the pyloric sphincter of a celiacs-spared animal. Scale bars for B, C, and D = 100 µm.
vagal afferent fibers of passage (for a more detailed account of WGA-HRP specificity when labeling vagal afferents, see Refs. 19, 24, 31).

Gastric infusion tests. In the infusion trials, the gastrics-spared animals, with innervation of the smooth muscle wall of the stomach nearly comparable to that of shams, exhibited only a nonsignificant attenuation of suppression. In contrast, the hepatic-spared group, which had only sparse innervation of the stomach, still exhibited significant, albeit substantially attenuated, inhibition of food intake. Still differently, the celiac-spared group, with only afferent innervation of the small intestines, did suppress slightly to the largest infusion volume used, but their suppression slope was not significantly different from that of completely vagotomized animals, which were entirely denervated and which did not respond to gastric loads. These findings demonstrate distinctly different roles for the vagal branches in the detection of the gastric volumes (and the distension produced by these volumes) tested in this study. The findings, particularly those for the completely vagotomized animals, also suggest that spinal afferents must not play a significant role in responding to the gastric distension signals such as those employed in the present experiment.

Distension-related feeding and stomach innervation patterns. In particular, the ability to detect the range of gastric volumes tested and to differentiate between these volumes was proportional to the density of the spared vagal afferent innervation of the stomach. If the stomach received even minimal afferent innervation, then animals were able to detect and differentiate between different volumes. Conversely, complete lack of vagal innervation appears to disrupt the ability to accurately monitor subtle changes in gastric volume (or distension). As a case in point, none of the selectively vagotomized animals detected either of the infusion volumes when their cuffs were open, unlike the sham-vagotomized animals that reliably suppressed to 10-ml gastric infusions with their cuffs open. This is particularly telling in the case of the gastrics-spared animals because their stomachs only lacked the modest innervation provided by the hepatic branch of the vagus, and yet without this limited and localized projection they were unable to respond to superficial distension.

With their cuffs closed, both the gastrics-spared animals and the hepatic-spared group had response profiles similar to those of shams, with both of these selectively vagotomized groups dose dependently suppressing to the infusion volumes tested. Whereas the gastrics-spared group’s suppression to infusions was only nonsignificantly attenuated in the absence of the innervation provided by the hepatic branch, the hepatic-spared animals’ detection of gastric infusions was significantly blunted without the innervation provided by the paired gastric branches. This is not surprising when you consider the pattern of innervation of the stomach by the gastrics and hepatic branch(es). The gastrics-spared animals appeared indistinguishable from shams with complete innervation of both the ventral and dorsal stomach walls by vagal afferents. In addition, the forestomach of this selective vagotomy group was well supplied with IMAs in both the longitudinal and circular muscles, and IGLEs were densely distributed throughout the myenteric plexus of the gastric compartment. In contrast, the stomach of hepatic-spared animals was minimally innervated at best. The hepatic innervation consists of fibers and endings located almost entirely within the forestomach of the ventral stomach wall with the few vagal afferents found on the dorsal stomach consisting of wraparounds from the ventral side (24). Interestingly, hepatic IMAs are primarily distributed in the longitudinal muscle wall of the forestomach along the greater curvature (24), and are sparse in density compared with the extensive complexity of IMAs in a completely innervated stomach. Similarly, hepatic branch IGLEs were also localized to the ventral forestomach, but did not have as concentrated a distribution as the IMAs.

This observation of the ability of hepatic-spared rats to respond so effectively to gastric infusions is in contrast to the finding of Gonzalez and Deutsch (10), who found no suppression by hepatic-spared animals. This inconsistency is most likely due to the methodological differences between the two studies (i.e., weight of animals, length of fast, infusion protocol, etc.). Notably though, even when they failed to suppress to gastric infusions, the rats in the Gonzalez and Deutsch study did detect withdrawal of gastric contents, indicating some sparing of mechanosensor function.

Unlike the sham vagotomy animals and the selectively vagotomized animals with their gastric branches spared or their hepatic branch spared, the other two groups, namely the completely vagotomized animals and animals with their paired celiac branches spared, did not show dose-dependent suppression to increasing gastric volume. This was not surprising because both of these latter groups lacked any vagal innervation of the stomach. The paired celiac branches appear, on the basis of the present findings on afferents and previous reports on efferents (2), to innervate only the small intestine, with the majority of this innervation occurring 5–6 cm distal to the pyloric sphincter (although a more thorough study is needed before any definitive statement can be made about the projection fields of the celiac branches). Nevertheless, the finding that celiac-spared group did suppress to 10-ml infusions when the pyloric cuff was closed seems to indicate that the celiac branches are able to monitor gastric distension through some circuitous route. Three possible mechanisms for detection of gastric distension by celiac afferent endings in the intestines are 1) hormonal changes in the intestine due to gastric distension; 2) gut motility initiated by gastric distension, propagated by the myenteric plexus, and detected by the celiac afferents; and 3) celiac mechanosensors detecting increasing abdominal pressure due to gastric fill. Without innervation of the
GI tract by the vagus, animals were unable to detect any of the infusion volumes used in this study. The suppression slope for completely vagotomized animals was the same whether their cuffs were open or closed: flat.

In assessing these patterns of suppression and their respective attenuations by selective vagotomy, it should be noted that some of the selectively vagotomized groups (the hepatic spareds, celiacs spareds, and completely vagotomized) exhibited lower baseline food intake during the suppression tests, thus potentially making it more difficult to detect residual sensitivity to gastric loads. Although such a scale-compression bias or “basement effect” needs to be considered, the pattern of results cannot easily be explained by an insensitive test. In particular, we observed that animals with only the hepatic branch spared are able to respond to gastric loads. Similarly, even the celiacs-spared animals were observed (perhaps surprisingly) to suppress their consumption after 10-ml infusions. It is conceivable that any putative bias might have produced some underestimate of the suppression, but the critical observation, not previously shown, is that such selectively vagotomized animals can respond to gastric distension.

Arguably, a basement effect might have masked residual sensitivity in the completely vagotomized group, which evidenced a lower baseline of food intake and did not respond to gastric loads; however, several observations make this unlikely. First, in the earlier Gonzalez and Deutsch experiment (10) observing that completely vagotomized animals do not respond to gastric loads, the experimenters established that their completely denervated animals did not respond even when gastric loads were augmented to offset any smaller meal size. Second, as widely observed, completely vagotomized animals typically have distended stomachs in ad libitum situations (because of ubiquitous emptying problems), even before meal taking; thus it is difficult to argue that slightly smaller meals plus gastric loads plus the chronic residue in the stomach (including solid-phase lipids from I socal and, frequently, bezoars) would have summed to less distension in vagotomized than in control animals. Third, although the different amounts of residual suppression of food intake produced by the gastric loads correlated well with the amount of vagal afferent innervation of the stomach spared in the present experiment, the different amounts of suppression obtained did not correlate well with the baseline meal sizes (i.e., hepatic-spared animals, celiacs-spared animals, and completely vagotomized animals exhibited different amounts of suppression, but they had similar-sized baseline meals). Finally, because all groups, including the completely vagotomized group, continued to consume significant quantities of their diet (=3 ml or more) in the test meal, all could have evidenced suppression.

Spontaneous feeding behavior after selective vagotomies. Immediately after vagotomy, animals with no vagal innervation of the GI tract (completes) and animals with minimal innervation of the GI tract (the hepatic-spared group) overate during their first meal and initial postsurgical daily intake (17-h food intake). At the same time points, the sham, gastrics-spared, and celiacs-spared rats did not overeat. Recently, Chavez et al. (5) demonstrated that rats will overeat a novel diet after destruction of visceral afferents by capsaicin. We extend these findings by showing that selectively vagotomized animals will overate even their maintenance diet. Because two of the five experimental groups reliably overate after surgery, the possibility of inhibitory effects of surgical trauma on food intake (cf. discussion in Ref. 5) cannot readily explain our findings. The specific projections spared in the three groups that did not overate presumably provided sufficient feedback to control meal size: gastrics-spared animals may have monitored food intake through detection of gastric distension and gastric emptying (33, 34), celiacs-spared animals may have received feedback from the chemospecific properties of the meal that emptied into the small intestine (36), and sham rats may have utilized both distension and chemodetection cues (25, 36). Thus, presumably through disparate means, these three groups did not overate. In contradistinction, hepatic-spared animals, with only vagal innervation of a limited region of the fundic wall of the stomach and first few centimeters of the small intestine, and completely vagotomized animals, which lacked any vagal innervation of the GI tract, were unable to accurately monitor and regulate food intake by GI negative feedback immediately following surgery.

In contrast to the pattern of food intake seen in the first hours after surgery, a different style of ad libitum food intake emerged within 48 h of surgery. Gastrics-spared animals ate similar amounts during the short term (first 30-min feeding bout), but the food intake of the celiacs-spared and hepatic-spared groups as well as the completely vagotomized animals was suppressed in comparison to shams. The small first meal size by the celiacs-spared group is possibly due to the aversive nature of the rapid dumping of a meal that occurs without vagal innervation of the gastric compartment by the gastric branches (33). Presumably, through their ability to monitor the chemical nature of the liquid diet (cf. Ref. 36), however, they are able to accurately maintain long-term needs and subsequently body weight. The daily intake and body weight of the complete and hepatic-spared groups were suppressed in comparison to shams, but the celiacs-spared group, like gastrics-spared animals, were not. It appears that with almost-complete innervation of either the stomach (gastrics spared) or intestines (celiacs spared), animals are able to properly regulate their daily food intake so as to maintain body weight. In contradistinction, animals with minimal (hepatic spared) or no vagal innervation (completes) are not able to monitor food intake in a manner that will allow for proper maintenance of body weight.

The equivalent response of hepatic-spared and completely vagotomized animals is surprising. One would think that with minimal afferent innervation of the stomach (allowing for substantial monitoring of gastric distension) and dense innervation of the proximal duodenum (function unknown, although possibly a
small role in chemodetection or intestinal motility; Refs. 11, 24), that hepatic-spared animals would be able to regulate their intake as well as gastrics-spared or celiacs-spared animals. This emphasizes the need for additional studies characterizing the role of the hepatic branch in feeding behavior.

Previous studies have repeatedly shown that rats with their pyloric sphincters occluded are still able to regulate meal size. This has been the case for intact animals (9, 10, 25) and animals with only the hepatic branch of the vagus left intact (10, 15). Consistent with these previous studies, we also found that after recovery from surgery, the sham, gastrics-spared, hepatic-spared, and completely vagotomized animals faithfully ate the same whether their pylori were occluded or unoccluded. Contrary to this reliable phenomenon, animals with only their paired celiac branches intact ate more when only the gastric compartment was stimulated (pylorus occluded) in comparison to when their whole GI tract was stimulated (pylorus unoccluded). There are two possible reasons for this finding: 1) having relied on information about the meal from intestinal chemoreceptors, they now overeat because of a lack of information from the denervated gastric compartment or 2) as previously discussed, the aversive dumping of the meal that occurs when the pylorus is open doesn’t occur when the pylorus is occluded, so the animal consumes more because of the lack of discomfort that normally follows a meal. Additional support for the latter comes from the Walls et al. (36) study in which evidence was found thatafferent components of the paired celiac branches played a role in the detection of a mucosal irritant. Furthermore, with their cuffs closed, celiacs-spared rats still ate 43% less Isocal than shams, so it is difficult to make the claim that the extra 0.97 ml consumed was a dramatic overconsumption of the diet.

Vagal afferent plasticity. The finding of vagal plasticity in the present study is troublesome not because of difficulty in differentiating between normal innervation patterns and new patterns but because changes in the innervation of the gastric compartment by regenerating axons or by reorganization of existing endings might alter, either repair or further distort, the consequences of the initial denervation. Our recent studies of vagal regeneration (Ref. 27 and R. J. Phillips, E. A. Baronowsky, and T. L. Powley, unpublished observations) have found that dramatic reinervation of denervated gastric smooth muscle occurs 18 wk after complete vagotomy, but that regenerating vagal afferents were distinctly different from the pattern of innervation found in controls. In addition, at 6 wk after selective vagotomy (in cases in which the hepatic branch was spared; Refs. 23, 26) vagal plasticity consisted primarily of ingrowing axons and growth cone profiles. The findings of the present experiment were consistent with these previous studies, which had been designed to specifically address the question of vagal plasticity. In the present study, we found that during the 5–6 wk period of testing, vagal plasticity consisting of regenerating axons and possible reorganization of existing axons had occurred, but these profiles were nonnormal in appearance and easily identifiable as plastic changes (cf. Fig. 7 of present study).

The larger issue raised by the findings of vagal plasticity in this study is the question of possible effects on function due to the changes that are occurring during testing. Unfortunately, at the present time, there is no clear-cut answer as to whether or not regenerating vagal afferents become functional or whether the reorganization of existing afferents after selective vagotomy affects function, but four observations suggest that such effects did not fundamentally distort the present results. 1) We only tested animals during the first 5–6 wk after vagotomy, an interval that previous observations suggest anticipates differentiation of new terminals (see above point). 2) In addition, in the current experiment, the observed presence of undifferentiated growth cone profiles instead of well-formed vagal endings at the time of histology substantiates the lack of functional recovery at time of testing. 3) In the gastric infusion tests, we randomized treatment conditions to provide a counterbalancing of conditions over time. 4) The distinctly different behavioral profiles associated with the different selective denervations argues that the effects of vagal plasticity appear to be minimal at most. It should be noted, however, that vagal plasticity is not only an issue that needs to be considered for this study but is also a potential concern for all past behavioral studies that have looked at feeding patterns postvagotomy (6, 10, 15, 33, 36) and those that follow.

In conclusion, we show that the detection of gastric distension that influences feeding behavior is primarily mediated by the paired gastric branches and hepatic branch of the vagus. In addition, the ability of these three branches to detect gastric volume is in direct proportion to the pattern of innervation (most likely terminals, specifically IMAs and IGLEs) they supply to the muscle wall and myenteric plexus of the stomach.

Perspectives

Recent morphological descriptions of the different vagal afferent terminals in the muscle wall of the GI tract now make it practical to formulate specific hypotheses about structure-function specializations of gastrointestinal mechanoreceptors. Although the gastric distension signals examined in the present experiment cannot be associated unequivocally with one type of ending, we would suggest that the present results probably reflect, at least most prominently, the activity of IMAs. More specifically, the results are consistent with the complementary hypotheses that 1) IMAs are specialized to detect stretch or tone maintained in the forestomach and other compartments that serve as reservoirs for ingested material, as well as in critical sphincter regions, and 2) IGLEs are specialized to translate local mechanical disturbances or changes in tension into alterations of motility, peristalsis, and emptying.

The IGLE was the first of the vagal endings in the wall of the alimentary canal to be clearly characterized.
paired. selective vagotomy), the sensitivity of the stomach to only the hepatic branch projection, with its small field other observation consistent with the suggestion that (Ref. 24; also present morphological observations). An-


conspicuous than the IGLEs associated with the branch


other contents of the GI tract at strategic loci such as patterns (e.g., peristalsis, migrating motor complexes,


extension, or more specific hypothesis, of Wang (Ref. 37;


that several observations are consistent with the recent extension, or more specific hypothesis, of Wang (Ref. 37;


also Refs. 29, 38) that IGLEs participate more particularly in the mechanosensory coordination of GI motility patterns (e.g., peristalsis, migrating motor complexes,


etc.), whereas IMAs participate more prominently in the monitoring of stretch generated by ingesta and other contents of the GI tract at strategic loci such as regions with reservoir functions (e.g., the stomach) and sphincters.

The results of the present experiment are congruent with this model associating IMAs with stretch-receptor specializations and IGLEs with local coordination of motility. The most suggestive of the present results are those of the residual sensitivity to gastric fill observed those of the residual sensitivity to gastric fill observed


Although this hypothesis assigning separate functional specializations to the two morphological types seems promising and can generate additional useful empirical tests, it is clearly provisional. For one thing, the fact that some individual vagal axons in the fore-


stomach have IGLEs on some collaterals and IMAs on others (4) suggests that any specializations of function and morphology may be overlapping and integrated, even at the level of the single afferent. Furthermore, more comprehensive inventories of GI tract afferents may identify additional structural categories and require additional refinements of classification.

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