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Protease-Activated Receptor 4: A Critical Participator in Inflammatory Response

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Abstract—Protease-activated receptors (PARs) are G protein-coupled receptors of which four members PAR1, PAR2, PAR3, and PAR4 have been identified, characterized by a typical mechanism of activation involving various related proteases. The amino-terminal sequence of PARs is cleaved by a broad array of proteases, leading to specific proteolytic cleavage which forms endogenous tethered ligands to induce agonist-biased PAR activation. The biological effect of PARs activated by coagulation proteases to regulate hemostasis and thrombosis plays an enormous role in the cardiovascular system, while PAR4 can also be activated by trypsin, cathepsin G, the activated factor X of the coagulation cascade, and trypsin IV. Irrespective of its role in thrombin-induced platelet aggregation, PAR4 activation is believed to be involved in inflammatory lesions, as show by investigations that have unmasked the effects of PAR4 on neutrophil recruitment, the regulation of edema, and plasma extravasation. This review summarizes the roles of PAR4 in coagulation and other extracellular protease pathways, which activate PAR4 to participate in normal regulation and disease.

KEY WORDS: protease-activated receptor 4; inflammation; mechanisms; coagulation.

INTRODUCTION

The protease-activated receptors (PARs) belong to a developing family of four transmembrane G protein-coupled receptors (GCPR) activated by a unique mode, underlined by the proteolytic cleavage of their extracellular N-terminal domain. After the interaction with various proteases, this cleavage site releases a new N-terminal domain that acts as a tethered ligand to induce an intracellular signal by linking the receptor itself to its second extracellular loop [1–5]. Four members of the PARs family, PAR1, PAR3, and PAR4, have been cloned [3, 6, 7] as thrombin receptors, since they have been shown to be responsible for thrombin-induced platelet activation. PAR1, 3, and 4 were initially recognized as receptors for the coagulation protease and are involved in thrombin signaling, mainly as a heterodimer [8–11]. In addition, PAR1 activation is induced by other coagulation proteases [12, 13], matrix metalloproteinase 1 [14], and microbial proteases [15]. PAR2 is activated by a series of extracellular proteases including coagulation factors, trypsin, tryptase, tissue kallikreins, and transmembrane serine proteases at epithelial interfaces and in the cardiovascular and immune system. So far, four PARs have been perceived in multiple cells affiliated in the inflammatory reaction such as macrophages, neutrophils, and mast cells [16].

As the last identified member of the PARs family, PAR4 generates physiological functions in the presence of thrombin, trypsin, and cathepsin G [17]. Moreover, PAR4 activation has been discovered in a variety of cells including endothelial cells, neutrophils, and sensory neurons [18, 19], as well as in mouse knee joints [20]. As previously reported, except for platelet activation and relaxation of esophageal smooth muscle, PAR4 is functionally active in the human lung, pancreas, heart, thyroid, small intestine, uterus, prostate, testicles, lymph nodes, the nervous system, and so on [21–23]. Currently, a proinflammatory role for PAR4 was studied when researchers found that PAR4 agonists could cause leukocyte rolling and adherence, full

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granulocyte recruitment, and edema [19, 23–25]. For example, the selective PAR4 agonist AYPGKF-NH_2 induces edema and enhanced blood flow, which result in leukocyte rolling and adhesion [19, 20, 24, 25], suggesting a proinflammatory character for this receptor [19, 24]. Russell *et al.* [26] proved that PAR4 is intricately connected to the B2 receptor regarding both its inflammatory and nociceptive roles. This review will focus on the role of PAR4 as a potent signaling mediator in inflammation.

PAR4-MEDIATED SIGNAL TRANSDUCTION

Platelet aggregation via PAR4-induced G protein signaling

G protein-coupled signaling is induced by the tethered ligand of PAR4 on the basis of effective collaboration with other factors, taking the effect of mediation of thrombin in platelets as an example. PAR4 is activated by higher concentrations of thrombin compared with PAR1 [27, 28], and PAR4 signaling has distinctive pathways for platelet activation [29–31]. Unlike PAR1 signaling, PAR4 does not need to be phosphorylated on key serine residues and internalizes slowly in comparison, resulting in prolonged degradation of the receptor [9, 32, 33]. Research in the field has indicated that PAR4, a receptor mediator with low affinity that is involved in thrombin signaling at high concentrations, has been shown to signal through at least two G protein signaling pathways ($G_{\alpha 12}$ and $G_{\alpha q}$) [34]. Similar to thromboxane A2, PAR4 and PAR1 can cause the release of ADP, which could trigger Gi pathways through initiation of the P2Y12 receptor [1, 9, 35, 36]. However, Kim *et al.* [37, 38] affirmed that platelet aggregation-mediating PAR4 does not depend on Gi stimulation; indeed, the physiological function of the Gi pathway is accomplished by secondary release of ADP, which works for the Gi-coupled ADP receptor, P2Y12 [37–39]. It is noteworthy that the platelet aggregation mediated by PARs has remained from unclear mechanisms relating to Gq signaling alone or coactivation of an elusive G protein-coupled pathway apart from Gq signaling. As already reported, PAR4 is not a high-affinity thrombin receptor [27, 28, 40]. The function of PAR4 can only be initiated with the help of PAR1 in environments where thrombin is low. On the contrary, PAR4 is adequate for the mediation of platelet aggregation when the concentration of thrombin is high enough. As a matter of fact, two independent studies have shown that PAR4 signaling is connected with PAR3. The inositol phosphate (IP) accumulation in response to thrombin was increased by about 1.7-fold in COS7 cells which expressed mouse PAR4 alone compared with COS7 cells which expressed mouse PAR4 as well as PAR3 [10]. Moreover, Mao *et al.* [41] revealed that PAR3 knockout ($PAR3^{-/-}$) mouse platelets had enhanced intracellular Ca^{2+} mobilization and platelet activation in response to plasmin in comparison with wild-type platelets [41]. These investigations indicate that PAR3 can affect PAR4 signaling more than heightening PAR4 activation. The consensus finding is that PAR3 adversely regulates PAR4-mediated Gq signaling via the down-regulation of Ca^{2+} mobilization instead of influencing the downstream signaling of the $G_{12/13}$ pathway [42].

PAR4 β-arrestin signaling

β-arrestins are a group of cytoplasmic proteins that were initially characterized due to their role of associating with agonist-activated G protein-coupled receptors (GPCRs) [43], inducing their internalization and desensitization [44]. Current research suggests that β-arrestins play excess roles in GPCR signaling, by acting as scaffolds for signaling complex recruitment to the receptor, and can lead to enhanced activation of G protein-coupled pathways [43, 45]. One of the arrestin-mediated pathways is the PI3K-induced activation of the Ser-Thr kinase, Akt [46, 47]. As the data display, arrestins have been considered to have a vital role localizing PI3K to GPCR complexes by collaborating with Src family kinases (SFKs) in fibroblasts, colorectal cells, and gastric carcinoma cells [48–50]. Thrombin-induced Akt phosphorylation associated with the activation of Gi as well as Gq-/Gi-dependent signaling to Akt requires RAS activation, whereas Gq-dependent Akt activation requires arrestin-2 [51]. In platelets, thrombin-mediated Akt phosphorylation trends to decrease by about 90 % when exposed to inhibitors for the Gi-coupled ADP receptor, P2Y12, and is prevented completely by inhibitors of PKC [37, 52].

Akt activation by thrombin is completely reliant on the PKC-induced release of ADP. However, the level of Akt phosphorylation induced by ADP is low. That is to say, the activation of P2Y12 is required, but not sufficient, for maximal Akt stimulation by PAR4 agonists or thrombin. Compared with the effect of specific G protein-subunits on thrombin with ADP-inducted signal pathway in mouse platelets, it can be seen that Gi2 is required solely for ADP signaling, while Gq is necessary for thrombin or ADP to induce Akt phosphorylation, specifically. The above facts suggest that there are some factors, not just ADP alone, that induce a secondary role of PAR4

stimulation. Moreover, new research has shown that PAR4 is involved in inducing Akt phosphorylation in platelets with P2Y12 knock-out [53]. To sum up, these studies manifest that the activation process of Akt stimulated by thrombin receptors versus P2Y12 is diverse, but synergistic to some degree. Besides, Akt phosphorylation partly relies on arrestin-2 in the formation of complexes with PI3Ks [51]. PAR4 agonist-induced Akt phosphorylation is arrestin-2-dependent, whereas Akt phosphorylation stimulated by ADP is not. Similarly, PAR4 agonist-induced fibrinogen binding is also arrestin-dependent, while fibrinogen binding induced by ADP is not. The involvement of arrestin-2 in platelet aggregation by PAR4 has been investigated by arrestin-2 knock-out mice, which have a mild defect in thrombus formation, suggesting that arrestin-2 might contribute to platelet function *in vivo* [54].

THE MAIN FACTORS CONNECTED WITH PAR4 PARTICIPATING IN INFLAMMATION

Tissue Factor

Tissue factor (TF) is a transmembrane glycoprotein that combines with the serine protease activated factor VII (FVIIa) to start the coagulation pathway. Two major forms of TF may exist: cell-bound form and the plasma or soluble form. The cell-bound form has been thought to take part in most of the known biological processes, but it is noteworthy that soluble forms of TF may participate in coagulation or hemostasis [55] and tissue inflammation [56]. Soluble tissue factor (sTF), when injected into mouse joints, is considered to cause inflammatory arthritis [57, 58]. In one regard, inflammatory response stimulates the coagulation cascade and is often seen as a prethrombotic state, but, on the contrary, coagulation can also start and perpetuate the inflammatory response. The factors related to the above phenomenon include TF, fibrin, thrombin, and PARs. TF-induced coagulation is attributed to fibrin formation and also evokes inflammatory responses through the activation of downstream coagulation proteases such as thrombin and FVIIa on PAR4. A recent study showed that injecting recombinant murine sTF into the mouse footpad leads to footpad swelling and an acute inflammatory response of extravascular tissues. This action is mainly regulated by the well-known pathway of coagulation activation, through thrombin and fibrin formation, as inflammatory responses were effectually attenuated by regulation of the thrombin inhibitor hirudin and an FVIIa inhibitor. The depletion of fibrinogen also blocked inflammatory responses in this model. Busso *et al.* [59] testified that fibrin formation was necessary to link coagulation and inflammatory responses. To verify whether PAR4 activation could also exert its effect, the mice that were deficient for the individual PARs and only PAR4-deficient mice were tested, which showed that these mice were entirely protected from sTF-induced inflammatory responses [59]. This is in contrast with glomerulonephritis and arthritis, which require an immune stimulus, where the activation of PAR1 and PAR2 seems to play a role [60, 61].

Bradykinin B2 Receptor

Bradykinin is a major inflammatory peptide that is formed at sites of injury and is known to activate sensory nociceptors, inducing a sensation of pain [62]. The inflammatory effects of activated PAR4 rely on the kallikrein-kinin system, which is based on the fact that AYPGKF-NH2-initiated edema could be abrogated by regulation of the bradykinin B2 receptor antagonist, HOE140 [20, 25]. Russell *et al.* [26] demonstrated that PAR4 is intricately linked to the B2 receptor with regard to both its inflammatory and nociceptive effects. The mechanisms of action of PAR4 in triggering joint inflammatory response and pain appear to be involved with the kallikrein-kinin system. The bradykinin antagonist HOE140 blocked edema, hyperemia, and painful responses to AYPGKF-NH2, which was consistent with the results of a previous research suggesting that PAR4-regulated edema could be blocked by pretreatment with a bradykinin B2 antagonist [25]. PAR4 is present on the surface of leukocytes, endothelial cells, and vascular smooth muscle cells [19], which are stimulated by PAR4-activating peptides to release kallikreins. The kallikreins released then cleave kininogens to generate active kinins, which eventually link to bradykinin receptors, leading to vasodilatation and enhanced vascular permeability. Actually, bradykinin is known to be vasoactive in knee joints, in which it leads to synovial vasodilatation and protein extravasation [63–65]. The effect of the kallikrein-kinin system on enhancing joint pain is also verified by other researches showing that bradykinin evokes peripheral sensitization of knee joint afferent nerves, resulting in increased pain sensation [66, 67]. Other PARs such as PAR1 and PAR2 cause painful and inflammatory responses by inducing the secondary release of proinflammatory neuropeptides from sensory neurons and by stimulating connective tissue mast cells [68–71]. On the contrary, however, the inflammatory response of PAR4 is not neurogenically driven and does not involve mast cell degranulation [24].

ERK/MAPK

PAR4-AP evokes the overexpression of CGRP via activation of the ERK1/2 signaling pathway, and PAR4 activation notably enhances the levels of CGRP messenger RNA (mRNA) and protein in DRG neurons in the presence of AYPGKF-NH2 both *in vivo* and *in vitro* [72]. Remarkably, this overexpression is significantly attenuated after inhibiting ERK1/2 phosphorylation. The activation of PAR4 is involved in the administration of CGRP expression in primary sensory neurons via activation of the ERK1/2 signaling pathway. In addition, the activation of PAR4 increases the number of CGRP, PAR4, or p-ERK1/2 immunopositive neurons in DRGs in the presence of AYPGKF-NH2 both *in vivo* and *in vitro*, characterized by small- and medium-sized painful neuronal cells [18, 73].

A previous research showed that harmful stimulation enhanced the level of p-ERK1/2 expression simultaneously in the cytoplasm and nucleus of DRG neurons [74]. PAR4 activation increases p-ERK1/2 staining in the cytoplasm and nuclei of DRG neurons with AYPGKF-NH2 treatment. The enhanced p-ERK1/2 staining in cytoplasm and nuclei represents cellular redistribution of p-ERK1/2 in DRG neurons after the activation of PAR4. However, nuclear translocation of ERK1/2 and an increase of p-ERK1/2 in the nucleus lead to numerous cell effects involving proliferation and gene transcription [75, 76]. Therefore, it is possible that the translocation of p-ERK1/2 to the nucleus induced by active PAR4 has a close relationship to CGRP gene transcription in DRG neurons [77, 78]. It is a closely relevant between the up-expression of cytoplasmic p-ERK1/2 in DRG neurons and harmful stimulation [74, 79].

Membrane depolarization and Ca^{2+} influx induced by harmful stimulation would result in ERK1/2 activation [80]. After phosphorylation, p-ERK1/2 is quickly localized from the cytosol into the nucleus, in which it phosphorylates variety of substrates, e.g., transcription factors [81–83]. The redistribution above is considered to be essential for the effective phosphorylation of transcription factors to mediate levels of mRNA [75, 76]. A rapid and continuing activation of ERK1/2 induced by PAR4 evokes activation of the transcriptional regulation factor cAMP-response element binding protein (CREB) which in turn causes gene expression [84–86]. Activation of p-ERK1/2 also induces CREB-initiated transcription and overexpression of CGRP mRNA in DRG neurons [77, 78]. It is connective between the activation of ERK and the expression of CGRP in sensory neurons [79, 87]. Harmful stimulation causes the rapid phosphorylation of ERK in sensory neurons that conduces to the facilitation of pain sensations and is often used as an instant marker for the excitation of spinal neurons. An intense harmful peripheral or C-fiber electrical stimulation triggers many p-ERK-positive neurons [88]. A recent study showed that harmful stimulation of the rat hind paw could evoke p-ERK1/2 activation in the cytoplasm and nucleus in L3–L5 DRG cells according to the time order exposed to stimulation [74], suggesting an immediate translocation of ERK1/2 from the cytoplasm to the nucleus. All of these data indicate that enhanced levels of p-ERK1/2 occur in advance of the increased expression of CGRP.

Accordingly, it is possible that PAR4 leads to activation of the ERK1/2 pathway which connects activation of the transcriptional regulation factor CREB to the production of CGRP. Depending on the activation of PAR4, p-ERK1/2 may be involved in the overexpression of CGRP mRNA through CREB-dependent transcription. Overexpression of CGRP mRNA and protein levels after AYPGKF-NH2 treatment in DRG neurons offers a molecular mechanism for PAR4 in painful and inflammatory responses. PAR4 activation can notably improve the levels of CGRP mRNA and protein in DRG neurons. The function of active PAR4 on CGRP expression seems to be mediated by the p-ERK signaling pathway.

PAR4 IN NOCICEPTION AND PAIN PATHWAYS

Nociception, mediated by sensory receptors on afferent neurons, is the neuronal sensation, transmission, and central procession of noxious stimulation. Usually, more sensitive nociceptors and enhanced pain sensation are expressed in inflammation. PAR4 protein and mRNA are commonly observed in small nociceptive dorsal root ganglion (DRG) neurons, the majority of which are peptidergic and are marked by substance P and calcitonin gene-related peptide (CGRP) [73, 89]. Furthermore, increasing experimental results prove that activated nociception and inflammation induce a rapid increase in protein and mRNA expression of CGRP in DRG neurons [90–92]. The expression of CGRP in DRG neurons plays a vital role in regulating neurogenic inflammation and pain [72, 93]. PAR4 has been recognized to be up-regulated in DRG neurons during nociceptor activation and inflammation pain [94, 95]. The PAR4 agonist does not evoke a Ca^{2+} signal in DRG neurons, but reduces the Ca^{2+} signal of DRG neurons, meaning that activated PAR4 can attenuate the nociceptive pathway in DRG neurons. Previous studies have suggested that, unlike PAR1 and

PAR2, edema induced by PAR4 is not reliant on a neurogenic signal relating to capsaicin-sensitive neurons [24], but is reliant on the kallikrein-kinin system activation [23, 25]. Asfaha *et al.* [18] identified a novel mechanism for the regulation of pain transduction, which demonstrated that PAR4 had an inhibitory role on pain and nociception. The finding that PAR4 exists in sensory neurons and that KCl-induced calcium mobilization in those neurons can be inhibited by a PAR4 agonist means that the activation of PAR4 has a direct effect on sensory nerves by inhibiting the transmission of nociceptive signal. The fact that PAR4 agonists directly activate DRG neurons mediates ascending nociceptive transduction, which is similar to that of opioid receptors, leading to abrogating nociception in DRG neurons.

Previous research has shown that injecting thrombin results in an analgesic effect under the condition of a mechanical stimulus, but hyperalgesia responds to a thermal stimulus, while selective PAR1 activation leading to analgesia under the action of both mechanical and thermal stimulus [96]. Similarly, the injection of a PAR4 agonist evokes analgesia in rat paw in the presence of both stimuli. With regard to the hyperpathia of thrombin in response to thermal stimulus, thrombin might work through a receptor different to PAR1 or PAR4, and/or might come into effect independently of its catalytic site [97–99]. In the joint, the activation of PAR4 on non-neuronal cells causes the release of tissue kallikreins secreted by multiple cell types [100]. This, in turn, results in the production of bradykinin, which can then enhance the sensitivity of knee joint primary afferents. The mechanism of action of PAR4 in triggering joint inflammatory reaction and pain appears to include the kallikrein-kinin system, as the bradykinin antagonist HOE140 weakens edema, hyperemia, and painful responses to AYPGKF-NH2 [20]. A previous finding that PAR4-dependent paw edema could be attenuated by pretreatment with a bradykinin B2 antagonist also manifested the similar conclusion [25]. Auge *et al.* [101] proved that PAR4 has an effect on the termination of primary afferent nerves in the mouse colon, suggesting that PAR4 activation had a direct effect on these afferent nerves.

PAR4 SIGNALING IN THE GASTROINTESTINAL INFLAMMATION

It has been confirmed that PAR4 participates in modulation of the motility of rat duodenum [102] or pig gall bladder [103]. However, Hollenberg *et al.* [2] indicated that PAR4 agonists evoke contraction of gastric longitudinal smooth muscle and nitric oxide-dependent relaxation of vascular smooth muscle. PAR4 is also moderately expressed in the human colon [104]. Furthermore, Auge *et al.* [101] proved that the colonic delivery of PAR4 agonists in mice reduces basal visceral pain and moderates visceral hypersensitivity triggered by PAR2. It was also demonstrated that PAR4 was detected in sensory neurons protruding from the colon and was co-expressed with PAR2 as well as TRPV4. PAR4 attenuates calcium mobilization evoked by PAR2 and TRPV4, while the use of PAR4 agonists alone did not cause any change in the free intracellular calcium mobilization in sensory neuron cells *in vitro*. A higher dose of the PAR4 agonist increased the visceral sensitivity to colorectal distension and was found to trigger a small increase in macroscopic damage scores and myeloperoxidase activity, indicating the existence of an inflammatory response. These observations are in accordance with the previous studies of a differential effect of PAR4 agonists on the development of inflammatory reaction and hyperalgesia, which suggest that PAR4 agonists may perform double pro- or anti-nociceptive roles in the somatic and visceral domains according to the dose used [105]. Mule *et al.* [106] have demonstrated that PAR4 is expressed in rat colon, the activation of which triggers contraction of the intestinal longitudinal smooth muscle. They found that PAR4 activation evoked contractile effects in rat colonic longitudinal muscle via treating with TTX, a neuronal transduction blocker, to evaluate the function of neuronal transmission in the induced effects, and it indicated that a neurogenic component was relevant to the PAR4 agonist-induced effects. PAR4 activation can trigger acetylcholine release via an action potential-dependent neuromechanism. Besides, NK1 and NK2 receptor antagonists, when mediated independently or in combination, reduce contraction because of PAR4 activation, indicating the involvement of these receptors in the functions induced by AYPGKF-NH2. The existence of these receptors mediating contraction in the rat colon has been previously proven by histochemistry and functional researches [107–109]. This functional mechanism may support a proinflammatory role for PAR4, which could conduces motility disturbances observed at the time of intestinal trauma and inflammation [106].

PAR4 AS MEDIATORS IN BRAIN MICROGLIAL INFLAMMATION

In the central nervous system (CNS), microglia are the major immune effector cells and reactive microgliosis

has been implicated in the pathogenesis of a broad range of CNS disorders. These include not only infectious CNS diseases but also acute CNS injuries such as traumatic brain injury (TBI) [110], spinal cord injury [111], stroke, and brain ischemia [112]. In addition, microgliosis is also intimately involved in several chronic neurodegenerative disorders such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) [113, 114]. Regarding the molecular mechanisms underlying thrombin-induced microglial activation, studies have suggested that PAR1 is not involved in the activation of microglia, using parameters such as inducible nitric-acid synthase [115]. However, Suo *et al.* [116] demonstrated that murine microglial cells constitutively express PAR4 at both the protein and mRNA levels. Functionally, a PAR4-specific agonist peptide mimics the thrombin-induced TNF-α production both *in vitro* and *in vivo*. The ineffectiveness of the scrambled PAR4AP on TNF-α induction confirms the specificity of the PAR4-AP effect. The inhibition of the effects of both thrombin and PAR4-AP on TNF-α induction following down-regulation of PAR4 with PAR4-specific antisense oligonucleotide further demonstrates the critical role of PAR4 in thrombin and PAR4-AP-induced TNF-α production. Therefore, PAR4 is at least one of the receptors that mediates the thrombin-induced TNF-α production in both mouse and rat microglia.

Experiments confirmed that PAR4 activation in microglial cells indeed induced a fourfold and twofold, rather than zero, increase of PAR4 binding to GRK5 and GRK2, respectively. This indicates that microglial PAR4, unlike fibroblastic PAR4, may be under the loose control of GRKs [111]. Besides the critically involved cellular thrombin receptors, Suo *et al.* [116] also revealed that, compared with PAR1, the PAR4 signaling pathway in microglia featured prolonged Ca^{2+} increase and p44/42 MAPK activation as well as subsequent NF-κB activation. This may explain why PAR1 primarily contributes to promoting microglial proliferation while PAR4 is fully engaged in activating microglia and inducing cytokine (i.e., TNF-α) production. These results are consistent with previously reported thrombin signaling in microglia [115, 117, 118], while adding more detailed features and clearer identification of upstream mediators.

CONCLUSION

PAR4-induced cell signaling remains a puzzle for most cell types. PAR4 activation regulates calcium signaling while PAR4-mediated NO release is independent of a Ca^{2+} mechanism in endothelial cells [96, 119]. In cardiomyocytes, PAR4 activation causes a certain amount of increased phospholipase C (PLC) and extracellular signal-regulated kinase (ERK) [120]. Furthermore, PAR4 evokes the strong activation of a non-receptor Src tyrosine kinase-p38-mitogen-activated protein kinase (MAPK) cascade in cardiomyocytes [121]. The studies discussed testify a main role of PAR4 signaling in critical pathways that induce regenerative processes and inflammation. These pathways have been demonstrated by the application of specific PAR4 agonist peptides in animal models with appropriate controls in PAR4-deficient animals, as demonstrated by the identification of a selective inhibitory antibody that blocks pathological PAR4 signaling in inflammation mouse models; however, the proteases concerned have not been well studied *in vivo. PAR4 seems to be expressed and able to inhibit* Ca^{2+} signaling in all sizes of neurons. Possible direct interactions of PAR with L-, N-, or P/Q-type voltage-gated Ca^{2+} pathways could explain the anti-nociceptive effects of PAR4 activation and its inhibitory effects against depolarization-induced Ca^{2+} signaling. Despite a direct inhibitory action of PAR4 activation on primary afferents explaining its analgesic effects, one cannot exclude the possibility that PAR4 might also exert indirect effects on other cell types [101]. To sum up, although the distribution, activation, and biological effects of PAR4 have been realized to a certain extent, deeper exploration of PAR4-mediated pathogenesis is still needed. Previous data suggest that PAR4 activated by thrombin, trypsin, tryptase, and so on play a pivotal role in pathological processes, defining that the crucial protease pathway associated with PAR4 signaling has underlying therapeutic value.

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