

How many kinds of visceral afferents?

M Costa, S H J Brookes, V Zagorodnyuk

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Most afferent signals from the viscera do not give rise to conscious experience and yet they participate in the complex neural control of visceral functions. Surprisingly little information is available on the origin, morphology, and receptor functional characteristics of the nerve endings of most primary afferent neurones to the digestive tract. This review deals with the morphological nature of the afferent neurones that supply the gastrointestinal tract specifically.

digestive tract and the correlation with their function is in its infancy.

INTRODUCTION

Only in specific circumstances do humans appear to have conscious experience of internal organs and yet the central nervous system is continuously bombarded by ongoing afferent traffic from viscera that is not associated with conscious experience.¹ This implies that most visceral afferent signals do not give rise to conscious perception. The primary afferent neurones that detect bodily functions can be distinguished as somatic and visceral. Those supplying blood vessels may be regarded as a separate component because of the ubiquitous nature of the blood supply.

This short review deals with the morphological nature of the afferent neurones that supply the gastrointestinal tract specifically. The major difficulty encountered by investigators in identifying the primary afferent neurones and their endings is that the digestive tract contains an extensive network of intrinsic neurones able to operate independently from the central nervous system. This "independent" enteric nervous system is composed of circuits that include intrinsic afferent neurones. Thus there are two types of afferent neurone involved in gastrointestinal functions—namely, IPANs, which are part of the enteric nervous system, and EPANs. Their nerve endings are mostly indistinguishable on the basis of their morphology. Similarly, the nerve endings of the axons of other enteric inter- and motor neurones, as well as the endings of the extrinsic preganglionic sympathetic and parasympathetic neurones that terminate within the gut wall, are mixed with the primary afferent nerve endings. Unravelling of these important nerve endings from the multitude of other nerve endings is in its infancy.

SUMMARY

Most afferent signals from the viscera do not give rise to conscious experience and yet they participate in the complex neural control of visceral functions. Studies of the extrinsic visceral afferent nerves supplying the digestive tract have been hampered because their nerve endings are intermingled with the extensive net of nerve fibres from the enteric nervous system, which also contains intrinsic primary afferent neurones (IPANs). The extrinsic afferent neurones (EPANs) to the digestive tract are unmyelinated and are located either in vagal or spinal ganglia where they are mixed with somatic afferent neurones and reach the gut via vagal, splanchnic, pelvic, and pudendal nerves. Anterograde fillings *in vivo* and *in vitro* have been used to reveal the mode of termination within the gut wall. The nerve endings of vagal afferents in the stomach are intraganglionic laminar endings (IGLEs) or intramuscular arrays (IMAs) and these supply almost all of the digestive tract. IGLEs are the site of mechanosensory transduction. Similar IGLEs supply the rectum (rectal IGLEs) running in the pelvic nerves. Spinal afferent neurones terminate as free nerve endings within the different gut layers. Different neurochemicals are present in the visceral afferents to the digestive tract but little is known of their specific function. Mucosal visceral afferent nerve fibres are activated and modulated by substances such as serotonin and cholecystokinin, released from mucosal enteroendocrine cells. Spinal visceral afferents provide collaterals to enteric and prevertebral ganglia and blood vessels where they are likely to play an efferent function. Intestino-fugal neurones provide another source of neural activity that leaves the digestive tract to affect neurones in prevertebral ganglia and in the spinal cord. Identification of afferent nerve endings in the

STRATEGIES FOR IDENTIFICATION OF EPANs

Axons of EPANs reach the digestive tract within nerve bundles. The major afferent connections occur via the vagus nerve (vagal afferent pathways), via the mesenteric nerves and the thoracic and lumbar splanchnics (spinal afferent pathways), and via the pelvic and pudendal nerves (sacral spinal afferent pathways). Most nerve fibres are unmyelinated.^{2,3} Two different

See end of article for authors' affiliations

Correspondence to:
Professor M Costa,
Department of Physiology,
School of Medicine,
Flinders University, PO Box
2100, Adelaide 5001, SA,
Australia; marcello.
costa@flinders.edu.au

Abbreviations: IPANs, intrinsic primary afferent neurones; EPANs, extrinsic primary afferent neurones; IGLEs, intraganglionic laminar endings; CGRP, calcitonin gene related peptide; IMA, intramuscular array; SP, substance P; CNS, central nervous system

questions arise with the extrinsic visceral afferents. Firstly, there is the question of where the nerve cell bodies of origin are located. The second relates to the structure and distribution of the nerve endings within the gut wall. The major problem in identifying the extrinsic afferent nerve endings, with the exception of some specialised terminals in the upper digestive tract and in the anal canal, has been that the other terminals may have no special morphological features that distinguish them from efferent or enteric nerve terminals.

There are several strategies to address these problems. Traditionally, the origin of neuronal pathways has been tackled with lesion studies to detect degeneration of nerve terminals when disconnected from their cell bodies of origin. However, degeneration of terminals after extrinsic denervation in the intestinal wall is hard to demonstrate.² In addition, extrinsic efferent nerve fibres also degenerate.

More recently, tracing studies have become the most effective way to study the origin of extrinsic afferent nerve terminals in the digestive tract. Only recently, retrograde and anterograde tracing techniques have been applied to these pathways. Retrograde labelling from the digestive tract confirms that the cell bodies are located in the nodose (vagal afferents) and dorsal root ganglia (spinal and sacral afferents). In these ganglia, they are mixed with all other primary afferent neurones supplying other viscera and blood vessels of the body wall (somatic). There appears to be no regional-topical distribution of the nerve cells. Also, each sensory ganglion supplies a large portion of the digestive tube. Anterograde tracing *in vivo* is performed by injecting neuronal markers into the sensory ganglia. These are then taken up by the nerve cells and transported to their terminals. The techniques have been pioneered in the laboratory of Terry Powley and Hans Berthoud, and have revealed for the first time details of sensory innervation of the gastrointestinal tract.

A modification of anterograde tracing suitable for visualising nerve endings of severed afferent axons has been developed in our laboratory for studies *in vitro*.⁴ This technique has been used to study extrinsic afferent terminals in the oesophagus and stomach.

Another strategy for localisation of primary afferent neurones is the use of histochemistry. If the chemical coding—that is, the specific combination of neurochemicals that are present in a functional class of primary afferent neurones—is established, the distribution of the specific markers can be used to map the distribution of these nerve terminals in the digestive tract. For example, the presence of the peptide calcitonin gene related peptide (CGRP) in a proportion of spinal afferent neurones to the guinea pig small intestine enabled the distribution of these fibres to be described.⁵

EXTRINSIC AFFERENT INNERVATION OF THE UPPER GASTROINTESTINAL TRACT

Using anterograde tracing methods *in vivo*, the oesophagus and stomach were found to be supplied by primary afferent neurones located in the nodose ganglia.^{6–8} Vagal afferent fibres form specialised nerve endings within the myenteric ganglia, termed IGLEs. These are distributed fairly homogeneously along the entire length of the oesophagus in the species studied so far (rat and mouse). In the rat, some similar structures have been reported in the mucosa of the upper oesophagus not associated with enteric ganglia.⁹ In the guinea pig oesophagus, IGLEs have been demonstrated to be the mechanosensitive terminals responsible for the stretch induced afferent activity.¹⁰ The stomach is also supplied by similar afferent nerve terminals of vagal origin.^{6,7} In the guinea pig, IGLEs are the site of transduction for passive and

active mechanical tension.¹¹ Their nerve endings are mainly located on the surface of ganglia with some lamellar endings deep in the ganglia.¹² They have a high density of mitochondria.¹³ An important type of primary afferent nerve ending of vagal origin is the IMA.^{6,7} These endings are more dense in the pyloric sphincter region. Despite their proposed role as tension receptors,^{14,15} evidence for this hypothesis is still lacking. Club-like afferent nerve endings of vagal origin are present in the thick muscle of the antrum. The stomach is also supplied by capsaicin sensitive spinal afferent fibres, identified tentatively as containing tachykinins and CGRP. Varicose nerve terminals appear to meander within the layers and enteric ganglia, with no specialised nerve endings.^{16,17}

SMALL INTESTINE

IGLEs and IMAs of vagal origin become more sparse along the small intestine.⁶ After the first suggestions based on lesion studies,¹⁸ anterograde tracing *in vivo* demonstrated that, in the mucosa, there are also primary afferent nerve endings of vagal origin.¹⁹ The spinal primary afferent neurones also supply the small intestine with terminals in the enteric ganglia and in the mucosa.³ However, up until now only one paper using an early method for anterograde tracing was published.²⁰

LARGE INTESTINE

There are still primary afferent fibres of vagal origin that reach the large intestine forming IGLEs⁶ and similarly, spinal afferent neurones supply the enteric plexuses and the mucosa of the colon.^{21,22} Afferent fibres from the colon reach the spinal cord via the lumbar colonic, hypogastric, and pelvic nerves.²³ The rectum and anal canal appear to receive the extrinsic afferent supply via the pelvic and pudendal nerves.^{24–26} Recent results show that there are special nerve endings similar to IGLEs (rectal IGLEs) which are sensitive to local mechanical stimuli and reaching the rectum via the pelvic nerves.²⁷ Earlier report of afferent nerve endings in the anal region in humans came from Duthie and Gairns.²⁴ They described free corpusculated nerve endings (Meissner, Krause, Golgi-Mazzoni, and other less definite endings) in the epithelium of the perianal skin and of the anal canal. Pacinian corpuscles are also present in human and cat mesentery.²⁸

CHEMICAL CODING

One of the most useful concepts developed from the explosion of neurochemical markers found in neurones by histochemical methods is the idea of chemical coding. It appears that functionally distinct classes of neurones are characterised by specific combinations of neurochemicals, some of which may be directly related to their transmitter functions, others to less clear properties.²⁹ The combination of neurochemicals varies significantly between regions of the body and species. The digestive EPANs are no exception. Some neuropeptides, including substance P (SP) and CGRP, have been demonstrated in spinal primary afferent neurones supplying viscera, including the intestine.^{30,31} Other neuropeptides are also present in some visceral sensory neurones.³² Chemical coding of spinal and vagal afferents is different. In the guinea pig, less than 10% of vagal afferents have the peptides CGRP, SP, or somatostatin.³³ IGLEs contain calretinin and calbindin in the rat but not in the guinea pig.^{9,10,34} A calcium binding protein, neurocalcin, has been demonstrated in rat IGLEs.¹² CGRP is present in IGLEs of the ferret (unpublished observations).

Among the many classes of molecules present on primary afferent endings that are likely to be determinant in the chemical coding are pharmacological receptors, ion, chemo- and mechano sensitive channels, calcium binding proteins,

and surface markers such as lectin binding sites, etc. For instance, in the rat there are at least two classes of small size spinal afferent neurones, namely those with neuropeptides and those with IB-4 lectin binding, but no neuropeptides, and with a high density of the tetrodotoxin resistant channel.³⁵⁻³⁶ The functional significance of the different calcium binding proteins is unclear although they may be involved in buffering cytoplasmic calcium, thus modulating calcium dependent potassium channels involved in after-hyperpolarisation.

Another difference in the extrinsic primary afferents is their dependence on growth factors. For instance, the neuropeptide containing spinal afferents in rats depend for their survival on nerve growth factor whereas IB4 positive neurones are dependent on glial derived neurotrophic factor.³⁵ More recently, it was shown that IGLEs in the mouse small intestine, but not those in the stomach, are sensitive to a deficiency in neurotrophin-4.³⁷ The *N*-methyl-D-aspartate glutamate receptor appears to be selectively localised on some spinal afferents to the colon.³⁸ Mouse vagal IGLEs are immunoreactive for the P2X2 receptor (fig 1).³⁹

RELATION BETWEEN PRIMARY AFFERENT FIBRES AND INTERSTITIAL CELLS OF CAJAL

In addition to their role as pacemaker cells and probably as intermediaries of nerve mediated motor excitation and inhibition of intestinal smooth muscle, the interstitial cells of Cajal have been proposed to be specifically associated with the nerve endings of IMAs³⁷ and with possible interstitial cells in the mucosa,¹⁹ and are thus potentially involved in afferent neural transduction. This fascinating possibility is one of the issues that needs to be clarified in future experiments.

RELATION BETWEEN PRIMARY AFFERENT FIBRES AND PARACRINE MUCOSAL CELLS

Primary afferent mucosal endings come in close proximity to many of the mucosal endocrine cells. Substances released from these cells by a variety of chemical and mechanical stimuli are also likely to act locally before entering the circulation. Nerve endings of IPANs and EPANs form one of the natural targets.⁴⁰ This possibility has been demonstrated for two such cell types. Cholecystokinin and the serotonin containing mucosal paracrine cells have been shown to activate vagal afferent neurones.⁴¹⁻⁴²

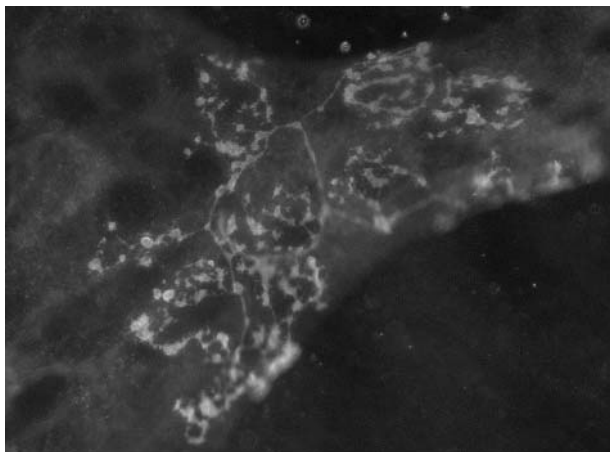


Figure 1 Whole mount preparation of mouse stomach with an intraganglionic laminar ending showing P2X2 receptor immunoreactivity.

EFFERENT FUNCTION OF PRIMARY AFFERENTS

Branching of primary afferent neurones suggests that action potentials which invade the branches antidromically may release transmitter substances from the peripheral endings. This has been shown to be the case for some of the peptide containing spinal afferent fibres. Collaterals of spinal afferent fibres cause release of peptides onto myenteric neurones and blood vessels.

A similar function has been suggested for some of the vagal afferent neurones. IGLE nerve endings form close connections with enteric neurones in the myenteric ganglia. In spite of some initial indications, there is little evidence that their activation leads to activity of myenteric neurones.

INTESTINOFUGAL NEURONES

Since the suggestion of Kunz and Saccomanno⁴³ that there are neurones with cell bodies in the enteric ganglia projecting to the prevertebral ganglia, these pathways have been extensively investigated.⁴⁴⁻⁴⁵ Also of potential importance for afferent information from the gut to the CNS are the rectospinal neurones whose cell bodies in the enteric ganglia project directly to the sacral spinal cord.⁴⁶

CONCLUSIONS

Surprisingly little information is available on the origin, morphology, and receptor functional characteristics of the nerve endings of most primary afferent neurones to the digestive tract. Even more surprising is the lack of identification of neurochemical markers in a vast population of primary afferents. The explosion of neurochemicals found in afferent neurones represents both a challenge and a unique opportunity to investigate ways to intervene in diseases involving primary visceral afferent pathways.

Authors' affiliations

M Costa, S H J Brookes, V Zagorodnyuk, Department of Physiology and Centre of Neuroscience, School of Medicine, Flinders University, Adelaide, Australia

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