Extrinsic primary afferent signaling in the gut

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Abstract

Visceral sensory neurons activate reflex pathways that control gut function and also give rise to important sensations, such as fullness, bloating, nausea, discomfort, urgency and pain. Sensory neurons are organised into three distinct anatomical pathways to the central nervous system (vagal, thoracolumbar and lumbosacral). Although remarkable progress has been made in characterizing the roles of many ion channels, receptors, and second messengers in visceral sensory neurons, the basic aim of understanding how many classes there are, and how they differ, has proven difficult to achieve. We suggest that there are just five structurally distinct types of sensory endings in the gut wall that account for essentially all of the primary afferent neurons in the three pathways. Each of these five major structural types of endings seems to show distinctive combinations of physiological responses. These types are: 'intraganglionic laminar' endings in myenteric ganglia; 'mucosal' endings located in the subepithelial layer; 'muscular mucosal' afferents, with mechanosensitive endings close to the muscularis mucosae; 'intramuscular' endings, with endings within the smooth muscle layers; and 'vascular' afferents, with sensitive endings primarily on blood vessels. 'Silent' afferents might be a subset of inexcitable 'vascular' afferents, which can be switched on by inflammatory mediators. Extrinsic sensory neurons comprise an attractive focus for targeted therapeutic intervention in a range of gastrointestinal disorders.

Introduction

Primary afferent neurons detect physical or chemical stimuli and convey information about them to the central nervous system (CNS). Exteroceptors detect external stimuli, proprioceptors encode bodily mechanics and interoceptors encode information from viscera. Visceral sensory neurons provide input to primitive circuits in the brainstem, and activate at least three ascending central pathways that traverse the thalamus en route to the cortex, where information is integrated and represented in the insula, cingulate gyrus and somatosensory cortices.[1–3] Gut sensations start with visceral sensory neurons. However, the CNS is not a passive recipient of sensory input; sensory processing into the dorsal horn is subject to complex modulation.[4] Psychological and experiential factors powerfully modify processing via descending spinal pathways.[2,3] Excellent reviews of central visceral sensory processing are available.[1,5] Even in the periphery, visceral sensory neurons do not operate in isolation. Enteroendocrine cells of the mucosa, and immune cells in the gut wall both have important roles in initiating sensation and in modulating reflex control of the gut.[6,7] Interactions between the microbiota of the gut lumen and the immune system modulate mood and sensation.[8–10] Understanding the molecular basis of such interactions is currently the subject of intense research effort. It is clear that many newly discovered mechanisms are not ubiquitously used by all visceral sensory neurons. To understand how to target sensory neurons therapeutically, a good understanding of the different types of sensory neurons and their roles in evoking sensation is important. In this Review, we provide a simplified account of the extrinsic sensory pathways that link the gut to the CNS, based on the structure of endings in the gut. This scheme is compatible with, and might extend, physiologically based classifications of extrinsic primary afferent neurons.

Sensory innervation of the gut

Primary afferent neurons are activated by physical or chemical stimuli and trigger reflex pathways controlling function—the subset of these neurons that gives rise to conscious sensation are called 'sensory neurons'. In most cases it is uncertain which types of primary afferents actually underlie sensation, so we use the terms 'sensory neuron' and 'primary afferent neuron' interchangeably. Most understanding of gut sensory innervation comes from studies in a small number of species of animals (mice, rats, guinea pigs, ferrets, cats, rabbits and sheep), although a few studies (including one dating back to the 1960s) have recorded from sensory neurons innervating human gut, *in vitro*.[11–13] To the extent that the sparse data enables conclusions, sensory nerves in human gut seem similar to those in animals.

Classes of extrinsic afferent neurons

The extrinsic sensory neurons that innervate the gastrointestinal tract appear to form a baffling number of types, classified in many ways by different investigators. Their neurotransmitters and modulators, neurochemical markers such as lectin binding sites or neurofilaments, their neurotrophic requirements and ion channels and receptors that they express, have all been used to distinguish different types of sensory neurons. Physiological features include basal firing rate, conduction velocity, adequate stimuli, thresholds, peak firing frequencies, stimulus-response functions and rates of adaptation. Sensitivity to transmitters, hormones and cellular mediators also vary markedly; afferents have been distinguished on the basis of their responses to capsaicin, bradykinin, purines, histamine or 5-hydroxytryptamine, among others. Morphological characteristics include cell body size and location and the microscopic structure of peripheral endings.

Using characteristics to distinguish classes of sensory neurons is valid, but two caveats must be kept in mind. First, many physiological and pharmacological characteristics show marked

plasticity, even during the course of a recording. Acute damage caused by ischaemia, hypoxia, dissection or repeatedly applying a blunt probe can modify responses. Second, physiological characteristics are rarely binary, that is either present or absent; they are usually graded. Rates of adaptation, sensitivity to distension or responses to chemical mediators might range from very low to very high in otherwise similar neurons. Bearing these points in mind, primary afferent neurons that mediate sensation from the length of the gastrointestinal tract have been classified in many different ways, which has led to what is a rather fragmented current understanding. Here, we propose a simplification. We suggest that there are just five basic types of sensory neurons innervating the gastrointestinal tract, each with specific anatomical endings in the gut wall. Between them, they form the three major extrinsic sensory pathways from the gut (vagal, thoracolumbar and lumbosacral). The five types are summarized in Figure 1 and include the following: type I : 'intraganglionic laminar' endings predominantly located in myenteric ganglia within the gut wall, that primarily detect innocuous, and possibly noxious, mechanical distortion; type II : 'mucosal' afferents, with subepithelial endings, sensitive to enteroendocrine cell mediators and light mechanical distortion; type III : 'muscular–mucosal' afferents, with mechanosensitive endings between the muscularis mucosae and the mucosa proper that detect both muscular activity and mucosal distortion; type IV : 'intramuscular' endings, with endings primarily in the smooth muscle layers in the gut wall that probably detect mechanical stimuli; and type V : 'vascular' afferents, with sensitive endings primarily on blood vessels, which are sensitive to intense mechanical stimulation, but are modulated by a wide range of chemical mediators of damage and inflammation. Their relative abundance in the three pathways is summarised in Table 1.

Dual innervation of each region of the gut

Each region of the gut receives dual sensory innervation. The oesophagus, stomach, small intestine and upper colon are innervated by sensory neurons that originate in the nodose and

jugular ganglia, and that project peripherally via the paired vagus nerves. These regions are also innervated by thoracolumbar spinal afferent neurons projecting via the splanchnic nerves. The lower colon and rectum are also dually innervated, by thoracolumbar spinal afferents and by lumbosacral spinal afferents projecting via pelvic and rectal nerves. Thoracolumbar spinal afferents run in parallel (and indeed, often in the same nerve trunks) as sympathetic efferent pathways to the gut, whereas vagal and sacral spinal afferents run in parallel with parasympathetic efferent pathways to the gut. This arrangement has functional relevance. Vagal afferents terminate largely in the nucleus tractus solitarius of the brainstem, where they make monosynaptic[14] and polysynaptic connections[15] with parasympathetic vagal efferents (in the dorsal motor nucleus of the vagus) that innervate the upper gut. Contacts are largely via glutamatergic synapses.[16] Sympathetic preganglionic neurons receive little monosynaptic input from spinal afferents, but there are potent polysynaptic inputs from the dorsal horn.[17] Some spinal afferents also give rise to collaterals, which directly synapse onto sympathetic post-ganglionic neurons in prevertebral ganglia.[18] Similarly, pelvic and/or sacral afferent fibres run in parallel to sacral parasympathetic outflow and are intimately connected by synapses in the spinal cord.[19] The anatomical sharing of peripheral nerve trunks by efferents and primary afferents is not just parsimonious—it reflects important functional connectivity.

Classification of primary afferent neurons

Type I: intraganglionic laminar afferents

Vagal intraganglionic laminar mechanoreceptors

The first sensory neurons to the gut to be recorded and physiologically characterized were low-threshold, tension-sensitive mechanoreceptors.[20,21] These are robustly activated by both distension and contraction.[22] A specialized combination of dye filling and recording techniques made it possible to identify the morphology of the sensory endings that belonged to these mechanoreceptors.[23,24] Identification was achieved by making extracellular recordings,

in vitro, from vagal nerve trunks within 1–2 mm of the specimen of gut. Mechanosensitive sites on the preparation were marked by fine carbon particles applied on the tips of von Frey hairs used for focal stimulation. At the end of the recording, biotinamide was applied in solution to the recorded nerve for a period of 4–20 h, during which it was taken up and transported to the axon terminals in the tissue.[23] After fixation, biotinamide was revealed by streptavidin-conjugated fluorophores. By comparing dye-filled nerve endings with carbon marks on the tissue, the sensory endings of low-threshold vagal mechanoreceptors were shown to correspond to intraganglionic laminar endings (IGLEs) located in myenteric ganglia.[24,25]

These IGLEs detect distortion of the surrounding tissue by as-yet-unidentified stretch-activated ion channels[26] and probably signal gastric distension after a meal. They end in-parallel with the smooth muscle fibres of the gut wall, yet respond mechanically as in-series tension receptors. This paradox is explained by their fine branching endings within myenteric ganglia, which might detect compression of the ganglia by surrounding layers of the gut wall.[22] Indeed, the mechanical environment of the endings leads to differences in mechanosensitivity between the compliant upper stomach and the less distensible, contractile lower stomach.[27] Although these endings certainly respond to changes in wall tension, they also fire for extended periods during maintained distension. IGLEs are found in the oesophagus, stomach, small intestine and in reduced numbers in the upper colon.[28,29] Each axon gives rise to several IGLEs within a small area[24,25] but might also innervate widely separated areas.[30,31] IGLEs have been suggested to detect shear forces between orthogonally arranged (longitudinal and circular) muscle bundles coupled to either face of the ganglion.[32]

An intriguing feature of IGLEs, shared by many other visceral afferents, is their sensitivity to a range of biological mediators. IGLEs are potently excited by ATP,[26,33] probably when this biochemical is released from damaged cells [34] ATP might also couple mechanosensitive epi-

thelia to excitation of mechanoreceptors;[35,36] however, the time course of ATP release and its effects preclude a role in mechanotransduction by IGLEs.[26] The same endings in ferret stomach and oesophagus express inhibitory $GABA_B$ receptors,[37] excitatory and inhibitory metabotropic glutamate receptors[38,39] and are inhibited by ghrelin. Responses of IGLEs to ghrelin show marked plasticity, being upregulated after overfeeding.[40] Such peripheral chemosensitivity might reflect the fact that these receptors have important roles at the endings of these sensory neurons in the central nervous system. Alternatively, there might be pathophysiological scenarios in which the hormone ghrelin or the transmitters glutamate, GABA or ATP accumulate within the gut wall to levels that modulate firing of these mechanoreceptors, perhaps after release from enteric nerve terminals.

Sacral spinal intraganglionic laminar mechanoreceptors

Similar, low-threshold, slowly adapting mechanoreceptors have been identified in sacral and/or pelvic pathways to the distal bowel. They respond to small distensions of the gut wall, within the physiological range caused by normal propulsion of faecal matter. Their transduction sites correspond to flattened branching endings in myenteric ganglia, called rectal IGLEs or rIGLEs. These are morphologically similar to vagal IGLEs, but they are simpler with less extensive branching. In the guinea pig, these rIGLEs are abundant in the rectum but are increasingly sparse further up the distal colon.[41] Each afferent has endings in multiple enteric ganglia. Electrophysiologically, they are typically silent at rest, but are powerfully activated by distension, with instantaneous firing frequencies up to 50 Hz.[42] The degree of distension at which their response saturates has not been determined, but they encode stretch over a wide dynamic range, into the noxious extremes[146]. They are activated by contraction of both the longitudinal and circular muscle layers, possibly via compressive forces acting on myenteric ganglia.[43] Like their vagal counterparts, these endings are rarely responsive to capsaicin, the activator of TrpV1 channels (transient receptor potential cation channel subfamily V member

1). Similar low-threshold mechanoreceptors have been recorded previously in pelvic pathways in the cat,[44] rat[45] and mouse,[46] where they were referred to as 'muscular' receptors. These primary afferents are probably excited by physiological distension of the distal colon and activate parasympathetic reflexes[47] that are important in defaecation.[48]

Type II: mucosal afferents

Vagal mucosal afferents

Originally described in the 1950s[51], vagal mucosal afferent fibres are not sensitive to distension or contraction of the upper gut, but can be activated by light stroking or compression of the mucosa. Many of these fibres are also sensitive to luminal osmotic, pH and chemical stimuli.[49–51] Morphologically, several types of vagal mucosal endings have been distinguished. In the small intestine there are endings that project along the length of the villi, ramifying beneath the epithelial layer[52]. Another type encircles the Crypts of Lieberkuhn and do not penetrate into the villi.[52]

Vagal mucosal afferents are often potently activated by mediators released from the extensive populations of enteroendocrine cells found in the mucosa. There are 10–20 classes of enteroendocrine cells, each of which releases a subset of about 20 mediators, often in response to nutrients.[53] Some of these mediators act in a paracrine fashion on primary afferent terminals, but some act as true hormones, coordinating secretory and other activity between different regions of the gut or by acting in the brain. The most abundant enterendocrine cells are the enterochromaffin cells that contain and release much of the body's 5-hydroxytryptamine, with potent effects on mucosal afferents.[54] In the mouse stomach, some vagal mucosal afferents are directly activated by bile salts.[33]

A subset of enteroendocrine mediators act as satiety factors, largely through local effects on vagal mucosal afferent terminals. These include cholecystokinin (CCK), glucagon-like peptide 1 (GLP1), apolipoprotein A-IV, enterostatin, gastrin-releasing peptide (a member of the bombesin family), oxyntomodulin, amylin and peptide YY. CCK administration causes reduced food intake,[55] while antagonists to CCK_A receptors substantially increase food intake;[56] thus, endogenous CCK has a role in controlling meal size. The satiety-inducing effects of CCK require an intact vagus nerve.[57] CCK excites vagal afferent fibres with mucosal endings,[58] and both long chain fatty acids and casein cause activation of vagal mucosal afferents, which is blocked by CCK_A antagonists.[59,60] PYY also excites vagal afferent neurons and its satiety-mediating effects might require intact vagal nerve connections,[61,62] although it also acts hormonally in the brain.[63] Even the prototypical hormone, secretin, activates a subset of vagal mucosal afferent neurons.[64] The action of gut hormones on vagal afferent endings is not surprising. The concentration of these mediators in the subepithelial space might reach higher orders of magnitude than ever occurs in the bloodstream, particularly when hepatic clearance is taken into account. Vagal mucosal afferents probably do not respond generically to all mediators: for example, mucosal afferents in the rat jejunum are excited by 5-hydroxytryptamine and histamine[65] but not by CCK.[66] How mucosal mechanical sensitivity relates to chemosensitivity is also unclear; however, mechanosensitivity does not depend on 5-hydroxytryptamine signalling from enterochromaffin cells.[67]

Spinal mucosal afferents

Low intensity mechanical stimuli applied to the mucosa of the distal bowel activate a class of spinal afferents that is insensitive to both distension and contraction. These so-called 'mucosal afferents' are probably the spinal equivalents of vagal mucosal afferents. They are excited by stroking the mucosa with very light von Frey hairs (10 mg) and are strongly activated by 5-hydroxytryptamine or 5-HT3 agonists.[68] Spinal mucosal afferents are more abundant in

lumbosacral than thoracolumbar spinal pathways,[46] but the detailed structure of their endings has not yet been reported. After biotinamide filling of rectal nerves, we observed branching varicose afferents in the subepithelial plexus of guinea pig rectum but their location has not yet been correlated with physiologically-mapped receptive fields.

Type III: vagal and spinal muscular–mucosal afferents

Low-threshold, distension-sensitive intraganglionic mechanoreceptors are readily distinguishable from mucosal afferent endings, which are not activated by distension or muscle contraction. However, some extrinsic sensory neurons are activated by both light mucosal distortion and by distension or contraction. Vagal 'tension-mucosal' receptors have been described in the gastro-oesophageal regions of ferrets. These receptors responded to both light mucosal stroking and to distension.[69] In spinal pathways, receptors that are sensitive to both mucosal stroking and to distension have been described in mouse pelvic/sacral pathways. It has been suggested that these sensory receptors have two separate transduction sites, in the muscularis externa and in the mucosal lamina propria.[46] Work from our laboratory suggests that they actually transduce both distension and mucosal distortion from endings in the subepithelial plexus (unpublished data). The role of these afferent fibres is open to speculation, but their remarkable sensitivity to mucosal shear suggests that they might detect movement of content over the surface of the gastrointestinal tract. In the rectum, we suggest that they might contribute to spinal defaecatory circuits and perhaps conscious sensation.

Type IV: vagal and spinal intramuscular afferents

Early studies on the morphology of vagal afferents revealed a second anatomical type of fibre located in the outer layers of the gut. These 'intramuscular arrays' consist of branching fibres extending parallel to bundles of muscle fibres in *muscularis externa*.[70] They are densest in the fundus and in the sphincteric regions of the stomach[71] where they run close to intramuscular

interstitital cells of Cajal (ICC).[52] This has led to the speculation that intramuscular arrays, intramuscular ICC, and possibly efferent nerve fibres, might form functional complexes, perhaps analogous to striated muscle spindles. To date, no electrophysiological activity has been recorded that can confidently be attributed to vagal intramuscular arrays.

Low-threshold, slowly adapting mechanoreceptors, with IGLEs as their transduction sites, are not the only vagal mechanoreceptors in the oesophagus. Another population of mechanosensitive afferents, with cell bodies in both nodose and jugular ganglia, has higher thresholds and lower net firing rates, a wide dynamic range and graded firing into the noxious range of intraluminal pressures. Importantly, many of the axons contain the capsaicin-sensitive TrpV1 channel, and are frequently peptidergic, similar to many spinal nociceptors.[72] Peptide-containing sensory neurons from the jugular ganglion provide extensive sensory innervation to the thoracic oesophagus, airways and heart, but less to the stomach or abdominal organs.[73] Some of these nociceptor-like afferents might give rise to intramuscular-array-like endings in the upper gut.

Although intramuscular arrays have been studied extensively in vagal pathways, similar types of endings have been described more distally, especially in the large intestine. Dye fills of extrinsic nerve trunks to the colon and internal anal sphincter label arrays of axons within circular and/or longitudinal muscle layers, particularly in the rectum.[41] In mutant mice that lack enteric ganglia in the distal bowel, stretch-sensitive mechanoreceptors that transduce mechanical stimuli from intramuscular endings are present.[74,75] In the smooth muscle of the internal anal sphincter, sacral afferents form arrays of intramuscular endings that are sensitive to both distension and to light von Frey hairs.[76] Although these endings are likely to be mechanosensitive, little physiological evidence exists to date as to whether they are likely to function as tension receptors, length receptors or a combination of the two.

Type V: Spinal vascular afferents

Spinal afferent innervation of the gut arises from dorsal root ganglia in thoracolumbar and lumbosacral segments, except for the upper oesophagus, which is innervated by cervical spinal afferents.[77,78] In the mouse colon, most of the thoracolumbar spinal afferent endings can be activated by strong local compression of the mesenteries or wall of the gut.[46] First described in the 1960s, these endings are associated with branch points of mesenteric arteries and encode both contraction and distension of the gut wall and traction on the mesenteries,[79] with relatively low sensitivity. This low sensitivity is particularly marked in the noncompliant, thick-walled mouse colorectum,[80] which might have led to their distension sensitivity being missed in some studies.[46,81] Similar afferent endings have been confirmed in other regions of the gut in several species.[82–84] These vascular afferent endings are also associated with other viscera, including the spleen, ovary, bladder and pancreas.[85] Endings of this type are sensitive to ischaemia, hypoxia and capsaicin,[86–88] and they are believed to comprise a major type of nociceptor. They are also sensitive to changes in perfusion rate, with an increased firing rate during reduced flow,[89] although this seems to depend more on mechanical factors than oxygen delivery.[90] Given that these receptors can be activated by blunt probing or compression on the outer wall of the gut, they were initially described as 'serosal' receptors.[50] Later, they were subdivided into 'mesenteric' and 'serosal', depending on the location of their mechano-sensitive sites[50].

Vascular afferents comprise about one-third of lumbosacral spinal afferents to the gut[46] but a much higher proportion of thoracolumbar spinal afferents. Intracellular recordings from lumbosacral dorsal root ganglion neurons revealed two populations of mechanosensitive neurons; one with relatively high thresholds to distension, slow firing rates and that frequently contain calcitonin gene related peptide (CGRP) and TrpV1,[91] which are likely to correspond to vascu-

lar afferents; the other class had lower thresholds and were more excitable, and probably correspond to rIGLEs.

The morphology of the peripheral endings of vascular afferent fibres has been determined in detail. They give rise to fine branching peri-arterial axons that are preferentially associated with arterial branch points. Importantly, they are not restricted to mesenteric vessels, but continue into the gut wall, innervating the arteries and second order arterioles in the submucosa,[92] but not finer branches or capillaries. The same afferent unit can have transduction sites on both mesenteric and submucosal vessels[92]. These afferents detect mechanical stimuli from mechanotransduction sites on both extramural and intramural blood vessels. They also give rise to collaterals to myenteric and submucosal enteric ganglia, which largely lack mechanosensitivity[42] but which provide excitatory synaptic connections onto enteric neurons.[93–95] They also give rise to axon branches in the mucosa and muscle layers but do not have collaterals in either the serosal membranes or mesenteric membranes. For this reason, the names 'serosal' and 'mesenteric' are misleading. Given that their transduction sites are on, or close to blood vessels, the term 'vascular' ending seems more appropriate. Vascular afferents do not seem to be present in vagal pathways but, as mentioned above, are present in lumbosacral pathways to the distal bowel.[46,91]

Vascular afferents: vasodilator role

Vascular afferents are likely to have an important sensory role, but they also have potent efferent effects on the blood vessels that they appose. The major resistance vessels of the gut are submucous arterioles, although the mesenteric feed-arteries also contribute.[96] Many arteries receive a prominent vasodilator input that can be stimulated from spinal ganglia.[97] These spinal sensory neurons are peptidergic, usually containing CGRP and substance P together,[98] and their axons form a distinctive peri-arterial plexus. Electrical stimulation or capsaicin cause

hyperpolarization and dilatation of mesenteric vessels,[99] mimicked by CGRP and blocked by a CGRP antagonist.[100,101] Synaptic transmission from collaterals in enteric ganglia excites enteric vasodilator neurons, adding to local hyperaemia.[102] Substance P (and neurokinin A) is also released onto blood vessels, where it increases vascular permeability and thereby causes plasma extravasation associated with neurogenic inflammation in many tissues throughout the body[101a]. Neurogenic inflammation also occurs in the gastrointestinal tract although not to the same extent as in the skin or other tissues.[103,104] The efferent function of these nerves has a protective role by increasing blood flow when tissue integrity is challenged. Thus, back-diffusion of gastric acid evokes a protective hyperaemia largely via activation of capsaicin-activated, H^+-sensitive TrpV1 ion channels.[96] Activation of peptidergic afferents by localized distension evokes an axon reflex, leading to peptide release and vasodilation in upstream mesenteric vessels.[105] When gut muscle contracts strongly, blood flow is significantly modified: the activation of sensory vasodilator mechanisms might therefore cause a compensatory vasodilation. It seems that activation of this vasodilator axon reflex occurs only during noxious stimulation: there is no evidence for CGRP-mediated systemic vasodilation at rest.[106]

Vascular afferents: activation by mediators

There are potentially complex interactions between the sensory and efferent functions of peptidergic thoracolumbar mechanoreceptor endings on mesenteric and submucosal blood vessels. This situation is further complicated by the bewildering range of chemosensitivities of these cells. They are activated or modulated by a range of algogenic mediators released during tissue damage, including ATP, bradykinin, glutamate, chemicals from mast cells (including mast cell proteases), nerve growth factor, prostaglandins, histamine and 5-hydroxytryptamine[106a]. They also have receptors for key inflammatory cytokines including IL-1β,[107] IL-6[108] and TNF.[109] The excitability of these neurons is profoundly modulated during experimental inflammation, particularly in the post-inflammatory period.[110] Consistent

with this finding, visceromotor responses to colonic distension are exaggerated during and after inflammation.[111,112]

TRP channels in vascular afferents

In the last decade, great progress has been made in identifying molecular mechanisms underlying afferent plasticity; much of this research has been carried out on vascular afferent nociceptors. In particular, progress has been made in revealing how inflammation and stress modify sensory neuronal activity. A number of TRP ion channels are worth specific mention, in particular TrpV1, TRPV4 and TRPA1.

TrpV1 is a cation channel that is activated by heat (>43˚C), low pH and by anandamide. It is abundant in peptidergic thoracolumbar vascular afferents,[113,114] and is detectable in the majority of their perivascular, intramuscular, ganglionic and mucosal axons in the gut wall.[115] TrpV1 might be directly activated by the acidosis that accompanies frank tissue damage[116] or by local increases in temperature during inflammation. The thermosensitivity of these channels might be supplemented by the heat activated calcium-dependent chloride channel, ANO-1, which is co-expressed in some nociceptors.[117] TrpV1 is also modulated by agents released during inflammation, including arachidonic acid metabolites, bradykinin, 5-hydroxytryptamine, nerve growth factor, purines and prostaglandins. Its expression is upregulated in nerve fibres in IBD[118] and in IBS.[119] TrpV1 mediates at least some of the sensitization of visceral afferents caused by experimental colitis,[120] in part by increased expression in spinal afferents.

TRPV4 is abundant in thoracolumbar spinal afferents to the gut.[121] This ion channel is gated by osmotic swelling, mechanical distortion, endogenous 5',6'epoxyeicosatrienoic acid, anandamide and 2-AG (2-arachidonoylglycerol) and, in a partially desensitizing fashion, by tem-

perature >27˚C.[122] Mechanically-induced firing of vascular afferents is attenuated in TRPV4-null mice and by a TRPV4 channel blocker.[121] Conversely, a TRPV4 agonist causes hypersensitivity to distension and enhanced afferent responses to distension.[121,123] TRPV4 is extensively co-localised with PAR2 (proteinase-activated receptor 2), which sensitizes thoracolumbar spinal afferents to the gut during neurogenic inflammation.[124] Activation of PAR2, by proteases released by mast cell degranulation, can lead to behavioural hypersensitivity and increased afferent firing via sensitization of TRPV4.[123,125]

TRPA1 is the third TRP channel known to have a key role in setting the sensitivity of vascular afferents. It might also mediate PAR2-evoked sensitization in the gut[126] and/or bradykinin-mediated hypersensitivity.[127] TRPA1 is directly mechanosensitive in gastrointestinal afferents and interacts with TrpV1.[127] It is opened by a wide variety of compounds, many of which might covalently modify its structure rather than acting at discrete receptor sites. These include pungent components of spices (such as mustard oil, wasabi, cinnamaldehyde, garlic, and menthol), irritant chemicals (including acrolein, pungent anaesthetics), hydrogen peroxide, nicotine,clotrimazole, dihydropyridines[128] and the gaseous mediator, hydrogen sulphide.[129]

Silent afferents: a subtype of vascular afferent?

In many studies, afferent units that were previously silent and insensitive to mechanical stimuli become active and mechanosensitive when exposed to capsaicin or inflammatory mediators (sometimes delivered in combinations, known as ‘inflammatory soup’). In a systematic study, it was estimated that about one-quarter of thoracolumbar and lumbosacral spinal afferents are mechanically insensitive in naive preparations.[130] This estimation was based on recordings made from either pelvic nerves or splanchnic nerves while electrically stimulating axons peripherally. These numbers might be an overestimate, as paravertebral sympathetic

efferent axons[131] might have been activated in these pathways by the same electrical stimuli. Nevertheless, zymosan treatment leads to a decrease in the proportion of mechanically insensitive ('silent') afferents both acutely, and several weeks later[81]. Interestingly this effect coincided with an increased proportion of mechanically sensitive 'serosal'-type afferents.[81] Other classes were variably affected: pelvic colorectal muscular–mucosal afferents were also sensitized, but muscular afferents (which correspond to pelvic afferents with intraganglionic laminar endings) were not affected.[81,110,132]

The suggestion that serosal vascular afferents are a major source of silent afferents is perhaps not surprising. Vascular afferents show a wide range of mechanosensitivities and consistently are among the least mechanically sensitive afferents in the gut. It seems likely that 'silent' afferents might comprise the extreme, insensitive end of the distribution of vascular afferents, rather than a distinct class. Like other vascular afferents, they are very prone to modulation by inflammatory mediators, both by acute actions and by long-term changes in gene expression,[133] which convert them from a silent to a mechanically sensitive phenotype.

Viscerofugal enteric primary afferents

One other type of primary afferent fibre, which is not extrinsic in origin, needs to be included in this brief survey. Many enteric neurons are directly excited by mechanical and chemical stimuli; they activate local enteric motor, secretory and vasomotor reflexes. Enteric primary afferent neurons have been reviewed elsewhere[134,135] and are generally accepted not to have any direct connections with the CNS. However, one particular class of enteric neuron has synaptic outputs that might enable them to contribute to conscious sensation. These are the enteric 'viscerofugal' neurons, which have cell bodies in myenteric ganglia and project out of the gut, to pre-vertebral ganglia.[134] At least some of these neurons in the distal bowel project directly to the spinal cord.[136–138] Viscerofugal neurons have been shown to be directly mecha-

nosensitive[139,140] but are also synaptically activated by enteric circuitry.[140,141] Their axons project in mesenteric nerves, pelvic nerves and possibly splanchnic nerves and contribute to recorded action potential discharge in colonic nerves.[140]

Extrinsic afferent pathways and sensation

Distension and contraction are powerful activators of pain pathways that can be monitored behaviourally by pseudaffective and visceromotor reflexes.[142] Colorectal distension has been widely studied. Lumbosacral afferents primarily mediate the visceromotor response to colorectal distension in normal mice, as severing pelvic pathways abolishes the response, but lesioning splanchnic pathways has little effect.[143] Lumbosacral dorsal rhizotomy abolishes visceromotor reflexes, but inflammation reinstates a thoracolumbar contribution,[144] which indicates that thoracolumbar vascular afferents can contribute to pain symptoms after inflammatory challenge. However, further up the colon, the situation might be quite different. Distension >16 cm above the anal sphincter in humans evoked pain, but this pain did not occur after bilateral sympathectomy. By contrast, sympathectomy did not abolish painful sensations evoked by distension within 16 cm of the sphincter.[145] The same study suggested that balloon distension of the upper colon and small intestine, with pressures of 15–40 mmHg, also evoked graded pain, which was abolished after bilateral sympathectomy from T7 to L3.[145] These results indicate that pain from the rectum is substantially mediated via pelvic pathways, while pain from more proximal regions of the gut is primarily mediated via thoracolumbar spinal afferents—the majority of which correspond to the vascular afferents. Evidence suggests that low-threshold, wide-dynamic range lumbosacral mechanoreceptors from the colorectum, which dominate pelvic nerve responses to rectal distension, are likely to be responsible for activation of pain pathways.[146] These mechanoreceptors include rIGLES and rectal muscular-mucosal endings. Interestingly, sensation evoked by slow ramp distension in the human rec-

tum was reduced by luminal application of the local anaesthetic, lidocaine, which suggests a possible role for muscular–mucosal afferents with endings close to the mucosal surface.[147]

Although pain from the gut is ubiquitous in response to high amplitude distension, other modalities of sensation are commonly experienced, particularly at the proximal and distal ends of the gastrointestinal tract. For example, humans can discriminate between solid, liquid and gaseous content in the distal rectum and anal canal,[148] although the sensory receptors responsible are not clear. There might be a 'sampling reflex' in which relaxation distally enables rectal content to move into the anal canal where different materials are distinguished. It is possible that the muscular–mucosal receptors, with their extraordinary sensitivity to mucosal distortion, might have a role in this discrimination. Low levels of distension of the rectum activate rectal contractions and anal relaxations;[48] largely via sacral parasympathetic pathways.[19] Rectal distension in humans evokes sensations of urge at low pressures, unpleasantness at high pressures and pain at still higher pressures.[149] Pain and unpleasantness co-vary more closely than either sensation with urge. This finding suggests that urge might be mediated by different sensory pathways from either unpleasantness or pain.[149] Consistent with this idea, studies in patients with spinal cord lesions suggest that painful rapid rectal distension might be preferentially detected by splanchnic pathways, whereas slow ramp distension activates pelvic pathways, with urge preceding discomfort.[147]

The proximal gastrointestinal tract is also dually innervated via vagal and splanchnic pathways. Gastric distension gives rise to sensations of fullness and has a potent effect on food intake, via gastric mechanoreceptors rather than chemoreceptors.[150] By contrast, satiety induced by nutrient infusion into the small intestine primarily reflects chemical content and is probably mediated via hormonal signals and via vagal mucosal afferents, excited by enteroendocrine cells in the duodenal mucosa.[150] Small intestinal nutrient signals modify sensation

evoked in humans by gastric distension, associated with effects on gastric motility,[151] suggesting complex central integration. It is well established that abdominal vagal afferents can contribute significantly to nausea and vomiting. At least some of this contribution is mediated by mucosal afferents that are sensitive to 5-hydroxytryptamine, released by a variety of toxins from enterochromaffin cells.[152,153] These same afferents might also be sensitive to nutrients,[154] including simple carbohydrates.

Some doubt exists as to whether gastric distension is sensed by vagal tension receptors or by vagal length receptors. Studies in humans have shown that changes in gastric muscle tone evoked by glucagon or erythromycin do not markedly affect the sensation of fullness evoked by a distension of fixed volume[155]. This finding suggests that volume, rather than pressure or wall tension, is sensed by afferents that mediate fullness.[155] It has been argued, on the basis of anatomical considerations, that there must be specialized volume receptors in the stomach that encode distension, largely independent of wall tension or pressure. However, to date, electrophysiological studies have failed to record specific length-sensitive extrinsic afferents. Indeed, afferent endings in the fundus of the ferret that seemed to encode distension rather than pressure, could also be activated by contraction, suggesting that they had a considerable tension-sensitive response.[27] It is possible that the receptors responsible for fullness during prolonged gastric distension are IGLEs, although a role for intramuscular arrays cannot be discounted. Consistent with this idea, maintained distension of strips of guinea pig corpus evoked increased levels of firing by IGLEs that persisted for several minutes.[25] IGLEs might not simply respond to wall tension, but rather a mixture of length and tension caused by distortion of the gut wall.[22]

Conclusions

Extrinsic afferent innervation of the gut has been the subject of study for more than 60 years. Over this period, many types of primary afferent sensory neurons have been characterized, using a wide variety of features. Reconciling findings from different studies has often proved difficult, and little consensus has emerged about the classes of afferents that mediate sensation from the gut. Despite this problem, major advances have been made in understanding the molecular mechanisms involved in transduction and modulation of sensory neurons, with potential clinical significance for understanding, diagnosing and treating gastrointestinal disorders. Here, we have tried to draw together the rather disparate literature about the types of gut sensory neurons, to provide a foundation for future molecular studies. Five basic types of extrinsic sensory ending in the gut, possibly supplemented by enteric viscerofugal neurons, might account for the full range of sensory information emanating from the mammalian gastrointestinal tract. This framework might be useful for understanding which ion channels, receptors and second messenger pathways interact within specific sensory neurons to give rise to both normal and pathological sensation from the gut.

Key points

- The gut is innervated by several classes of extrinsic sensory neurons, which have distinct combinations of properties that make them sensitive to particular mechanical and chemical stimuli
- Progress has been made in identifying the morphology of sensory endings in the gut wall, possibly providing a more robust means to classify sensory innervation
- Five different morphological types of endings can be distinguished by their structure; these account for the great majority of sensory nerves to the gastrointestinal tract and seem to correspond to distinct major physiological classes

- The physiological properties of extrinsic afferent nerves innervating the gut are characterized by variability and by plasticity, which can make it difficult to reliably distinguish the classes of sensory neurons that underlie gut sensation

Figure Legend

Figure 1: Five morphological types of extrinsic sensory neurons to the gut. The five types of sensory endings in the gut wall are outlined, together with enteric viscerofugal neurons (for the sake of completeness). Transduction sites are shown as open circles; transmitter release sites are shown as open triangles. Vascular afferents are the most complex afferents, with both intramural and extramural perivascular axons, and collaterals in enteric ganglia, mucosa, muscularis externa and in prevertebral ganglia. Intraganglionic axons provide intraganglionic laminar endings, mostly in myenteric ganglia. Mucosal afferents innervate the subepithelial mucosa. Muscular-mucosal afferents have endings deep in the mucosa, close to the muscularis mucosae, and intramuscular afferents have nerve endings within longitudinal and/or circular muscle layers. Abbreviations: CM: circular muscle, LM: longitudinal muscle, MP: myenteric plexus

Table 1. Distribution of endings of extrinsic primary afferents between the three major anatomical pathways to the gut*

	Enteric viscerofugal	Vascular	Intraganglionic laminar	Mucosal	Muscular mucosal	Intra-muscular
Vagal	+	0	+++	+++	+++	+++
Thoracolumbar	+++	+++	0	+	0	0
Lumbosacral	+++	+++	+++	+++	+++	+++

*Enteric viscerofugal neurons have been included, for the sake of completeness. Vagal pathways include neurons originating in paired nodose and jugular ganglia. Thoracolumbar pathways have cell bodies in thoracolumbar dorsal root ganglia and project via splanchnic nerves and mesenteric/colonic/hypogastric nerves. Lumbosacral pathways have cell bodies in lumbosacral dorsal root ganglia and project via pelvic nerves and rectal nerves to the distal bowel. 0: absence of afferent type in pathway, + denotes moderate abundance; +++ denotes high abundance.

Review criteria

Extensive use was made of Medline and Pubmed databases to supplement a general familiarity with the literature built up by tracking the published literature. Numerous searches were carried out using general terms: "sensory", "afferent", "gastric", "stomach", "esophagus", " duodenum", " ileum", " intestine", "colon", " rectum", " rectal", "retrograde", " anterograde", "immunohistochemisty", "immunocytochemistry", "antibody" or "antiserum" together with more specialized terms, for example "CGRP or calcitonin gene related peptide" etc. Papers were restricted to full text papers, published in English. In some cases references were followed back from reference lists in papers.

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