Chapter

Intervention of PAR-2 Mediated CGRP in Animal Model of Visceral Hyperalgesia

Manoj Shah

Abstract

Protease-activated receptor-2 (PAR-2) mediates calcitonin gene-related peptide (CGRP) release and collectively plays a crucial role in inflammation-induced visceral hyperalgesia (VH). The present review chapter outlines the substantial advances that elucidated the underlying role of PAR-2 and CGRP in gut inflammation-induced VH and highlights their relevancies in the management of VH. PAR-2 is expressed in a wide range of gastrointestinal cells and its activation on primary afferent nerves by tryptase, trypsin or cathepsin-S is the key mechanism of sensitization during intestinal inflammation. The activated PAR-2 sensitizes transient receptor potential vanilloid subtype-1 receptors and triggers the release of substance-P (SP) and CGRP that are involved both in the transmission and modulation of VH. Approximately, two-thirds of sensory neurons express PAR-2 and 40% of the PAR-2-expressing sensory neurons also express SP and CGRP. Accumulating set of experiments devised that the blockade or antagonism of PAR-2 in inflammatory diseases of the gut depicts double advantages of reducing inflammation and VH. Simultaneously, the uses of CGRP-antagonists inhibit VH and completely suppress PAR-2-agonists-induced intestinal inflammation in animals. However, further study is imperative to improve our understanding of the blockade or antagonism of PAR-2 and CGRP release before its implication as a novel therapeutic for the clinical management of VH in human patients.

Keywords: PAR-2, CGRP, intestinal inflammation, visceral hyperalgesia, activation

1. Introduction

Visceral hyperalgesia (VH) is a pathological state of inflammatory bowel diseases (IBDs) and irritable bowel syndrome (IBS) or other functional bowel disorders, in which sensory threshold for abdominal pain and discomfort decreases due to tissue injury, inflammation, and persistent exposure of tissues/organ to noxious stimuli. In this state, the continuous release of inflammatory mediators results in sensitization of primary afferents and abdominal pain, both during the acute flare of diseases and their remission [1, 2]. Despite several proposed factors including inflammation, psychology and aberrant sensory-motor function of the gut contribute to peripheral and central sensitization [3], the exact underlying mechanism of VH has not been

fully elucidated. The cell-membrane protease-activated receptor-2 (PAR-2) mediates calcitonin gene-related peptide (CGRP) release, and their associated roles in neurogenic inflammation-induced sensitization could be of great interest for the researchers to address this persistent nature of VH.

A G-protein coupled receptor PAR-2, distributed throughout the gastrointestinal (GI) tract, is activated particularly by proteases such as tryptase, trypsin, and cathepsin-S [4–6]. PAR-2 activation on several cells (epithelial cells, endothelial cells, neutrophils, macrophages, monocytes, mast cells, fibroblasts, neurons, dendritic cells, lymphocytes, etc.) could lead to the release of cytokines, chemokines, prostaglandins [7], as well as CGRP and substance-P (SP) in the enteric neurons and afferent neurons [8, 9]. Numerous reports indicated the diverse SP and CGRP expressions within the dorsal root ganglia (DRG) and spinal neurons during colitis and ileitis [10–15]. The expressions of SP and CGRP within the gut not only excite extrinsic afferents but also perpetuate the central transmission of nociceptive traffic between afferent neurons and higher-order neurons in the spinal cord and brainstem [16]. Thus, it is worthwhile to consider the key role of PAR-2 in the release of CGRP, which subsequently triggers neurogenic inflammation mediated VH.

Currently, the pharmacotherapy for VH is unsatisfactory because of its unknown precise mechanism. Earlier study suggests that the blockade of PAR-2at the periphery and/or the inhibition of luminal protease activity may be of interest for treating the VH [17]. Likewise, the administration of CGRP antagonists inhibits VH in animals [18, 19]. Therefore, the blockade or antagonism of either PAR-2 or CGRP may be a promising therapeutic target for VH. This review chapter explores the important roles of PAR-2 and PAR-2-mediated CGRP during inflammatory gut and their antagonism or blockade for the treatment of VH.

2. PAR-2 activation in the gastrointestinal tract

PAR-2 is activated through proteolytic cleavage by specific serine proteases, such as trypsin and mast cell (MC)-tryptase [4] and lysosomal macrophagic cysteine protease cathepsin-S [5, 6]. PAR-2 is generally expressed in the basolateral and apical side of epithelial cells [20], fibroblasts, MCs, smooth muscle cells, endothelial cells of the GI tract [21], enteric sensory neurons, terminals of mesenteric afferent nerves, and immune cells [17]. The higher number of mast cells and mast cell tryptase in biopsied colonic tissues enhanced the PAR-2 activity to regulate CGRP, SP, and VIP expressions resulting in symptoms associated with IBD [22]. Recently, Hassler et al. [23] suggested that PAR2-expressed sensory neurons are a key target for mechanical and spontaneous pain triggered by the release of endogenous proteases from the many immune cells. In-vitro study exhibits the up-regulation of PAR-2 expression in cultured endothelial cells of human umbilical vein treated with TNF-α, IL-1α, and bacterial lipopolysaccharide in a dose-dependent manner [24]. Therefore, it is important to note that PAR-2 activation on intestinal immunocytes induces acute enteritis [9, 25] while its neuronal expression incites neurogenic inflammation [26, 27].

2.1 Role of PAR-2 in inflammation

PAR-2 seems essential in the interplay between nerves, immunocytes, MCs, and epithelial cells within the luminal wall during GI diseases [17]. Histopathologically, PAR-2-agonists (SLIGRL) induced acute colitis has been observed with erythema,

granulocyte infiltrations and thickened colonic wall [25, 28], the colonic tissue sampled from the PAR-2 knockout mice that are infused intracolonically with 2,4,6-trinitrobenzene sulfonic acid (TNBS) showed lower myeloperoxidase activities, microscopic- and macroscopic-damage scores [29]. Mediators such as intracellular- and vascular cell adhesion-molecule-1 were decreased while cyclooxygenase-1 was increased in the PAR-2 knockout mice, which clearly confirms the pro-inflammatory role of PAR-2. Notably, PAR-2, inactive during colitis, has been expressed for inducing VH after resolution of colitis [30]. Furthermore, PAR-2 has also been over-expressed in biopsies obtained from ulcerative colitis (UC) and CD patients, which strongly suggests its intricate role in IBDs [31–33].

2.2 Effects of PAR-2 on gastrointestinal functions

PAR-2 modulates GI functions, such as motility, ionic exchange, paracellular permeability, sensory functions, and inflammation [34]. The excitatory, as well as inhibitory actions of PAR-2-agonists on isolated smooth muscles, have been devised earlier [35, 36]. In-vitro, PAR-2 activation shows a region-specific role because it enhances the contractibility of gastric smooth muscles and reduces the contractility of circular and longitudinal colonic smooth muscles in mice [35, 37]. However, the intraperitoneal administration of PAR-2-agonists accelerated GI transit in mice [38]. Moreover, Mall et al. (2002) reported that PAR-2 activation on the enterocytes triggers intestinal water secretion through a direct cellular mechanism, while Kong et al. [20] described the same by a prostaglandin E2-dependent mechanism. Additionally, activated PAR-2 stimulates mucus secretion by a nerve-mediated mechanism [39]. It weakens the intestinal barrier, resulting in an increased passage of fluids or even microorganisms across the gut mucosa. The intracolonic administration of PAR-2-agonist in mice increases colonic permeability and results in a general inflammatory response [25, 34].

3. CGRP-receptors and their distribution

CGRP-receptor is a heterotrimeric complex, composed of calcitonin receptor-like receptor (CLR), receptor activity-modifying protein-1, and a small intracellular protein component and receptor component protein. CLR, a classical G-protein linked receptor, couples through adenylyl cyclase [40]. CGRP is expressed throughout the peripheral and central nervous systems (CNS). Of the two forms, α-CGRP is mainly expressed in the CNS, especially in striatum, amygdalae, hypothalamus, colliculi, brainstem, cerebellum, and trigeminal complex [41–43], while β-CGRP is primarily expressed in the enteric neurons and vascular smooth muscle cells [44, 45]. Interestingly, α-CGRP is also found to be expressed in primary spinal afferent C- and Aδ-fibers [46].

The majority of spinal afferents innervated into the GI tract express CGRP and SP [47]. CGRP has been reported to be expressed markedly higher in the lumbosacral DRG and spinal cord dorsal horn (SCDH) during visceral inflammation [11, 48]. Zhang et al. [49] confirmed the absence of secondary hyperalgesia in the mice missing α-CGRP expression in the CNS. The SP and CGRP released from afferent terminals lead to neurogenic inflammation at the peripheral sites, resulting in MCs degranulation, plasma extravasation, and arteriolar vasodilation [50]. CGRP causes vasodilatation via its receptors on the smooth muscle cells at peripheral synapses. However, at

central synapses, it acts postsynaptically on the second-order neurons to transmit pain via the brainstem and midbrain to higher cortical pain regions [51].

3.1 CGRP modulates mast cell functions

CGRP is secreted from non-myelinated C-fibers and thinly myelinated Aδ-fibers originating from DRG neurons [52]. Sun et al. [53] showed peak CGRP levels in the colonic tissues, spinal cord, and hypothalamus of rats with IBS, and its correlation with VH. Our earlier studies also demonstrated the remarkably higher CGRP expression in DRG and spinal cord that was correlated with VH in the TNBS-induced ileitis rats and goats, respectively [13, 15]. Therefore, CGRP and CGRP-receptors are found to be involved in the transmission and modulation of pain in the periphery and CNS [54, 55].

MCs that reside near the nerve fibers are true candidates for modulating neural activity and nociception [56]. The mediators such as SP, CGRP, vasoactive intestinal protein (VIP), dopamine, and arachidonic acid are able to influence MCs activation. The aforementioned mediators act on nociceptors, send signals to the CNS, and cause the simultaneous central release of SP and CGRP [57], which further activate MCs, and create a bidirectional positive feedback-loop for resultant neurogenic inflammation [58].

3.2 CGRP-release mediated by PAR-2

Activated PAR-2 sensitizes Transient Receptor Potential Vanilloid subtype-1 receptors (TRPV-1) and triggers the release of sensory CGRP and SP [59]. CGRP and SP released from intestinal afferent terminals cause vascular dilatation, plasma extravasation, granulocyte infiltrations, and neurogenic inflammation [8, 9, 60]. An earlier study [8] reported that PAR-2-agonists-induced edema was entirely mediated by the release of SP and CGRP from sensory neurons and further activation of neurokinin-1 (NK-1)- and CGRP-receptors on endothelial cells. In DRG, PAR-2 co-expresses with TRPV-1, TRPV-4, TRPA-1 (Transient Receptor Potential Cation Channel, Subfamily-A, Member-1), SP and CGRP [8, 61, 62]. It is also reported that 63% of sensory neurons express PAR-2 and up to 40% of them express both SP and CGRP [8]. Activated PAR-2 transmits C-fiber afferent input to the SCDH for the release of excitatory amino acids and neuropeptides from the central terminals [63].

3.3 Role of CGRP in sensitization

Afferent fibers innervating the gut vessels have cell bodies in the DRG. These fibers are peptidergic, containing both CGRP and SP, and have collaterals in enteric ganglia, mucosa, muscularis externa, and sympathetic prevertebral ganglia [64]. SP, CGRP, VIP, and somatostatin act as mediators of neurogenic inflammation in IBDs [65–67]. After stimulation, TRPV-1 depolarizes sensory neurons either directly or indirectly to initiate the release of these neuropeptides from the afferent terminals [68]. TRPV-1-positive nerve fibers co-express with SP, NK-1, and CGRP in mucosa, submucosal layer, deep muscular plexus, circular muscle, myenteric plexus, and longitudinal muscle layer in the rectum and colon of mice [69]. CGRP which is expressed largely in splanchnic afferents and CGRP-immunoreactivities from the GI tract disappears with capsaicin treatment [70]. Interestingly, about 50% of CGRP-immunoreactive extrinsic afferent neurons express SP- or NK-1-immunoreactivities [71] and their expressions fluctuate during

colitis [72]. The earlier decrease of the above neuropeptides may be due to their depletion from the peripheral nerve terminals or the damaged nerves at the initial inflammatory stage. CGRP and SP increase during inflammation or afferent nerve stimulation. TNBS-induced colitis/ileitis and or colorectal distension (CRD) results in higher expression of neural activation markers (such as c-Fos, pERK) as well as releases of SP and CGRP in the SCDH that are commonly linked with pain signaling [15, 73, 74].

Plourde et al. [75] confirmed the role of CGRP in pain modulation because intravenously administered CGRP-1-receptor-antagonists (h-CGRP8-37) reversed the sensitization provoked by infusion of intracolonic acetic acid. SP and CGRP may either increase the peripheral sensory gain of extrinsic afferents within the gut or contribute to primary afferent transmission within the CNS [16, 76]. Despite irritation, immune challenge and inflammation cause the release of CGRP and SP from extrinsic afferents and intrinsic neurons within the gut [45, 77], the precise site at which CGRP-receptor and NK-1 mediate visceral pain is not known.

4. Role of PAR-2 in VH

PAR-2 activation in GI resident cells such as MCs, macrophages, or neutrophils induces the release of tiny amounts of inflammatory mediators that sensitizes primary afferents. It regulates vascular tone and causes immense pro- or anti-inflammatory as well as pro-nociceptive effects in somatic or visceral pain [78]. PAR-2 expressed at the peripheral afferent neurons is more importantly involved in inflammation-induced VH [29, 30]. The glial cells of the enteric nervous system play pivotal roles in neuroimmune interactions and modulate enteric neurotransmission, inflammation, and intestinal barrier functions as they express receptors for purines and contain precursors for neurotransmitters such as GABA and NO. They can produce cytokines (TNF-α, IL-1β, IL-6), NGF, and neuropeptides (NK-1, SP, CGRP) after their activation. Both PAR-2 and proinflammatory cytokines impair the epithelial barrier by decreasing tight junction protein expression and consequently facilitate the entry of luminal aggressors perpetuating inflammation and pain [9].

PAR-2 expressed in enterocytes increases permeability, which is linked with the immune activation and generation of VH [25, 79]. It is found that PAR-2-agonists evoke the transient depolarization of submucosal enteric neurons with long-lasting hyperexcitability in guinea pigs [80]. Similarly, intracolonically administered PAR-2 agonist (SLIGRL-NH2, 100 μg/mouse) increased intestinal permeability and VH in mice [81]. The intracolonic administration of sub-inflammatory doses of PAR-2-agonist led to prolonging the VH in response to CRD in rats [78]. PAR-2 activation on enteric neurons is also directly responsible for the development of VH as it conveys nociceptive signals for the excitability of submucosal neurons, colonic projections of DRG, and jejunal afferent neurons [7]. Shi et al. [82] reported PAR-2 activation and higher CGRP levels in the serum and colonic tissue during VH in a rat model of IBS. Accumulating set of evidence suggests that protease activity is remarkably prominent in diarrheic-IBS and UC patients. The fecal supernatant or colonic biopsies from these patients when infused intracolonally into rodents resulted in higher intestinal permeability, mucosal inflammation, and subsequent VH through a PAR-2 activation mechanism [83–86], while the same treatment failed to cause the VH in the PAR-2 knockout mice [83]. **Table 1** summarizes the findings of preclinical studies that intervened in the effects of PAR-2 on underlying VH.

PAR	Agonist/ antagonist	Species (hypersensitivity model)	Study type	Effects	Ref.
PAR-2	Agonist (SLIGRL-NH2)	Mice (PAR2-agonist)	In vivo	↑ hyperalgesia	[39]
PAR-2	Agonist (SLIGRL-NH2, Tc-NH2, trypsin, tryptase)	Mice, rat (PAR-2-agonist)	KO	↑ hyperalgesia, absent in KO mice	[87]
PAR-2	Agonist (SLIGRL-NH2, trypsin)	Rat (PAR2-agonist)	In-vivo	↑ hyperalgesia	[78]
PAR-2	PAR-2 agonists (trypsin, tryptase, and a selective PAR-2-activating peptide)	Mice received intracolonically PAR-2 agonists	KO	Colonic administration of PAR-2 agonists up-regulated PAR-2 expression and induced colonic inflammatory reaction and permeability.	[25]
PAR-2	Agonist (SLIGR)	Intracolonic infusion to mice	In-vivo	Colonic inflammation and enhanced colonic permeability, while the intravenous injection of CGRP antagonist, i.e., CGRP (8–37) prevented PAR-2 induced colonic inflammation.	[9]
PAR-2	Agonist (SL-NH2, trypsin, tryptase)	Guinea pig submucosal neurons (PAR-2-agonist)	Ex-vivo	↑ neuron excitability	[80]
PAR-2	Agonist (SLIGR)	Intracolonic infusion of SLIGR (5 and 100 μg per mouse)	In-vivo	At lower dose, SLIGRL increased colonic permeability while higher dose resulted in colonic inflammation	[79]
PAR-2	Agonist (2-furoyl-LIGRL-NH2)	Mice (capsaicin)	KO	↑ hyperalgesia, absent in KO	[88]
PAR-2	Antagonist (ENMD-1068)	Mice (IBS-supernatant)	KO	↓ hypersensitivity, absent in KO	[83]

PAR	Agonist/ antagonist	Species (hypersensitivity model)	Study type	Effects	Ref.
PAR-2	PAR-2 deficient	TNBS- and dextran sodium sulfate-induced colitis in mice	KO	Endogenous PAR-2 activation controls leukocyte recruitment in the colon and thus possesses a new potential therapeutic target for the treatment of IBD.	[29]
PAR-2	PAR-2 activation	TNBS-induced colitis rats	In-vivo	PAR-2 activation resulted in colitis and VH	[30]
PAR-2	Mediators from colonic biopsies of diarrhea-predominant IBS patients	Mice DRG (IBS-D supernatant)	KO	↑ neuron excitability, absent in KO mice	[89]
PAR-2	Colono-scopic biopsies	IBS-D and IBS-C patients	In-vivo	Elevated PAR-2 expression to regulate the expression of CGRP, VIP and SP resulting in symptoms associated with IBD	[22]
PAR-2	IntracolonicPAR-2 agonist (SLIGRL-NH2, 100 μg/mouse)	PI-IBS Mouse Model	In-vivo	↑ intestinal permeability and VH	[81]
PAR-2	PAR-2 activation	TNBS-induced post-inflammation irritable bowel syndrome (PI-IBS) rats	In--vivo	↑ visceral hypersensitivity	[90]
PAR-2	PAR-2 activation	TNBS-induced ileitis goat	In-vivo	↑ visceral hypersensitivity	[15]

Abbreviations: DRG, dorsal root ganglia; IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome; KO, Knock-out; PAR-2, Protease-activated receptor-2.

Table 1.
Preclinical studies investigating the effects of protease activated receptor-2 on visceral hyperalgesia.

Currently, the role of cathepsin-S is considered insightful because it activates spinal nociceptive neurons through a PAR-2-dependent mechanism and amplifies VH. Over the years, studies reported that cathepsin-S released from spinal microglial cells during nerve injury or colitis secretes fractalkine, thereby intensifying and maintaining the chronic pain [91, 92].

5. Role of PAR-2 in pain transmission

Proteases directly activate PAR-2 as well as assist other pronociceptive mediators for the subsequent sensitization of afferent fibers [83]. **Figure 1** illustrates the important role of PAR-2 in pain transmission during GI disorders. PAR-2 activation on afferent neurons leads to specific calcium signals that could participate in conveying pain messages [93]. Elmariah et al. [6] reported that cathepsin-S played a role in molecular signaling either alone or together with activated PAR-2. Activation of PAR-2 on DRG by its agonists enhances potassium chloride ions and the capsaicin (TRPV-1 agonist)-evoked release of CGRP [8, 94]. Protease-activated receptor-1 and PAR-2 on enteric afferent fibers facilitate nociceptive input to the CNS, while spinal PAR-2 activation aggravates pain behaviors [21]. These findings strongly suggest that visceral activation of PAR-2 has an important role in sensitizing the second-order neurons at spinal level.

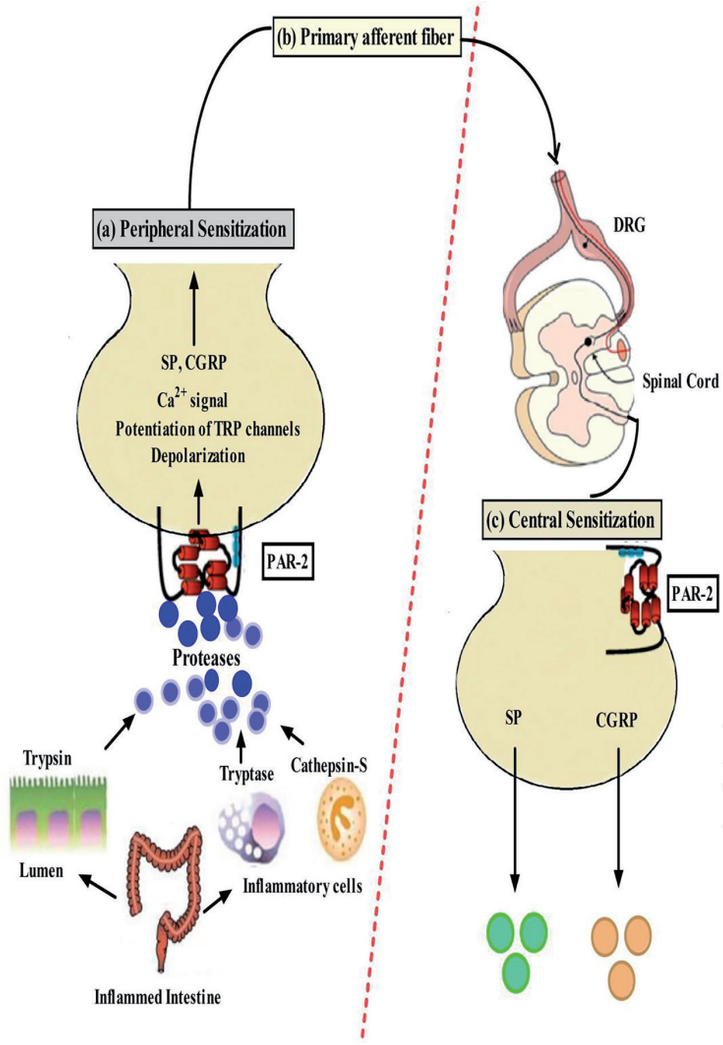

Figure 1.
Role of PAR-2 in pain transmission. (a) Peripheral sensitization. PAR-2 is activated by proteases released from inflammatory and immune cells as well as from mediators of the intestinal lumen. Proteases sensitize neurons to innocuous stimuli. After stimulation, TRPV-1 depolarizes sensory neurons either directly or indirectly to initiate the release of SP and CGRP from the afferent terminals. PAR-2 activation on afferent neurons leads to specific calcium signals. (b) Primary afferent fiber. Pain signal is transmitted along primary afferent fibers to the spinal dorsal horn and subsequently to the brain. (c) Central sensitization. Persistent small-afferent input leads to a central sensitization associated with local release of SP and CGRP. PAR-2, protease-activated receptor-2;TRP, Transient Receptor Potential; Ca^{2+}, calcium ion; SP, substance-P; CGRP, calcitonin gene-related peptide;TRPV-1, Transient Receptor Potential Vanilloid subtype-1.

6. Therapies targeting PAR-2 and CGRP for VH

Researchers have come a long way in terms of understanding and controlling the inflammation-induced VH in experimental animals. An overview of the studies described in the following paragraph is shown in **Table 2**. It is worth mentioning that the oral administration of PAR-2-antagonists (GB88) ameliorates acute and chronic colitis induced by PAR-2-agonists and TNBS, respectively, in rats [28]. Several studies have demonstrated that protease inhibitors and PAR-2-antagonists relieve the inflammation and resultant VH in animals [78, 83, 86, 87, 95, 96, 102]. In chronic inflammation and pain syndromes, the blockade of PAR-2 inhibits both pain signals and inflammatory responses [7]. The intraperitoneal administration of PAR-2 antagonist (FSLLRY-NH2, 3 mg/kg daily for 5 days) reversed intestinal permeability and also attenuated VH in PI-IBS mice which confirms the therapeutic potential of PAR-2antagonist in VH [81].

Studies utilizing both CGRP knockout mice and antagonist hCGRP8-37 have confirmed the protective role of CGRP in colitis and devised its insightful roles in hyperalgesia [18, 97, 98, 103]. Intravenously administered hCGRP8-37 attenuated distension-evoked pain responses and completely reversed the sensitization effects in acetic acid-induced acute colitis rats [18]. Julia and Bueno [99] reported that hCGRP8-37 also suppressed the pain in rats provoked by intraperitoneal injection of acetic acid. Furthermore, its intrathecal administration reversed the CGRP expressions and alleviated the VH in both acetic acid-induced acute and TNBS-induced chronic colitis rats [18, 19]. Recently, Noor-Mohammadi et al. [101] reported that the single dose of intraperitoneally administered anti-CGRP, i.e., $F(ab')_2$ fragment antibody attenuated the stress-induced colonic hypersensitivity in rats which confirms the prevailing role of CGRP in persistent visceral pain.

Nowadays, alternative therapies have been attracting attention due to their potential in the treatment of VH. Sun et al. [53] described that electroacupuncture (EA) attenuates VH in rats with diarrheic-IBS by suppressing spinal CGRP. EA therapy also alleviated the VH symptoms through downregulation of the PAR2, SP, and CGRP levels in colon tissues in post-inflammation-IBS rats [90]. Likewise, Deng et al. [100] exhibited that the EA at ST-37 and ST-25 relieved the VH in IBS rats by decreasing the number of MCs and suppressing the expression of PAR-2, TRPV1, CGRP, SP and Try proteins in the colonic tissues. Our recent study also reported the effectiveness of repetitive EA for treating both acute and chronic pain because it down-regulated the PAR-2-mediated CGRP release in the spinal cord [15]. Shi et al. [82] administered Shugan decoction (herbal extracts) intragastrically in rats in IBS model and found that it abolished VH by attenuating the release of PAR-2-mediated CGRP.

7. Conclusions

GI tract is the organ that is exposed frequently to proteases both during physiological and pathophysiological conditions. Besides degradative enzymatic roles, the proteases also act as signaling molecules in various gut diseases. Understanding the exact mechanism of VH is pivotal to identifying the novel efficacious therapy for IBDs. PAR-2 activation by tryptase, trypsin, and cathepsin-S causes the release of CGRP and SP in extrinsic primary afferent fibers and intrinsic enteric neurons [45, 77]. Both CGRP and SP facilitate the excitation of extrinsic afferents as well as participate in the

Targeted substances	Antagonist/ inhibitors	Species (VH model)	Study type	Effects	Ref.
PAR-2	PAR-2 antagonist (GB88)	PAR-2-agonists and TNBS-induced colitis rats	In-vivo	Acute and chronic colitis ↓	[28]
PAR-2	PAR-2 gene deletion	Paw inflammation in Rats and Mice	In-vivo	Hyperalgesia ↓	[87]
PAR-2	PAR-2 agonist (SLIGRL-NH2)	PAR-2 agonist induced colitis and VH	In-vivo	Increased intestinal permeability and the activation of NK_1 receptors.SLIGRL-NH2 induced hyperalgesia was inhibited by a NK_1 receptor antagonist (SR 140333).	[78]
PAR-2	PAR-2 agonist	Intrapancreatic administration of PAR-2	In-vivo	PAR-2 expression in all thoracic DRG. Increased c-FOS expression and pain behaviors.	[95]
PAR-2	PAR-2 antagonist PAR-2 knockout	Colonic biopsy from IBS patients	In-vivo	Supernatants from colonic biopsies of IBS patients showed VH. Serine protease inhibitors and a PAR-2 antagonist inhibited VH. However, VH was absent in PAR-2 knockout mice.	[83]
Protease	Fecal protease	Fecal proteases from IBS-D patients	In-vitro	Increased fecal protease and amylase in patients with IBS-D.	[86]
PAR-2	Serene protease inhibitor and PAR-2 antagonist Knockout	Fecal supernatant from IBS-D patients infused into the colon of mice	In-vivo	Increased VH in mice infused with fecal supernatant while VH was suppressed in mice infused with intracolonic serene protease inhibitor and PAR-2 antagonist.	[96]
PAR-2	PAR-2 antagonist (FSLLRY-NH2, 3 mg/kg daily intraperitoneally for 5 days)	PI-IBS Mouse Model	In-vivo	Intestinal permeability and VH ↓	[81]
CGRP	Intravenous antagonist CGRP [human CGRP-(8–37) Intrathecal administration of hCGRP-(8–37) (mid-lumbar)	Acetic acid induced colitis Intravenous CGRP to induce VH	In-vivo	VH ↓	[18]
CGRP	CGRP antagonist (h-CGRP 8–37)	TNBS-induced colitis	In-vivo	VH ↓	[97]

Targeted substances	Antagonist/ inhibitors	Species (VH model)	Study type	Effects	Ref.
CGRP	Mutant mice lacking α-CGRP or β-CGRP expression	DSS induced colitis	In-vivo	α-CGRP and β-CGRP play a protective role in the generation of spontaneous colitis, supporting a role for both extrinsic and intrinsic CGRP-containing neurons.	[98]
CGRP	CGRP antagonist (hCGRP8–37)	Intraperitoneal acetic acid-induced VH	In-vivo	VH ↓	[99]
CGRP	CGRP antagonist (hCGRP8–37)	TNBS-induced acute colitis rats	In-vivo	Intrathecal administration of hCGRP8–37 reversed the CGRP expressions and alleviated the VH.	[19]
CGRP	EA	Chronic and acute stressed rats with IBS-D	In-vivo	EA attenuates VH in rats with IBS-D through suppressing spinal CGRP.	[53]
CGRP	Shugan decoction (herbal extracts)	A rat model of IBS induced by chronic water avoidance stress	In-vivo	Intragastrically administered Shugan decoction abolished VH by attenuating the PAR-2 and CGRP.	[82]
PAR-2 and CGRP	EA at ST-37 and ST-25	A rat model of IBS induced by chronic water avoidance stress	In-vivo	Attenuation of VH attributed due to decreasing number of MCs and down-regulation of PAR-2, TRPV1, CGRP, SP and Try proteins in the colonic tissues.	[100]
PAR-2 and CGRP	EA at ST-25 and ST-37	TNBS instilled into anus to induce post-inflammation visceral hypersensitivity	In-vivo	EA alleviated visceral hypersensitivity symptoms through downregulation of the PAR-2, SP and CGRP in colonic tissues in post inflammation-IBS rats.	[90]
PAR-2 and CGRP	EA at ST-36	TNBS-induced ileitis	In-vivo	Repetitive EA therapy attenuated visceral hypersensitivity through the suppression of spinal PAR-2 and CGRP in goats.	[15]
CGRP	$F(ab')_2$ fragment antibody (30 mg/kg intraperitoneally)	Chronic Adult Stress in rats Induced by Water Avoidance Stress	In-vivo	A single dose of $F(ab')_2$ fragment antibody inhibited stress-induced colonic hypersensitivity	[101]

Abbreviations: PAR-2, protease-activated receptor-2;TNBS, 2,4,6-trinitrobenzene sulfonic acid; DRG, dorsal root ganglia; NK-1, neurokinin-1;IBS, irritable bowel syndrome; IBS-D, irritable bowel syndrome with diarrhea; CGRP, calcitonin gene-related peptide; α-CGRP, alpha-calcitonin gene-related peptide; β-CGRP, beta-calcitonin gene-related peptide; VH, visceral hyperalgesia; EA, electroacupuncture.

Table 2.
Preclinical studies targeting the antagonism or blockade of PAR-2 and CGRP as a therapeutic strategy for the management of inflammation and visceral hyperalgesia.

central transmission of nociceptive traffic between afferent neurons and higher-order neurons in the spinal cord and brainstem. The blockade and/or antagonism of PAR-2 and CGRP release can effectively relieve VH in IBDs, IBS, or other functional bowel disorders. Further research is required to deepen our understanding of the blockade or antagonism of PAR-2 or CGRP before these potential therapies can be clinically translated for the management of VH in humans.

Author contributions

The author confirms sole responsibility for the conception, drafting, revision, and the approval of final review chapter.

Conflict of interest

The author declares no conflict of interest.

Abbreviations

CGRP	calcitonin gene-related peptide
CNS	central nervous system
CRD	colorectal distension
DRG	dorsal root ganglia
GI	gastrointestinal
IBD	inflammatory bowel diseases
IBS	irritable bowel syndromes
MC	mast cell
NK-1	neurokinin-1
PAR-2	protease-activated receptor-2
SCDH	spinal cord dorsal horn
SP	substance-P
TNBS	2,4,6-trinitrobenzene sulfonic acid
UC	ulcerative colitis
VH	visceral hyperalgesia
VIP	vasoactive intestinal protein

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Author details

Manoj Shah
Department of Surgery and Pharmacology, Agriculture and Forestry University, Rampur, Nepal

*Address all correspondence to: mkshah@afu.edu.np

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