Summary

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Neural information from the viscera to the central nervous system (CNS) plays a very important role in health and disease. Afferent neural activity from the gastrointestinal tract signals the CNS but the nature of the stimuli required to activate the visceral afferent neurones is still not well defined. Greater comprehension of the anatomy of visceral afferents and of the underlying mechanisms relating to visceral mechano- and chemoreception is required in order to identify rational therapeutic targets for the treatment of functional gastrointestinal diseases.

Neural information from the viscera to the central nervous system (CNS) plays a very important role in health and disease and yet much of the visceral afferent information does not reach conscious experience, except when it produces unpleasant sensations such as discomfort, pain, or nausea. Afferent neural activity from the gastrointestinal tract signals the CNS about states of inflammation, presence of harmful luminal stimuli, or abnormal muscle activity, and yet the nature of the stimuli required to activate the visceral afferent neurones, even in physiological conditions, is still not well defined.

The paper by Costa and colleagues¹ in this supplement (*see page ii1*) gives an updated picture of what is known about the anatomy and types of visceral afferents. The primary afferent neurones that are concerned with bodily functions are somatic and visceral. The gastrointestinal tract has two types of afferent neurones—the intrinsic primary afferent neurones (IPANs), which are part of the enteric nervous system, and the extrinsic primary afferent neurones (EPANs), with their cell bodies in the dorsal root ganglia and nodose ganglia. Tracing studies combined with histochemistry have confirmed that the cell bodies of vagal afferents are in the nodose ganglia and those of the spinal and sacral afferents in the dorsal root ganglia. The oesophagus and stomach are supplied by EPANs located in the nodose ganglia, and within the pyloric sphincter region many EPANs form intramuscular arrays (IMAs). In the small intestine, intraganglionic laminar endings (IGLEs) and IMAs are of vagal origin although there are primary afferents of spinal origin. Afferents from the colon travel in the spinal cord via the lumbar colonic, hypogastric, and pelvic nerves while the rectum and anal canal receive extrinsic innervation via the pelvic and pudendal nerves. Peripheral afferent neurones forming IMAs are also associated with pacemaker cells or

intermediaries—the interstitial cells of Cajal. Their nerve endings come into close contact with many mucosal endocrine cells (see Lundgren² in this supplement, *page ii16*) that are capable of releasing substances such as 5-hydroxytryptamine (5-HT) and cholecystokinin (CCK), which act locally before entering the circulation, acting on nerve endings of the IPANs and EPANs. Extrinsic spinal primary afferents, particularly peptide containing ones, also have some efferent function, as suggested by their branching and their ability to conduct action potentials antidromically and to release neuropeptides. It seems that functionally distinct classes of neurones are characterised by specific combinations of neurochemicals, some of which may be directly related to transmission, others having a less clear role. These neurochemical combinations vary significantly between regions of the body, probably related to some differences in their functions, and this opens up the possibility for selective intervention in diseases involving visceral afferent pathways (figs 1, 2).

Activation of visceral afferents (fig 2) is explored in the paper by Grundy³ in this supplement (*see page ii5*). Vagal and spinal afferents convey sensory information from the gut to the CNS and they are sensitive to both mechanical and chemical stimuli. Vagal muscle mechanoreceptors, located in the gut wall, have low thresholds of activation when the gut is distended and reach maximal responses within physiological levels of distension while spinal afferents are able to respond beyond the physiological range and can encode both physiological and noxious levels of stimulation. Two types of vagal ending have been attributed to mechanosensory function: firstly, IMAs found in both circular or longitudinal muscle layers—these are probably “in series” tension receptor endings—and secondly, IGLEs within myenteric ganglia and which may also be chemosensitive. The spinal afferents respond to distension over a wide dynamic range, with some contributing to the signalling of visceral pain by recognising the intensity of distension or contraction while others respond only to noxious levels of distension—that is, the so-called “sleeping” or silent nociceptors that can be “awakened” under conditions of injury or inflammation. Visceral

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Abbreviations: CNS, central nervous system; IPANs, intrinsic primary afferent neurones; EPANs, extrinsic primary afferent neurones; IMAs, intramuscular arrays; IGLEs, intraganglionic laminar endings; 5-HT, 5-hydroxytryptamine; CCK, cholecystokinin; ASIC, acid sensing ion channel; IBS, irritable bowel syndrome; NGF, nerve growth factor; EC, enterochromaffin cells

Figure 1 Gastrointestinal extrinsic afferent pathways. Summary of the extrinsic afferent pathways from the gastrointestinal tract (GIT) with their major connections.

afferents are influenced by an enormous range of pharmacological agents and endogenous substances via numerous receptors. These substances produce their effects on visceral afferents in three distinct ways:

- (1) by direct activation, usually by opening ion channels;
- (2) by sensitisation, where afferents become hyperexcitable to both chemical and mechanical modalities; and
- (3) by altering the phenotype of the afferent nerve—for example, by altering the expression of mediators, channels, or receptors.

In contrast with specific signalling pathways that exist in vagal mucosal afferents, many mediators released under conditions of inflammation, injury, or ischaemia can influence the sensitivity of spinal afferents.

The paper by Wood⁴ in this supplement (*see page ii9*) covers recent advances in the understanding of the molecular mechanisms behind primary afferent activation. Over the past decade, the molecular structures involved in transducing the signals that cause pain in response to thermal, mechanical, or chemical stimuli have been identified by molecular cloning, and thresholds for excitation and transmission of electrical signals have been studied, to a considerable extent in vitro, using the rat dorsal root ganglion preparation. Nociceptor neurones can be activated by heat, cold, low pH, and mechanical stimulation and it is now known that the effect of heat is mediated through a cation selective ion channel that is also gated by capsaicin. The existence of other vanilloid (VR1-like) receptors and the large number of transient receptor potential family members suggest that they may be activated by factors other than noxious heat and probably also play a role in mechanosensory transduction. Damaged tissue is associated with lower extracellular pH values, and protons are known to activate vanilloid receptors and cause pain. However, other proton gated channels also

exist within the peripheral neurone system, the first to be identified being the acid sensing ion channel (ASIC). Using homology cloning techniques, the ASIC homologue (DRASIC) was found to be present in sensory neurones and is thought to be expressed in the spinal cord. There seem to be two mechanosensitive channels on sensory neurones; P2Y₁ and P2X₃ receptors are activated by mechanical distortion in the rat. Channels that underlie mechanosensation remain unknown although ASIC cation channels have been proposed to detect cutaneous stimuli in mammals and DRASIC may also participate. There is mounting evidence that a sodium channel that is resistant to tetrodotoxin plays a unique role in the transmission of nociceptive information to the spinal cord. Although there seem to be no unique markers for visceral afferents, many aspects of nociceptor activation are common to visceral and other primary afferents. Selective gene ablation will hopefully permit definition of the roles of individual receptors in the development of visceral pain.

The plasticity of visceral afferents—that is, alterations in their ability to encode and transmit sensory information—is discussed in the paper by McMahon⁵ in this supplement (*see page ii13*). In the case of sensory neurones, an increase in their excitability can have major consequences in terms of perceived pain or reflex control. Modulation of their excitability may be particularly relevant in diseases such as irritable bowel syndrome (IBS). There are two main types of sensory neurone plasticity: peripheral sensitivity or desensitisation, which is rapid in onset and does not involve altered gene expression, and slower onset phenotypic changes in neurones, as a consequence of altered gene expression. In the case of peripheral sensitisation, tissue injury, inflammation, and algogenic chemicals produce changes in the stimulus response, leading to either sensitisation or desensitisation. The stimuli that trigger this may act via G protein coupled receptors in the nociceptor terminal or by activating ligand gated receptors. Several trophic factors and cytokines may also cause sensitisation. The final effector mechanism underlying sensitisation of nociceptors is variable. It can involve modulation of Na⁺, K⁺, or Ca²⁺ channels, which adjust the threshold of the membrane and it can also involve modulation by phosphorylation of some receptors such as VR1. Unfortunately, there are still no strong data on the molecular nature of the mechanical transducer. Phenotypic changes in neurones are slower in onset but more persistent, and there is a large body of evidence suggesting that these changes occur as a consequence of tissue injury. Several potentially important signals for plasticity of gene expression include nerve growth factor (NGF), which is upregulated in many experimental models of inflammation. It probably initiates signalling via its complex with trkA (NGF/trkA), which activates gene transmission factors. Glial cell line derived neurotrophic factor is another neurotrophic factor that can regulate gene expression in some primary sensory neurones. Elucidation of the mechanisms involved in neural sensitisation may offer novel targets for treatment interaction in conditions such as IBS.

In the paper by Lundgren² in this supplement (*see page ii16*), possible mechanisms by which the intestinal contents may influence afferent nerve fibres in the intestinal mucosa are discussed. Mucosal surfaces in the gastrointestinal tract are the interface with the microbiological environment and thus have an important role in controlling the entry of microbiological pathogens into the host. They constitute an important mechanical barrier that separates the host's internal milieu from the external environment. The intestinal epithelium has a high permeability to lipophilic substances but is less permeable to hydrophilic ones. Thus lipid soluble compounds may readily diffuse across enterocytes to influence afferent neurones. Hydrophilic substances on the other

Figure 2 Activation of gastrointestinal extrinsic afferents. Symbolic neurones and paracrine cells represent several different populations with extensive but specific distribution along the gastrointestinal tract. The symbolic inflammatory and immune cells are also represented, together with other cell types—any cell that releases active substances capable of activating or sensitising extrinsic afferent neurones. 5-HT, 5-hydroxy-tryptamine; CCK, cholecystokinin; CK, cytokines; PARs, proteinase activated receptors.

hand cross the epithelium by passive diffusion through “pores” in the tight junctions between enterocytes. There is experimental evidence that tight junction permeability is influenced by intestinal contents—probably pores increase in size in response to solute transport. Recently, it has been observed that epithelial cells play an integral role in generating and transmitting signals between invasive and non-invasive microbial pathogens and adjacent/underlying cells in the mucosa. When epithelial cells are exposed to various strains of bacteria, there is increased expression and secretion of some cytokines with chemoattractant and proinflammatory functions. The response of enterocytes to luminal bacteria is more or less the same regardless of the microbe involved and many of the compounds released by them may (either alone or together) activate afferent neurones in the villi. There are numerous specialised hormone secreting cells dispersed among enterocytes, the most thoroughly investigated being CCK and enterochromaffin (EC) cells. Digestive products of fat and protein stimulate CCK secretion and one type of CCK receptor (CCK-A) has been localised to peripheral autonomic afferent neurones. EC cells contain 5-HT and various peptide hormones that are released by stroking the intestinal mucosa and by stimulating muscarinic receptors. This release is inhibited by adrenergic stimulation. The epithelial lining of the gastrointestinal tract is also scattered with specialised cells, known as brush cells, whose function is currently unknown although their morphology suggests that they may have a chemosensory function. It is

postulated that luminal contents sensed by brush cells may activate villous afferent neurones via release of nitric oxide although this remains to be demonstrated experimentally.

Despite the considerable advances in knowledge regarding the basic mechanisms underlying visceral pain, no new effective therapies for abdominal pain have been discovered. In his clinical review, Collins⁶ attempts to integrate this knowledge into novel therapeutic approaches for the treatment of chronic abdominal pain (see page ii19). It is emphasised however that although in conditions such as IBS pain is largely attributed to visceral hyperalgesia, the latter cannot be demonstrated in all patients. The idea that events such as acute infection may trigger IBS and that low grade inflammation or immune activation may be responsible for prolonged gut dysfunction, akin to asthma, is now being given greater credence. An in depth study of sensory nerves revealed the dynamic and flexible state of sensory input, usually described as “neural plasticity”, which may have potential for therapeutic manipulation. An altered neurotransmitter environment may affect nociception—for example, an altered chemical milieu around the myenteric ganglia may sensitise and contribute to visceral hyperalgesia. This is supported by evidence that there is lymphocytic infiltration of myenteric ganglia and an increased number of mast cells in some patients with severe IBS. Sensitisation may result from activation of PAR-2 receptors on primary afferents by mast cell tryptase. The basis for sensitisation by neurotransmitters has yet to be established. A number of

receptors, chemicals, and channels have been alluded to in this workshop and the role of NGF is of considerable interest as it is elevated in conditions such as IBD and can induce persistent as well as transient sensitisation of neurones.

There is increasing interest in the role of bacteria in IBS and the influence, if any, of manipulating the gastrointestinal flora. Bacteria can interact with intestinal epithelial cells and generate mediators, including cytokines, that can influence sensory nerve endings, and they can also influence intestinal permeability. The role of non-granular “brush cells” in the sensitisation process remains speculative but it is possible that they act as chemosensors of the luminal contents. Luminal antigen may also be transduced to the nervous system via the immune system and in the case of viruses, the circuitry involves vagal pathways to the brain.

Collins⁶ urges caution in the use of “acute” inflammation models to elucidate visceral hypersensitivity. He suggests that new approaches looking at chronic low grade inflammation may be more relevant to disease states such as IBS, and that an understanding of the influence of different inflammatory infiltrates on sensory endings may lead to effective therapeutic intervention.

In conclusion, the consensus was that greater comprehension of the anatomy of visceral afferents and of the

underlying mechanisms relating to visceral mechano- and chemoreception is required, in order to identify rational therapeutic targets for the treatment of functional gastrointestinal diseases.

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