British Journal of Pharmacology (2004) 141, 1264–1274 
www.nature.com/bjp

REVIEW

# Modulation of visceral pain and inflammation by protease-activated receptors

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The gastrointestinal (GI) tract is exposed to a large array of proteases, under both physiological and pathophysiological conditions. The discovery of G protein-coupled receptors activated by proteases, the protease-activated receptors (PARs), has highlighted new signaling functions for proteases in the GI tract, particularly in the domains of inflammation and pain mechanisms. Activation of PARs by selective peptidic agonists in the intestine or the pancreas leads to inflammatory events and changes in visceral nociception, suggesting that PARs could be involved in the modulation of visceral pain and inflammation. PARs are present in most of the cells that are potentially actors in the generation of irritable bowel syndrome (IBS) symptoms. Activation of PARs interferes with several pathophysiological factors that are involved in the generation of IBS symptoms, such as altered motility patterns, inflammatory mediator release, altered epithelial functions (immune, permeability and secretory) and altered visceral nociceptive functions. Although definitive studies using genetically modified animals, and, when available, pharmacological tools, in different IBS and inflammatory models have not yet confirmed a role for PARs in those pathologies, PARs appear as promising targets for therapeutic intervention in visceral pain and inflammation processes.
*British Journal of Pharmacology* (2004) **141,** 1264–1274. doi:10.1038/sj.bjp.0705750

**Keywords:** Inflammation; pain; visceral hypersensitivity; irritable bowel syndrome; thrombin; trypsin; tryptase; proteases

**Abbreviations:** CGRP, calcitonin gene-related peptide; ENS, enteric nervous system; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; NK-1 R, tachykinin-1 receptor; PARs, protease-activated receptors; $PAR_1$, protease-activated receptor-1; $PAR_2$, protease-activated receptor-2; $PAR_3$, protease-activated receptor-3; $PAR_4$, protease-activated receptor-4; PSTI, pancreatic secretory trypsin inhibitor

## Introduction

Proteases represent 2% of the human genome and are present at particularly high levels in the gastrointestinal (GI) tract (Caughey, 1995). Thus, it is not surprising if, in addition to their role in protein degradation and/or digestion, certain proteases exert also a role as signaling molecules, regulating cell functions by cleaving receptors activated upon proteolysis. Those receptors constitute a family of G protein-coupled receptors (four members have been cloned thus far), which are called protease-activated receptors (PARs). Activation of those receptors has been shown to interfere with inflammatory and nociceptive pathways in different tissues (Vergnolle *et al*., 2001b). Understanding the functional role of PARs in a system as exposed to proteases as the GI tract represents an important and exciting challenge that could lead to define new pharmacological targets for GI diseases in general, and in particular for pathologies associating inflammatory and visceral pain disorders.

## PAR structure and activation

PARs are activated by a unique mechanism that first involves recognition of an extracellular domain of the receptor situated on the N-terminus, by a protease (Rasmussen *et al*., 1991; Vu *et al*., 1991; Ishihara *et al*., 1997; Xu *et al*., 1998; Coughlin, 1999). Some proteases such as thrombin, bind the receptors. Others, such as trypsin or tryptase, do not need to bind the receptor in order to cleave it. Then, cleavage by proteolysis of this recognized site occurs, which exposes a new N-terminus domain that acts as a tethered ligand domain interacting with domains situated in the second extracellular loop of the receptor, to induce an intracellular signal (see Figure 1a). PARs respond to a variety of proteases, although thrombin for $PAR_1$, $PAR_3$ and $PAR_4$, and trypsin for $PAR_2$ and $PAR_4$ are usually regarded as the main activators of PARs. Proteases of the coagulation cascade such as factor Xa and VIIa can activate $PAR_2$ and $PAR_4$ (Dery *et al*., 1998; Coughlin, 1999; Hollenberg & Compton, 2002). However, co-factors such as tissue factor are needed to enhance the ability of factor VIIa to activate $PAR_2$. Cathepsin G released from neutrophils triggers $PAR_4$ activation and can activate $PAR_1$. Tryptase released from mast cells can activate $PAR_2$, while bacterial proteases such as gingipains can activate $PAR_1$, $PAR_2$ and $PAR_4$ (Dery *et al*., 1998; Coughlin, 1999; Hollenberg & Compton, 2002). Proteases released by dust mites are also able to activate $PAR_2$ (see Table 1) (Lourbakos *et al*., 2001; Asokananthan *et al*., 2002). Soluble forms of integral membrane proteases such as membrane-type serine protease 1 can also activate PARs in general, and $PAR_2$ in particular. Since $PAR_2$ and membrane-type serine protease 1 have similar tissue distribution, this further suggests a role for intra-membrane-type serine protease

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Advance online publication: 29 March 2004

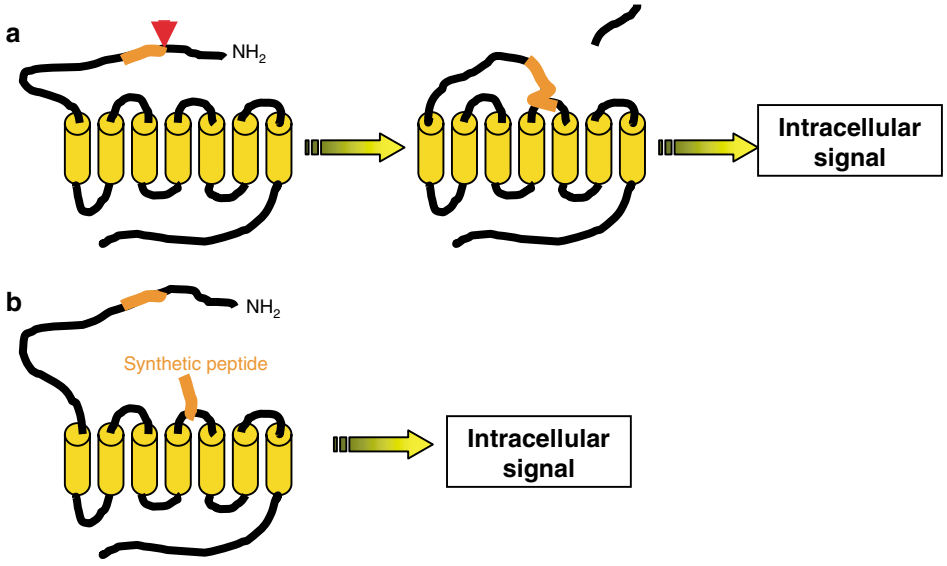


**Figure 1** Mechanism of activation of PARs. (a) Proteases cleave the extracellular N-terminus domain to release a new N-terminus domain that acts as a tethered ligand binding and activating the receptor to induce an intracellular signal. (b) Synthetic peptides corresponding to the tethered ligand domain can mimic the effects of the proteolytically cleaved N-terminal domain to specifically activate the receptor.

1 as an endogenous activator of $PAR_2$. Although some proteases are common to the activation of different PARs, a single protease agonist activates distinct receptors with different potencies. For instance, thrombin can activate $PAR_1$, $PAR_3$ and $PAR_4$, with a highest potency for $PAR_1$, then a lower potency for $PAR_3$, and is finally weaker for the activation of $PAR_4$ (for reviews, see Dery *et al*., 1998; Coughlin, 1999; Hollenberg & Compton, 2002). *In vivo* activation of PARs requires the release of active proteases in the vicinity of the receptors. Depending on the physiological or pathophysiological situation, different proteases might activate the same receptor. Determining which proteases are endogenous activators of PARs in selected pathologies would be of great importance for the potential use of protease inhibitors as therapeutic options.

With the exception of $PAR_3$, synthetic peptides corresponding to the tethered ligand domain released upon proteolytic activation of $PAR_1$, $PAR_2$ and $PAR_4$ can directly activate the receptor (see Figure 1b) (Hollenberg & Compton, 2002). Those peptides constitute important pharmacological tools to understand the functions of PARs. Pharmacological studies have determined optimum peptidic sequences for selectivity and potency of PAR activation (see Table 1). For example, the tethered ligand peptide SFLLR, which corresponds to the human $PAR_1$, has been shown to also activate $PAR_2$, but a simple replacement of the serine residue by a threonine residue (TFLLR peptide) gives a more selective peptidic agonist for $PAR_1$ activation. Synthetic peptide corresponding to the mouse $PAR_4$ (GYPGKF) constitutes a selective agonist for mouse $PAR_4$, but substitution of the G residue by an A residue (AYPGKF peptide) creates a selective $PAR_4$ agonist, at least 10 times more potent than the tethered ligand peptide (Faruqi *et al*., 2000).

The understanding of the physiological and pathophysiological role of PARs has been hampered by difficult pharmacologic intervention to inhibit the activation of PARs. Different strategies and extensive chemical synthetic efforts have been employed to develop PAR antagonists (reviewed in Derian *et al*., 2003). Several $PAR_1$ antagonists are now available (see Table 1), and their use has demonstrated the prime role for $PAR_1$ in thrombotic and restenotic events. However, no studies have reported the use of $PAR_2$ or $PAR_3$ antagonists in bioassays or *in vivo* studies. Two peptidic $PAR_4$ antagonists have been tested in platelet assays (see Table 1), but their pharmacological properties in other tissues and bioassays still have to be established. Gene-deletion approach with the use of PAR-deficient mice appears as an alternate reliable approach to understand the role of PARs.

## Proteases and PARs in the GI tract

PARs have been detected in several cell types throughout the entire GI tract and pancreas (see Figure 3 and Table 2). $PAR_1$, $PAR_2$ and $PAR_4$ have been detected in enterocytes (Kong *et al*., 1997; Buresi *et al*., 2001; Mule *et al*., 2004), where $PAR_1$ and $PAR_2$ have been shown to be functional both on the apical and baso-lateral membranes (Kong *et al*., 1997; Chin *et al*., 2003). $PAR_1$ and $PAR_2$ are also expressed in human colon cancer cell lines where their activation modulates proliferation and motility (Darmoul *et al*., 2001; 2003). $PAR_1$ and $PAR_2$ have been detected in enteric neurons (extrinsic and intrinsic submucosal and myenteric neurons), where they co-express with neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) (reviewed in Vergnolle *et al*., 2003c). Although its expression on enteric neurons has not been clearly established, functional $PAR_4$ seems to be present on enteric neurons, which respond to $PAR_4$ agonists by evoking a depolarizing response in the guinea-pig small intestine (Gao *et al*., 2002). $PAR_1$ and $PAR_2$ have also been shown to be expressed in intestinal myofibroblasts (Seymour *et al*., 2001; 2003) and in mast cells (D'Andrea *et al*., 2000). The fact that $PAR_1$, $PAR_2$ and $PAR_4$ agonists cause contraction and/or relaxation in isolated GI segments suggest that those receptors

**Table 1** Structure and activation of protease-activated receptors

| | *Number of amino acids* | *Tethered ligand sequence* | *Activating protease* | *Peptidic selective agonists* | *Antagonists* |
|---|---|---|---|---|---|
| $PAR_1$ | 425 aa | Human: SFLLR<br>Mouse/rat: SFFLR | Thrombin, trypsin, cathepsin G, granzyme A, factor VIIa, factor Xa, gingipain, plasmin | TFLLR<br>ApfFRChaCitY | RWJ-56110<br>RWJ-58259<br>SCH-79797<br>SCH-73754<br>FR-171113<br>BMS-200261<br>'Merck isoxazole 1'<br>*Trans*-cinnamoyl-(*p*-F-Phe)-(*p*-guanidino-Phe)-LRR |
| $PAR_2$ | 397 aa | Human: SLIGKV<br>Mouse/rat: SLIGRL | Trypsin, tryptase, factor Xa, factor VIIa, tissue factor, acrosin, trypsin IV, membrane-type serine protease 1, dust mite proteases | SLIGRL<br>Trans-cinnamoyl-LIGRLO<br>2-furoyl-LIGRLO | |
| $PAR_3$ | 374 aa | Human: TFRGAP<br>Mouse: SFNGGP | Thrombin, trypsin | | |
| $PAR_4$ | 385 aa | Human: GYPGQV<br>Mouse: GYPGKF | Thrombin, trypsin, cathepsin G, factor VIIa, factor Xa, gingipain, trypsin IV | GYPGKF<br>GYPGFK<br>GYPGQV<br>AYPGKF | *Trans*-cinnamoyl-YPGKF<br>Palmitoyl-SGRRYGHALR |

are expressed in smooth muscle cells (Cocks *et al*., 1999; Kawabata *et al*., 2000a, b; Gao *et al*., 2002; Mule *et al*., 2002b; 2004; Zhao & Shea-Donohue, 2003). However, only the expression of $PAR_2$ in intestinal myocytes and $PAR_1$ in irradiated intestinal smooth muscle cells has been clearly established (Corvera *et al*., 1997; Wang *et al*., 2002). $PAR_1$, $PAR_2$ and $PAR_4$ expression both on endothelial surfaces and leukocytes has also been demonstrated (Hou *et al*., 1998; Vergnolle *et al*., 2002). Messengers of RNA for $PAR_1$ and $PAR_2$ have been found in parotid, sublingual and submaxillary glands, and $PAR_2$ protein has been detected in the pancreatic duct epithelium and pancreatic acinar cells (Nguyen *et al*., 1999; Kawabata *et al*., 2000c, d; 2002; Kawabata, 2003).

The GI tract and pancreas are particularly exposed to a large array of proteases (see Figure 2). Trypsin is released in the upper GI tract lumen, and in pancreatic duct under its inactive (proactive) form trypsinogen, for physiological digestive purposes. On mucosal surfaces, a balance between proteolytic activity and the presence of protease inhibitors such as pancreatic secretory trypsin inhibitor (PSTI) is constantly present. PSTI, which is released by mucus-secreting cells throughout the GI tract, prevents premature activation of pancreatic proteases, and protects mucosal surfaces from exposure to active proteolytic enzymes (Marchbank *et al*., 1996; 1998). Thus, the balance between proteolytic activity in the center of the lumen and the presence of protease inhibitors at mucosal surfaces warrants in the upper intestine efficient digestive processes and mucosal protection. In the lower GI tract, trypsin is not released into the lumen for digestive purposes. However, inflammatory bowel disease (IBD) patients showed an increased trypsin activity in their colonic luminal content, suggesting that the balance between tryptic proteolytic activity and protease inhibitors is broken in this particular pathophysiological situation, and that trypsin is present in the lumen of the lower GI tract, associated with inflammatory conditions. Trypsinogen can also be synthesized by several different extrapancreatic cell types including endothelium (Koshikawa *et al*., 1997; 1998). Trypsinogen IV and trypsin IV, which have been shown to activate $PAR_2$ and $PAR_4$ (see Table 1), have been found in epithelial cell lines from prostate, colon and airways (Cottrell *et al*., 2003; 2004). Thus, it is conceivable that endothelium- or epithelium-derived trypsin can also be present on the basolateral side of the intestinal barrier. Tryptase, which is expressed by almost all subsets of human mast cells (Caughey, 1995), is released upon mast cell degranulation. Although tryptase is considered poorly diffusible, in pathologies such as intestinal inflammation, allergy, or even stress, large amounts of tryptase have been found in the vasculature and the gut lumen of patients or animals (Plebani *et al*., 1992; Miller, 1996; Santos *et al*., 1998; 1999; Gelbmann *et al*., 1999). Proteases of the coagulation cascade such as thrombin, factor VIIa and Xa are also potentially present in the GI tract during inflammation or tissue trauma. These events have been shown to lead to the presence of pro-thrombin and active thrombin into the intestinal lumen and deeper within the intestinal tissues (McHugh *et al*., 1996), where it could activate PARs. Cathepsin G, which is released upon neutrophil activation, can also be massively present in GI tissues associated with inflammatory conditions. The transepithelial migration of neutrophils towards mucosal surfaces upon inflammation (Edens *et al*., 2002) also renders plausible the presence of cathepsin G into the gut lumen. Finally, mucosal surfaces are constantly exposed to bacterial products, and particularly to bacterial proteases. Although no studies have reported the effects of intestinal bacteria (pathogens or nonpathogens) on PAR activation, studies have reported the possible activation of $PAR_1$, $PAR_2$ and $PAR_4$ by proteases from pathogens such as *Porphyromonas gingivalis* (Lourbakos *et al*., 2001) or dust mites (Sun *et al*., 2001; Asokananthan *et al*., 2002). Thus, a variety of proteases may act on PARs and influence GI functions at several levels depending on the PAR-expressing target cells. Whether PARs are activated in

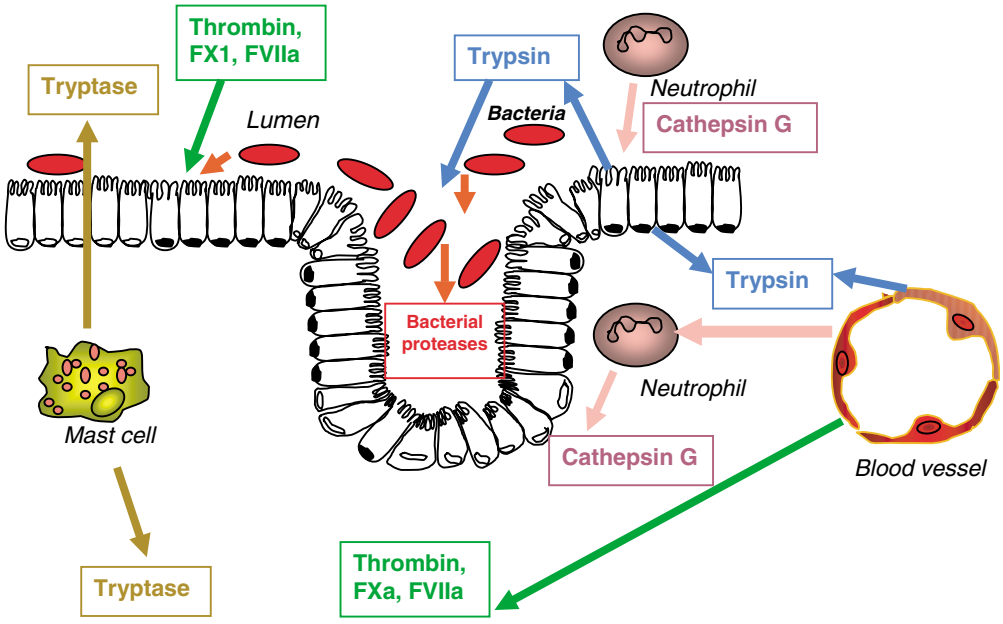


**Figure 2** Presence of proteases in the GI tract. Trypsin is released in the lumen of the GI tract for digestive purposes, but can also be present deeper into the tissues released from endothelial or epithelial cells. Proteases of the coagulation cascade such as thrombin, factor Xa and VIIa can be released into the lumen or the GI tissues upon tissue damage. When they degranulate, mast cells release massive amounts of tryptase both in the gut lumen and vasculature. Neutrophils present either in the tissues or translocated in the lumen release cathepsin G. Epithelial cells are exposed to bacterial proteases from the luminal side.

**Table 2** Localization and function of protease-activated receptors in the GI tract

| | *Localization in the GI tract* | *Known functions* |
|---|---|---|
| $PAR_1$ | Enterocytes | Increase permeability, apoptosis, chloride secretion, prostaglandin release |
| | Human colon cancer epithelium | Proliferation and motility |
| | Myenteric neurons | Suppression of fast excitatory postsynaptic potential |
| | Submucosal neurons | Inhibition of chloride secretion, |
| | Fibroblasts | Prostaglandin release |
| | Mast cells | |
| | Smooth muscle | Relaxation/contraction |
| | Endothelium | Gap formation |
| $PAR_2$ | Enterocytes | Chloride secretion, prostaglandin production, eicosanoid production |
| | Human colon cancer epithelium | Proliferation |
| | Myenteric neurons | Neuropeptide release, increased excitability, suppression of fast excitatory postsynaptic potential |
| | Submucosal neurons | Neuropeptide release, stimulate epithelial ion secretion, hyperexcitability |
| | Fibroblasts | Prostaglandin release, proliferation |
| | Mast cells | |
| | Smooth muscle | Relaxation/contraction |
| | Pancreatic duct epithelium | Ion channel activation |
| | Pancreatic acinar cells | Amylase secretion |
| | Endothelium/leukocyte interface | Rolling, adhesion, transmigration, gap formation |
| $PAR_3$ | Detected by RT–PCR in whole GI tissues (stomach and small intestine), but unidentified cell type | |
| $PAR_4$ | Enterocytes | |
| | Submucosa | Contraction of longitudinal muscle |
| | Enteric neurons | Depolarization |
| | Endothelium/leukocyte interface | Rolling, adhesion, transmigration |

physiological or pathophysiological settings might depend on the proximity of the receptors to digestive enzymes, inflammation-associated protease or proteases released by pathogen or nonpathogen organisms. However, several studies using different PAR agonists and antagonists point to a role for PARs in inflammatory and nociceptive mechanisms of the GI tract.

## Proteases and PARs: role in inflammation of the GI tract

Direct injection of thrombin, trypsin, tryptase or selective agonists for $PAR_1$ and $PAR_2$ into the paw of rodents produces edema and granulocyte infiltration, two of the main features of inflammation (Cirino *et al*., 1996; Vergnolle *et al*., 1999a, b). Since those receptors and proteases are also highly present in the GI tract, they could be involved in GI inflammatory processes.

### *Intestine*

Recently, we have shown that luminal administration of selective peptidic agonists for $PAR_1$ (TFFLR), $PAR_2$ (SLIGRL) and $PAR_4$ (AYPGKF) provoked a colonic inflammation within a few hours (from 4 to 24 h). This inflammation was characterized by an increased wall thickness, the presence of erythema and significant infiltration of granulocytes (Cenac *et al*., 2002; Ferazzini *et al*., 2004; Vergnolle *et al*., 2004). $PAR_2$-induced colitis was dependent on sensory neuron activation, substance P and CGRP release (Cenac *et al*., 2003; Nguyen *et al*., 2003). Intracolonic administration of $PAR_2$ also resulted in increased paracellular permeability, as observed by the passage of $^{51}$Cromium-EDTA from the lumen to the blood and by the presence of translocated bacteria in different intraperitoneal organs (Cenac *et al*., 2002). Tight junction blockers and inhibitors of myosin-light-chain kinase blocked this increased permeability without affecting the level of granulocyte recruitment (Cenac *et al*., 2003). This suggests that although increased permeability and granulocyte infiltration might both participate in the generation of the inflammatory response, they constitute separated events that can occur independently. Activation of $PAR_1$ also caused increased intestinal permeability both *in vivo* (intracolonic administration of TFLLR) and *in vitro* (on enterocytes monolayers), by a mechanism involving the induction of apoptosis of the intestinal epithelium, and the activation of tyrosine and myosin-light-chain kinases (Chin *et al*., 2003). The fact that $PAR_1$ activation can compromise the epithelial barrier function suggests that $PAR_1$ could be implicated in the pathogenesis of a number of disorders affecting mucosal surfaces, including IBD and infectious diseases. Using $PAR_1$-deficient mice, we have been able to show that $PAR_1$ activation is implicated in the pathogenesis of trinitrobenzene sulfonic acid (TNBS)-induced colitis. $PAR_1^{-/-}$ mice showed significantly less inflammatory damage than wild-type controls after the induction of TNBS colitis (Vergnolle *et al*., 2003a). Another recent study showed that $PAR_1$ agonist-induced epithelial cell ion transport was altered after nematode infection, also suggesting a role for $PAR_1$ in infectious intestinal diseases (Fernandez *et al*., 2003). The fact that $PAR_2$-induced colonic inflammation is regulated by a neurogenic mechanism is in favor of a role for $PAR_2$ in infectious intestinal diseases, if we consider the fact that enteric infections are largely mediated by neurogenic mechanisms (Spiller, 2002; Vergnolle *et al*., 2003c) and the fact that $PAR_2$ can be activated by bacterial proteases (Lourbakos *et al*., 2001; Sun *et al*., 2001; Asokananthan *et al*., 2002). However, no study has reported yet such role for $PAR_2$. In IBD models, however, activation of the enteric nervous system (ENS) and further release of neuropeptides protects against inflammatory damage (Collins, 2000). Thus, in the setting of chronic inflammation, $PAR_2$-induced ENS activation might exert protective effects rather than pro-inflammatory effects. This hypothesis is further supported by the findings of Fiorucci *et al*. (2001), who have observed that daily systemic treatments with $PAR_2$ agonists diminished inflammatory damage caused by the intracolonic injection of TNBS. These results suggest that $PAR_2$ agonists might be beneficial in the setting of chronic inflammation such as IBD, where they might exert protective effects though ENS activation. However, the use of such agonists might also enhance visceral hypersensitivity (see next chapter), which then would be detrimental, potentially causing more pain to patients. The use of $PAR_2$-deficient mice in different models of infectious colitis or IBD should help, in the very near future, to clarify the role of $PAR_2$ in those pathologies. However, the overexpression of $PAR_2$ observed in biopsies from ulcerative colitis patients strongly suggests a role for $PAR_2$ in IBD (Kim *et al*., 2003). We have reported that treatment of mice with the $PAR_4$ antagonist palmitoyl-SGRRYGHALR reduced inflammation induced by dextran sodium sulfate (Ferazzini *et al*., 2003). However, the specificity of such antagonist could still be questioned and only experiments performed with $PAR_4$-deficient mice would completely clarify the role of $PAR_4$ in IBD models.

### *Stomach*

$PAR_2$ can potentially modulate a variety of gastric functions, through its ability to induce the secretion of mucus and pepsinogen, to suppress acid output, to increase mucosal blood flow and to induce gastric strip contraction/relaxation (reviewed in Kawabata, 2003; Nishikawa & Kawabata, 2003). Except for pepsinogen secretion, all these effects of $PAR_2$ in the stomach favor a protective role for $PAR_2$ in gastric mucosa. As a matter of fact, treatment of rats with the selective $PAR_2$ agonist SLIGRL has been shown to be protective in models of gastritis induced by indomethacin or ethanol and HCl (Kawabata *et al*., 2001a). Here again, this protective effect of $PAR_2$ agonist has been shown to be mediated, at least in part, by the activation of sensory neurons (Kawabata *et al*., 2001a). The selective agonist for $PAR_1$ also reveals protective effects for gastric mucosa, inhibiting acid secretion and increasing gastric mucosal blood flow (Kawabata, 2003). However, this protective effect of $PAR_1$ activation does not seem to be mediated by a neurogenic mechanism, but rather by an enhancement of endogenous protective prostaglandin production (Kawabata, 2003).

### *Pancreas*

Inflammation of the pancreas leads to the premature activation of trypsin, which can then signal to pancreatic acini and duct cells through the activation of $PAR_2$ (Cottrell *et al*., 2003). Trypsin stimulates fluid and electrolyte secretion in the pancreatic ducts through a mechanism involving the activation of $PAR_2$ (Nguyen *et al*., 1999). $PAR_2$ selective agonist causes a prompt increase, followed by transient decrease of pancreatic juice secretion, and also facilitates amylase secretion (Kawabata *et al*., 2000d; 2002; Kawabata, 2003). These $PAR_2$-induced effects in the pancreas may also be protective, although *in vivo* studies using $PAR_2$-deficient mice and/or

$PAR_2$ antagonists still have to be performed to demonstrate such hypothesis.

## Proteases and PARs: mediators of visceral perception

Afferent sensory fibers of the ENS convey sensory data to the central nervous system, and the presence of $PAR_1$ and $PAR_2$ on those fibers has suggested a role for those receptors in visceral nociception mechanisms.

### *$PAR_2$*

It has been shown that peripheral (intraplantar) administration of sub-inflammatory doses of the $PAR_2$ agonist SLIGRL, and also trypsin and tryptase, provoked nociceptor activation at a spinal level, together with a severe and prolonged ($>24$ h) thermal and mechanical hyperalgesia (Vergnolle *et al*., 2001a). Transient receptor potential vanilloid-like 1 (TRPV1) mediated $PAR_2$-induced thermal, but not mechanical hyperalgesia (N. Vergnolle, unpublished work). When injected into the colon lumen, sub-inflammatory doses of $PAR_2$-activating peptide and trypsin caused visceral hyperalgesia, as observed by an increased number of abdominal contractions in response to colorectal distension (Coelho *et al*., 2002). The increased Fos expression observed in the superficial laminae of the dorsal horn in response to intracolonic administration of $PAR_2$ agonist also suggests that visceral activation of $PAR_2$ induces the activation of second-order neurons at a spinal level (Coelho *et al*., 2002). This was confirmed by a similar study, which showed that pancreatic activation of $PAR_2$ also provoked an increased Fos expression in the superficial laminae of the dorsal horn (Hoogerwerf *et al*., 2001). Since in both cases (colonic lumen or pancreatic duct exposure to $PAR_2$ agonists) increased Fos expression was observed primarily in laminae I and II of the dorsal horn, which contain nociceptive nerve terminals, this suggests that a central nociceptive signal is triggered by visceral activation of $PAR_2$. Although these experiments showed that peripheral activation of $PAR_2$ caused nociceptor activation, they did not unequivocally show that this effect was due to a direct activation of $PAR_2$ on sensory neurons. The presence of functional $PAR_2$ on dorsal root ganglia neurons (Steinhoff *et al*., 2000) and the fact that, in those cells, $PAR_2$ agonists enhanced KCl- and capsaicin (TRPV1 agonist)-evoked release of CGRP (a spinal mediator of nociception) (Hoogerwerf *et al*., 2001) strongly suggest that $PAR_2$ agonists directly activate primary afferents to induce a nociceptive signal. Other evidence suggesting that $PAR_2$ agonists signal directly to neurons to induce hyperalgesia comes from a study by Reed *et al*. (2003), which showed that trypsin, tryptase and $PAR_2$-activating peptide induced prolonged hyperexcitability of submucosal neurons isolated from the guinea-pig ileum. In a recent study, we have shown that not only peripheral but also spinal activation of $PAR_2$ can participate in inflammatory visceral hyperalgesia (Vergnolle *et al*., 2003b). Intrathecal injection of the selective $PAR_2$-activating peptide SLIGRL, but not the control peptide or their vehicle, increased (in a dose-dependent manner) the number of writhing behaviors in response to intraperitoneal injection of acetic acid. Intrathecal injections of tachykinin-1 receptor and CGRP receptor antagonists were able to block this $PAR_2$-induced enhancement of visceral hyperalgesia, suggesting that spinal release of substance P and CGRP are involved in $PAR_2$-induced spinal effect (Vergnolle *et al*., 2003b). These results suggest that $PAR_2$ activation from the periphery, but also at a spinal level, might play an important role in states of hypersensitivity.

Although a clear role for $PAR_2$ in inflammation-induced somatic hyperalgesia and particularly in mast cell degranulation-induced hyperalgesia has been demonstrated using $PAR_2$-deficient mice (Steinhoff *et al*., 2000), such role in visceral hyperalgesia has yet to be demonstrated.

### *$PAR_1$*

In contrast to what has been shown for $PAR_2$, sub-inflammatory doses of $PAR_1$ agonist did not provoke hyperalgesia when injected into the rat paw, but increased nociceptive threshold and significantly inhibited inflammatory hyperalgesia induced by intraplantar injection of carrageenan (Asfaha *et al*., 2002). Intraperitoneal injection of the selective $PAR_1$ agonist TFLLR also inhibited visceral pain behaviors induced by the intraperitoneal injection of acetic acid, by a mechanism involving the activation of opioid receptors (Vergnolle *et al*., 2003a). Intracolonic administration of thrombin and TFLLR has also been shown to produce visceral analgesia, reducing the number of abdominal contractions in response to colorectal distension (Coelho & Bunnett, 2003). Here again, there are no evidences that such analgesic effect of $PAR_1$ activation is due to a direct activation on primary afferents. Further *in vitro* studies would be necessary to determine whether or not $PAR_1$ agonists could cause hyperpolarization of the sensory neuron membrane.

## Proteases and PARs in the pathogenesis of irritable bowel syndrome (IBS)

The IBS is characterized by abdominal pain or discomfort associated with disturbed defecation and often bloating. Several pathophysiological factors are involved in the generation of symptoms of IBS: psychological factors, altered motility patterns, inflammatory mediator release, altered epithelial functions (permeability, ion exchanges and immune) and altered visceral nociceptive functions (hypersensitivity) (for a review, see Mayer & Collins, 2002). PARs are present in most of the cells that are potentially actors in the generation of IBS symptoms, and PAR activation interferes with several components of the pathogenesis of IBS (see Figure 3).

First, as discussed in the precedent paragraph, PARs are present on sensory neurons, where they can potentially interfere in a direct manner with the transmission of nociceptive signal. Activation of PARs on enteric neurons ($PAR_1$ and $PAR_2$) can also provoke the release of neuropeptides such as substance P and CGRP (Steinhoff *et al*., 2000; de Garavilla *et al*., 2001), which in turn activate their receptors present on endothelium to induce plasma extravasation. Such PAR-induced micro-inflammation might participate in the generation of IBS symptoms, as low levels of inflammation have been proposed to be involved in the pathogenesis of hypersensitivity (Collins, 2001; Collins *et al*., 2001; Bueno & Fioramonti, 2002). Activation of PARs on cells that are involved in inflammatory or immune responses, such as

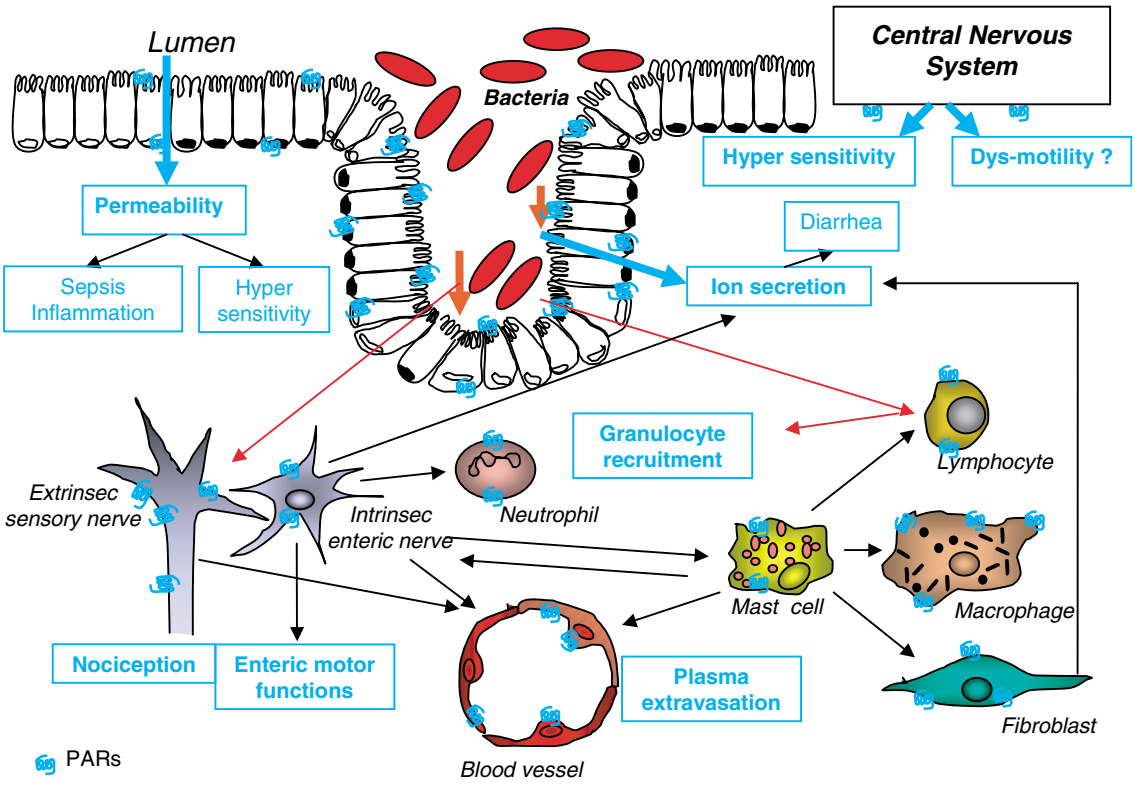


**Figure 3** Interactions of PARs with components of the pathogenesis of IBS. $PAR_1$ and $PAR_2$ are present on enterocytes, where their activation potentially induces an increase in permeability, which could lead to bacterial translocation, sepsis and inflammation, as well as hypersensitivity. Direct activation of $PAR_1$ and $PAR_2$ on enteric neurons interferes with nociceptive and motor functions. Activation of PARs on cells involved in inflammatory responses (mast cells, neutrophils, lymphocytes, macrophages, fibroblasts, endothelium or neuropeptides containing neurons) could lead to the generation of inflammatory mediators and provokes further signs of inflammation such as granulocyte recruitment and plasma extravasation. Ion secretion and potentially water flux disturbances (diarrhea) could be evoked by PAR activation on enterocytes, fibroblasts or enteric nerves. PAR activation in the central nervous system modulates nociceptive responses to peripheral stimulation, playing a potential role in hypersensitivity states and eventually (although never studied) in enteric motor dysfunctions. Activation of PARs on enterocytes, enteric neurons or lymphocytes by bacterial proteases from luminal or infiltrated pathogens could also participate in the generation of symptoms associated with IBS.

neutrophils, mast cells, lymphocytes, macrophages, or fibroblasts, might also be involved in causing hypersensitivity, by indirect stimulation of primary afferents (through the release of prostaglandins, neuropeptides or cytokines). Such indirect pro-algesic effect is in accordance with the hyperalgesia and nociceptor activation that have been demonstrated in response to visceral exposure to $PAR_2$ agonists (Hoogerwerf *et al*., 2001; Coelho *et al*., 2002). However, $PAR_1$ agonists did not cause hyperalgesia, but decreased nociceptive threshold and inhibited painful behaviors (Asfaha *et al*., 2002; Coelho & Bunnett, 2003; Vergnolle *et al*., 2003a). A possible explanation is that sub-inflammatory doses were used for $PAR_1$ agonists to cause analgesia, while larger doses of TFLLR were necessary to cause inflammation.

Compromised intestinal barrier function has been associated with IBS in child and adult patients (Barau & Dupont, 1990; Spiller *et al*., 2000), suggesting that increased intestinal permeability plays a role in the generation of the symptoms associated with IBS. Since both $PAR_1$ and $PAR_2$ activation disrupted the integrity of the intestinal barrier, it can be hypothesized that PAR-induced increased permeability plays a role in visceral hypersensitivity states. As a matter of fact, a recent study has shown that a tight junction blocker (2,4,6 triaminopyrimidine – TAP) was able to inhibit $PAR_2$-induced permeability, but also at the same time inhibited $PAR_2$-induced rectal hypersensitivity (Moriez *et al*., 2003). The fact that $PAR_1$ agonists induce increased permeability, and also analgesia, might also depend on the doses of $PAR_1$ agonists used. In order to cause bacterial translocation and significant increase of $^{51}$CrEDTA passage from the lumen to the blood, doses of 200 $\mu$g per mouse were used (Chin *et al*., 2003), while doses of only 1–100 $\mu$g per animal were used in rats to observe analgesia (Coelho & Bunnett, 2003; Vergnolle *et al*., 2003a). This suggests that at low doses $PAR_1$ activation induces analgesia independently of permeability dysfunctions, while high doses of $PAR_1$ agonists might cause hyperalgesia through a mechanism dependent on the integrity of intestinal barrier.

Enterocytes that secrete electrolytes, such as chloride, promote the movement of water in the intestinal lumen, and can thereby regulate IBS symptoms such as diarrhea. Recent studies have demonstrated that $PAR_1$ activation in human non-transformed intestinal epithelia cell line (SCBN) results in calcium-dependent chloride secretion through a pathway that involves MAP-kinase and cyclooxygenase (Buresi *et al*., 2001). In contrast, activation of $PAR_1$ in isolated segments of mouse colon resulted in a decrease of neurally evoked ion secretion

(Buresi *et al*., 2003). Thus, depending on the target cells where $PAR_1$ is activated, this activation can lead to different diarrheal or anti-diarrheal symptoms. $PAR_2$ activation in isolated intestinal segments also provoked chloride secretion (Vergnolle *et al*., 1998). Several cells could constitute the primary target for $PAR_2$-induced chloride secretion: enterocytes which express functional $PAR_2$ (Kong *et al*., 1997), fibroblasts which stimulate $PGE_2$ release in response to $PAR_2$ agonists (Seymour *et al*., 2003), or enteric neurons which have been shown to be involved at least in part in $PAR_2$-induced ion transport in porcine ileum (Green *et al*., 2000). It is not yet known whether or not $PAR_1$ and $PAR_2$ activation participate in the generation of diarrhea symptoms associated with IBS, but considering the effects of PAR agonists on epithelial secretory functions PARs appear as potential therapeutic targets in the treatment of secretory dysfunctions.

Thrombin, trypsin and mast cell tryptase, as well as selective agonists for $PAR_1$, $PAR_2$ and $PAR_4$, enhanced the excitability and firing of enteric neurons (Gao *et al*., 2002). In that study, the authors suggest that these PARs can be activated on inhibitory motor enteric neurons, which would lead to inhibited contractile and motor activity. Several studies have reported the effects of PAR agonists on motor functions of the GI tract (Kawabata *et al*., 2001b; Mule *et al*., 2002a, b; 2004; Zhao & Shea-Donohue, 2003), suggesting that PAR activation could participate in motor dysfunctions associated with IBS. Whether direct activation of PARs on smooth muscle cells or on enteric neurons is involved in PAR-induced GI motor changes still has to be determined.

Finally, bi-directional interactions between the central nervous system and the gut-directed pathogenetic mechanisms are playing a major role in the development of IBS symptoms. Whether or not proteases and PARs are implicated in such interactions still has to be investigated. However, a recent study reports that central activation of $PAR_1$ by thrombin or selective $PAR_1$ agonist inhibits NMDA-mediated nociception (both somatic and visceral), by a pathway involving endothelin type A receptors (Fang *et al*., 2003). We also reported that spinal activation of $PAR_2$ exacerbated visceral pain behaviors (Vergnolle *et al*., 2003b). These results suggest an important role for central activation of PARs in states of hypersensitivity, in accordance with a pro-algesic role for $PAR_2$ activation and an analgesic role for $PAR_1$ activation.

## Conclusions

The development of drugs for IBS remains a therapeutic challenge. Scientists are constantly in search of potential new therapeutic targets for the treatment of IBS, and PARs appear as interesting receptors that clearly interfere with visceral nociceptive pathways. PARs are expressed in diverse cell types of the GI tract and their activation is associated with several pathophysiological factors that are involved in the generation of IBS symptoms, such as altered motility patterns, inflammatory mediator release, altered epithelial functions and altered visceral nociceptive functions. Further studies using genetically modified animals, and, when available, pharmacological tools, in different IBS and IBD models would definitively contribute to our understanding of the role of these receptors and their activating proteases in visceral pain and inflammation processes.

Dr Vergnolle is an Alberta Heritage Foundation for Medical Research Scholar. Her work is supported by the Canadian Association of Gastroenterology, the Crohn's and Colitis Foundation of Canada, and the Canadian Institute for Health Research.

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*(Received January 27, 2004*
*Revised February 19, 2004*
*Accepted February 19, 2004)*