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Structural basis of sensory nerve pathways from the gut

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The gut is innervated by a number of classes of sensory neurons with cell bodies located in three major sources: the nodose ganglia, thoracolumbar spinal ganglia (dorsal root ganglia) and lumbosacral spinal ganglia. It is now well established that there are distinct differences in the properties and sensitivities of neurons running in the three different pathways. Since the 1950's, many studies have used electrophysiological methods to record the activity of afferents in these pathways, and have distinguished different types on the basis of their threshold, mechanosensitivity, rate of adaptation, chemosensitivity and pharmacology. However, there is often much overlap between classes of sensory neurons characterised in this way. In addition, studies have often concentrated on different species, regions of gut, experimental paradigms, and stimulus regimes, making it difficult to identify systematically all types of sensory innervation of the gut. We have attempted to identify different types of sensory neurons by the location, morphology and immunohistochemistry of their sensory endings in the gut and combine this information with detailed electrophysiology, pharmacology and mechanical stimulation. Morphological information adds a powerful, independent criterion for distinguishing different functional classes of sensory innervation and has proven valuable in identifying sites and mechanisms of sensory transduction.

We have modified conventional recording techniques to be able to identify morphologically the endings of electrophysiologically characterised extrinsic afferent nerves to the gut. First, studies have been carried out in isolated preparations in vitro, in order to allow fine microdissection and controlled, repeatable stimulation of endings. Second, techniques have been developed to make recordings very close to the site where extrinsic nerve trunks enter the gut wall; this makes it possible to work on small pieces of gut tissue in which mechanical activity can be closely controlled and/or recorded. Third, a technique has been developed in which biotinamide can be used to label axons in the nerve trunk from the point at which recordings were made, filling takes only 4-12 hours. Lastly, analysis of video recordings of preparations has also been carried out in order to relate smooth muscle contractility with firing of identified extrinsic afferents.

Vagal afferents are concentrated in the upper gut and are known to consist of two major types: mechanoreceptors which act largely as in-series tension receptors, and mucosal receptors which respond to mechanical stimulation of the mucosa and to a range of chemicals, mediators and nutrients. While the endings of mucosal receptors have been well described[1], the identity of low threshold vagal mechanoreceptors had not been ascertained. Recordings from small preparations of the outer muscle layers of guinea pig oesophagus or stomach revealed mechanoreceptors which responded to distension and also to focal distortion of the tissue with a light (0.1-1 mN) von Frey hair. Vagal mechanoreceptors had 2-6 sensitive sites ("hotspots") each of which was surrounded by a mechanically insensitive area. Dye fills revealed intraganglionic laminar endings (IGLEs) underlying marked "hotspots": IGLEs were significantly closer to hotspots than any other dye filled structures or randomly generated sites. Thus IGLEs were identified as the transduction sites of in-series vagal tension receptors[2,3]. Morphological studies have revealed another class of vagal afferent nerve endings in the wall of the stomach: intramuscular arrays (IMAs); their role is uncertain[4].

The structure of spinal afferent nerve endings in the gut were then systematically studied using the same techniques. Throughout most of the small and large intestine, low threshold mechanoreceptor endings were not located in outer muscle layers of the gut. This indicates that most of the small and large

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intestine of guinea pigs does not receive substantial innervation from vagal mechanoreceptors. However, the rectum was an exception. Here, low threshold mechanosensitive afferent endings were abundant. Each ending had about 8-10 small, focal mechanosensitive "hotspots" which corresponded to a new type of ending in myenteric ganglia (rectal IGLEs or rIGLEs). RIGLEs are the transduction site of low threshold, slowly adapting mechanoreceptors in the rectum[5], which arise from sacral spinal pathways and which are sparse in the distal colon[6].

Previous studies have shown that the small and large intestine are innervated by mechanosensitive spinal afferents from thoracolumbar spinal ganglia, often with high thresholds: their transduction sites have not been identified in the gut. As reported previously[7,8], numerous high threshold mechanosensitive endings were detected in the mesenteries. Dye filling revealed that these were exclusively associated with varicose branching axons (VBAs) located on mesenteric arteries and, to a lesser extent, on mesenteric veins. These endings are responsive to strong traction on the mesenteries. They are frequently immunoreactive for the neuropeptide, CGRP, and also express TRPV1 channels and are powerfully activated by capsaicin.

Thus the mechanotransduction sites of mechano-nociceptors, which are abundant in the membranes surrounding the intestines, have been identified. However, it seems intuitively unlikely that gut distension could mechanically activate such remote endings, given the high compliance of the mesenteries. In addition, it is unclear how mucosal inflammation could affect mechano-nociceptor sensitivity if their transduction sites are located outside the gut. We searched for high threshold mechanosensitive endings within the gut wall. Very few such endings could be located in the external muscle layers, but in preparations with all layers present, numerous "hotspots" could be found. Microdissection revealed that these were located in the submucosa, and did not require the mucosa, external muscle layers, myenteric plexus or serosa/mesentery to be present. Fine mapping revealed that their mechanosensitive sites were exclusively located on submucosal blood vessels; these endings are directly mechanosensitive to distension of the gut wall and are well placed to be sensitised by mucosal inflammation. Thus mesenteric and serosal mechano-nociceptors are actually located on blood vessels outside and within the gut wall respectively.

By knowing the location and morphology of mechanosensitive endings, it has been possible to study their mechanosensitivity[9] and transduction mechanisms in fine detail, in vitro. All three major classes of mechanoreceptor endings are directly sensitive to mechanical stimuli and do not rely on the release of chemical mediators from other cells for their responses[10,11]. However, each class also shows a distinct combination or code of pharmacological sensitivities, which can powerfully modulate their excitability. Put together, these studies have provided an account of the structure of the majority of the sensory nerve endings of the gut wall, allowing a picture of the different types of information carried in each pathway to be assessed.

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