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Structure–function relationship of sensory endings in the gut and bladder

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ABSTRACT

Visceral afferents play a key role in neural circuits underlying the physiological function of visceral organs. They are responsible for the detection and transmission of a variety of visceral sensations (e.g. satiety, urge, discomfort and pain) from the viscera to the central nervous system. A comprehensive account of the different functional types of visceral sensory neurons would be invaluable in understanding how sensory dysfunction occurs and how it might be diagnosed and treated. Our aim was to explore the morphology of different nerve endings of visceral afferents within the gastrointestinal tract and urinary bladder and how the morphology of these nerve endings may relate to their functional properties. Morphological studies of mechanosensitive endings of visceral afferents to the gut and bladder correlated with physiological recordings have added a new dimension to our ability to distinguish different functional classes of visceral afferents.

1. Introduction

Visceral afferents play a key role in neural circuits underlying the physiological function of visceral organs. They are responsible for the detection and transmission of a variety of visceral sensations (e.g. satiety, urge, discomfort and pain) from the viscera to the central nervous system. These sensations, particularly pain, are of major medical significance, causing much human suffering and considerable economic impact. In each case, the first component of the pathways mediating visceral sensation are sensory neurons with endings in the body's organs. A comprehensive account of the different functional types of sensory neurons to the gut, bladder, urogenital system, airways, blood vessels and other organs would be invaluable in understanding how sensory dysfunction occurs and how it might be diagnosed and treated. Achieving this has proved remarkably difficult.

In contrast, the sensory innervation of cutaneous and somatic tissues has been well characterised. The major types of sensory endings in glabrous and hairy skin have been identified and the types of information that they encode has been determined. For visceral afferent neurons, our understanding lags far behind. This is probably because viscera receive a complex innervation from autonomic neurons giving rise to many different types of efferent and afferent nerve endings. This has made it difficult to identify which sensory axons give rise to each pattern of firing, let alone how many different classes may contribute to sensation overall.

Intuitively, the different functional classes of sensory neurons innervating an organ should be most readily discriminated by their responses to a range of specific stimuli. The development of single unit recordings by Adrian (1933) made this theoretically possible. Many attempts have been made to identify classes of afferent units by their firing patterns. Some consistent features have emerged, but clear demarcation of functional classes has been elusive. Results differ between laboratories probably due to variations in recording techniques, stimulation paradigms, anatomical areas, species etc. Recently it has become possible to identify the morphology of individual axons responsive to specific visceral stimuli (Zagorodnyuk and Brookes, 2000; Zagorodnyuk et al., 2001, 2008; Lynn et al., 2003; Spencer et al., 2008a,b; Song et al., 2009). Since such anatomical features are likely to be relatively stable over time, potentially providing a reliable additional criterion to distinguish different classes of sensory endings. Indeed, morphological studies correlated with physiological recordings are beginning to clarify some of the uncertainties that were not resolved by functional studies alone. This review explores how morphological data can help distinguish different classes of sensory neurons innervating the viscera.

Visceral afferents have been variously been classified by the location of their receptive fields (mucosal afferents, muscle afferents), their function (mechanoreceptors, chemoreceptors, nociceptors), their rates of adaptation (slow and fast adaptive), their conduction velocities (C fibres, and Aδ and Aβ fibres), thresholds for mechanical activation (low and high threshold), range of responses (wide dynamic or saturated), the magnitude of their responses (low and high responders) and their neurochemical coding (peptidergic and non-peptidergic afferents) (Grundy and Scratcherd, 1989; Janig and Koltzenburg, 1993; Cervero, 1994; Sengupta and Gebhart, 1994; Leek, 1977; Costa et al., 2004; Janig, 1996, 2006). In addition, many visceral afferents have been described as

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"polymodal afferents", since they respond to a range of mechanical and chemical stimuli (Grundy and Scratcherd, 1989; Page and Blackshaw, 1998; Su and Gebhart, 1998; Zagorodnyuk et al., 2007; Xu and Gebhart, 2008). Clearly, there is considerable diversity in the stimuli that can activate visceral afferents. Some of this diversity may be due to specific morphology and location of their endings within their target organs. This review will focus on the morphology of different nerve endings of visceral afferents within the gastrointestinal tract and urinary bladder and how the morphology of these nerve endings may relate to their functional properties. The techniques used to identify the morphology of mechanosensory transduction sites have been described in a number of recent papers (Zagorodnyuk and Brookes, 2000; Zagorodnyuk et al., 2001, 2008; Lynn et al., 2003; Spencer et al., 2008a,b; Song et al., 2009), and have been supplemented with a number of older anatomical tracing studies (eg: Neuhuber, 1987; Berthoud and Powley, 1992; Clerc and Mazzia, 1994; Berthoud et al., 1995, 1997; Wang and Powley, 2000).

2. Vagal afferents to the gut

The anatomy of vagal afferent pathways innervating gastrointestinal tract via the vagus nerve have been studied using neuroanatomical tracing techniques *in vivo* (Neuhuber, 1987; Berthoud and Powley, 1992; Berthoud et al., 1995, 1997; Wang and Powley, 2000). These studies demonstrated that vagal afferents to the gut form at least three different morphological types of specialised endings: mucosal endings, intraganglionic laminar endings (IGLEs) in myenteric ganglia and intramuscular arrays (IMAs) in the muscularis externa. Electrophysiologically, at least three types of vagal afferents have been identified in the upper gut. These are mucosal afferents which respond to mucosal stroking and luminal chemical stimuli, low threshold stretch-sensitive muscular afferents and high threshold distension-sensitive mechanoreceptors (Iggo, 1955; Davison and Clarke, 1988; Sengupta and Gebhart, 1995; Page and Blackshaw, 1998; Zagorodnyuk et al., 2001; Page et al., 2002; Yu et al., 2005).

2.1. IGLEs

Most studies of distension-sensitive vagal afferents have identified mechanoreceptors that fire action potentials roughly in proportion to wall tension, reflecting a combination of passive distension and active contraction of the gut wall (Iggo, 1955; Blackshaw et al., 1987; Davison and Clarke, 1988; Zagorodnyuk et al., 2001). A modified dye-filling technique *in vitro* (anterograde labelling of extrinsic axons from a single fine nerve trunk) (Tassicker et al., 1999) has been combined with "close-to-target" *in vitro* extracellular recordings from the fine nerve trunks (Zagorodnyuk and Brookes, 2000). Using this approach made it possible to correlate dye-filled structures with mechanically sensitive sites of single vagal units in the upper gut (Zagorodnyuk and Brookes, 2000; Zagorodnyuk et al., 2001). This revealed that IGLEs, located in oesophagus and stomach, are the transduction sites of low threshold slowly adapting vagal tension receptors (Zagorodnyuk and Brookes, 2000; Zagorodnyuk et al., 2001; Brookes et al., 2005) (Fig. 1). IGLEs are located in myenteric ganglia and form flattened plate-like structures often concentrated in the edges and surfaces of the ganglia beneath the ganglionic sheath. In the guinea pig upper gut, IGLEs-type afferents, most of which are activated by P2X agonists but not capsaicin, have axons corresponding to Aδ fibres arising from the nodose ganglion, with saturating response to distension (Page and Blackshaw, 1998; Zagorodnyuk et al., 2003; Yu et al., 2005). They are found in decreasing densities throughout the small and large intestine (Berthoud et al., 1997; Wang and Powley, 2000).

2.2. IMAs

A second class of vagal afferent endings, IMAs terminates in the muscle layers rather than ganglia (Berthoud and Powley, 1992; Wang and Powley, 2000). IMAs consist of branching varicose nerve fibres running for several millimetres parallel to bundles of either longitudinal or circular muscle. They mainly concentrated in the upper stomach and the lower oesophageal sphincter and pylorus (Kressel et al., 1994; Wang and Powley, 2000). It has been suggested that IMAs might be specialised "length" receptors in the oesophagus and stomach, perhaps sensing distension irrespective of intraluminal pressure and wall tension (Phillips and Powley, 2000). However, to date, there is no functional evidence to support this speculation. Another possibility is suggested by the observation that several types of high threshold capsaicin-sensitive vagal afferent fibres have been shown to innervate the guinea pig oesophagus: these are non-peptidergic nodose C fibres and peptidergic jugular Aδ and C fibres (Yu et al., 2005). Removal of the mucosa did not affect mechanosensitivity of these fibres, so it is likely that they have their endings in the muscular layers of the oesophagus and they may serve a nociceptive function. Their mechanosensitive endings can be located along the whole oesophagus (Yu et al., 2005) whereas oesophageal IMAs are concentrated in the vicinity of the lower oesophageal sphincter (Wang and Powley, 2000; Phillips and Powley, 2000). The functional role of IMAs is yet to be determined.

2.3. Vagal sensory endings in the mucosa

Vagal afferent nerve endings in the mucosal layers of the upper gut have been identified after anterograde tracing *in vivo*. Relatively non-specialised arrays of endings were observed in villi, with some fibres approaching the basal side of epithelial cells and some dense networks of arborising terminal fibres in neighboring villi (Berthoud et al., 1995; Williams et al., 1997). These endings are highly likely to form the morphological substrate for the vagal mucosal afferents which include stretch-insensitive mucosal mechanoreceptors (Clarke and Davison, 1978; Blackshaw and Grundy, 1990; Page and Blackshaw, 1998), osmo-sensitive afferents (Mei and Garnier, 1986; Blackshaw and Grundy, 1990; Zhu et al., 2001), thermo-sensitive fibers (El Ouazzani and Mei, 1982) and amino-acid- and glucose-sensitive afferents (Mei, 1978; Zhu et al., 2001). It is likely that vagal mucosal afferents express polymodal sensitivity to a variety of chemical and light mechanical stimuli, including gentle stroking or compression, but not distension or stretch (Grundy and Scratcherd, 1989; Page and Blackshaw, 1998). Their sensitivity to movement across the mucosal surface may allow them to detect a food bolus in the lumen (Grundy and Scratcherd, 1989; Sengupta and Gebhart, 1994). Some rapidly adapting mucosal mechanoreceptors have receptive fields in multiple distant sites in the upper gut and about one third of them respond to capsaicin (Berthoud et al., 2001).

3. Spinal afferents to the gut

Visceral spinal afferents project to the intestine via the thoracic and lumbar splanchnic nerves, then via mesenteric nerves. In addition, sacral afferents project to the distal bowel via the pudendal and pelvic/rectal nerves (Schofield, 1968; Cervero and Sharkey, 1988; Costa et al., 2004). Electrophysiological studies have revealed that thoraco-lumbar and lumbo-sacral populations of afferent nerves contain markedly different proportions of a number of classes of mechanosensitive afferent fibres (Brierley et al., 2004).

3.1. Thoraco-lumbar splanchnic afferents

Relatively few *in vivo* anatomical studies of spinal sensory projections to the viscera have been reported, probably due to the inaccessibility of dorsal root ganglia. However, the spinal afferent innervation of the gastro-oesophageal junction has been described (Clerc and Mazzia, 1994), as has innervation of mesenteric ganglia (Aldskogius et al., 1986) and pylorus (Lindh et al., 1989). These studies