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

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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 PAR_2 is important to neurogenic inflammation in the intestine. Moreover, PAR_2 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*

Le système nerveux entérique dans l'inflammation et la douleur : Le rôle des récepteurs activés par la protéinase

Le système nerveux entérique (SNE) joue un rôle critique dans les processus inflammatoires et nociceptifs. Les médicaments qui interagissent avec le SNE ont récemment suscité un intérêt considérable en raison de leur capacité de réguler de nombreux aspects de la physiologie et la physiopathologie intestinales. Le présent article résume des recherches récentes sur les protéinases et les récepteurs activés par la protéinase (RAP) à titre de molécules de signalisation dans le SNE. En particulier, des expériences sur des modèles animaux laissent supposer que les RAP_2 sont importants pour l'inflammation neurogène de l'intestin. De plus, les agonistes des RAP_2 semblent induire une hypersensibilité intestinale et des états hyperalgésiques, ce qui laisse croire que ce récepteur joue un rôle dans la perception de la douleur viscérale. Cependant, les RAP, conjointement avec les protéinases qui les activent, représentent des nouvelles cibles intéressantes d'intervention thérapeutique sur le SNE.

The gut is extensively innervated: the enteric nervous system (ENS) contains approximately 10^8 neurons (as many as in the entire spinal cord). The ENS is characterized by an extensive and elaborate network of nerves extending from the esophagus to the anal sphincter. Intrinsic neurons have cell bodies within the submucous and myenteric plexuses, while the cell bodies of extrinsic sensory nerves are in the dorsal root ganglia of the spinal cord and in the nodose ganglia. The ENS controls motility, secretion, absorption, microcirculation, sensation and immune function in the gut. The present review focuses on the role of proteinases as signalling molecules for both the extrinsic and intrinsic nerves of the ENS, through the activation of proteinase-activated receptors (PARs).

NEUROGENIC INFLAMMATION IN THE GUT

The ENS modulates inflammatory processes in several distinct ways. One way is through the release of neuropeptides upon stimulation of enteric nerves by noxious agents. Intrinsic and extrinsic enteric nerves contain neuropeptides, such as substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP), that modulate various components of the inflammatory process. The release of neuropeptides from both intrinsic and extrinsic enteric neurons can initiate neurogenic inflammation.

Vasodilation and increased vascular permeability are 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 neurons 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 immunglobulin A synthesis by B-lymphocytes (1).

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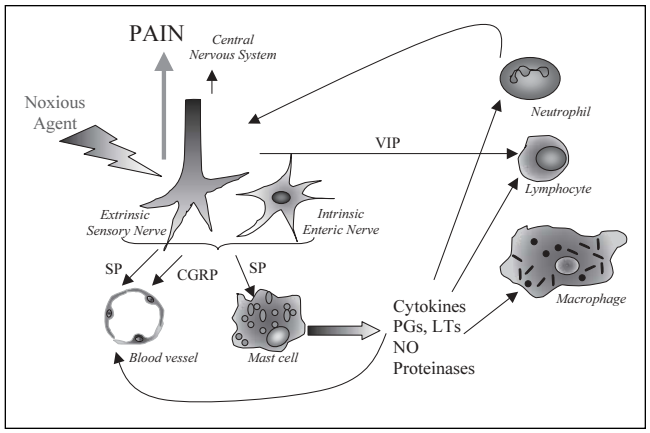

Figure 1) *Interactions of the enteric nervous system (ENS) with components of the inflammatory process. Upon activation by noxious agents, the ENS (both extrinsic and intrinsic enteric nerves) release neuropeptides (substance P [SP] and calcitonin gene-related peptide [CGRP]) that, by acting on vascular 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*

Thus, by releasing proinflammatory neuropeptides, the ENS is capable of initiating and amplifying inflammatory reactions. What could initiate a neurogenic mechanism of inflammation by triggering activation of the ENS and subsequent release of neuropeptides? We propose that PARs act as important mediators of ENS activation.

PARS IN THE ENS

PARs constitute a group of seven transmembrane G protein-coupled receptors that are activated by the proteolytic cleavage of their *N*-terminal domain. Proteolysis releases a new *N*-terminal domain, which acts as a tethered ligand, binding and activating the receptor itself (2). Small synthetic peptides, corresponding to the tethered ligand domains released by proteolysis, are selective agonists for these receptors, and thus serve as useful pharmacological tools for understanding the physiology of these receptors. Three PARs (PAR_1, PAR_3 and PAR_4) are activated by thrombin, and have been cloned in the course of studies that aimed at understanding the role of thrombin in platelet activation (3). A fourth receptor (PAR_2) can be activated by trypsin and mast cell tryptase, as well as by pathogen proteinases, but not by thrombin (2,4,5).

The four PARs are expressed throughout the gastrointestinal tract in different cell types (2). For example, immunoreactive PAR_1 and PAR_2 have been detected in more than 60% of neurons from the myenteric plexus of the guinea pig small intestine, both in primary cultures and in whole mounts of myenteric tissues (6). A large proportion of neurons expressing substance P, a primary excitatory neuropeptide, also expressed PAR_1 (89%) and PAR_2 (50%). Similarly, a large percentage of neurons expressing VIP, which acts as an inhibitory motor transmitter, also expressed PAR_1 (44%) and PAR_2 (90%) (6,7). More than 50% of neurons expressing PAR_1 also expressed PAR_2 (6).

PAR_1 and PAR_2 are also expressed by extrinsic enteric neurons. PAR_2-like immunoreactivity and PAR_2 mRNA expression have also been detected in rat isolated dorsal root ganglia neurons (8). Among the sensory neurons expressing PAR_2, approximately 40% coexpressed CGRP and 30% coexpressed substance P (8). PAR_1 is also located in dorsal root ganglia neurons, where it often coexpressed with substance P (9).

PAR_1 and PAR_2 are also functional in ENS cells. Linden and colleagues (10) have shown that PAR_2 agonists signal guinea pig myenteric neurons, inducing depolarization and increasing the number of action potentials in isolated cells. Reed and colleagues (7) have shown that PAR_2 agonists evoked depolarization and long term excitation in submucosal neurons isolated from the guinea pig ileum. Both PAR_1 and PAR_2 agonists stimulate calcium mobilization in isolated guinea pig myenteric neurons (6) and dorsal root ganglia neurons (8,9). Although the presence of PAR_1, PAR_2 and PAR_4 was not clearly established, a study by Gao et al (11) suggested that those receptors act on neurons that regulate enteric motor function in the guinea pig small intestine.

This large expression of PARs strongly suggests that they play a crucial role in the physiology and pathophysiology of the ENS. Among the functions of the ENS, it appears that proteinases and PARs are especially likely to regulate inflammation and pain.

PAR_2 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 PAR_2 agonists (12-14). Because proteinases that are able to activate PAR_2 (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 PAR_2 agonist (PAR_2-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 PAR_2 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 PAR_2 agonists is under neural control, and is affected by the release of neuropeptides such as substance P and CGRP. The release of substance P and CGRP, as a direct result of activation of PAR_2 on ENS neurons, was not demonstrated in this study. Evidence supporting this idea include the facts that functional PAR_2 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 PAR_2 agonists.

Taken together, these results showed that, upon PAR_2 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 PAR_2 in the gut would be to investigate to what extent PAR_2-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 proteinases that activate PAR_2 on enteric neurons, and that this activation leads to mucosal inflammation. This hypothesis is supported by the fact that PAR_2 can be activated by proteinases from pathogens such as dust mites (5) or *Porphyromonas gingivalis* (4). PAR_2 activation by pathogens would support a proinflammatory role for PAR_2 in enteric infections and would suggest that PAR_2 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, PAR_2-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 PAR_2 agonist was protective in a model of trinitrobenzene sulfonic acid-induced colitis. The lack of readily available PAR_2 antagonists has hampered progress in this field. The availability of PAR_2-deficient mice should help, in the very near future, to clarify this hypothesis.

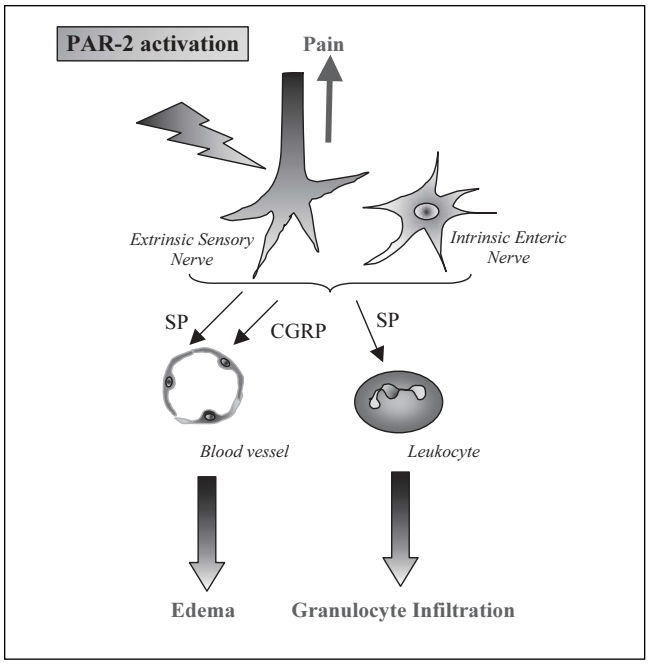

Figure 2) *Proteinase-activated receptor-2 (PAR_2) activation causes neurogenic inflammation in the mouse colon. Upon PAR_2 activation, the enteric nervous system releases substance P (SP) and calcitonin gene-related peptide (CGRP), two proinflammatory neuropeptides, which induce increased vascular permeability and vasodilation, thereby causing edema. PAR_2-induced SP release, through the activation of neurokinin-1 receptors, is involved in granulocyte recruitment*

DOES PAR_2 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 PAR_2, on enteric afferent sensory fibres might also send a nociceptive signal to the central nervous system. It has been shown that subinflammatory doses of PAR_2 agonists (PAR_2-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, PAR_2 agonists (PAR_2-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 PAR_2 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 PAR_2 agonists were due to a direct activation of PAR_2 on the ENS. The study by Reed and colleagues (7), however, showed that transient exposure of enteric submucosal neurons to PAR_2 agonists evoked long term hyperexcitability. Similar hyperexcitability of extrinsic afferent neurons could be responsible for the PAR_2-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 PAR_2 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 PAR_2 activation has been demonstrated in the establishment of mast cell degranulation-induced somatic hyperalgesia, using PAR_2-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 NK-1 receptor activation has been demonstrated for PAR_1 agonist-induced paw edema (9), there is currently no evidence that such a mechanism also occurs in the gut. Contrary to the situation with PAR_2, subinflammatory doses of PAR_1 agonists did not induce hyperalgesia after intraplantar 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 PAR_1 activation on enteric neurons. Buresi and colleagues (27) have recently shown that PAR_1 activation in mouse intestinal tissues, mounted in Ussing chambers, decreased secretory responses to neural stimulation. This suggests that PAR_1 could contribute to disorders of secretory function associated with the development of colitis. It is interesting to note that PAR_2 agonists also regulate intestinal secretion, but, unlike PAR_1, PAR_2 agonists have been shown to stimulate chloride secretion by a neuro-

genic process (28). No studies have yet demonstrated a role for PAR_3 or PAR_4 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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