Articles in PresS. Am J Physiol Gastrointest Liver Physiol (April 6, 2017). doi:10.1152/ajpgi.00401.2016

# Transient Receptor Potential Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades Underlying Visceral Hypersensitivity

*Running title:* **TRP channels in visceral hypersensitivity**

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**Abstract**

Visceral hypersensitivity is an important mechanism underlying increased abdominal pain perception in functional gastrointestinal disorders (FGID) including functional dyspepsia, irritable bowel syndrome (IBS) and inflammatory bowel disease in remission. Although the exact pathophysiological mechanisms are poorly understood, recent studies described upregulation and altered functions of nociceptors and their signaling pathways in aberrant visceral nociception, in particular the transient receptor potential (TRP) channel family. A variety of TRP channels are present in the gastrointestinal tract (TRPV1, TRPV3, TRPV4, TRPA1, TRPM2, TRPM5 and TRPM8) and modulation of their function by increased activation or sensitization (decreased activation threshold) or altered expression in visceral afferents, have been reported in visceral hypersensitivity. TRP channels directly detect or transduce osmotic, mechanical, thermal and chemosensory stimuli. In addition, pro-inflammatory mediators released in tissue damage or inflammation can activate receptors of the G-protein coupled receptor (GPCR) superfamily leading to TRP channel sensitization and activation, which amplify pain and neurogenic inflammation. In this review, we highlight the current knowledge on the functional roles of neuronal TRP channels in visceral hypersensitivity and discuss the signaling pathways that underlie TRP channel modulation. We propose that a better understanding of TRP channels and their modulators may facilitate the development of more selective and effective therapies to treat visceral hypersensitivity.

**Key words**

pain; nociceptor; hyperalgesia; sensitization; TRP channels; visceral hypersensitivity; inflammatory mediators; G-protein coupled receptor

## Introduction

Visceral hypersensitivity (VHS) is defined as abnormal abdominal pain perception to intestinal distention and is considered as the most disturbing and therapy resistant hallmark of functional gastrointestinal disorders (FGID). Over 50% of patients suffering from irritable bowel syndrome (IBS) (64; 113) or functional dyspepsia (FD) (96), two chronic disorders of the upper and lower gastrointestinal tract respectively, suffer from hypersensitivity to gastric or colonic balloon distention. Also approximately one third of ulcerative colitis (UC) patients and half of Crohn's disease (CD) patients in remission report IBS-like symptoms including VHS (86; 87) that cannot be linked with identifiable inflammatory disease activity (45). Even though VHS has undoubtedly a complex and multifactorial etiopathology involving both peripheral and central mechanisms, accumulating evidence shows that the onset of chronic VHS is often preceded by an infectious gastroenteritis or acute inflammatory episode. It is currently hypothesized that a subgroup of these patients fail to resolve this initial inflammation leading to persistent immune activation, in particular mast cell activation and the subsequent release of pro-inflammatory mediators that activate or sensitize visceral nociceptors (14; 129). This finding is further underscored by the fact that supernatant of IBS intestinal biopsies contains more mast cell mediators such as tryptase, serotonin and histamine that can activate (22) or sensitize (128) human enteric neurons, leading to aberrant pain perception. So far, numerous mediators have been shown to directly activate gut afferents by binding to various cell surface receptors and channels expressed on their peripheral endings (20). In particular transient receptor potential (TRP) cation channels seem to play a key role in visceral nociception as they can be directly activated or act as secondary transducers of various G-protein coupled receptors (GPCRs) that are activated by pro-inflammatory mediators. In this review, we highlight the current knowledge on the functional roles of TRPV1, TRPA1, TRPV4, TRPM2 and TRPM8 in VHS. We discuss the signaling pathways that contribute to TRP sensitization and propose potential novel therapeutic strategies to treat VHS.

## TRP channels in the viscera

Visceral (nociceptive) stimuli are sensed by a specialized set of neurons with their cell body in the dorsal root ganglion and free sensory nerve endings in the intestinal wall. These nerve terminals reside in a complex signaling environment where they are subjected to mechanical distortion during distension and a changing milieu of neuroactive signaling molecules that can be modulated by stress, immune cells and the microbiome (20). The peripheral nerve endings in the gut are equipped with numerous receptors and ion channels that allow them to detect and respond to diverse chemical, mechanical, and thermal stimuli. These visceral signals are then transduced to interneurons in the

dorsal horn of the spinal cord which transmit the signal to the brainstem and if intensely enough, to the cortex for conscious perception. The best studied set of molecular sensors are the TRP channels as they can bind many endogenous lipids and exogenous natural or synthetic compounds (17).

Somatic pain studies repeatedly showed that direct activation of TRP channels in sensory nerves triggers protective mechanisms that lead to withdrawal from danger (pain), removal of irritants (itch, cough), and resolution of infection (neurogenic inflammation) (114). These physiologic processes are essential for survival and are normally under tight control and cease when the initial trigger, for example inflammation, is removed. However, in the diseased state, longer-lasting and sometimes even persistent neuronal hypersensitivity is maintained by TRP channel sensitization (49; 114). This process is characterized by aberrant pain responses to noxious and non-noxious stimuli and is a major cause of chronic disorders such as asthma, psoriasis and FGIDs. The exact mechanisms involved are not fully understood but it seems that TRP channels act as targets for major downstream effectors of GPCR signaling. Stimulation of GPCR signaling by inflammatory mediators enhances the response to TRP agonists via sensitization, making them very attractive therapeutic targets in various disorders that are characterized by neuronal hypersensitivity.

To date, there are 28 TRP genes described in mammals that are grouped into six TRP channel subfamilies: TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPML (mucolipin) and TRPP (or PKD; polycystin) (130). TRP channels non-selectively conduct cations and, when activated, lead to increased intracellular $Na^{+}$ and $Ca^{2+}$ concentrations and the initiation of neuronal excitation and a plethora of cellular responses that are relevant to chemo-, thermo- and/or mechano-sensation.

In the gastrointestinal tract, multiple cells express a variety of TRP channels (TRPV1, TRPV3, TRPA1, TRPM2, TRPM5 and TRPM8) that are crucial in tasting seasoned food, thermoregulation of the gut, peristalsis, secretion, mucosal homeostasis, tissue protection, epithelial restitution, controlling of the membrane potential and excitability of neurons, epithelial cells, muscle cells and interstitial cells of Cajal and visceral sensation (49). Emerging clinical evidence demonstrates aberrant TRP channel expression or function in FGIDs (4; 5; 128) while preclinical models using TRP agonists and transgenic mouse models lacking TRP channels confirmed the crucial role of TRP channels in the development and maintenance of colonic afferent hypersensitivity (27; 31; 49; 112). In the following paragraphs, we present an overview of the function and mediators involved in sensitization of TRPV1, TRPV4, TRPA1, TRPM2 and TRPM8 channels in the pathophysiology of VHS as seen in FGIDs.

*TRPV1*

The best characterized and most studied nociceptor in VHS is TRPV1. TRPV1 is a voltage-gated outwardly rectifying cation channel activated by noxious heat, acidosis (pH < 6) (110), exogenous irritants such as capsaicin (the active component of hot peppers)(25), allyl isothiocyanate (AITC, aka mustard oil) (34) and a variety of endogenous lipid compounds including anandamide (140) and some lipoxygenase metabolites of arachidonic acid (53).

In the gastrointestinal tract, TRPV1 is highly expressed by extrinsic sensory neurons and by intrinsic enteric neurons (7; 8; 121). An overview of clinical and preclinical studies providing evidence for the role of TRPV1 in VHS is presented in Table 1. For example, Akbar et al. showed that, in comparison with healthy individuals, quiescent IBD patients with IBS-like symptoms(4) and IBS patients(5) showed increased numbers of TRPV1 positive nerve fibers that correlate with abdominal pain scores (4; 5). Others provided rather functional evidence for TRPV1 deregulation, as ingestion of capsaicin capsules caused increased pain responses in patients with diarrhea-predominant IBS and FD patients compared to healthy individuals (41; 46; 68). These findings were corroborated by our group; visceral hypersensitive IBS patients, identified by colorectal balloon distention, experienced more pain during rectal application of capsaicin compared to normo-sensitive patients and healthy individuals (113). Even though hypersensitive patients reported more pain to rectal capsaicin application, rectal TRPV1 mRNA and protein expression was similar between IBS patients and healthy individuals, suggesting that TRPV1 is sensitized rather than upregulated (113). In a follow-up study, TRPV1 responses to capsaicin were indeed potentiated in rectal submucosal neurons of IBS patients but not in healthy subjects (128). Additionally, murine primary sensory DRG neurons revealed an increased capsaicin-induced intracellular $Ca^{2+}$ response after overnight incubation with rectal biopsy supernatants of IBS patients but not of healthy subjects, indicating that submucosal biopsies of IBS patients release mediators that can sensitize TRPV1 (128).

The involvement of TRPV1 in VHS has also been demonstrated in various preclinical models of visceral hypersensitivity. For example, increased TRPV1 immunoreactivity was detected in mouse DRG neurons of a post 2,4,6-Trinitrobenzenesulfonic acid (TNBS) induced colitis model and was linked to chemical (capsaicin) and mechanical (colonic distention) VHS (78). Moreover, mice deficient in TRPV1 failed to develop post-inflammatory VHS following acute colitis induced by dextran sulfate sodium (DSS) (66). Finally, in a rat stress model of maternal separation, visceral hypersensitivity in adult rats was reversed by a TRPV1 antagonist (112) further underscoring the role of TRPV1 in VHS.

*TRPV4*

The fourth member of the vanilloid subfamily of TRP channels, TRPV4, is a $Ca^{2+}$-permeable cation channel that has been detected in both sensory and non-sensory cells. In the gastrointestinal tract, TRPV4 has been reported to be primarily expressed on extrinsic afferent nerve fibers and a variety of non-neuronal cells such as epithelial and endothelial cells. Although TRPV4 was originally identified as a channel activated by hypo-osmotic swelling (69; 105; 127), recent evidence indicates that the channel can be activated by diverse stimuli including shear stress(38), non-noxious warm temperatures (44; 124), acidity(108), phorbol esters (both protein kinase C–activating and non-activating phorbol esters) (38; 122; 131), and downstream metabolites of arachidonic acid (epoxyeicosatrienoic acids) (29; 119; 123).

Accumulating evidence indicates that TRPV4 activation triggers VHS (overview in Table 2). For example, Cenac et al. elegantly demonstrated that the levels of the TRPV4 agonist 5,6-EET, but not of TRPV1 or TRPA1 agonists, were increased in IBS biopsies compared with controls, and that these increased levels correlated with abdominal pain and bloating scores (29). Intracolonic infusion of supernatants from IBS biopsies, but not from controls, induced VHS in mice, while knockdown of TRPV4 in mouse primary afferent neurons by siRNA inhibited the hypersensitivity caused by supernatants from IBS biopsies (29). Moreover, polyunsaturated fatty acid (PUFA) metabolites extracted from IBS biopsies or colons of mice with VHS activated mouse sensory neurons *in vitro*, an effect that was mediated by TRPV4 activation. Intriguingly, the supernatants of IBS biopsies itself did not contain 5,6-EET but triggered the production of 5,6-EET by mouse sensory neurons via a mechanism that involved the proteinase-activated receptor-2 (PAR-2) and cytochrome epoxygenase (29), indicating that sensory neurons themselves produce TRPV4 agonists upon activation by proteases. Moreover, recently it was shown that human serosal nociceptor mechanosensitivity was attenuated by application of the TRPV4 antagonist HC067047, further underscoring the potential role of TRPV4 in VHS (74). Using live imaging of rectal biopsies, we recently found increased $Ca^{2+}$ responses to TRPV4 agonist GSK1016790A in submucosal neurons of IBS patients compared to healthy controls, an effect that could be mimicked by histamine in submucosal neurons of healthy subjects. As no increased TRPV4 messenger RNA (mRNA) was found, we hypothesize that TRPV4 is rather sensitized than upregulated (11). Also in patients suffering from acute IBD, TRPV4 mRNA is highly enriched in colonic sensory neurons (21) and in colonic biopsies obtained from patients with Crohn's disease and ulcerative colitis compared with healthy subjects (36). Data on TRPV4 expression in IBD patients in remission and suffering from VHS are lacking so far.

In addition to the clinical data indicating a potential role for TRPV4 in VHS, various preclinical models already provide functional evidence. Activation of TRPV4 by the TRPV4 agonist 4α-phorbol 12,13-didecanoate (4α-PDD) in colonic projections of DRG neurons induced mechanical VHS in a dose-

dependent manner (27). Moreover, mechano-sensory responses of colonic serosal and mesenteric fibers were enhanced by the TRPV4 agonist 5,6-EET, and significantly reduced by targeted deletion of TRPV4 or by the TRPV4 antagonist, ruthenium red (21). Others showed that intervertebral pretreatment of mice with TRPV4 directed small interfering RNA (siRNA) reduced basal visceral nociception, as well as 4α-PDD agonist-induced hypersensitivity (27). Furthermore, selective blockade of TRPV4 in the TNBS colitis mouse model alleviated colitis and pain associated with acute intestinal inflammation (36). Based on these data, TRPV4 seems an important colonic nociceptor that mediates both mechanical and chemical hyperalgesia. Despite these findings, more clinical studies investigating the role of TRPV4 in VHS in IBS, FD and IBD patients in remission are warranted.

*TRPA1*

In mammals, TRPA1 is the sole member of the TRPA gene subfamily. TRPA1 is a cold- and mechanosensitive TRP channel activated by cooling to the noxious cold range of temperatures (<17°C) (104). TRPA1 is best known as an irritant sensor and is activated by a wide variety of pungent compounds, such as cinnamaldehyde (12), AITC (57), allicin (15), menthol (59), inflammatory fatty acids, prostaglandin metabolites and hydrogen peroxide(9; 72). In addition, TRPA1 acts as a sensor of bacterial lipopolysaccharides (77; 102). In the gastrointestinal tract of mammals, TRPA1 has been shown to be expressed on extrinsic primary afferent nerves as well as in intrinsic enteric neurons (84; 104). Besides neuronal cells, TRPA1 is also highly expressed in non-neuronal 5-hydroxytryptamine-releasing enterochromaffin cells (80), cholecystokinin-releasing endocrine cells (91) and intestinal epithelial cells (63).

Recent reports identified TRPA1 as a target for the noxious and inflammatory irritant AITC in peripheral sensory neurons, implicating a functional role in pain and neurogenic inflammation (overview in Table 3). Although the majority of the literature on TRPA1 in VHS is based on preclinical studies, a recent study reported upregulation of TRPA1 mRNA expression in biopsies of active IBD patients, but not in quiescent IBD patients (65). This effect on acute pain perception was already described by Meseguer et al. who found that TRPA1 channels mediate acute neurogenic inflammation and pain produced by LPS (77). Also in mice, intracolonic administration of a TRPA1 agonist increased the visceromotor response, an effect that was absent in TRPA1 deficient mice (19; 26). Others showed upregulation of TRPA1 expression in colonic DRGs of mice suffering from acute TNBS-induced colitis that led to an enhanced visceromotor response to colorectal distention, an effect that was prevented by intrathecal pretreatment with a TRPA1 antisense oligodeoxynucleotide (134) and TRPA1 blockade (115). In addition, the TRPA1 agonist AITC induced colonic hypersensitivity in a mild DSS colitis model that was prevented by treatment with a TRPA1 antagonist (79).

Besides its role in acute pain perception, preclinical models showed that intracolonic treatment of newborn mouse pups with the TRPA1 agonist AITC triggers a permanent increase in the percentage of TRPA1-positive DRG neurons and results in adult VHS (31). Increased responses of mechano-senstive colonic afferent neurons by TRPA1 agonists has been suggested to result from upregulation of TRPA1 mRNA in a model of mustard oil induced colitis in mice (61). Moreover, in a model of chronic exposure to water avoidance stress, the increased visceromotor response to colorectal distention correlated with a significant protein upregulation of TRPA1 and TRPV1 in DRG neurons (135), indicative of a crucial role for TRPA1 and TRPV1 in VHS. Indeed, in sensory neurons, TRPA1 has been shown to act in concert with TRPV1 (see details below). Finally, we recently demonstrated an increased TRPA1 agonist induced $Ca^{2+}$ response in rectal submucosal neurons of IBS patients compared to those of healthy controls (11). Furthermore histamine was able to potentiate TRPA1 responses in submucosal neurons of healthy subjects. Again, TRPA1 mRNA expression was not upregulated in rectal biopsies of IBS patients compared to healthy individuals, suggesting that also TRPA1 is sensitized in IBS (11). Even though these studies are promising, more clinical studies are required to better understand the role of TRPA1 in VHS in functional gastrointestinal disorders.

*TRPM2*

TRPM2 is a heat-sensitive TRP channel that belongs to the melastatin subgroup of the TRP channel superfamily. It can be activated by intracellular adenosine diphospate (ADP)-ribose and extracellular stimuli such as reactive oxygen species (47; 85; 125). TRPM2 channels are expressed by intrinsic and spinal primary afferent neurons innervating the distal colon in rat (73). Besides neuronal cells, TRPM2 is also expressed in mucosal macrophages and mast cells and contributes to the progression of experimental colitis and food allergy in mice (81; 133).

Several reports show that TRPM2 deficiency has anti-allodynic effects in a wide variety of inflammatory and neuropathic pain mouse models (101), suggesting that TRPM2 may be a new therapeutic target for controlling chronic pain. Furthermore a recent study found evidence for a role of TRPM2 in visceral nociception and hypersensitivity (73) (Overview in Table 4). TRPM2 expression was increased in distal colon of a TNBS-colitis rat model and oral administration of TRPM2 antagonist or TRPM2 deficiency reduced the visceromotor response to noxious colorectal distention in rats. These data suggest that TRPM2 is involved in VHS and may present a novel therapeutic target for VHS triggered by intestinal inflammation. To date, clinical studies investigating the role of TRPM2 in visceral pain sensation in FGID are completely lacking, but definitely warranted to establish preclinical evidence.

*TRPM8*

The cold and menthol receptor TRPM8 is activated by cooling, menthol, and cooling compounds such as spearmint, eucalyptol, and icilin (75; 83). Recent evidence suggests that TRPM8 is also expressed by peripheral sensory neurons of visceral organs (48) and may be involved in the development of VHS (overview in Table 5). Although the role of TRPM8 in VHS in FGIDs has hardly been studied yet, preclinical models suggest that activation of TRPM8 results in a diminished visceral pain perception. Transgenic mice deficient for TRPM8 exhibit loss of acute innocuous cold sensation, impaired responses to noxious cold temperatures, and deficits in nocifensive responses to cooling compounds and impaired inflammatory and neuropathic cold allodynia. For example, post-TNBS -induced colonic mechano-hypersensitivity was significantly reduced by a mixture of the TRPM8 agonists peppermint and caraway oil (3). Also pilot clinical trials wherein IBS patients are treated with enteric-coated peppermint oil decreased abdominal pain together with an increase in life quality (23; 62; 76). The mechanism underlying these clinical findings is not fully understood but one study showed that TRPM8 activation on colonic afferents triggers mechanical desensitization combined with diminished agonist-evoked responses to TRPA1 and TRPV1, indicating that TRPM8 couples to TRPV1 and TRPA1 to inhibit their downstream chemosensory and mechanosensory actions(48). Others propose that TRPM8 exerts anti-inflammatory properties. Pretreatment with the TRPM8 agonist icilin decreased inflammatory cytokines and mucosal damage in a TNBS and DSS experimental colitis model, suggesting an anti-inflammatory role for TRPM8 activation, in part due to an inhibition of neuropeptide release (93). Of note, not all authors confirm these anti-nociceptive and anti-inflammatory findings, as Hosoya et al. showed increased expression of TRPM8 in the distal colon mucosa of a TNBS and DSS mouse colitis model and treatment with the TRPM8 agonist WS-12 induced increased visceral pain responses compared to controls, which was prevented by pretreatment with a TRPM8 antagonist (50).

Taken together, depending on the context, TRPM8 functions in innocuous cool sensation, nociception and analgesia. How TRPM8 may be able to convey these different sensory modalities is still unclear and awaits further investigation.

**Mechanisms underlying sensitization of TRP channels in visceral hypersensitivity**

Given that TRP channels are crucial in the development and maintenance of VHS, it is clear that insight in the mechanisms contributing to persistent TRP channel activation or sensitization is key for the development of novel therapeutic strategies. In general, TRP channels play three distinct cellular roles: (1) TRP channels operate as molecular sensors, that is primary detectors and transducers of chemical and physical stimuli from the micro-environment; (2) TRP channels act as downstream or secondary transducers of cell activation mediated by GPCRs or ion channel activation; and (3) TRP

channels function as ion transport channels, e.g., for $Ca^{2+}$ and $Mg^{2+}$ responsible for cellular homeostasis. Within primary afferent neurons, translation of signals detected by TRP channels into effector responses is carried out by the local release of neuropeptides from the peripheral fibers of TRP-expressing afferent neurons, which causes changes in local tissue function; and on the other hand, by transmission of the signals to the central nervous system resulting in (nociceptive) sensation.

Long-term deregulation and disease can lead to chronic TRP channel sensitization, thereby triggering VHS. However, the exact mechanisms underlying long-term sensitization of TRP channels in FGIDs are not fully understood. From somatic pain studies and studies in the skin we know that the GPCR-TRP axis plays a central role in TRP sensitization (114). Indeed, GPCRs enable sensory neurons to detect diverse stimulants and inhibitors, including amines (histamine, serotonin), peptides (kinins, tachykinins, opioids), purines and nucleotides (adenosine, ATP), lipids (prostaglandings), steroids (bile acides) and proteases (serine, cysteine). The capacity of GPCRs to excite primary sensory neurons requires activation of TRP channels and the activities of many GPCRs converge on a small number of TRP channels that are vitally important for sensory signaling. GPCRs can stimulate TRPs by two general mechanisms: Gα mediated activation of phospholipases that relieve phosphatidylinositol 4,5-biphosphoate (PIP2)-dependent channel inhibition and generate endogenous TRP agonists and stimulation of kinases (protein kinase C, PKA, tyrosine kinases) that phosphorylate TRPs to increase cell-surface expression and interactions with adaptor proteins. These mechanisms lead to TRP channel sensitization or activation (114; 118) (overview in Table 6). The net result is that TRP channels can amplify the effects of GPCRs and mediate their contributions to transmission of pain and neurogenic inflammation. Below we review the current knowledge of mediators and receptors involved in TRP sensitization and VHS.

*TRPV1*

TRPV1 contains numerous phosphorylation sites for serine and threonine protein kinases such as PKA, PKC, $Ca^{2+}$/calmodulin dependent kinase (CaMK)II and sarcoma (Src) kinase, all of which regulate TRPV1 activity by a dynamic interplay between receptor phosphorylation and dephosphorylation (52). This complex system allows TRPV1 sensitization by various inflammatory mediators and receptors *in vitro, ex vivo* and in models of somatic pain (30; 51; 55; 58; 95; 107; 109; 111; 137). For example, TRPV1 is potentiated by bradykinin in a PKC-dependent way (107; 109), by PAR-2 in a phospholipase C (PLC), PKA and PKC dependent manner (30), by histamine through activation of the histamine 1 receptor (Hrh1) and PLC and PKC activation (58), by prostaglandin E2 (PGE2) through PKA-dependent phosphorylation (51; 95), by extracellular ATP secreted by damaged cells (111), and

by nerve growth factor (NGF) acting on the TrkA receptor, activating a signaling pathway in which PI3 kinase and Src kinase bind and phosphorylate TRPV1 (55; 137), etc.

Recent literature provided evidence that TRP sensitization by GPCR activation may also contribute to aberrant visceral pain perception. *In vitro* exposure of murine colonic afferents to an acidic inflammatory soup containing bradykinin, serotonin (5-HT), histamine and $PGE_2$ induced mechanical sensitization, an effect that was not observed in TRPV1 knockout mice (56). In particular 5-HT exerts TRPV1 sensitizing effects as pre-exposure of mouse sensory neurons in lumbosacral DRG neurons receiving colonic input to 5-HT augmented TRPV1 activation in a 5-HT2 and 5-HT4 but not 5-HT3 dependent manner (106). Downstream signaling required G-protein activation and phosphorylation, as intracellularly administered PKA inhibitors and an A-kinase anchoring protein inhibitor significantly blocked serotonergic facilitation of TRPV1 function. 5-HT2 receptor-mediated facilitation was also inhibited by a PKC inhibitor and the authors concluded that the facilitation of TRPV1 by metabotropic 5-HT receptor activation may contribute to hypersensitivity of primary afferent neurons (106). Indeed, also Qin et al. showed that depletion of 5-HT from the colon reduced the excitability of DRG neurons by capsaicin (92).

We recently demonstrated that TRPV1 responses to capsaicin are potentiated in rectal submucosal neurons of IBS patients compared to those of healthy subjects (128), an effect that was histamine dependent. We showed an increased capsaicin-induced response of sensory DRG neurons after overnight incubation with rectal biopsy supernatants of IBS patients compared to healthy subjects. As this effect was also mediated via activation of the Hrh1 (128), we speculate that mediators released by immune cells, most likely mast cells, in the submucosa trigger TRP sensitization and neuronal hypersensitivity. A small pilot study in 55 IBS patients assessing the effect of the Hrh1 antagonist ebastine for 12 weeks revealed that ebastine improved global symptom relieve in about 46% of patients with the maximal effect from 8 weeks onwards (128).

*TRPV4*

TRPV4 is regulated by serine/threonine phosphorylation in a similar manner to TRPV1. Both PKC and PKA, as downstream molecules of GPCR activation by inflammatory mediators such as histamine, 5-HT, bradykinin, PGE2 and proteases, can enhance the activation of TRPV4 via phosphorylation at specific residues and the phosphorylation depends on the assembly of PKC and PKA (35). Besides phosphorylation, incubation of DRGs with histamine or serotonin triggered the translocation of TRPV4 to the membrane, an effect that can also contribute to neuronal potentiation (28).

As for TRPV1, the function of TRPV4 in visceral pain is strongly modulated by pro-inflammatory mediators that act on upstream GPCRs. For example, TRPV4 was found to be co-expressed with the

protease receptor PAR-2 on nociceptive neurons (43; 100) and pre-treatment of sensory DRG neurons with a PAR-2 agonist resulted in an enhanced TRPV4 activity, which was prevented by PKA and PKC inhibition (43). *In vivo* administration of sub-nociceptive doses of serotonin and histamine potentiated TRPV4-induced hypersensitivity in response to colorectal distention in mice, which was prevented by intravertebral injection of TRPV4 siRNA (28). Also intraluminal administration of PAR-2 agonists resulted in an increased visceromotor response to colorectal distention, which was not observed in TRPV4 knockout mice (27; 100). In contrast, activation of PAR-4 significantly reduced the visceromotor response to colorectal distention in mice and inhibited PAR-2 agonist- and TRPV4 agonist-induced allodynia and hyperalgesia (10). These results are of particular interest as they demonstrate that activation of inhibitory GPCR receptor subunits can bind to inhibitory secondary signaling molecules, preventing and potentially reversing TRP potentiation.

*TRPA1*

Several studies on somatic pain already demonstrated that also TRPA1 activation is potentiated by GPCR activation. Bradykinin potentiates TRPA1 in PKA and PLC dependent way *in vitro* and *in vivo* (97; 120). Also activation of PAR-2 by mast cell tryptase can trigger sensitization of TRPA1 involving PKA and PKC signaling leading to somatic cold hyperalgesia in mice (30). In addition, TRPA1 sensitization by PAR-2 activation was observed in human embryonic kidney-293 (HEK-293) cells and DRG neurons, an effect that was mediated by PLC activation and phosphatidylinositol biphosphate ($PIP_2$) (32). Degradation of $PIP_2$ into diacylglycerol (DAG) and inositol triphosphate ($IP_3$) leads to $Ca^{2+}$ release from internal stores, and this intracellular $Ca^{2+}$ mobilization may directly activate TRPA1 (139). Finally, inflammatory signals or acute activation of TRPA1 by mustard oil induces translocation of the TRPA1 channels to the membrane in sensory neurons which is PKA-and PLC-dependent (97).

In parallel, activation of colonic afferents with the inflammatory mediator bradykinin resulted in increased mechanosensitivity of these neurons, which was absent in TRPA1 knockout mice (19). Similary, intracolonic administration of a PAR-2 agonist resulted in an increased visceromotor response to colorectal distention which is abrogated by TRPA1 gene deletion (26). This finding was not confirmed by Brierley et al., who did not find evidence to support an interaction of TRPA1 and PAR-2 in splanchnic colonic afferents (19). Although the role of TRPA1 sensitization seems well established in somatic pain, its role in VHS in FGIDs remains to be elucidated.

*TRPM8*

Unlike TRPV1, TRPV4 and TRPA1 whose activation is enhanced by phosphorylation, modulation of TRPM8 by protein kinases appears to function as a negative regulator. Although there are no data available on TRPM8 in VHS, somatic pain studies showed that PKC and PKA activation initiates de-phosphorylation of TRPM8 via phosphatase activation (16; 88). Various studies in murine DRG neurons show that treatment with pro-inflammatory mediators bradykinin and PGE2 led to a reduction in the amplitude of the TRPM8 response to cooling resulting in a shift of the threshold to colder values. These effects were mediated by PKC and PKA, respectively (16; 71; 88). Another report confirmed the involvement of PKC in TRPM8 desensitization in HEK-293 cells (2). Others showed that a subset of sensory neurons co-express TRPM8 ion channels and 5-HT1B receptors (5-HT1BR). The 5-HT1BR has previously been reported to exert an anti-nociceptive influence (42; 60). 5-HT1BRs signal through PLD and PIP2 to potentiate TRPM8 activation, thereby amplifying TRPM8 attenuation of neuronal hypersensitivity (117). The authors also showed that 5-HT1BR activation led to the amplification of TRPM8 mediated analgesia in behavioral models of chronic pain (117).

On the other hand, there is recent evidence that the inflammatory mediators bradykinin and histamine can also inhibit TRPM8 activation independent of protein kinases via activation of the G-protein subunit Gαq that binds TRPM8 and directly inhibits the ion channel activity (138). Thus, inflammatory mediators not only enhance the activation of pro-nociceptive TRP channels (see above) but also desensitize TRPM8, resulting in even further enhanced inflammatory pain perception. Altogether, depending on the micro-environment and activated upstream GPCR, TRMP8 can exert both anti-and pro-nociception and may serve as an interesting target to treat VHS.

*Cross-talk between TRP channels*

As various TRP channels are co-expressed on sensory neurons and often simultaneously upregulated or sensitized potentially downstream of various activated GPCRs in preclinical and clinical VHS, it has been proposed that TRP channels may also cross-sensitize each other. Indeed, 97% of TRPA1-positive sensory neurons co-express TRPV1, while 30% of the TRPV1-positive neurons also express TRPA1, suggesting that these two TRP channels can interact (104). Several studies indeed showed that activation of TRPA1 can modulate TRPV1 activity. For example activation of TRPA1 in DRG neurons results in sensitization of TRPV1, which involves activation of adenylyl cyclase, cyclic adenosine monophosphate (cAMP) and subsequent activation of PKA leading to phosphorylation of TRPV1 (103). Furthermore intraperitoneal injection of a TRPV1 antagonist combined with a TRPA1 antagonist in a mouse model of experimental colitis results in a significant decrease of VHS compared

462 to injection of the antagonists separately, suggesting a synergistic effect (115). Another study shows
463 desensitization of TRPA1 in sensory neurons due to $PIP_2$ depletion by activation of TRPV1 by
464 capsaicin (6). Furthermore cannabinoid-induced TRPV1 dephosphorylation in sensory neurons is
465 absent if TRPA1 is knocked down, suggesting interaction between TRPA1 and TRPV1 (54). Of
466 interest, activation of TRPM8 resulted in a decrease of agonist-evoked responses to TRPA1 and
467 TRPV1 in colonic afferents, suggesting co-expression and cross talk between TRPM8, TRPV1 and
468 TRPA1 (48). Taken together these data suggest that cross-(de)sensitization of TRP channels can
469 contribute to pain sensitivity in inflamed tissues and may serve as novel therapeutic targets.
470
471 **TRP channels: implications for therapy**
472 Mounting evidence indicates that TRP channels are attractive targets for novel analgesics effective in
473 a wide range of pathophysiological conditions including VHS amongst many others and numerous
474 companies initiated research tracks to identify TRP modulators. However, antagonizing TRP channels
475 is challenging as they are often not only expressed by visceral sensory neurons but also by a
476 multitude of tissues including higher brain structures, non-sensory neurons and non-neuronal cells
477 leading to severe side-effects. Recently, a number of small molecule TRPV1 antagonists have been
478 advanced into clinical trials. The systemic use of TRPV1 antagonists revealed two major drawbacks,
479 namely hyperthermia and impaired noxious heat sensation, leading to their withdrawal from clinical
480 trials (33; 39; 40; 90). Some TRPV1 antagonists patented in recent years overcame the known
481 undesirable side effects, making the development of TRPV1 antagonists much more promising (67;
482 98). Besides TRPV1 antagonists, prolonged intake of capsaicin also appears to desensitize afferent
483 nerves against noxious stimuli. For example, ingestion of capsaicin capsules by healthy volunteers 3
484 times per day for 4 weeks have been shown to decrease the pain response evoked by duodenal
485 capsaicin administration and balloon distention (37). In line with these results, treatment of FD
486 patients with capsaicin capsules for 5 weeks resulted in a significant reduction of visceral pain (18).
487 The challenge for an effective and safe therapy however will be to rather suppress the pathological
488 contribution of TRPV1 to pain while preserving its physiological function.
489 TRPA1 may be a better candidate for therapeutic intervention as it is specifically expressed in a
490 subclass of TRPV1-expressing nociceptors (104). TRPA1 antagonists do not have the same
491 temperature regulation safety concerns as TRPV1 antagonists and may therefore be a more suitable
492 target (89). Although the TRPA1 antagonist GRC17536 has shown efficacy in patients with painful
493 diabetic neuropathy in Phase 2a proof-of-concept studies (89), studies on TRPA1 antagonism in VHS
494 are completely lacking. To date there is also no clinical evidence available for TRPV4 antagonism,
495 although new blockers have been developed and await to be assessed for their therapeutic efficacy
496 and safety (116).

TRPM8 is often thought of as an ion channel giving rise to only non-painful sensations but more recent evidence suggests that TRPM8 channel agonists may have analgesic effects. Several double-blind placebo-controlled clinical trials indeed showed that ingestion of peppermint-oil in IBS patients resulted in a significant decrease of abdominal pain perception while significantly improving quality of life in 75% of patients compared to 40% in patients treated with placebo (23; 62; 76). Moreover, a recent double-blind placebo-controlled clinical trial showed that ingestion of a novel formulation of peppermint-oil with sustained release resulted in a 40% reduction of the total IBS symptom score (abdominal pain, bloating, urgency, etc.) after 4 weeks treatment compared to 24.3% decrease in the placebo group (24). In addition herbal preparations containing peppermint successfully relieve FD-related symptoms such as epigastric/abdominal pain, bloating and heartburn in 78% of the treated patients (94).

Altogether, even though there are some preliminary positive reports the direct blockade of TRP channels often leads to severe side-affects. It has been suggested that indirect action on the modulation of these channels may be a more promising approach. As described above, inflammatory mediators lower the threshold of several pro-nociceptive TRPs via activation of the corresponding GPCR. Hence, VHS may be counteracted by interacting with this process. In this line of thinking, we recently showed that Hrh1 antagonism prevents sensitization of TRPV1 on sensory neurons resulting in significantly decreased abdominal pain in a proof-of-concept clinical trial (128). Since activation of PAR-2 has also been shown to sensitize TRPV1, TRPV4 and TRPA1 (30), blocking PAR-2 may also a promising therapeutic approach, however so far clinical evidence is lacking. Stimulation of GPCRs results in activation of several protein kinases that phosphorylate and sensitize TRP channels. Inhibition of the downstream pathways of GPCRs activation may represent an interesting alternative therapeutic approach for VHS. The success of kinase inhibitors in the treatment of cancer showcased their therapeutic potential (136). This success, coupled with a greater understanding of inflammatory signalling cascades, led to kinase inhibitors taking centre stage in the pursuit for new anti-inflammatory agents for the treatment of (auto-)immune-mediated diseases (70). To date, only a handful of kinase inhibitors have reached the stage of FDA approval, while others have had mixed results in clinical trials. It remains to be determined whether protein kinases are good drug targets to treat FGIDs.

Recently, somatic pain studies have indicated that resolvins (Rv), a new class of compounds known for their anti-inflammatory properties, prevent activation of TRP channels including TRPA1, TRPV1 and TRPV4 (1; 13; 82; 132). Resolvins are endogenous lipid mediators produced by immune cells, including eosinophils and neutrophils, and drive the resolution phase of inflammation even at concentrations in the nanomolar range (99). Understandably, evidence is quickly growing for their pain-relieving potential too. To date, especially RvE1, RvD1 and RvD2 have been studied for their

analgesic properties. Increasing evidence shows that these resolvins potently interfere with TRP channel function independent of their effect on the immune system (99). RvE1 and RvD1 were shown to normalize inflammatory pain by central and peripheral actions (132). Furthermore, resolvins inhibited acute pain evoked by intraplantar injection of TRPV1, TRPV4 and TRPA1 agonists. Also in vitro, in DRG neurons and HEK cells, TRPV1, TRPV4 and TRPA1 signaling could be inhibited by RvD1, RvD2 and RvE1 (13; 82; 132). Besides TRP channel activation it can be speculated that resolvins also prevent TRP channel sensitization. The mechanism by which resolvins inhibit TRP channel activation and sensitization is not entirely unraveled. It is proposed that resolvins activate inhibitory GPCR (Gαi) that antagonize the GPCR-mediated sensitization of TRP channels. Activation leads to inhibition of adenylyl cyclase dependent cAMP production and subsequent down-regulation of PKA-mediated TRP sensitization (126). Therefore this signaling mechanism is potentially a very interesting approach to resolve visceral hypersensitivity in FGID, mediated by TRP channel sensitization.

## Conclusions

Relief of chronic pain in functional gastrointestinal disorders including FD, IBS and IBD in remission, is a largely unmet medical need. This review underscores the critical role of TRP ion channels in peripheral neuronal sensitization, generating and sustaining chronic pain by the increase in neuronal excitability in primary sensory neurons. TRP channels not only function as detectors of thermal, chemical and mechanical stimuli but also serve as secondary transducers in which activation of various GPCRs by pro-inflammatory mediators triggers TRP sensitization leading to aberrant pain perception (Figure 1). Therefore TRP channels as well as the GPCRs and downstream signaling molecules are promising drug targets for the management of VHS as seen in several FGIDs. Since multiple inflammatory mediators have been identified that can individually result in TRP channel modulation via GPCR signaling, identifying the mediator signature in individual patients with VHS is key to predict which treatment would be beneficial to relieve symptoms.

## Tables

**Table 1:** Implications of TRPV1 in the pathophysiology of visceral hypersensitivity in FGID

| Tissue (disease) | Species | Tissue or cell type | Technique | Result | Reference |
|---|---|---|---|---|---|
| **Expression profiles** | | | | | |
| Colon (IBD) | Human | Rectosigmoid biopsies | Immunohistochemistry | Increased $TRPV1^+$ nerve fibers | (4) |
| Colon (IBS) | Human | Rectosigmoid biopsies | Immunohistochemistry and symptom questionnaires | Increased $TRPV1^+$ nerve fibers correlating with abdominal pain | (5) |
| Colon (IBS) | Human | Rectal biopsies | Immunohistochemistry, RT-qPCR | No upregulation of TRPV1 | (113) |
| Colon (DSS colitis) | Mice | HEK-293 cells and dorsal root ganglia | Immunohistochemistry, RT-qPCR, western blot | Upregulation TRPV1 by substance P | (66) |
| Colon (TNBS colitis) | Rats | Dorsal root ganglia | Immunohistochemistry | Increased TRPV1 immunoreactivity | (78) |
| **Functional data** | | | | | |
| Colon (IBS-D) | Human | | Symptom questionnaires | Increased pain sensation to capsaicin capsules | (41) |
| Stomach and small intestine (FD) | Human | | Symptom questionnaires | Increased pain sensation to capsaicin capsules | (46; 68) |
| Colon (IBS) | Human | | Symptom questionnaires | Rectal capsaicin application induced increased pain perception. | (113) |

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|---|---|---|---|---|---|
| Rectum and colon (IBS) | Human and mice | Submucosal neurons (human) and dorsal root ganglia (mice) | Calcium imaging and symptom questionnaires | Increased TRPV1 sensitivity in IBS mediated by histamine and H1R. Symptom reduction after treatment with H1R antagonist | (128) |
| Colon (DSS colitis) | Mice | | Colorectal distention | TRPV1 deficiency prevents post-inflammatory VHS | (66) |
| Colon | Mice | Serosal afferent nerves | Colorectal distention and afferent nerve recording | Inflammatory mediators sensitize TRPV1 resulting in VHS, an effect lacking in TRPV1 knock-out mice | (56) |
| Colon | Mice | Dorsal root ganglia | Patch-clamp | TRPV1 sensitization by 5-HT | (106) |
| Colon | Rat | | Maternal separation: colorectal distention | VHS after maternal separation reversed by TRPV1 antagonist | (112) |
| Colon | Rat | Dorsal root ganglia | Colorectal distention and patch-clamp | Depletion 5-HT decreases capsaicin response and VHS | (92) |

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**Table 2:** Implications of TRPV4 in the pathophysiology of visceral hypersensitivity in FGID

| Tissue (disease) | Species | Tissue or cell type | Technique | Result | Reference |
|---|---|---|---|---|---|
| **Expression profiles** | | | | | |
| Colon (IBD) | Human | Surgical resections and colonic biopsies | Immunohistochemistry and RT-qPCR | Upregulation TRPV4 in sensory neurons, serosal blood vessels and colonic biopsies | (21; 36) |
| **Functional data** | | | | | |
| Ilium, colon and rectum | Human | Serosal afferent nerves | Afferent nerve recording | Application of TRPV4 antagonist HC067047 attenuated serosal nociceptor mechanosensitivity | (74) |
| Rectum and colon (IBS) | Human and mice | Submucosal neurons (human) and dorsal root ganglia (mice) | Calcium imaging | Increased TRPV4 sensitivity in IBS mediated by histamine and H1R. | (11) |
| Colon | Human and mice | Colonic biopsies (human) and dorsal root ganglia (mice) | Calcium imaging | Knockdown of TRPV4 inhibited hypersensitivity caused by supernatants from IBS biopsies | (29) |
| Colon (TNBS colitis) | Mice | | Evaluation of pain-related behavior | TRPV4 antagonists alleviates colitis and inflammatory pain | (36) |

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|---|---|---|---|---|---|
| Colon | Mice | | Colorectal distention | Intracolonic administration of TRPV4 agonists induces VHS which was inhibited by TRPV4 siRNA treatment | (27) |
| Colon | Mice | Serosal and mesenteric afferent nerves | Colorectal distention and afferent nerve recording | TRPV4 knock-out mice or treatment with TRPV4 siRNA decreases visceromotor response. Serosal and mesenteric afferent nerves respons to TRPV4 agonist 5,6-EET | (21) |
| Colon | Mice | Dorsal root ganglia | Colorectal distention and calcium imaging | Intracolonic administration of histamine and serotonin potentiated TRPV4-induced VHS, absent mice treated with TRPV4 siRNA. Histamine and serotonin potentiate the TRPV4 response on mouse dorsal root ganglia | (28) |
| Colon | Mice | Dorsal root ganglia | Colorectal distention and calcium imaging | Intracolonic administration of PAR-2 agonists induces VHS, absent in TRPV4 knock-out mice. PAR-2 agonists potentiate the TRPV4 response in mouse dorsal root ganglia | (27; 100) |
| | | | | | (10) |

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|---|---|---|---|---|
| Colon | Mice | Dorsal root ganglia | Colorectal distention and calcium imaging | Intracolonic PAR-4 agonist inhibits PAR-2 agonist and TRPV4 agonist induced VHS. PAR-4 agonist inhibited calcium response to PAR-2 and TRPV4 agonist in mouse dorsal root ganglia |

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**Table 3:** Implications of TRPA1 in the pathophysiology of visceral hypersensitivity in FGID

| Tissue (disease) | Species | Tissue or cell type | Technique | Result | Reference |
|---|---|---|---|---|---|
| **Expression profiles** | | | | | |
| Colon (IBD) | Human | Colonic biopsies | Immunohistochemistry and RT-qPCR | Upregulation TRPA1 | (65) |
| Colon (mustard oil colitis) | Mice | Colonic tissue | RT-qPCR | Upregulation TRPA1 on colonic afferent nerves | (61) |
| Colon | Mice | Dorsal root ganglia | Immunohistochemistry | Treatment mice pups with TRPA1 agonist increases TRPA1 expression | (31) |
| Colon (TNBS) | Mice | Dorsal root ganglia | | Upregulation TRPA1 | (115; 134) |
| Colon | Rats | Dorsal root ganglia | Western-blot | TRPA1 upregulation in stress-induced VHS | (135) |
| **Functional data** | | | | | |
| Rectum and colon (IBS) | Human and mice | Submucosal neurons (human) and dorsal root ganglia (mice) | Calcium imaging | Increased TRPA1 sensitivity in IBS mediated by histamine and H1R. | (11) |
| Colon | Mice | | Colorectal distention | Intracolonic administration of TRPA1 agonists induces VHS, absent in TRPA1 knock-out mice | (19; 26) |

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|---|---|---|---|---|---|
| Colon | Mice | | Colorectal distention | Treatment mice pups with TRPA1 agonist results in adult VHS | (31) |
| Colon | Mice | | Colorectal distention | Intracolonic PAR-2 agonist administration induces VHS, absent in TRPA1 knock-out mice | (26) |
| Colon | Mice | Serosal and mesenteric afferent nerves | Colorectal distention and afferent nerve recording | Bradykinin increases mechanosensitivity in afferent nerves and VHS, absent in TRPA1 knock-out mice | (19) |
| Colon | Mice | Serosal and mesenteric afferent nerves | Afferent nerve recording | No interaction of PAR-2 and TRPA1 in splanchnic afferents | (19) |
| Colon (TNBS and DSS colitis) | Rats and mice | | Colorectal distention | VHS absent in TRPA1 knock-out mice and by TRPA1 blockade | (26; 79; 115; 134) |

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**Table 4:** Implications of TRPM2 in the pathophysiology of visceral hypersensitivity in FGID

| Tissue (disease) | Species | Tissue or cell type | Technique | Result | Reference |
|---|---|---|---|---|---|
| **Expression profiles** | | | | | |
| Colon (TNBS- colitis) | Rat | Distal colon | Immunohistochemistry | Increased TRPM2 expression | (73) |
| **Functional data** | | | | | |
| Colon (TNBS- colitis) | Rat | Distal colon | Colorectal distention | Treatment with TRPM2 antagonist restores VHS | (73) |

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**Table 5:** Implications of TRPM8 in the pathophysiology of visceral hypersensitivity in FGID

| Tissue (disease) | Species | Tissue or cell type | Technique | Result | Reference |
|---|---|---|---|---|---|
| **Expression profiles** | | | | | |
| Colon (IBD, TNBS and DSS colitis) | Human and mice | Colonic tissue | RT-qPCR | Upregulation TRPM8 | (93) |
| Colon (TNBS and DSS colitis) | Mice | Colonic tissue | Immunohistochemistry | Increased TRPM8 expression | (50) |
| **Functional data** | | | | | |
| Colon (IBS) | Human | | Symptom questionnaires | Treatment with TRPM8 agonist decreases IBS symptoms such as abdominal pain and increases quality of life | (23; 24; 62; 76) |
| Colon (TNBS and DSS colitis) | Mice | | Evaluation of pain-related behavior | Treatment with TRPM8 agonist increases visceral pain | (50) |
| Colon | Mice | Serosal and mesenteric afferent nerves | Afferent nerve recording | TRPM8 activation desensitizes TRPV1 and TRPA1 | (48) |
| Colon (TNBS and DSS colitis) | Mice | Colonic tissue and HEK-293 cells | Calcium imaging | TRPM8 agonist has anti-inflammatory effect and inhibits capsacin-induced responses | (93) |

| | | | | |
|---|---|---|---|---|
| Colon (TNBS post-inflammatory VHS) | Rats | Colorectal distention | TRPM8 agonists decrease post-inflammatory VHS | (3) |

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**Table 6: GPCR signaling-mediated sensitization of TRP channels**

| | Receptor (Ligand) | Signaling mechanism | Reference |
|---|---|---|---|
| **TRPV1** | $BK_1$ (bradykinin) | PKC | (107; 109) |
| | PAR-2 (protease) | PLC, PKC, PKA | (30) |
| | $Hrh_1$ (histamine) | PLC, PKC | (58; 128) |
| | PGR (prostaglandin $E_2$) | PKA | (51; 95) |
| | TrkA (NGF) | PI3, Src kinase | (55; 137) |
| | $5HT_2R$, $5HT_4R$ (serotonin) | PKA, PKC | (106) |
| **TRPV4** | PAR-2 (protease) | PKA, PKC | (43) |
| | $Hrh_1$ (histamine) | PKC, PLC, $PLA_2$, MAPK | (11; 28) |
| | $5HT_3R$ (serotonin) | PKC, PLC, $PLA_2$, MAPK | (28) |
| | $BK_2$ (bradykinin) | PLC, PKC | (35) |
| **TRPA1** | $BK_2$ (bradykinin) | PLC, PKA | (97; 120) |
| | PAR-2 (protease) | PKA, PKC, PLC, $PIP_2$ | (30; 32) |
| | $Hrh_1$ (histamine) | - | (11) |
| **TRPM8** | PGR (prostaglandin $E_2$) | PKA | (71) |
| | $BK_2$ (bradykinin | PKC | (88) |
| | $5HT_{1B}R$ (serotonin) | PLD, $PIP_2$ | (117) |

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## Figure Legends

**Figure 1:** GPCR mediated TRP channel sensitization contributing to visceral hypersensitivity

Activation of nociceptive Gαs-linked receptors such as bradykinin receptor ($BK_1$), 5-hydroxytryptamine receptor ($5HTR_4$), histamine receptor ($Hrh_2$), prostaglandin receptor (PGR), results in the production of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (AC). This leads to an increase of protein kinase C (PKC) activity resulting in TRP channel sensitization by phosphorylation. On the other hand, activation of nociceptive Gαq-linked receptors such as bradykinin receptor ($BK_2$), protease-activated receptor 2 (PAR-2), 5-hydroxytryptamine receptor ($5HTR_2$), histamine receptor ($Hrh_1$), prostaglandin receptor (PGR), leads to the production of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$) by phospholipase C (PLC), resulting in protein kinase A (PKA) activation which leads to TRP channel phosphorylation and sensitization. In parallel, Gαq activates phospholipase A2 ($PLA_2$), leading to the production of arachidonic acid (AA) and downstream polyunsaturated fatty acids (PUFAs) that can directly activate TRP channels. Activation and sensitization by phosphorylation of TRP channels, contribute to aberrant pain perception and visceral hypersensitivity (VHS).

Activation of Gαi-linked receptors by resolvins inhibits adenylyl cyclase, with subsequent down-regulation of PKA antagonizing Gαs-mediated sensitization. Legend: $PIP_2$: phosphatidylinositol 4,5-bisphosphate, P: phosphate, $Ca^{2+}$: Calcium, $Na^{+}$: Natrium, TRP: transient receptor potential, VHS: visceral hypersensitivity, GPCR: G-protein coupled receptor

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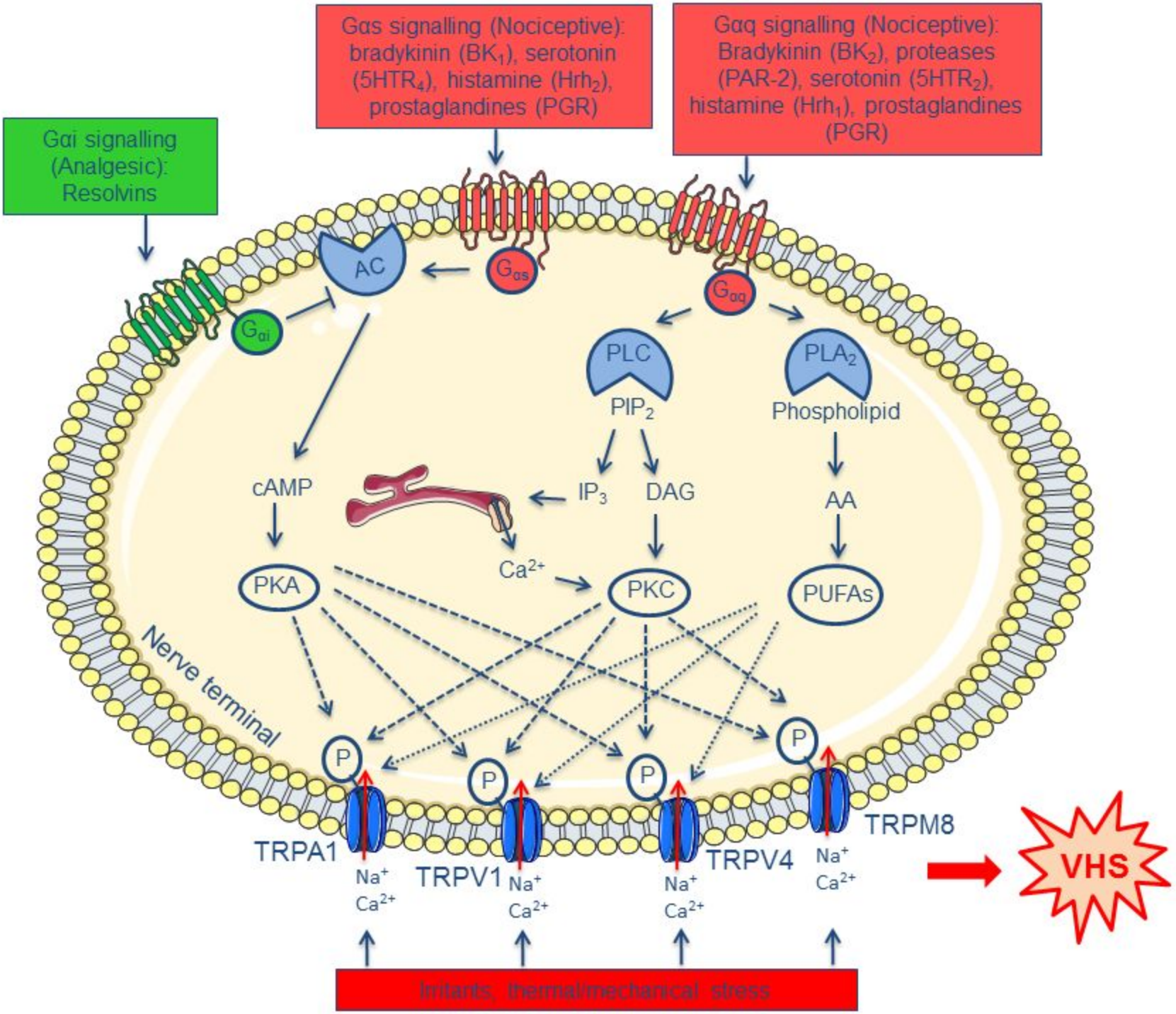
Gαs signalling (Nociceptive): bradykinin ($BK_1$), serotonin ($5HTR_4$), histamine ($Hrh_2$), prostaglandines (PGR)
Gαq signalling (Nociceptive): Bradykinin ($BK_2$), proteases (PAR-2), serotonin ($5HTR_2$), histamine ($Hrh_1$), prostaglandines (PGR)
Gαi signalling (Analgesic): Resolvins
AC
$G_{\alpha s}$
$G_{\alpha q}$
$G_{\alpha i}$
PLC
$PIP_2$
$PLA_2$
Phospholipid
cAMP
$IP_3$
DAG
AA
$Ca^{2+}$
PKA
PKC
PUFAs
Nerve terminal
P
TRPA1
TRPV1
TRPV4
TRPM8
$Na^+$
$Ca^{2+}$
VHS
Irritants, thermal/mechanical stress