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PAR4: A new role in the modulation of visceral nociception

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Abstract

Protease-activated receptors (PARs) are a family of G-protein coupled receptors with a widespread distribution that are involved in various physiological functions including inflammation and nociception. In a recent study in *Neurogastroenterology and Motility*, Augé et al describe for the first time the presence of PAR4 on visceral primary afferent neurons and its role in modulating colonic nociceptive responses, colonic hypersensitivity and primary afferent responses to PAR2 and Transient Receptor Potential Vanilloid-4 (TRPV4). Using the model of visceromotor response (VMR) to colorectal distension (CRD), they show that a PAR4 agonist delivered into the colon lumen decreases basal visceral response to CRD and reduces the exacerbated VMR to CRD induced by treatment with PAR2 or TRPV4 agonists. In isolated sensory neurons, they show that a PAR4 agonist inhibits calcium mobilization induced by PAR2 or TRPV4 agonists. Finally, they describe increased pain behavior evoked by luminal application of mustard oil in PAR4 deficient mice compared to wild type controls. The newly discovered role of PAR4 in modulating visceral pain adds to our growing understanding of the contribution of colonic proteases and PARs to the mechanisms involved in colonic hypersensitivity as well as their potential role as therapeutic targets for irritable bowel syndrome.

Keywords

PAR4; sensory neurons; visceral nociception

The protease-activated receptors (PARs), a novel subfamily of G-protein-coupled receptors (GPCRs), and their agonist proteases, have been first discovered and characterized more than a decade ago. 1 The cloning of PAR1, PAR2, PAR3 and PAR4, was only completed in 1998. 2-5 Their presence throughout the gastrointestinal (GI) tract has stimulated a field of investigation on the role of PARs in modulating GI functions. PAR1 and PAR2 have been mainly involved in the regulation of the GI epithelium function, GI motility via an action on smooth muscles, and inflammation and pain. However, while PAR4 is highly expressed in the gut, the role of this receptor in GI functions has been only scarcely explored and its role in visceral nociception has not been investigated.

In their study, Augé et al. 6 provide the first evidence for a role of PAR4 in the modulation of visceral nociception, showing that colonic delivery of a PAR4 agonist in mice decreases basal visceral nociceptive response and inhibits visceral hypersensitivity induced by PAR2 or TRPV4 agonists. They demonstrate that PAR4 is present in sensory neurons projecting from the colon and it is co-expressed with PAR2 and TRPV4. While PAR4 agonist exposure

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alone has no effect on free intracellular calcium mobilization in sensory neurons *in vitro*, it inhibits calcium mobilization induced by PAR2 and TRPV4. The role of endogenous PAR4 in visceral pain processing was confirmed using PAR4 deficient mice, which exhibit increased nociceptive behaviors in response to intracolonic administration of mustard oil compared to controls.

PROTEASE-ACTIVATED RECEPTORS

PARs have a unique mechanism of activation by which proteases cleave part of their extracellular N-terminal sequence, allowing the tethered ligand that is formed from the truncated portion of the N-terminus to bind to the receptor itself, leading to activation.⁷ While PAR2 can be activated by trypsin and mast cell tryptase, PAR1, 3, 4 are considered thrombin receptors. However, PAR4, the receptor of interest in this study, has low affinity for thrombin, requiring high concentration of this agonist for activation. PAR4 can also be activated by trypsin, cathepsin G, the activated factor X of the coagulation cascade and trypsin IV.⁸ Except for PAR3, PARs can also be activated by short synthetic peptides that mimic the tethered ligand. These PAR-activating peptides have been key in studying the physiological functions of PAR subtypes. PARs are involved in a wide range of processes including endothelial cell functions, platelet activation and homeostasis, tumor cell growth and metastasis, response to joint injury, as well as somatic and visceral nociceptive response and gastrointestinal functions including intestinal motility and inflammatory response.⁹ Studying the specific functions of each receptor is complicated by the fact that multiple receptors can be expressed on the same tissues, often with opposing effects upon activation, depending on the doses. It is further complicated by the ability of certain proteases to bind to several receptor subtypes and the apparent difficulty in developing specific antagonists.

ROLE OF PARS IN INFLAMMATION AND PAIN

Both PAR1 and PAR2 have been shown to play a role in pro-inflammatory responses in somatic and visceral structures. However, while a PAR2 agonist can induce colonic inflammation in mice¹⁰, PAR2 activation may also induce anti-inflammatory responses, depending on the condition studied. For example, a PAR2 agonist has been shown to protect against gastric mucosal injury produced by HCl/ethanol in rats¹¹ and to reduce colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in mice.¹²

PAR1 and PAR2 have also been shown to modulate nociceptive responses: while sub-inflammatory doses of PAR1 agonists can induce mechanical and thermal analgesia, hyperalgesic response have been observed in response to sub-inflammatory doses of PAR2 agonists. A pro-nociceptive role of PAR2 activation has also been identified in models of visceral pain where the injection of PAR2 agonists into the pancreatic duct or into the lumen of the colon has been shown to induce visceral hyperalgesia and c-fos expression in spinal neurons.¹³

PAR4, in addition to its role in platelet activation, has been involved in inflammatory responses, in particular in leukocyte recruitment and plasma extravasation.¹⁴⁻¹⁵ In prior studies, Vergnolle's group has demonstrated that, consistent with observations of edema and granulocyte infiltration after injection into the hindpaw¹⁶, intra-articular injection of a PAR4 agonist leads to pro-inflammatory changes in the knee joint in mice.¹⁷ Interestingly, whereas the PAR4 agonist had antinociceptive effects in the hind paw model, it produced an allodynic and hyperalgesic response in the joint inflammation model. Since the analgesic effect in the hind paw model was observed at doses below the threshold needed to cause inflammation, the authors speculated that low dose of the PAR4 agonist has analgesic properties whereas higher concentrations of the peptide are pro-algesic. In their recent paper⁶, they report for the first time, a visceranalgesic effect of a PAR4 agonist. Intracolonic

administration of a sub-inflammatory dose of the PAR4 agonist peptide in mice reduced the visceromotor response to colorectal distension and inhibited visceral hyperalgesia induced by a prior treatment with a PAR2 agonist or a TRPV4 agonist. A higher dose of the PAR4 agonist increased the visceral sensitivity to colorectal distension and was found to induce a small increase in macroscopic damage scores and myeloperoxidase (MPO) activity, suggesting the presence of inflammation. These data are consistent with the prior observations of a differential, dose related effect of PAR4 agonists on the development of inflammation and hyperalgesia. The authors proposed that at pro-inflammatory doses, PAR4 may induce the release of a range of mediators by infiltrated cells contributing to the sensitization of nociceptors and increased pain response, thereby masking the potential direct inhibitory effect of PAR4 activation on sensory neurons. In a prior study, they showed that PAR4-induced inflammation in the paw is not mediated by a neurogenic mechanism but involves the activation of the kallikrein pathway in leucocytes, leading to the release of bradykinin and increased vascular permeability, supporting this hypothesis.¹⁸

Taken together, these data indicate that PAR4 agonists may exert dual pro- or anti-nociceptive effects in the somatic and visceral domains, depending on the dose (pro-inflammatory versus sub-inflammatory) used. While its analgesic effect lends support to a potential use for the treatment of hyperalgesia, the pro-inflammatory component in the effects of PAR4 remains an important issue and a better understanding of the mechanisms of action involved in the modulation of nociceptive responses is required.

MECHANISMS OF ACTION OF PAR4 IN THE MODULATION OF VISCERAL NOCICEPTION

Although different intracellular signaling pathways have been described in response to PAR4 in various cell types, there are no prior reports available in neurons. Intracellular calcium mobilization was found in response to PAR4 in platelets, but was not observed in endothelial cells. In cardiomyocytes, pathways engaging both Phospholipase C/Extracellular signal-regulated kinase (PLC/ERK) and p38 Mitogen-Activated Protein Kinase (MAPK) have been described (reviewed in 8). In their report, Augé et al 6 demonstrate that PAR4 is expressed on sensory neurons in dorsal root ganglia (DRGs) projecting to the colon, and it is co-localized with PAR2 and TRPV4. They showed, for the first time, that while neuronal signaling events induced par the PAR4 agonist are independent of calcium signaling in basal condition, the PAR4 agonist was able to inhibit calcium mobilization induced by PAR2 or TRPV4 agonists in all sizes of isolated neurons. A direct interaction of PAR4 with L, N, or P/Q-type voltage-gated calcium channels was proposed as a mechanism involved in the inhibitory effect of PAR4 against depolarization-evoked calcium signal, although there is no direct evidence supporting this hypothesis.

Another important observation from this paper is the confirmation that PAR4 are expressed on nerve endings in the colon, and that they co-localize with peripherin, a marker of nociceptive neurons. Based on these data combined with the observations of a visceral analgesic effect of the PAR4 agonist delivered into the colon lumen, the authors propose that the modulatory effect of the PAR4 agonist on visceral nociception is mediated by a peripheral local effect on sensory nerves endings, inhibiting the transmission of pro-nociceptive signals. However, a big question left unanswered is the accessibility to PAR4 on afferent endings of agents delivered at low doses into the colon lumen and the electrophysiological confirmation that such delivery method can trigger a response from colonic sensory neurons.

PAR4: A TARGET FOR THE MODULATION OF VISCERAL NOCICEPTION IN PATHOLOGICAL CONDITIONS?

A previous report from Vergnolle et al showed increased expression and release of trypsin and tryptase from colonic biopsies from irritable bowel syndrome (IBS) patients compared with controls and increased levels of proteolytic activity in colonic biopsies and colonic washes from IBS patients 19. They also demonstrated that the supernatant of colonic biopsies from IBS patients, containing high levels of proteases, triggers visceral hyperalgesia in animal models. These effects were inhibited by serine protease inhibitors, or a PAR2 antagonist, and were absent in PAR2-deficient mice. 19 Together, these data, combined with the new findings that a PAR4 agonist can inhibit both cellular and pain behavior responses evoked by PAR2 in mice, suggest the potential use of PAR4 agonists to treat visceral hyperalgesia in conditions associated with increased PAR2 activity, such as IBS.

However, despite the attractiveness of the concept, a number of issues need to be addressed before such a strategy can be endorsed. Convincing evidence is needed confirming the role of proteases released by IBS biopsies in the actual physiopathology of visceral hyperalgesia in IBS patients. It remains unclear whether the release of endogenous proteases is an important component in the development of IBS symptoms. In addition, while PARs have been found to play a significant role in the regulation of epithelial ions transport in the gut 20, we need a better understanding on how disruption of the proteases balance in the intestinal epithelial cell microenvironment may contribute to pathological conditions. Together, given the complexity and the plasticity of the proteases signaling system in the lumen of the gastrointestinal tract, potential unpredictable effects of exogenously delivered PAR agonists or antagonists on mucosal immune function or on the epithelial barrier may occur. Interference with these systems may impede the potential therapeutic use of PAR modulating agents for visceral pain in IBS.

CONCLUSION

The work from Augé et al 6 provides important new observations on the role of PARs in the modulation of visceral nociception, showing the first evidence of an analgesic and anti-hyperalgesic effect of a PAR4 agonist in a mouse model. The discovery of PAR4 as new modulator of colonic hypersensitivity widens the array of potential therapeutic targets for the treatment of visceral pain symptoms in IBS patients.

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