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Protease-Activated Receptors as Therapeutic Targets in Visceral Pain

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Abstract: The protease-activated receptors (PARs) play a pivotal role in inflammatory and nociceptive processes. PARs have raised considerable interest because of their capacity to regulate numerous aspects of viscera physiology and pathophysiology. The present article summarizes research on PARs and proteases as signalling molecules in visceral pain. In particular, experiments in animal models suggest that PAR_2 is important for visceral hypersensitivity. Moreover, endogenous PAR_2 agonists seem to be released by colonic tissue of patients suffering from irritable bowel syndrome, suggesting a role for this receptor in visceral pain perception. Thus, PARs, together with proteases that activate them, represent exciting targets for therapeutic intervention on visceral pain.

Keywords: Calcium channels, IBD, IBS, neuromediators, Protease, Pain, Inflammation, Protease activated receptor.

1. INTRODUCTION

Normal individuals do not perceive sensory signals coming from their viscera, however once visceral afferents become hypersensitive and the patients then perceive pain. This hypersensitization occurs in several diseases such as functional bowel syndromes or in diseases associated with inflammation such as inflammatory bowel disease and pancreatitis. Unexplained abdominal pain accounts for 40% of gastroenterology practice in the United Kingdom. Mostly abdominal pain is due to functional gastrointestinal disorders; irritable bowel syndrome, and functional dyspepsia [1]. Visceral pain is defined as nociceptive inputs that result from the activation of nerve terminals of the thoracic, pelvic, or abdominal viscera (organs). Visceral structures are highly sensitive to distension (stretch), ischemia and inflammation, but relatively insensitive to other stimuli that normally evoke somatic pain such as cutting or burning. Visceral pain is diffuse, difficult to localize and often referred to a distant, usually superficial, structure. It may be accompanied by symptoms such as nausea, vomit, changes in vital signs as well as emotional manifestations. One of the major differences between somatic and visceral pain is that not all internal organs are sensitive to pain and some can be extensively damaged without pain sensation. For instance, the large majority of diseases affecting the liver, the lungs or the kidneys are totally painless. The pain symptoms associated to these diseases are the consequences of the organ malfunction. Alternatively, hollow viscera or viscera in contact with the external environment are characterized by pain sensation associated with damage of the organs. This discrepancy is linked to the inherent innervation of the internal organs. Visceral pain is related to the innervation of the organ by sensory neurons expressing nociceptors. The extrinsic primary afferent innervation of the viscera serves both efferent and afferent functions, but the major function of those neurons is to transfer information from the viscera to the central nervous system [2]. Both vagal and spinal afferent nerve fibers transmit sensory information from the viscera to the central nervous system [2]. Terminal receptors of visceral afferents are located in the mucosa, muscle and serosa of tissues, where they respond to chemical and mechanical stimuli [3]. Sensory transduction ultimately depends on the activation or modulation of nociceptors located at nerve terminals. Some visceral nociceptors become active only after inflammation or infection of the mucosa of the organs that they innervate [3, 4]. They are particularly important in signaling pain from inflamed and sensitized viscera. In that setting, proteases occupy a place of choice, being released by all the cellular actors of inflammation or even pathogens, to cause or modulate pain signals [5]. Proteases can act on pain perception by activating families of receptors such as the PARs [6]. PARs are present and functional on sensory neurons, where they participate either directly or indirectly in the transmission and/or inhibition of nociceptive messages. Taken together, the results discussed in this review highlight PARs as important potential targets for the development of analgesic drugs in the treatment of visceral pain.

2. PROTEASE-ACTIVATED RECEPTORS: STRUCTURE AND ACTIVATION

Four PARs have been cloned so far: PAR_1, PAR_2, PAR_3 and PAR_4. PARs belong to a family of seven transmembrane domains, G-protein-coupled receptors that are activated by proteolytic cleavage of their N-terminal domain [6]. The unmasked new N-terminal sequence acts as a tethered ligand that binds and activates the receptor itself [7]. On the contrary, some proteases can also inactivate PARs by a cleavage on another site and the production of a disabled receptor that cannot respond to its activation [8]. For example, PAR_1 is activated by thrombin, coagulation factor

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Xa, Granzyme A, Ginpain-R and trypsin, and is inactivated by Cathepsin G or Elastase [9]. Short synthetic peptides based on the tethered ligand sequences of the different PARs, PAR-activating peptides (PAR-APs), bind to and activate the uncleaved receptor [9]. Like these peptides are specific for a receptor they are very important pharmacological tools to understand the physiology and the physiopathology of PARs. PAR_1 and PAR_2 are the most studied receptors within this family, wherein numerous studies concerning their expression and their effects in several types of tissues and cells have been performed so far. Very little is known about PAR_3 since in cells transfected with this receptor, no intracellular signal transduction has been detected in response to either thrombin or its tethered ligand peptide [9]. Interestingly, one study showed the implication of PAR_3 as an accessory protein binding thrombin by its hirudin-like domain for PAR_4 activation [10]. Like PAR_4 was the most recent member of the PARs family to be cloned, very little is known about the physiological and pathophysiological importance of this receptor in visceral pain.

3. IMPLICATION OF PROTEASE-ACTIVATED RECEPTORS IN VISCERAL PAIN, WHAT WE LEARN FROM ANIMAL MODELS

Serine proteases, which are released upon inflammation, can activate PARs. A number of studies have focused on the effect of PARs on activation or inhibition of pain pathways, by using *in vivo*, sub-inflammatory doses of PAR-APs [4, 11]. As a whole, these different studies suggest that activation of PAR_1, PAR_2 and PAR_4 can interfere with the conduction of pain, independently of their pro-inflammatory effects [11]. The function of each PAR in the modulation of pain pathways seems to be very different, and sometimes opposite. As a matter of fact, PAR_2 could be considered as a pro-nociceptive receptor causing pain and hypersensitivity, while PAR_1 and PAR_4 are able to decrease nociceptive signals, causing analgesia [11].

3.1. Protease-Activated Receptors and Pancreas Hypersensitivity

PAR_2 is expressed and functional at all levels of the dorsal root ganglia (DRG) chain. In adult rat thoracic DRG neurons, PAR_2 agonists such as trypsin and PAR_2-AP promote calcium mobilization [12]. Like for somatic activation of PAR_2 in the paw, administration of PAR_2-AP into the pancreatic duct also increases Fos expression in the spinal segments, T9 and T10 [12]. The most prominent Fos protein expression is observed in interneurons located at laminas I and II which receive nociceptive inputs from the proximal nerve terminals of primary sensory neurons. In this study, authors demonstrate that PAR_2 activation by PAR_2-AP mediated sensitization of primary afferent neurons *in vitro* and *in vivo* and therefore supports a novel role for PAR_2 in pancreas nociceptive signaling [12]. By performing electromyographic recordings from the acromiotrapezius, a superficial dorsal skeletal muscle group, it is possible to evaluate a quantifiable nociceptive response to pancreatic noxious stimuli [13]. The use of this technique demonstrated that the administration of sub inflammatory doses of trypsin or PAR_2-AP into the pancreatic duct can induce a behavioral pain response characterized by an increase in contraction of the acromiotrapezius muscle [13]. In the same study, authors showed that trypsin increases Fos expression in neurons from laminas I and II, similarly to PAR_2-AP [13]. In the inflamed pancreas, where trypsin can be prematurely activated, PAR_2 can be activated on peripheral sensory nerves resulting in their excitation and in the release of neurotransmitters such as substance P and CGRP in the spinal cord. Recently, trypsin and its minor isoforms have been described as mediators of pancreatic pain and inflammation [14]. In particular, the inhibitor-resistant isoforms trypsin IV and P23 may be important in mediating prolonged pancreatic inflammatory pain in pancreatitis [14]. Nevertheless, studies will be needed to corroborate a role for this system in pancreatitis with both experimental and clinical studies.

3.2. PARs and Bladder Hypersensitivity

PAR_2 and PAR_4 are expressed in bladder nerve fibers as well as in bladder afferent cells in lumbosacral DRG in rats [15]. In contrast, in mice PAR_4 but not PAR_2 is expressed in bladder nerves and plexus cell bodies [16]. On a bladder inflammatory model, PARs expression is increased suggesting their involvement in pain related to cystitis [15, 16]. Although PAR_2 has been implicated in bladder contraction [17], the functional relevance in hypersensitivity of PARs expression in urinary bladder under normal and inflamed conditions needs further investigation.

3.3. Protease-Activated Receptors and Hypersensitivity of the Gastrointestinal Tract

Within the oral cavity, administration of PAR_2-AP but not PAR_1-AP into the parotid duct increased Fos expression in nociceptive neurons located in laminas I and II of the caudal part of the spinal trigeminal nucleus, which corresponds functionally to the spinal cord dorsal horn [18]. Thus, PAR_2 seems to be involved in the development of parotid pain [18]. Co-administration of PAR_1 and PAR_2-AP into the parotid duct did not suppress the increase in Fos expression due to PAR_2 activation, suggesting that PAR_1 could not be antinociceptive in parotid pain [18]. Similarly, intracolonic injections of PAR_2-AP increase Fos expression in neurons from laminae I and II of the spinal cord (segment L4-L6) in rat [19]. The record of the number of abdominal contractions of the external oblique musculature by electromyography showed a hypersensitive response to colorectal distention, 10 hours after PAR_2-AP or trypsin administration [19]. Moreover, this PAR_2-induced hyperalgesia is not associated with an inflammatory reaction, as no major signs of inflammation were observed after intracolonic administration of the doses of PAR_2-AP used in this study. As observed for somatic pain, treatment with an NK1 antagonist suppressed the visceral hyperalgesia induced by PAR_2 activation [19, 20]. While the colonic hypersensitivity induced by PAR_2 activation was dependent on NK1 activation at the periphery, skin hyperalgesia was shown to be dependent on central NK1 activation [19, 20]. Moreover, indomethacin, a COX-1 and -2 inhibitor has no effect on PAR_2-related visceral pain [19], while it was able to reduce PAR_2-induced somatic pain [20]. In the case of colonic activation of PAR_2, the release of neuropeptides such as SP

occurs peripherally, which could further amplify mast cells degranulation, thereby participating to sustain hypersensitivity. However, we cannot rule out that the release of neuropeptide also occurs in spinal projections of sensory neurons, further leading to activation of nociceptive pathways and provoking the increase in Fos expression in laminae I and II of the dorsal horn.

In contrast to PAR_2, the role of PAR_1 and PAR_4 activation has been mostly characterized at the somatic level. Nevertheless, several results obtained in somatic inflammatory pain could be transferred to visceral pain. In opposition to PAR_2, activation of PAR_1 or PAR_4 in models of paw inflammation seemed to be protective against the development of hyperalgesia. Likewise activation of PAR_1 by selective agonists attenuated nociception, caused analgesia in non-inflammatory conditions and reduced inflammatory hyperalgesia independently of the inflammatory response [21]. Interestingly, in this study the authors demonstrated that PAR_1-AP was able to decrease inflammatory mechanical and thermal hyperalgesia induced by an intraplantar injection of carrageenan, whereas thrombin had an opposite effect on nociception in response to a thermal stimulus [21]. In fact, intraplantar injection of thrombin, as PAR_1-AP, inhibited carrageenan-induced mechanical hyperalgesia, but increased carrageenan-induced thermal hyperalgesia [21]. The hyperalgesic effects of thrombin cannot be explained by a pro-inflammatory effect of thrombin since doses of this protease that caused hyperalgesia did not increase MPO activity or induce oedema [21]. Consistent with this, co-injection of thrombin with carrageenan did not amplify the inflammatory response caused by carrageenan [21]. Moreover, if inflammation underlined the thrombin hyperalgesic effect in response to a thermal noxious stimulus, hyperalgesia instead of analgesia would have been observed in response to a mechanical stimulus. The hyperalgesic effect of thrombin may be explained by the fact that thrombin is not a selective agonist for PAR_1. Thrombin's actions are mediated by activation of PAR_1, but also by activation of PAR_3 and PAR_4. Thrombin's actions can also be mediated by its non-catalytic site [22-24]. Thus, it is possible that thrombin causes thermal hyperalgesia by activating a receptor in nerve fibers other than PAR_1. This study highlights one of the major efforts that needs to be done to understand the implication of PARs in pain: define which endogenous proteases are present at inflammatory sites and that could modulate pain pathways. Even though PAR_1 is expressed in primary culture of sensory neurons extracted from dorsal root ganglia of mice, its activation seems to have no effect on calcium mobilization [25] or on the secretion of neuromediators [26]. Interestingly, PAR_1-AP triggers the production of proenkephalin and the activation of opioid receptors, suggesting that PAR_1 endogenously controls inflammatory pain, by activating opioid pathways. In non-inflammatory condition, activation of PAR_1 induced an increase in opioid mRNA in the rat paw [25]. Several studies have demonstrated that endogenous opioids are extensively produced by immune cells [27, 28] and also by cells from the skin [28, 29]. PAR_1 activation did not induce proenkephalin expression in isolated dendritic cells and macrophages *in vitro*, both in inflammatory and non-inflammatory conditions [25]. However, proenkephalin expression was increased in keratinocytes and fibroblasts after PAR_1 activation *in vitro*, signifying that these cells could be the source of proenkephalin release into mouse paws following PAR_1 activation [25]. Nevertheless, secretion of proenkephalin by other types of immune cells present at the site of inflammation can not be excluded.

Similar to PAR_1 specific agonists, administration of sub-inflammatory doses of PAR_4-AP was able to significantly reduce carrageenan-induced thermal and mechanical hyperalgesia [30]. However, in contrast to PAR_1, PAR_4 was functional on DRG neurons, wherein its activation was able to reduce capsaicin, PAR_2-AP, TRPV4 agonist or KCl-induced calcium mobilization [30, 31]. Study of neuronal currents following PAR_4 agonist peptide treatment provides a direct support for the concept of a direct effect on neurons and unequivocal evidence that suppression of intrinsic excitability can contribute to the observed antinociceptive effect of PAR_4 [32]. At the colonic level, PAR_4 agonists modulate colonic nociceptive response, inhibit colonic hypersensitivity and primary afferent activation to pro-nociceptive mediators [31]. Intracolonic administration of mustard oil provoked a significant increase in the number of pain behaviors compared with saline administration in mice [33]. Interestingly, the increased number of pain behaviors was significantly enhanced in PAR_4-deficient mice compared to wild-type littermates [31]. In parallel, the latency for the appearance of the first pain behavior after mustard oil intracolonic administration was significantly reduced in PAR_4-deficient mice compared to wild-type littermates [31].

In conclusion, the different PAR-APs helped us to understand the implication of each receptor in inflammatory pain. Those studies have demonstrated opposite effects of the different PARs on pain signaling pathways, PAR_2 being pro-nociceptive, PAR_1 and PAR_4 being anti-nociceptive. Further, evidence has been raised for a possible direct effect for PAR_2 and PAR_4 activation on sensory neurons (respectively activating or inhibiting nociceptors), and an indirect effect for PAR_1, which seemed to act through the release of endogenous opioids by resident cells. One major and still open question is the nature of endogenous proteases that are released at inflammatory sites and active on pain pathways. Characterization of these proteases and their effects on visceral pain needs to be the future direction of research in this field. This would be essential for the classification of PARs as potential therapeutic targets in chronic visceral pain.

4. IMPLICATION OF PROTEASE-ACTIVATED RECEPTORS IN VISCERAL PAIN, RELEVANCE IN FUNCTIONAL BOWEL DISORDER

Release of mast-cell–derived proteases and their effect on PAR_2 activation might represent a crucial element in the bi-directional regulatory pathways between mast cells and sensory neurons. Irritable bowel syndrome (IBS) is characterized by chronic abdominal pain associated with the presence of micro-inflammation in colonic tissues [34]. In this human disease, the distance between mast cells and nerve terminals was significantly reduced in patients with IBS, compared to healthy subjects [35]. Moreover, they demonstrate that supernatants from IBS biopsies were able to

increase nerve discharge of rat mesenteric sensory nerves [36]. These supernatants were characterized by an increase in tryptase, histamine and PGE_2 concentration compared to healthy control [36]. Ketotifen, a mast cell stabilizer and an H1 receptor antagonist, improved IBS symptoms and quality of life [37]. This study performed in human reinforced the possibility of the use of mast cell stabilizers as a therapeutic approach in the treatment of patients with IBS [37].

This favored crosstalk between neurons and mast cells in the setting of a painful pathology could implicate the release of mast cell proteases and further activation of PAR_2 on sensory neurons of those patients. Such hypothesis has been addressed, at least in part, in a study published by Cenac *et al*. Biopsies from IBS patients were taken during colonoscopy, and mediators that are released by those fresh tissues into culture media were analyzed [38]. Increased proteolytic activity was found in the culture media of biopsies harvested from IBS patients compared to control patients [38]. Further, this proteolytic activity was able to signal to sensory neurons and to reproduce hypersensitivity symptoms when injected into the mouse colonic lumen [38]. Finally, it was shown that calcium signals in sensory neurons and hypersensitivity symptoms in mice (both hyperalgesia and allodynia), induced by supernatants obtained from IBS patient biopsies, were entirely PAR_2-dependent [38]. Neurons from PAR_2-deficient mice failed to respond to those supernatants, and PAR_2-deficient mice did not develop hypersensitivity symptoms when administered with supernatants from IBS patient biopsies [38]. In addition, the pivotal role of PAR_2 in IBS patient biopsies-induced hypersensitivity was observed whether experiments were performed with biopsies from patients with diarrhea-predominant, constipation-predominant, or alternating-type IBS [38]. This study identified PAR_2 as the first common possible mediator in all IBS patients, and raised evidence that PAR_2-activating proteases are released in a human disease associated with visceral pain symptoms. The nature of those proteases still has to be fully investigated, but increased levels of tryptase and trypsin, two PAR_2-activating proteases, were observed in biopsies and biopsy supernatants [36, 38]. In addition, a recent study has confirmed that trypsinogen IV expression was enhanced in tissues from IBS patients [39]. The effects and pharmacology of mediators released from the mucosa of IBS patients were also studied on the activity of human submucosal enteric neurons. In this study, authors demonstrated that mediators released from colonic mucosal biopsy of patients with IBS excited neurons of the human submucosal plexus [40]. As demonstrated previously in mice, the excitation was not related to IBS subtypes and may therefore represent a general feature in IBS [40]. Finally, IBS supernatant-evoked excitation was inhibited by proteases inhibitor and histamine or serotonin receptor antagonists [40]. Remarkably, the use of protease inhibitor blocked the spike discharge in 100% of human enteric neurons demonstrating that proteases play a dominant role [40]. Although an increase in tryptase protein was quantified in IBS supernatant in this study, the link with PARs was not studied. The same group describes the effects of PARs activation in the human enteric nervous system in comparison to guinea pig submucous plexus. They demonstrated that stimulation of PARs by PAR-APs and by the endogenous proteases thrombin or tryptase activated neurons and glia in the human submucous plexus [41]. In opposition to rodent enteric system, most neurons and glia in human submucous plexus responded to PAR_1-AP and thrombin application, whereas activation of PAR_2 and PAR_4 generated minor responses in a limited number of neurons and glia [41]. In opposition to human, PAR_2-AP evoked a much stronger response in guinea pig submucous neurons [41]. The same discrepancy was also observed in the myenteric plexus [42]. Even if these results demonstrate the importance to consider PAR_1 as a drug target in gut diseases associated with increased protease levels, they do not exclude the possibility that PAR_2 is important in the regulation of pain sensation. In fact, enteric nervous system and thus PAR_1 regulate mostly muscle and epithelial function and not directly the nociceptive signal which is dependent on spinal and on a lesser extent on vagal projections. With that said, the characterization of proteases released by intestinal tissues under pathologic conditions and their respective PARs counterparts are a field of major interest to be further explored.

5. ENDOGENOUS PROTEASES AND PAIN RELATED TO PROTEASE-ACTIVATED RECEPTORS ACTIVATION

One of the major obstacles in defining a physiological role for PARs resides in the question of the endogenous proteases responsible for their activation. Under physiological conditions, colonic trypsin activity is constantly counter-balanced by the presence of trypsin inhibitors [43]. On the contrary, in patients with inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease, there is a reduction of the expression of the trypsin inhibitor [44] [45] and thus, trypsin activity is increased in IBD patients [46]. Indeed, this phenomenon has also been observed in animal models of colitis [47]. Trypsin was also detected in the pancreatic parenchyma and pancreatic secretions of patients with pancreatitis, as well as in rodent models of experimental pancreatitis [48]. Thus, in pancreatitis, trypsin secretion by pancreatic acinar cells could constitute the major candidate in mediating pain related to this disease. Recently, increased elastolytic activity (elastase-like proteolysis) in colon tissue of IBD patients has been characterized [49]. This increase in activity was coupled with a decrease of mucosal expression of elafin (endogenous elastase inhibitor) in patients with IBD [49]. In mouse colitis, macrophage cathepsins are activated during colitis, and Cat-S activates nociceptors to induce visceral pain *via* PAR_2 [50]. These different studies demonstrated an increase in proteolytic activity in IBD patients or in mice under experimentally-induced colitis. Nevertheless, a direct relationship between this increase and visceral pain was not investigated. Tryptase, another protease that can activate PAR_2, constitutes the major protein released upon mucosal mast cell degranulation in humans, and is released in the setting of inflammation [47]. Trypsin and tryptase constitute good candidates to activate PAR_2 on DRG and could be responsible for the pain related to IBD. In patients with irritable bowel syndrome (IBS) the number of mucosal mast cells (MMC) in terminal ileum and caecum is increased [51, 52]. The degranulation rate of MMC in close proximity to nerves was reported to be significantly higher

than degranulation of MMC distant from nerve fibers in the colorectal specimens of diarrhea predominant IBS patients [53]. Moreover, a positive correlation was found between the severity of abdominal pain and the proximity of MMC and nerve fibers in IBS patients [35]. In a model of IBS in rats [54], pretreatment with the mast cells stabilizer, doxantrazole, reduced visceral hypersensitivity to rectal distension while no effects were observed in control rats, further showing the possible implication of mast cells in the hypersensitivity related to IBS [55]. Thus, in IBS, tryptase released by mast cells constitutes the major candidate to mediate visceral pain *via* PAR_2 activation. Serine proteases of bacteria such as the gingipains-R, can also activate PARs [56]. Likewise, an increase of proteolytic activity has been described from samples of post-infectious IBS patients or mouse models [36, 38, 57]. Even though, the source of these serine-proteases, whether the host epithelial cells or pathogens, needs to be characterized. Serine proteases of the coagulation cascade can also activate PARs. As thrombin can activate PAR_1 but not PAR_2, this serine-protease could be a good candidate as the endogenous PAR_1 agonist mediating this analgesic effect. Coagulation factors such as the coagulation factor Xa can activate both PAR_1 and PAR_2, thus the effects of factor Xa on nociceptive functions might depend on the ratio of expression between these receptors expressed in the vicinity and potentially on the cells targeted by this protease. Clearly, more studies are necessary to determine the implication of the coagulation factor in the regulation of pain.

6. INTERACTION OF PROTEASE-ACTIVATED RECEPTORS WITH TRANSIENT RECEPTOR POTENTIAL, IMPLICATION IN VISCERAL PAIN

The molecular mechanism of PAR_2-mediated hyperalgesia is largely unknown. However, it is likely to involve signaling events that regulate activity or expression of ion channels. The most prominent candidate in this regard is the transient receptor potential vanilloid-1 (TRPV1). TRPV1 is a member of the transient receptor potential (TRP) family of channels. TRPV1 is a nonselective cation channel that is activated by protons, elevated temperature, and certain lipids, as well as by exogenous vanilloid molecules such as capsaicin [58, 59]. For instance, PAR_2 is co-expressed with TRPV1, in sensory neurons of dorsal root ganglia [60, 61] on nerve fibers of the bladder [15] or of pancreas [12]. On the nerve fibers arising from the pancreas, PAR_2 and TRPV1 are also functionally coupled, since activation of PAR_2 sensitized DRG neurons responses to capsaicin, as measured by an enhancement of capsaicin-evoked CGRP release [12]. The TRPV1 antagonist capsazepine blocks PAR_2-mediated thermal hyperalgesia, but not spontaneous nociceptive behavior or Fos expression, showing that sensitization/transactivation of TRPV1 by PAR_2 might be involved in thermal hyperalgesia but not in the primary pain message [62]. In a model of visceral pain induced by intracolonic administration of capsaicin, intracolonic injection of PAR_2 agonist, 6 or 18h before capsaicin, produces a delayed sensitization of capsaicin receptors, resulting in facilitation of visceral pain and referred hyperalgesia in response to von Frey filaments stimuli [63]. In the same study, authors have shown that PAR_1 activation appears to play an antinociceptive role in processing of visceral pain in this model [63]. To study the relationship between PAR_2 and TRPV1, Amadesi and colleagues used four different approaches: calcium signaling in HEK cells co-transfected with PAR_2 and TRPV1, calcium signals in DRGs, electrophysiology approach, and *in vivo* experiments [60]. All these different approaches pointed to the same mechanism, demonstrating that PAR_2 activation potentiates the capsaicin response in a PKC-dependent manner [60, 64]. In HEK cells and DRG neurons, PAR_2 and TRPV1 are functionally coupled, application of PAR_2 specific agonists, trypsin or tryptase potentiated capsaicin-induced currents [60, 64]. Dai and colleagues confirmed a functional interaction between TRPV1 and PAR_2 in HEK293 and in mouse DRG neurons by a patch-clamp technique [61]. Further, Amadesi *et al*. have shown that PAR_2 agonists in sensory neurons promoted translocation of the epsilon form of PKC and protein kinase A catalytic subunits from the cytosol, to the plasma membrane [64]. Studies on the interactions of PARs and channels such as the TRP family could be the challenges and opportunities of future research in molecular mechanism of PARs-regulated inflammatory pain.

PAR_2 is also co-expressed with other members of the TRP family in sensory neurons such as TRPV4 [65, 66] and TRPA1 [67, 68]. In sensory neurons, PAR_2 activation potentiates the response of those cells to agonists of TRPA1 [68] or TRPV4 [66]. Moreover, TRPV4 potentiation was also dependent on histamine and serotonin receptors activation [69]. Inhibitors of protein kinases A, C and D were able to suppress PAR_2 agonist-induced sensitization of TRPV4-mediated calcium signals in sensory neurons [66]. Interestingly, the mechanism of TRPA1 sensitization by PAR_2 activation is independent of PKC [68]. In sensory neurons, the increased TRPA1 sensitivity is due to activation of PLC, which releases TRPA1 from PIP2 inhibition [68]. Direct activation of TRPV4 or TRPA1 causes somatic and visceral hypersensitivity to mechanical stimuli [38, 66-68]. Moreover, TRPV4 and TRPA1 mediate PAR_2-induced somatic and visceral hyperalgesia *in vivo* [38, 66-68]. For TRPV4, the interaction between this receptor and PAR_2 was confirmed by the fact that direct responses of splanchnic colonic afferents to PAR_2 activation were totally lost in TRPV4-deficient mice [70]. However, the same group observed that the loss of TRPA1 did not affect the magnitude or proportion of direct responses to PAR_2 specific agonist [71]. Authors suggest that the strong interaction between TRPV4 and PAR_2 in sensory neurons projecting from the colon is due to a tight co-localization, whereas TRPA1 and PAR_2 are localized in different cellular microdomains, rendering this channel inaccessible to products of PAR_2 activation [71]. Thus, the electrophysiological recordings from splanchnic afferents did not reveal that PAR_2 sensitizes TRPA1 [71] but the behavioral studies of awake animals showed sensitization [67]. In a very recent study, authors demonstrated a functional coupling between PAR_2 and TRPV4 [72]. In fact, PAR_2 activation induced the production of endogenous activators of TRPV4 *via* cytochrome epoxygenase activation [72]. This functional coupling was not observed between PAR_2 and TRPA1 [72].

As all of these studies have been performed with activation of PAR_2 by its agonist peptide, we can not

Table 1. Functions of PARs Activation in Visceral Pain

	Rodent		HUMAN
	Sensory Neurons	**Animal Models**	
PAR_1	No effect on: - calcium flux - release of neuropeptides	ND	Activates enteric neurons
PAR_2	Increase: - calcium flux - release of neuropeptides	Induce: - Pancreatic pain - Parotid pain - Colonic hypersensitivity	Do not activate enteric neurons. Proteases released by biopsies of IBS patients activate PAR_2 in mice
PAR_3	ND	ND	ND
PAR_4	Decrease excitability	Decrease colonic hypersensitivity	Do not activate enteric neurons

extrapolate these results to the pathophysiology of proteases in inflammatory pain [4]. Taking trypsin as an example, which can activate both PAR_2 and PAR_4 on sensory neurons, one could raise the question whether trypsin would sensitize TRPV4 through a functional interaction with PAR_2 or inhibit TRPV4 signaling through PAR_4 activation [31]. In this way, studies of the effect of endogenous protease on this sensitization pathway are necessary to understand such mechanism and to develop potential therapeutic drug against inflammatory pain.

7. CONCLUSIONS

The ability of PARs to act at different levels of the visceral pain pathways demonstrates their value as potential therapeutic targets in this phenomenon (Table **1**). Even if basic science results point to a crucial role for PARs in visceral pain, there is a lack of knowledge concerning the nature of proteases released upon human pain-associated diseases. As a matter of fact, future directions in this field as in the field of inflammatory pain would have to include the determination of the nature and specific functions of protease released in human pathologies [4].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Thompson, W. G. The road to Rome. *Gut*, **1999**, 45 Suppl 2, II80.

[2] Davis, M. P. Drug management of visceral pain: concepts from basic research. *Pain Res. Treat.*, **2012**, *2012*, 265605.

[3] Vergnolle, N. Visceral afferents: what role in post-inflammatory pain? *Auton Neurosci*., **2010**, 153, 79-83.

[4] Cenac, N.: Proteases and Inflammatory Pain. In: *Proteases and Their Receptors in Inflammation.* Edited by Vergnolle N, Chignard M: Springer Basel; **2011**, 253-274.

[5] Vergnolle, N. Review article: proteinase-activated receptors-novel signals for gastrointestinal pathophysiology. *Aliment. Pharmacol. Ther.,* **2000**, *14*, 257-266.

[6] Vergnolle, N.; Ferazzini, M.; D'Andrea, M. R.; Buddenkotte, J.; Steinhoff, M. Proteinase-activated receptors: novel signals for peripheral nerves. *Trends Neurosci*., **2003**, *26*, 496-500.

[7] Hollenberg, M. D. Protease-mediated signalling: new paradigms for cell regulation and drug development. *Trends Pharmacol. Sci.,* **1996**, *17*, 3-6.

[8] Hollenberg, M. D.; Compton, S. J. International Union of Pharmacology. XXVIII. Proteinase-activated receptors. *Pharmacol. Rev.,* **2002**, *54*, 203-217.

[9] Bunnett, N. W. Protease-activated receptors: how proteases signal to cells to cause inflammation and pain. *Semin. Thromb. Hemost.,* **2006**, 32 Suppl 1, 39-48.

[10] Nakanishi-Matsui, M.; Zheng, Y. W.; Sulciner, D. J.; Weiss, E. J.; Ludeman, M. J.; Coughlin, S. R. PAR3 is a cofactor for PAR4 activation by thrombin. *Nature*, **2000**, *404*, 609-613.

[11] Vergnolle, N. Protease-activated receptors as drug targets in inflammation and pain. *Pharmacol. Ther.,* **2009***, 123*, 292-309.

[12] Hoogerwerf, W. A.; Zou, L.; Shenoy, M.; Sun, D.; Micci, M. A.; Lee-Hellmich, H.; Xiao, S. Y.; Winston, J. H.; Pasricha, P. J. The proteinase-activated receptor 2 is involved in nociception. *J Neurosci.,* **2001**, *21*, 9036-9042.

[13] Hoogerwerf, W. A.; Shenoy, M.; Winston, J. H.; Xiao, S. Y.; He, Z.; Pasricha, P. J. Trypsin mediates nociception *via* the proteinase-activated receptor 2: a potentially novel role in pancreatic pain. *Gastroenterology*, **2004**, *127,* 883-891.

[14] Ceppa, E. P.; Lyo, V.; Grady, E. F.; Knecht, W.; Grahn, S.; Peterson, A.; Bunnett, N. W.; Kirkwood, K. S.; Cattaruzza, F. Serine proteases mediate inflammatory pain in acute pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2011**, *300*, G1033-1042.

[15] Dattilio, A.; Vizzard, M. A. Up-regulation of protease activated receptors in bladder after cyclophosphamide induced cystitis and colocalization with capsaicin receptor (VR1) in bladder nerve fibers. *J. Urol.,* **2005**, *173*, 635-639.

[16] D'Andrea, M. R.; sabam, M. R.; Nguyen, N. B.; Andrade-Gordon, P.; Saban, R. Overriding participation of protease activated receptor (PAR)s 1, 2, 3, and 4 in experimental bladder inflammation. *Am. J. Pathol.,* **2003**, *162,* 907-923.

[17] Shimizu, N.; Nakahara, T.; Saito, M.; Ishii, K. Role of capsaicin-sensitive sensory nerves in protease-activated receptor-2-mediated contraction of rat urinary bladder. *Eur. J. Pharmacol.*, **2007***, 569*, 145-148.

[18] Kawabata, A.; Kuroda, R.; Nishida, M.; Nagata, N.; Sakaguchi, Y.; Kawao, N.; Nishikawa, H.; Arizono, N.; Kawai, K. Protease-activated receptor-2 (PAR-2) in the pancreas and parotid gland: Immunolocalization and involvement of nitric oxide in the evoked amylase secretion. *Life Sci.,* **2002**, *71*, 2435-2446.

[19] Coelho, A. M.; Vergnolle, N.; Guiard, B.; Fioramonti, J.; Bueno, L. Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. *Gastroenterology,* **2002**, *122* 1035-1047.

[20] Vergnolle, N.; Bunnett, N. W.; Sharkey, K. A.; Brussee, V.; Compton, S. J.; Grady, E. F.; Cirino, G.; Gerard, N.; Basbaum, A.

I.; Andrade-Gordon, P.; Hollenberg, M. D.; Wallace, J. L. Proteinase-activated receptor-2 and hyperalgesia: A novel pain pathway. *Nat. Med.*, **2001**, *7*, 821-826.

[21] Asfaha, S.; Brussee, V.; Chapman, K.; Zochodne, D. W.; Vergnolle, N. Proteinase-activated receptor-1 agonists attenuate nociception in response to noxious stimuli. *Br. J. Pharmacol.*, **2002**, *135,* 1101-1106.

[22] Bar-Shavit, R.; Kahn, A.; Mudd, M. S.; Wilner, G. D.; Mann, K. G.; Fenton, J. W. Localization of a chemotactic domain in human thrombin. *Biochemistry*, **1984**, *23*, 397-400.

[23] Bar-Shavit, R.; Kahn, A.; Wilner, G. D.; Fenton, J. W. Monocyte chemotaxis: stimulation by specific exosite region in thrombin. *Science,* **1983**, *220*, 728-731.

[24] Herbert, J. M.; Dupuy, E.; Laplace, M. C.; Zini, J. M.; Bar-Shavit, R.; Tobelem, G. Thrombin induces endothelial cell growth *via* both a proteolytic and non-proteolytic pathway. *Biochem. J*, **1994**, *303*, 227-231.

[25] Martin, L.; Auge, C.; Boue, J.; Buresi, M. C.; Chapman, K.; Asfaha, S.; Andrade-Gordon, P.; Steinhoff, M.; Cenac, N.; Dietrich, G.; Vergnolle, N. Thrombin receptor: An endogenous inhibitor of inflammatory pain, activating opioid pathways. *Pain*, **2009**, *146,* 121-129.

[26] de Garavilla, L.; Vergnolle, N.; Young, S. H.; Ennes, H.; Steinhoff, M.; Ossovskaya, V. S.; D'Andrea, M. R.; Mayer, E. A.; Wallace, J. L.; Hollenberg, M. D.; Andrade-Gordon, P.; Bunnett, N. W. Agonists of proteinase-activated receptor 1 induce plasma extravasation by a neurogenic mechanism. *Br. J. Pharmacol*., **2001**, *133*, 975-987.

[27] Jaume, M.; Laffont, S.; Chapey, E.; Blanpied, C.; Dietrich, G. Opioid receptor blockade increases the number of lymphocytes without altering T cell response in draining lymph nodes *in vivo*. *J. Neuroimmunol.,* **2007**, *188,* 95-102.

[28] Nissen, J. B.; Kragballe, K. Enkephalins modulate differentiation of normal human keratinocytes *in vitro*. *Exp. Dermatol*., **1997**, *6,* 222-229.

[29] Bottger, A.; Spruce, B. A. Proenkephalin is a nuclear protein responsive to growth arrest and differentiation signals. J.Cell Biol., 1995, 130, 1251-1262.

[30] Asfaha, S.; Cenac, N.; Houle, S.; Altier, C.; Papez, M. D.; Nguyen, C.; Steinhoff, M.; Chapman, K.; Zamponi, G. W.; Vergnolle, N. Protease-activated receptor-4: a novel mechanism of inflammatory pain modulation. *Br. J. Pharmacol*., **2007**, *150*, 176-185.

[31] Auge, C.; Balz-Hara, D.; Steinhoff, M.; Vergnolle, N.; Cenac, N. Protease-activated receptor-4 (PAR 4): a role as inhibitor of visceral pain and hypersensitivity. *Neurogastroenterol. Motil.,* **2009**, *21*, 1189-e1107.

[32] Karanjia, R.; Spreadbury, I.; Bautista-Cruz, F.; Tsang, M. E.; Vanner, S. Activation of protease-activated receptor-4 inhibits the intrinsic excitability of colonic dorsal root ganglia neurons. Neurogastroenterol.Motil., 2009, 21, 1218-1221.

[33] Laird, J. M.; Martinez-Caro, L.; Garcia-Nicas, E.; Cervero, F. A new model of visceral pain and referred hyperalgesia in the mouse. *Pain,* **2001**, *92*, 335-342.

[34] Salzmann, J. L.; Peltier-Koch, F.; Bloch, F.; Petite, J. P.; Camilleri, J. P. Morphometric study of colonic biopsies: a new method of estimating inflammatory diseases. *Lab. Invest.,* **1989**, *60*, 847-851.

[35] Barbara, G.; Stanghellini, V.; De, G. R.; Cremon, C.; Cottrell, G. S.; Santini, D.; Pasquinelli, G.; Morselli-Labate, A. M.; Grady, E. F.; Bunnett, N. W.; Collins, S. M.; Corinaldesi, R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology*, **2004**, *126*, 693-702.

[36] Barbara, G.; Wang, B.; Stanghellini, V.; De, G. R.; Cremon, C.; Di, N. G.; Trevisani, M.; Campi, B.; Geppetti, P.; Tonini, M.; Bunnett, N. W.; Grundy, D.; Corinaldesi, R. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology,* **2007**, *132*, 26-37.

[37] Klooker, T. K.; Braak, B.; Koopman, K. E.; Welting, O.; Wouters, M. M.; van der Heide, S.; Schemann, M.; Bischoff, S. C.; van den Wijngaard, R. M.; Boeckxstaens, G. E. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut,* **2010**, *59*, 1213-1221.

[38] Cenac, N.; Andrews, C. N.; Holzhausen, M.; Chapman, K.; Cottrell, G.; ndrade-Gordon, P.; Steinhoff, M.; Barbara, G.; Beck, P.; Bunnett, N. W.; Sharkey, K. A.; Ferraz, J. G.; Shaffer, E.; Vergnolle, N. Role for protease activity in visceral pain in irritable bowel syndrome. *J. Clin. Invest.,* **2007**, *117*, 636-647.

[39] Kerckhoffs, A. P.; Ter Linde, J. J.; Akkermans, L. M.; Samsom, M. Trypsinogen IV, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. *Neurogastroenterol. Motil.,* **2008**, *20,* 900-907.

[40] Buhner, S.; Li, Q.; Vignali, S.; Barbara, G.; De Giorgio, R.; Stanghellini, V.; Cremon, C.; Zeller, F.; Langer, R.; Daniel, H.; Michel, K.; Schemann, M. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology*, **2009**, *137,* 1425-1434.

[41] Mueller, K.; Michel, K.; Krueger, D.; Demir, I. E.; Ceyhan, G. O.; Zeller, F.; Kreis, M. E.; Schemann, M. Activity of protease-activated receptors in the human submucous plexus. Gastro-*enterology,* **2011**, *141*, 2088-2097 e2081.

[42] Kugler, E. M.; Mazzuoli, G.; Demir, I. E.; Ceyhan, G. O.; Zeller, F.; Schemann, M. Activity of protease-activated receptors in primary cultured human myenteric neurons. *Front. Neurosci.,* **2012**, *6,* 133.

[43] Motta, J. P.; Magne, L.; Descamps, D.; Rolland, C.; Squarzoni-Dale, C.; Rousset, P.; Martin, L.; Cenac, N.; Balloy, V.; Huerre, M.; Frohlich, L. F.; Jenne, D.; Wartelle, J.; Belaaouaj, A.; Mas, E.; Vinel, J. P.; Alric, L.; Chignard, M.; Vergnolle, N.; Sallenave, J. M. Modifying the protease, antiprotease pattern by elafin overexpression protects mice from colitis. *Gastroenterology,* **2011**, *140*, 1272-1282.

[44] Bustos, D.; Negri, G.; De Paula, J. A.; Di Carlo, M.; Yapur, V.; Facente, A.; De Paula, A. Colonic proteinases: increased activity in patients with ulcerative colitis. Medicina (B Aires), **1998**, *58*, 262-264.

[45] Playford, R. J.; Hanby, A. M.; Patel, K.; Calam, J. Influence of inflammatory bowel disease on the distribution and concentration of pancreatic secretory trypsin inhibitor within the colon. *Am. J. Pathol.,* **1995**, 146, 310-316.

[46] Tarlton, J. F.; Whiting, C. V.; Tunmore, D.; Bregenholt, S.; Reimann, J.; Claesson, M. H.; Bland, P. W. The role of up-regulated serine proteases and matrix metalloproteinases in the pathogenesis of a murine model of colitis. *Am. J. Pathol*., **2000**, *157,* 1927-1935.

[47] Raithel, M.; Winterkamp, S.; Pacurar, A.; Ulrich, P.; Hochberger, J.; Hahn, E. G. Release of mast cell tryptase from human colorectal mucosa in inflammatory bowel disease. *Scand. J. Gastroenterol.,* **2001**, *36*, 174-179.

[48] Luthen, R.; Niederau, C.; Grendell, J. H. Intrapancreatic zymogen activation and levels of ATP and glutathione during caerulein pancreatitis in rats. *Am. J. Physiol.*, **1995**, *268*, G592-604.

[49] Motta, J. P.; Bermudez-Humaran, L. G.; Deraison, C.; Martin, L.; Rolland, C.; Rousset, P.; Boue, J.; Dietrich, G.; Chapman, K.; Kharrat, P.; Vinel, J. P.; Alric, L.; Mas, E.; Sallenave, J. M.; Langella, P.; Vergnolle, N. Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis. *Sci. Transl. Med.,* **2012**, *4,* 158ra144.

[50] Cattaruzza, F.; Lyo, V.; Jones, E.; Pham, D.; Hawkins, J.; Kirkwood, K.; Valdez-Morales, E.; Ibeakanma, C.; Vanner, S. J.; Bogyo, M.; Bunnett, N. W. Cathepsin S is activated during colitis and causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. *Gastroenterology*, **2011**, *141*, 1864-1874 e1861-1863.

[51] Weston, A. P.; Biddle, W. L.; Bhatia, P. S.; Miner, P. B., Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. *Dig. Dis. Sci.*, **1993**, *38,* 1590-1595.

[52] O'Sullivan, M.; Clayton, N.; Breslin, N. P.; Harman, I.; Bountra, C.; McLaren, A.; O'Morain, C. A. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol. Motil.,* **2000**, *12*, 449-457.

[53] Park, C. H.; Joo, Y. E.; Choi, S. K.; Rew, J. S.; Kim, S. J.; Lee, M. C. Activated mast cells infiltrate in close proximity to enteric nerves in diarrhea-predominant irritable bowel syndrome. *J. Korean Med. Sci.*, **2003**, *18*, 204-210.

[54] La, J. H.; Kim, T. W.; Sung, T. S.; Kang, J. W.; Kim, H. J.; Yang, I. S. Visceral hypersensitivity and altered colonic motility after subsidence of inflammation in a rat model of colitis. *World J. Gastroenterol.,***2003**, *9*, 2791-2795.

[55] La, J. H.; Kim, T. W.; Sung, T. S.; Kim, H. J.; Kim, J. Y.; Yang, I. S. Role of mucosal mast cells in visceral hypersensitivity in a rat model of irritable bowel syndrome. *J. Vet. Sci.,* **2004**, *5*, 319-324.

[56] Lourbakos, A.; Yuan, Y. P.; Jenkins, A. L.; Travis, J.; Andrade-Gordon, P.; Santulli, R.; Potempa, J.; Pike, R. N. Activation of protease-activated receptors by gingipains from Porphyromonas gingivalis leads to platelet aggregation: a new trait in microbial pathogenicity. *Blood,* **2001**, *97*, 3790-3797.

[57] Ibeakanma, C.; Ochoa-Cortes, F.; Miranda-Morales, M.; McDonald, T.; Spreadbury, I.; Cenac, N.; Cattaruzza, F.; Hurlbut, D.; Vanner, S.; Bunnett, N.; Vergnolle, N. Brain-gut interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. *Gastroenterology,* **2011**, *141,* 2098-2108 e2095.

[58] Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, **1997**, *389*, 816-824.

[59] Julius, D.; Basbaum, A. I. Molecular mechanisms of nociception. *Nature*, **2001**, *413*, 203-210.

[60] Amadesi, S.; Nie, J.; Vergnolle, N.; Cottrell, G. S.; Grady, E. F.; Trevisani, M.; Manni, C.; Geppetti, P.; McRoberts, J. A.; Ennes, H.; Davis, J. B.; Mayer, E. A.; Bunnett, N. W. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. *J. Neurosci.*, **2004**, *24,* 4300-4312.

[61] Dai, Y.; Moriyama, T.; Higashi, T.; Togashi, K.; Kobayashi, K.; Yamanaka, H.; Tominaga, M.; Noguchi, K. Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain. *J. Neurosci.,* **2004**, *24,* 4293-4299.

[62] Kawao, N.; Shimada, C.; Itoh, H.; Kuroda, R.; Kawabata, A. Capsazepine inhibits thermal hyperalgesia but not nociception triggered by protease-activated receptor-2 in rats. *Jpn. J. Pharmacol.,* **2002**, *89*, 184-187.

[63] Kawao, N.; Ikeda, H.; Kitano, T.; Kuroda, R.; Sekiguchi, F.; Kataoka, K.; Kamanaka, Y.; Kawabata, A. Modulation of capsaicin-evoked visceral pain and referred hyperalgesia by protease-activated receptors 1 and 2. *J. Pharmacol. Sci.,* **2004***, 94,* 277-285.

[64] Amadesi, S.; Cottrell, G. S.; Divino, L.; Chapman, K.; Grady, E. F.; Bautista, F.; Karanjia, R.; Barajas-Lopez, C.; Vanner, S.; Vergnolle, N.; Bunnett, N. W. Protease-activated receptor 2 sensitizes TRPV1 by protein kinase C- (epsilon) and A-dependent mechanisms in rats and mice. *J. Physiol.,* **2006**, 575(Pt2), 555-71.

[65] Cenac, N.; Altier, C.; Chapman, K.; Liedtke, W.; Zamponi, G.; Vergnolle, N. Transient receptor potential vanilloid-4 has a major role in visceral hypersensitivity symptoms. *Gastroenterology*, **2008**, *135*, 937-946, 946 e931-932.

[66] Grant, A. D.; Cottrell, G. S.; Amadesi, S.; Trevisani, M.; Nicoletti, P.; Materazzi, S.; Altier, C.; Cenac, N.; Zamponi, G. W.; Bautista-Cruz, F.; Lopez, C. B.; Joseph, E. K.; Levine, J. D.; Liedtke, W.; Vanner, S.; Vergnolle, N.; Geppetti, P.; Bunnett, N. W. Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. *J Physiol.,* **2007**, *578*, 715-733.

[67] Cattaruzza, F.; Spreadbury, I.; Miranda-Morales, M.; Grady, E. F.; Vanner, S. J.; Bunnett, N. W. Transient Receptor Potential Ankyrin-1 has a Major Role in Mediating Visceral Pain in Mice. *Am. J. Physiol Gastrointest. Liver Physiol.,* **2009**, *298*(1),G81-91.

[68] Dai, Y.; Wang, S.; Tominaga, M.; Yamamoto, S.; Fukuoka, T.; Higashi, T.; Kobayashi, K.; Obata, K.; Yamanaka, H.; Noguchi, K. Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J. Clin. Invest,* **2007**, *117*, 1979-1987.

[69] Cenac, N.; Altier, C.; Motta, J. P.; d'Aldebert, E.; Galeano, S.; Zamponi, G. W.; Vergnolle, N. Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. *Gut*, **2010***, 59,* 481-488.

[70] Brierley, S. M.; Page, A. J.; Hughes, P. A.; Adam, B.; Liebregts, T.; Cooper, N. J.; Holtmann, G.; Liedtke, W.; Blackshaw, L. A. Selective role for TRPV4 ion channels in visceral sensory pathways. *Gastroenterology*, **2008**, *134*, 2059-2069.

[71] Brierley, S. M.; Hughes, P. A.; Page, A. J.; Kwan, K. Y.; Martin, C. M.; O'Donnell, T. A.; Cooper, N. J.; Harrington, A. M.; Adam, B.; Liebregts, T.; Holtmann, G.; Corey, D. P.; Rychkov, G. Y.; Blackshaw, L. A. The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. *Gastroenterology*, **2009**, *137,* 2084-2095 e2083.

[72] Poole, D. P.; Amadesi, S.; Veldhuis, N. A.; Abogadie, F. C.; Lieu, T.; Darby, W.; Liedtke, W.; Lew, M. J.; McIntyre, P.; Bunnett, N. W. Protease-activated Receptor-2 (PAR2) and Transient Receptor Potential Vanilloid 4 (TRPV4) Coupling is Required for Sustained Inflammatory Signaling. *J. Biol. Chem*., **2013**, *288(8),* 5790-802.

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