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Review Article

PAR2 as a target for gastric mucosal protection

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Abstract

Protease-activated receptors (PARs), especially PAR2, are abundantly expressed throughout the gastrointestinal tract, including the stomach. PAR2 plays multiple roles in the modulation of gastric functions. Exogenously applied PAR2 agonists stimulate the secretion of gastric mucus and pepsinogen, suppress gastric acid output, enhance gastric mucosal blood flow and modulate the motility of gastric smooth muscle. Most of these gastric functions of PAR2, except for the stimulation of pepsinogen secretion, are thus protective in the stomach. Indeed, PAR2 agonists protect against gastric mucosal lesions induced by ethanol/HCl or indomethacin in laboratory animals. The PAR2-mediated protective mechanisms appear to primarily involve activation of capsaicin-sensitive sensory nerves in the gastric mucosa, although PAR2 stimulation would also trigger activation of other multiple pathways. PAR2 would thus appear to be a novel target for the development of drugs for the treatment of gastric mucosal diseases.

Introduction

G-protein-coupled 7-transmembrane-domain receptors (GPCRs) comprise the largest group of receptors in mammalian physiological processes, and a large number of homologous receptors and associated ligands have been identified. A recently described novel classification of this group, protease-activated receptors (PAR), has been shown to have unique mechanisms of activation. Serine proteases cleave an extracellular domain of the receptor. This serves to unmask a tethered ligand that presumably binds to the extracellular loop 2 of the receptor itself and activates the receptor (Fig. 1) (1-3). Since PAR1 was discovered as a thrombin receptor in 1991 (4), four distinct subtypes of PARs (PAR1, PAR2, PAR3 and PAR4) have been cloned. PAR1, PAR3 and PAR4 are activated by thrombin, whereas PAR2 is activated not by thrombin but by trypsin, mast cell tryptase and coagulation factors VIIa and Xa (Fig. 1) (1, 5-7). Interestingly, excpepct for PAR3, synthetic peptides based on the receptor-activating sequence of the tethered ligand, known as PAR-activating peptides, are capable of activating the parent receptors directly by a mechanism independent of proteolysis (Fig. 1) (1-4, 8).

PAR1 and PAR2 are expressed in a variety of tissues and cells, especially throughout the gastrointestinal tract, including the salivary glands, esophagus, stomach, small and large intestine, and pancreas (9-12). PARs, especially PAR2, are now considered particularly important receptors in the alimentary system, modulating the motility of gastric smooth muscle, stimulating salivary and pancreatic exocrine secretion, and affecting gastric mucosal functions (1, 2, 9, 12-14). In this review, we focus on the roles played by PAR2 in the stomach and describe the possibility that PAR2 could be a novel target for the development of drugs for the treatment of gastric mucosal disease.

Neurally mediated gastric mucus secretion and mucosal protection by PAR2 activation

Since PAR2 activation was shown to trigger mucus secretion in the isolated rat sublingual glands (15), we hypothesized that activation of this receptor might also induce gastric mucus secretion and possibly lead to subsequent gastric mucosal protection *in vivo*. Indeed, we found that PAR2-activating peptides such as SLIGRL-

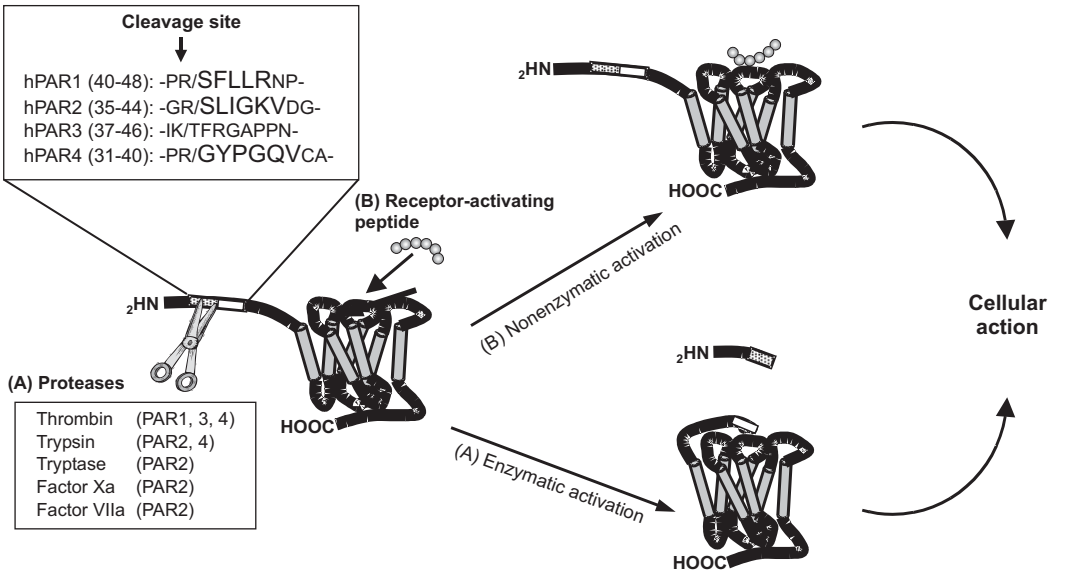

Fig. 1. Schematic diagram for activation mechanisms of protease-activated receptors (PARs). Serine proteases cleave an *N*-terminal extracellular domain of the receptor. This serves to unmask a tethered ligand that presumably binds to the extracellular loop 2 of the receptor itself and activates the receptor (A). Synthetic peptides, based on the tethered ligand sequence, function as agonists independently of *N*-terminal cleavage (B).

NH_2 and its derivatives, when administered systemically in combination with amastatin, an inhibitor of the peptide-degrading enzyme aminopeptidase, caused a rapid increase in gastric luminal mucus content in anesthetized pylorus-ligated rats (14).

Although PAR2 activation often induces prostanoid formation in various organs/tissues (16, 17), the gastric mucus secretion caused by PAR2-activating peptides is considered to be independent of endogenous prostanoid production, one of the protective factors in the gastric mucosa, based on the lack of inhibitory effect of diclofenac, an inhibitor of cyclooxygenase (14). Given the abundant expression of PAR2 in capsaicin-sensitive sensory nerves (18), we have examined possible neuronal mechanisms underlying the PAR2-triggered gastric mucus secretion and found that ablation of capsaicin-sensitive neurons abolished the effect of PAR2-activating peptides as gastric mucus secretagogues. The C-fiber neurotransmitters calcitonin gene-related peptide (CGRP), neurokinin A (NKA) and substance P, administered systemically, also induced gastric mucus secretion with an order of potency of: NKA > CGRP > substance P (14). These findings are in agreement with independent evidence that CGRP increases the synthesis of mucus in the gastric mucosa (19). The PAR2-triggered gastric mucus secretion is most probably mediated by endogenous CGRP and/or the endogenous NK_2 receptor agonist NKA (Fig. 2), as it was blocked by specific $CGRP_1$ or NK_2 receptor antagonists (14). Also, upon activation, PAR2 receptors present in the gastric mucosal C-fiber neurons cause release of CGRP and tachykinins, especially NKA, which in turn trigger the secretion of mucus in the gastric mucosa (Fig. 2). Our experiments have further shown that

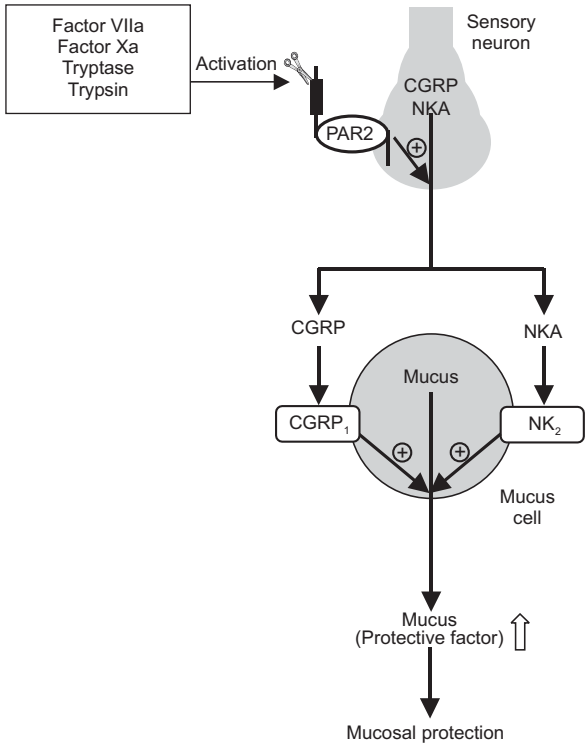

Fig. 2. The mechanism of PAR2-triggered mucus secretion in the gastric mucosa. PAR2 activators (trypsin, tryptase, coagulation factors VIIa and Xa, and agonist peptides such as SLIGRL-NH_2) activate PAR2 in sensory neurons. This triggers release of calcitonin gene-related peptide (CGRP) and tachykinins, especially neurokinin A (NKA), which in turn cause secretion of mucus from gastric mucus cells via activation of $CGRP_1$ and NK_2 receptors, respectively, leading to mucosal protection.

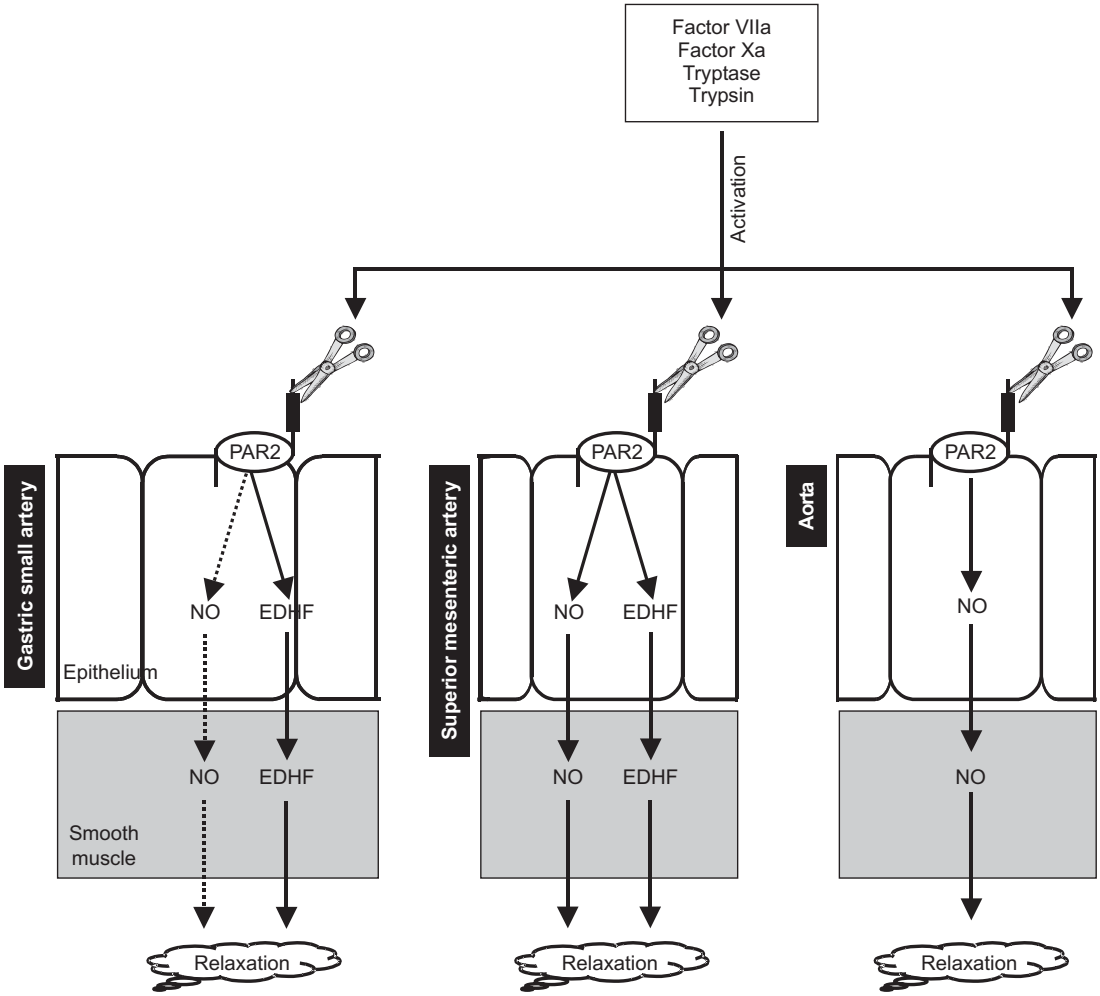

Fig. 3. Roles for PAR2 in the modulation of vascular tone. In the the gastric small artery, endothelial PAR2 activation causes vasodilatation dependent on both nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). However, PAR2-mediated vasorelaxation in smaller arterioles might be exclusively dependent on EDHF and independent of NO. In the superior mesenteric artery, PAR2 triggers vasodilatation via formation of NO and EDHF. In the aorta, PAR2 activation causes endothelial NO-dependent relaxation.

PAR2 agonists exhibit mucosal cytoprotective activity in distinct rat models of gastric mucosal injury induced by ethanol/HCl or indomethacin (14). Further evidence for the involvement of PAR2 in gastric mucosal cytoprotection was obtained in a more recent study employing PAR2 knockout mice (20).

Modulation of gastric mucosal blood flow

PAR2 is abundantly expressed in vascular endothelium and modulates vascular tone. In isolated rat aorta, PAR2-activating peptides and the endogenous PAR2 activator trypsin produce endothelial nitric oxide (NO)-mediated relaxation (Fig. 3), without affecting the tension of endothelium-denuded preparations (21, 22). A recent study suggested that endothelium-derived hyperpolarizing factor (EDHF) plays an important role in the PAR-mediated vascular relaxation in small blood vessels (23). In isolated rat superior mesenteric artery, PAR2-activating peptides elicit endothelium-dependent relaxation, which is only partially inhibited by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) alone, but is abolished by L-NAME in combination with apamin, an inhibitor of small-conductance, Ca^{2+}-activated K^+ channels, plus charybdotoxin, an inhibitor of large- and intermediate-conductance, Ca^{2+}-activated K^+ channels, known to block the EDHF pathway (24). This suggests the involvement of both NO and EDHF pathways in PAR2 regulation of vascular tone in the rat superior mesenteric artery (Fig. 3). A role for EDHF in the relaxation of gastric small arteries has also been described (25). In isolated rat gastric small arteries, PAR2-activating peptides cause vasorelaxation in a manner dependent on both NO and EDHF pathways (26) (Fig. 3). An *in vivo* study has shown that systemically administered PAR2 agonists cause a transient increase in gastric mucosal blood flow accompanied by rapid hypotension in anesthetized rats. The PAR2-triggered

gastric blood flow increase can be blocked by a combination of apamin and charybdotoxin, but not by either agent alone (27). In contrast, neither inhibition of the NO pathway by L-NAME nor ablation of capsaicin-sensitive sensory neurons affects PAR2 modulation of gastric blood flow (14, 27, 28), which is inconsistent with the evidence for the involvement of both NO and EDHF in isolated rat gastric artery (Fig. 3). It is thus likely that the increase in gastric mucosal blood flow induced by PAR2 agonists *in vivo* might reflect EDHF-dependent vasorelaxation in gastric arterioles smaller than the isolated gastric artery preparations used *in vitro*.

Suppression of gastric acid output and modulation of pepsinogen secretion

Systemically administered PAR2-activating peptides produce a dose-dependent decrease in gastric acid secretion produced by carbachol, pentagastrin or 2-deoxy-D-glucose in anesthetized pylorus-ligated rats (29). Although capsaicin-sensitive sensory neurons in the gastric mucosa are known to function to suppress acid secretion, the inhibitory effect of the PAR2-activating peptides on carbachol-induced gastric acid secretion is not altered by ablation of sensory neurons by pretreatment with large doses of capsaicin (29), suggesting the involvement of mechanisms distinct from those for PAR2-triggered mucus secretion. Endogenous prostanoids also do not appear to be involved in PAR2-mediated inhibition of gastric acid output (29). The precise mechanisms underlying the PAR2-mediated inhibition of gastric acid secretion remain to be elucidated. Nonetheless, this effect of PAR2 activation might contribute to PAR2-mediated protection against gastric mucosal injury (Fig. 4).

An immunohistochemical study has shown that PAR2 is expressed abundantly in the mucosal chief cells, but not parietal cells (30). PAR2-activating peptides, when administered repeatedly at 1-h intervals 4 times in all, gradually increase the secretion of pepsinogen in anesthetized pylorus-ligated rats (30). The pepsinogen secretion triggered by PAR2 activation is not secondary to gastric acid secretion, since it was unaffected by pretreatment with the proton pump inhibitor omeprazole at a dose that completely inhibits gastric acid output (30). PAR2-mediated pepsinogen secretion is also resistant to the NO synthase inhibitor L-NAME, atropine or ablation of sensory neurons (30). Taken together, these findings suggest that activation of PAR2 expressed in chief cells would directly trigger pepsinogen secretion (Fig. 4). Although PAR2-triggered pepsinogen secretion could be an aggressive factor in the gastric mucosa, suppression of gastric acid secretion by PAR2 activation (29) might reduce the conversion of pepsinogen to pepsin, which might overcome the aggressive role of pepsinogen in the gastric mucosa (Fig. 4).

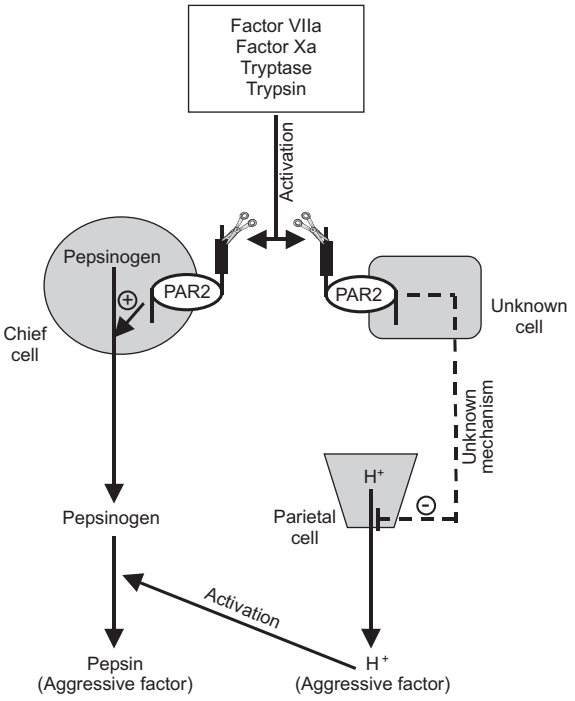

Fig. 4. Roles for PAR2 in regulating the secretion of pepsinogen and gastric acid. Activation of PAR2 present in unknown cells suppresses gastric acid output, which might contribute to mucosal protection. Activation of PAR2 expressed in mucosal chief cells triggers the secretion of pepsinogen.

Modulation of motility of gastric smooth muscle following activation of PAR2

PAR2 is also expressed in gastric smooth muscle (2, 3) and. PAR2 modulation of gastric smooth muscle motility has been extensively investigated. Early studies showed that PAR2 agonists produce contractile responses in isolated rat gastric longitudinal smooth muscle (31). This PAR2-mediated gastric smooth muscle contraction can be blocked by the L-type Ca^{2+} channel blocker nifedipine, the cyclooxygenase inhibitor indomethacin and the tyrosine kinase inhibitor genistein (31). PAR2 agonists also contract mouse gastric longitudinal smooth muscle strips, an effect which is partially reduced in the presence of indomethacin and also by C-fiber desensitization with capsaicin (32). In mouse gastric preparations precontracted with carbachol, however, PAR2 agonists cause relaxation responses that are abolished by apamin, implying the involvement of small-conductance, Ca^{2+}-activated K^+ channels (32, 33). This relaxation caused by PAR2 agonists in smooth muscle preparations does not involve endogenous NO, prostanoids or neuronal mechanisms (32, 33). Of note is that PAR2-activating peptides cause neither contractile nor relaxant responses in gastric smooth muscle preparations from PAR2 knockout mice (32). Regardless of the complex effects of PAR2 agonists *in vitro*, systemic administration of PAR2 agonists facilitates gastrointestinal transit, an effect which is enhanced by pretreatment with apamin

(34). This is consistent with evidence that PAR2 modulates the motility of isolated mouse duodenal, jejunal and ileal smooth muscle in a manner partially sensitive to apamin (32). Nevertheless, the precise physiological and/or pathophysiological roles played by PAR2 in the modulation of gastrointestinal smooth muscle motility have not yet been elucidated.

Other functions of PAR2 in the gastrointestinal tract

PAR2 also plays a variety of roles in gastrointestinal organs/tissues other than the stomach. PAR2-mediated salivary and pancreatic exocrine secretion has been described *in vitro* and *in vivo* (9, 11, 15). In the pancreas, PAR2 appears to play a protective role during pancreatitis (35, 36), although conflicting evidence has also been reported (37). There is evidence that PAR2 regulates intestinal ion transport (38). In the colon, PAR2 appears to play a dual role, being both anti- and proinflammatory (39, 40). A nociceptive role for PAR2 in the processing of visceral pain has also been described (41, 42). PAR2 is thus considered a key molecule in the modulation of gastrointestinal functions.

Conclusions

As described above, PAR2 plays complex multiple roles in the stomach. PAR2 is considered to be primarily protective in the gastric mucosa, because activation of PAR2 enhances protective factors, such as mucus secretion and mucosal blood flow, and attenuates aggressive factors, such as acid output. Therefore, we propose that PAR2 could be a novel target for the development of therapeutic agents for gastric mucosal injury, such as gastritis and peptic ulcer.

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