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REVIEW

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Gastrointestinal roles for proteinase-activated receptors in health and disease

A Kawabata, M Matsunami and F Sekiguchi

Division of Pharmacology and Pathophysiology, Kinki University School of Pharmacy, Higashi-Osaka, Japan

It has been almost a decade since the molecular cloning of all four members of the proteinase-activated receptor (PAR) family was completed. This unique family of G protein-coupled receptors (GPCRs) mediates specific cellular actions of various endogenous proteinases including thrombin, trypsin, tryptase, etc. and also certain exogenous enzymes. Increasing evidence has been clarifying the emerging roles played by PARs in health and disease. PARs, particularly PAR1 and PAR2, are distributed throughout the gastrointestinal (GI) tract, modulating various GI functions. One of the most important GI functions of PARs is regulation of exocrine secretion in the salivary glands, pancreas and GI mucosal epithelium. PARs also modulate motility of GI smooth muscle, involving multiple mechanisms. PAR2 appears to play dual roles in pancreatitis and related pain, being pro-inflammatory/pro-nociceptive and anti-inflammatory/anti-nociceptive. Similarly, dual roles for PAR1 and PAR2 have been demonstrated in mucosal inflammation/damage throughout the GI tract. There is also fundamental and clinical evidence for involvement of PAR2 in colonic pain. PARs are thus considered key molecules in regulation of GI functions and targets for development of drugs for treatment of various GI diseases.
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Keywords: proteinase-activated receptor; PAR; proteinase; gastrointestinal function; exocrine secretion; pancreatitis; colitis; pain; smooth muscle

Abbreviations: GERD, gastroesophageal reflex disease; GI, gastrointestinal; GPCR, G-protein-coupled receptor; HEEC, normal human oesophageal epithelial cell; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; PAR, proteinase-activated receptor; PPI, proton-pump inhibitor; TNBS, 2,4,6-trinitrobenzene sulphonic acid

Introduction

The proteinase-activated receptor (PAR) is a unique family of G-protein-coupled seven transmembrane domain receptors (GPCRs) that have relatively long N-terminal domains, compared with other GPCRs. It has been more than a decade and a half since the first molecular cloning of PAR1, a thrombin receptor, was carried out (Vu *et al*., 1991a, b). Cloning of all other members of the PAR receptor family, that is, PAR2, PAR3 and PAR4, was not completed until 1998 (Nystedt *et al*., 1994; Ishihara *et al*., 1997; Kahn *et al*., 1998; Xu *et al*., 1998). PAR3 and PAR4 are also activated by thrombin, whereas PAR2 does not respond to thrombin at all (Hollenberg *et al*., 1997; Kawabata *et al*., 1999b; Kawabata, 2002). Although PAR2 was originally believed to be a receptor just for trypsin and mast cell tryptase (Nystedt *et al*., 1994; Molino *et al*., 1997), a number of endogenous and exogenous proteinases including kallikreins and mite allergens are now known to stimulate PAR2 (Sun *et al*., 2001; Kawabata, 2002; Ossovskaya and Bunnett, 2004; Hansen *et al*., 2005; Hollenberg, 2005; Oikonomopoulou *et al*., 2006a, b). The unique activation mechanisms for PARs are as follows: (1) agonist proteinases unmask the cryptic receptor-activating peptide sequence present in the extracellular N-terminal domain of each PAR, leading to cell signalling via interaction of the exposed tethered ligand with the body of the receptor itself; and (2) synthetic peptides as short as 5–6 amino acids, on the basis of tethered ligand sequences, are capable of binding to PARs, mimicking the actions of agonist proteinases, in the case of PAR1, PAR2 and PAR4 (Kawabata, 2002; Ossovskaya and Bunnett, 2004; Hollenberg, 2005). In contrast, synthetic peptides based on the presumed N-terminal PAR3-activating sequence are incapable of causing PAR3 signalling, and the physiological significance of PAR3 is not well understood (Kawabata, 2002; Ossovskaya and Bunnett, 2004; Hollenberg, 2005). Interestingly, PAR3 could be a cofactor for activation of PAR4 by thrombin (Nakanishi-Matsui *et al*., 2000), and might also regulate PAR1 signalling by receptor dimerization (McLaughlin *et al*., 2007). Common major cell signals triggered by activation of distinct PARs are activation of phospholipase C_β

Correspondence: Dr A Kawabata, Division of Pharmacology & Pathophysiology, Kinki University School of Pharmacy, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan.
E-mail: kawabata@phar.kindai.ac.jp
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via $G_{q/11}$ proteins, leading to the formation of inositol triphosphate followed by Ca^{2+} mobilization and diacylglycerol-mediated activation of PKC. However, it is now known that a variety of signalling pathways other than activation of phospholipase C_{β} can also be stimulated by the activation of PARs, which are different depending on types of PARs and cells/tissues (Kawabata, 2002; Ossovskaya and Bunnett, 2004; Hollenberg, 2005; Kawao *et al.*, 2005; Sekiguchi *et al.*, 2007).

Since selective and potent antagonists of PARs, particularly PAR2, have not been easily available, lessons from studies using receptor-activating peptides and genetically receptor-deficient mice have greatly contributed to elucidation of functions of PARs. Increasing evidence has demonstrated emerging roles for PARs in the mammalian body. PARs, particularly PAR1 and PAR2, are distributed throughout the gastrointestinal (GI) tract, and are now considered key molecules in regulation of GI functions and in the pathogenesis of GI diseases. Here we focus on GI roles of PARs in health and disease. Currently available clinical aspects on PARs are also mentioned in this review.

Major GI functions of PARs

PAR2 and exocrine secretion

One of the most important functions of PAR2 in the mammalian body, particularly in the GI system, is regulation of glandular exocrine secretion (Figure 1). PAR2-mediated release of amylase from isolated rat pancreatic acini was first described by Bohm *et al.* (1996), and PAR2 is now recognized as one of the key molecules in regulation of pancreatic exocrine secretion (Nguyen *et al.*, 1999; Kawabata *et al.*, 2000c, d, 2002b; Singh *et al.*, 2007). PAR2 agonists, in a manner dependent on cytosolic Ca^{2+} mobilization, enhance not only protein secretion by acinar cells (Sharma *et al.*, 2005b), but also transport of ions such as Cl^- and K^+ in pancreatic ductal epithelial cells, possibly through interaction with basolateral PAR2 (Nguyen *et al.*, 1999). Basolateral application of PAR2 agonists also increases bicarbonate (HCO_3^-) secretion by pancreatic ductal cells (Namkung *et al.*, 2004) (Figure 1), although activation of apical PAR2 might suppress ductal secretion of HCO_3^- (Alvarez *et al.*, 2004).

PAR2-activating peptides cause prompt salivation *in vivo* (Kawabata *et al.*, 2000c) and secretion of proteins including amylase and mucin in isolated rat parotid and sublingual glands, respectively, *in vitro* (Kawabata *et al.*, 2000c, d) (Figure 1). Ultimate evidence for roles of PAR2 in salivary exocrine secretion has been obtained by a study employing PAR2-knockout mice (Kawabata *et al.*, 2004b). Interestingly, PAR2-mediated salivary exocrine secretion is enhanced in M_3-acetylcholine receptor-deficient mice (Nishiyama *et al.*, 2007), implying that PAR2 might compensate for impaired salivary function due to M_3 receptor deficiency. If this is the case in humans, PAR2 could be a target for development of drugs for treatment of dysfunctions of salivary secretion such as dry mouth. Further, since PAR2-related peptides are capable of causing tear secretion through both PAR2-dependent and -independent mechanisms (Nishikawa *et al.*, 2005), PAR2 agonists might be suitable for the treatment of exocrine dysfunction such as Sjögren syndrome.

Evidence suggests that PAR2 plays an emerging role in the regulation of exocrine secretion in gastric mucosa (Figure 1). Our immunohistochemical study (Kawao *et al.*, 2002a) indicates that PAR2 is particularly abundant in rat gastric mucosal chief cells. Actually, PAR2 agonists elicit secretion of pepsinogen into the gastric lumen *in vivo*, an effect resistant to omeprazole, a proton-pump inhibitor (PPI), N^G-nitro-L-arginine methyl ester, an NOS inhibitor, or atropine, a muscarinic receptor antagonist (Kawao *et al.*, 2002a). PAR2-triggered pepsinogen secretion has also been confirmed in guinea pig gastric-isolated chief cells, and involvement of cytosolic Ca^{2+} mobilization, and activation of the MEK–ERK pathway in the secretory mechanisms has been suggested (Fiorucci *et al.*, 2003). Functional PAR2 appears to be expressed in capsaicin-sensitive sensory neurons in rat gastric mucosa (Kawabata, 2002), although PAR2 immunostaining of the nerve endings in the gastric mucosa has not been successful (Kawao *et al.*, 2002a). PAR2 agonists trigger gastric mucus secretion in anaesthetized rats, an effect that is abolished by ablation of sensory neurons by pretreatment with capsaicin, and by antagonists of $CGRP_1$ receptors and of NK_2 receptors for tachykinins (Kawabata *et al.*, 2001b). These findings are consistent with evidence that exogenously applied CGRP and neurokinin A stimulate synthesis and/or release of gastric mucus (Ichikawa *et al.*, 2000; Kawabata *et al.*, 2001b). In contrast, systemic administration of PAR2 agonists suppresses gastric acid secretion caused by carbachol, pentagastrin or 2-deoxy-D-glucose, an effect that is resistant to pretreatment with indomethacin or ablation of capsaicin-sensitive sensory neurons (Nishikawa *et al.*, 2002). The precise mechanisms for the PAR2-mediated suppression of acid secretion are still open to question.

PAR2 is also expressed in intestinal epithelial cells (Kong *et al.*, 1997; Green *et al.*, 2000). In isolated segments of rat jejunum, serosal application of agonists for PAR2 stimulates Cl^- secretion through prostanoid formation, which is independent of enteric nerves (Vergnolle *et al.*, 1998) (Figure 1). There is also evidence that basolateral PAR2 stimulation induces neurally independent Cl^- secretion in human and mouse colon *in vitro* (Cuffe *et al.*, 2002; Mall *et al.*, 2002) (Figure 1). In contrast, luminal activation of PAR2 in mouse colon appears to increase colonic paracellular permeability (Cenac *et al.*, 2004; Roka *et al.*, 2007). Most recently, PAR2 regulation of electrolyte secretion has also been described in the gallbladder. In the gallbladder of wild-type, but not PAR2-knockout mice, serosally applied PAR2 agonists cause HCO_3^- secretion (Figure 1), which is independent of prostanoids (Kirkland *et al.*, 2007). Thus, PAR2 is considered as a key molecule in regulation of epithelial ion transport in the alimentary system.

Do thrombin receptors (PAR1 and PAR4) play roles in regulation of exocrine secretion?

Unlike PAR2, none of the thrombin receptors including PAR1 and PAR4 is involved in the regulation of salivary or pancreatic exocrine secretion (Nguyen *et al.*, 1999; Kawabata *et al.*, 2000c, d). In gastric mucosa, however, agonists of PAR1

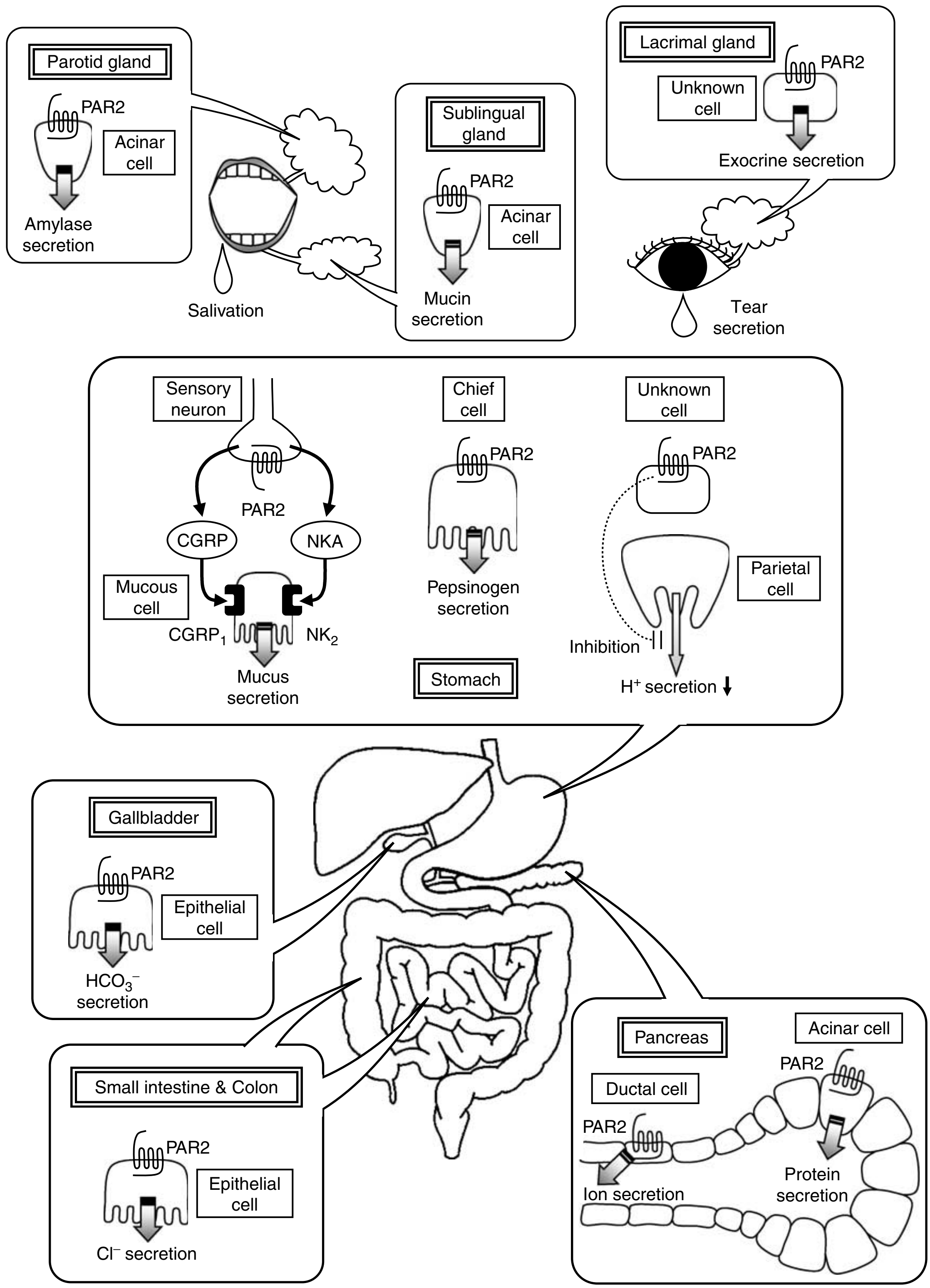

Figure 1 The role of PAR2 in exocrine secretion. It is of note that functions of PAR2 shown here have not necessarily been demonstrated in humans. NKA, neurokinin A; NK_2, neurokinin NK_2 receptor; $CGRP_1$, $CGRP_1$ receptor; PAR, proteinase-activated receptor.

suppress carbachol-evoked gastric acid secretion through COX-1-dependent formation of prostaglandins (Kawabata *et al*., 2004d). Since immunoreactive PAR1 and COX-1 are colocalized in the muscularis mucosae of rats and humans (Kawabata *et al*., 2004d), it is hypothesized that prostanoids derived from the muscularis mucosae in response to PAR1 stimulation might contribute to suppression of the gastric acid secretion. Although PAR1 agonists also facilitate pepsinogen secretion *in vivo* (Kawao *et al*., 2003), gastric mucosal chief cells themselves do not appear to express PAR1. PAR1 is expressed on both the basolateral and apical sides of SCBN, a novel nontransformed human duodenal epithelial cell line. Stimulation of basolateral PAR1 causes apically directed Cl^- secretion (Buresi *et al*., 2002), while stimulation of apical PAR1 results in apoptosis and increases in epithelial monolayer permeability (Chin *et al*., 2003). PAR1 is also expressed on submucosal secretomotor neurons in mouse colon, and its activation suppresses neurally evoked Cl^- secretion (Buresi *et al*., 2005). To our best knowledge, PAR4 does not appear to play significant roles in GI exocrine secretion.

PARs and modulation of GI smooth muscle motility

PAR1, PAR2 and PAR4 are expressed in smooth muscle cells and/or their adjacent cells in the GI tract, modulating smooth muscle motility. The roles of PARs in motility modulation are highly complex, and are greatly different depending on species and organs. Both PAR2 and PAR1 agonists cause strong constriction in isolated mouse gastric longitudinal smooth muscle strips, whereas they produce transient relaxation in the same preparations when precontracted by carbachol (Cocks *et al*., 1999b; Sekiguchi *et al*., 2006). In isolated mouse small intestine, agonists for PAR2 or PAR1 elicit transient relaxation followed by contraction (Sekiguchi *et al*., 2006). It has been confirmed that any responses to PAR2 agonists, as shown in GI smooth muscle preparations from wild-type mice, completely disappear in the preparations from PAR2-deficient animals (Sekiguchi *et al*., 2006). In rat duodenal preparations, PAR2 agonists produce slowly developing and persistent contraction, while PAR1 agonists cause prompt relaxation followed by strong contraction (Kawabata *et al*., 1999a). There is also evidence that either PAR2 or PAR1 agonists elicit contraction and/or relaxation in colonic smooth muscle preparations (Corvera *et al*., 1997; Mule *et al*., 2002a, b; Sato *et al*., 2006). Of note is that PAR4 agonists also contract rat colonic tissue strips (Mule *et al*., 2004). In rat oesophageal muscularis mucosae preparations, PAR1 agonists produce contraction, while PAR4 agonists induce relaxation (Kawabata *et al*., 2000a). These observations are in agreement with evidence that thrombin produces contraction and relaxation at high and low concentrations, respectively (Kawabata *et al*., 2000a). Thus, modulation of GI smooth muscle motility by PAR1, PAR2 and PAR4 is complex, and its physiological and pathophysiological relevance is still largely open to question. Of interest is that both PAR2 and PAR1 agonists, administered systemically, facilitate GI transit in mice (Kawabata *et al*., 2001c), which might predict protective roles for those receptors activated by endogenous proteinases during inflammation. PAR2-mediated relaxation in the colonic smooth muscle is impaired after colonic inflammation induced by dextran sodium sulphate (DSS) in rats (Sato *et al*., 2006), implying involvement of altered PAR2 functions in abnormal intestinal motility during intestinal inflammation.

The mechanisms for modulation of GI motility by PARs are also very complex, involving multiple pathways (Figure 2). Primarily, PAR1, PAR2 and PAR4 present in muscular cells are considered to mediate the contractile activity of agonists for each receptor in the GI smooth muscle. Activation of the $G_{q/11}$-phospholipase C_β pathway following activation of each PAR should play a central role in causing smooth muscle contraction (Kawabata, 2002; Mule *et al*., 2002b; Ossovskaya and Bunnett, 2004; Hollenberg, 2005). In some GI preparations, however, endogenous prostanoids formed by activation of PAR might contribute to the evoked

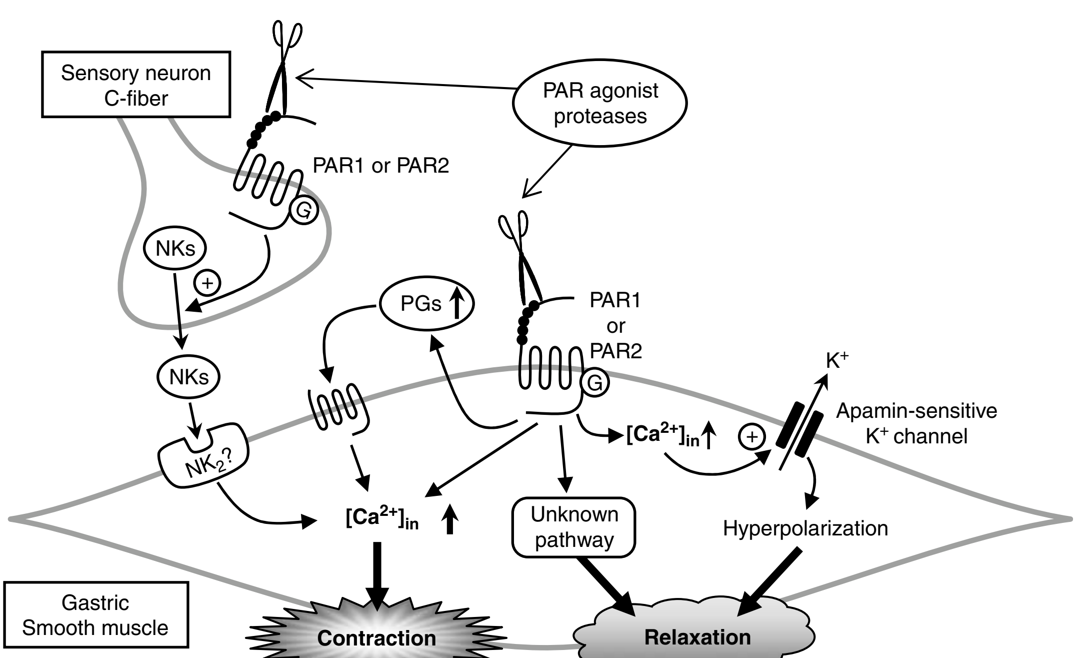

Figure 2 Mechanisms for regulation of GI smooth muscle motility by PARs. $[Ca^{2+}]_{in}$, intracellular (cytosolic) Ca^{2+} concentration; NKs, neurokinins; PGs, prostaglandins; NK_2, neurokinin NK_2 receptor; G, G protein; GI, gastrointestinal.

muscular constriction through autocrine and/or paracrine mechanisms (Saifeddine *et al*., 1996; Zheng *et al*., 1998; Sekiguchi *et al*., 2006). Further, involvement of sensory neurons has also been suggested in the contractile activity of PAR agonists in certain regions of the GI tract (Mule *et al*., 2003, 2004; Zhao and Shea-Donohue, 2003;Q9 Sekiguchi *et al*., 2006). The relaxant activity of PAR agonists in mouse gastric and rat duodenal and colonic smooth muscle segments is predominantly attributable to activation of apamin-sensitive K^+ channels, that is, small-conductance Ca^{2+}-activated K^+ channels (Cocks *et al*., 1999b; Kawabata *et al*., 1999a; Mule *et al*., 2002a; Sekiguchi *et al*., 2006). The accelerated GI transit by agonists for PAR2 and PAR1 is also further enhanced by pretreatment with apamin, suggesting dual roles (suppression and excitation) of these receptors in regulation of GI motility *in vivo* (Kawabata *et al*., 2001c). However, other unknown mechanisms should also be involved in the relaxant effects of PAR agonists in the GI tract, since apamin exerts partial and no inhibition of the PAR-mediated relaxation in some intestinal preparations and rat oesophagenal muscular segments, respectively (Kawabata *et al*., 2000a; Mule *et al*., 2003; Sekiguchi *et al*., 2006). Physiological and pathological significance of these complex mechanisms for PAR modulation of GI smooth muscle motility has yet to be investigated.

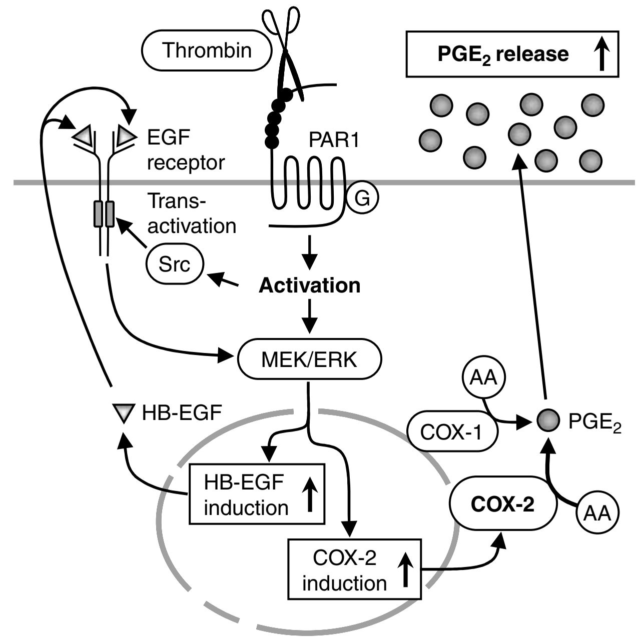

Figure 3 Signal transduction for PAR1-triggered prostaglandin E_2 formation in RGM1 cells. HB-EGF, heparin-binding EGF; AA, arachidonic acid; G, G protein; PGE_2, prostaglandin E_2.

Cellular signalling triggered by PARs in GI epithelial cells

Cellular signal transduction following activation of PAR1 or PAR2 has been investigated pharmacologically in GI smooth muscle segments (Zheng *et al*., 1998; Kawabata *et al*., 2000b; Mule *et al*., 2002b). Apart from cancer cell lines (Darmoul *et al*., 2004a, b; Nguyen *et al*., 2005), cellular signalling triggered by activation of PARs in normal GI epithelial cells has not been well understood. As described for the airway or lung epithelial cells/tissues (Cocks *et al*., 1999a; Asokananthan *et al*., 2002; Kawao *et al*., 2005), activation of PARs causes prostanoid formation in the GI tissues/cells (Kong *et al*., 1997; Toyoda *et al*., 2003; Kawabata *et al*., 2004d; Kubo *et al*., 2006; Sekiguchi *et al*., 2007). In a rat normal gastric mucosal epithelial cell line, RGM1, which is useful for analysis of functions of noncancer gastric mucosal epithelial cells, PAR1 agonists, but not PAR2 agonists, cause delayed formation of prostaglandin E_2 (PGE_2) accompanied with COX-2 upregulation, although both PAR1 and PAR2 agonists elicit cytosolic Ca^{2+} mobilization (Toyoda *et al*., 2003; Sekiguchi *et al*., 2007). The signal transduction mechanisms for PAR1-triggered upregulation of COX-2 in RGM1 cells involve persistent activation of the MEK–ERK pathway and EGF receptors, while other multiple signalling molecules including Src, heparin-binding EGF, and COX-1, are also considered responsible for the PGE_2 formation and/or COX-2 upregulation (Sekiguchi *et al*., 2007) (Figure 3). Similarly, in SCBN duodenal epithelial cells, Src, EGF receptors, the MEK–ERK pathway, cytosolic phospholipase A_2, and both COX-1- and COX-2-derived products other than $PGF_{2\alpha}$ or PGE_2 are involved in Cl^- secretion caused by activation of PAR1 (Buresi *et al*., 2002). These complex signalling mechanisms, particularly activation of the MEK–ERK pathway and EGF receptors, following PAR1 stimulation in GI epithelial cells are similar, in part, to PAR2-triggered cell signals in lung epithelial cells (Kawao *et al*., 2005).

Roles of PARs in diseases

PAR2 and pancreatic inflammation/pain

As described above, PAR2 is expressed in pancreatic acinar cells (Kawabata *et al*., 2002b) and ductal epithelium (Nguyen *et al*., 1999), and its activation stimulates pancreatic juice secretion (Kawabata *et al*., 2000d). Although PAR2 might not play critical roles in pancreatic exocrine secretion under physiological conditions, increasing evidence suggests the emerging roles played by PAR2 during pancreatitis (Olejar *et al*., 2001; Namkung *et al*., 2004; Maeda *et al*., 2005; Sharma *et al*., 2005a; Matej *et al*., 2006; Kawabata *et al*., 2006b; Singh *et al*., 2007). PAR2 expression in the pancreas appears to increase during taurocholate-induced acute pancreatic lesion development in rats, although the physiological relevance of PAR2 upregulation remains to be determined in this model (Olejar *et al*., 2001). Systemic administration of PAR2 agonists suppresses caerulein-induced acute pancreatitis in rats and mice (Namkung *et al*., 2004; Sharma *et al*., 2005a; Kawabata *et al*., 2006b). PAR2-deficient mice exhibit more severe inflammatory signs than wild-type animals in a relatively severe pancreatitis model induced by 12 hourly injections of caerulein at $50\,\mu g\,kg^{-1}$ (Sharma *et al*., 2005a), suggesting a protective role for activation of PAR2 by endogenous proteinase such as trypsin. However, the difference in the severity of inflammatory symptoms between PAR2-deficient and wild-type animals is not clear in a mild pancreatitis model induced by 6 hourly injection of caerulein at the same dose (Kawabata *et al*., 2006b). The protective mechanisms for PAR2 in pancreatitis appear to, at least in part, involve inhibition of translocation of phosphorylated ERK to the nucleus in pancreatic cells (Sharma *et al*., 2005a), although phosphorylation of ERK in whole cells is unaffected or rather facilitated by PAR2 activation (Namkung *et al*., 2004; Sharma *et al*., 2005a). The most recent evidence indicates that trypsin released during the early stages of pancreatitis activates PAR2 on the acinar cells and stimulates secretion of digestive enzymes including trypsinogen from these cells, leading to decreased intrapancreatic enzyme levels and limitation of the severity of pancreatitis (Singh *et al*., 2007) (Figure 4). In contrast, a study using anti-PAR2-antibodies implies a pro-inflammatory role for PAR2 in caerulein-induced pancreatitis in rats (Maeda *et al*., 2005), which is inconsistent with evidence from studies employing PAR2-activating peptides and PAR2-knockout mice. Although the discrepancy has yet to be explained, it is likely that trypsin-induced activation of PAR2 present in intrapancreatic sensory neurons (Steinhoff *et al*., 2000; Hoogerwerf *et al*., 2001) might promote inflammation, since pancreatitis appears to involve neurogenic inflammation (Nathan *et al*., 2001, 2002; Hutter *et al*., 2005). There are plenty of clinical and fundamental studies showing that inhibitors of pancreatic proteinases that are capable of activating PAR2 improve acute pancreatitis (Iwaki *et al*., 1986; Otsuki *et al*., 1990; Harada *et al*., 1991; Takeda *et al*., 1996; Chen *et al*., 2000; Maeda *et al*., 2005; Ishikura *et al*., 2007).

Clinically, acute pancreatitis is accompanied with a sharp and severe pain from the upper abdominal area to the back, and treatment of the pancreatitis-related pain is very important. Apart from pancreatitis itself, PAR2 expressed in sensory neurons is involved in pancreatic pain (Hoogerwerf *et al*., 2001, 2004; Kawabata *et al*., 2006b; Ishikura *et al*., 2007). Administration of PAR2-activating peptides and trypsin into the pancreatic duct causes activation of nociceptive neurons, as measured by expression of Fos protein, in the superficial layers of the thoracic spinal cord in anesthetized rats, and induces a behavioural pain response in awake rats (Hoogerwerf *et al*., 2001, 2004; Ishikura *et al*., 2007). The ductal trypsin-evoked spinal Fos expression can be blocked by pretreatment with camostat mesilate, a proteinase inhibitor (Ishikura *et al*., 2007). The mice with mild pancreatitis caused by 6 hourly repeated systemic administration of caerulein exhibit referred hyperalgesia in the skin of the upper abdomen. This referred hyperalgesia during the mild pancreatitis can be abolished by not only repeated but also single administration of the proteinase inhibitor, camostat mesilate (Ishikura *et al*., 2007), and nafamostat mesilate (Kawabata *et al*., a manuscript in preparation). This suggests a possibility that endogenous proteinases including trypsin might directly stimulate PAR2 present in intrapancreatic sensory neurons during pancreatitis, resulting in pancreatic pain/referred hyperalgesia (Figure 4). Nonetheless, the referred hyperalgesia during the pancreatitis in PAR2-knockout mice is more severe than that in wild-type animals, while the inflammatory symptoms in this mild pancreatitis model are not significantly different between the PAR2-knockout and wild-type animals (Kawabata *et al*., 2006b). Further, repeated co-administration of PAR2-activating peptides with caerulein suppressed the referred hyperalgesia in wild-type animals, but not PAR2-knockout mice. Thus, the role of PAR2 in pancreatitis-related pain is very complex. One possibility is, as mentioned above, that trypsin released during the early stages of pancreatitis

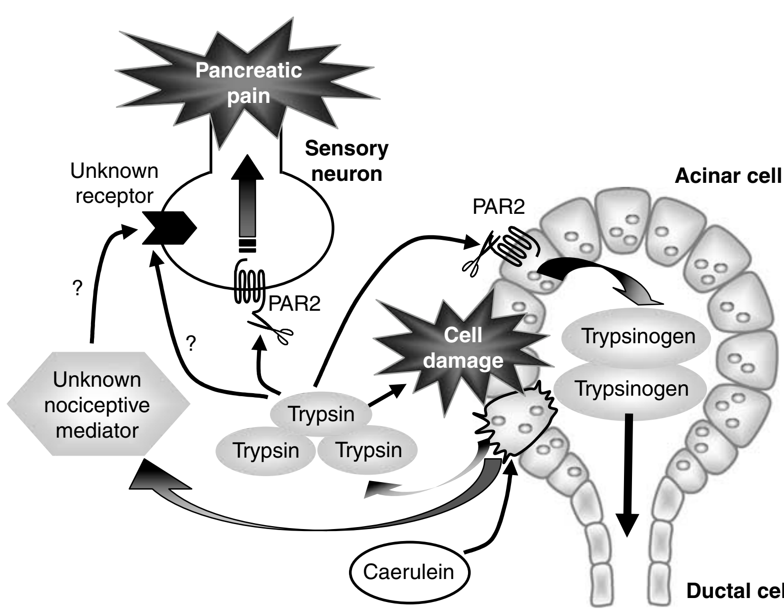

Figure 4 A scheme for roles of PAR2 expressed on sensory neurons and acinar cells during pancreatitis. Trypsin, released from acinar cells in response to cytotoxic stimulation such as caerulein, causes pancreatic cell damage by proteolytic digestion, and would also activate neuronal PAR2, leading to pancreatic pain. In the early stages of pancreatitis, trypsin could activate PAR2 on the acinar cells and decrease intrapancreatic trypsin levels by stimulating exocrine secretion of trypsinogen into the duodenum, limiting the extent of pancreatitis and related pain. Unknown non-PAR2 receptors on sensory neurons should mediate pancreatic pain in response to trypsin and/or unknown nociceptive mediators, derived from acinar cells, particularly in PAR2-knockout mice.

might stimulate PAR2 on the acinar cells and decrease intrapancreatic levels of nociceptive mediators including trypsin through enhancement of exocrine secretion of acinar cell contents such as trypsinogen into the duodenum (Figure 4). In PAR2-knockout mice, however, receptors other than PAR2 expressed in intrapancreatic sensory neurons should mediate the actions of trypsin and/or the other unknown nociceptive messengers present in the acinar cells (Figure 4). It is likely that PAR4 might mediate the nociceptive actions of trypsin and kallikrein released from the acinar cells, since PAR4 can be activated directly by those acinar cell enzymes (Kawabata, 2002; Ossovskaya and Bunnett, 2004; Oikonomopoulou *et al*., 2006a, b). Bradykinin B_2 receptors could also mediate the nociception through the kallikrein–bradykinin pathway, known to be activated during pancreatitis (Griesbacher and Lembeck, 1992; Griesbacher *et al*., 2002), since even a single dose of HOE-140, a B_2 receptor antagonist, partially inhibited the established referred hyperalgesia during pancreatitis in mice (Kawabata *et al*., unpublished data). These hypotheses have yet to be evaluated by more in-depth studies. Together, proteinase inhibitors and PAR2 antagonists, if available, might be clinically useful for the treatment of pain accompanying established acute pancreatitis, although the use of PAR2 antagonists might not be recommended in the early stages of acute pancreatitis. Interestingly, there is clinical evidence that proteinase inhibitors such as nafamostat mesilate and gabexate mesilate are highly effective against established acute pancreatitis-related pain (Harada *et al*., 1991; Takeda *et al*., 1996; Chen *et al*., 2000).

PARs and mucosal injury/protection in the oesophagus, stomach and colon

PARs, particularly PAR2 and PAR1, play emerging roles in maintenance of mucosal integrity and/or pathogenesis of mucosal inflammation/injury throughout the GI tract including the oesophagus (Kawabata, 2002, 2003; Ossovskaya and Bunnett, 2004). Systemic administration of PAR2 agonists exerts gastric mucosal cytoprotection in rat gastric injury models induced by HCl/ethanol and by indomethacin, an effect that is abolished by ablation of capsaicin-sensitive sensory nerves (Kawabata *et al*., 2001b). This is in agreement with evidence that PAR2 agonists stimulate neurally-mediated gastric mucus secretion in rats (Kawabata *et al*., 2001b), as mentioned above. It is also noteworthy that PAR2 stimulation causes vasorelaxation in isolated gastric artery *in vitro* and enhances gastric mucosal blood flow *in vivo* (Kawabata *et al*., 2001b, 2003, 2004c). Inhibition of gastric acid secretion by PAR2 agonists (Nishikawa *et al*., 2002) might also contribute to prevention of gastric mucosal injury in certain models. Ultimate evidence for involvement of PAR2 in gastric mucosal protection has been obtained from a study showing that protective effects of PAR2 agonists on HCl/ethanol-induced gastric mucosal injury is detectable in wild-type mice, but not PAR2-knockout mice, although the extent of the evoked gastric mucosal damage is not different between wild-type and PAR2-knockout animals (Kawabata *et al*., 2005). PAR1 agonists also protect against gastric mucosal injury produced by HCl/ethanol in rats (Kawabata *et al*., 2004d). Interestingly, the protective effect of PAR1 agonists, unlike PAR2 agonists, is independent of sensory neurons, but is mediated by COX-1-derived endogenous prostanoids (Kawabata *et al*., 2004d). It is noteworthy that PAR1 agonists exert prostanoid-dependent suppression of carbachol-evoked acid secretion in rats (Kawabata *et al*., 2004d), and that PAR1 stimulation is also capable of relaxing isolated rat gastric artery *in vitro* and enhancing gastric mucosal blood flow in rats *in vivo* (Kawabata *et al*., 2004c, d). Thus, both PAR2 and PAR1 are considered protective in gastric mucosa, at least, in animal models. Although there is limited clinical evidence for roles of PARs in human gastric mucosa (Fujimoto *et al*., 2006; Arisawa *et al*., 2007), studies using human cancer-derived cell lines imply that PARs are associated with cancer cell proliferation (Caruso *et al*., 2006) and involved in inflammatory responses, particularly after infection with *Helicobacter pylori* (*H. pylori*) (Yoshida *et al*., 2006b; Seo *et al*., 2007). As described above, delayed upregulation of COX-2 followed by prostaglandin E_2 formation in response to stimulation of PAR1, but not PAR2, is detectable in RGM1 cells, a rat noncancer gastric mucosa epithelial cell line (Toyoda *et al*., 2003; Sekiguchi *et al*., 2007). This evidence is not necessarily consistent with our *in vivo* finding that PAR1 agonists exerted gastric mucosal protection in a manner dependent on COX-1, but not on COX-2, in a rat model (Kawabata *et al*., 2004d).

Recently, involvement of PAR2 in oesophageal inflammation has been suggested by studies using laboratory animals (Naito *et al*., 2006) and cultured normal human oesophageal epithelial cells (HEECs) derived from an established cell line (Yoshida *et al*., 2007). Therapeutic usefulness of camostat mesilate, a proteinase inhibitor, has been emphasized in these studies. Gastroesophageal reflex disease (GERD) is one of the most common GI diseases in the Western and Asian countries. PPIs recognized as the mainstay of medical therapy for GERD, may not completely improve oesophageal mucosal breaks and symptoms such as heartburn, and some patients, even if treated with PPIs for maintenance therapy, may have a relapse of oesophagitis (Chiba, 1997; Naito *et al*., 2006). In this context, PAR2 and/or its agonist proteinases may be promising therapeutic targets for the treatment of GERD including erosive and nonerosive reflux diseases.

PAR2 and/or PAR1 play dual roles in the development of intestinal inflammation, given that they are pro- and anti-inflammatory. There is evidence that intracolonic administration of PAR2-activating peptides dissolved in ethanol is capable of inducing colitis (Cenac *et al*., 2004). The pro-inflammatory role of PAR2 and its agonist proteinases has been described in mouse models for *Citrobacter rodentium*-induced colitis (Hansen *et al*., 2005) and for *Clostridium difficile* toxin A-induced enteritis (Cottrell *et al*., 2007). In contrast, the anti-inflammatory/protective role of PAR2 has been suggested in a mouse model for inflammatory bowel disease (IBD) induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS) (Fiorucci *et al*., 2001) and in rat and mouse models for ischaemia/reperfusion-induced intestinal tissue injury (Cattaruzza *et al*., 2006). Nonetheless, there is evidence that intracolonic administration of a proteinase inhibitor, nafamostat mesilate, improves TNBS-induced colitis (Isozaki *et al*., 2006). Clinical studies show that PAR2 might be involved in the pathogenesis of IBD, particularly ulcerative colitis (Kim *et al*., 2003; Yoshida *et al*., 2006a), and that anti-tryptase therapy using a daily nafamostat mesilate enema for 2 weeks has beneficial effects for the treatment of human IBD (Yoshida *et al*., 2006a). PAR1's pro-inflammatory role has been reported in animal models and cultured epithelial cells, that is, PAR1 agonists induce epithelial apoptosis and increases intestinal permeability (Chin *et al*., 2003). In contrast, anti-inflammatory roles for PAR1 have been described in a rat model for intestinal ischaemia/reperfusion injury (Tsuboi *et al*., 2007) and in a mouse model for colitis mediated by a type II immune response (Cenac *et al*., 2005). Thus, PAR2 and PAR1 are considered as key molecules in the maintenance and/or disruption of intestinal mucosal integrity.

PAR2 and colonic pain

As described above, PAR2 is expressed in capsaicin-sensitive sensory neurons, and involved in the processing of either somatic or visceral pain (Vergnolle *et al*., 2001; Kawabata *et al*., 2001a, 2002a, 2004a; Coelho *et al*., 2002; Kawao *et al*., 2002b, 2004). Intracolonic administration of PAR2-activating peptides or trypsin produces delayed (10–24 h after administration) hyperalgesia in a rat colorectal distension model (Coelho *et al*., 2002), and also delayed (6 h or more after administration) hypersensitivity to intracolonic administration of capsaicin in mice (Kawao *et al*., 2004). The delayed hyperalgesia to capsaicin after PAR2-activating peptides and trypsin is not detectable in PAR2-knockout mice (Kawabata *et al*., 2006a). Activation of PAR2 on colonic nociceptive neurons causes sustained hyperexcitability through activation of PKC and ERK (Kayssi *et al*., 2007). However, the extremely slow (6 h or more) onset of hyperalgesia after intracolonic administration of PAR2 agonists implies the possibility that activation of non-neuronal PAR2 might cause release of nociceptive messengers, leading to delayed and sustained neuronal hyperexcitability. The bradykinin-B_2 receptor pathway might mediate the PAR2-triggered delayed hyperalgesia (Kawabata *et al*., 2006a). Most recently, two independent clinical studies indicate that mucosal mast cell mediators in colonic biopsies from patients with irritable bowel syndrome (IBS) excite rat nociceptive visceral sensory nerves (Barbara *et al*., 2007), and that intracolonic administration of human colonic biopsy supernatants from IBS patients, but not controls, causes delayed visceral hyperalgesia in a mouse colorectal distension model (Cenac *et al*., 2007). In the latter study, the pro-nociceptive effect of IBS patients' biopsy supernatants is blocked by proteinase inhibitors or a PAR2 antagonist, and is absent in PAR2-knockout mice (Cenac *et al*., 2007). These studies strongly suggest that proteinases released from colonic mucosa generate hypersensitivity symptoms through activation of PAR2 in IBS patients.

Summary and conclusions

As described so far, PARs and their agonist proteinases are involved in a variety of GI functions. In addition to the original roles for PAR1 and PAR4 in mediating thrombin-induced aggregation in human platelets (PAR3 and PAR4 in rodent platelets), we now have to consider modulation of ion secretion, smooth muscle motility and mucosal integrity by these receptors in the GI systems, when agonist proteinases including thrombin become accessible to the target receptors, for instance, during inflammation. PAR2, a receptor activated by trypsin, tryptase and many other endogenous and exogenous proteinases, plays an extensive and critical role in regulation of GI exocrine secretion. In addition to PAR2 present in non-neuronal cells, PAR2 expressed on sensory neurons is involved in regulation of GI smooth muscle motility and exocrine secretion, and in modulation of GI mucosal integrity and processing of visceral pain sensation. Together, PARs are considered key molecules in regulation of GI functions, and could be targets for development of drugs for treatment of various GI diseases.

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Conflict of interest

The authors state no conflict of interest.

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