The enteric nervous system in inflammation and pain: The role of proteinase-activated receptors

Nathalie Vergnolle PhD


The enteric nervous system (ENS) plays a pivotal role in inflammatory and nociceptive processes. Drugs that interact with the ENS have recently raised considerable interest because of their capacity to regulate numerous aspects of the gut physiology and pathophysiology. The present article summarizes recent research on proteinases and proteinase-activated receptors (PARs) as signalling molecules in the ENS. In particular, experiments in animal models suggest that PAR₂ is important to neurogenic inflammation in the intestine. Moreover, PAR₂ agonists seem to induce intestinal hypersensitivity and hyperalgesic states, suggesting a role for this receptor in visceral pain perception. Thus, PARs, together with the proteinases that activate them, represent exciting new targets for therapeutic intervention on the ENS.

Key Words: Inflammation; Pain; Proteinases; Thrombin; Trypsin; Tryptase

The ENS also influences vasodilation and vascular permeability. It is observed in response to CGRP and substance P release, through direct endothelial activation of the type I CGRP receptor and the neurokinin-1 receptor (NK-1), respectively. Activation of endothelial receptors for CGRP and NK-1 causes not only extravasation of plasma from the blood into the tissues, but also facilitates the recruitment of inflammatory cells (1) (Figure 1). Moreover, enteric neurons can stimulate mast cell degranulation by the release of substance P (Figure 1).

Mast cell degranulation causes the release of numerous inflammatory mediators: cytokines, proteinases, nitric oxide and lipid mediators of inflammation, including prostaglandins, leukotrienes and thromboxane. Those molecules stimulate other inflammatory cells, such as macrophages, neutrophils and lymphocytes, which can then further promote the release of neuropeptides from enteric nerves, thereby amplifying the inflammatory response (Figure 1).

The ENS also influences vasodilation and vascular permeability indirectly, through the release of mast cell mediators (1). Because enteric nerves are closely associated with macrophages and lymphocytes in the gut wall, the release of VIP from those neurons seems to aggravate inflammation by stimulating cytokine release from B and T lymphocytes, as well as by increasing immunoglobulin A synthesis by B-lymphocytes (1).

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PAR2 TRIGGERS ENTERIC NERVES TO CAUSE INFLAMMATION

It is known that all the hallmarks of inflammation – swelling, increased blood flow and granulocyte infiltration – are induced by exposure of various tissues (paw, airways, skin, etc) to PAR2 agonists (12-14). Because proteinases that are able to activate PAR2 (such as trypsin and mast cell tryptase) are particularly abundant in the gut of patients with inflammatory bowel diseases (IBD) (15-17), we hypothesized that high concentrations of proteinases in the lumen of the gut might induce intestinal inflammation. We have shown that, in the rat colon, the intracolonic injection of a selective PAR2 agonist (PAR2-activating peptide), as well as of trypsin and mast cell tryptase, caused edema, granulocyte infiltration and intestinal barrier breakdown, as demonstrated by bacterial translocation from the gut to peripheral organs (18). Furthermore, we have shown that this PAR2 agonist-mediated colitis could be inhibited by a pretreatment with capsaicin, a neurotoxin that depletes sensory neurons of their neuropeptide content, or pretreatment with NK-1 or CGRP receptor antagonists (19). These results showed that the intestinal inflammation induced by PAR2 agonists is under neural control, and is affected by the release of proteinases such as substance P and CGRP. The release of substance P and CGRP, as a direct result of activation of PAR2 on ENS neurons, was not demonstrated in this study. Evidence supporting this idea include the facts that functional PAR2 has been identified on ENS neurons that also expressed substance P and CGRP, and that isolated sensory afferents can release substance P and CGRP upon direct application of PAR2 agonists.

Thus, by releasing proinflammatory neuropeptides, the ENS is capable of initiating and amplifying inflammatory reactions. What could initiate a neurogenic mechanism of inflammation? Neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) that, by acting on vascular endothelium, cause vasodilation and increased vascular permeability, two major features of the inflammatory reaction. Neuropeptides can also provoke mast cell activation/degranulation, which subsequently activates the other actors of inflammation: vascular endothelium, macrophages, lymphocytes and neutrophils, through the release of various inflammatory mediators (cytokines, prostaglandins [PGs], leukotrienes [LTs], proteinases and nitric oxide [NO]). Inflammatory cells could, in turn, further induce the ENS to release neuropeptides, thus amplifying the inflammatory reaction. Through the release of vasoactive intestinal peptide (VIP), enteric neurons can also interact with inflammatory cells. Activation of nociceptive enteric neurons also conveys nociceptive information, especially pain, to the central nervous system.

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Figure 1) Interactions of the enteric nervous system (ENS) with components of the inflammatory process. Upon activation by noxious agents, the ENS (both extrinsic and intrinsic enteric nerves) release neuropeptides (substance P [SP] and calcitonin gene-related peptide [CGRP]) that, by acting on vascular endothelium, cause vasodilation and increased vascular permeability, two major features of the inflammatory reaction. Neuropeptides such as SP also provoke mast cell activation/degranulation, which subsequently activates the other actors of inflammation: vascular endothelium, macrophages, lymphocytes and neutrophils, through the release of various inflammatory mediators (cytokines, prostaglandins [PGs], leukotrienes [LTs], proteinases and nitric oxide [NO]). Inflammatory cells could, in turn, further induce the ENS to release neuropeptides, thus amplifying the inflammatory reaction. Through the release of vasoactive intestinal peptide (VIP), enteric neurons can also interact with inflammatory cells. Activation of nociceptive enteric neurons also conveys nociceptive information, especially pain, to the central nervous system.
Taken together, these results showed that, upon PAR₁ activation, the ENS releases neuropeptides (substance P and CGRP) that initiate neurogenic inflammation, characterized by edema and granulocyte infiltration (19) (Figure 2).

An important step in understanding the role of PAR₂ in the gut would be to investigate to what extent PAR₂-induced ENS activation is associated with bowel disease. Because inflammation driven by enteric infections has been shown to be largely mediated by a neurogenic mechanism involving extrinsic sensory nerves, substance P and CGRP receptors (20), it could be hypothesized that pathogens release proteases that activate PAR₂ on enteric neurons, and that this activation leads to mucosal inflammation. This hypothesis is supported by the fact that PAR₂ can be activated by proteases from pathogens such as dust mites (5) or Porphyromonas gingivalis (4). PAR₂ activation by pathogens would support a proinflammatory role for PAR₂ in enteric infections and would suggest that PAR₂ antagonists might be helpful in the treatment of enteric infections. In IBD models, however, activation of the ENS and further release of neuropeptides have been shown to be protective (21). Thus, in the setting of chronic intestinal inflammation, PAR₂-induced ENS activation might have beneficial and protective effects. This hypothesis is further supported by the findings of Fiorucci and colleagues (22), who have observed that daily systemic treatment with a PAR₂ agonist was protective in a model of trinitrobenzene sulfonic acid-induced colitis. The lack of readily available PAR₂ antagonists has hampered progress in this field. The availability of PAR₂-deficient mice should help, in the very near future, to clarify this hypothesis.

**DOES PAR₂ TRIGGER ENTERIC NERVES TO CAUSE VISCERAL HYPERSENSITIVITY?**

Afferent sensory fibres of the ENS not only release inflammatory neuropeptides, but they also convey sensory data. One hypothesis is that activation of PARs, and particularly PAR₂, on enteric afferent sensory fibres might also send a nociceptive signal to the central nervous system. It has been shown that subinflammatory doses of PAR₂ agonists (PAR₂-activating peptides but also trypsin and tryptase), when injected into the rat or mouse paw, provoked nociceptor activation at a spinal level, together with severe and prolonged hyperalgesia (23). Within 2 h after their intracolonic administration, PAR₂ agonists (PAR₂-activating peptides and trypsin) also caused nociceptor activation at a spinal level, as demonstrated by increased fos protein expression in the superficial laminae of the spinal dorsal horn (24). Subinflammatory doses of PAR₂ agonists from the colonic lumen provoked a significant increase in the number of abdominal contractions in response to rectal distension, which is characteristic of visceral hyperalgesia (24). Here again, these experiments could not unequivocally show that these pro-nociceptive and hyperalgesic effects of PAR₂ agonists were due to a direct activation of PAR₂ on the ENS. The study by Reed and colleagues (7), however, showed that transient exposure of enteric submucosal neurons to PAR₂ agonists evoked long term hyperexcitability. Similar hyperexcitability of extrinsic afferent neurons could be responsible for the PAR₂-induced hyperalgesia we observed in vivo in a rat model of visceral nociception (23).

Recent studies have shown that patients with the irritable bowel syndrome (IBS) exhibit an increased spontaneous release of mast cell tryptase from colonic tissues (25). It could be hypothesized that tryptase-induced PAR₂ activation of enteric sensory nerves induces a long term hyperexcitability of these neurons, resulting in the visceral pain and hyperalgesia experienced by IBS patients. Although a clear role for PAR₂ activation has been demonstrated in the establishment of mast cell degranulation-induced somatic hyperalgesia, using PAR₂-deficient mice (23), such a role in visceral hyperalgesia has yet to be demonstrated.

**THE ROLES OF OTHER PARs IN THE ENS**

Although a neurogenic mechanism involving neurokinin-1 receptor activation has been demonstrated for PAR₁ agonist-induced paw edema (9), there is currently no evidence that such a mechanism also occurs in the gut. Contrary to the situation with PAR₂, subinflammatory doses of PAR₁ agonists did not induce hyperalgesia after intraperitoneal injection, but they increased nociceptive threshold in rats and significantly inhibited inflammatory hyperalgesia induced by carrageenan (26). Here again, there is no evidence yet for such a mechanism in the gastrointestinal tract. However, other neurally controlled functions of the gut seem to be regulated by PAR₁ activation on enteric neurons. Buresi and colleagues (27) have recently shown that PAR₁ activation in mouse intestinal tissues, mounted in Ussing chambers, decreased secretory responses to neural stimulation. This suggests that PAR₁ could contribute to disorders of secretory function associated with the development of colitis. It is interesting to note that PAR₁ agonists also regulate intestinal secretion, but, unlike PAR₂, PAR₁ agonists have been shown to stimulate chloride secretion by a neuro-
genic process (28). No studies have yet demonstrated a role for PAR$_1$ or PAR$_2$ in neurally evoked intestinal functions.

CONCLUSIONS
This review summarized recent evidence that proteinases, through the activation of PARs, are able to interact with the ENS, thereby affecting neurally evoked intestinal functions. Inflammation and pain perception appear to be two major functions that are regulated by neuronal PARs. There has been considerable interest recently in the development of agents that modify these processes. PARs that are expressed on nerves represent, together with the proteinases that activate them, exciting new targets for therapeutic intervention.

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