British Journal of Pharmacology

DOI:10.1111/bph.12641
www.brjpharmacol.org

Themed Section: The pharmacology of TRP channels

REVIEW

Transient receptor potential cation channels in visceral sensory pathways

L Ashley Blackshaw

Wingate Institute for Neurogastroenterology, Centre for Digestive Diseases, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Correspondence
L. Ashley Blackshaw, Wingate Institute, 26 Ashfield Street, London E1 2AJ, UK. E-mail: a.blackshaw@qmul.ac.uk

Keywords
TRP channels; pain; mechanosensitivity; inflammation

Received
12 September 2013
Revised
9 January 2014
Accepted
20 January 2014

The extensive literature on this subject is in direct contrast to the limited range of clinical uses for ligands of the transient receptor potential cation channels (TRPs) in diseases of the viscera. TRPV1 is the most spectacular example of this imbalance, as it is in other systems, but it is nonetheless the only TRP target that is currently targeted clinically in bladder sensory dysfunction. It is not clear why this discrepancy exists, but a likely answer is in the promiscuity of TRPs as sensors and transducers for environmental mechanical and chemical stimuli. This review first describes the different sensory pathways from the viscera, and on which nociceptive and non-nociceptive neurones within these pathways TRPs are expressed. They not only fulfil roles as both mechano- and chemo-sensors on visceral afferents, but also form an effector mechanism for cell activation after activation of GPCR and cytokine receptors. Their role may be markedly changed in diseased states, including chronic pain and inflammation. Pain presents the most obvious potential for further development of therapeutic interventions targeted at TRPs, but forms of inflammation are emerging as likely to benefit also. However, despite much basic research, we are still at the beginning of exploring such potential in visceral sensory pathways.

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Abbreviations
AITC, allyl isothiocyanate; ASIC, acid sensing ion channel; CRD, colorectal distension; DRG, dorsal root ganglion; IBS, irritable bowel syndrome; IGLE, intraganglionic laminar endings; IMA, intramuscular arrays; NCCP, non-cardiac chest pain; PAR2, protease activated receptor-2; TRP, transient receptor potential; TRPA1, TRP ankyrin-1; TRPM8, TRP melastatin-8; TRPV1, TRP vanilloid 1; TRPV4, TRP vanilloid 4; TTX, tetrodotoxin

Introduction

It is difficult to estimate exactly how many papers have been written on the association between the transient receptor potential cation channels (TRP channels) and visceral afferents, especially when one considers the large range of channels in the TRP family combined with the diversity of visceral organs innervated by many different types of sensory fibres. The number is almost certainly in the thousands, especially when we include the early use of capsaicin as a neurotoxin before its mechanism of action via TRPV1 channels was known, the use of mustard oil as an inflammatory stimulus before its role as a TRPA1 agonist was discovered, and the use of menthol as a cooling agent before its main target, the TRPM8 channel, became evident (receptor nomenclature follows Alexander *et al.*, 2013a.

It is not the intention of this article to chronicle the development of understanding of TRPs in visceral sensory pathways, but to point out areas I believe to be important for improving knowledge in the future, and the state of the art in general. It is often assumed that the role of TRPs in sensory neurones is similar across populations, particularly in nociceptive fibres. However, it is clear that the expression and function of TRPs differs considerably between visceral and somatic types, and not merely because of the prevalence of pain as a visceral sensation. Therefore, it is misleading to generalize and likely to give rise to a view of visceral afferent function that is not only incorrect but also counterproductive if we are to target TRP channels in visceral afferents therapeutically.

The author is more expert in the innervation of the gastrointestinal tract than other visceral organs, but the full

range of thoracic, abdominal and pelvic viscera will be covered albeit not in as much detail. The vasculature of the entire body is smooth muscle and is therefore, by definition, visceral. Blood vessels receive a dense sensory innervation, which may give rise to a range of symptoms, most of which are poorly understood, but some, such as headache, are an area of research in their own right. This is a large field, and cannot be given fair space in this account, so the reader is referred to seminal reviews addressing TRP involvement in headache by Dux *et al.*, 2012 and Strassman and Levy, 2006.

What are the sensory pathways from the viscera?

Vagal afferents

The vagus, the 10th pair of cranial nerves, innervates all cervical, thoracic and abdominal viscera including parts of the colon. Vagal afferents vastly outnumber vagal efferent fibres, and transmit a huge array of mechanosensory and chemosensory information that is critical to reflexes and behaviours essential for maintaining life. The abdominal vagus nerves used to be cut therapeutically in order to reduce acid production by the stomach, but this led to major disruption of gut function and is no longer practised. Cervical vagotomy is invariably fatal, demonstrating the importance of vagal cardiovascular and respiratory afferents in vital functions. This generalization of vagal afferents subserving homeostatic and regulatory functions applies to the vast majority throughout the viscera, with the possible exception of oesophageal vagal afferents discovered by the Johns Hopkins group, which may give rise to pain (Yu *et al.*, 2005), although these are defined as nociceptive not based on their ability to evoke pain, but on their responses to high-intensity mechanical stimuli and activation of TRPV1 channels.

Anatomically, vagal afferents have their cell bodies in the nodose and jugular ganglia, and innervate many tissue types including glomus tissue, epithelium, visceral muscle and blood vessels. Their function is determined largely by their location. Whether this is simply because of their local environment and/or because of their molecular specialization is not clear. Attempts have been made to classify afferents neurochemically according to their target tissue type, but so far, this has only been successful in the airways, where, based on their neurochemical coding, morphology, location and origin, three sensory receptor end organs are currently morphologically well characterized: smooth muscle-associated airway receptors, neuroepithelial bodies and visceral pleura receptors (Brouns *et al.*, 2012). In the oesophagus, afferents innervating multiple layers have been described (Page and Blackshaw, 2009), which may transduce stimuli applied to both muscle and mucosa, but their anatomical correlate has not yet been found. Otherwise, in the gastrointestinal tract endings tend to be associated with either the mucosal or muscular layers, not both, as far as the rectum and anal canal (Brierley *et al.*, 2004). Mucosal endings are in close proximity to epithelial cells and respond to release of mediators from enteroendocrine cells or intraepithelial immune cells. Muscular endings come in two classes – intraganglionic laminar endings (IGLE) and intramuscular arrays (IMA). IGLE are associated with myenteric ganglia of the enteric nervous system, and form basket-like structures around the ganglion. They are the transduction sites for stretch and contractile force (Zagorodnyuk and Brookes, 2000). IMA have not yet been ascribed a function since their locations do not correlate well with mechanotransduction sites (Zagorodnyuk and Brookes, 2000).

In the rat heart, anterogradely labelled vagal afferent terminals have been shown to consist of dense pericellular varicose endings within the four major ganglionated plexuses of the epicardium, simple endings near muscle fibres of the myocardium, and more complex 'flower-spray' and 'end-net' endings in the ¨endocardium (Cheng *et al.*, 1997a). In addition, similar complex terminals were found in the wall of the aortic arch, and carotid artery and near glomus cells at these locations, suggesting both mechano- and chemoreceptive functions (Cheng *et al.*, 1997b). Innervation of glomus tissue within paraganglia associated with the laryngeal nerve has also been reported (Dahlqvist *et al.*, 440; Kummer and Neuhuber, 1989). See Berthoud and Neuhuber, 2000 for references.

Spinal afferents

Unlike the vagal innervation, which diminishes in density caudally, the spinal innervation of the viscera is relatively evenly distributed. In general, the region innervated corresponds with the level of dorsal root ganglia where their cell bodies are located, with the exception of L2–L5, which does not contain appreciable numbers of visceral afferent cell bodies. Although spinal afferents terminate in regions of the gut that overlap with vagal afferents, they tend to be more frequently associated with deeper structures like the adventitia of blood vessels, locations that are most effective in evoking pain. Vagal afferents, on the other hand, are more commonly associated with superficial structures like epithelia. It is also notable, at least in the gastrointestinal tract, that spinal afferents terminate directly on peripheral neurones, including those in the prevertebral and myenteric ganglia (Gautron *et al.*, 2011). They are therefore more likely than vagal afferents to participate in peripheral axon collateral reflexes in addition to transmitting information centrally.

Expression of TRPs on visceral sensory pathways

Afferent fibres

TRP channels in families A, C, M and V have been demonstrated to exist on visceral afferents (Zhang *et al.*, 2004). The more recently characterized TRPP family of pH-sensitive channels has not yet been investigated. In keeping with the greater role of spinal afferents in mediating pain, they generally express higher levels of TRP channels associated with pain, but this rule has many exceptions, and the distinction is never absolute. In many cases, it is not known what the relative expression of a given channel is between vagal and spinal afferent innervation of an organ since non-quantitative methods were used to detect them. There is also the conundrum of whether absolute levels of TRP mRNA in a ganglion of afferent cell bodies are representative of the func-

tion of the particular TRP. Perhaps a more representative measure is the proportion of afferents that express the protein above a given level, suggesting that they will respond to the natural stimuli for the channel. In this respect, there is a good agreement with the expression of TRPV1 in vagal and spinal afferents innervating the upper and lower gut respectively. About one third of upper gastrointestinal vagal afferents express TRPV1, and the same proportion respond to capsaicin with increases in action potential firing (Blackshaw *et al.*, 2000). Likewise, about three quarters of colonic splanchnic afferents express TRPV1 and show capsaicin responsiveness (Brierley *et al.*, 2005a).

There is invariably co-expression of TRP channels on any given afferent neurone, both within and between families. In other systems, the co-expression and functional co-dependence of TRPA1 and TRPV1 channels is considered important. Why a cold- and a hot-sensitive channel should coexist is perhaps puzzling, but as they have in common the property of evoking pain, it makes more sense to detect painful heat and cold in the same neurone. TRPA1 and V1 channels also coexist in visceral afferents, but since the viscera are never exposed to extremes of temperature, we must consider other roles for them. Both are activated by a host of endogenous inflammatory mediators and chemical compounds that may access the viscera from the environment, such as in noxious smoke, herbs and spices. Thus, they may take on different roles in internal organs.

Non-neural cells

The list of epithelial and immune cell types that express TRPs is growing continually, which is in keeping with the role of TRPs as vanguards of the body. In many cases, these cells are in direct communication with sensory nerves, so the sensory apparatus is arranged in a similar manner to the gustatory and visual systems, with two primary components. The epithelia of the urinary bladder and oesophagus are notably rich in expression of TRPV1 and TRPV4 and it is clear that mice lacking these channels have reduced sensory and reflex function from these organs. Capsaicin (at TRPV1) and acid [at both TRPV1 and acid sensing ion channel (ASIC)] induce ATP release from the rat bladder mucosa. This ATP appears to be principally of urothelial origin (Sadananda *et al.*, 2009). Furthermore, TRPV4 channels are involved in mechanically and chemically evoked Ca^{2+} influx and ATP release in primary urothelial cell cultures (Mochizuki *et al.*, 2009), and in mouse oesophageal keratinocytes (Mihara *et al.*, 2011). Enteroendocrine cells of the gastrointestinal tract also express TRPs (Purhonen *et al.*, 2008), which may be important in their endocrine role, but it is not yet clear to which dietary stimuli, if any, they respond.

TRPS as primary sensors

Mechanosensors

Proving that a particular molecule is itself a mechanotransducer is extremely difficult. In many cases, creating a knockout mouse that lacks the gene for a particular channel will show that the molecule is essential for normal mechanotransduction. However, this does not tell us whether it is the channel or associated proteins that are the responsive element, or even if the channel contributes merely towards excitability of the peripheral ending. Expressing the channel in a recombinant system is not much help as it may require several associated molecules to function correctly, which would have to be co-expressed in the right proportions. Even studies in native cells are fraught with problems since mechanotransduction takes place at sensory endings, not at cell bodies, where the cytoskeleton may be specialized to support a role for mechanotransduction molecules. Our best attempt at this challenge was to observe responses in cultured sensory neurones to deformation of their distal neurites, then to over- or under-express the channel in question using adenoviral vectors, germ-line knockouts and finally by pharmacological agonists and antagonists. Using this combination of approaches, we were able to demonstrate a role for TRPA1 channels in mechanotransduction in both high and low threshold colonic afferents innervating blood vessels and mucosal tissue respectively (Brierley *et al.*, 2009b; 2011). In particular, the gain of function seen upon overexpressing TRPA1 suggests it plays a direct role in mechanotransduction in the processes of mouse thoracolumbar dorsal root ganglia dorsal root ganglion (DRG) neurones. Table 1 lists the evidence for a role of TRP channels as mechanotransducers in visceral sensory neurones. The picture that emerges is not clear because different classes of fibres were studied in different pathways with slightly different methods. What we can say with certainty, nonetheless, is that members of the TRPA, M and V families all have a role in modulating visceral mechanotransduction and/or contributing directly to it. One of the more exciting observations, in my opinion, was that TRPV4 channels seem to be preferentially expressed on nociceptive visceral afferents, and to mediate the responses to algesic mediators (see coupling with GPCRs below), thus placing it in the position of a selective target for analgesic therapies. Indeed, evidence is emerging of TRPV4 antagonists that are able to reduce mechanical sensitivity of nociceptors in human bowel (Peiris *et al.*, 2011; McGuire *et al.*, 2013), so why have not these drugs reached clinical trials for mechanically induced visceral pain? The answer probably lies in the non-neural expression profile of TRPV4 and other TRPs, which are found in epithelia, endothelia and in some cases, areas of the CNS unconnected with pain. However, whether or not this distribution is a source of adverse effects of antagonists in the clinic is largely untested.

Sensors of environmental chemicals

TRPs have a strong reputation as sensors of environmental chemical hazards, such as those found in smoke and toxic materials. In these circumstances, TRPs are coupled to defensive mechanisms, mainly in skin, mucous membranes and airways, which are the first points of contact with the environment. Whether or not TRPs in cardiovascular or gut tissue have any function in this regard is unclear since they are not as directly exposed to the environment. These tissues are definitely innervated by TRP-expressing sensory neurones, but the main function of TRPs in these endings may be as mechanosensors or as coupling mechanisms (see below).

The cough reflex is driven by vagal afferents innervating the airway epithelium (Canning *et al.*, 2004). Research on the role of TRPs on these airway afferents has been intense, in the pursuit of novel anti-tussive agents (Muroi and Undem,

Table 1

Effects of TRP channel knockouts and antagonists on visceral afferent mechanosensory function

Channel	Vagal gastro-oesophageal afferents		Vagal/spinal jejunal afferents			Splanchnic distal colon afferents		Pelvic colorectal afferents				Response *in vivo* to CRD
	Mucosal	Tension	Low threshold	Wide dynamic range	High threshold	Mesenteric	Serosal	Serosal	Mucosal	Muscular	Muscular/ mucosal	
TRPV1												
−/−	–	→	↕	→	↕	–	–	↕	↕	↕	⇉	⇉
Antagonist	–	–	→	→	→	–	–	↕	–	–	⇉	–
TRPV4												
−/−	↕	↕	–	–	–	⇉	⇉	⇉	↕	↕	↕	⇉
Antagonist	–	–	–	–	–	⇉	⇉	⇉	–	–	–	–
TRPA1												
−/−	→	↕	–	–	–	⇉	⇉	⇉	→	↕	↕	–
Antagonist	–	–	–	–	–	–	–	–	–	–	–	–

Number and direction of arrows indicate degree of change in mechanosensory function. Empty cells indicate not tested. Adapted from Hughes *et al.* (2009).

2011). It is very logical that, given the fact many environmental airborne irritants are effective at activating TRPA1 channels due to their peculiar structure, these channels should be of interest (see Geppetti *et al.*, 2010). However, TRPV1 agonists are the most potent in activating airway afferents (Brozmanova *et al.*, 2012).

A summary of the sensitivity of TRPs to natural agonists is shown in Table 2 and in recent reviews (Blackshaw *et al.*, 2010; Brozmanova *et al.*, 2012).

TRPs as downstream sensory signalling mechanisms

As if it were not enough for nature to provide a wealth of specific targets for direct therapeutic modulation of sensory function in the form of TRPs, the very same channels also function as transducers for a range of other receptors. Thus, a GPCR or cytokine receptor may transactivate or modulate opening of a TRP channel adjacent to it in the cell membrane, providing a broader repertoire of functions for each TRP channel. This is an emerging field, and exactly how promiscuous a particular TRP channel is in a particular cell is still unclear. One thing is certain, however, and that is TRPs form a gateway to the generation of pain by many inflammatory mediators, and this may in fact be their major role in some circumstances.

Coupling with GPCRs

5-HT enhances sensitivity to heat in colonic DRG neurones, so that they are activated at normal body temperature (Sugiuar *et al.*, 2004), which is lost in TRPV1 knockout mice, although how 5-HT receptors and TRPV1 channels are arranged relative to one another is yet to be shown. In somatosensory neurones, there is evidence also for coupling of TRPV1 to cannabinoid CB_1 and protease-activated receptors (PAR2; receptor nomenclature follows Alexander *et al.*, 2013b) and that PAR2 interacts with TRPA1 channels (Dai *et al.*, 2007) but these associations remain to be determined in visceral afferent neurones. There is evidence for interaction of PAR2 with TRPV4 in viscerosensory neurones, which contributes to visceral hypersensitivity (Cenac *et al.*, 2008; Sipe *et al.*, 2008). These studies also found TRPV4 and PAR2 are often co-expressed in colonic sensory neurones, and that a PAR2-activating peptide (AP), sensitizes TRPV4-induced currents. The PAR2-AP also directly activated colonic high-threshold afferent endings, an effect that was totally absent in TRPV4 knockouts (Sipe *et al.*, 2008). Intra-colonic administration of PAR2-AP increased the abdominal contraction response to noxious colorectal distension (CRD), an effect also lost in TRPV4 knockout mice (Cenac *et al.*, 2008; Sipe *et al.*, 2008). These findings suggest a more exclusive relationship of PAR2 with TRPV4 channels in viscerosensory than in somatosensory neurones. Hypersensitivity to CRD induced by intraluminal 5-HT or histamine were both significantly inhibited by interference with TRPV4 expression at the spinal level, which provides indirect evidence that other GPCR may activate or sensitize sensory neurones via TRPV4 channels (Cenac *et al.*, 2010).

TRPA1 channels are likely to mediate bradykinin B_2 receptor-induced mechanical hypersensitivity in guinea pig

Table 2

TRPs discussed in this paper, their natural ligands, physical stimuli and localization, where known

Channel	Natural ligand	Physical stimuli	Direct endogenous stimuli	Indirect endogenous stimuli	Relevant locations
TRPV1	Capsaicin, acid	Thermal >43°C	Acid, anandamide, reactive oxygen species	Cannabinoids, bradykinin, 5-HT, proteases	Sensory neurones, oesophageal epithelium
TRPV4	*Andrographis paniculata* (Chinese herbal medicine)	Mechanical, Thermal >25°C	Anandamide, arachidonic acids, epoxyeicosatrienoic acid metabolites	Proteases	Sensory neurones, vascular endothelium
TRPA1	Mustard, oregano, cinnamon, garlic	Mechanical, Thermal <17°C	4-Hydroxnonenal, reactive oxygen species	Cannabinoids, bradykinin, prostaglandins, proteases	Sensory neurones, enteroendocrine cells
TRPM8	Peppermint, eucalyptus	Thermal <28°C	Lysophospholipids	–	Sensory neurones, bladder epithelium

oesophageal afferents (Yu and Ouyang, 2009). Similarly, B_2 receptor activation induced mechanical hypersensitivity of colonic splanchnic high-threshold afferents (but not pelvic afferents; Brierley *et al.*, 2005b), which was lost in TRPA1 knockouts (Brierley *et al.*, 2009a).

Therefore, it appears that TRPV1, TRPV4 and TRPA1 channels all interact with inflammatory GPCRs. In direct contrast to the excitatory actions just described, inflammatory mediators such as bradykinin and histamine inhibit TRPM8 in intact sensory nerves, but do so through a novel signalling pathway. The G-protein subunit $G\alpha_q$ binds to TRPM8 channels, and when activated by a Gq-coupled receptor directly inhibits ion channel activity (Zhang *et al.*, 2012). This may be connected with the anti-itch and antinociceptive actions of TRPM8, which would be suppressed during inflammation to make way for activation of pain and defence mechanisms.

Although TRPs function as effectors for many pathways, single TRP knockouts normally retain some response to GPCR agonists (except for PAR2 effects in TRPV4 knockouts), suggesting there are parallel mechanisms – either other TRPs or completely distinct pathways. Therefore, there is considerable redundancy among TRP channels in these pathways.

Coupling with cytokine receptors

It is clear that the pro-inflammatory cytokine TNF-α sensitizes sensory neurones via interactions between their receptors (see Alexander *et al.*, 2013c) and TRPV1, but also via sodium channels and/or potassium channels (Jin and Gereau, 2006; Hagenacker *et al.*, 2010; Ibeakanma and Vanner, 2010). We confirmed the effect of TNF-α in visceral sensory neurones, showing an increase in mechanosensory responses after incubation, but no direct response to TNF-α itself (Hughes *et al.*, 2013). Selective antagonism of TRPV1 did not affect sensitization by TNF-α. However, the TRPA1 antagonist HC-030031 blocked the sensitizing effects of TNF-α, indicating in colonic afferent endings TNF-α signals via interactions between tumour Necrosis Factor receptor-1 and TRPA1 but not TRPV1. In contrast to TNF-α, IL-1β caused both direct firing and mechanical hypersensitivity that was unchanged after inhibition of TRPA1 channels (Hughes *et al.*, 2013). However, this was blocked by TTX, implying a selective coupling of IL-1R1 to TTX-sensitive Na_V channels, and distinct signalling pathways for different pro-inflammatory cytokines within the same population of neurones. How other cytokines interact with TRPs in visceral sensory neurones remains to be seen, but clearly, TRPs represent a point of convergence for a range of different types of receptor-mediated events (Figure 1).

TRPs in disease

Pain and sensory dysfunction

Chronic pain and discomfort from the viscera occur in response to inflammation or injury, but pain in the absence of major pathological markers affects up to 10% of the general population, and therefore represents a large unmet need for treatment and consequent economic impact. No systemically active drugs are yet available for clinical use in chronic visceral pain that target TRP channels, although some have been through clinical trials that hold promise for development of targets for treatment of visceral pain.

Visceral pain of the most concern is in the form of angina pectoris, which is normally caused by cardiac ischaemia, and therefore of immediate threat to life, although there may be other causes. Ischaemia causes local increases in the tissue levels of hydrogen ions, reactive oxygen species, bradykinin and other algogenic mediators, all of which excite cardiac nociceptive afferents (Fu and Longhurst, 2009). The role of TRP channels in the direct mediation of these responses is not known, but it is clear that cardiac nociceptors express TRP channels, and that desensitization of them attenuates responsiveness (Pan and Chen, 2004). Clearly, it is of dubious clinical benefit to reduce pain of the type that signals imminent cardiac arrest, so cardiac afferents are less of a therapeutic target than those elsewhere. Non-cardiac chest pain (NCCP) has identical symptoms to that caused by ischaemia, and is a major burden not just in terms of the need for urgent and correct diagnosis, but also in terms of chronicity and severity, and thus quality of life. Many NCCP patients ultimately receive a diagnosis of oesophageal origin for their pain, so there is interest in oesophageal pain for the treatment of

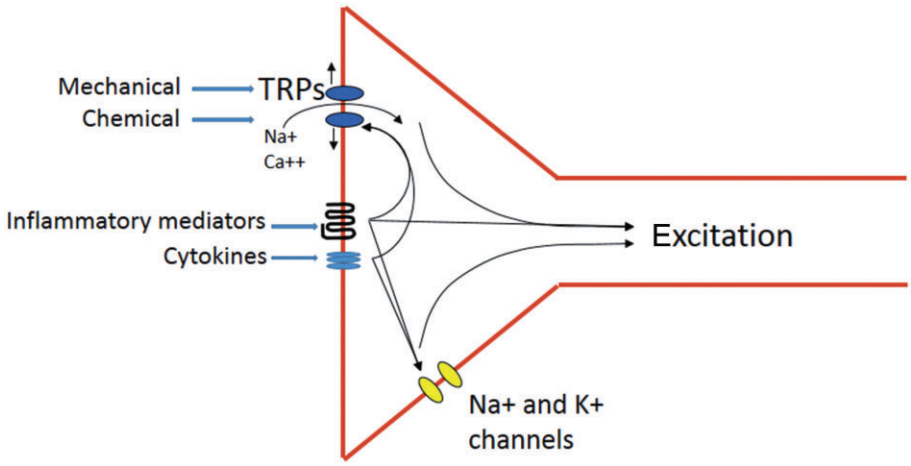

Figure 1

Diagram of ways in which TRPs on visceral afferents may become activated. Environmental mechanical and chemical stimuli may act directly on the channel to increase its probability of opening, allowing cation influx. Alternatively, extracellular mediators such as cytokines and inflammatory mediators may activate them via specific receptors, which recruit a range of intracellular cascades (these are discussed in other articles in this theme). We have evidence that some receptors may preferentially act via opening of sodium channels and some via TRPs. There is also the possibility of closure of potassium channels as a means of increasing excitability.

NCCP and of heartburn. Oesophageal pain differs markedly from that evoked from other organs since the primary stimulus is unbuffered gastric acid (pH 1–2), whereas elsewhere, pH rarely falls below 5 or 6. The pH sensitivity of TRPV1 channels has made it a major target for investigation in oesophageal afferents, and indeed vagal afferents can be categorized based on their responses to TRPV1 activation (Yu *et al.*, 2005). We found that the activation of cervical and thoracic DRG neurones by intra-oesophageal acid *in vivo* was lost in TRPV1 knockout mice, as was the downstream activation of dorsal horn neurones (Harrington *et al.*, 2013), suggesting TRPV1 channels are required for acid-induced oesophageal pain. Although it is clear that TRPV1 channels are expressed in nociceptive neurones, in the oesophageal and bladder epithelium, they are expressed also in squamous cells (Birder *et al.*, 2002; Cheng *et al.*, 2009). In both these locations, they can act as a transducer in much the same way as in neurones. Perhaps because of this particular localization, TRPV1 has become a target for treatment of oesophageal pain (Krarup *et al.*, 2013) and overactive bladder (Chancellor and de Groat, 1999; Birder *et al.*, 2013), although other TRPs in epithelia may fulfil similar roles with similar clinical implications (Andersson *et al.*, 2010).

My group has focused on the distal colon and rectum as sources of visceral pain since pain from these regions is one of the most common reasons for clinical consultation in the form of irritable bowel syndrome (IBS). However, we expect many of the principles that apply here will apply elsewhere. What has become clear from ours and others' studies is that multiple TRPs are expressed on gut sensory neurones. Our data indicate that TRPA1, V1, V4, and M8 most likely coexist on the same neurones, and they interact in the orchestration of a nociceptive response. For example, in the TRPA1 knockout mouse, the desensitizing effect of capsaicin on colonic afferents is lost (Brierley *et al.*, 2009b). This indicates TRPA1 channels are required for the effects of TRPV1 on mechanosensitivity. Also in these fibres, the TRPM8 agonist icilin reduced the direct chemosensory response to capsaicin and prevented mechanosensory desensitization induced by capsaicin. Icilin also prevented sensitization by the TRPA1 agonist allyl isothiocyanate (AITC) (Harrington *et al.*, 2011). Therefore, TRPM8 couples to TRPV1 and TRPA1 channels to inhibit their downstream chemosensory and mechanosensory actions. These findings may explain the use of natural TRPM8 agonists such as menthol to alleviate gut symptoms. The concentrations of these agonists reaching the intestine would, however, barely reach threshold levels for TRPM8 activation. However, a delayed release formulation was effective in a recent trial in IBS patients (Merat *et al.*, 2010).

The pancreas is another source of visceral pain that is often very severe and resistant to medication. Zhu *et al.* showed that nociceptor activation and pain in chronic pancreatitis are mediated via nerve growth factor, which in turn up-regulates TRPV1 channels (Zhu *et al.*, 2011). TRPV1-expressing neurones may in turn act as a means by which multiple stimuli can exacerbate experimental pancreatitis, but TRPV1 knockout mice are not protected from it, suggesting that there are overlapping mechanisms within these neurones (Romac *et al.*, 2008), and other TRPs may play important roles. There is a transition from acute to chronic pancreatitis, before which antagonists of TRPV1 and TRPA1 channels may ameliorate disease, but after which antagonism of these TRP channels is ineffective (Schwartz *et al.*, 2013). Deletion of TRPV4 or TRPA1 suppressed activation of pain behaviour in pancreatitis, and deletion of TRPA1 channels attenuated pancreatitis. Thus, TRPV4 and TRPA1 contribute to pancreatic pain, and TRPA1 channels also mediate pancreatic inflammation (Ceppa *et al.*, 2010). All these findings suggest at least three TRPs are involved in pancreatic pain and inflammation in different ways.

A major unanswered question in the field of TRPs on visceral sensory function is what happens to them in chronic

pain states? A novel approach using a transient noxious stimulus in the neonatal period showed a potentially important role for TRPV1 channels in initiation and maintenance of persistent visceral hypersensitivity in rats (Winston *et al.*, 2007). A water-avoidance stress-induced chronic pain model also showed that TRPV1 channels were up-regulated, and that a TRPV1 antagonist could prevent the development of hyperalgesia (Hong *et al.*, 2009). In the urinary bladder, cyclophosphamide-induced cystitis reduces ASIC channel but enhances TRPV1 receptor function in sensory neurones (Dang *et al.*, 2013). Translation from chronic pain models in animals to clinical trials is notoriously difficult, due to interpretation of readouts and species differences, and the case is no different for visceral pain (Blackshaw, 2012). My group chose to use a post-inflammatory chronic hypersensitivity model in mice using intracolonic trinitrobenzene sulphonic acid. This showed a selective sensitization of high-threshold afferents (Hughes *et al.*, 2009). The role of different TRPs in this sensitization is currently under investigation, but preliminary data indicate a decrease in TRPV1 channel responsiveness and expression in colonic afferents. This is difficult to reconcile with the observations above, but agrees with observations in somatic neuropathic pain (Staaf *et al.*, 2009). Jones *et al*. on the other hand, found that zymosan-induced sensitization of colonic afferents is absent in TRPV1 knockouts (Jones *et al.*, 2005). There is not enough space here to document all of the direct and indirect evidence for the changes in TRP function in experimental models of visceral pain, but it is clearly a controversial area, and may depend on the model, species, pathway and subtype of afferent fibre.

Inflammation

Many of the studies discussed above are of inflammatory or post-inflammatory models of pain, so it is often difficult to disentangle the effects of inflammation on TRPs from the effects of TRPs on inflammation. In this section, inflammation is treated as the end point rather than as the trigger for pain.

As a general rule, visceral inflammation in response to a variety of pathogens is worse in TRP knockouts, indicating that they serve a protective role, and yet some selective TRP agonists are capable of inducing inflammation. In many cases, this discrepancy can be explained by the way sensory endings are affected by TRP agonists. Stimulation of TRPs by ongoing inflammation (via the mechanisms discussed earlier) is enough to release neuropeptides locally, such as calcitonin gene-related peptide, which in turn modulate immune and vascular function to prevent damage and promote healing. Desensitization or destruction of these endings by agonist overdose, or deletion of TRPs, prevents this important protective axon reflex. The best example of this is in the way TRPV1 channels can provide protection from acid-induced gastric damage (Holzer, 2007). There is also evidence for this process extending between organs, where potent afferent activation in the duodenum by the TRPA1 agonist AITC promotes acute pancreatitis via axon collaterals, which may provide a mechanism by which alcohol induces pancreatitis (Li *et al.*, 2013; plus see section above on pancreatic pain). The inflammatory response in chronic obstructive pulmonary disease produces a range of chemical mediators, which similarly activates a vicious circle that through TRPA1 activation contributes to the severity of the disease (Geppetti *et al.*, 2010). Also, experimental colitis was inhibited or reduced in TRPA1–/– mice and by a pharmacological inhibitor of TRPA1 channels (Engel *et al.*, 2011). Recent evidence indicates TRPM8 channels provide a protective role in the colonic mucosa via activation of sensory endings (Ramachandran *et al.*, 2013), which is of interest given its potential also in visceral pain discussed earlier.

Conclusions

I have attempted to restrict the discussion in this review to papers specifically on TRPs along visceral afferent pathways. It is clear now that there are sufficient of these to form a critical mass upon which drug development programmes can be re-launched. This was not the case a few years ago, and most summaries in the literature relied heavily on evidence from studies of TRPs in somatosensory pathways. Although I have not explicitly catalogued the differences between visceral and somatic pathways in terms of TRP expression and function, the main message is that they are abundant in visceral sensory pathways and perform a wide variety of functions, which merits their consideration as targets in this domain as a primary focus. The sometimes marked differences in the expression and function of TRPs in visceral and somatic pathways (e.g. TRPV4 channels) may have led to some being overlooked in target validation programmes in the pharmaceutical industry. This in turn may well be a reason for their lack of exploitation as therapeutic targets.

Conflict of interest

A. B. receives grant funding from Grunenthal Pharmaceuticals, and his position is funded by the Wellcome Trust.

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