TRP channels: new targets for visceral pain

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INTRODUCTION
How often do gastroenterologists advise patients to “avoid spicy food” without really knowing why? The answer may lie in the family of transient receptor potential (TRP) channels, which includes receptors for compounds found in common herbs and spices. This aim of this review is not really to answer this question, but it will shed light on why spices may cause symptoms. Instead, its aim is to explore the TRP family for potential targets that may in fact reduce gut symptoms. Chronic pain and discomfort of unknown origin in functional gastrointestinal disorders represent a large unmet need for treatment and consequent economic impact. There are also features of other gut disease which generate symptoms with obscure origins. In order to understand how symptoms are generated in the gut and transmitted to the central nervous system, we need to know at least three principles of extrinsic sensory nerve function—first, what types are there and what do they signal?; secondly, what is the molecular basis of sensory transduction?; and thirdly, how does all of this change in disease? These questions are a key focus of this article, with a particular emphasis on the role of TRP channels in each case. Although there are no drugs yet available for clinical use that target TRP channels, some of the early pointers are identified that hold promise for their use in pharmacotherapy of gastrointestinal sensory dysfunction.

DETECTION AND SIGNALLING OF GUT SYMPTOMS
Subtypes of sensory fibres
Peripheral endings of sensory afferent fibres can be classified into five subtypes in the mouse gastrointestinal tract according to the location of their mechanoreceptive fields.1 2 These are: mucosal, muscular (or tension receptor), muscular–mucosal (or tension–mucosal), serosal and mesenteric afferents (fig 1).3–4 Mucosal afferents respond exclusively to fine tactile stimulation of the luminal surface. Anatomically, they appear as bare endings in the lamina propria of villi.1 5 Muscular afferents (or tension receptors) respond to distension at physiological levels (<20 mm Hg), and have specialised terminals that surround myenteric ganglia.6–8 Serosal and mesenteric afferents respond at noxious levels of distension (>40 mm Hg),1 9 10 and correspond to varicose branching axons close to blood vessels.11 Muscular–mucosal afferents respond to both tactile and distension stimuli, but their anatomy has not been confirmed. These five classifications were made based primarily on functional observations, and we are still in the process of matching structure with function. This has probably led to the overlap in terminology between tension-sensitive afferents in the upper gastrointestinal tract (whose adequate stimulus defines their nomenclature), and afferents sensitive to distortion of muscle layers (defined according to location of receptive field). Other classifications have arisen in this and other species, but the consensus appears to be that there are fibres with low and high thresholds to distension, fibres that are distension insensitive (probably mucosal afferents) and a separate class of genuine mechanically insensitive fibres that respond only to chemical stimuli.12 13 To name fibres based on the layer of the gut wall from which responses are most readily evoked is probably misplaced until the structural identity is confirmed anatomically. Thus, high threshold afferents are not exclusively in the serosa and mesentery, and may also be located in the submucosa.11

Mucosal afferents are abundant throughout the innervation of the gut, although scarce in the mid-colon.1 The role of mucosal receptors is unlikely to be in direct conscious perception of events in the gut, except in the anal and pharyngeal regions, where discrimination of solid, liquid and gas is required. It is likely that elsewhere they are important in refining the perception of other events such as distension and contraction, and/or in subliminal autonomic reflexes controlling gut function and behaviour. Stimulation of mucosal afferents in the stomach and small intestine is commonly associated with reduced food intake behaviour, nausea and vomiting, particularly after activation by enteroendocrine mediators.14 Their functions in the lower bowel remain to be elucidated. Muscular–mucosal (or tension–mucosal) receptors exist in the oesophagus and distal colon/rectum which detect both tactile and distending stimuli, combining the functions of muscular and mucosal afferents in the same fibre. Their functional role is undetermined, but it is possible they may play a specialised role in detecting bolus or stool passage. The roles of other populations of sensory fibres are perhaps easier to envisage, with muscular (or tension) receptors providing input into perception of distension or contraction, and serosal mesenteric afferents detecting overdistension, high amplitude contraction, and distortion of blood vessels.10 16 Although the structure and localisation of afferent endings are very important in determining their physiological and pathophysiological roles, the range of ion channels, receptors and enzymes they express may be equally important.
Pathways to the central nervous system

Sensory nerves follow three main pathways from the gastrointestinal tract to the central nervous system—the vagal, splanchnic and pelvic nerves. Vagal afferent fibres have neuronal cell bodies in the nodose and jugular ganglia, whilst the splanchnic and pelvic innervations have cell bodies in spinal thoracolumbar and lumbosacral dorsal root ganglia (DRGs), respectively. Gastrointestinal afferents in the vagal pathway (tension and mucosal) are typically associated with sensations such as satiety and nausea, although a subpopulation of oesophageal vagal afferents responds at high thresholds, implicating them in pain. Spinal pathways innervate all viscera and are associated with sensations of pain, discomfort, bloating and urgency to void. Correspondingly, the majority of vagal afferents innervating smooth muscle respond over a restricted range of distension pressures, whereas spinal afferents respond over a wide dynamic range. There are differences within the spinal innervation, such that the pelvic pathway contains both non-nociceptive and nociceptive afferents, whilst splanchnic afferents have generally higher mechanical thresholds, with few mucosal and muscular afferents, constituting primarily a nociceptive pathway. Understanding the specialised roles of TRP channels in visceral pain will depend on determining which of the afferent subtypes and pathways express particular channels, and the consequence of blocking their function in each subtype.

ROLES OF TRP CHANNELS IN SENSORY SIGNALLING

The range available, and their localisation

The TRP family comprises five subfamilies (TRPA, TRPC, TRPM, TRPP and TRPV) in mammals, which share several key properties: they are non-selective cation channels and most have six transmembrane domains (fig 2). As their name suggests, they were first identified as channels mediating brief excitatory events in non-mammalian sensory systems, although their roles have outgrown this original label as evident from the discussion below. They are grouped according to their structural similarities, and are discussed below in order of the interest that has been expressed in them in visceral sensory pathways. For a more complete review of their functions in various physiological systems, see reviews by Clapham, Dhaka et al and Christensen and Corey. TRP channels have received fame and/or notoriety in a number of fields, including pain, respiratory and cardiac function, ion metabolism and absorption.

TRPV

The TRPV, or vanilloid family, is probably the best known subfamily of TRP channels, notably TRPV1...
which is activated by the vanilloid chilli extract capsaicin. TRPV1 expression is greater in gut-innervating neurons that in those innervating the skin, with 50–60% of lumbosacral and 82% of thoracolumbar colonic DRG neurons displaying TRPV1 immunoreactivity, similar to the proportion of thoracic gastric DRG neurons. TRPV1 is also found in peripheral terminals throughout the gut, and in 40–80% of nodose ganglion neurons innervating the stomach.

TRPV4 is the mammalian homologue of the Caenorhabditis elegans osmosensory gene Osm-9; it responds to extracts of the herb Andrographis paniculata. TRPV4 is located at sites of mechanical stimulus detection including inner-ear hair cells, sensory neurons and somatic mechanosensory structures. TRPV4 appears to be enriched within certain populations of sensory neurons in the DRGs. There is greater TRPV4 mRNA expression in colonic sensory neurons than in whole DRGs. Within the thoracolumbar (splanchnic) DRGs, TRPV4 mRNA in identified colonic neurons is 20 times that in whole DRGs. Differences between pathways are also evident, with colonic sensory neurons expressing 3–8 times the levels of TRPV4 in identified gastric vagal sensory neurons. TRPV4 is found in 38% of gastro-oesophageal vagal afferents, 54% of splanchnic colonic afferents and 58% of pelvic colonic afferents.

In the periphery, TRPV4 protein co-localises with calcitonin gene-related peptide (CGRP) in colonic nerve fibres in the outermost (serosal and mesenteric) layers but is scarce in intramuscular or mucosal endings. This pattern of localisation is apparent in both mouse and human tissue, and correlates well with the functional role of TRPV4 (see below). Other members of the vanilloid family include TRPV2, 3, 5 and 6, whose function in the gut has not yet been investigated. TRPV2 is expressed in gastric vagal afferents, but not TRPV3.

TRPA
The ankyrin, or TRPA family has only one member—TRPA1—which is the mammalian orthologue of the Drosophila gene painless, where it is expressed in polymodal nociceptor endings and contributes to the detection of intense mechanical stimuli. It has a characteristic large number of ankyrin protein repeats, thought to act as a spring and intracellular anchor, which may contribute to mechanosensory transduction (fig 2). It responds to a range of environmental irritants and extracts of mustard, cinnamon, garlic and oregano (table 1). TRPA1 is localised in 55% of gastro-oesophageal vagal afferents, 54% of splanchnic colonic afferents and 58% of pelvic colonic afferents.

TRPM
The melastatin, or TRPM family, was originally named for the antimelanoma properties of TRPM1. There are two members of interest: TRPM8 is now widely researched because it mediates the cooling and soothing sensation of menthol, icilin and eucalyptol. Thus, TRPM8 may be critical in alleviating pain. TRPM8 is localised in DRG neurons that do not co-localise with the “algesic” TRP channels V1 and A1. It would be most valuable to determine TRPM8 expression and function in specific subpopulations of visceral afferents, which, by analogy with skin, may have an antinociceptive function. TRPM5 is localised in taste buds, where it is part of the pathway leading to release of neurotransmitters involved in various taste modalities. It is also found in the gut epithelium, where it may serve a similar function.

TRPC
The canonical, or TRPC family has six members, most of which operate as classical calcium channel effectors, rather than sensors. Their sensory role in the gut is currently being investigated. TRPC1 was of interest, since it was shown to play a role in mechanosensation in recombinant systems. However, this evidence has been refuted, and the role of TRPC1 in mechanosensation has been cast in doubt.
Table 1  A select list of transient receptor potential (TRP) channels, their natural ligands, physical stimuli and localisation, where known

<table>
<thead>
<tr>
<th>Channel</th>
<th>Natural ligand</th>
<th>Physical stimuli</th>
<th>Direct endogenous stimuli</th>
<th>Indirect endogenous stimuli</th>
<th>Locations found</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV1</td>
<td>Capsaicin, acid</td>
<td>Thermal &gt; 43 °C</td>
<td>Acid, anandamide</td>
<td>Cannabinoids, bradykinin, serotonin, proteases</td>
<td>Sensory neurons, ossephageal stratum granulosum and muscularis mucosa</td>
</tr>
<tr>
<td>TRPV4</td>
<td>Andrographis paniculata (Chinese herbal medicine)</td>
<td>Mechanical, thermal &gt; 25 °C</td>
<td>Anandamide, arachidonic acids, epoxyeicosatrienoic acid metabolites</td>
<td>Proteases</td>
<td>Sensory neurons, inner ear, colonic brush-bordered epithelial cells</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Mustard, oregano, cinnamon, garlic</td>
<td>Mechanical, thermal &lt;17 °C</td>
<td>4-Hydroxononenal</td>
<td>Cannabinoids, bradykinin, prostaglandins, proteases</td>
<td>Sensory neurons, inner ear, enteroendocrine cells</td>
</tr>
<tr>
<td>TRPM8</td>
<td>Peppermint, eucalyptus</td>
<td>Thermal &lt;28 °C</td>
<td>Lysophospholipids</td>
<td></td>
<td>Sensory neurons, bladder, prostate</td>
</tr>
<tr>
<td>TRPM5</td>
<td></td>
<td></td>
<td>Phospholipase C/J2</td>
<td>Sugars</td>
<td>Taste cells, enteroendocrine cells</td>
</tr>
<tr>
<td>TRPC1</td>
<td>Mild mechanical</td>
<td></td>
<td></td>
<td></td>
<td>Sensory neurons, brain</td>
</tr>
<tr>
<td>TRPP2</td>
<td>Acid</td>
<td>Mild mechanical</td>
<td></td>
<td>Central canal spinal cord</td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Osmo-sensory and chemosensory properties of visceral mechanoreceptors.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Stimulus</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV1</td>
<td>Osmotic</td>
<td>Low threshold</td>
</tr>
<tr>
<td>TRPV4</td>
<td>Osmotic</td>
<td>High threshold</td>
</tr>
<tr>
<td>TRPM5</td>
<td>Chemo</td>
<td>Medium threshold</td>
</tr>
<tr>
<td>TRPM8</td>
<td>Chemo</td>
<td>High threshold</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Chemo</td>
<td>High threshold</td>
</tr>
</tbody>
</table>


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gastrointestinal vagal afferents, which may be relevant to symptoms generated in humans, discussed later. Genetic deletion of TRPV1 has no significant effect on mechanosensory function of somatic nociceptors, but there are deficits in visceral mechanoreceptors (table 2). In the gastro-oesophageal region TRPV1 knockouts had reduced vagal afferent responses to distension. TRPV1 knockouts also have significantly reduced responses of jejunal wide-dynamic range afferents to distension, but had normal function of low or high threshold afferents. In the distal colon/rectum TRPV1 deletion significantly reduced the distension responses of pelvic muscular and mucosal–mucosal afferents, but not responses to mucosal stroking. Effects on serosal afferents were not reported. Therefore, TRPV1 deletion consistently reduces the mechanosensitivity of distension-sensitive gastro-oesophageal, jejunal and pelvic colonic afferents. Similar defects were found in bladder afferents. Capsaicin potently activates splanchnic and pelvic colonic serosal afferents, which is followed by pronounced mechanical desensitisation in splanchnic but not pelvic afferents. This suggests that the coupling of TRPV1 differs between pathways. Whereas TRPV1−/− mice had normal somatic mechanical pain sensitivity, their behavioural responses to colorectal distension were reduced. Therefore, TRPV1 is important in the signalling of physiological mechanical stimuli from throughout the viscerum.

Since TRPV1 normally responds to low pH, this makes it a prime candidate for the sensing of refluxed acid in the oesophagus, and giving rise to the sensation of heartburn. Indeed it has been detected in fibres innervating the human oesophageal mucosa. Mice lacking TRPV1 channels develop significantly less oesophagitis after acid exposure compared with wild-type mice, suggesting a role beyond sensory function. In the guinea-pig, oesophageal tension-sensitive vagal afferents termed “nociceptive-like” are distinguishable from “non-nociceptive-like” afferents by their responsiveness to capsaicin. These “nociceptive-like” tension receptors in the oesophagus are stimulated by acid. In contrast, the responses of “non-nociceptive” tension receptors to acid are less well defined. Rong et al showed that responses of jejunal afferents to acid were reduced up to 50% in TRPV1 knockouts, indicating a major, but not exclusive role for TRPV1 in detecting low pH.

TRPV4 is involved in the transduction of osmotic stimuli and is also implicated in mechanosensory function, since TRPV4−/− mice show reduced behavioural sensitivity to noxious somatic mechanical stimuli. The role of TRPV4 in the viscera is more discrete (table 2): deletion of TRPV4 had no effect on jejunal afferent function, consistent with low TRPV4 expression in gastro-oesophageal vagal afferents. However, in colonic afferents, where TRPV4 is enriched, mechanosensory responses were dramatically reduced in TRPV4 knockouts, and mechanosensory thresholds were increased. This was the case for both

Together as a heterodimer to act as a signalling complex (also known as PKD1L3 and PKD2L1), which is unlike other TRP channels where homotetramers are the normal configuration. They recently received interest in connection with a role in acid sensing, although such a role in the gut remains to be tested.

TRPML

The mucolipin, or TRPML family has three members, one of which is associated with mucolipidosis. They have an intracellular function putatively in vesicle formation and will not be considered further here.

TRP channels as detectors

TRP channels are proposed to be primary transducers of thermal, mechanical and chemical stimuli. Thermal transduction is unlikely to be important in the gut and will not be discussed in detail. There are several mechanosensory and chemosensory properties (table 1 and outlined below) that make particular TRP channels important targets for investigation in gastrointestinal sensory pathways. In contrast to their established roles as chemosensors, conclusive evidence that TRP channels function as mechanosensors has been elusive as this requires mechanosensory function studies from isolated channels to intact sensory fibres to intact animals.

TRPV

TRPV1 is activated by capsaicin, noxious heat, low pH and a range of herb and spice extracts (table 1). We are all familiar with the burning sensation evoked by capsaicin applied to the mouth or mucous membranes, and when administered intracolonically it causes pronounced pain. However, capsaicin also activates non-nociceptive upper
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Table 2  A summary of the effects of selected transient receptor potential (TRP) channel deletion, utilising knockout mice, on intestinal afferent mechanosensory function and of TRP channel antagonists on mouse afferent function

<table>
<thead>
<tr>
<th>Channel</th>
<th>Vagal gastro-oesophageal afferents</th>
<th>Vagal/spinal jejunal afferents</th>
<th>Splanchnic distal colon afferents</th>
<th>Pelvic colorectal afferents</th>
<th>Response to colorectal balloon distension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low threshold</td>
<td>Wide dynamic range</td>
<td>High threshold</td>
<td>Mesenteric</td>
<td>Serosal</td>
</tr>
<tr>
<td>TRPV1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPV4</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Antagonist</td>
<td></td>
<td></td>
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<tr>
<td>TRPA1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Antagonist</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Arrows indicate the degree of change in mechanosensory function. Empty cells indicate not tested.

splanchnic and pelvic high threshold (serosal and mesenteric) afferents, which was consistent with TRPV4 expression in the periphery. These deficits were restricted to mechanosensory function, since afferent excitability, electrical thresholds and conduction velocities were identical in TRPV4−/− and TRPV4+/+ mice. However, as was the case in the vagal pathway, pelvic mucosal and muscular afferents were normal. Thus, TRPV4 makes a specific and major contribution to colonic high threshold mechanosensory afferent function, and as such is the only nociceptor-specific TRP channel as yet identified.

Opening the TRPV4 channel pharmacologically increases the afferent response to mechanical stimuli. The endogenous arachidonic acid metabolite 5,6-EET is an agonist of TRPV4, which causes significant potentiation of mechanosensory responses in wild-type mice, but not in TRPV4 knockouts. Pharmacological blockade with the non-selective TRP channel blocker ruthenium red correspondingly reduces mechanosensitivity only in wild-type mice. Consistent with effects of TRPV4 activation in colonic afferent endings, 4α-PDD, a synthetic TRPV4 agonist, caused significant TRPV4-mediated calcium influx in isolated colonic DRG neurons. From these data it is clear that alterations in the pharmacology of splanchnic and pelvic colonic afferents parallel the changes in mechanosensitivity in TRPV4 knockouts, indicating that there may be potential for development of a nociceptor-specific analgesic.

The selective role of TRPV4 in high threshold afferents would be expected to translate into a role in pain perception in vivo, which was indeed the case. There were decreased abdominal electromyo- graphic (EMG) responses to noxious colorectal distension in TRPV4 knockouts or in mice with downregulated TRPV4 expression (via intervertebral small interfering RNA (siRNA) delivery). This was apparent particularly at higher distension pressures. Intracolonic administration of 4α-PDD in wild-type mice caused neuronal activation in the lumbosacral spinal cord and caused dose-dependent visceral hypersensitivity to colorectal distension.

TRPA

TRPA1 was originally thought to play a role in hair cells of the inner ear, but TRPA1−/− mice have normal hearing. One line of TRPA1-deficient mice has increased pain thresholds to somatic mechanical stimuli, but another line had normal responses to noxious stimuli, so the contribution of TRPA1 to somatosensory function is unresolved. Its role in the gut is becoming clearer, although results are still preliminary. TRPA1−/− mice have reduced mechanosensitivity of mucosal afferents in vagal and pelvic pathways. TRPA1 deletion also reduced the stimulus–response functions of high threshold colonic splanchnic and pelvic serosal and mesenteric afferents. However, the distension responses of vagal tension receptors and pelvic muscular and muscular–mucosal afferents were unaltered. Taken together these data suggest paradoxically that TRPA1 contributes to mechanosensation in both tactile and nociceptive afferents, but not in others.

A nociceptive role for TRPA1 was also apparent in vivo; intrathecal administration of TRPA1 antisense oligodeoxynucleotide in rats (to reduce TRPA1 expression in DRGs) reduced the colitis-induced hyperalgesia to nociceptive colonic distension and intracolonic mustard oil. Although TRPA1 may not be as specific in its role as TRPV4, it is clearly evident as a target for reducing signalling of pain from the colon. When its role in mucosal afferents emerges, this may provide important insight into not only the functional role of the channel, but also the role of this subtype of afferents in sensation from the lower gut.

TRPM

Evidence for or against TRPM8 as a mechanosensor is lacking. Activation of a subpopulation of cutaneous sensory neurons by TRPM8 agonists elicits analgesia in chronic pain models in rats, which is blocked by siRNA knockdown of TRPM8. This analgesic effect of TRPM8-sensitive afferents is probably centrally mediated through activation of inhibitory interneurons in the spinal dorsal horn. This is similar to a somatosensory
process known as “gate control”, which underlies the analgesic effects of massage and transcutaneous electrical nerve stimulation. Although the exact mechanisms involved are unknown, the role of TRPM5 in gate control is potentially important. TRPM8 agonists are already known to activate non-nociceptive vagal afferent neurons innervating the gut.

TRPP

TRPP1 and 2 are recent mechanosensory candidates in the primary cilium of kidney cells and are suggested to be important in ciliated mechanosensory structures that detect very small forces. Therefore, this may be important in colonic mucosal afferents which are highly tactile. It was recently shown that PKD1L3 and PKD2L1 are activated by various acids at pH <3 when co-expressed in heterologous cells but not by other classes of tastants. These results suggest that TRPPs may function as sour taste receptors, which begs the question as to whether they may be involved in the detection of refluxed acid in the oesophagus. Since knockouts of these channels are lethal, advances in the field may well depend on novel pharmacology in recombinant systems.

TRP channels as effectors

TRP channels can also function as effectors, mediating excitation of cells in response to activation of G-protein-coupled receptors and other channels (table 1). This is an important aspect of the biology of most TRP subfamilies, and in some cases it is their only identified role, for example in some members of the TRPC family. The effector function of TRP channels is, however, important in sensory nerve function, such as inflammatory pain evoked by the mediators bradykinin and mast cell proteases. TRPV1 is not considered to be directly mechanically gated; therefore, the effects on mechanosensation may be mainly due to indirect effects on neuron excitability or via interactions with other receptors or TRP channels. For example, 5-hydroxytryptamine (5-HT) enhances sensitivity to heat in colonic DRG neurons via TRPV1, which leads to excitation at normal body temperature. Protease-activated receptor 2 (PAR2) sensitises TRPV1 through defined intracellular pathways. Cannabinoids modulate TRPV1 either by a direct interaction of the eicosanoid mediator anandamide with the channel itself, or by coupling through intracellular pathways after acting on G-protein-coupled cannabinoid receptors.

In addition to its coupling with TRPV1, there is also evidence for a strong interaction of PAR2 with TRPV4, which may contribute to visceral hypersensitivity. TRPV4 and PAR2 are co-expressed in a large proportion of colonic sensory neurons. In isolated colonic DRG neurons, PAR2-activating peptide (AP) sensitises TRPV4-mediated currents, whilst PAR2 AP also activates splanchnic serosal colonic afferent endings, but not in TRPV4 knockouts. Intracolonic administration of PAR2 AP increases the behavioural response to noxious colorectal distension, an effect also lost in TRPV4−/− mice.

PAR2 interacts with TRPA1 via similar intracellular mechanisms to those involved with TRPV1. TRPA1 can also be indirectly activated by bradykinin, and mediates bradykinin-induced mechanical hypersensitivity in the guinea-pig oesophagus. In the colon bradykinin induces mechanical hyper-sensitivity in splanchnic serosal afferents, which is lost in fibres from TRPA1−/− mice.

Other TRP channels are also associated with bradykinin activation, including TRPC1, whereby specific signalling from the BK2 receptor opens TRPC1. Thus TRPC1, like TRPV4 and TRPA1, interacts with inflammatory G-protein-coupled receptors. However, in contrast to TRPV1, TRPA1 and TRPC1, which are all sensitised by BK2 receptor activation, TRPM5 function is inhibited by bradykinin via a reduction in intracellular phosphoinositide (PIP2) levels.

In addition to its role in neurons, there is emerging evidence for involvement of TRPA1 in mediator release from enteroendocrine cells. Enterochromaffin cells express TRPA1 and release serotonin cholecystokinin (CCK) in response to mustard oil, which also releases CCK from the enteroendocrine cell line STC-1, suggesting that dietary spices may augment release of mucosal mediators in response to nutrients.

Although the role of TRPM5 is best established in the tongue, as a channel that couples taste receptor activation with cation influx, via the G-protein–phospholipase C pathway, it is also known to be expressed in the gut epithelium, where it may serve a similar “tasting” function. In keeping with this, its expression is regulated by changes in luminal or systemic glucose concentration.

Although TRP channels function as effectors for many pathways, single TRP knockouts normally retain some response to the initial stimulus, for example in bradykinin-evoked excitation of airway afferents, suggesting that there are parallel mechanisms—either other TRP channels or completely distinct pathways.

TRP channels may interact with one another, for example TRPA1 and TRPV1 are often co-localised in nociceptive DRG neurons, where a TRPA1 agonist causes cross-desensitisation of responses to capsaicin, and vice versa. Whether or not TRP channels may couple together positively, and therefore act as effectors for one another, is not yet evident.

CHANGES IN SENSORY SIGNALLING IN DISEASE

The effects of inflammation on visceral afferent endings have been studied by several groups, with a view to finding a model for postinfectious irritable bowel syndrome (eg, Mayer and Gebhart). The majority of electrophysiological studies in the literature have focused on the responses of afferents to colorectal distension and how this is altered by inflammation. A recent study by DeSchepper et al showed that acute...
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trinitrobenzene sulfonic acid (TNBS) colitis in rats increased the response to colorectal distension of pelvic afferent C-fibres in vivo; the increased sensitivity to distension was reduced by a TRPV1 antagonist. An earlier study by Sengupta et al\textsuperscript{93} using an identical in vivo rat TNBS protocol recording from the same population of afferents did not reveal any changes in mechanosensitivity, so there is clearly controversy in this area. In agreement with Sengupta’s\textsuperscript{93} findings in rat, Jones et al\textsuperscript{92} saw no change in mechanosensitivity of pelvic muscular or muscular–mucosal afferents in a mouse zymosan colonic inflammation model. The behavioural response to colorectal distension was, however, increased after zymosan, and this remained to an extent in TRPV1 knockout mice, so the mechanism underlying pain hypersensitivity was not revealed. In a model of colonic hypersensitivity induced by colonic irritation in neonatal rats,\textsuperscript{95} TRPV1 inhibitors were effective in reducing pain responses to colorectal distension. Increased expression of TRPV1 in DRGs is evident in this model, as well as in a TNBS colitis model,\textsuperscript{94} suggesting that TRPV1 upregulation may be a mechanism by which the effect of a TRPV1 antagonist becomes manifest. In a model of pancreatic inflammation this mechanism was confirmed.\textsuperscript{96} The upregulation of function via TRPV1 can be long lasting, and may initiate other events leading to hypersensitivity.\textsuperscript{95–98} Alternatively, the translucency of TRPV1 to the cell membrane may have an important influence on its function independent of changes in expression.\textsuperscript{99} Studies of biopsies from patients with visceral hypersensitivity, either in irritable bowel syndrome (IBS)\textsuperscript{97} (fig 3) or in non-erosive reflux disease,\textsuperscript{100} show that mucosal fibres containing TRPV1 may be increased in number up to 3.5-fold, and that this increase may correlate with symptoms. Chan et al\textsuperscript{100} showed that increased rectal sensitivity was associated with increased TRPV1 fibres in all layers of the gut wall. Mustard oil, which we now know to be a selective TRPA1 agonist, has long been used as a visceral inflammatory model to sensitise nociceptors and provoke tissue damage.\textsuperscript{101} This suggests that TRPA1 has a role not only in activation of sensory pathways, but also in local neurogenic inflammation, presumably after evoking release of peptides such as substance P from peripheral endings. Studies are ongoing to determine if TRPA1 function or expression is increased in inflammatory models or patients with IBS.

From the perspective of active inhibition of visceral pain the observation is worth noting that a combination of peppermint oil (a TRPM8 agonist) and caraway oil attenuates postinflammatory visceral hyperalgesia in rats,\textsuperscript{102} which may correlate with its therapeutic effects in patients described below.

**THERAPEUTIC OPPORTUNITIES**

A hallmark of functional gastrointestinal disorders is allodynia and hyperalgesia to mechanical events,\textsuperscript{103–105} and low-grade inflammatory status.\textsuperscript{106–108} Therefore, treatments are needed that reduce signalling of nociceptive mechanosensory and inflammatory events. TRP channels are clearly involved in both of these processes. Normally, there is a constant stream of subliminal information from the gut, which is involved in autonomic reflexes controlling motor and secretory function. It is therefore important to distinguish this information from nociceptive signals in targeting visceral pain to avoid altering normal gut function. So far, TRPV4 is the only TRP channel that appears to be nociceptor specific, but ironically is the least well served pharmacologically. As with many other TRP channels, its localisation is not, however, confined to sensory neurons,\textsuperscript{109} so the potential exists for off-target adverse effects. Such effects have applied powerful brakes to the progress of other types of treatments for visceral pain, but it could be argued that in the case of TRP channels, there are fewer other target tissues to be concerned about. We hope this article may provide a stimulus for further development of selective drugs, either as pharmacological treatments or as tools to investigate the roles of TRP channels more effectively. Another interesting application of TRP channel pharmacology is in diagnostic testing for functional gastrointestinal disorders. Administration of a capsaicin capsule produces more symptoms including pain, warmth and pressure in patients with functional dyspepsia compared with controls.\textsuperscript{110} Although the predictive value is far from ideal—only half the patient cohort were responsive—their promise for development of better provocative tests for functional disorders, considering we have very few options currently.

Herbal preparations containing peppermint have been successful in the treatment of functional dyspepsia,\textsuperscript{111} although whether this is a sensory or motor effect and whether it is mediated via TRPM8 remain to be seen. Several clinical trials have been conducted with selective TRP channel antagonists for pain,\textsuperscript{112} such as the TRPV1 antagonist, AMG-517, which was discontinued due to causing hyperthermia after systemic administration. Pharmaceutical companies are currently...
determining whether this liability for TRPV1 antagonists can be managed. Site-specific administration of TRPV1 desensitisation gets around this issue, and has shown promise in other visceral neurological disease, reviewed elsewhere, but whether the gut is an appropriate place for site-specific interventions remains a difficult question. By far the greatest attention has been given to TRPV1 as a therapeutic target, and there is still specific interventions remains a difficult question. By far the greatest attention has been given to TRPV1 as a therapeutic target, and there is still

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