Review article

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Review article: transient receptor potential channels as possible therapeutic targets in irritable bowel syndrome

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Summary

Background: Abdominal pain in irritable bowel syndrome (IBS) remains challenging to treat effectively. Researchers have attempted to elucidate visceral nociceptive processes in order to guide treatment development. Transient receptor potential (TRP) channels have been implied in the generation (TRPV1, TRPV4, TRPA1) and inhibition (TRPM8) of visceral pain signals. Pathological changes in their functioning have been demonstrated in inflammatory conditions, and appear to be present in IBS as well.

Aim: To provide a comprehensive review of the current literature on TRP channels involved in visceral nociception. In particular, we emphasise the clinical implications of these nociceptors in the treatment of IBS.

Methods: Evidence to support this review was obtained from an electronic database search via PubMed using the search terms "visceral nociception," "visceral hypersensitivity," "irritable bowel syndrome" and "transient receptor potential channels." After screening the abstracts the articles deemed relevant were cross-referenced for additional manuscripts.

Results: Recent studies have resulted in significant advances in our understanding of TRP channel mediated visceral nociception. The diversity of TRP channel sensitization pathways is increasingly recognised. Endogenous TRP agonists, including poly-unsaturated fatty acid metabolites and hydrogen sulphide, have been implied in augmented visceral pain generation in IBS. New potential targets for treatment development have been identified (TRPA1 and TRPV4,) and alternative means of affecting TRP channel signalling (partial antagonists, downstream targeting and RNA-based therapy) are currently being explored.

Conclusions: The improved understanding of mechanisms involved in visceral nociception provides a solid basis for the development of new treatment strategies for abdominal pain in IBS.

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1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by chronic recurrent abdominal pain and alterations in bowel habit. The pathophysiology of IBS is incompletely understood, which poses obstacles in the search for effective therapeutic approaches. While the defecation pattern can generally be managed adequately with pharmacotherapy, abdominal pain tends to be difficult to treat effectively in IBS patients. In the search for new therapeutic strategies, accumulating interest has been given to peripheral mechanisms of nociception as a key target to develop novel analgesics for IBS-related pain. It is now widely accepted that an altered visceral sensitivity through abnormal endogenous pain processing plays an important role in the pathogenesis. This can result both from peripheral and central sensitization processes.[1] By virtue of peripheral sensitization of nociceptive afferents, increased nociceptive discharge can result in the generation of pain symptoms.[2] The responsiveness of these nociceptive afferents or nociceptors, is determined by the expression of specific channels sensing noxious stimuli.[3] The discovery of sensory transducer molecules, including the transient receptor potential (TRP) channel family has opened a new horizon in understanding peripheral nociceptive processes. TRP channels constitute a family of nonselective cation channels. Several members of this family, of which transient receptor potential vanilloid 1 (TRPV1) has been studied most extensively, have been identified to function as integrators and transducers of nociceptive signals in both somatic and visceral pain. However, as nearly all sensory neurons and several non-neuronal cell types express TRPV1, its role is not limited to nociception. TRP channels indeed appear to have a broad spectrum of functions in the human body, a topic that has been reviewed in detail, elsewhere.[4] This review will focus on current knowledge with regards to the potential role of TRP channels in the pathogenesis of pain symptoms in IBS, with particular emphasis on visceral nociception. Specifically, we will summarise their clinical implications and discuss the future of TRP channel targeted therapy.

2 | METHODS

Evidence to support this review was obtained from an electronic database search via PubMed by two of the authors (AB and ZW) using the search terms "visceral nociception," "visceral hypersensitivity," "irritable bowel syndrome" and "transient receptor potential channels." The last date of the search was 21st of July 2017. After screening the abstracts the articles deemed relevant were cross-referenced for additional manuscripts.

3 | IRRITABLE BOWEL SYNDROME PATHOPHYSIOLOGY

Several mechanisms have been hypothesized to play a role in the pathogenesis of IBS, including disturbances in microbiota, low-grade inflammation, immune activation, intestinal barrier dysfunction and altered bile salt absorption. Discussing these mechanisms separately is beyond the scope of this article. A thorough overview is provided in a recent review article.[5] We would like to emphasise that IBS is a heterogeneous disease. Even identical symptoms are likely caused by different processes.[5] Grouping IBS patients on the basis of stool pattern thus promotes heterogeneity, resulting in varying results with different cohorts. This aspect is also relevant when studying the role of TRP channels in visceral pain generation in IBS. Indeed, low-grade mucosal inflammation has been proposed as an important pathophysiological factor in IBS.[6] Researchers have since demonstrated a sensitizing effect of inflammatory mediators on various TRP channels, as will be discussed below. It is important to note that inflammation does not seem to be required to maintain visceral sensitization, as two recent clinical studies investigating the effects of mesalazine in IBS failed to demonstrate any benefits.[7,8] On the other hand, post-inflammatory sensitization can provide a theoretical explanation for IBS-like symptoms after gastro-enteritis, known as post-infectious IBS, and after achieving endoscopic and biochemical remission in inflammatory bowel disease (IBD). However, it would be inadequate to assume inflammation as the sole driving factor of visceral hypersensitivity. Shortcomings of our current knowledge on TRP channel sensitization should be recognized.

4 | SENSORY INNERVATION OF THE INTESTINE

Nociceptive signalling from the oesophagus to the proximal colon is conducted through the vagal nerve. Information from mid to distal colon and rectosigmoid is carried by the lumbar splanchnic and sacral pelvic nerves. The vagal nerve contains the peripheral terminals of pseudo-unipolar neurons with their cell bodies located in the nodose ganglia. Visceral sensory information from the vagal nerve supply is transduced into the solitary nuclei located in the medulla oblongata. The splanchnic and pelvic nerves contain axons of pseudo-unipolar neurons arising from a dorsal root ganglion (DRG). Peripheral sensory information from this supply is transduced into the dorsal horn of the spinal cord and ascends via the spinothalamic tract.[9] Peripheral nociceptive signalling is extensively modulated by the central nervous system, resulting in suppression or augmentation of the nociceptive input. These central processes determine whether nociceptive signalling (sensing and transmitting noxious stimuli) is perceived as pain (unpleasant experience).[10]

Sensory afferents of the vagal and spinal nerves have previously been divided in different subclasses.[11] Based on their sensitivity to mechanical stimuli, afferents were divided in mucosal, muscular, serosal and mesenteric fibre classes.[2] Mucosal afferents were defined as responsive to fine tactile and chemical stimuli, whereas serosal and mesenteric afferents respond to noxious mechanical stimuli.[1] Sensing of intermediate (physiological) distension was attributed to muscular afferents. An additional class specific to the pelvic pathway, the muscular-mucosal class, responds to tactile stimuli and

distension. It should be noted that evidence supporting the suspected anatomical distribution described above is currently lacking. Song et al have attempted to morphologically identify specialised afferent axonal structures in the guinea pig intestine.[12] They were able to demonstrate mechanosensitive fibres in the mesenteries and preparations of isolated mucosa/submucosa of the ileum and colon. However, no mechanosensitive fibres were observed in preparations of isolated muscle layers (with intact myenteric ganglia and serosa). Afferent functioning therefore appears to depend on molecular characteristics rather than the location within the gut wall.[1,13] Functional differences in nociceptor transducer molecules and their divergent expression along sensory afferents determine the physiological role of these afferents.[13] Understanding the functioning of individual TRP channels may provide further insights into nociceptive processes and sensitization mechanisms. Below we will discuss in detail the TRP channels that have been identified as key players in visceral nociception. These include TRPV1, TRPV4, TRPA1 and TRPM8. An overview of current data on these channels and their implications in IBS is provided in Table 1.

5 | TRP CHANNEL REGULATION

In order to understand TRP channel functioning, one must be aware of the complex molecular modulation that these channels are subjected to. Modulatory processes can either result in sensitization or desensitization of the respective afferent.[14] While desensitization prevents nociceptive signaling, sensitization of nociceptors enhances their discharge (ie potentiates the nociceptor response to a second stimulus).

Several mechanisms are involved in TRP channel regulation.[14] First, gene expression can be altered through DNA methylation, resulting in gene silencing. Second, posttranslational modifications (eg phosphorylation and dephosphorylation) affect channel functioning. Phosphorylation cascades can be initiated by various sensitizing agents (discussed below). Depending on the agent, different phosphorylation pathways are involved (eg protein kinase A, protein kinase C, calmodulin-dependent kinase).[15] In contrast, dephosphorylation reduces TRP channel sensitivity to stimuli. Finally, TRP channels can be degraded by movement to intracellular lysosomes,[16] or mobilized from intracellular pools to the cell membrane (translocation).[15] All of these processes are kept in balance in physiological conditions, but can be disrupted in disease. Currently, we are only beginning to understand the role of these modulatory processes in IBS. Considering our growing knowledge on TRP channels in visceral pain, future studies may focus on this unexplored field to guide treatment development.

6 | TRPV1

Of all TRP channels, TRPV1 has been studied most extensively. Studies investigating the expression patterns of TRPV1 in mice have demonstrated the channel's presence along the entire gastrointestinal tract.[17] Although human studies are more scarce, the expression of TRPV1 in the oesophagus and colon is now well documented, and the channel is suspected to be present in the human small intestine as well.[18] Immunostaining of human colon biopsies has demonstrated TRPV1-positive fibres throughout the mucosa, with a particular abundance in the submucosal plexus.[19,20] Activation of these fibres by noxious stimuli results in action potential generation and pain sensation. TRPV1 is activated by noxious heat (>42°C), protons (pH <6) and the vanilloid capsaicin, the pungent principle in hot peppers.[20] In addition, several compounds have been identified as endogenous agonists. These include inflammatory mediators such as lipoxygenase products and prostaglandins, and endocannabinoids such as anandamide (Table 2). Furthermore, TRPV1 seems to be involved in afferent signalling of mechanical stimuli,[3] but its exact mechanism is still poorly understood. Whether the mechanosensory properties of TRPV1 are related to indirect effects on neuronal excitability or interactions with other TRP channels, remains to be established.[21]

The use of potent chemical activators such as capsaicin has provided valuable information on the functioning of TRPV1-expressing afferents. Upon activation, in addition to the generation of an action potential, these sensory afferents release pro-inflammatory sensory neuropeptides; calcitonin-gene related peptide (CGRP) produces local vasodilation and substance P (SP) increases venular and capillary permeability leading to plasma protein extravasation and oedema formation, collectively referred to as neurogenic inflammation.[22] TRPV1-expressing sensory neurons can therefore influence GI vascular, immune and smooth muscle function, as well as sensitize surrounding nociceptors.[3] Under physiological conditions, these effects are counteracted by the anti-inflammatory effects of somatostatin, which has also been shown to be released by capsaicin-sensitive afferents.[23] Sustained disruptions in the balance of these pro- and anti-inflammatory neuropeptides may result in the pathological sensitization of nociceptive afferents, as well as local tissue inflammation (Figure 1). Importantly, these processes do not seem to be limited to TRPV1, but also apply to the other TRP channels discussed in this review.

Sensitization of TRPV1-expressing afferents has been demonstrated in IBS patients by increased perceptive responses to capsaicin in multiple studies.[20,24,25] Gonlachanvit et al demonstrated that diarrhoea predominant IBS (IBS-D) patients experience greater abdominal burning after a single ingestion of a spicy meal or standard meal in combination with a capsaicin capsule, compared to healthy controls.[24] As symptoms developed within one hour after ingestion, proximal gut hypersensitivity to capsaicin was suggested to exist in these patients. Schmulson et al showed a significantly decreased rectal pain threshold in IBS patients after a 7-day chilli rich diet compared to a diet without chilli, suggesting TRPV1-induced visceral hyperalgesia.[25] More recently, van Wanrooij et al studied the effects of rectal capsaicin application in IBS patients.[20] Patients reported increased pain intensity, a similar effect lacking in healthy volunteers. Furthermore, the pain response appeared to be

TABLE 1 Data on TRP channels in IBS

Channel	Implications in IBS	Study type	Reference
TRPV1	Sensitized and/or upregulated in colonic tissue samples of IBS patients, resulting in enhanced capsaicin sensitivity.	Human studies	19, 20, 26-28
	Sensitized by inflammatory mediators.	Animal study	32
		Combined study	34
	Expression profiles regulated epigenetically, likely influenced by psychological stress through glucocorticoids and/or catecholamines.	Human study	27
		Animal studies	36, 37
	Indirectly involved in mechanosensation.	Animal studies	21, 48
TRPV4	Elevated levels of endogenous agonist 5,6-EET in the supernatant in of colonic biopsies from IBS-D patients.	Human study	31
	Sensitized by inflammatory mediators.	Animal studies	42-44
	Putative direct mechanosensitive nociceptor.	Human study	41
TRPA1	Functional coupling with TRPV1.	Animal study	52
	Activated by hydrogen sulphide, of which higher amounts are present in IBS-D patients with small intestinal bacterial overgrowth.	Human study	57
	Sensitized by inflammatory mediators.	Animal studies	53, 54
	Likely to act as a directly mechanosensitive nociceptor in hyperalgesia.	Animal studies	48, 49
		Human study	50
TRPM8	Inhibits chemo- and mechanosensory responses of TRPA1 and TRPV1.	Animal study	61
	Potentially protective against nociceptor sensitization through anti-inflammatory effects.	Animal study	62
	TRPM8 polymorphisms are associated with slower colonic transit and an increased risk of IBS-C and IBS-M.	Human study	58

TABLE 2 Identified agonists and physical stimuli of TRP channels discussed in this paper[16,55,56,110-112]

Channel	Exogenous agonists	Physical stimuli	Endogenous agonists
TRPV1	Capsaicin (red pepper), polygodial (mountain pepper), piperine (black pepper), gingerol (ginger), olvanil, resiniferatoxin, camphor, diphenylboronic anhydride, double-knot toxin (DkTx), vanillotoxin (tarantula toxin), phenylacetylrinvanil, 2-aminoethoxydiphenyl borate (2-APB), evodiamine, cannabidiol, cannabigerol	(Thermal >42°C)	Acid (pH <6), lipoxygenase products (eg 12-(S)-hydroperoxyeicosatetraenoic acid (12S-HPETE), 15-(S)-hydroperoxyeicosatetraenoic acid (15S-HPETE), leukotriene B4 (LTB4), 5-(S)-hydroxyeicosatetraenoic acid (5S-HETE)), reactive oxygen species (ROS), adenosine, ATP, lysophosphatidic acid, polyamines (eg spermine, spermidine, and putrescine) and conjugates of biogenic amines (eg N-arachidonylethanolamine (anandamide), N-arachidonoyldopamine (NADA), N-oleoyldopamine, N-oleoylethanolamine (OLEA), N-arachidonolylserine, N-hexadecanamide, and various N-acyltaurines and N-acylsalsolinols)
TRPV4	Bisandrographolide A (BAA), alpha-phorbol 12,13-didecanoate (4α-PDD), phorbol 12-myristate 13-acetate (4α-PDH), apigenin, GSK1016790A and RN1747	Mechanical and thermal (>24°C)	Citric acid, dimethylallyl pyrophosphate, anandamide, arachidonic acids and epoxyeicosatrienoic acid metabolites (eg 5,6-epoxyeicosatrienoic acid (5,6-EET) and 8,9-EET, which also mediate TRPV4 activation by cell swelling)
TRPA1	Allyl isothiocyanate (AITC), cinnamaldehyde (cinnamon), allicin (garlic), carvacrol and thymol (oregano), curcumin (turmeric), capsiate (capsinoid), acrolein, menthol, icilin, nicotine, URB597, chlorobenzylidene malononitrile (tear gas), formalin, α, β-unsaturated aldehydes, auranofin, PF-4840154, cannabichromene, cannabidiol (CBD), tetrahydrocannabinol (THC) and apomorphine (agonist in low micromolar range and antagonist in higher concentration)	Mechanical and thermal (<15°C and >25°C)	Prostaglandins (eg prostaglandin A1, 8-iso-prostaglandin A2, and 15-deoxy-Δ-prostaglandin J2), 4-Hydroxnonenal (4-HNE, a lipid peroxidation product), 4-oxononenal (4-ONE), methylglyoxal, reactive oxygen species (ROS), cytokines (eg TNF-α and IL-6), bradykinin and hydrogen sulphide
TRPM8	Menthol, icilin, linalool, geraniol, WS-3, WS-12, WS-23, PMD38, hydroxycitronellal, FrescolatMGA, FrescolatML, CoolactP, Cooling agent 10, cis- and trans-p-menthane3 and CPS-36	Thermal (<28°C)	Unknown

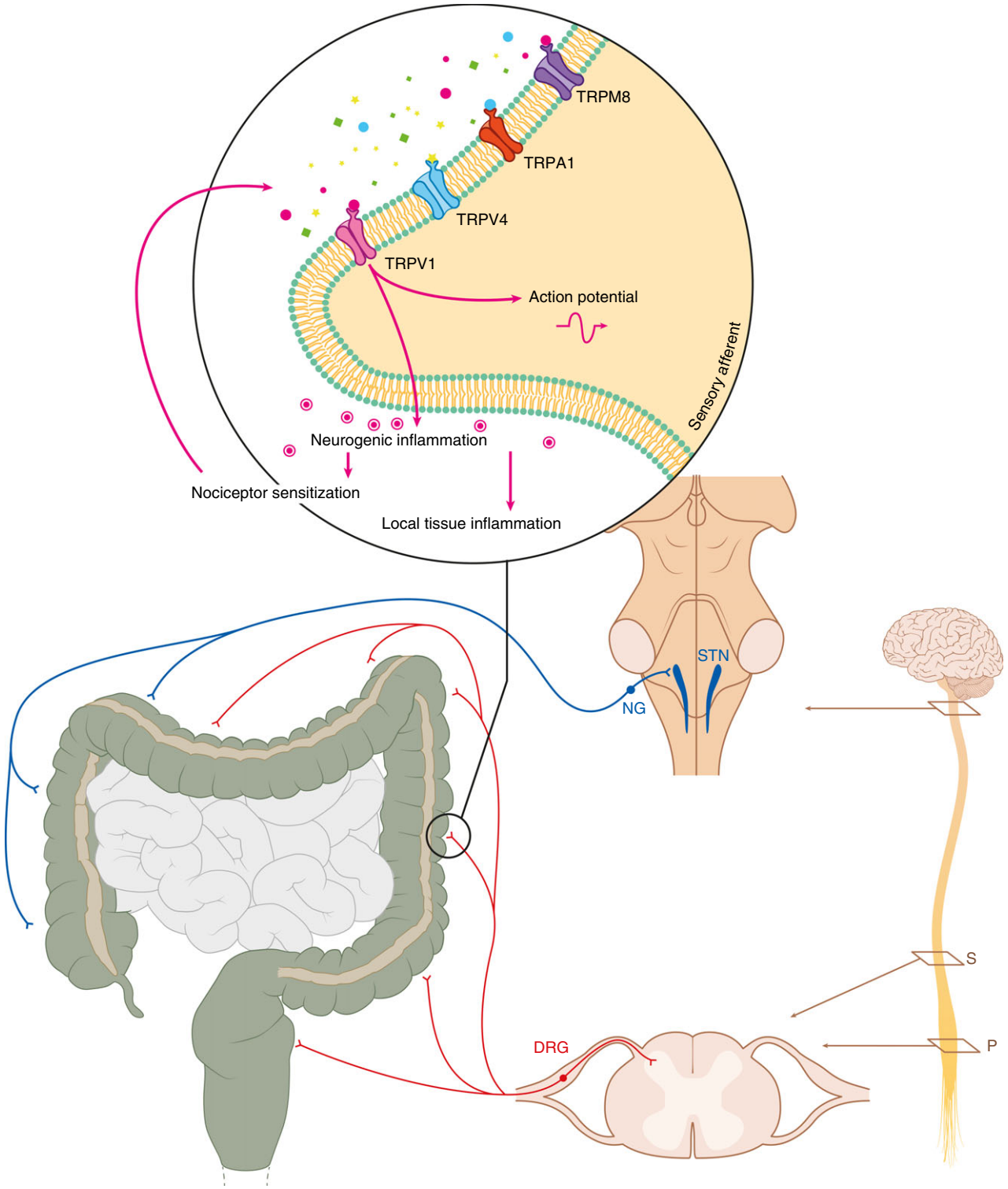

FIGURE 1 Schematic depiction of nociceptive afferent innervation of the intestine. Proximal (blue) neurons travel through the vagal nerve. These neurons transduce sensory information through the nodose ganglia into the solitary tract nuclei. Distal (red) neurons travel through the splanchnic and pelvic nerves (two distinct systems). Both the splanchnic and pelvic nerves' somata reside in the dorsal root ganglia. In addition, splanchnic nerves travel through prevertebral ganglia (not shown). The nociceptive afferents within these neurons presumably have their nerve endings (top inset) in the mucosa/submucosa and mesenteries.[12] Various stimuli (shown at the top) can activate the nociceptors, depending on the expressed TRP channels (Table 2). Stimuli include exogenous agonists (eg capsaicin or menthol), physical stimuli (eg mechanical or thermal) and endogenous agonists (eg prostaglandins or lipoxygenase products). Activation of nociceptors by these stimuli results in action potential generation and pain sensation. In addition, inflammatory mediators are released (neurogenic inflammation), which can result in TRP channel sensitization and local tissue inflammation. NG = nodose ganglion, STN = solitary tract nucleus, DRG = dorsal root ganglion, S = splanchnic nerves, P = pelvic nerves

independent of anticipatory anxiety, suggesting a direct capsaicin effect on nociceptive mucosal afferents.

Mechanisms underlying the increased capsaicin sensitivity in IBS patients have been studied extensively. Akbar et al demonstrated upregulation in sigmoid mucosal samples of IBS patients.[19] This increase correlated with symptom severity, suggesting that an increase in afferent discharge through TRPV1 activation might be directly related to pain symptom generation. Our earlier study corroborated these findings, demonstrating increased transcription of TRPV1 in IBS patients, which also strongly correlated with symptom severity.[26] More recently, two studies confirmed the augmented expression of TRPV1 in colonic biopsies of IBS-D patients.[27,28] It should be noted that the overall density of innervation has been shown to be increased in IBS patients. Increased TRPV1 sensitivity may therefore be due to axonal sprouting rather than isolated TRPV1 upregulation, possibly as a result of increased nerve growth factor expression.[28,29] On the other hand, van Wanrooij et al were unable to objectify increased numbers of mucosal TRPV1 in colonic biopsies of IBS patients, as compared to healthy controls.[20] Even when the IBS patient group with visceral hypersensitivity (defined by decreased discomfort threshold during rectal distension) was analysed separately, no significant upregulation of TRPV1 was found. The question whether increased capsaicin responsiveness in IBS relates to individual TRPV1 sensitivity or TRPV1 expression thus remains without a decisive answer.

6.1 | TRPV1 functioning in (post-)inflammatory conditions

As discussed above, the mediators of neurogenic inflammation are known to sensitize TRPV1. In addition, systemic inflammatory mediators have been shown to be involved in both sensitization and activation of TRPV1 (Table 2).[30] Their potential relevance to IBS pathophysiology is evident, as many have postulated a role for subclinical inflammation in IBS.[5] However, inflammation has been argued to be within the physiological range in IBS.[6] Moreover, a study measuring poly-unsaturated fatty acids in colon biopsy material from IBS-D patients and healthy controls, failed to demonstrate differences in concentrations of lipoxygenase products (TRPV1 agonists).[31] It is possible that the role of inflammatory mediators in TRP channel sensitization is limited to post-inflammatory hyperalgesia, as encountered in post-infectious IBS and IBD in remission. Animal studies have provided evidence for TRPV1 mediated post-inflammatory hyperalgesia, using experimental colitis models induced by dextran sodium sulphate. After recovery from colitis, TRPV1 deficient mice showed no pain-related behavioural responses or increased visceromotor responses to colorectal distension, whereas these responses were readily observed in wildtype mice.[32]

Another explanation for inflammation mediated hyperalgesia in IBS could be related to histamine. Barbara et al observed increased numbers of mucosal mast cells in close proximity to sensory nerves in colon biopsies of IBS patients,[33] and these findings have been confirmed in a more recent study.[28] Moreover, Wouters et al demonstrated an increased Ca^{2+} response and increased number of responding neurons to capsaicin in histamine pre-treated biopsy specimens of healthy volunteers.[34] Immunostaining showed co-expression of histamine receptor H1 (HRH1) and TRPV1 on submucosal neurons in both IBS patients and healthy controls. A functional coupling of these receptors therefore appears likely. In a proof-of-concept trial, the same study group demonstrated a significant decrease in abdominal pain scores in IBS patients after 12 weeks of treatment with the HRH1 antagonist ebastine, as compared to placebo. Unfortunately, not all patients reported pain relief, emphasising the heterogeneity of the IBS patient population.

6.2 | Chronic stress and epigenetics

IBS is often described to be a disorder of the brain-gut axis. In this model, psychological stress is generally accepted as a key factor influencing GI symptoms and vice versa. Importantly, animal models have suggested both glucocorticoid- and catecholamine-mediated TRPV1 upregulation.[35,36] In addition, epigenetic mechanisms may regulate the effects of chronic stress on TRPV1 expression. Increased histone acetylation of the TRPV1 promoter has been demonstrated in chronic stress models in rats, resulting in TRPV1 upregulation in DRG derived neurons.[37] Furthermore, the epigenetics of visceral pain perception have been investigated in diarrhoea predominant IBS patients.[27] Two miRNAs known to decrease TRPV1 expression, miR-199a and miR-199b, were found to be significantly downregulated and shown to correlate with pain scores. Taken together, these results indicate epigenetic alterations, possibly under the influence of psychological stress, modulate TRPV1 functioning in IBS.

6.3 | Activation of sensitized nociceptors

Currently identified endogenous agonists of TRPV1, as well as the other TRP channels discussed in this review, are primarily related to inflammation (Table 2). As already noted, (subclinical) inflammation is not the sole underlying mechanism in IBS. Furthermore, it is unknown whether the concentrations of endocannabinoids known to activate TRPV1 in vitro are high enough in vivo in order to achieve activation.[38] This constitutes a significant gap in our knowledge of peripheral nociception in IBS, as it remains unclear what stimuli ultimately activate sensitized nociceptors in vivo. Although capsaicin is a common dietary constituent, it is unlikely to be a major factor in abdominal pain generation in IBS. Current understanding of intestinal signalling suggests that nociceptive signals are generated by exciting sensitized nociceptors as a result of mechanical stimulation or distension. These mechanical stimuli could be related to physiological motor responses of the intestine.[6] High amplitude colonic contractions have been shown to be of a magnitude above nociceptive thresholds in visceral hypersensitivity. Therefore, mechanical stimuli generated by the gut itself may be responsible for the generation of pain symptoms through sensitized nociceptors.

7 | TRPV4

Studies investigating expression patterns of transient receptor potential vanilloid 4 (TRPV4) in the human colon have demonstrated immunoreactivity in the submucosa and serosa.[39] Initially termed vanilloid receptor-related osmotically activated channel (VR-OAC), this channel has been implicated in the detection of osmolarity changes.[40] In addition, TRPV4 is now known to sense strong acidosis, temperatures >24°C and, among others, the synthetic phorbol ester alpha-phorbol 12,13-didecanoate (4αPDD) (Table 2). Currently identified endogenous agonists include anandamide and the poly-unsaturated fatty acid metabolites 5,6-epoxyeicosatrienoic acid (5,6-EET) and (8,9-EET).

Accumulating evidence points toward a role of TRPV4 in mechanosensation.[3,41] Under basal conditions, TRPV4 is thought to primarily sense high threshold mechanical stimuli.[42] Comparing TRPV4 knockout and wildtype mice, responses to noxious distension pressures were diminished in TRPV4 knockouts. In contrast, responses did not differ at innocuous pressures. TRPV4 is however considered to play a major role in visceral hypersensitivity.[42,43] Intracolonic administration of 4αPDD has been shown to induce hyperalgesia in mice.[42] In IBS, several pathways have been proposed to result in visceral hypersensitivity through TRPV4 sensitization. The effects on TRPV4 of known mediators of visceral hypersensitivity, serotonin and histamine, were investigated in one study.[44] Serotonin and histamine administration was demonstrated to result in potentiated TRPV4 responses to 4αPDD. The same research group postulated a role for proteases in mediating visceral hypersensitivity.[45] Subsequent studies have supported this theory.[42,43] Activation of protease-activated receptor (PAR_2), a channel that is also co-expressed with TRPV4 in afferents innervating the colon, resulted in visceral hyperalgesia in wildtype mice. Because hyperalgesia was lacking in TRPV4 knockout mice, this channel was suspected to be the downstream effector of PAR_2 mediated visceral hypersensitivity.[42]

Human studies on visceral TRPV4 functioning are currently limited. In one study researchers acquired supernatant of colonic biopsies from IBS-D patients.[31] Intracolonic administration of the supernatant resulted in visceral hypersensitivity in mice. This was concluded to be TRPV4 mediated, as injection of TRPV4 targeted small interfering RNA prevented the effect. Subsequently, potential TRPV4 agonists in the supernatant were quantified. The concentration of 5,6-EET was found to be significantly elevated and correlated with patients' abdominal pain severity and frequency. Interestingly, 5,6-EET production was linked to PAR_2 activation, as PAR_2 agonist peptide induced 5,6-EET synthesis in sensory neurons. The authors therefore suggested 5,6-EET to be an endogenous TRPV4 agonist with a major role in visceral hypersensitivity in IBS-D patients. Again, the heterogeneity of IBS should be emphasised as the above solely relates to IBS-D patients. No significant differences in poly-unsaturated fatty acid metabolite concentrations in supernatants have been observed in constipation predominant IBS (IBS-C) patients, mixed bowel habit IBS (IBS-M) patients and healthy volunteers.

8 | TRPA1

To date, our knowledge on transient receptor potential ankyrin 1 (TRPA1) functions in visceral nociception is mostly limited to animal models. TRPA1 is thought to primarily act as a chemosensor, responding to various irritants and spices, among others cinnamaldehyde (cinnamon) and allyl isothiocyanate (AITC), the pungent compound in mustard oil, horseradish and wasabi.[3] Currently identified endogenous agonists include prostaglandins and products of oxidative stress (Table 2). Furthermore, TRPA1 has been implicated in temperature sensation, although its responsiveness has long been debated. Recently, a U-shaped temperature-activation curve was demonstrated using human TRPA1 in lipid bilayer and whole-cell patch-clamp recordings.[46] This study group observed TRPA1 activation in temperatures below 15°C, as well as temperatures above 25°, but little activation to temperatures in between.

In addition to the above, TRPA1 has been studied intensively for its suspected mechanosensitive properties. It appears to have no role in sensing high pressure distension under basal conditions.[47] However, TRPA1 has been demonstrated to be an important mediator of visceral hyperalgesia.[48,49] Several animal studies have indicated that the mechanical stimulus threshold can be decreased upon chemical activation with mustard oil. These findings have recently been confirmed in an ex vivo study using human colonic tissue.[50] Moreover, TRPA1 can be sensitized via PAR_2 activation. Similar to TRPV4, TRPA1 may therefore be one of the effectors of protease mediated visceral hypersensitivity.

Several characteristics of TRPA1 add to the complexity of its functioning. TRPA1 is almost exclusively expressed in TRPV1-positive neurons. Both channels have been shown to interact with each-other.[51] Similar to TRPV1, TRPA1 can be desensitized upon repeated stimulation.[52] In addition, TRPA1 activation can be reduced upon repeated capsaicin application, a process referred to as cross-desensitization.[52] Indeed, Brierley et al showed that TRPA1 knockout mice were responsive to capsaicin, but lacked mechanical desensitization that normally follows afterwards.[48] Thus, whereas TRPV1 is responsible for the direct response to capsaicin, the subsequently reduced mechanosensory function appears to be TRPA1-mediated. These results are in line with the general belief that TRPV1 itself is not directly mechanically gated,[21] as the reduced response to mechanical stimuli upon chemical desensitization of TRPV1 relies on TRPA1.

8.1 | TRPA1 functioning in (post-)inflammatory conditions

TRPA1 expression has previously been investigated in inflamed human colonic tissue, showing upregulation in IBD patients with active inflammation.[51] Evidence explaining the role of TRPA1 in inflammation however remains contradictory, with reports of both pro- and anti-inflammatory effects. Although its effect on inflammation is enigmatic, TRPA1 itself is undoubtedly affected by inflammatory mediators. Indeed, the endogenous agonists of TRPA1 are

related to inflammation (Table 2). Furthermore, studies investigating the effects of chemically induced colitis in mice demonstrated sensitization of visceral afferents to mechanical stimuli. These effects were observed in wildtype mice, but not in TRPA1 deficient mice.[47,53,54]

Since TRPV1, TRPV4 and TRPA1 have all been shown to be involved in inflammation induced visceral hyperalgesia, one could assume that combined sensitization of these channels provides a particularly potent mechanism to induce hypersensitivity. Synergistic effects of TRPV1 and TRPA1 inhibition have indeed been demonstrated in the attenuation of colorectal distension-associated pain behaviour at high pressures in rats.[54] Unfortunately, this concept has not yet been proven in IBS. Cenac et al have measured endogenous agonists of TRPA1, TRPV1 and TRPV4 (primarily inflammatory mediators) in the supernatant of colon biopsy material of IBS patients.[31] They demonstrated elevated levels of endogenous agonists of TRPV4, but not TRPA1 or TRPV1. Another study involved peripheral blood mononuclear cell supernatants from IBS-D patients.[55] The supernatants were shown to induce mechanical hypersensitivity in vitro in colonic afferent neurons. Cytokine concentrations in the supernatants were subsequently measured, showing elevated levels of TNF-α, soluble IL-2, IL-6, IL-10, IL-1β and the chemokines CCL3 and CCL4. Combined with the expression profiles of the receptors of these signalling molecules in colonic nerves, the authors proposed TNF-α, IL-6, IL-10 and IL-1β as possible mediators of mechanical hypersensitivity in IBS-D. The mechanism of action of TNF-α was demonstrated to be TRPA1 dependent, as its sensitizing effect was abolished in the presence of a TRPA1 antagonist. In contrast, the selective inhibition of TRPV1 using low doses of capsazepine had no effects on mechanical hypersensitivity induced by TNF-α. In addition, a more recent study by the same group demonstrated IL-6 mediated mechanical hypersensitivity to be TRPA1 dependent as well.[56] Whether an interaction of sensitized TRP channels plays an important role in IBS thus remains to be elucidated.

Future studies should include different approaches covering the diversity of potential pathophysiological mechanisms in IBS. For example, elevated levels of hydrogen sulphide, an endogenous TRPA1 agonist, were recently demonstrated in IBS-D patients with small intestinal bacterial overgrowth.[57] These findings demonstrate that mechanisms of TRP channel sensitization may vary among patients.

9 | TRPM8

Transient receptor potential melastatin 8 (TRPM8) appears to be one of the least studied TRP channels in humans. Only very recently TRPM8 polymorphisms have been demonstrated to be associated with slower colonic transit and an increased risk of IBS-C and IBS-M in humans.[58] However, data on TRPM8 functioning is mainly based on animal studies, limiting our understanding of its role in visceral pain generation. Our current knowledge of TRPM8 is that it has a role in thermosensation (primarily low temperatures).[59] Several chemical compounds are able to activate TRPM8, among others menthol and icilin. Many will acknowledge that the sensation of mentholated liniments is difficult to describe. Cold and burning perceptions alternate upon application. Because of these opposing sensory inputs, menthol is thought to also activate channels other than TRPM8. Indeed, some authors have pointed to menthol-induced TRPA1 activation in order to explain the diverse psychophysical sensations after topical application of menthol.[60] Moreover, coupling of TRPM8 to both TRPV1 and TRPA1 has been demonstrated previously.[61] As mentioned above, AITC is able to cause mechanical hypersensitivity through TRPA1 activation. This effect, however, does not occur after pre-treatment with icilin. In contrast, icilin-induced mechanical desensitization is absent in TRPA1 deficient mice, indicating that the effect was TRPA1 mediated. Likewise, capsaicin is able to cause mechanical desensitization, but not after icilin pre-treatment.[61] TRPM8 is therefore thought to inhibit chemo- and mechanosensory responses of TRPA1 and TRPV1, and thus provide antinociceptive effects through cross-desensitization.

In addition, visceral TRPM8 is thought to have a role in inflammation. Human studies have revealed increased TRPM8 expression in colonic biopsy material from IBD patients as compared to healthy controls.[62] Several experimental colitis models in mice have suggested protective effects of TRPM8 activation. In these studies, icilin treatment significantly attenuated induced colitis in wildtype mice, but not in TRPM8 deficient ones.[62-64] The protective effects of TRPM8 activation have been linked to CGRP, which co-localizes with TRPM8 in the human colon.[64] Although the pro-inflammatory effects of CGRP in neurogenic inflammation are evident, primarily consisting of vasodilation, anti-inflammatory effects of the neuropeptide have been observed as well.[65,66] De Jong et al demonstrated expression of the components of the CGRP receptor, calcitonin receptor-like receptor (CLR) and receptor activity modifying protein-1 (RAMP1), on CD11c+ dendritic cells in the murine spleen.[64] CGRP knockout mice were shown to have higher levels of pro-inflammatory cytokines (including TNF-α and IL-6) in chemically induced acute colitis, as compared to wildtype mice. Moreover, CD11c+ dendritic cells were found to be co-localized with CGRP positive fibres in the murine colon. CGRP was therefore thought to exert a protective role in colitis via inhibition of the release of pro-inflammatory cytokines, through interaction with local dendritic cells. Indeed, TRPM8 deficient mice showed significant improvement in disease activity after treatment with recombinant CGRP, whereas no effect of treatment was observed in wildtype mice with induced colitis. Paradoxically, enhanced CGRP expression levels have been observed in mucosal fibres of TRPM8 deficient mice.[62] This discrepancy has yet to be clarified, although it is likely that a disrupted colonic CGRP release prevents the neuropeptide from reaching its effector. Taken together, these results suggest that TRPM8 upregulation is a protective mechanism aimed at mitigating tissue inflammation. Therefore, TRPM8 could theoretically protect against nociceptor sensitization by inflammatory mediators.

10 | NON-NEURONAL TRP CHANNEL EXPRESSION

As already mentioned, TRP channels are expressed by a multitude of cell types including those of non-neuronal origin (both intestinal and extra-intestinal).[67] Examples of non-neuronal cells expressing TRP channels include vascular smooth muscle cells, endothelial cells, keratinocytes and intestinal epithelial cells. The possible involvement of the latter in IBS pathophysiology should not be overlooked. Increased intestinal permeability has been demonstrated in IBS-D patients,[68] and has been associated with visceral hypersensitivity.[69] In two studies, TRPV4 activation with 5,6-EET and 4αPDD resulted in increased intestinal permeability.[70,71] One of the proposed mechanisms was via downregulation of tight junction proteins. However, conflicting results have been obtained for the role of TRPV1 in regulating intestinal permeability. Capsaicin has previously been shown to increase permeability. In contrast, the endocannabinoid-like compound oleoylethanolamine has been shown to be able to both increase and decrease intestinal permeability via TRPV1.[72] Similarly, the role of TRPA1 in the regulation of intestinal permeability remains controversial. Fothergil et al demonstrated decreased trans-mucosal resistance in colon tissue from mice after AITC and cinnamaldehyde administration.[73] We demonstrated no effects on small intestinal permeability with the administration of cinnamaldehyde in twelve healthy controls.[74] It therefore remains unclear to what extent TRP channels contribute to IBS pathophysiology via altering intestinal permeability.

11 | MOTILITY EFFECTS OF TRP CHANNEL ACTIVATION

Although we here focus on the role in visceral nociception, it should be noted that TRP channels are known to affect gut motor function as well.[21] The effects of TRP channel activation on motility are not only channel dependent, but also location dependent. For example, TRPV1-positive fibres located in the lower oesophageal sphincter that are exposed to gastric acid cause a local inhibitory reflex, lowering the intraluminal pressure.[75] On the other hand, application of capsaicin in the distal colon and rectum in mice has been shown to cause fast transient colonic contractions followed by a delayed sustained contraction.[76] These results indicate that different effector pathways are involved depending on the location. Suggested effectors are the tachykinin receptors (mainly NK1 and NK2), which respond to neuropeptides released upon TRP channel activation, as discussed above.[77] Indeed, the contractility lowering effect in the oesophagus was demonstrated to be NK1 dependent via local substance P release, whereas NK2 activation was shown to be responsible for long lasting contractility in the distal colon.[75,76] Additional pathways are likely to be involved however, as fast transient colonic contractions were shown to be inhibited by NK1 antagonists. Other TRP channels have also been implicated in motility. Whereas TRPV4 has previously been demonstrated to inhibit colonic motility in mice via reduced NO-dependent calcium release, TRPA1 has been implicated in both reduced and increased motor activity.[39,78,79] Taken together, the effects on gut motility emphasise the involvement of TRP channels in IBS pathophysiology, as they may account for both the altered defecation pattern as well as pain symptoms encountered in the syndrome.

12 | CLINICAL IMPLICATIONS

Although all of the TRP channels discussed above are suggested to have a role in visceral pain generation, only two (TRPV1 and TRPM8) have been implied in the treatment of IBS. Generally, two strategies are exploited in blocking TRP channels. One is the direct inhibition by administration of antagonists, the other one by repeated stimulation in order to desensitize the channel and its respective nerve terminal. In addition, several alternative techniques for TRP channel targeted therapy have been developed, that will be discussed hereafter. A summary of the optional therapeutic strategies related to each TRP channel is provided in Table 3.

12.1 | TRP antagonists

The discovery of TRPV1 as a key player in nociception led to the development of TRPV1 antagonists as novel therapeutics in pain control. However, investigators soon encountered a major hurdle, as the first compounds interfered with thermoregulation. Several first-generation compounds were stranded in pre-clinical trials as they elicited marked hyperthermia.[80,81] Moreover, interspecies differences in TRPV1 functioning further complicated research. While one TRPV1 antagonist appeared to be safe in animals, its first human clinical trial was prematurely halted as hyperthermia was observed in three out of four patients.[82] Interestingly, while TRPV1 has been identified as a thermosensor, its temperature threshold is well above physiological body temperature. It is possible that adverse effects are not TRPV1 related and represent an off-target effect. Additionally, classical TRPV1 antagonists impair the noxious heat sensation and may therefore increase burn risk. Together, these results disfavoured the development of non-selective TRPV1 antagonists. Therefore, new strategies are being explored in order to tackle thermoregulation related adverse events in TRPV1 targeted therapy. Several second generation modality specific antagonists have been developed, as it has been observed that hyperthermia is less severe with compounds that are full antagonists for capsaicin, but not for protons.[83] Two recent phase-I trials reported no side effects of two modality-selective TRPV1 antagonists.[84,85] Their potency as analgesics however remains to be established in future studies.

12.2 | Desensitization

The desensitizing properties of TRPV1 agonists such as capsaicin have been exploited for many years by topical preparations in the treatment of neuropathic pain.[86]

TABLE 3 Summary of therapeutic strategies in relation to the TRP channels discussed in this paper

	TRPV1	TRPV4	TRPA1
Antagonists	Potent analgesics, but thermoregulation interference with first generation compounds[80-82] Modality-selective antagonists currently being developed[108]	Reduction of human nociceptor mechanosensitivity ex vivo[41]	Tested in neuropathies with good safety profiles[105] Several antagonists currently evaluated in clinical trials[105]
Desensitization	Six wk of chilli treatment effective in IBS-D patients, but short-term adverse effects may limit adherence[87]	Evidence currently lacking	Repeated TRPA1 stimulation results in desensitization[52]
Cross-desensitization	Inhibition of chemo- and mechanosensory responses with peppermint oil[a]	Inhibition of chemo- and mechanosensory responses with peppermint oil[a]	Reduced mechanosensitivity through repeated capsaicin administration[52]
Downstream targets	NK1 antagonists may provide anti-hyperalgesic effects[96] Improved abdominal pain and stool pattern in female IBS-D patients with NK2 antagonist ibodutant[94] Somatostatin analogues may provide anti-hyperalgesic effects[98-101] Improved abdominal pain scores in IBS patients with HRH1 antagonist ebastine[34]	Evidence currently lacking	Evidence currently lacking
RNA-based therapy	Plausible[102]	Plausible[102]	Plausible[102]

[a]Effects mediated by TRPM8 activation[61]

At this moment, only one small randomized crossover study investigated the effects of six weeks of chilli treatment in IBS-D patients.[87] At the end of treatment, patients reported significantly decreased post-prandial abdominal burning sensations. Similar positive effects have been demonstrated in epigastric pain in patients with functional dyspepsia.[88] An important drawback of capsaicin therapy however, is its short-term aggravating effects, possibly limiting adherence in the clinical setting. Therefore, more studies are needed to evaluate efficacy and feasibility in a larger IBS population. In addition, it remains to be established at which dose, frequency and length of administration repeated capsaicin is able to, if all, induce desensitization of TRPV1-positive nerve endings in the gut.

Ultra-rapid desensitization using potent agonists offer a theoretical background for achieving a fast analgesic response. One of these ultra-potent TRPV1 agonists is resiniferatoxin, which causes sustained calcium influx, resulting in a cytotoxic intracellular free calcium concentration and consequent axonal damage of TRPV1-positive neurons.[89] Unfortunately, its potency also poses challenges. Similar to first generation TRPV1 antagonists, resiniferatoxin increases the heat pain threshold. Moreover, high or repeated systemic doses of resiniferatoxin induce long-lasting damage to TRPV1-positive neurons,[90] rendering it not suitable for therapeutic applications in IBS.

12.3 | Cross-desensitization

Another mechanism aimed at analgesia is related to TRPV1 channel cross-desensitization. As mentioned above, TRPM8 activation is thought to provide antinociceptive effects through subsequent inhibition of TRPV1 and TRPA1. Peppermint oil, containing the TRPM8 agonist menthol, exploits these beneficial effects and is registered for the use in IBS in several countries.[91] It should be noted that the exact mechanism of action remains to be elucidated. Moreover, its effects appear to reach beyond TRP channel signalling as calcium channel mediated smooth muscle relaxation has been observed in vitro in human colon tissue without the involvement of TRPM8.[92]

Multiple small trials have evaluated the efficacy of peppermint oil in IBS. Side effects of this herbal therapy are rather mild, with heartburn being the most common.[91] One meta-analysis reported that 75% of IBS patients experience improvement in abdominal pain compared with only 27% in patients receiving placebo.[91] In a more recent trial, patients received either a sustained release peppermint oil preparation ensuring drug release in the small intestine, or a placebo. Patients receiving peppermint oil reported a significantly greater reduction in abdominal pain or discomfort compared with patients receiving placebo after a treatment duration of 28 days.[93] New preparations have been developed recently ensuring peppermint oil release in the colon, trials are ongoing and data on the efficacy of these new formulations will be reported in the near future.

12.4 | Downstream targets of therapy

Drugs that target molecules downstream of TRPV1 provide an attractive pharmacological alternative to direct TRPV1 inhibition. As TRPV1 activation is accompanied by the release of sensory neuropeptides inducing neurogenic inflammation and sensitization of surrounding nociceptors, it makes sense to target mediators (or their respective receptors) of this process. Indeed, antagonists of the tachykinin NK1 and NK2 receptors of substance P and neurokinin A respectively, are being developed for various purposes, among others

for symptom relief in IBS. For example, ibodutant, a neurokinin-2 receptor antagonist, has been shown to improve abdominal pain and stool pattern in female IBS-D patients.[94] Moreover, neurokinin-1 receptor antagonists such as aprepitant are currently being used as anti-emetics and have been suggested as analgesics as well.[95,96] Unfortunately, several pre-clinical studies demonstrated that NK1 antagonists lacked analgesic effects.[97] It should be noted that animal models studying the effects of NK1 antagonists simulated somatic pain, whereas the NK1 receptors are more relevant in visceral pain.[96] Moreover, NK1 antagonists are thought to function as anti-hyperalgesic agents rather than analgesics as they do not affect baseline nociception, but attenuate nociceptive responses sensitized through inflammation.[97] Although at first sight this does not appear favourable, inhibiting the sensitized state rather than the normal resting state may actually make NK1 antagonists excellent candidates for treating visceral pain. The most important advantage would be that physiological functions may remain unaltered. In fact, similar mechanisms likely explain the beneficial effects of the HRH1 antagonist ebastine in IBS patients, which has been discussed above.[34] As TRPV1 sensitization after histamine pre-treatment has been shown to be HRH1 mediated, the effects of ebastine on abdominal pain are thought to be related to the inhibition of the sensitized state of TRPV1.

One additional mediator of neurogenic inflammation has been implicated as a potential target in the development of analgesic therapies. Somatostatin, originating from capsaicin-sensitive sensory afferents, counteracts pro-inflammatory neuropeptides CGRP and substance P. Indeed, the potent analgesic effects of somatostatin have long been recognized.[98] Early studies using the somatostatin analogue octreotide have demonstrated increased thresholds of visceral sensory perception in IBS patients.[99,100] Moreover, anti-hyperalgesic effects of selective agonists of somatostatin receptor 1 and 2 have been reported in induced visceral hypersensitivity in mice.[101] Therefore, somatostatin analogues or receptor agonists may provide additional therapeutic strategies in visceral pain. Clinical studies will need to substantiate their applicability in IBS patients.

12.5 | RNA-based therapy

The observation of decreased expression of several TRPV1 targeting miRNAs in IBS patients suggests an opportunity for RNA-based therapy. The therapeutic potential of post-transcriptional gene silencing by RNA interference is increasingly being recognized.[102] The major benefit of synthetic siRNAs is their high target specificity, preventing the suppression of unrelated genes. Unfortunately, several challenges have yet to be overcome before RNA-based therapy can become a therapeutic option in IBS. The oral bioavailability of oligonucleotides is limited, confining the possible modes of administration to more invasive approaches. Furthermore, the effects are only transient, demanding repeated treatment. Therefore, there is a need for stable TRPV1 targeting siRNAs that are readily absorbed in the GI tract.

12.6 | Exploiting new targets

Our current knowledge on the role of visceral TRPV4 and TRPA1 in humans is limited. Nonetheless, data from animal studies, discussed above, suggest that these channels are viable targets for IBS therapy. The previous discovery of elevated levels of the endogenous TRPV4 agonist 5,6-EET in IBS-D patients and the putative implication of TRPV4 in mechanical hypersensitivity justify the need for further research. TRPA1-targeted therapy has been explored in animal models of visceral pain. However, contrasting evidence was found demonstrating attenuated visceral nociception in rodents with both TRPA1 antagonists as agonists.[54,103,104] It is unclear whether the mechanism of action of the latter was based on desensitization. Arguing against such a mechanism is the observation that reduced abdominal contractions were observed in mice after a single oral dose of a TRPA1 agonist. Furthermore, one TRPA1 antagonist has recently shown efficacy in patients with painful diabetic neuropathy, and several other TRPA1 antagonists have entered the phase of testing in clinical trials.[105] Although the true potential of these drugs will need to be explored, their safety profile appears to be more favourable than that of early TRPV1 antagonists. Given this advantage, we expect a boost in TRPA1 targeted therapy development in the near future.

New targets may continue to be identified. TRP channel regulatory processes in IBS constitute relatively unexplored terrain (see Section 5). Improved understanding of the underlying molecular processes may help identify targets that modulate TRP channel functioning.

13 | CHALLENGES OF TRP CHANNEL TARGETED THERAPY

Although the possibilities of TRP channel targeted therapy appear endless, treatment development has been plagued by many challenges over the past years.[16] As mentioned above, the first TRPV1 antagonists were associated with thermo-regulatory side effects. To date, the mechanisms by which these side effects arise are unknown. New selective agents may provide a solution to this issue. However, other challenges are to be expected.[16] This is mainly provoked by two factors. First, TRP channels are expressed in a wide range of tissues (see Section 10). Targeting TRP channels consequently affects systems other than nociceptors. For example, a systemically administered TRPV4 agonist resulted in endothelial dysfunction and cardiovascular collapse in one study.[106] TRPV1 expressed in the central nervous system is thought to have a role in mood disorders. Although conflicting data exists, TRPV1 antagonism may exacerbate depressive symptoms.[38] Regulatory relations between organ systems further complicate the matter. TRPV1-expressing neurons are thought regulate immunological functions, and interference with this function could prove detrimental in systemic inflammatory conditions.[107] The other major issue of TRP targeted treatment development is related to the large diversity of possible stimuli of each channel. Even

selective agents will not immediately overcome this hurdle. For example, a new TRPV1 antagonist that does not cause hyperthermia, was shown to potentiate proton-induced calcium influx in one study.[108] This unforeseen effect could prove problematic in the upper gastrointestinal tract, as it may result in excessive TRPV1 activation by gastric acid (eg physiological gastro-oesophageal reflux).

In addition, several limitations currently exist in the study of TRP channels and their role in nociception. Basic research is complicated by the lack of quality reagents. Antibodies used to investigate expression patterns possess poor specificity. A similar issue is encountered with agonists/antagonists used to investigate TRP channel functioning, which presents a major problem in understanding TRP biology.[109] Furthermore, studies regarding TRP channel involvement in visceral pain generation mostly focus on inflammation. Although animal colitis models have provided a wealth of information, alternative mechanisms of TRP channel sensitization should be explored. As discussed above, IBS is highly heterogeneous. Further insights in the mechanisms underlying IBS are needed in order to expand our knowledge of peripheral nociception and ultimately guide IBS treatment development.

14 | CONCLUSIONS

In summary, TRPV1, TRPV4, TRPA1 and TRPM8 have been shown to play important roles in visceral pain generation and inhibition, making them potential targets in the treatment of IBS. TRPV1 antagonists have proven to be potent analgesics, but it remains difficult to produce compounds with an acceptable safety profile. Different strategies targeting TRPV1 (ie modality-selective antagonism) or downstream molecules (NK1 or NK2 antagonists or somatostatin agonists) may solve this issue. TRPV1 desensitization strategies may provide suitable alternatives, yet short-term adverse effects may limit treatment adherence. In contrast, TRPV1 cross-desensitization with peppermint oil is attractive because of the low prevalence of adverse effects. The mechanism of action of peppermint oil however appears to reach beyond TRP channel signalling. In addition, TRPV4 and TRPA1 provide promising new targets. In our opinion, TRPA1 represents an important candidate for the development of new treatments of visceral pain. Its putative implication in mechanosensation in hyperalgesia but apparent lack of such function under basal conditions suggests a major role in IBS. Moreover, early TRPA1 antagonists have proven to be safe in clinical trials, rendering TRPA1 targeted therapy less of a pharmaceutical challenge than TRPV1 inhibition.

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