Strathprints Institutional Repository

Proteinase-activated receptor 2: Are common functions in glial and immune cells linked to inflammation-related CNS disorders?

Trevor J. Bushell[*], Margaret R. Cunningham, Kathryn A. McIntosh, Serge Moudio and Robin Plevin.

Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK.

[*] Address correspondence to this author at the Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK. Tel/Fax: +44 (0)141 548 2856, +44 (0)141 552 2562; Email: trevor.bushell@strath.ac.uk.

Abstract.

Protease-activated receptors (PARs) are a novel family of G-protein coupled receptors (GPCRs) whose activation requires the cleavage of the N-terminus by a serine protease. However recent evidence reveals that alternative routes of activation also occur and that PARs signal via multiple pathways and that pathway activation is activator-dependent. Given our increased understanding of PAR function both under physiological and pathophysiological conditions; one aspect that has remained a constant is the link between PAR2 and inflammation. PAR2 is expressed in immune cells of both the innate and adaptive immune system and has been shown to play a role in several peripheral inflammatory conditions. PAR2 is similarly expressed on astrocytes and microglia within the CNS and its activation is either protective or detrimental to CNS function depending on the conditions or disease state investigated. With a clear similarity between the function of PAR2 on both immune cells and CNS glial cells, here we have reviewed their roles in both these systems. We suggest that the recent development of novel PAR2 modulators, including those that show biased signalling, will further increase our understanding of PAR2 function and the development of potential therapeutics for CNS disorders in which inflammation is proposed to play a role.

1. INTRODUCTION

Protease-activated receptors (PARs) are a family of G-protein coupled receptors that are unique in their mechanism of activation. Cleavage of the N-terminus by serine proteases unveils a 'tethered ligand' which binds to the second extracellular loop of the receptor leading to the activation of signalling pathways (Figure 1). PARs were first discovered following investigations into the role of thrombin in platelet aggregation which lead to the identification of PAR1 and its unique mechanism of activation [1,2] which was closely followed by the discovery of PAR2 [3,4] and PAR3 and 4 [5-8]. Of the PAR family members cloned to date, research into PAR1 has been the most progressive. The PAR1 crystal structure has recently been resolved [9] and in 2014, the first PAR1 selective antagonist, Vorapaxar, was FDA approved for clinical use in the US to reduce the risk of heart attack, stroke and other cardiovascular-related deaths [10]. Whilst efforts have been made to generate antagonists for the other PAR family members, it is clear that further understanding of their roles and regulation are required.

A key feature of all the PARs is that they can be cleaved by serine proteases, for example thrombin, trypsin, tryptase and kallikreins [11-13], at an arginine residue in the N-terminus which reveals a tethered ligand that is specific to each receptor. Identification of these tethered ligand sequences helped increase our understanding of the roles and functions of PARs through using synthetic activating peptides based on these sequences (PAR-APs) but diligence is required when using such peptides and their use for CNS investigations in vivo is limited due to poor bioavailability [12]. In addition to their activation by serine proteases, it is now accepted that PARs can be activated by other non-canonical activators which cleave the PAR N-terminus at regions different from the canonical serine protease cleavage sites. The first evidence of this came with matrix metalloprotease-1 (MMP-1) being shown to induce signalling via PAR1 by cleaving its N-terminus upstream of the serine protease site but still initiating signalling similar to that observed via the canonical pathway [14,15]. This non-canonical activation has now been shown for PAR1, PAR2 and PAR3 with activated protein C (APC), neutrophil elastase (NE) and neutrophil protease-3 being shown to activate PARs [16-20]
.
PARs have been shown to signal via multiple G-proteins with PAR activation leading to coupling with $G\alpha_q$, $G\alpha_i$ and $G\alpha_{12/13}$ depending on the activator or cellular localisation (Figure 1).

Evidence exists for PAR1 & PAR2 activation leading to signalling via all these G-protein subunits whereas PAR4 signals via $G\alpha_q$ and $G\alpha_{12/13}$ and PAR3 signalling remains elusive but current theory indicates it acts as a co-factor for PAR1 and PAR4 [21,22]. Furthermore, PAR2 can initiate signalling via G-protein-independent mechanisms that involve the recruitment of β-arrestins (Figure 1) [23,24]. In addition to the co-factoring by PAR3, it is now acknowledged that homologous and heterologous PAR dimerization exists [25,26] and that PARs can transactivate and communicate with other receptors including EGFR and TLR4 [13]. Our increased understanding of the complexity of PAR activation has also lead to the appreciation that PARs signal via multiple pathways and that pathway activation is activator-dependent. Given that many GPCRs can display functional selectivity or biased agonism [27,28], it was proposed that biased agonism at PARs was an explanation for the varying results seen in these and many other studies. Indeed, numerous studies have now shown that this is the case for PAR1 and PAR2 [16,29]. Given our ever increasing knowledge regarding the multiplicity of function for PARs and their activation of signalling pathways, one aspect that has remained a constant is the link between PAR2 and inflammation.

2. PAR2 and the immune system.

PAR2 are expressed on the majority of immune cells spanning both the innate and the adaptive immune system with receptor activity being proposed to be involved in inflammatory-related conditions ranging from arthritis to obesity and metabolic syndrome.

2.1. The innate immune system.

Neutrophils: Within the innate immune system, PAR2 was initially shown to be expressed on neutrophils where its activation lead to an elevation in intracellular Ca^{2+} and promoted neutrophil shape changes with exposure to a PAR2-AP and a neutrophil activator leading to a synergistic increased expression of Macrophage-1 antigen (Mac-1) [30]. In addition PAR2 activation in human neutrophils increases cell motility, elevates the expression of Mac-1 and integrin VLA-4 and stimulates the release of a number of cytokines and glycoproteins known to be required for neutrophil function [31,32]. As to the role of PAR2 in neutrophil function, previous studies have shown that neutrophil protease 3 (PR3) can cleave and activate PAR2 [33]. The action of PR3 via PAR2 was latterly proposed to underlie the non-opsonic phagocytosis of bacteria by

neutrophils [34]. Furthermore, PAR2-dependent neutrophil activation has recently been shown to modulate IFN-γ anti-viral activity in human neutrophils (Table 1) [35].

Eosinophils: Human eosinophils express PAR2 where its activation leads to degranulation, generation of reactive oxygen species and modulation of eosinophil function (Table 1) [36,37]. These findings are important with regard to the activation of PAR2 by proteases released by allergenic organisms and their link to airway diseases including asthma but recent conflicting reports regarding the role of PAR2 in house dust mite allergen-induced lung inflammation [38,39] indicate further investigation is required.

Monocytes: Human blood monocytes have been shown to express PAR2 as well as other PARs with its activation resulting in intracellular Ca^{2+} increases and stimulating the release of interleukin (IL)-1β, IL-6 and IL-8 (Table 1) [40-42]. Recent evidence has revealed elevated PAR2 expression levels in monocytes isolated from patients with rheumatoid arthritis [43] adding further weight to the critical role of PAR2 in this disease. PAR2 monocyte expression has also been implicated in arteriogenesis with studies in PAR2$^{-/-}$ mice highlighting a role in promoting the repair-associated responses in ischemic tissues [44].

Macrophages: Monocyte-derived macrophages express PAR2, the activation of which leads to increases in intracellular Ca^{2+} but prolonged exposure to IL-4 leads to a reduction in PAR2 expression [40]. In addition, human alveolar macrophages and vascular macrophage-derived foam cells also expressing PAR2 (Table 1). Inhibition of PAR2 has recently been demonstrated to limit macrophage infiltration associated with arthritogenesis [45] and PAR2$^{-/-}$ mice are protected from viral infection [46] revealing that PAR2 activation negatively regulates the TLR3 antiviral pathway in macrophages. Furthermore, PAR2 activation is reported to promote an anti-inflammatory M2-like macrophage phenotype in LPS-treated macrophages [47]. PAR2 expression has also been shown in microglial cells, the macrophages of the brain but this will be explored in more detail when introducing PAR2-associated glial function.

Dendritic cells (DC): Initial studies confirmed the expression of PAR2 mRNA in monocyte-derived DCs however studies have failed to detect PAR2 protein expression on the surface of these cells [40] with blood DC also lacking PAR2 protein expression [48]. Despite these early findings, PAR2 activation promotes DC maturation (Table 1) [33,49,50] with proposed roles in DC trafficking to the lymph nodes [51]. PAR2 activity in myeloid DC is also important in the

regulation of allergic airway responses [52] with PAR2 activation by tissue factor suppressing T cell priming [53].

Mast cells (MC): The critical role of MC in innate immunity is well established but recently a role in adaptive immunity has been proposed including in response to infection by a variety of organisms [54]. MC express PAR2 [55-57] with receptor activation in most cases resulting in MC activation and degranulation (Table 1) [56,58] whereas this is not the case for peritoneal MCs [59,60]. Importantly, the serine protease released by MC, mast cell tryptase, is a known activator of PAR2 [61-63] and has been proposed to be involved in numerous inflammatory reactions and diseases [12,13,64] and be a major contributor to postoperative nociception through its ability to activate PAR2 [65,66].

2.2. The adaptive immune system

PAR2 is expressed in several lymphocyte cell types associated with the adaptive immune system but is not expressed in B cells either at the mRNA or protein level [42]. In contrast PAR2 is expressed in both human and mouse T cells [67-69] with a recent study indicating PAR2 expression on human CD4+ and natural killer cells but not CD8+ or ɣδ T cells (Table 1) [42]. With regard to the function of PAR2 on lymphocytes, PAR2 activation has been shown to increase leukocyte rolling and adhesion [70] which was confirmed in PAR2 deficient mice [71,72] with the PAR2–T cell interaction being linked to several inflammatory conditions and diseases [12,13,64].

2.3. What roles does PAR2 play in peripheral inflammatory diseases?

Given the presence of PAR2 on cells both of the innate and adaptive immune system, it is no surprise that evidence exists for PAR2 being involved in inflammatory diseases. As we will focus on central nervous system (CNS) cells and disorders later in the review, here we introduce evidence supporting a role for PAR2 in peripheral inflammatory diseases.

2.3.1. Neurogenic inflammation: Sensory afferent neurones express PAR2 and is largely co-localised with neuropeptides including CGRP and substance P [72,73]. Release of these neuropeptides is known to induce a number of responses including oedema and granulocyte infiltration associated with a site of inflammation. Similar responses were observed following

PAR2 activation thus revealing that PAR2 has a role to play in neurogenic inflammation through the stimulation of neuropeptide release [69,72,73]. This effect is mediated by direct neuronal activation rather than via mast cell activation as mast cell stabilisation did not alter these observed effects [70]. Further to its role in neurogenic inflammation, PAR2 has been implicated in nociceptive signalling with PAR2 activation activating nociceptors and inducing hyperalgesia with the underlying mechanisms proposed to include a reduction in the excitation threshold of certain transient receptor potential (TRP) channels [75,76] and BDNF/trkB/aPKC signalling [77].

2.3.2. Arthritis: Extensive evidence exists to support the role of PAR2 in arthritis with genetic and pharmacological manipulation revealing its role in both rheumatoid and osteoarthritis [78-80]. The use of PAR2 deficient mice emphasised the importance of PAR2 in arthritis. Mice lacking PAR2 show a complete abrogation of disease pathology associated with this condition, such as knee joint swelling, influx of immune cells and hyperemia. Indeed PAR2 expression is up-regulated following induction of chronic inflammation in both disease models thus highlighting that inhibiting PAR2 function is a novel therapeutic target for treating these inflammatory diseases.

2.3.3. Gastrointestinal tract (GIT): PAR2 is widely expressed in the GIT with its activation shown to induce the secretion of electrolytes and fluids as well as regulating motility of colonic cells [81,82]. Given the expression of PAR2 in the GIT and the elevated levels of potential activators in inflammatory bowel disease, PAR2 is proposed to play a role in GIT inflammatory diseases. Indeed, PAR2 appears to have a duality of function in pancreatitis depending on the disease model used and extensive evidence links PAR2 to colitis with PAR2 being shown to regulate endothelial permeability and leukocyte recruitment [83-85].

2.3.4. Skin: PAR2 and its signalling pathways are established to play a role in regulating epithelial function in the skin with PAR2 activating proteases being anchored to the epithelial cell membrane. Matriptase, a known PAR2 activator, has been implicated in subcellular trafficking and cell surface targeting associated with epithelial cell differentiation [86]. The role of PAR2 in skin inflammation is closely related to the function of serine protease inhibitor

Kazal-type 5 (SPINK5), a negative regulator of tissue kallikrein 5 (KLK5) which is a known activator of PAR2 [87,88]. Loss of function mutations in the SPINK5 gene are the cause of the genetic skin disease, Netherton syndrome. Studies in SPINK5/PAR2 double knock out mice have confirmed involvement of KLK5/PAR2 cascades in the early proinflammatory signalling but highlight other non-PAR2 pathways in the inflammatory phenotype of this disease [88-90].

2.3.5. Cancer: Upregulation of PAR2 and its activators has been shown in several cancers including prostate and colon cancer [91,92]. Potential activators, such as kallikreins, are upregulated in colon cancer cells specific to the tumour cells with minimal expression in surrounding tissue [91,93].. In addition, tissue factor (TF) can play an important role in the progression of certain cancers with the TF-VIIa complex being able to cleave and activate PAR2. [64]. Both TF and PAR2 are upregulated in human breast cancer with receptor activation promoting breast cancer angiogenesis [94] and migration [95]. Furthermore, a recent study proposed a novel mechanism by which PAR2 promotes cancer cell migration through the repression of miRNA expression [96].

3. PAR2 and CNS glial cells.

Having outlined the role for PAR2 in the innate and adaptive immune systems in addition to its proposed role in inflammatory-related peripheral diseases, we will now introduce the role of PAR2 on the glial cells of the CNS, namely microglia and astrocytes and highlight their potential physiological and pathophysiological roles.

3.1. Astrocytes

PAR2 is expressed in astrocytes as evidenced using both rodent and human tissue (Table 2) [11,12,97]. Astrocytic PAR2 activation is known to play a number of roles including modulation of synaptic transmission, release of chemokines, cytokines and gliotransmitters and having both a neuroprotective and degenerative effect depending on the CNS disease type examined [11,12]. Astrocytic PAR2 expression was first reported by the Reiser group which revealed that PAR2 activation leads to increases in intracellular Ca^{2+} levels [98]. Since these initial findings, astrocytic PAR2 expression has been shown to be modulated by inflammatory mediators [99] with PAR2 activation inducing the production and release of gliotransmitters including

chemokines and cytokines [100-103], nitric oxide [104] and glutamate [102,105] and increasing the expression of astrocytic αA-crystallin [106]. Indeed, the production of chemokines as gliotransmitters has been proposed to underlie the neuroprotective role shown by PAR2 activation when investigating C2-ceramide-induced cell death [100,101] and kainate-induced neurotoxicity in organotypic hippocampal slice cultures [102]. In both cases, the protective effect of astrocytic PAR2-mediated release of chemokines, which is independent of increases in intracellular Ca^{2+} [100], is mediated via the CXCR2 chemokine receptor as the selective antagonist, reparixin blocks the PAR2-mediated effects [101,102]. Regarding the PAR2-mediated increases in αA-crystallin, it is proposed that the cytoprotective effect is mediated via p38 MAPK and ERK activation [107]. PAR2-induced glutamate release also contributes to the neuroprotective effects of PAR2-APs as the broad spectrum metabotropic glutamate receptor antagonist, LY341495, inhibited the PAR2-mediated neuroprotection [102]. In contrast to other studies, a reduction in p38 MAPK and ERK activity is proposed to underlie the PAR2-mediated neuroprotection as it was mimicked by pharmacologically inhibiting p38 MAPK and ERK activity [102]. In addition to PAR2-induced glutamate release being neuroprotective, it is also responsible for the indirect modulation of hippocampal synaptic transmission following PAR2 activation [105]. Spontaneous action potential frequency is reduced in primary hippocampal cultures following PAR2 activation in an astrocytic-dependent manner as the glial toxin, fluoroacetate, abolishes these effects. Furthermore, PAR2 activation in acute hippocampal slices leads to a long lasting depression of synaptic transmission which is dependent on GluN2B receptor activation via the astrocytic-dependent release of glutamate [105]. Hence a common theme throughout these studies is the integral role that astrocytes play in the effects of PAR2 activation observed within the CNS.

3.2. Microglia

Microglia are the innate immune system of the CNS and similar to its expression in the peripheral innate immune system, PAR2 is expressed in microglia [104, 108], however the role of PAR2 on microglia has been somewhat neglected until recently. In contrast to the neuroprotective role observed with astrocytic PAR2 activation, recent studies have revealed that PAR2 activation of microglia leads to the release of mediators including nitric oxide, reactive oxygen species and inflammatory cytokines (Table 2) [103,109-111] that contribute to neuronal

cell death. In addition, microglial PAR2 activation induces an increased expression of P2X4 receptor [112] and induces the release of brain-derived neurotrophic factor (BDNF) in a time-dependent manner [110,112-114] both of which are suggested to contribute to the role of microglia in neuropathic pain.

3.3. Is PAR2 expression in astrocytes and microglia linked to CNS diseases?

Having highlighted the link between PAR2 on immune cells and peripheral inflammatory diseases, does astrocytic and/or microglial PAR2 play a role in CNS disorders? Evidence indicates that PAR2 is indeed linked to inflammation-related CNS disorders with a duality of function evident depending on whether the primary action of PAR2 is either neuronal (protective) or glial (degenerative) [11,12]. Much of this evidence indicates that under disease conditions, PAR2 expression is increased with this first being shown in organotypic slice cultures where PAR2 expression was transiently increased following experimental ischaemia [115].

3.3.1. Multiple sclerosis: With regard to inflammation-related disorders, PAR2 up-regulation was reported in experimental autoimmune encephalomyelitis (EAE). Increased receptor expression was observed in astrocytes and infiltrating macrophages but not neurons with PAR2 regulating the expression of inflammatory mediators in these cell types [109]. In addition, increased microglial activation was observed in PAR2$^{+/+}$ mice when compared to PAR2$^{-/-}$ mice in the EAE model with reduced neurobehavioural phenotype severity in PAR2$^{-/-}$ mice. Significantly, similar expression changes were observed in post-mortem MS tissue when compared to that of non-MS patients thus implicating glial PAR2 in MS disease progression (Table 2).

3.3.2. *Alzheimer's disease:* In addition to the link to MS, PAR2 expression was also altered in frontal lobe tissue taken from patients suffering from Alzheimer's disease (AD) [110]. Whilst total PAR2 mRNA levels were significantly reduced in AD tissue, PAR2 immunoreactivity was more evident in glial cells of AD brains with a concomitant elevation of several pro-inflammatory genes. A role for PAR2 in AD pathology was supported by experiments examining the consequence of PAR2 activation on macrophages and astrocytes against β-

amyloid-induced neurotoxicity.   Conditioned media from PAR2-activated macrophages exacerbated β-amyloid-induced neurotoxicity in human foetal neurons whereas conditioned media from astrocytes was without effect. In contrast, it should be noted that neuronal PAR2 activation impaired β-amyloid-induced neurotoxicity.However, using an animal model of AD to examine the early stages of AD rather than the late stages as investigated with the post-mortem tissue, PAR2 mRNA levels were significantly increased in TgCRND8 mice as was the astrocytic marker, GFAP. Finally, investigating the toxicity of β-amyloid in PAR2$^{-/-}$ mice revealed reduced glial activation in PAR2$^{-/-}$ mice and that neurobehavioural phenotypes, as shown using the Morris water maze, were more severe in PAR2$^{+/+}$ mice when compared to PAR2$^{-/-}$ mice. These data indicate that PAR2 glial cell activation contributes to β-amyloid-induced neurotoxicity and AD via induction of inflammatory pathways within the CNS but the role of neuronal PAR2 highlights the complex role of PAR2 in AD pathology (Table 2).

3.3.3. *Parkinson's disease:* Another CNS disorder that has neuroinflammatory links is Parkinson's disease (PD) and previous evidence indicates a role for PAR1 in its progression [116,117], however a recent study indicates that PAR2 may also play a role [118]. PAR2 expression was increased in the substantia nigra of MPTP-treated rats with PAR2 blockade leading to reduced α-synuclein synthesis and reduced the MPTP-induced motor dysfunction. Importantly, NF-ƙB signalling was reduced following PAR2 blockade and given the connection between NF-ƙB and inflammatory cytokine production, this study suggests that the PAR2-NF-ƙB signalling pathway may play a role in PD disease progression. However, this study did not determine in which cell types the PAR2 up-regulation was observed so whether PAR2 glial expression was responsible for these effects cannot be confirmed.

3.3.4. Non-glial PAR2 and CNS diseases: It should be noted that in contrast to the detrimental role of glial PAR2 activation outlined above, PAR2 activation has also been shown to be neuroprotective in models and post-mortem tissue from patients who have suffered a stroke [119] or from HIV-associated dementia [120]. In both these cases, the PAR2-mediated neuroprotection was suggested to be via increased PAR2 expression and activation specifically in neurons, thus highlighting the duality of function associated with PAR2 depending on cell type location [11].

4. Is PAR2 a realistic therapeutic target for the treatment of CNS disorders?

Given the evidence that glial PAR2 activation is involved in disease progression in several inflammation-related CNS disorders, the development of PAR2 antagonists for the treatment of these diseases could be beneficial as a novel therapeutic strategy. However, despite the progress made with PAR1 antagonists [12,28], the development of PAR2 antagonists has proven difficult, especially small molecule antagonists that have the ability to cross the blood brain barrier (BBB) as would be required for the treatment of CNS disorders. ENMD1068 is a PAR2 antagonist that has been shown to reduce joint inflammation in models of rheumatoid arthritis (Figure 2) [121] but its lack of potency has prevented its widespread use (Figure 2). Despite the apparent difficulty in developing PAR2 antagonists, a number of compounds that inhibit PAR2 Ca^{2+} signalling have emerged that are purported to act as biased antagonists.

A PAR2-specific peptoid, GB88, was initially described as a surmountable competitive antagonist of PAR2-AP but a non-competitive antagonist of trypsin [122]. So far studies have shown that GB88 does not elicit increases in intracellular Ca^{2+} but blocks increases in intracellular Ca^{2+} initiated by PAR2 activation in a number of preparations (Figure 2) [122-125]. It has since emerged that modulation of PAR2 activity is pathway specific [126]. Despite its ability to block PAR2 Ca^{2+} signalling, GB88 is able to promote PAR2 β-arrestin interaction and stimulate ERK signalling. An unusual aspect of GB88's action is that despite it stimulating these interactions and signalling, it does not mediate receptor internalisation. The therapeutic potential for PAR2 antagonism has been highlighted with GB88 preventing several inflammatory diseases including experimental colitis, collagen-induced arthritis and diet-induced obesity [123,124,126]. Whilst these studies indicate that GB88 may prove useful in the treatment of peripheral inflammatory diseases, our preliminary data indicates that GB88 does not cross the BBB (T. Bushell, unpublished observations) and therefore would not be useful in the treatment of inflammation-related CNS disorders. However it may prove useful to further our understanding of PAR2 function in CNS diseases using in vitro methods. The use of GB88 for selective activation of the ERK pathway, which many studies show to be neuroprotective, may aid in the

design of small molecule analogues which act as biased agonists for this pathway but are able to cross the BBB.

Another proposed PAR2 antagonist is the cell-penetrating pepducins is P2pal-18S pepducin (Figure 2) [127]. P2pal-18S pepducin is based on the third intracellular loop of PAR2 and is proposed to specifically target PAR2 signalling by interfering with receptor-G-protein coupling. Indeed, it blocks PAR2 signalling induced by trypsin and mast cell tryptase and inhibits PAR2-dependent inflammatory signalling in mouse models of paw oedema and mast cell driven inflammation [127]. However it has been suggested that P2pal-18S pepducin may act in a similar manner to GB88 as its action does not prevent trypsin-mediated PAR2 interactions with β-arrestin [28]. Whether it specifically blocks the PAR2 Ca^{2+} signalling pathway whilst other pathway activation remains intact is as yet unknown. How useful this method of inhibiting PAR2 activation will be for the treatment of CNS disorders remains to be elucidated. Despite systemic administration of P2pal-18S pepducin having beneficial effects in mouse models of peripheral inflammation, it does not cross the BBB.. Finally and as outlined above, it should be noted that PAR2 activation plays a neuroprotective role under certain conditions [102,119,120]. The development of PAR2 agonists that penetrate the CNS would allow further investigation into this. Recently developed compounds including AC264613 may fit the bill (Figure 2) [128-130] but caution needs to be taken given the consequence that PAR2 activation has on immune cells and their well-established role in many CNS disorders.

5. Conclusion

There is ever increasing evidence that PAR2 plays a significant role in inflammatory diseases both in the periphery and in the CNS. There is a clear similarity between PAR2 expression and activation on cells of the immune system and those cell types that are proposed to play a role within the CNS, astrocytes and microglia. Activation of both leads to the release of pro-inflammatory cytokines which contributes to the detrimental aspects of diseases ranging from arthritis and colitis through to MS. Recent studies indicate that PAR2 antagonism has therapeutic potential when targeting peripheral inflammatory diseases but the verification that PAR2 antagonism is a viable target for CNS diseases remains elusive. Genetic studies indicate that PAR2 antagonism would be useful, for example in MS, as PAR2$^{-/-}$ mice have a reduced

neurobehavioural phenotype in the EAE model. A major factor that is limiting progress is the lack of available small molecule antagonists capable of crossing the BBB and therefore be effective in treating these CNS disorders. Furthermore, given that studies have also revealed a neuroprotective and an anti-inflammatory role with regard to PAR2 activation, it also needs to be considered whether PAR2 agonists may prove beneficial in a disease-dependent manner. Indeed, recent developments regarding biased agonism and antagonism in relation to PAR signalling may indicate that developing compounds that selectively activate beneficial signalling pathways may be the way forward for targeting PAR2. If so, careful consideration will be required to the potential adverse effects elicited as a consequence of central and peripheral PAR2 activation. Nonetheless, clear evidence exists for PAR2 being a target in a variety of inflammation-related diseases and further development of novel compounds will fully elucidate its therapeutic potential.

References.

[1] Rasmussen UB, Vouret-Craviari V, Jallat S, et al. cDNA cloning and expression of a hamster alpha-thrombin receptor coupled to Ca^{2+} mobilization. FEBS Lett 1991; 288: 123–128.

[2] Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 1991; 64: 1057–1068.

[3] Nystedt S, Emilsson K, Wahlestedt C, Sundelin J. Molecular cloning of a potential protease activated receptor. Proc Natl Acad Sci USA 1994; 91: 9208–9212.

[4] Bohm SK, Kong WY, Bromme D, et al. Molecular cloning, expression and potential functions of the human protease-activated receptor-2. Biochem J 1996; 314:1009–1016.

[5] Connolly AJ, Ishihara H, Kahn ML, Farese RV Jr, Coughlin SR. Role of the thrombin receptor in development and evidence for a second receptor. Nature (Lond) 1996; 381:516–519.

[6] Ishihara H, Connolly AJ, Zeng D, et al. Protease-activated receptor 3 is a second thrombin receptor in humans. Nature (Lond) 1997; 386:502–506.

[7] Kahn ML, Zheng YW, Huang W, et al. A dual thrombin receptor system for platelet activation. Nature (Lond) 1998; 394: 690–694.

[8] Xu WF, Andersen H, Whitmore TE, et al. Cloning and characterization of human protease activated receptor 4. Proc Natl Acad Sci USA 1998; 95: 6642–6646.

[9] Zhang C, Srinivasan Y, Arlow DH, et al. High-resolution crystal structure of human protease-activated receptor 1. Nature 2012; 492: 387-92.

[10] French SL, Arthur JF, Tran HA, Hamilton JR. Approval of the first protease-activated receptor antagonist: Rationale, development, significance, and considerations of a novel anti-platelet agent. Blood Rev. 2014 Nov 6. pii: S0268-960X(14)00088-5.

[11] Bushell TJ. The emergence of protease-activated receptor-2 as a novel target for the treatment of inflammation-related CNS disorders. J. Physiol. 2007; 581: 7–16.

[12] Ramachandran R, Noorbakhsh F, Defea K, Hollenberg MD. Targeting protease-activated receptors: therapeutic potential and challenges. Nat Rev Drug Discov 2012; 11: 69–86.

[13] Gieseler F, Ungefroren H, Settmacher U, Hollenberg MD, Kaufmann R. Protease-activated receptors (PARs) - focus on receptor-receptor-interactions and their physiological and pathophysiological impact. Cell Commun Signal. 2013; 11: 86.

[14] Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell 2005; 120: 303–313.

[15] Trivedi V, Boire A, Tchernychev B, et al. Platelet matrix metalloprotease-1 mediates thrombogenesis by activating PAR1 at a cryptic ligand site. Cell 2009; 137: 332–343.

[16] Russo A, Soh UJ, Paing MM, Arora P, Trejo J. Caveolae are required for protease-selective signaling by protease-activated receptor-1. Proc Natl Acad Sci U S A 2009; 106: 6393–6397.

[17] Mosnier LO, Sinha RK, Burnier L, Bouwens EA, Griffin JH. Biased agonism of protease-activated receptor 1 by activated protein C caused by noncanonical cleavage at Arg46. Blood 2012; 120: 5237–5246.

[18] Schuepbach RA, Madon J, Ender M, Galli P, Riewald M. Protease activated receptor-1 cleaved at R46 mediates cytoprotective effects. J Thromb Haemost 2012; 10: 1675–1684.

[19] Mihara K, Ramachandran R, Renaux B, Saifeddine M, Hollenberg MD. Neutrophil elastase and protease-3 trigger G-protein biased signaling through protease activated receptor-1 (PAR1). J Biol Chem 2013; 288: 32979–32990.

[20] Stavenuiter F, Mosnier LO. Noncanonical PAR3 activation by factor Xa identifies a novel pathway for Tie2 activation and stabilization of vascular integrity. Blood 2014; 124(23):3480-9.

[21] Nakanishi-Matsui M, Zheng YW, Sulciner DJ, Weiss EJ, Ludeman MJ, Coughlin SR. PAR3 is a cofactor for PAR4 activation by thrombin. Nature 2000; 404: 609–613.

[22] Soh UJ, Dores MR, Chen B, Trejo J. Signal transduction by protease-activated receptors. Br J Pharmacol. 2010; 160: 191-203.

[23] DeFea K. Beta-arrestins and heterotrimeric G-proteins: collaborators and competitors in signal transduction. Br J Pharmacol. 2008; 153 Suppl 1: S298-309.

[24] Grimsey N, Lin H, Trejo J. Endosomal signaling by protease-activated receptors. Methods Enzymol. 2014; 535: 389-401.

[25] Cunningham MR, McIntosh KA, Pediani JD, et al. Novel role for protease-activated receptor 2 (PAR2) in membrane trafficking of protease-activated receptor 4 (PAR4). J Biol Chem. 2012; 287:16656-69.

[26] Lin H, Liu AP, Smith TH, Trejo J. Cofactoring and dimerization of protease-activated receptors. Pharmacol Rev. 2013; 65: 1198-213.

[27] Kenakin TP. Biased signalling and allosteric machines: new vistas and challenges for drug discovery. Br J Pharmacol. 2012; 165: 1659-69.

[28] Wisler JW, Xiao K, Thomsen AR, Lefkowitz RJ. Recent developments in biased agonism. Curr Opin Cell Biol. 2014; 27: 18-24.

[29] Hollenberg MD, Mihara K, Polley D, et al. Biased signalling and protease-activated receptors (PARs): targeting inflammatory disease. Br J Pharmacol. 2014; 171: 1180-94.

[30] Howells GL, Macey MG, Chinni C, et al. Protease-activated receptor-2: expression by human neutrophils. J. Cell Sci. 1997; 110: 881–887.

[31] Shpacovitch VM, Varga G, Strey A, et al. Agonists of protease-activated receptor-2 modulate human neutrophil cytokine secretion, expression of cell adhesion molecules, and migration within 3-D collagen lattices. J. Leukoc. Biol. 2004; 76, 388–398.

[32] Wang H, He S. Induction of lactoferrin and IL-8 release from human neutrophils by tryptic enzymes via protease activated receptor-2. Cell Biol. Int. 2006; 30: 688–697.

[33] Csernok E, Ai M, Gross WL, et al. Wegener autoantigen induces maturation of dendritic cells and licenses them for Th1 priming via the protease-activated receptor-2 pathway. Blood. 2006; 107: 4440-8.

[34] Kim YC, Shin JE, Lee SH, et al. Membrane-bound protease 3 and PAR2 mediate phagocytosis of non-opsonized bacteria in human neutrophils. Mol Immunol. 2011; 48: 1966-74.

[35] Feld M, Shpacovitch V, Ehrhardt C, et al. Protease-activated receptor-2 agonist activates anti-influenza mechanisms and modulates IFNγ-induced antiviral pathways in human neutrophils. Biomed Res Int. 2013; 2013:879080.

[36] Miike S, McWilliam AS, Kita, H. Trypsin induces activation and inflammatory mediator release from human eosinophils through protease-activated receptor-2. J. Immunol. 2001; 167: 6615–6622.

[37] Bolton, S. J., McNulty, C. A., Thomas, R. J., Hewitt, C. R., Wardlaw, A. J. Expression of and functional responses to protease-activated receptors on human eosinophils. J. Leukoc. Biol. 2003; 74: 60–68.

[38] de Boer JD, Van't Veer C, Stroo I, et al. Protease-activated receptor-2 deficient mice have reduced house dust mite-evoked allergic lung inflammation. Innate Immun. 2013; 20: 618-625.

[39] Post S, Heijink IH, Petersen AH, de Bruin HG, van Oosterhout AJ, Nawijn MC. Protease-activated receptor-2 activation contributes to house dust mite-induced IgE responses in mice. PLoS One. 2014; 9: e91206.

[40] Colognato R, Slupsky,JR, Jendrach M, Burysek L, Syrovets T, Simmet T. Differential expression and regulation of protease- activated receptors in human peripheral monocytes and monocyte-derived antigen-presenting cells. Blood 2003; 102: 2645–2652.

[41] Johansson U, Lawson C, Dabare M, et al. Human peripheral blood monocytes express protease receptor-2 and respond to receptor activation by production of IL-6, IL-8, and IL-1β. J. Leukoc. Biol. 2005; 78: 967–975.

[42] López ML, Soriano-Sarabia N, Bruges G et al. Expression pattern of protease activated receptors in lymphoid cells. Cell Immunol. 2014: 288: 47-52.

[43] Crilly A, Burns E, Nickdel MB, et al. PAR(2) expression in peripheral blood monocytes of patients with rheumatoid arthritis. Ann Rheum Dis. 2012; 71: 1049-54.

[44] van den Hengel LG, Hellingman AA, Nossent AY, et al. Protease-activated receptor (PAR)2, but not PAR1, is involved in collateral formation and anti-inflammatory monocyte polarization in a mouse hind limb ischemia model. PLoS One. 2013; 8: e61923.

[45] Lohman RJ, Cotterell AJ, Barry GD, et al. An antagonist of human protease activated receptor-2 attenuates PAR2 signaling, macrophage activation, mast cell degranulation, and collagen-induced arthritis in rats. FASEB J. 2012; 26: 2877-87.

[46] Nhu QM, Shirey K, Teijaro JR, et al. Novel signaling interactions between proteinase-activated receptor 2 and Toll-like receptors in vitro and in vivo. Mucosal Immunol. 2010: 3: 29-39.

[47] Nhu QM, Shirey KA, Pennini ME, Stiltz J, Vogel SN. Proteinase-activated receptor 2 activation promotes an anti-inflammatory and alternatively activated phenotype in LPS-stimulated murine macrophages. Innate Immun. 2012: 18: 193-203.

[48] Johansson U, Lawson C, Dabare M, et al. Human peripheral blood monocytes express protease receptor-2 and respond to receptor activation by production of IL-6, IL-8, and IL-1β. J. Leukoc. Biol. 2005; 78: 967–975.

[49] Fields RC, Schoenecker JG, Hart JP, Hoffman MR, Pizzo SV, Lawson JH. Protease-activated receptor-2 signaling triggers dendritic cell development. Am J Pathol. 2003; 162: 1817-22.

[50] Jiang B, Grage-Griebenow E, Csernok E, et al. The role of protease 3 (PR3) and the protease-activated receptor-2 (PAR-2) pathway in dendritic cell (DC) maturation of human-DC-like monocytes and murine DC. Clin Exp Rheumatol. 2010; 28(Suppl 57):56-61.

[51] Ramelli G, Fuertes S, Narayan S, Busso N, Acha-Orbea H, So A. Protease-activated receptor 2 signalling promotes dendritic cell antigen transport and T-cell activation in vivo. Immunology. 2010; 129: 20-7.

[52] Lewkowich IP, Day SB, Ledford JR, et al. Protease-activated receptor 2 activation of myeloid dendritic cells regulates allergic airway inflammation. Respir Res. 2011; 12: 122.

[53] Shrivastava S, Ma L, Tham el-L, H McVey J, Chen D, Dorling A. Protease-activated receptor-2 signalling by tissue factor on dendritic cells suppresses antigen-specific CD4+ T-cell priming. Immunology. 2013; 139: 219-26.

[54] Urb M, Sheppard DC. The role of mast cells in the defence against pathogens. PLoS Pathog. 2012; 8: e1002619.

[55] D'Andrea MR, Rogahn CJ, Andrade-Gordon P. Localization of protease-activated receptors-1 and -2 in human mast cells: indications for an amplified mast cell degranulation cascade. Biotech. Histochem. 2000; 75: 85–90.

[56] He SH, Xie H, Fu YL. Activation of human tonsil and skin mast cells by agonists of protease activated receptor-2. Acta Pharmacol Sin. 2005; 26: 568-74.

[57] Moormann C, Artuc M, Pohl E, et al.W., Luger, T. A., Steinhoff, M. (2006) Functional characterization and expression analysis of the protease-activated receptor-2 in human cutaneous mast cells. J. Invest. Dermatol. 2006; 126, 746–755.

[58] Dugina TN, Kiseleva EV, Glusa E, Strukova SM. Activation of mast cells induced by agonists of protease-activated receptors under normal conditions and during acute inflammation in rats. Eur J Pharmacol. 2003; 471: 141-7.

[59] Nishikawa H, Kawabata A, Kuroda R, Nishida M, Kawai K. Characterization of protease-activated receptors in rat peritoneal mast cells. Jpn. J. Pharmacol. 2000; 82, 74–77.

[60] Stenton GR, Nohara O, Dery RE, et al. Protease-activated receptor (PAR)-1 and -2 agonists induce mediator release from mast cells by pathways distinct from PAR-1 and PAR-2. J. Pharmacol. Exp. Ther. 2002; 302, 466–474.

[61] Molino M, Barnathan ES, Numerof R, et al. Interactions of mast cell tryptase with thrombin receptors and PAR-2. J Biol Chem. 1997; 272: 4043-9.

[62] Compton SJ, Renaux B, Wijesuriya SJ, Hollenberg MD. Glycosylation and the activation of protease-activated receptor 2 (PAR(2)) by human mast cell tryptase. Br J Pharmacol. 2001; 134: 705-18.

[63] Ribatti D, Ranieri G. Tryptase, a novel angiogenic factor stored in mast cell granules. Exp Cell Res. 2015; 332: 157-162.

[64] Rothmeier AS, Ruff W. Protease-activated receptor 2 signaling in inflammation. Semin Immunopathol. 2012; 34: 133-49.

[65] Oliveira SM, Silva CR, Ferreira J. Critical role of protease-activated receptor 2 activation by mast cell tryptase in the development of postoperative pain. Anesthesiology. 2013; 118: 679-90.

[66] Chatterjea D, Martinov T. Mast cells: versatile gatekeepers of pain. Mol Immunol. 2015; 63: 38-44.

[67] Mari B, Guerin S, Far DF, et al. Thrombin and trypsin-induced Ca^{2+} mobilization in human T cell lines through interaction with different protease-activated receptors. FASEB J. 1996; 10: 309–316.

[68] Bar-Shavit R, Maoz M, Yongjun Y, Groysman M, Dekel I, Katzav S. Signaling pathways induced by protease-activated receptors and integrins in T cells. Immunology 2002; 105: 35–46.

[69] Li T, He S. Induction of IL-6 release from human T cells by PAR-1 and PAR-2 agonists. Immunol. Cell Biol. 2006; 84: 461–466.

[70] Vergnolle N. Protease-activated receptor-2-activating peptides induce leukocyte rolling, adhesion, and extravasation in vivo. J Immunol. 1999; 163: 5064-9.

[71] Lindner JR, Kahn ML, Coughlin SR, et al. Delayed onset of inflammation in protease-activated receptor-2-deficient mice. J Immunol. 2000; 165: 6504-10.

[72] Lim SY, Tennant GM, Kennedy S, Wainwright CL, Kane KA. Activation of mouse protease-activated receptor-2 induces lymphocyte adhesion and generation of reactive oxygen species. Br J Pharmacol. 2006; 149: 591-9.

[73] Vergnolle N. The enteric nervous system in inflammation and pain: the role of protease-activated receptors. Can J Gastroenterol. 2003; 17: 589-92.

[74] Steinhoff M, Vergnolle N, Young SH, et al. Agonists of protease-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med. 2000; 6: 151-8.

[75] Amadesi S, Cottrell GS, Divino L, et al. Protease-activated receptor 2 sensitizes TRPV1 by protein kinase Cepsilon- and A-dependent mechanisms in rats and mice. J Physiol. 2006; 575: 555-71.

[76] Poole DP, Amadesi S, Veldhuis NA, et al. Protease-activated receptor 2 (PAR2) protein and transient receptor potential vanilloid 4 (TRPV4) protein coupling is required for sustained inflammatory signaling. J Biol Chem. 2013; 288: 5790-802.

[77] Tillu DV, Hassler SN, Burgos-Vega CC, et al. Protease activated receptor 2 (PAR2) activation is sufficient to induce the transition to a chronic pain state. Pain, 2015; in press.

[78] Ferrell WR, Lockhart JC, Kelso EB, et al. Essential role for protease-activated receptor-2 in arthritis. J Clin Invest. 2003; 111: 35-41.

[79] Ferrell WR, Kelso EB, Lockhart JC, Plevin R, McInnes IB. Protease-activated receptor 2: a novel pathogenic pathway in a murine model of osteoarthritis. Ann Rheum Dis. 2010; 69: 2051-4.

[80] McIntosh KA, Plevin R, Ferrell WR, Lockhart JC. The therapeutic potential of protease-activated receptors in arthritis. Curr Opin Pharmacol. 2007; 7: 334-8.

[81] Kong W, McConalogue K, Khitin L M, et al. Luminal trypsin may regulate enterocytes through protease-activated receptor 2. Proc. Natl. Acad. Sci. USA 1997; 94: 8884–8889.

[82] Sekiguchi F, Hasegawa N, Inoshita K, et al. Mechanisms for modulation of mouse gastrointestinal motility by protease-activated receptor (PAR)-1 and -2 in vitro. Life Sci. 2006; 78: 950-7.

[83] Cenac N, Garcia-Villar R, Ferrier L, et al. Protease-activated receptor-2-induced colonic inflammation in mice: possible involvement of afferent neurons, nitric oxide, and paracellular permeability. J Immunol. 2003; 170: 4296–4300.

[84] Hansen KK, Sherman PM, Cellars L, et al. A major role for proteolytic activity and protease-activated receptor-2 in the pathogenesis of infectious colitis. Proc Natl Acad Sci. U S A. 2005; 102: 8363-8.

[85] Hyun E, Andrade-Gordon P, Steinhoff M, Vergnolle N. Protease-activated receptor-2 activation: a major actor in intestinal inflammation. Gut. 2008; 57: 1222-9.

[86] Antalis TM, Buzza MS, Hodge KM, Hooper JD, Netzel-Arnett S. The cutting edge: membrane-anchored serine protease activities in the pericellular microenvironment. Biochem J. 2010; 428: 325–346.

[87] Stefansson K, Brattsand M, Roosterman D, et al. Activation of protease-activated receptor-2 by human kallikrein-related peptidases. J Invest Dermatol. 2008; 128: 18-25.

[88] Briot A, Lacroix M, Robin A, Steinhoff M, Deraison C, Hovnanian A. PAR2 inactivation inhibits early production of TSLP, but not cutaneous inflammation, in Netherton syndrome adult mouse model. J Invest Dermatol. 2010; 130: 2736-42.

[89] Sales KU, Masedunskas A, Bey AL, et al. Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. Nat Genet. 2010; 42: 676-83.

[90] Frateschi S, Camerer E, Crisante G, et al. PAR2 absence completely rescues inflammation and ichthyosis caused by altered CAP1/Prss8 expression in mouse skin. Nat Commun 2011; 2: 161.

[91] Gratio V, Loriot C, Virca GD, et al. Kallikrein-related peptidase 14 acts on protease-activated receptor 2 to induce signaling pathway in colon cancer cells. Am J Pathol. 2011; 179: 2625-36.

[92] Ramsay AJ, Dong Y, Hunt ML, et al. Kallikrein-related peptidase 4 (KLK4) initiates intracellular signaling via protease-activated receptors (PARs). KLK4 and PAR-2 are co-expressed during prostate cancer progression. J Biol Chem. 2008; 283: 12293-304.

[93] Chung H, Hamza M, Oikonomopoulou K, et al. Kallikrein-related peptidase signaling in colon carcinoma cells: targeting protease-activated receptors. Biol Chem. 2012; 393: 413-20.

[94] Schaffner F, Versteeg HH, Schillert A, et al. Cooperation of tissue factor cytoplasmic domain and PAR2 signaling in breast cancer development. Blood. 2010; 116: 6106-13.

[95] Su S, Li Y, Luo Y, et al. Protease-activated receptor 2 expression in breast cancer and its role in breast cancer cell migration. Oncogene. 2009; 28: 3047-57.

[96] Yang L, Ma Y, Han W, Li W, Cui L, Zhao X, Tian Y, Zhou Z, Wang W, Wang H. Proteinase-Activated Receptor 2 Promotes Cancer Cell Migration Through RNA Methylation-Mediated Repression of MiR-125b. J Biol Chem. 2015 doi: 10.1074/jbc.M115.667717.

[97] Wang H, Ubl JJ, Reiser G. Four subtypes of protease-activated receptors, co-expressed in rat astrocytes, evoke different physiological signaling. Glia 2002; 37: 53–63.

[98] Ubl JJ, Vohringer C, Reiser G. Co-existence of two types of $[Ca^{2+}]$i-inducing protease-activated receptors (PAR-1 and PAR-2) in rat astrocytes and C6 glioma cells. Neuroscience 1998; 86: 597–609.

[99]. Sokolova E, Aleshin S, Reiser G. Expression of protease-activated receptor (PAR)-2, but not other PARs, is regulated by inflammatory cytokines in rat astrocytes. Neurochem Int. 2012; 60: 276-85.

[100] Wang Y., Luo W. and Reiser G. Activation of protease-activated receptors in astrocytes evokes a novel neuroprotective pathway through release of chemokines of the growth-regulated oncogene/cytokine-induced neutrophil chemoattractant family. Eur. J. Neurosci. 2007; 26: 3159–3168.

[101] Wang Y., Luo W. and Reiser G. Protease-activated receptor-1and -2 induce the release of chemokine GRO/CINC-1 from rat astrocytes via differential activation of JNK isoforms, evoking multiple protective pathways in brain. Biochem. J. 2007; 401: 65–78.

[102] Greenwood SM, Bushell TJ. Astrocytic activation and an inhibition of MAP kinases are required for protease-activated receptor-2-mediated protection from neurotoxicity. J Neurochem. 2010; 113: 1471–1480.

[103] Zeng X, Zhang S, Xu L, Yang H, He S. Activation of protease-activated receptor 2-mediated signaling by mast cell tryptase modulates cytokine production in primary cultured astrocytes. Mediators Inflamm. 2013; 2013: 140812.

[104] Park GH, Jeon SJ, Ko HM, et al. Activation of microglial cells via protease-activated receptor 2 mediates neuronal cell death in cultured rat primary neuron. Nitric Oxide. 2010; 22: 18-29.

[105] Gan J, Greenwood SM, Cobb SR, Bushell TJ. Indirect modulation of neuronal excitability and synaptic transmission in the hippocampus by activation of protease-activated receptor-2. Br J Pharmacol. 2011; 163: 984-94.

[106] Li R, Rohatgi T, Hanck T, Reiser G. Alpha A-crystallin and alpha B-crystallin, newly identified interaction proteins of protease-activated receptor-2, rescue astrocytes from C2-ceramide- and staurosporine-induced cell death. J Neurochem. 2009; 110: 1433-44.

[107] Li R, Zhu Z, Reiser G. Specific phosphorylation of αA-crystallin is required for the αA-crystallin-induced protection of astrocytes against staurosporine and C2-ceramide toxicity. Neurochem Int. 2012; 60: 652-8.

[108] Balcaitis S, Xie Y, Weinstein JR, et al. Expression of protease-activated receptors in mouse microglial cells. Neuroreport. 2003; 14: 2373-7.

[109] Noorbakhsh F, Tsutsui S, Vergnolle N, et al. Protease-activated receptor 2 modulates neuroinflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. J Exp Med. 2006; 203: 425-35.

[110] Afkhami-Goli A, Noorbakhsh F, Keller AJ, et al. Protease-activated receptor-2 exerts protective and pathogenic cell type-specific effects in Alzheimer's disease. J Immunol. 2007; 179: 5493-503.

[111] Chen CW, Chen QB, Ouyang Q, et al. Transient early neurotrophin release and delayed inflammatory cytokine release by microglia in response to PAR-2 stimulation. J Neuroinflammation. 2012; 9: 142.

[112] Zhang S, Zeng X, Yang H, Hu G, He S. Mast cell tryptase induces microglia activation via protease-activated receptor 2 signaling. Cell Physiol Biochem. 2012; 29: 931-40.

[113] Yuan H, Zhu X, Zhou S, et al. Role of mast cell activation in inducing microglial cells to release neurotrophin. J Neurosci Res. 2010; 88: 1348-54.

[114] Fan Y, Chen J, Ye J, Yan H, Cai Y. Protease-activated receptor 2 modulates corticotropin releasing hormone-induced brain-derived neurotrophic factor release from microglial cells. Cell Biol Int. 2014; 38: 92-6.

[115] Striggow F, Riek-Burchardt M, Kiesel A, et al. Four different types of protease-activated receptors are widely expressed in the brain and are up-regulated in hippocampus by severe ischemia. Eur. J. Neurosci. 2001; 14: 595–608.

[116] Ishida Y, Nagai A, Kobayashi S, Kim SU. Upregulation of protease-activated receptor-1 in astrocytes in Parkinson disease: astrocyte-mediated neuroprotection through increased levels of glutathione peroxidase. J Neuropathol Exp Neurol. 2006; 65: 66-77.

[117] Cannon JR, Keep RF, Schallert T, Hua Y, Richardson RJ, Xi G. Protease-activated receptor-1 mediates protection elicited by thrombin preconditioning in a rat 6-hydroxydopamine model of Parkinson's disease. Brain Res. 2006; 1116: 177-86.

[118] Liu P, Sun L, Zhao XL, Zhang P, Zhao XM, Zhang J. PAR2-mediated epigenetic upregulation of α-synuclein contributes to the pathogenesis of Parkinson′s disease. Brain Res. 2014; 1565: 82-9.

[119] Jin G, Hayashi T, Kawagoe J, et al. Deficiency of PAR-2 gene increases acute focal ischemic brain injury. J. Cereb. Blood Flow Metab. 2005; 25: 302-313.

[120] Noorbakhsh F, Vergnolle N, McArthur JC, et al. Protease-activated receptor-2 induction by neuroinflammation prevents neuronal death during HIV infection. J Immunol. 2005; 174: 7320-9.

[121] Kelso EB, Lockhart JC, Hembrough T, et al. Therapeutic promise of protease-activated receptor-2 antagonism in joint inflammation. J Pharmacol Exp Ther. 2006; 316: 1017-24.

[122] Suen JY, Barry GD, Lohman RJ, et al. Modulating human protease activated receptor 2 with a novel antagonist (GB88) and agonist (GB110). Br J Pharmacol. 2012; 165: 1413–1423.

[123] Lohman RJ, Cotterell AJ, Barry GD, et al. An antagonist of human protease activated receptor-2 attenuates PAR2 signaling, macrophage activation, mast cell degranulation, and collagen-induced arthritis in rats. FASEB J. 2012; 26:
2877–2887.

[124] Lohman RJ, Cotterell AJ, Suen JY, et al. Antagonism of protease activated receptor 2 protects against experimental colitis. J Pharmacol Exp Ther. 2012; 340: 256–265.

[125] Suen JY, Cotterell A, Lohman RJ, et al. Pathway-selective antagonism of protease activated receptor 2. Br J Pharmacol. 2014; 171: 4112-24.

[126] Lim J, Iyer A, Liu L, et al. Diet-induced obesity, adipose inflammation, and metabolic dysfunction correlating with PAR2 expression are attenuated by PAR2 antagonism. FASEB J. 2013; 27: 4757-67.

[127] Sevigny LM, Zhang P, Bohm A, et al. Interdicting protease-activated receptor-2-driven inflammation with cell-penetrating pepducins. Proc Natl Acad Sci U S A. 2011; 108: 8491-6.

[128] Gardell LR, Ma JN, Seitzberg JG, et al. Identification and characterization of novel small-molecule protease-activated receptor 2 agonists. J Pharmacol Exp Ther. 2008; 327: 799-808.

[129] Seitzberg JG, Knapp AE, Lund BW, et al. Discovery of potent and selective small-molecule PAR-2 agonists. J Med Chem. 2008; 51: 5490-3.

[130] Ma JN, Burstein ES. The protease activated receptor 2 (PAR2) polymorphic variant F240S constitutively activates PAR2 receptors and potentiates responses to small-molecule PAR2 agonists. J Pharmacol Exp Ther. 2013; 347: 697-704.

Figure legends.

Figure 1. PAR2 activation leads to the activation of multiple signalling pathways.

PAR2 N terminal cleavage by serine proteases leads to the exposure of a tethered ligand, which binds to the second extracellular loop and activates signalling pathways either via multiple G-protein subtypes or β-arrestin-dependent signalling. With the recent discovery of PAR2 biased signalling, pathway activation will depend on the molecule by which it is activated.

Figure 2. PAR2 inhibition or selective activation of signalling pathways via biased agonism may prove to be beneficial in inflammation-related disorders.

A + B) P2pal-18S pepducin and ENMD1068 are PAR2 inhibitors that have therapeutic potential in peripheral diseases but have as yet to be tested in CNS disorders. C) GB88 is a proposed biased antagonist as it activates PAR2 mediated ERK signalling but blocks PAR2-mediated Ca^{2+} signalling. D) AC264613 is a PAR2 small molecule agonist that may be useful in determining the neuroprotective properties of PAR2 activation under certain conditions.

Table legends

Table 1. Activation of PAR2 in immune cells leads to the activation of a number of immune responses.

Table 2. Activation of PAR2 in CNS glial cells leads to the activation of a variety of signalling pathways and is proposed to be involved in the progression of multiple sclerosis and Alzheimer's disease.
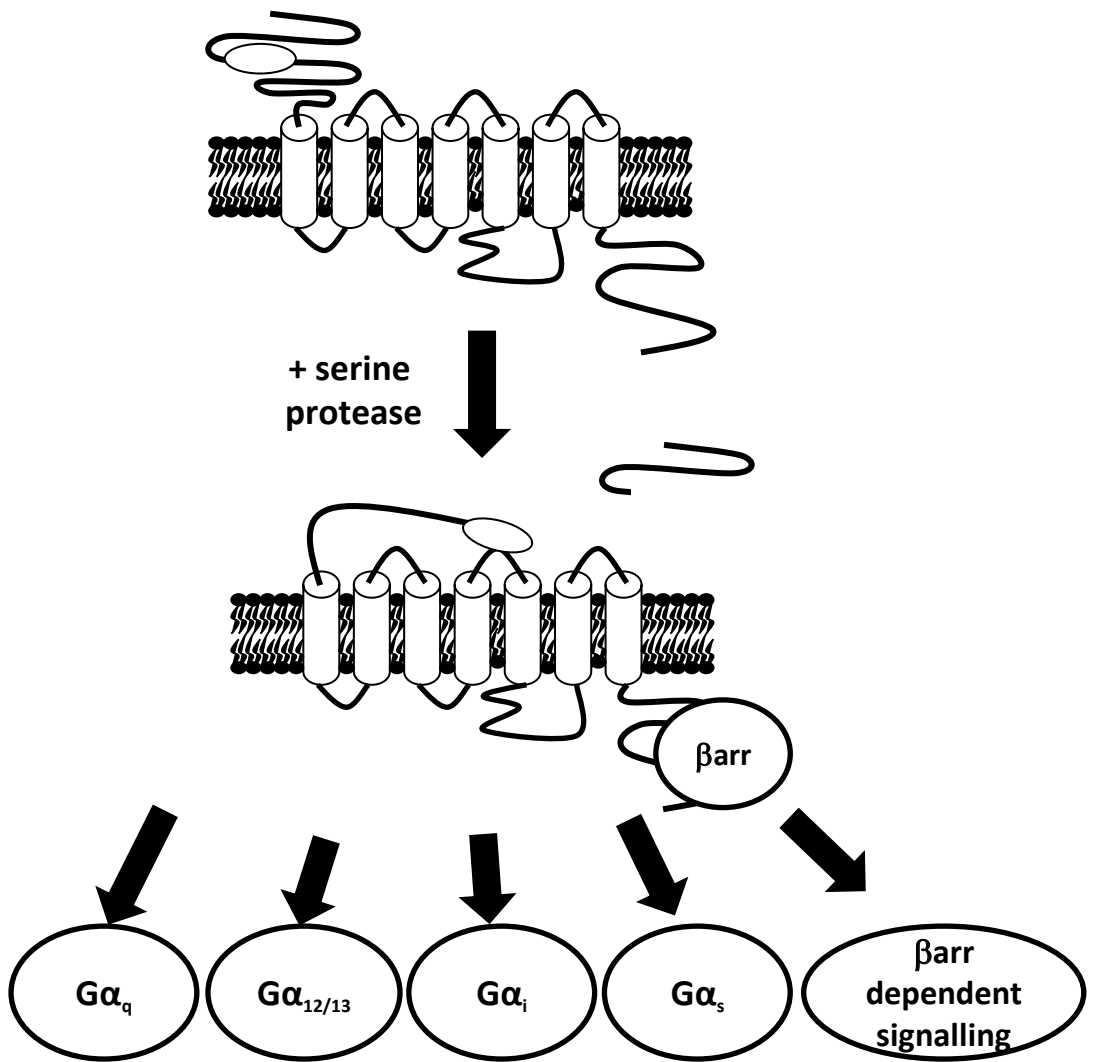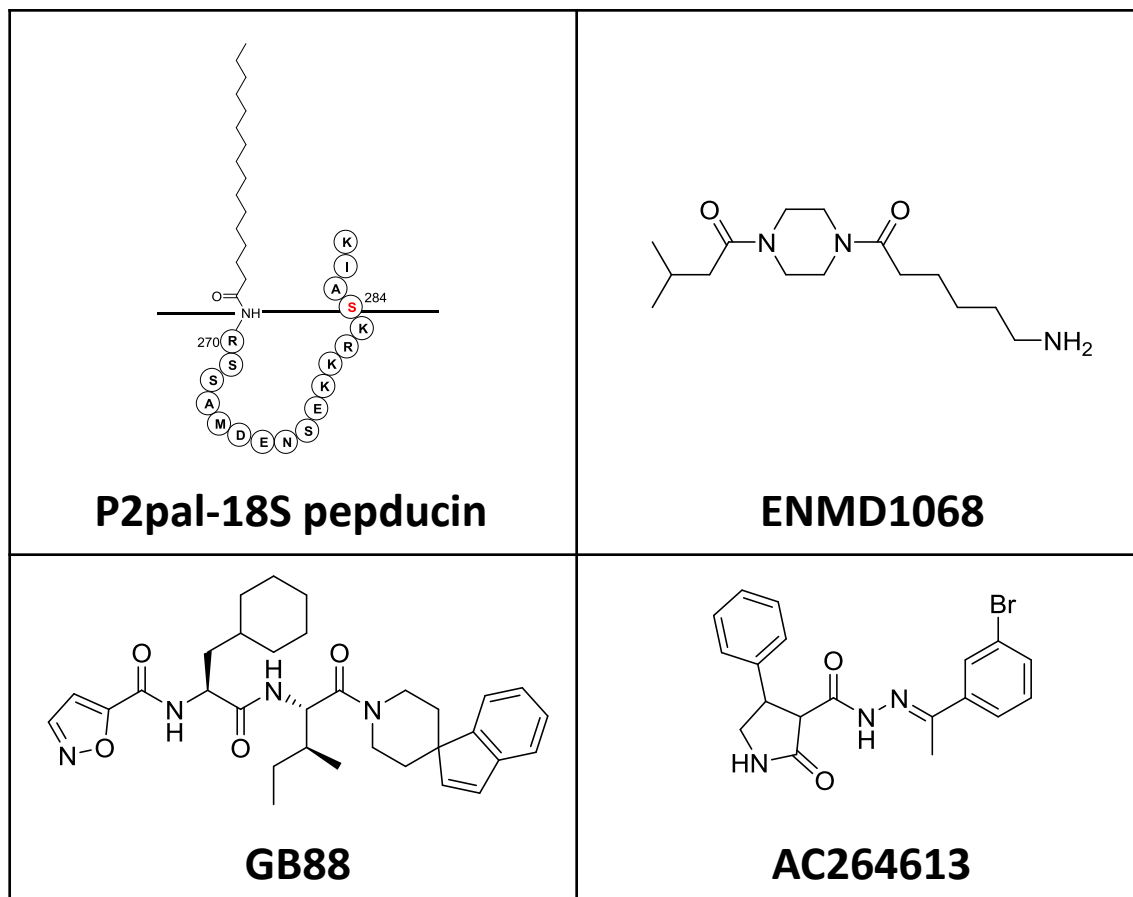
Figure 1.

+ serine
protease

βarr

Gα_q Gα_{12/13} Gα_i Gα_s βarr
dependent
signalling

Figure 2.

P2pal-18S pepducin

ENMD1068

GB88

AC264613

Table 1.

Immune cell type	Cellular response to PAR2 activation
Neutrophils	$\uparrow$ [Ca^{2+}]$_i$ $\uparrow$ shape change & motility $\uparrow$ Mac-1 & VLA-4 $\uparrow$ Il-1β, IL-6, IL-8 $\uparrow$ MPO $\uparrow$ ROS $\uparrow$ lactoferrin
Eosinophils	$\uparrow$ degranulation $\uparrow$ ROS $\uparrow$ IL-6, IL-8
Monocytes	$\uparrow$ [Ca^{2+}]$_I$ $\uparrow$ Il-1β, IL-6, IL-8
Macrophages	$\uparrow$ [Ca^{2+}]$_I$
Dendritic cells	$\uparrow$ maturation $\uparrow$ trafficking
Mast cells	activation & degranulation
T cells	$\uparrow$ rolling & adhesion $\uparrow$ tyrosine phosphorylation
B Cells	PAR2 not expressed

Table 2.

CNS glial cell type	Cellular response to PAR2 activation	PAR2 and Multiple Sclerosis	PAR2 and Alzheimer's disease
Astrocytes	↑ $[Ca^{2+}]_i$ ↑ glutamate release ↑ CINC-1/3 release ↑ αA-crystallin ↑ IL-6 ↑ TNF-α ↑ MAPK activity ↑ proliferation	↑astrocytic PAR2 expression in MS brains ↓ Release of inflammatory mediators in PAR2^{-/-} astrocytes ↓ neuroinflammation in EAE PAR2^{-/-} mice ↓ neurobehavioural phenotype in EAE PAR2^{-/-} mice	↑astrocytic PAR2 immunoreactivity in AD brains ↑ neuroinflammtory response and toxicity following astrocytic PAR2 activation ↓ astrocytic activation in β-amyloid injected PAR2^{-/-} mice ↓ neuroinflammatory respnse in β-amyloid injected PAR2^{-/-} mice ↓ neurobehavioural phenotype in β-amyloid injected PAR2^{-/-} mice
Microglia	↑ NO ↑ ROS ↑ P2X$_4$ ↑ BDNF	↓ microglial activation in EAE PAR2^{-/-} mice	↑microglial PAR2 immunoreactivityin AD brains