Vagal and spinal mechanosensors in the rat stomach and colon have multiple receptive fields

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Berthoud, Hans-Rudolf, Penny A. Lynn, and L. Ashley Blackshaw. Vagal and spinal mechanosensors in the rat stomach and colon have multiple receptive fields. Am J Physiol Regulatory Integrative Comp Physiol 280: R1371–R1381, 2001.—Mechano- and chemosensitive extrinsic primary afferents innervating the gastrointestinal tract convey important information regarding the state of ingested nutrients and specific motor patterns to the central nervous system via splanchnic and vagal nerves. Little is known about the organization of peripheral receptive sites of afferents and their correspondence to morphologically identified terminal structures. Mechano- and chemosensory characteristics and receptive fields of single vagal fibers innervating the stomach as well as lumbar splanchnic nerves innervating the distal colon were identified using an in vitro perfusion system. Twenty-three (17%) of one-hundred thirty-six vagal units identified were found to have multiple, punctate receptive fields, up to 35 mm apart, and were distributed throughout the stomach. Evidence was based on similarity of generated spike forms, occlusion, and latency determinations. Most responded with brief bursts of activity to mucosal stroking with von Frey hairs (10–200 mg) but not to stretch, and 32% responded to capsaicin (10⁻⁵ M). They were classified as rapidly adapting mucosal receptors. Four (8%) of fifty-three single units recorded from the lumbar splanchnic nerve had more than one, punctate receptive field in the distal colon, up to 40 mm apart. They responded to blunt probing, particularly from the serosal side, and variously to chemical stimulation with 5-hydroxytryptamine and capsaicin. We conclude that a proportion of gastrointestinal mechanosensors has multiple receptive fields and suggest that they integrate mechanical and chemical information from an entire organ, constituting the generalists in visceral sensation.

visceral afferents; electrophysiology; chemoreceptors; gastrointestinal innervation; vagus

BRAIN-GUT INTERACTIONS ARE increasingly recognized as major players in physiological and pathophysiological regulation of the gastrointestinal tract and associated organs. The afferent limb of the bidirectional neural connections signals important information from the gastrointestinal tract to the brain. Although this area has recently been the subject of intense investigation, there are still major gaps in a comprehensive description of morphology, chemistry, and functional significance of the visceral afferent system. For example, little is known about the specificity of visceral sensors and the correspondence of specific functions with morphology and distribution of afferent terminals. The physical nature and viscerotopic origin of input to a single sensory unit is a fundamental question in sensory physiology because it is the first step of the integration process that makes sensory input interpretable to the higher functions of the brain.

The peripheral organization of primary afferent vagal fibers innervating the stomach wall has been studied using both electrophysiological recording and anatomical tracing methods. Studies recording single-unit gastric mechanosensor activity in various species generally reported the presence of only one receptive field (12, 14, 20, 26), although the rare exception with two receptive fields was noticed by some researchers (14). The size of the receptive fields for slowly adapting gastric tension receptors has been reported to be 1–3 mm² in the esophagus and stomach of the ferret (26), ~10 mm² in rats (15, 29), and up to 400 mm² in cats (27). In contrast, recording from serosal receptors with single units in the splanchnic nerves supplying the small and large intestines, Morrison (22) reported up to six separate receptive fields that could be as far as 100 mm apart in the cat and dog (for reviews, see also Refs. 1, 18, 29). Neural tracing studies with Dil (a red fluorescent carbocyanine dye) or horseradish peroxidase (HRP) injected into the rat nodose ganglia found some individual vagal afferent fibers to produce several collaterals with characteristic terminal structures in the myenteric plexus [intraganglionic laminar endings (IGLEs)] and in the external smooth muscle layers [intramuscular arrays (IMAs)] at the end of each collateral, suggesting the possibility of multiple receptive fields (6). Because the mucosa is not typically preserved in whole mounts of gastrointestinal wall, the branching characteristics of afferent fibers with mucosal endings are not known.

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With the recently developed in vitro preparation allowing single-unit recording from extrinsic nerves of superfused open sheets or pieces of gut wall, it became feasible to systematically stimulate both mucosal and nonmucosal mechanono- and chemosensors in a controlled fashion (10, 21, 26, 35, 36). At least three types of mechanosensitive vagal and spinal afferent fibers have been identified in the alimentary canal of rat and mouse. Vagal afferent fibers that respond with brief or prolonged bursts to light stroking of the esophageal (26) or colonic (21) mucosa but not circular or longitudinal stretch of the entire wall are suspected to have receptive terminals in the mucosa. Some of these afferents are also sensitive to chemical stimulation with 5-hydroxytryptamine (5-HT), bradykinin, and capsaicin (10, 21, 26). A second type of vagal afferent fiber responds primarily to stretching of the tissue and may also respond to probing with light to moderate forces (26, 36). The likely sites of mechanosensitive terminals for this type of afferents are in the external muscle layer, with the morphological specializations of IGLEs (37) and IMAs (6). The third type of afferents is sensitive to probing with relatively strong forces, and because the threshold is typically lower when stimulated from the serosal side, they have been designated serosal receptors. These receptors have been mainly described in the lower gastrointestinal tract (18, 21, 22), and the morphological analog for endings of this type has not yet been identified.

The aim of the present study was to identify the receptive fields and mechanosensory characteristics of single afferent fibers teased from the vagal trunks using an in vitro perfusion system. For comparison, a similar characterization was performed for single afferent fibers in the lumbar splanchnic nerve supplying the distal colon.

**MATERIALS AND METHODS**

**Animals**

Adult female Sprague-Dawley rats weighing 180–350 g were obtained from the Institute of Medicine and Veterinary Sciences, University of Adelaide. Animals were kept under standard laboratory conditions (22 ± 3°C). All experiments were carried out according to guidelines of the Animal Ethics Committees of the University of Adelaide and the Institute for Medical and Veterinary Science.

**Dissection of Stomach with Attached Vagal Supply**

The rats were deeply anesthetized with pentobarbital sodium (80 mg/kg ip). They were placed on ice after cervical dislocation and bleeding. The entire stomach, proximal duodenum, and esophagus were dissected with care not to damage any vagal nerve fibers running along the esophagus and distributing on the stomach wall. The entire piece was then transferred into a sylgard-coated Petri dish containing ice-cold, thoroughly carbogenated Krebs solution for further dissection. Starting at a midtoracic level, both vagal trunks were marked with a black silk tie and separated from the esophagus all the way down to the cardia of the stomach. The hepatic and celiac branches were cut as they left the respective subdiaphragmatic trunks. The esophagus was then severed about 3 mm from the lower esophageal sphincter. The stomach was stripped of any excess adipose or connective tissue, cut along the greater curvature, and the mucosa was rinsed with Krebs. The stump of the esophagus was then pinned down near the hole connecting the perfusion chamber with the recording chamber so that the separated vagal nerve trunks were near the entry to the recording chamber, through which one of them was pulled (Fig. 1). The butterfly-shaped stomach wall was then pinned, mucosa-up, in the perfusion chamber.

**Dissection of the Colon with Attached Neurovascular Bundle**

Dissection was carried out according to the protocol described in detail earlier (21). Briefly, after a midline laparotomy, 4–5 cm of distal colon lying oral to the rim of the pelvis were removed along with the lumbar colonic nerves and the neurovascular bundle containing the inferior mesenteric ganglion, the intermesenteric nerve, and the lumbar splanchnic nerves, according to the classification of Baron et al. (2), and transferred into ice-cold carbogenated modified Krebs bicarbonate buffer. The distal colon was opened longitudinally, off-center to the antimesenteric border, to orientate lumbar colonic nerve insertions to lie along the edge of the opened preparation. Connective tissue was dissected away from the neurovascular bundle, and the latter was cut and tied at the level of insertion of the inferior mesenteric artery into the abdominal aorta. The preparation was then pinned flat with mucosa up in the perfusion chamber, and the neurovascular bundle was pulled through the hole into the adjacent paraffin-filled recording chamber.

**Perfusion Chamber and Perfusate**

The organ bath used for both preparations consisted of two adjacent compartments machined from clear acrylic (Fig. 1). One organ compartment was superfused with Krebs solution, and the recording compartment containing the nerve and electrodes was filled with paraffin oil. The perfusion chamber was further subdivided into a basement (serosal) and an upper (mucosal) chamber by a nylon mesh on which the opened preparation rested. This allowed superfusion of the serosal and mucosal sides with different media if appropriate.

For stomach superfusion and the colon serosal chamber, modified Krebs solution containing (in mM) 117.9 NaCl, 4.7 KCl, 25 NaHCO3, 1.3 NaH2PO4, 1.2 MgSO4 (H2O)7, 2.5 CaCl2, and 11.1 α-D-glucose was used. For the colon mucosal chamber, glucose was replaced by the short-chain fatty acids butyrate (2 mM) and acetate (20 mM). In all colon and in most gastric preparations, the cyclooxygenase inhibitor indomethacin (1–3 µM) was added to suppress potential inhibitory actions of endogenous prostaglandins. In some gastric preparations, the calcium-channel blocker nifedipine (5 µM) was added to the solution to suppress spontaneous or induced smooth muscle contractions. The perfusion medium was always bubbled with carbogen (O2 95%, CO2 5%). In some gastric preparations, calcium-free Krebs was used by replacing CaCl2 with MgCl2 (H2O)7. After identification of receptive fields, the perfusion solutions were warmed to 32–34°C in a heating coil, and the flow rate was typically set at 15 ml/min.

In some experiments, drugs were either added to the perfusion medium or were locally applied by means of a metal cylinder of 10-mm diameter placed around a specific receptive field.
Experimental Protocol

The ventral or dorsal vagal trunk was stretched and anchored with a pin in the paraffin-filled recording chamber and freed from the surrounding connective tissue and blood vessels for a length of at least 5 mm. The trunk was then carefully desheathed, and small strands of fibers were separated with fine forceps. The strand was laid on one of the platinum wire electrodes, and a piece of connective tissue of similar diameter was laid on the other electrode. With the amplifier and acoustic monitor turned on, mucosal stroking then began, first with a wide soft brush. When receptive fields were found, stimulation continued with a smooth rounded glass rod (tip radius \( \leq 3 \) mm) and/or with calibrated von Frey hairs of 10, 50, 200, or 1,000 mg nominal force. When the entire surface of the respective ipsilateral stomach side was searched and more than one area was found to generate spike activity, each site was stroked one to three times every 10 s and with intervals of 30 s between sites with a given probe. After a resting period of 2 min, the procedure was repeated with another probe, usually of increasing force. Collision tests were carried out with two concentric electrodes (outer diameter 2 mm) positioned by micromanipulators directly over the receptive fields suspected to belong to the same afferent fiber. They were connected to two square wave stimulators via stimulus-isolation units and programmed to fire synchronously. Stimulus intensity for each stimulator was first set above threshold for activation of the respective receptive fields, and then the intensity of one stimulator was systematically varied up and down in a ramp-like fashion. Conduction velocities were also calculated from the delay between stimulation at the receptive fields and the arrival of a spike at the electrode, assuming straight projections.

Protocol for Recordings from Colon

Receptive fields were identified by systematically stroking the mucosal surface with calibrated von Frey hairs, as for the stomach, and probing with a blunt glass probe with an undetermined force. Results for afferents with single receptive fields in mucosa and muscle layers of this study were reported elsewhere (21). In all cases of multiple receptive fields, only the application of pressure with the blunt glass probe elicited burst activity. This was taken to indicate that recordings were from afferents with receptive fields in the serosal layer. This was confirmed by reflecting the ends of the preparation, whereupon thresholds to probing (the serosa) were found to be considerably lower than those to mucosal probing.

Data Collection and Analysis

The signal from the platinum electrode was fed into a differential amplifier, filtered, and monitored on an oscilloscope. The amplified signal was also used for the audio monitor and stored on tape for later use. The analog signal was sampled at a rate of 20 kHz in a 1401 data interface (CED, Cambridge, UK) and stored on the hard drive of an Apple Macintosh computer using Spike II software (CED). The records were later analyzed using Spike II software. To check for similarity of spikes, templates were generated for specific time periods, usually only including one or several bursts evoked by stroking a particular receptive field. This yielded relatively narrow templates, which recognized \( \geq 90\% \) of the action potentials within the burst around which they were generated. These site-specific templates were then cross-correlated with spike activity generated by stimulating all other receptive fields. The percentage of spikes of a burst at any other stimulation that was recognized was thus a measure of similarity. If \( <50\% \) of spikes were recognized, similarity was rejected. If \( >70\% \) of the spikes were recognized by comparison in both directions, similarity was assumed. In addition, similarity of spike forms was confirmed by manually superimposing images of action potentials.

RESULTS

Upper Gastrointestinal Tract

General features of mechanosensitivity. From a total of 22 rats, recordings from 136 units in 54 strands were obtained (Fig. 2). Sixty-eight percent of units that were systematically tested with von Frey probes responded to light mucosal stroking with 10–200 mg, and most were located in the gastric corpus. The rest of the units responded to stroking with the glass probe and/or stretching. Although no attempt was made to measure the exact dimensions of receptive fields, they were
often very small (~1–5 mm²), and stroking areas surrounding the hot spot did not trigger any activity. Stroking with probes exerting forces as little as 10 mg in many cases produced brief bursts of firing, and firing could not be sustained when the probe was held in place with continuous pressure. Stretching the area containing the receptive field in either direction typically did not trigger spiking activity (Fig. 3A). Units with receptive fields of this type were classified as mucosal and were found throughout the stomach as well as in the pylorus and proximal duodenum (Fig. 3). In some cases, bursting activity continued for a few seconds and, in a few cases, up to 60 s after the initial touch (Fig. 3B). These sites could also be conveniently and more reliably activated with the blunt glass probe, because the greater contact surface was more likely to hit the small field. In a few cases, stimulation with von Frey hairs or the glass probe was relatively ineffective, but either circular or longitudinal pull from the edge of the tissue, or with the two limbs of a fine forceps pulling in opposite directions, was very effective (Fig. 3E). In these cases, the unit fired with a high initial rate followed by a sustained lower rate during continued pull and ceased on termination of the stimulus.

**Multiple receptive fields.** For 113 (83%) units, only one mechanoreceptive field was identified, whereas for 23 units (17%), various analytical evidence indicated that they could be activated from at least two and, in a few cases, three or more distinctively located small receptive fields. The largest distance between punctate receptive fields exciting the same single unit was 35 mm (Fig. 4). In this preparation, muscular activity was blocked by addition of nifedipine to the perfusion medium. Gentle stroking of the mucosa with the blunt glass probe at one site in the ventral corpus and two sites in the antrum elicited bursts of action potentials of similar shapes in a strand dissected from the ventral vagal trunk. Stroking of two additional sites in the fundus generated bursts with a different spike shape, and probing the pyloric sphincter muscle produced a third spike form. Spike-form analysis using template generation and superimposition of randomly chosen spikes from each receptive field showed that the three receptive fields in the corpus and antrum indeed produced indistinguishable spike forms (Fig. 4). The two receptive fields in the fundus also produced indistinguishable spike forms (not shown).

In another case using calcium-free medium and indomethacin throughout, stroking with the glass probe of sites ~10 mm apart within the dorsal corpus elicited bursts of action potentials within a strand isolated from the dorsal vagal trunk (Fig. 5). Stroking between the two sites did not evoke any spikes, and the thresholds for activation were 10 and 50 mg, respectively (not shown). Superimposition of 12 randomly chosen action potentials generated at one site produced action potentials that fell within a narrow envelope, however, with considerable variation. Superimposition of spikes from both sites did not further extend the width of the individual envelopes. With the use of electrical stimulation of each receptive field, latencies of 148 ms for field 1 and 120 ms for field 2 were obtained, corresponding to conduction velocities of 0.16 and 0.29 m/s (at 22°C), respectively. Interestingly, the field that was further away from the recording electrode (field 2) had the shorter latency. Most importantly, when the electrical stimulus intensity of field 1 was gradually increased, the action potential recorded switched from the latency of field 2 (120 ms) to that of field 1 (148 ms) at a certain threshold stimulus intensity (Fig. 5). Stimulation of field 1 thus blocked propagation of the action potential generated by stimulating site 2. This indicates occlusion of the two spikes within the axon collateral that connects field 2 with the parent fiber. Collision tests and latency determinations in one other case involving two receptive fields in the antrum provided similar results, showing generation of identical spike forms within one vagal afferent unit with two axon collaterals innervating distinctive receptive fields.

In addition to the above cases with collision tests and superimposition of spikes, in 19 cases, evidence for single units with multiple receptive fields was obtained.
on the basis of similarity of action potentials using the Spike II template software. In most of these cases, site-specific templates were cross-correlated with the other sites. As an example, this analysis provided strong evidence that mechanical stimulation at three distinct focal points generated activity of the same single unit in the ventral vagal trunk (see Fig. 7A, right). Stroking the mucosa near the cardia and near the lesser curvature ~10 mm from the cardia with a 50-mg von Frey hair as well as probing near the pylorus with the blunt glass probe all generated a burst of spike activity in the same unit. Stroking the pylorus with 50 or 200 mg was not sufficient to activate the unit, and although the exact force was not determined, considerable pressure (>5 g) had to be applied to the blunt glass probe to activate the unit. However, using the same probe proximal and distal to the pylorus did not activate the unit, suggesting that it was not merely
mechanical distortion of the axon triggering unit activity. A fourth responsive site was found in the fundus near the greater curvature on stroking with 200 mg or the glass probe, but it produced a clearly different spike form.

Overall, for 21 units, evidence for multiple receptive fields was based on similarity of waveforms, and for two units, it was based on collision test. In an additional eight units, wave forms generated from different receptive fields showed some similarity, but the similarity was judged not sufficient. A map depicting the location of the clearly multiple receptive fields is shown in Fig. 7A.

Chemosensitivity of upper GI vagal afferents. Of 36 receptive fields tested with $10^{-6}$ M CCK either added to the perfusion medium or locally, only four (8%) showed a moderate increase in firing rate. Capsaicin ($10^{-5}$ or $10^{-6}$ M) produced a clear increase in firing rate in 6 (32%) of 19 units tested. With the higher dose, very strong firing was usually followed by a quiescent period (Fig. 3), in some cases without recovery.

Colon

Of a total of 39 preparations with 53 units recorded, 4 single units in separate preparations were found to have two or more separate receptive fields. Evidence for multiple receptive fields was based on similarity of action potentials as assessed by the template algorithm of Spike II. In one preparation, the distinct punctate receptive fields were 40 mm apart, and both responded to probing but not to stretching or stroking (Fig. 6). A map depicting all cases of multiple receptive fields in the colon is provided in Fig. 7B.
A Responses to mechanical stimulation

probing with glass rod  single action potential  12 spikes superimposed  both sites, 24 spikes

Location of 2 distinct receptive fields

B Responses to electrical stimulation - collision

Modeling of axonal geometry  Latency of spikes after electrical stimulation  Latency plots

Latency plots
DISCUSSION

We found evidence for multiple receptive fields in the stomach in 17% of units recorded from the ventral or dorsal vagal trunk. In the colon, 8% of recorded units in the lumbar splanchnic nerves had multiple receptive fields. In most cases, two receptive fields and, in a few cases, three or more receptive fields were found to belong to the same afferent fiber. Even in cases of multiple receptive fields <10 mm apart, we are confident they were distinct receptive fields because stroking between the fields did not trigger action potentials. The true percentages for both organs are most likely larger because the adequate stimulus intensity and the exact location could be easily missed for potential additional receptive fields of a given unit.

With all the units recorded and taken into consideration in this study, the results also show that small strands teased from the vagal trunks (estimated to contain ~10–100 afferent axons) innervate vastly different regions of the rat stomach and beyond. This suggests that there is little or no viscerotopic organization within this large nerve. This absence of spatial organization is in contrast to findings from the optic nerve (13) and to a recent report describing a certain degree of viscerotopy of vagal afferent primary perikarya within the nodose ganglia (19).

The in vitro superfused rat stomach is a useful tool in the study of peripheral vagal sensory mechanisms, as has been shown earlier for the stomach (10, 26, 35), esophagus (26), small intestine (11), and colon (21). In contrast to the in vivo methodology used by many before (7, 8, 12, 14–16, 20, 27, 32), the in vitro technique allows easy and precise manipulation of mucosal receptors, which are less accessible in vivo, and offers the prospect of controlled chemical and pharmacologi-

Fig. 6. Evidence based on similarity of spike form that single afferent fiber recorded from the lumbar splanchnic nerves has 3 distinct punctate receptive fields (color coded with blue, red, and green) in distal colon of rat. A: probing of each site with blunt glass rod generates brief bursts of spikes of similar amplitude and waveform. Light stroking with von Frey hairs and stretching did not evoke responses (not shown). B: template generated by Spike II software used to identify similar spike forms. C: sketch of the 3 receptive fields on schematic outline of distal colon. The position of the neurovascular bundle running along the mesentery attachment and the exit of the lumbar splanchnic nerves containing the inferior mesenteric ganglion are also shown. D: all 3 receptive fields were sensitive to local application of capsaicin (10^{-4} M), but only 2 sites were sensitive to 5-hydroxytryptamine (5-HT; 10^{-4} M).
cal access to all vagal sensors in the stomach. For example, our preliminary results with calcium-free Krebs solution show that extracellular calcium is not necessary for mechanical stimuli to generate action potentials in vagal mechanosensors. This does not rule out a role for calcium released from stores within the afferent axon terminals. To determine this, additional experiments with chelating agents and inhibitors of intracellular Ca release will be necessary.

Before we discuss the possible physiological significance of these findings, let us examine some of the important methodological issues.

**Methodological Issues**

**Discriminating spike forms using templates and other techniques.** There are several factors that could potentially lead to false positive conclusions about multiple receptive fields driving the same primary afferent fiber. First, insufficient analytical power to discriminate two spikes that are only slightly different in shape and amplitude. The Spike II software creates templates from a mixture of different spike forms that can be used to identify spikes with a high degree of similarity and distinguish them from spikes with different shapes and amplitudes. Because the amplitude and even the shape of single units can vary considerably over time, the criteria to produce the templates cannot be set too restrictively, and so must therefore leave some room for erroneous conclusions. This possibility is more likely if the templates are generated on the basis of extended periods of recording, by averaging over a great number of spikes. Although such templates are representative for the variation of individual spikes over time, they tend to be broad and nondiscriminative.

We have consistently generated templates over very short periods, during bursts of activity produced by stimulating a particular receptive field, and subsequently assessed spikes generated by stimulating the other receptive fields to determine whether they fit the template. Templates generated over short periods were typically much narrower, and the process of cross-correlation provided a more conservative determination of spike similarity. In fact, with this method, it was possible that changes in amplitude and shape of one unit precluded it from being recognized as the same unit at different time points of the recording. This procedure is in fact likely to lead to false negative conclusions, in which units appearing to be different were in fact the same unit that changed the waveform and/or amplitude of its action potential over time.

Despite the rigorousness of template analysis we used, even a very high degree of similarity between spike forms does not completely rule out the possibility that they do, in fact, belong to separate afferent fibers. To rule out this possibility, the collision test is necessary.

**Collision test.** The collision test is a more conclusive proof that action potentials are generated within the morphological confines of a single neuron. It is based on the fact that action potentials traveling in opposite directions along a single axon cancel each other on collision (20). As shown in Fig. 5, the action potential generated by the receptive site that has the fastest conducting connection with the parent axon will antidromically invade the other axon collateral first and collide with the action potential generated by that second receptive site. Electrical stimulation was chosen because the stimuli can be exactly timed, which is not possible with mechanical stimulation. Because of the electrical stimulation, it could be argued that not a receptive field, but an axon passing by is actually stimulated. We found that electrical stimulation between receptive fields was ineffective, probably because the axon runs deeper in the tissue than the terminal receptive field. However, because the exact path of the axon between the receptive fields is not known, we could have missed it. Therefore, in itself, the collision test does not unequivocally prove the presence of multiple receptive fields.

**Activation of fibers of passage.** It is possible that not only electrical, but also mechanical stimulation of afferent fibers passing through a particular area generate action potentials in a unit. We have shown that an afferent nerve fiber can only be activated by stimulating its mechanosensory transduction site at a specialized ending, although injurious stimuli or cutting a fiber can produce bursts of activity (unpublished observations). This is because systematic probing of the preparation only generated action potentials at certain points at which only weak forces of <200 mg were required.

If inadvertent activation of fibers of passage had played a role, we would also expect to find the receptive fields in some topological relation to the distribution of extrinsic nerve fibers within the gastric or colonic wall. Whereas in several instances, receptive field 1 was
indeed found near the predicted fiber path of receptive field 2 (Fig. 7), this clearly would not account for all cases. In a few cases, we measured conduction time for each presumptive receptive field driving the same unit. This analysis showed that in at least two cases, the conduction time was not shorter from the site suspected to lie near the fiber path of the other site. Furthermore, in at least two cases, although one receptive field was lying near the expected fiber path, the weak stimulus forces of 10–50 mg would not be expected to activate a fiber of passage in the myenteric plexus of the corpus with its relatively thick mucosa.

Comparison with Morphological Tracing Results

An assessment of the peripheral morphological characteristics of primary afferent endings has only recently become available. At least three different types of vagal afferent endings have been described on the basis of tracing experiments with injection of Dil or HRP into the nodose ganglia. IGLEs throughout the myenteric plexus from esophagus to colon (4–6, 23, 28), intramuscular endings or IMAs in both the longitudinal and circular muscle layers of primarily the gastric fundus and corpus (6, 28, 34), and intramucosal endings primarily in the stomach and proximal small intestine (3, 4). All three types of endings are potentially mechano-sensitive; the mucosal endings may represent the electrophysiologically identified, rapidly adapting mucosal touch receptors (7, 8, 12), the IMAs may represent the in-series tension receptors (9, 15, 20), and the IGLEs may be additional mecanosensors that primarily detect the shearing forces between the longitudinal and circular muscle layers (23–25), and/or the compression of the gut wall during distension. Tracing of individual vagal afferent fibers has shown that they can produce multiple collaterals and terminal structures sometimes covering areas of hundreds of square millimeters (Ref. 6 and unpublished observations). Because it is difficult to follow individual fibers from their entry into the myenteric plexus to their terminals, it is also very likely that the true innervation territory of single fibers is much underestimated.

It is thus not surprising from a morphological point of view that individual fibers have multiple receptive fields. The most recent report by Zagorodnyuk and Brookes (37), using a combination of electrophysiolog- ical recording and subsequent anterograde fiber tracing, lends additional support for the idea. These authors have demonstrated that punctate mechanical stimulation in or near up to three separately located IGLEs activates the same vagal afferent unit. Although the largest distance between IGLEs was only 2 mm in their study, it is very likely that the complete axonal tree was not contained in the relatively small esophageal segment they recorded from in vitro.

Perspectives

Clearly, the most interesting aspect of widely distributed receptive fields belonging to a single fiber is its physiological significance. What could be gained from such an arrangement as opposed to one fiber, one receptive field? To answer this question, we may first look at information processing by second-order neurons in the caudal brain stem. A number of studies suggests that convergence of sensory input from primary afferents is widespread (9, 17) and is in fact a major component of organizing vago-vagal and other reflexes. To generate meaningful adaptive responses, the brain is likely to recognize patterns of sensory activity, not bursts from single discrete sensory neurons. It would not therefore be surprising to find some of the integration being delegated to primary afferents. Such afferents would integrate luminal tactile stimuli from various parts of the stomach and even the pylorus and proximal duodenum and could thus provide a more global signal of gastric contents. Just as in other sensory systems such as taste and smell (e.g., Ref. 30), these would be the generalist sensors with a broad tuning for various mechanical events as opposed to the specialists that are narrowly tuned.

It is already acknowledged that primary afferents of vagal (16, 20, 31) and dorsal root origin (33) can, at the same time, sense mechanical, chemical, and/or temperature stimuli (polymodal afferents). It may be significant but not surprising that they sense mechanical and chemical stimuli from different receptive fields.

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