The role of thrombin and protease-activated receptors in pain mechanisms

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Summary

As our knowledge of the mechanisms underlying the sensation of pain continues to expand, researchers are constantly searching for novel therapeutic targets. One such novel pain pathway involves thrombin and its associated protease-activated receptor (PAR). Besides its traditional role in haemostasis, thrombin has multiple roles in both the central and peripheral nervous system including activation of microglia, regulation of neuronal death and neurite outgrowth, and influencing the transmission of pain signals in the nociceptive circuitry. Eventually therapeutic modalities directed at these targets could provide novel therapeutic approaches for treating chronic pain. The thrombin-associated PARs also have roles in inflammation, neurodevelopment, and conducting pain, both in conjunction with thrombin and independently. Recent laboratory evidence suggests that the PARs can attenuate pain mediated by the enteric nervous system in animal models (for example in pancreatitis and colitis). This review highlights several pathways in the mediation of pain sensation that can be influenced by thrombin.

Keywords

Thrombin, protease activated receptor, pain, hemostasis, coagulation, inflammation

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Introduction

Despite decades of research, many patients continue to suffer from chronic pain that may be refractory to current medical regimens (1, 2). New pharmacologic targets for alleviating pain are under investigation, and the interrelationships between pain perception and other physiologic pathways are becoming better understood (3–5). Only recently, has the overlap between the haemostatic system and the pain pathways been appreciated (6, 7). Thrombin (factor IIa), a key pathway protein in the coagulation cascade, initiates fibrin formation, platelet activation, and clot formation (8–10). Recently, the interactions between thrombin and its receptors, the protease-activated receptors (PARs), have been implicated in inflammation (11), neurodevelopment (12) and in the nociceptive circuitry (13), providing other important potential targets in the pathophysiology of pain. The interplay between coagulation and inflammation is an important facet in host defense; initially, to stop bleeding, then to signal injury has occurred (i.e. pain), and to activate host inflammatory systems against invading organisms. The purpose of this review is to summarise the interrelationships among haemostasis, inflammation, and pain pathways, with a focus on thrombin as a therapeutic target to treat pain syndromes.

Implication statement

The understanding of pain pathways continues to expand. This review discusses thrombin and its associated protease-activated receptors that represent potential therapeutic targets.

Thrombin's role in the nervous system

Thrombin is a promiscuous molecule with multiple binding sites (exosites) that allow thrombin to serve as the intermediary in systems besides coagulation; including the immune system and the nervous system (Fig. 1) (14–16). Thrombin dynamically modulates cell growth, development and response to injury in the central and peripheral nervous systems, which underlie the involvement of this important molecule in the mediation of pain sensation (5).

The dynamic physiologic concentrations of free thrombin during coagulation reactions is estimated to vary from 1 nM (0.1 U/ml) to over 100 – 500 nM (17) depending on detection methods and experimental conditions (18, 19). Typically low concentrations (<10 nM) are associated with platelet activation and thick, loosely organised fibrin strands susceptible to fibrinolysis, while higher concentrations produce tightly-packed fibrin strands
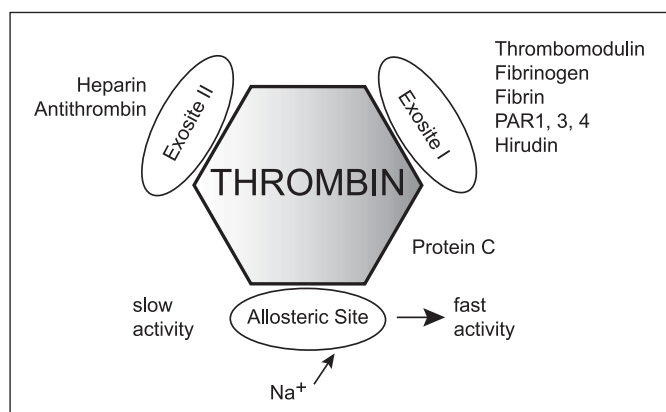
Figure 1: The complexity of thrombin's regulatory systems lies in the dynamics of its exosites. At thrombin's exosite I, not only is fibrinogen cleaved and clot initiated, but inhibitors of thrombin, including thrombomodulin and hirudin, can cease coagulation [15]. Further control occurs at exosite II, where both heparin and antithrombin III act to inhibit thrombin [16]. Allosteric effects of thrombin are managed by a sodium ion which increases thrombin's activity [15].

in vitro (23, 24). Regeneration of the motor neuron after injury requires thrombin as well as neurotrophic factors for appropriate regrowth (20). These neurotrophic factors and thrombin inhibitors, like hirudin, can modulate thrombin's effects in motoneuron cultures (21) suggesting that thrombin's role after peripheral neuronal injury is complex.

Following CNS vascular injury, neuronal damage may be attributed to inflammation and to the direct effects of thrombin (25). Thrombin infused into the rodent basal ganglia can produce glial scars similar to that observed after head injury, can cause brain oedema, and precipitate seizures (26–29) . Furthermore, lowering the concentration of thrombin through the use of thrombin inhibitors (*in vivo* and *in vitro*) may be neuroprotective fostering neuronal survival under oxidative or hypoglycaemic stresses (28). It has even been suggested that an imbalance between thrombin and serine protease inhibitors (serpins) may be involved in the neuronal cell death characteristic of Alzheimer's and other neuropathological diseases (30, 31).

capable of a stable clot (17). Similarly, the concentration of thrombin in the central nervous system (CNS) appears to control its function in neurogenesis (20). Concentrations of thrombin as low as 1–10 nM can influence glial cell mitosis and neuronal outgrowth, and presumably neurodevelopment during the embryonic period (21). Thrombin (100 nM) has been shown to induce apoptosis in motor neurons, and this effect can be blocked by co-application of thrombin inhibitors (22). The effects of thrombin on the important parts of peripheral nervous system (PNS) involved in mediating pain have also been studied. Thrombin inhibits developmental neurite outgrowth from the dorsal root ganglion (DRG)

Protease-activating receptor localisation and mechanism of action in relaying pain information by the nervous system

Thrombin's effects on the nervous system are mediated through activation of protease-activating receptors (PARs). However, not all PARs are specific for thrombin, rather, multiple serine proteases, including thrombin, can initiate a cascade of intracellular events, though a G-protein-coupled mechanism, by freeing the tethered ligand, thereby, enabling it to interact with the receptor (Fig. 2). Four PARs (PAR1–4) have been identified and reviewed pre-
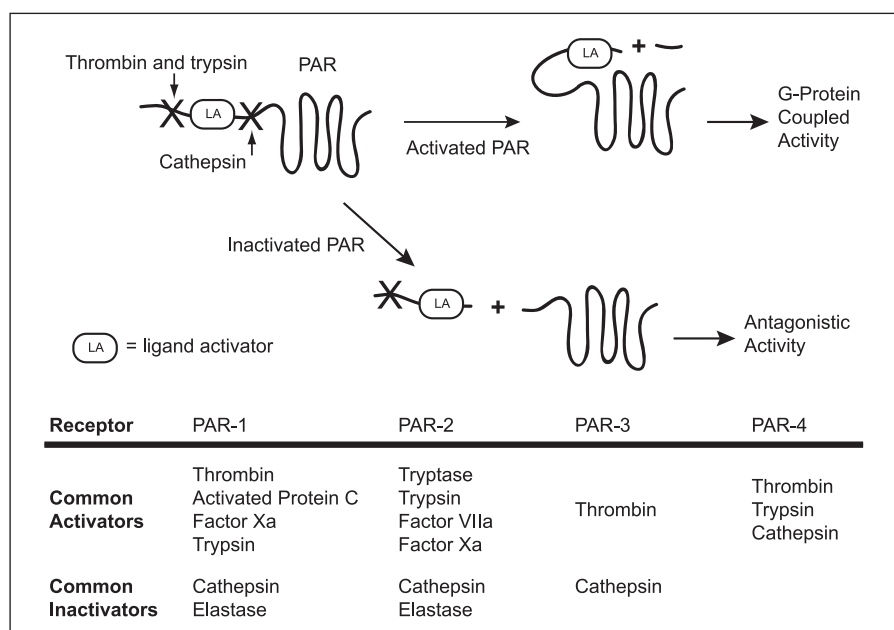

Receptor	PAR-1	PAR-2	PAR-3	PAR-4
Common Activators	Thrombin Activated Protein C Factor Xa Trypsin	Tryptase Trypsin Factor VIIa Factor Xa	Thrombin	Thrombin Trypsin Cathepsin
Common Inactivators	Cathepsin Elastase	Cathepsin Elastase	Cathepsin	

Figure 2: Activators of PAR's cleave the receptor so that the tethered ligand can activate the PAR. Inactivators cleave the ligand. Various activators and inactivators have been described, with some of the most common, and as related to the pain pathways shown [33].

viously (32–37). As predicted by the known associations between thrombin and neurodevelopment, the PAR family has been linked to the development and regrowth of CNS pathways related to memory, neurodegenerative diseases and the dopaminergic reward pathway. Activation of the PARs by thrombin or other ligands has been associated with increased apoptosis of dopaminergic neurons in the substantia nigra (38, 39) and degeneration of hippocampal cells (39). Thrombin potentiates the activity of NMDA (N-methyl-D-aspartate) receptors in hippocampal cells through PAR-1, impacting synaptic and neuronal development and neuroprotection during early injury (40). Similarly, an excess of thrombin, or a lack of serpins, has been shown to lead to increased neuroexcitability and neuronal cell damage after injury to the cerebellum (41).

Of the known PARs, PAR-1 and PAR-3 are strongly activated by thrombin, while PAR-4 shows weak activation by thrombin, and PAR-2 is not activated by thrombin (42). Although, thrombin does not activate PAR-2, this receptor can be activated by many other proteases, including trypsin, tryptase, and other members of the coagulation cascade, such as TF-VIIa complex (43). While each receptor can be distinguished pharmacologically with a specific activating peptide, direct receptor antagonists have been more difficult to identify (33). The mRNA for all four PAR receptors has been demonstrated in the nervous system (31) and even in the lung (44). Specific PARs appear to also have important functions in mediating sensory pain information in the colon (PAR-4) (45), endothelial cells (PAR-4) (46), and tight junctions among epithelial cells (PAR-3) (47).

All four of the described PARs are G-protein coupled receptors that span the entire cell membrane (48). They are activated by various proteases by a tethered-ligand mechanism, i.e. once a protease cleaves the amino terminal of the PAR, the remaining portion of the amino terminal of the protein activates the receptor (42). This is an efficient mechanism because protease is not required to remain complexed to the receptor for activation (the PAR has its own ligand). One protease can activate multiple PARs and multiple proteases can activate the same PAR. Furthermore receptor agonists

need not be related to the activating proteases, but need only possess the amino acid sequence of the PAR's own activating tethered ligand. Once the PAR is activated, it is uncoupled from signalling after a certain time interval via phosphorylation (33), and then the receptor is internalised, with most of the receptors degraded by lysozymes (48).

Research on the role of thrombin and its receptor family in the nervous system has focused on the first two PARs discovered (PAR-1 and PAR-2) (49–51). Figure 3 summarises the role of PAR-1 and PAR-2 in the nervous system. While all four receptors are located in multiple neuronal cells, PAR-1 and PAR-2 receptors are important in pain pathways for their potential as drug targets (52) by either the activating peptide or by manipulation of serine protease concentrations by serpins such as protease nexin.

PAR-1 and PAR -2 and neurogenic inflammation

Both PAR-1 and PAR-2 have been implicated in neurogenic inflammation in the PNS. We define neurogenic inflammation as the combination of vasodilation, oedema, and leakage of proteins, leukocyte adhesion, and pain responses (53). This constellation of pathological mechanisms is closely associated with PAR –1 and PAR-2 activity. Both PAR-1 and PAR-2 exist on sensory afferent nerve (C and Aδ fibers) endings in the PNS (33, 48). Activation of these receptors results in indirect release of substance P and calcitonin gene related peptide (CGRP) in *vivo* (54, 55). These neuropeptides mediate edema through loosening of the epithelial gap junctions (54).

Activating the PAR-2 receptors may play an important role in not only neurogenic inflammation but also airway and enteric inflammatory responses (56). Various trypsins (from pancreatic and extrapancreatic sources), tryptase (from mast cells), and coagulation factors (VIIa and Xa) can activate the PAR-2 (49). The diversity of stimuli allows the receptor to control several functions at

Figure 3: The location and concentration of thrombin's (and other serine proteases) activity via the PARs plays a key role in nociception. For example, intrathecal injection of thrombin into the mouse spinal cord appeared to inhibit hyperalgesia mediated by NMDA receptors [49]. In contrast, peripheral activation of the PARs in the peripheral nervous system of a rat resulted in potentiation of NMDA mediated hyperalgesia [50]. Other effects of NMDA may also be modified by thrombin in the CNS, including long-term potentiation and nociception [51].
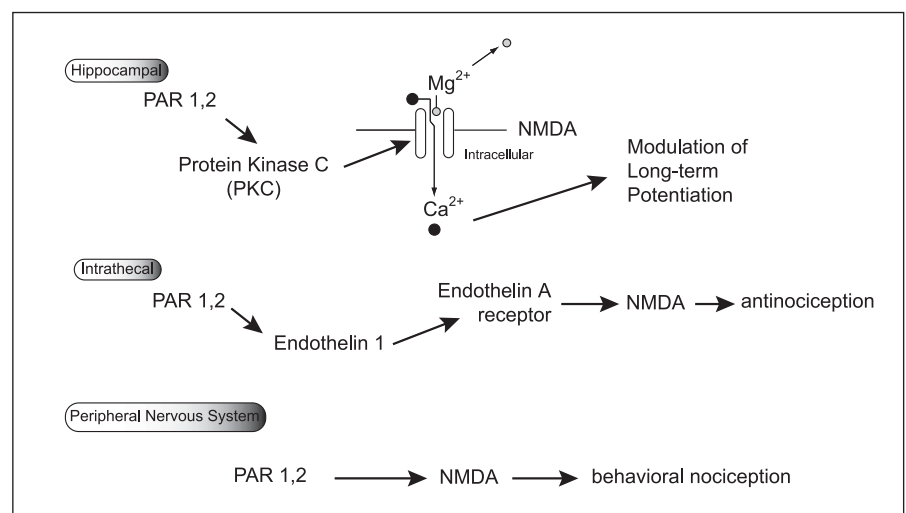
multiple locations. Neurogenic inflammation mediated in the intestine may contribute to the pathophysiology of inflammatory bowel diseases (57). However, activation of PAR-2 may protect gastric mucosa (58). Similarly, trypsin mediated responses from PAR-2 receptors in the respiratory system may lead to either bronchoconstriction or bronchodilation (33, 58). Thus, PAR-2 likely mediates both pro- and anti inflammatory effects.

PARs: Pain and nociception

PARs are located on many sensory afferent nerve endings, and appear to mediate pain responses. Asfaha et al. demonstrated that activation of the PAR-1 receptor by thrombin or selective agonists (at sub-inflammatory doses) mediated analgesia to mechanical stimulation of a rat paw (59). Furthermore, PAR-1 agonists contribute to analgesic properties in dorsal root ganglion (DRG) neurons (60). The activation of PAR-1 in intraplantar paws of rats may also reduce the hyperalgesic response to pain stimulation by carrageenan, a polysaccharide known to cause pain when injected in the rat paw (61).

Narita et al. investigated the role of the PAR-1 receptor at the spinal cord level. Thrombin may be a mediator of neuropathic pain in a rat pain model with partial lesion of the sciatic nerve (62). Intrathecal hirudin prevented the development of neuropathic pain and curbed pain responses for seven days after the injury. The authors discovered a geographical association between platelet-derived growth factor (PDGF) receptors and PARs in the dorsal horn of the spinal cord. Inhibiting PDGF also reduced the pain response and neuropathic pain resulting from intrathecally administered thrombin. This led the authors to conclude that thrombin may activate the PAR-1 receptor in the spinal cord (lamina I and II), which may influence the release of PDGF, modulating neuropathic pain in the spinal cord (62). Similarly, thrombin regulation by protease nexin has been shown to decrease brain oedema following intracranial haemorrhage (63) and potentially could mitigate chronic pain caused by inflammation of nervous tissue.

The role of PAR-2 in the mediation of pain information has also been examined in animal models. Ding-Pfennigdorff evaluated the effects of activating PAR-2 on unmyelinated C fibers of the rat saphenous nerve (64). In this study, activating the PAR-2 receptor enhanced the response to thermal stimuli and decreased the activation temperature needed for a pain response. A mechanism of sensitisation (PAR-2 stimulating release of substance P or neurokinin-1 in the CNS) of spinal neurons may lead to a hyperalgesic response to peripheral stimuli.

Examining transmission of the pain signal by unmyelinated C-fibers, Dai et al. and Amadesi et al. evaluated coupling of the capsaicin receptor (also known as the transient receptor potential vanilloid, TRPV-1), and the PAR-2 (60, 65). The TRPV-1 is activated by heat (43°C and above and by protons, H^+) as well as capsaicin. Dai et al. found PAR-2 and TRPV-1 were coupled in DRG neurons. Activating the PAR-2 receptor sensitised the TRPV-1 receptor, initiating a larger electrical response to capsaicin, a sensitization that lasted for minutes. Furthermore, the sensitisation by PAR-2 led to a decreased threshold to temperature for a pain response (65).

Because PAR-2 is located in many organs including the visceral organs, trypsin and tryptase were found to activate PAR-2 and cause sensitisation of the TRPV-1 receptor to painful stimuli (60). Injecting PAR-2 agonists on the intraplantar surface of a mouse model caused sustained hyperalgesia to thermal pain stimuli via the TRPV-1 pathway (43, 60), thus suggesting this pathway may be
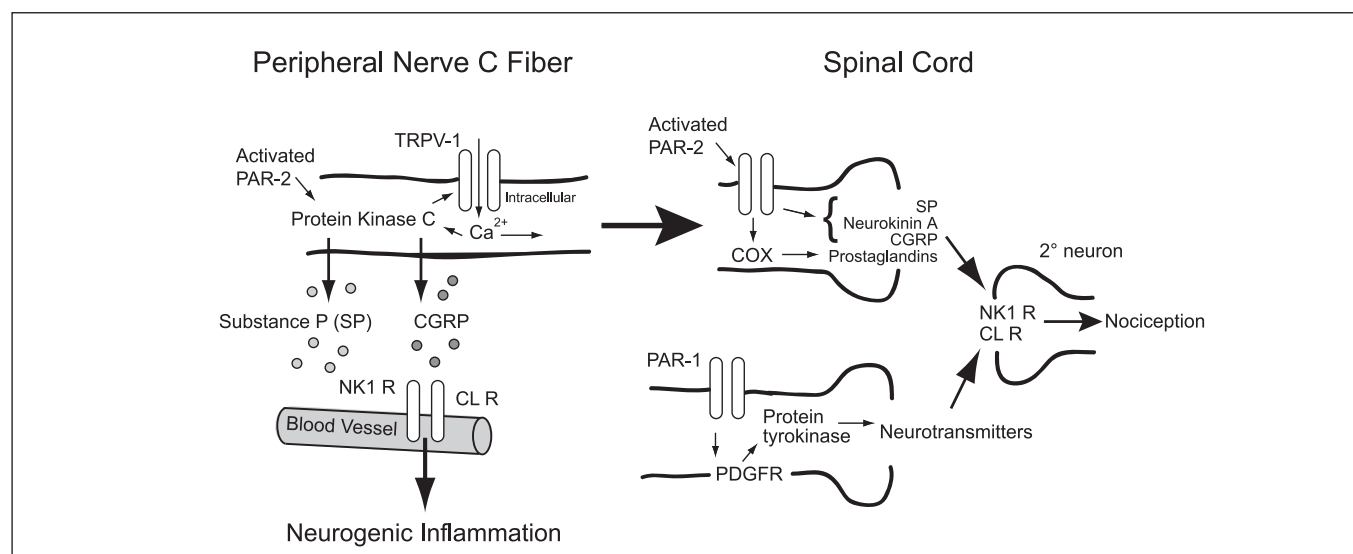

Figure 4: Shown is the coupling of PAR-1, 2 to various receptors in modulation of the nociceptive pathways [48, 58, 73]. The response on C fiber nerve endings propagates to the spinal cord to the second order neurons in the pain pathway [48, 62]. Abbreviations: NK1 R (neurokinin receptor), CL R (CGRP receptor), PDGFR (platelet derived growth factor receptor), COX (cyclooxygenase enzyme), TRPV-1 (transient receptor potential vanilloid), PAR (protease activated receptor).

modifiable pharmacologically in preventing the progression of neuropathic pain.

As discussed earlier, thrombin and its associated PARs (predominantly PAR-1) were associated with NMDA receptors. Similarly, the PAR-2 receptor may sensitise the NMDA-glutamate pathway in the spinal cord. Kawabata et al. found a link between the PAR-2 receptor on C-fibers and the spinal NMDA receptors, resulting in behavioral nociception and thermal hyperalgesia initiated by PAR-2 agonists (66). The authors suggested that activation of NMDA receptors in the spinal cord may be nitric oxide-mediated, while in the periphery, PAR-2 may be coupled directly to NMDA receptors. A summary of PAR pathways in pain is presented in Figure 4.

PARs as a model for pain

Experimental research models of specific pain syndromes (i.e. trigeminal neuralgia and chronic abdominal pain) have been associated with the PARs. Kawabata evaluated PAR-2 receptors in the parotid gland of rats, and reported PAR-2 receptor activation at the trigeminal nociceptive sensory neurons led to transmission of pain (67). They conclude that exocrine secretions in the salivary glands, like tryptase, may lead to inflammation and parotitis-related pain mediated by PAR-2. Similarly, Holzhausen et al. further delineated PAR-2's involvement as a putative mediator in periodontitis. PAR-2 may be activated by external sources of serine proteases, i.e. by bacteria such as *Porphyromonas gingivalis,* leading to pain, acute inflammation, and bone destruction during periodontitis (68).

In the enteric nervous system, the PARs are associated with cells that release calcitonin gene-related peptide (CGRP), substance P, and vasoactive intestinal peptide (VIP), all neurotransmitters known to modulate pain (56). Because proteases, such as trypsin, are used in the digestive function of the gut it is not surprising to discover a role for them in inflammation and inflammatory bowel diseases.

Inflammation and pain are closely coupled in the enteric nervous system as in the peripheral nervous system (69). Biopsies in humans with Crohn's disease show a seven-fold increase in the expression of PAR-1 and activation of the PAR-1 initiates colitis via an antibody response in an animal model for inflammatory bowel disease (IBD) (60). However, activation of PAR-1 receptors may have a protective role in inflammation mediated by helper-T cells (type 2 response) (43). Activating PAR-2 may also lead to inflammation and colitis in mice (56, 57). The activation of PAR-1 can result in analgesia in the colon (60, 70). PAR-2 receptors in the colon and jejunum initiate hyperalgesic responses and increase activation of pain receptors in spinal cord lamina I and II (43, 71). These hyperalgesic responses may be mitigated with naproxen, and are related to dose of PAR agonist. Capsaicin-evoked visceral pain and referred hyperalgesia can involve activation of PAR-1s or PAR-2s (67). PAR activation by proteases can either result in analgesia with sub-inflammatory activation or hyperalgesia with concentrations of agonist that predispose to inflammation (48, 58, 72, 73).

The initiation of pancreatic pain by intraductal administration of PAR-2 agonists may actually contribute to the pathophysiology of pancreatitis and not just the painful sequelae (70). Trypsin and other agonists result in increased CGRP release, and activation of TRPV-1 receptors (74). Maeda et al. showed that PAR-2 agonists also initiate release of cytokines, such as interleukin-6 and interferon-γ, in the progression of acute pancreatitis in rats (75). Hoogerwerf et al. suggest a role for mast cells activating PAR-2 and TRPV-1, resulting in pancreatic pain (76). However, mild PAR-2 activation demonstrates a protective role in the progression of early pancreatitis (77).

Conclusion

Theories regarding pain mechanisms that arise through thrombin signaling and PARs activation are being developed. Modifying pain and inflammation through these mechanisms represents a novel approach for therapy. Statins have been shown to decrease thrombin generation and to reduce PAR-1 expression, and this knowledge may result in improved management of pain (78). Serine protease inhibitors may also have a potential role in interfering with transmission of pain signals (79), and may provide novel targets for pain control. As the role of the balance between thrombin and related proteases and their associated receptors in the pathophysiology of pain and inflammation becomes better defined, novel therapeutic agents and targets to better modulate pain can be developed.

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