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REVIEW

Proteinases and signalling: pathophysiological and therapeutic implications via PARs and more

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Proteinases like thrombin, trypsin and tissue kallikreins are now known to regulate cell signaling by cleaving and activating a novel family of G-protein-coupled proteinase-activated receptors (PARs 1–4) via exposure of a tethered receptor-triggering ligand. On their own, short synthetic PAR-selective PAR-activating peptides (PAR-APs) mimicking the tethered ligand sequences can activate PARs 1, 2 and 4 and cause physiological responses both *in vitro* and *in vivo*. Using the PAR-APs as sentinel probes *in vivo*, it has been found that PAR activation can affect the vascular, renal, respiratory, gastrointestinal, musculoskeletal and nervous systems (both central and peripheral nervous system) and can promote cancer metastasis and invasion. In general, responses triggered by PARs 1, 2 and 4 are in keeping with an innate immune inflammatory response, ranging from vasodilatation to intestinal inflammation, increased cytokine production and increased or decreased nociception. Further, PARs have been implicated in a number of disease states, including cancer and inflammation of the cardiovascular, respiratory, musculoskeletal, gastrointestinal and nervous systems. In addition to activating PARs, proteinases can cause hormone-like effects by other signalling mechanisms, like growth factor receptor activation, that may be as important as the activation of PARs. We, therefore, propose that the PARs themselves, their activating serine proteinases and their associated signalling pathways can be considered as attractive targets for therapeutic drug development. Thus, proteinases in general must now be considered as 'hormone-like' messengers that can signal either *via* PARs or other mechanisms.
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Abbreviations: APC, activated protein C; CNS, central nervous system; GPCR, G-protein coupled receptor; PAR, proteinase-activated receptor; PAR-AP, proteinase-activated-receptor-activating peptide; PGE2, prostaglandin E2; SERPIN, serine proteinase inhibitor; TE, tethered ligand; hPAR, human-PAR; mPAR, murine PAR

Introduction

Proteinases as signal generating enzymes

While traditionally regarded as digestive protein-degrading enzymes, proteinases are now gaining recognition as versatile and multifunctional hormone-like signalling molecules that are implicated in a number of physiological and pathophysiological events. Proteinases can regulate cellular signalling events through their interaction with a large variety of targets, including pro-hormones, kininogens, chemokines precursors and proteinase zymogens to name a few. In addition, proteinases can also potentially regulate integrin-extracellular matrix signalling and activate growth factor receptors like the one for insulin. Of key interest in terms of proteinase-mediated signalling is the ability of proteinases to regulate cell function by cleaving and activating the proteinase activated receptor (PAR) family of G-protein-coupled receptors (GPCRs).

Classes of proteinases that can trigger cell signalling

Proteinases may be divided into five different classes based on their mechanism of catalysis, namely the aspartate, mettalo, cysteine, serine and threonine proteinases. The aspartate and metalloproteinases use an activated water molecule as a nucleophile to attack peptide bonds, while a catalytic amino-acid residue in the active site of the proteinase serves as the nucleophile for the remaining classes of proteinases. The completion of the human genome sequencing project revealed approximately 550 genes encoding proteinase, with the metalloproteinases and serine proteinases making up the bulk of these (Puente *et al.*, 2003). In principle, both of these classes of proteinases can signal via the mechanisms to be discussed in the following sections, which will focus in large part, but not entirely on PARs as

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targets for proteinase-mediated signal transduction. Thus, the proteinase-mediated signalling to be considered can proceed via both PAR and non-PAR mechanisms.

PARs and proteinase signalling

Many physiological responses mediated by serine proteinases can occur through the PAR family of GPCRs (for a comprehensive list, see review by Steinhoff *et al.*, 2005). The PAR receptors, named PARs 1 to 4 in order of their discovery, have a unique mechanism of activation (Figure 1) that distinguishes them from other seven transmembrane GPCRs. Most GPCRs are activated reversibly by small hydrophilic molecules to elicit cellular responses (Wettschureck *et al.*, 2005). PARs on the other hand effectively carry their own activating molecule in a masked state, with receptor activation achieved through proteolytic cleavage of a specific site within the receptor N-terminus to reveal a cryptic tethered ligand (TL) that binds to and activates the receptor (Hollenberg and Compton, 2002; Coughlin, 2005; Steinhoff *et al.*, 2005). In addition, PARs, with the exception of PAR_3, are also activated by short synthetic peptide sequences derived from the sequences of the proteolytically revealed TL (Vu *et al.*, 1991; Scarborough *et al.*, 1992). As outlined briefly in the following section and more extensively elsewhere (Hollenberg and Compton, 2002), these PAR-activating peptides (PAR-APs) have been very valuable tools in teasing out specific receptor function in systems that express more than one member of the PAR family. In addition to the cleavage/activation of PARs, proteinases can also negatively regulate functioning through the PARs by 'disarming' the receptor by cleavage at a non-receptor activating site to remove the TL (Figure 2). These truncated receptors nonetheless remain responsive to PAR-APs but would be unable to signal in a physiological setting.

Discovering pathophysiological roles for PARs: a pharmacological approach with receptor-selective agonists and antagonists

Receptor-selective PAR-activating peptides as probes for PAR function in bioassay systems: using structure–activity relationships to establish PAR-mediated responses

Studying PAR function with proteinase activation has been challenging especially in systems where more than one receptor is expressed. As alluded to above, synthetic peptides with sequences based on those of the proteolytically revealed PAR TLs can selectively activate the receptors without the need for proteolysis and have been key to teasing out responses mediated by these receptors. Importantly, it has also been possible to develop synthetic 'scrambled' TL peptide sequences that are not able to activate the PARs, so as to serve as appropriate 'control peptides' for studies done with cultured cells or tissues *in vivo*. Receptor-selective PAR-APs for PARs 1, 2 and 4 as well as the corresponding control peptides are outlined in Table 1. It has turned out surprisingly that PAR_3 on its own does not appear to signal and does not respond either to thrombin or to the PAR-APs based on the thrombin-revealed PAR_3 'tethered ligand sequence'. Rather, peptides with sequences derived from the thrombin-revealed PAR_3 N-terminus are able to activate either PAR_1 or PAR_2 (Hansen *et al.*, 2004). Studies have also provided evidence for a possible cofactor like role for PAR_3 through its interaction with PAR_1 and PAR_4 (Nakanishi-Matsui *et al.*, 2000; McLaughlin *et al.*, 2007). The PAR-selective activating peptides, along with the appropriate PAR-inactive peptide sequences, have served as key reagents to explore the impact of activating a specific PAR in a tissue or cell, without the need for proteinase-stimulated activation of the receptor, which might result in effects other than simply activating a PAR.

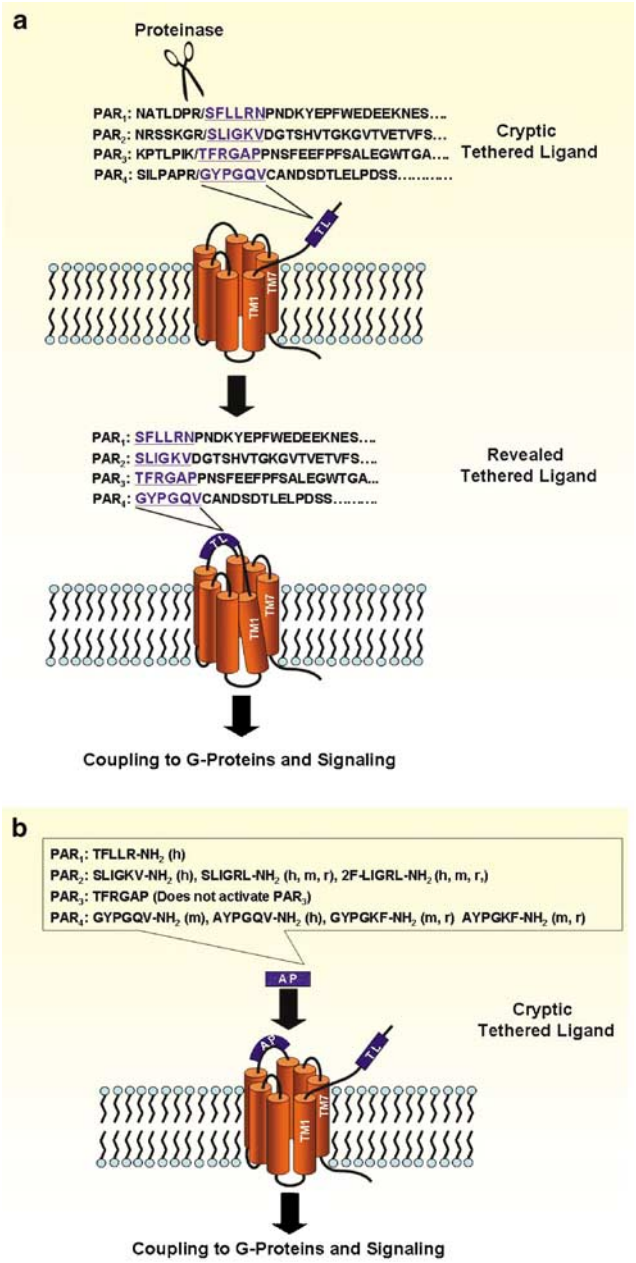

Figure 1 Mechanisms of PAR activation. (**a**) Activation of PAR signalling by proteinase-mediated cleavage of receptor N-terminus to reveal tethered ligand (TL). (**b**) Activation of PAR signalling by exogenous application of synthetic PAR agonist peptide (AP) without the need for proteolytic revealing of the TL. PAR, proteinase-activated receptor.

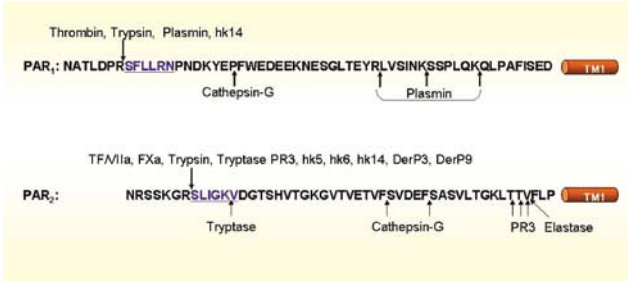

Figure 2 Proteinases targeting PAR_1 and PAR_2. Proteinases can activate PARs by cleaving the receptor to reveal the tethered ligand (TL) (↓) or disarm them by cleaving at other sites (↑), making the TL unavailable to the receptor. Disarmed receptors remain responsive to AP-stimulated activation. hk, human kallekrien; TF, tissue factor; PAR, proteinase-activated receptor; PR3, proteinase 3.

Table 1 PAR activating peptides and corresponding control peptides

Receptor	*PAR-APs*	*Inactive control peptides*
PAR_1	TFLLR-NH_2	*FTLLR-NH_2*
PAR_2	SLIGRL-NH_2; 2-furoyl-LIGRLO-NH_2	*LSIGRL-NH_2; 2-furoyl-OLRGIL-NH_2*
PAR_3	TFRGAP: does not activate PAR_3	
PAR_4	AYPGKF-NH_2	*YAPGKF-NH_2*

Abbreviations: PAR, proteinase-activated receptor; PAR-AP, PAR-activating peptide.

An example of the use of such receptor-selective PAR-APs can be seen in studies of PAR_2-mediated calcium signalling in a PAR_2-expressing KNRK cell line. In this test system, the relative potencies of the PAR_2-selective agonist peptides, SLIGRL-NH_2, *trans*-cinnamoyl-LIGRLO-NH_2, 2-furoyl-LIGRLO-NH_2 and of a potent PAR_1-selective PAR_1AP, AparafluoroFRChaChaCitY-NH_2 would be 2-furoyl-LIGRLO-$NH_2 \gg$ *trans*-cinnamoyl-LIGRLO-$NH_2 \cong$ SLIGRL-$NH_2 \gg$ AparafluoroFRChaChaCitY-NH_2. A completely reversed structure–activity relationship (SAR) would be expected of a PAR_1-mediated response, in which the three PAR_2-activating peptides would be essentially inactive. The same order of peptide agonist potencies would be expected for the activation of PAR_2 in tissue preparations. This principle has been used to establish in a variety of tissue and cell preparations, the PAR-mediated responses that would be anticipated upon activating the receptors by a proteinase. The use of these receptor-selective agonists, along with PAR-null animals, has led to the discovery of the ability of the PARs to regulate pain and inflammation and to affect a variety of tissues ranging from nerve cells to the vasculature and intestinal tract. In this manner, a number of physiological roles have been proposed for the PARs as summarized in Table 2 and outlined in more detail below.

Responses triggered by PAR-activating peptides that cannot be attributed to PAR activation

In the context of using the PAR-APs, it must be emphasized that the same SAR principle (Ahlquist, 1948) that can verify PAR-related responses to the peptides can also be used to establish that, in certain circumstances, the PAR-APs can stimulate a receptor other than one of the PARs. It thus came as a surprise that the SAR for these PAR_2 agonists observed in a rat jejunal ion transport assay (SLIGRL-NH_2 > *trans*-cinnamoyl-LIGRLO-NH_2 > AparafluoroFRChaChaCitY-NH_2) was different from the SAR expected of either PAR_2 or PAR_1 (Vergnolle *et al*., 1998). A plausible conclusion was that the short-circuit current response in the jejunal Ussing chamber due to the serosal application of the PAR-APs and trypsin was mediated by a receptor different from either PAR_1 or PAR_2. In a similar manner, some recent work with PAR_4-derived agonists has been able to verify the presence of PAR_4 in rat platelets, using a platelet aggregation assay, while pointing to a non-PAR_4-mediated response in a rat gastric longitudinal muscle assay (Hollenberg *et al*., 2004). The work with the PAR_4-derived peptides illustrates that a judicious choice of standard PAR-APs as well as a standard PAR-inactive peptide is required to establish whether or not a given response can be attributed to a given PAR.

Table 2 Potential physiological roles for PARs

Potential role	*Comment*	*Reviewed by*
CNS neuronal and astrocyte function	Upregulation of PARs observed in the setting of CNS inflammation	Noorbakhsh *et al*., 2003
Endothelial cell function: (PARs 1, 2 and 4)	Regulate release of NO, von Willebrand factor; increase neutrophil adherence; promote cell migration	Coughlin, 2005
Intestinal function: (PARs 1, 2 and 4)	Regulation of motility (GI smooth muscle) and secretion (GI epithelial cell)	Vergnolle, 2005a
Myenteric neuron function	Also affects GI motility and inflammatory response	Vergnolle, 2003
Vascular smooth muscle function	Activation of contractility; angiogenesis?	Coughlin, 2005
Renal vascular function	Regulation of flow and afferent arteriolar function	Vesey *et al*., 2007
Skin pigmentation	Proteinase inhibition affects skin pigmentation. Involvement of PAR_2 in ethnic skin color phenotypes	Seiberg, 2001
Hyperalgesia and analgesia	PAR activation can increase (PAR_2 mediated) or decrease (PAR_1 and PAR_4 mediated) pain sensation	Vergnolle, 2005b
Platelet activation, haemostasis: Thrombin-activated receptors (PARs 1, 3, 4)	Regulate both secretion and aggregation; PARs 1 and 4 can play opposing roles possibly due to differential coupling to g-protiens	Coughlin, 2005
Response to joint injury	Use of PAR_2 null mice and PAR antagonists suggests key role for PAR_2 in arthritis	McIntosh *et al*., 2007
Tumour cell growth and metastasis	Both PARs 1 and 2 may play roles, activated by tumor-derived serine proteinases and matrix metalloproteinases (e.g. MMP-1)	Ruf *et al*., 2006; Soreide *et al*., 2006

Abbreviations: CNS, central nervous system; PAR, proteinase-activated receptor.

Proteinase signalling by mechanisms other than PARs

Although the main focus of this review is on PARs as a target for proteinase signalling, it is important to recognize that

quite a number of other mechanisms can account for the ability of proteinases to regulate cell function. Thus, at this point, having dealt with an overview of the PARs, it is of interest to consider briefly some alternate targets that can result in signal transduction.

Regulation of growth factor receptors

One of the first indications that proteinases can activate hormone-like cellular signals came from the observations in the early 1960s that trypsin and pepsin can exhibit an insulin-like action in rat diaphragm tissue (Rieser *et al*., 1964; Rieser, 1967). This hormone-like action of trypsin in striated muscle and adipocytes (Cuatrecasas, 1971; Kono *et al*., 1971) cannot be attributed to the activation of PARs, but is rather due to the effect of trypsin on the receptor for insulin. By cleaving at a dibasic residue of the insulin receptor alpha-subunit, trypsin generates a truncated receptor that has intrinsic signalling activity (Shoelson *et al*., 1988). In principle, this kind of action of proteinases, either activating or disarming growth factor receptors (for example at higher concentrations, trypsin can abolish the ability of the insulin receptor to bind to insulin) (Cuatrecasas, 1971) can in principle modulate cell function in a variety of settings, for example via the insulin-like growth factor-1 receptor. Another proteolytic mechanism that can lead to the activation of a growth factor receptor involves the proteolytic generation of a growth factor agonist in the cell environment. For instance, the *trans*-activation of the epidermal growth factor receptor can result from the metalloproteinase-mediated release from the cell surface of a receptor agonist (heparin-binding epidermal growth factor) (Prenzel *et al*., 1999). Thus, in principle, any of the receptors for growth factors or other comparable agonists like cytokines or interleukins (ILs) can be regulated either by activation or inactivation by proteinase action.

Non-receptor signalling targets

Apart from classical pharmacological receptors that exhibit the dual property of selective agonist recognition and signalling, other 'non-receptor' targets can also result in signalling by proteinases. For instance, disruption of extracellular matrix–integrin signalling by proteolysis of either the matrix or integrin molecules would in principle alter cell behaviour. In this regard, the ability of thrombin to activate metalloproteinases (Lafleur *et al*., 2001) could in principle lead to PAR-independent signalling via remodelling of the extracellular matrix. Another novel mechanism for proteinase-triggered signalling can be seen in the action of plasmin, which in addition to regulating PAR activity (below) can signal via an annexin A2 target mechanism, in which plasmin-mediated proteolysis of annexin A2 triggers chemotaxis in human monocytes (Laumonnier *et al*., 2006; Li *et al*., 2007). It can be presumed that serine proteinases other than plasmin will also be found to regulate cell behaviour via this novel annexin A2 proteolytic process. The signalling mechanism whereby annexin cleavage regulates chemotaxis or other cell responses remains to be determined. Whether thrombin at high concentrations might mimic the annexin A2 cleavage caused by plasmin, in the manner that plasmin mimics the action of thrombin on the PARs, is also an issue to be explored.

Non-catalytic mechanisms for proteinase-mediated signalling

Protein–protein interactions in addition to their catalytic function must also be considered in evaluating proteinase-mediated signalling. For instance, apart from its ability to signal catalytically via the PARs, thrombin can also yield from within its structure, chemotactic-mitogenic peptides released by proteolytic processing of its non-catalytic domain (Bar-Shavit *et al*., 1984, 1986). These thrombin-derived peptides cause their effects via receptors that are not PARs (Glenn *et al*., 1988). The ability of proteinases to affect signalling *via* their non-catalytic domains is an issue that can be often overlooked. The essence of the previous sections is that proteinases, apart from targeting the PARs, can affect signalling by a diverse set of mechanisms. This diversity of hormone-like signalling roles played by proteinases is exceeded only by the diversity of the proteinase families themselves. The next question, therefore, is which proteinases might be candidates for signalling via the PARs and other mechanisms?

Searching for the proteinases responsible for PAR activation in physiological settings

Although the approaches described in the preceding sections have led to an understanding of the potential physiological roles the PARs may play, identifying the proteinases that may activate the PARs in a variety of physiological settings has proved to be a challenge. The following sections deal with the proteinases that can potentially fulfill the roles of 'physiological' PAR regulators.

Coagulation cascade proteinases

The coagulation cascade mediated by a variety of serine proteinases is one of the best characterized systems where such enzymes play key roles. Haemostasis is characterized essentially by a fine balance between the formation and the lysis of clots. The level of coagulation factors circulating in the blood, if totally activated, would significantly shift this fine balance towards the uncontrolled clotting of blood (Esmon, 2004). The coagulation cascade allows the body to control this process and consists of a number of serine proteinase zymogens and their glycoprotein cofactors, which are activated in sequence to propagate the cascade (Davie and Ratnoff, 1964; Macfarlane, 1964). Quite apart from their critical involvement in the clotting of blood, a number of coagulation cascade proteinases are reported to target the PAR family of GPCRs, and there is now little doubt that a number of the coagulation cascade proteinases represent physiological regulators of PARs 1, 2 and 4 (Coughlin, 2005). Indeed, the initial impetus for the search for PARs came from the inability of known mechanisms to explain the numerous functions that thrombin performed in various cellular environments (Weksler *et al*., 1978; Bar-

Shavit *et al*., 1983a, b; Babich *et al*., 1990). As it turns out, thrombin is one of the most potent agonists for PAR_1 and to a lesser extent PAR_4 (Vu *et al*., 1991; Kahn *et al*., 1998; Xu *et al*., 1998). While thrombin is also known to cleave PAR_3, the ambiguity surrounding the function of this receptor makes it difficult to assign physiological relevance to this event. PAR_2, the remaining member of the PAR family, is not activated by thrombin and was thought not to be a target for the coagulation proteinases. However, PAR_2 is now known to be activated by the Tissue Factor/VIIa binary complex and by Factor Xa, which can cleave PAR_2 either independently, or with greater efficiency as part of a ternary complex with TF and VIIa (Camerer *et al*., 2000; Riewald *et al*., 2001). PAR_2 activation by coagulation proteases is now known to initiate a number of important signalling events, including signalling and migration of cancer cells (Morris *et al*., 2006), induction of angiogenesis (Belting *et al*., 2004; Uusitalo-Jarvinen *et al*., 2007) and signalling in Osteosarcoma cells (Daubie *et al*., 2007).

In addition to the proteinases involved in clot formation, trypsin-related serine proteinases of the fibrinolytic system such as plasmin can also regulate signalling in part via the PARs. However, the role of plasmin is complex since this enzyme can both activate and disarm a PAR like PAR_1 (Kimura *et al*., 1996; Kuliopulos *et al*., 1999) and can also activate PAR_4 (Quinton *et al*., 2004). Further, as mentioned above, plasmin can activate cell signalling by a mechanism that, apart from involving the PARs, signals via the cleavage of the heterotetramer composed of annexin A2 and S100A10. Yet another clotting cascade-associated serine proteinase with anticoagulant and anti-inflammatory activities, activated protein C (APC), can exert its cytoprotective/anti-inflammatory effects by activating PAR_1 employing a mechanism that involves both binding to the endothelial cell surface via an APC-targeted endothelial adsorption site (endothelial cell protein C receptor) and a specific interaction with PAR_1 via a specific APC exosite domain (Riewald *et al*., 2002; Yang *et al*., 2007). The impact that APC has on the disarming of PAR_2 or the potential activation of PAR_4 has not yet been evaluated. Apart from these coagulation-associated proteinases, for which a physiological PAR-regulatory role can be seen, the tissue-localized enzymes that may regulate PARs *in vivo*, including PARs 2 and 4, which are both potently activated by trypsin (and presumably other serine proteinases), have yet to be identified.

Proteinases in the immune system

Neutrophils are immune cells that are well known to carry a variety of proteinases to aid in their essential roles in host defence against invading pathogens. Neutrophil proteinases consist mainly of proteinase-3, cathepsin-G and elastase (Pham, 2006). Neutrophils can be seen to play a 'signalling role' in terms of their processing and enhancing the chemotactic activity of chemokines such CXCL8, CXCL5, and CCL15 as well as proteolytically activating cytokines like IL-1 and tumour necrosis factor. Neutrophil proteinases can also negatively regulate cytokine function with elastase and cathepsin-G reported to degrade tumour necrosis factor, while proteinase-3, cathepsin-G and elastase are reported to inactivate IL-6 (Pham, 2006).

Proteinase-3 is reported to activate PAR_2 on epithelial cells (Uehara *et al*., 2002, 2003). Of relevance to PAR signalling as well is the recent report that proteinase-3 can cleave and inactivate the endothelial cell protein C receptor, consequently inhibiting the ability of APC to regulate PAR_1 activation (Villegas-Mendez *et al*., 2007). Cathepsin-G preferentially activates PAR_4 and has been reported to activate this receptor in a number of cell types (Sambrano *et al*., 2000; Ramachandran *et al*., 2007). In addition, cathepsin-G is known to disarm PAR_1 (Molino *et al*., 1995) and in murine platelets to inactivate PAR_3 and abolish its cofactor like function (Cumashi *et al*., 2001). The elastase-mediated regulation of PARs occurs through disarming the receptors, with cleavage of the PAR_2 receptor reported at Ser37-Leu38 and Gly52-Val53 sites (Dulon *et al*., 2003, 2005), while PAR_1 is susceptible to elastase cleavage at the Val72-Ser73 and Ile74-Asn75 sites downstream of the activation site (Renesto *et al*., 1997).

Proteinases in the nervous system

The expression of a number of PARs and their possible activating proteinases has been described in both the central (CNS) and the peripheral nervous system. The potential activating proteinases in the peripheral nervous system are essentially as found in the organ system being innervated, while the CNS is also seen to express a number of known serine proteinases that may regulate PAR function, including thrombin, plasmin, urokinase plasminogen activator, tissue plasminogen activator and tissue kallikreins. In the CNS, such proteinases are believed to play an important role in neural plasticity (Yoshida *et al*., 1999; Shiosaka *et al*., 2000). Thrombin expression in the brain is well established (Yoshida *et al*., 1999; Rohatgi *et al*., 2004) and would constitute a key regulator of the PARs in the CNS. As outlined above, the plasmin–tissue plasminogen activator system is also reported to activate PAR_1 in the brain (Nagai *et al*., 2006). In a number of neuropathological conditions, levels of thrombin are seen to be upregulated (Turgeon *et al*., 1997), whereas levels of plasmin are reduced in Alzheimer's disease (Dotti *et al*., 2004). The differential expression and levels of proteinases in the brain could thus potentially result in selective activation of the various PARs expressed in CNS tissue and may work in concert with the upregulation of PAR expression as seen in disease settings (Boven *et al*., 2003; Noorbakhsh *et al*., 2006). The regulators of PAR_2 in the brain are harder to describe; however, recent evidence has pointed to the involvement of the kallikrein family of serine proteinases as key regulators of PAR_2 function (Oikonomopoulou *et al*., 2006). A number of kallikreins have been described in the brain with an important role indicated for neuropsin (KLK8) and neurosin (KLK6) (Yousef *et al*., 2003). Neurosin levels are downregulated in Alzheimer's disease (Ogawa *et al*., 2000), while elevated levels of neuropsin and neurosin are reported in experimental autoimmune encephalomyelitis (Terayama *et al*., 2005) and spinal chord injury (Terayama *et al*., 2004).

In addition to the kallikreins, a number of trypsin species are reported to be expressed in the brain and to target PARs (Minn *et al*., 1998; Wang *et al*., 2006). Further, following an inflammatory event, as is seen in a number of neurological disorders, immune cells are detectable in the CNS and therefore the potential exists for immune cell proteinases described above to regulate PAR function in the brain.

Proteinases in the airways

The airways are exposed to numerous proteinases both from resident cells and from infiltrating immune cells. In addition, the airways are constantly exposed to airborne microorganisms, a number of which can also present proteinases to the airways. Proteinases produced by mast cells, tryptase, chymase and cathepsin-G, are some of the most abundant serine proteinases detectable in the airways. Mast cell proteinases are also implicated in a variety of cellular responses, including inflammation, matrix destruction and remodelling, hydrolysing chemokines and cytokines as well as inactivating allergens and neuropeptides to name but a few. Importantly, mast cell proteinases are also reported to regulate the PARs, both through activating and disarming the receptors (Reed *et al*., 2004).

Tryptase is expressed in the airways as both soluble (α, β and γ tryptase) and membrane-anchored (δ tryptase) forms. Only the β form of human mast cell tryptase is believed to play important roles in the extracellular environment (Caughey, 2007) and is known to promote allergic inflammation, airway hyperresponsiveness and tissue remodelling (Cairns, 1998; Berger *et al*., 1999). Tryptase is also reported to cleave and activate PAR_2 (Molino *et al*., 1997) and a number of the tryptase-stimulated cellular responses have been attributed to PAR_2 activation (Compton *et al*., 1999; Akers *et al*., 2000; Frungieri *et al*., 2002; Berger *et al*., 2003). Mast cell chymase is predominantly involved in matrix destruction by cleaving fibronectin and collagens and is also reported to activate PAR_1 (Schechter *et al*., 1998). Cathepsin-G on the other hand has been reported to activate PAR_4 (Sambrano *et al*., 2000) as well as disarming PAR_1 and PAR_2 (Ramachandran *et al*., 2007).

In addition to mast cell proteinases, the expression of PAR-activating extrapancreatic trypsins, such as trypsin, trypsin IV and human airway trypsin, have also been reported in the airways (Cocks *et al*., 1999a; Cottrell *et al*., 2004; Matsushima *et al*., 2005).

PARs are also targeted by proteinases produced by the house dust mite. Der-p1 is reported to cleave the PAR_2 activation site and disarm PAR_1 (Asokananthan *et al*., 2002b). Der-p3 and Der-p9 are also shown to have the potential to cleave the PAR_2 activation site (Sun *et al*., 2001), although there are alternative interpretations to those data, since Der-p1 responses have been reported to result clearly from mechanisms that do not involve PAR_2 (Adam *et al*., 2006). Under inflammatory conditions, the airways are also infiltrated with large numbers of polymorphonuclear neutrophils. The proteinases produced by these cells also contribute to regulating responses via the PARs as discussed above.

Proteinases in the gastrointestinal system

The gastrointestinal tract is exposed to very high levels of proteinases, including digestive gland proteinases involved in digestion of food as well as exogenous proteinases produced by the commensal gut microflora or by invading pathogenic microorganisms (Antalis *et al*., 2007). It is believed that in the gastrointestinal tract, members of the trypsin family represent the major physiological regulators of the PARs (Kong *et al*., 1997). In a murine model of infectious colitis, colon-derived trypsin family members triggered by the infectious process have been identified and have been shown to be capable of activating PAR_2 (Hansen *et al*., 2005). In a study on colonic tissue from individuals with inflammatory bowel syndrome, it was shown further that levels of trypsin, tryptase and other unidentified serine proteinase that favoured cleavage at an arginine site are significantly upregulated (Cenac *et al*., 2007). These proteinases have been shown to target PAR_2. High concentrations of trypsin like proteinases are also potentially activators of PAR_1 (Knecht *et al*., 2007) and PAR_4 (Xu *et al*., 1998) and increased levels of trypsin are reported in patients with conditions such as inflammatory bowel disease (Playford *et al*., 1995).

Pathogen-derived proteinases, such as gingipains, arginine-specific proteinases produced by the oral pathogen *Poryphyromonas gingivalis*, are also known to activate PARs 1, 2 and 4 (Lourbakos *et al*., 1998, 2001; Holzhausen *et al*., 2006).

Inflammation is a key event in a number of disorders of the gut, including conditions such as inflammatory bowel disease. The resultant infiltration of mast cells and immune cells would allow the presence of proteinases secreted by these cells to regulate PARs in the gastrointestinal tract as described in the sections above. The precise relationship between PARs, their regulating proteinases present in the enteric tract and gastrointestinal pathophysiology is currently a topic of intensive investigation.

PAR-mediated signal transduction pathways

PAR_1 is known to couple with multiple G-proteins, including G_i, $G_{q/11}$ and $G_{12/13}$ families (Macfarlane *et al*., 2001; Steinhoff *et al*., 2005). However, our knowledge of PAR interaction with G proteins is still far from complete. Evidence for PAR_1 interaction with G_i has come from multiple cell types, such as HEL cells (Brass *et al*., 1991), osteosarcoma cells (Babich *et al*., 1990), vascular smooth muscle cells (Kanthou *et al*., 1996), fibroblasts (Hung *et al*., 1992a), platelets (Kim *et al*., 2002), astrocytes (Wang *et al*., 2002) and endothelial cells (Vanhauwe *et al*., 2002). Coupling of PAR_1 to $G_{q/11}$ was revealed through the use of G-protein-specific antibodies, which inhibited PAR_1-mediated calcium signalling in CCL-39 fibroblast cells (Baffy *et al*., 1994) and through co-immunoprecipitation of $G_{q/11}$ with PAR_1 (Ogino *et al*., 1996). Interaction between PAR_1 and $G_{q/11}$ was also reported in astrocytes (Wang *et al*., 2002) and indicated in astrocytoma cells (LaMorte *et al*., 1993). Evidence for PAR_1 coupling to $G_{12/13}$ comes from studies on thrombin-stimulated platelets (Offermanns *et al*., 1994) and for coupling to G_{12} in astocytoma cells (Aragay *et al*.,

1995; Post *et al.*, 1996). PAR_1 is also known to activate multiple signalling pathways downstream of coupling to G-proteins, including the activation of PI3 kinase (Malarkey *et al.*, 1995), Src family tyrosine kinases (Sabri *et al.*, 2000) and the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway (Vouret-Craviari *et al.*, 1993). PAR_1 signalling to nuclear factor (NF)-κB (Rahman *et al.*, 2002) has also been reported, but the precise signal pathways involved are not known.

PAR_2 signalling has been studied in less detail than for PAR_1. The activation of PAR_2 leads to an elevation of intracellular calcium subsequent to the release of inositol tris-phosphate along with diacyl-glycerol (Nystedt *et al.*, 1995). This result suggests that PAR_2 signals via $G_{q/11}$ and possibly G_i (Macfarlane *et al.*, 2001). Coupling of PAR_2 with $G_{12/13}$ has not been reported. Another very important aspect of cell activation via PAR_2 relates to its ability to signal via an arrestin-mediated process that can be independent of G_q–protein interactions (Zoudilova *et al.*, 2007). This arrestin-dependent mechanism may explain the 'dual' actions that PAR_2 can have in certain settings, triggering either inflammatory or anti-inflammatory responses. The ability of arrestin-mediated, G-protein-independent signalling that might involve PAR heterodimers (for example PAR_2/PAR_1) is a topic that merits an in-depth evaluation.

PAR_2 activation on smooth muscle and neuronal cells has been observed to activate phospholipase C (Berger *et al.*, 2001b) and protein kinase C, respectively (Okamoto *et al.*, 2001). A recent study has revealed further that regions within the PAR_2 C-terminal tail are critical for PLC-mediated IP3 formation and calcium signalling as opposed to the activation of MAPkinase that is triggered by other intracellular PAR_2 sequences (Seatter *et al.*, 2004). PAR_2 was also reported to activate the MAPK cascade in rat aortic smooth muscle cells (Belham *et al.*, 1996) and in human keratinocytes PAR_2-mediated activation of JNK and p38 MAPK was shown to mediate NF-κB DNA binding (Kanke *et al.*, 2001; Buddenkotte *et al.*, 2005). Further, in keratinocytes, intracellular calcium mobilization is an important determinant of PAR_2 NF-κB signalling (Macfarlane *et al.*, 2005). PAR_2-mediated activation of NF-κB has also been demonstrated in epithelial cells (Vliagoftis *et al.*, 2000) and coronary artery smooth muscle cells (Bretschneider *et al.*, 1999). PAR_2 activation of ERK1/2 is now established in a number of cell including cardiomyocytes (Sabri *et al.*, 2000) and smooth muscle cells (Belham *et al.*, 1996). Factor Xa-mediated activation of ERK1/2 in coronary artery smooth muscle cells was also shown to be PAR_2 mediated (Koo *et al.*, 2002). Endothelial PAR_2 stimulation can trigger the activation of the 'small G-proteins' such as Rho-A, Rac leading to the regulation of p21-activated kinase. These responses are believed to be involved in mediating cytoskeletal effects (Vouret-Craviari *et al.*, 2003), possibly involving arrestin-mediated signalling (Zoudilova *et al.*, 2007).

The signalling role of PAR_3 is not yet clear, except for its ability to act as a cofactor for PAR_1 activation. On its own, PAR_3 does not appear to signal, and its TL sequence can activate other PARs, like PARs 1 and 2 (Hansen *et al.*, 2004; Kaufmann *et al.*, 2005). However, the ability of PAR_3 to dimerize with other PARs (McLaughlin *et al.*, 2007) points to another mechanism whereby PAR_3 can regulate signalling, apart from employing its TL.

PAR_4 is known to activate calcium signalling, presumably via G_q (Kahn *et al.*, 1998; Xu *et al.*, 1998; Camerer *et al.*, 2002) and PAR_4 activation of MAPK signalling has been observed in vascular smooth muscle cells (Bretschneider *et al.*, 2001). PAR_4-mediated Src-dependent p38 phosphorylation and activation of ERK and PLC has been described in cardiomyocytes derived from PAR_1-knockout mice (Sabri *et al.*, 2003). A recent study has also reported PAR_4 activation of p38 MAPK in endothelial cells (Fujiwara *et al.*, 2005). Whether or not this PAR_4-mediated activation of MAPkinase involves G_i or a separate arrestin-mediated process remains to be determined.

PARs in physiology and pathophysiology

Since their identification within the last 16 years or so, a myriad of physiological and pathophysiological events have been attributed to PAR-mediated signalling. The specifics of their involvement in various processes, however, remain unknown. The complex interplay within PARs, multiple proteinases, proteinase inhibitors and other molecules that may be present in different organ systems, and under different pathological conditions, makes the understanding of PAR-mediated events a complex exercise. The lack of potent, readily available antagonists for all of the PARs and the challenge of extrapolating data obtained from animal models to human disease are some of the other issues to deal with in determining the precise role that PARs may play in humans. Notwithstanding, the results of work with PAR-null animals and the documented expression and activation of PARs in various organs in humans point to key roles for the PARs in many pathophysiological settings.

PARs in the cardiovascular and circulatory system

PARs are expressed by multiple cells in the cardiovascular and circulatory system, including the circulating cells as well as the vascular endothelial and smooth muscle cells. Human platelets express PAR_1 and PAR_4 (Kahn *et al.*, 1999), while murine platelets express PAR_3 and PAR_4 (Kahn *et al.*, 1998). PAR_1 (Vu *et al.*, 1991), PAR_2 (Hwa *et al.*, 1996; Mirza *et al.*, 1996), PAR_3 (Schmidt *et al.*, 1998) and PAR_4 (Fujiwara *et al.*, 2005) are expressed on endothelial cells, while vascular smooth muscle cells express PAR_1 (McNamara *et al.*, 1993; D'Andrea *et al.*, 1998) and PAR_2 (D'Andrea *et al.*, 1998; Molino *et al.*, 1998). Both PAR_3 (Bretschneider *et al.*, 2003) and PAR_4 (Bretschneider *et al.*, 2001) are also found on vascular smooth muscle cells.

Activation of PAR_1 or PAR_4 on human platelets is sufficient to trigger calcium signalling and aggregation (Vu *et al.*, 1991; Xu *et al.*, 1998; Kahn *et al.*, 1999). At low thrombin concentrations, PAR_1 appears to be responsible for mediating thrombin-mediated responses in platelets, while PAR_4-mediated responses are limited to systems where PAR_1 is not expressed or where high thrombin concentrations are employed (Hung *et al.*, 1992b; Kahn *et al.*, 1999). It has also been claimed that PAR_4 may be involved in responding to

proteinases other than thrombin, and $hPAR_4$ activation by the neutrophil proteinase cathepsin-G has been revealed (Sambrano *et al*., 2000). It has also been suggested that $hPAR_4$ might mediate slow and prolonged responses to thrombin, while $hPAR_1$-mediated signalling events may be more rapid. Support for this hypothesis comes from different kinetics observed in $hPAR_1$ and $hPAR_4$ desensitization (Shapiro *et al*., 2000). A differential function of PARs 1 and 4 for regulating human platelets can be seen in the ability of PAR_1 activation to trigger the release of vascular endothelial growth factor, whereas PAR_4 activation causes the release of endostatin, which would be counter-regulatory for vascular endothelial growth factor action (Ma *et al*., 2005). Recent studies in human platelets have indicated that activation of PAR_1 and PAR_4 triggers distinct pathways following thrombin cleavage (Bilodeau *et al*., 2007; Holinstat *et al*., 2007; Voss *et al*., 2007) and may form the basis for the differential functions observed. Murine platelets express $mPAR_3$ and $mPAR_4$, yet $mPAR_4$ is the sole functional thrombin receptor, with $mPAR_3$ serving as a cofactor to enhance thrombin action (Nakanishi-Matsui *et al*., 2000). The presence of both receptors is, however, essential for haemostasis, since genetically deleting either receptor in mice results in an increased tail bleeding time and compromised thrombosis (Sambrano *et al*., 2001). Moreover, the administration of the peptide pepducin P4pal-10 PAR_4 antagonist also prolongs tail bleeding times in mice, providing further evidence for a key role for PAR_4 in thrombin signalling in rodents (Covic *et al*., 2002b). In other mammals, however, PAR_1 appears to be more important than PAR_4 for regulating platelet aggregation. Thus, by inhibiting PAR_1 function selectively with specific antagonists, it is possible to prevent thrombus formation in monkeys and presumably also in humans (Derian *et al*., 2003).

Activation of either rat or human endothelial PAR_1 and PAR_2 in organ bath preparations causes vascular relaxation, which is mediated, at least in part by the release of endothelial nitric oxide (Al-Ani *et al*., 1995; Hamilton *et al*., 2001, 2002). Comparable results have been obtained for observations done *in vivo* in rats and mice (Cicala *et al*., 2001). A study done *in vivo* in humans has revealed further that PAR_2-AP causes vasodilation in an NO- and prostaglandin-dependent manner (Robin *et al*., 2003). Moreover, activation of PAR_1 and PAR_2 can cause proliferation of cultured vascular smooth muscle (McNamara *et al*., 1993; Chaikof *et al*., 1995; Bretschneider *et al*., 1999; Koo *et al*., 2002) and PAR_2 activation can also stimulate endothelial cell proliferation (Mirza *et al*., 1996). Another report has shown that PAR_2 can stimulate angiogenesis *in vivo* in a murine model of hindlimb ischaemia (Milia *et al*., 2002). PAR_4 activation has also been implicated in vascular smooth muscle proliferation (Bretschneider *et al*., 2001).

PAR_1, PAR_2 and PAR_4 have also been implicated in mediating vascular responses in the setting of models of inflammation (for example increased leukocyte endothelial adherence and rolling; migration of leukocytes from the vasculature into inflamed tissues). The increased expression of proteinases from inflammatory cells and from activation of the coagulation cascade very likely serve as triggers for the PARs in this type of setting. Activation of rat paw PAR_1 in response to the intraplantar injection of a PAR_1-activating peptide causes oedema that is, in part, due to the extravasation of plasma into the peripheral tissue via a breach in endothelial integrity (Vergnolle *et al*., 1999b). The involvement of PAR_4 in inflammatory responses has also been implicated by observations *in vivo* demonstrating that thrombin and a PAR_4-AP (but not a PAR_1-AP) induce leukocyte endothelial adhesion and rolling in rat mesenteric venules (Vergnolle *et al*., 2002). Like PAR_1, PAR_2 activation resulting from the intraplantar administration of a PAR_2-AP also causes oedema and granulocyte infiltration from the vasculature (Vergnolle *et al*., 1999a). The oedema caused by PAR_2 activation has been found to depend on the release of neuropeptides from sensory nerves, whereas the extravasation of granulocytes is believed to be due to a direct effect of activating PAR_2 on endothelial cells.

PARs in the immune system

PAR expression has been revealed on many immune cells. PAR_1 is expressed on macrophages (Colognato *et al*., 2003), monocytes (Colognato *et al*., 2003) and mast cells (D'Andrea *et al*., 2000), while other immune cells such as T cells (Mari *et al*., 1996) and Jurkat-T leukaemic cells (Bar-Shavit *et al*., 2002) are seen to be thrombin responsive presumably via PAR_1. PAR_2 expression has been detected on mast cells (D'Andrea *et al*., 2000), eosinophils (Miike *et al*., 2001), monocytes (Colognato *et al*., 2003; Roche *et al*., 2003), macrophages (Colognato *et al*., 2003) and neutrophils (Howells *et al*., 1997; Lourbakos *et al*., 1998; Shpacovitch *et al*., 2004), but the function of the PARs in these cells is not always clear. PAR_3 expression has been detected on eosinophils (Miike *et al*., 2001) and Jurkat-T leukaemic cells (Bar-Shavit *et al*., 2002), while PAR_4 expression is reported to be functional on circulating leukocytes (Vergnolle *et al*., 2002).

The primary response mediated by PARs in the immune system appears to be related to chemotaxis and cytokine release from inflammatory cells. Thrombin is known to stimulate the release of IL-8, IL-6 (Naldini *et al*., 2000) and monocyte chemoattractant protein 1 (Colotta *et al*., 1994). Thrombin also activates natural killer cells to stimulate IL-2 release (Naldini *et al*., 1996). Eosinophil PAR_2 activation results in degranulation and superoxide production (Miike *et al*., 2001), while exposure to mast cell tryptase also induces eosinophil IL-8 release implicating an involvement for PAR_2 in mediating this response (Temkin *et al*., 2002). Neutrophil PAR_2 activation causes shape changes and enhanced CD11b/CD18 expression on these cells (Howells *et al*., 1997). PAR_2 agonists are also known to cause calcium signalling in neutrophils and to enhance neutrophil motility in 3D collagen gel lattices through upregulation of cell surface integrins (Shpacovitch *et al*., 2002).

PARs in the nervous system

The expression of all four PARs has been detected in CNS of rodents (Striggow *et al*., 2001), as revealed in rat brain tissue and in cultured rat glioma cells and astrocytes (Ubl *et al*.,

1998). PAR_1 expression has also been documented in human astrocytoma cells (Grishina *et al.*, 2005) and PAR_2 expression was been found on normal human astrocytes and neurons (D'Andrea *et al.*, 1998). PAR_4 expression has also been reported in human astrocytes (Kaufmann *et al.*, 2000). Further, PAR_1 and PAR_2 are also found in the spinal cord and in isolated rat dorsal root ganglia (Noorbakhsh *et al.*, 2003) as well as in guinea pig myenteric (Corvera *et al.*, 1999) and submucosal neurons (Reed *et al.*, 2003).

Both thrombin and PAR_1-AP have been revealed to have effects on astrocyte morphology and proliferation. Also, for some time, thrombin has been known to affect the morphology and outgrowth of neurites in nerve cells, very likely by activating PAR_1 (Gurwitz and Cunningham, 1990; Suidan *et al.*, 1992; Olianas *et al.*, 2007). At low concentrations, thrombin is observed to reverse astrocyte process extension perhaps leading to compromised integrity of the blood–brain barrier (Noorbakhsh *et al.*, 2003). Higher concentrations of thrombin on the other hand, can cause cell proliferation (Noorbakhsh *et al.*, 2003), and indeed an astrocyte proliferation-dependant disorder, astrogliosis, is revealed to be triggered by PAR_1 activation (Nicole *et al.*, 2005). Thrombin-mediated PAR_1 activation has also been shown to protect neuronal cells and astrocytes from hypoglycaemia or oxidative stress-induced cell death (Vaughan *et al.*, 1995). Thrombin and PAR_1-APs were also reported to exert cytotoxic effects, affecting the morphology of neuronal cells, particularly with respect to shortening the length of the secondary and tertiary neurites (Suidan *et al.*, 1992; Debeir *et al.*, 1998). Indirect PAR_1 involvement in synaptic function has also been indicated through potentiating the effects of *N*-Methyl-D-aspartate receptors by activation of protein kinase C (Gingrich *et al.*, 2000). Both PAR_1 and PAR_4 have also been implicated in activation of microglial cells (Suo *et al.*, 2002, 2003a).

An important role for thrombin in CNS injury is indicated by a number of studies. Increased thrombin levels have been observed at sites of cerebrovascular injury (Smirnova *et al.*, 1996) and high levels of thrombin have been detected in plaques of Alzheimer's disease patient brains (Akiyama *et al.*, 2000). Further, thrombin, very likely acting via PAR_1 in concert with metalloproteinase 9 activity, has been observed to exacerbate neurotoxicity in a murine model of intracerebral haemorrhage (Xue *et al.*, 2006). In this regard, it is of interest that thrombin can either protect neurons and astrocytes from cell death produced by environmental insults or can promote neuronal and astrocytes apoptosis, presumably acting via PAR_1 (Vaughan *et al.*, 1995; Donovan *et al.*, 1997). The importance of thrombin signalling in Alzheimer's disease pathology is further underlined by the observed decrease in levels of protease nexin-1, a thrombin inhibitor, in the Alzheimer's disease affected brain (Vaughan *et al.*, 1994; Choi *et al.*, 1995). *In vitro* studies on cultured neurons show that low concentrations of thrombin and PAR_1-AP can reduce β-amyloid neurotoxicity and astrocytes stellation while also increasing secretion of basic fibroblast growth factor, an index of neurotoxicity (Pike *et al.*, 1996), while PAR_4 activation by thrombin is thought to partly mediate rapid tau aggregation and delayed hippocampal neuronal death (Suo *et al.*, 2003b), thus suggesting that thrombin at least in part through PAR_1 and PAR_4 is involved in Alzheimer's disease pathophysiology.

Recent studies have also revealed a role for PAR_1 in HIV-associated neurodegenerative disorders (Boven *et al.*, 2003; Noorbakhsh *et al.*, 2005). Increased levels of PAR_1 were observed in astrocytes through staining of brain sections from patients with HIV encephalitis. Cultured human astrocytes stimulated with thrombin or PAR1-AP revealed increased levels of IL-1β and nitric oxide synthase (NOS), with levels of PAR1 mRNA also being upregulated in these cells (Boven *et al.*, 2003). PAR_2 expression is increased by tumour necrosis factor α and IL-1β in the HIV-associated dementia brains, and PAR_2 activation exerts a neuroprotective effect, preventing HIV-encoded protein Tat-mediated neuronal death (Noorbakhsh *et al.*, 2005). High levels of mast cells are also seen in brains and cerebrospinal fluid of multiple sclerosis patients (Roznieckl *et al.*, 1995; Noorbakhsh *et al.*, 2003). Thus, PAR_2 activation could potentially play a role in this disease, but it remains to be seen whether these effects are protective or degenerative. PAR_2-mediated coronary vasodilation appears to involve a neurogenic mechanism (McLean *et al.*, 2002), but it is not clear if PAR_2 activation occurs directly on the neuronal elements or on other cells in the nerve cell environment (or both).

Studies on PAR function in the peripheral nervous system have pointed to a role for these receptors in neurogenic inflammation (Steinhoff *et al.*, 2000), hyperalgesia, analgesia (Vergnolle *et al.*, 2001) and itching (Steinhoff *et al.*, 2003), as well as playing roles in nerve regeneration (Vergnolle *et al.*, 2003), gastric epithelial ion secretion (Green *et al.*, 2000) and mucous secretion function (Kawabata *et al.*, 2001a, 2002). Recent evidence has also implicated a role for PAR_2 in potentiating transient receptor potential vanilloid-1 (TRPV-1) and TRPV-4 ion channel function to mediate hyperalgesia and inflammation (Amadesi *et al.*, 2004; Dai *et al.*, 2004; Grant *et al.*, 2007).

PARs in the respiratory system

PAR expression is detectable in a wide range of airway cells, and evidence so far suggests that the PARs play critical roles in pulmonary physiology and pathology. Signalling via PARs may be regulated by a large number of potential PAR proteinase agonists in the airways, including, but not limited to trypsin (Cocks *et al.*, 1999a), mast cell tryptase (Molino *et al.*, 1997), neutrophil proteinases (Uehara *et al.*, 2003), allergens (Asokananthan *et al.*, 2002b) and human airway trypsin (Matsushima *et al.*, 2005). PAR_1 is found on pulmonary fibroblasts (Trejo *et al.*, 1996; Chambers *et al.*, 1998; Ramachandran *et al.*, 2006, 2007), epithelial cells (Asokananthan *et al.*, 2002a), endothelial cells (D'Andrea *et al.*, 1998; Kataoka *et al.*, 2003) and smooth muscle cells (Lan *et al.*, 2000; Walker *et al.*, 2005). PAR_2 expression is also widely distributed in the respiratory system and has been detected on epithelial cells (Knight *et al.*, 2001; Vliagoftis *et al.*, 2001; Ubl *et al.*, 2002; Asokananthan *et al.*, 2002a), smooth muscle cells (Schmidlin *et al.*, 2001; Berger *et al.*, 2001a), endothelial cells (D'Andrea *et al.*, 1998; Schmidlin *et al.*, 2001) and fibroblasts (Akers *et al.*, 2000; Matsushima *et al.*, 2005; Ramachandran *et al.*, 2006, 2007). PAR_3

expression in the airways has not been clearly demonstrated. However, PAR_3 mRNA expression is reported in airway smooth muscle cells (Hauck *et al.*, 1999), epithelial cells (Shimizu *et al.*, 2000) and fibroblasts (Ramachandran *et al.*, 2006). PAR_4 expression appears to be limited to respiratory epithelial (Shimizu *et al.*, 2000; Asokananthan *et al.*, 2002a), endothelial (Kataoka *et al.*, 2003) and smooth muscle cells (Lan *et al.*, 2000).

An important function of PARs in the respiratory system involves the regulation of airway tone by causing either a contraction or relaxation of smooth muscle cells (Lan *et al.*, 2002). Their involvement has also been demonstrated in remodelling of the lung, through promoting secretion of pro-inflammatory and profibrotic mediator release (Mercer *et al.*, 2007), the production of extracellular matrix components and through stimulating cell mitogenesis (Moffatt *et al.*, 2004). An important role for PARs is also indicated in regulating the inflammatory responses in the airways through recruitment of inflammatory cells (Cocks *et al.*, 2001; Lan *et al.*, 2002).

PAR_1 involvement in the airways appears to be important in mediating cell mitogenesis (Blanc-Brude *et al.*, 2005; Walker *et al.*, 2005; Ramachandran *et al.*, 2007), although in some reports thrombin but not PAR_1-AP-mediated mitogenesis has been reported, suggesting a non-PAR mechanism for this action of thrombin (Tran *et al.*, 2003; Walker *et al.*, 2005). Thrombin acting through PAR_1 is also shown to stimulate fibroblast procollagen production (Chambers *et al.*, 1998) and upregulates connective tissue growth factor (Chambers *et al.*, 2000), an autocrine agent which promotes fibroblast mitogenesis and extracellular matrix production. The importance of PAR_1 in mediating fibrotic responses described above is supported further by findings that PAR_1 expression is significantly increased in a bleomycin induced model of pulmonary fibrosis, and that thrombin inhibitors can reduce connective tissue growth factor gene expression and collagen accumulation (Howell *et al.*, 2001, 2002). Interestingly, intratracheal administration of APC, a coagulation cascade inhibitory protein that can activate PAR_1, is reported to be protective in bleomycin induced pulmonary fibrosis (Yasui *et al.*, 2001). A further study has confirmed that when activating PAR_1, APC can stimulate signalling pathways distinct from those activated by thrombin (Riewald *et al.*, 2005). Thus, depending on the activating proteinase, thrombin or APC, PAR_1 activation could potentially cause fibrosis or protect from fibrotic lung disease. PAR_1-mediated thrombin signalling is also shown to upregulate IL-8 secretion from lung fibroblasts as well as IL-6, IL-8 and prostaglandin E2 release from lung epithelial cells (Asokananthan *et al.*, 2002a). Thrombin, acting via PAR_1 in lung epithelial cells is also known to stimulate expression of platelet-derived growth factor (Shimizu *et al.*, 2000). Studies of the ability of PAR_1 to regulate airway tone have revealed a dual role that may be species-dependent. In isolated human bronchial tissue, thrombin and a PAR_1-AP causes contraction (Hauck *et al.*, 1999). A contractile response to PAR_1 activation is also observed *in vivo* for guinea pig bronchi (Cicala *et al.*, 1999). In contrast, in mouse trachea, PAR_1 activation causes an epithelial-dependent relaxation through prostaglandin E2 release (Lan *et al.*, 2001).

PAR_2 plays an important role in regulating airway tone, with studies showing both a protective bronchodilatory response as well as detrimental bronchoconstriction. Pulmonary epithelial PAR_2 activation in isolated pre contracted bronchi from mice (Cocks *et al.*, 1999a; Lan *et al.*, 2001), rats (Cocks *et al.*, 1999a; Chow *et al.*, 2000), guinea pigs (Cocks *et al.*, 1999a) and humans (Cocks *et al.*, 1999a) results in relaxation. Similar results were demonstrated through *in vivo* studies where PAR_2-AP administration was found to attenuate drug-induced bronchoconstriction in mice (Cocks *et al.*, 1999a). On the other hand, activation of PAR_2 on isolated human bronchi has been reported to cause contraction (Schmidlin *et al.*, 2001) and studies done *in vivo* showed that intratracheal, intravenous or intranasal administration of PAR_2 agonists causes bronchoconstriction (Ricciardolo *et al.*, 2000; Schmidlin *et al.*, 2002). A possible explanation for these contradictory results was provided by another study which found that PAR_2 activation results in relaxation in the main bronchi and the trachea, but causes a contractile response in tissues isolated from smaller intrapulmonary bronchi (Ricciardolo *et al.*, 2000). Thus, PAR_2 may be protective in the larger airways, but activation in the smaller bronchioles might increase airway resistance and may thus be detrimental. PAR_2 involvement in mediating eosinophil infiltration into the airways has also been reported (Schmidlin *et al.*, 2002).

One of the major features of airway disease is increased structural cell proliferation and an associated narrowing of airways with reduced airflow. PAR_2 is implicated in mediating some of these responses through inducing proliferation of smooth muscle cells (Berger *et al.*, 2001a), epithelial cells (Cairns *et al.*, 1996) and fibroblasts (Akers *et al.*, 2000; Matsushima *et al.*, 2005). Mast cell tryptase, thought to be acting via PAR_2, is also known to cause hyperresponsiveness and increased mast cell infiltration into subepithelial tissue in isolated human bronchi (Berger *et al.*, 1999). Similar results are reported in *in vivo* studies where airway hyperresponsiveness and inflammatory cell infiltration have been observed in response to PAR_2 agonist administration (Schmidlin *et al.*, 2002; Ebeling *et al.*, 2005). Moreover, studies using airway epithelial cells grown *in vitro* have shown PAR_2-mediated release of IL-6, IL-8 and prostaglandin E2 (Asokananthan *et al.*, 2002a) and allergen stimulated (possibly PAR_2 mediated), GM-CSF and eotaxin release from pulmonary epithelial cells (Sun *et al.*, 2001). Recent reports in cultured airway fibroblasts have also suggested a role for PAR_2 in stimulating cytokine production and adhesion molecule expression (Ramachandran *et al.*, 2006).

PARs in the gastrointestinal system

Of all the body systems, the gastrointestinal tract is most exposed to proteinases, both from physiologically relevant processes such as digestion and from exposure to the bacterial flora of the gut. Under pathological conditions, the gut is also exposed to proteinases released by infectious agents and immune cells. Thus, expression and function of PARs may be especially relevant in the gastrointestinal system.

All of the PARs have been found to be expressed in the gastrointestinal tract, with PAR_1 present on endothelial cells

(Vergnolle *et al*., 2004), epithelial cells (Buresi *et al*., 2001, 2002), smooth muscle cells (Kawabata *et al*., 2004), myofibroblasts (Seymour *et al*., 2003) and on enteric neurons (Corvera *et al*., 1997). PAR_2 expression has been observed on colonic myocytes (Cenac *et al*., 2002), enterocytes (Cenac *et al*., 2002), epithelial cells (Bohm *et al*., 1996), smooth muscle cells (Al-Ani *et al*., 1995), endothelial cells (Vergnolle, 2005a), neurons (Reed *et al*., 2003) and myofibroblasts (Seymour *et al*., 2005). PAR_3 mRNA has also been detected in a variety of tissues including the stomach and small intestine (Ishihara *et al*., 1997), but the cellular expression patterns have not been studied. Mechanical responses to PAR_4-activating peptides indicate that functional PAR_4 is present in rat gastric and colonic tissue (Hollenberg *et al*., 1999; Mule *et al*., 2004).

PAR activation in the gastrointestinal system is involved in ion transport, permeability, motility and inflammation. Activation of both PAR_1 (Buresi *et al*., 2001, 2002) and PAR_2 (Vergnolle *et al*., 1998; Nguyen *et al*., 1999; Green *et al*., 2000; Cuffe *et al*., 2002) can induce intestinal epithelial chloride secretion, thus enhancing the accumulation of intralumenal fluid, possibly contributing to diarrhoea as well as potentially serving a protective function by flushing away pathogens and toxins. Both PAR_1 and PAR_2 are reported to be involved in mediating intestinal barrier permeability (Chin *et al*., 2003; Cenac *et al*., 2004) and thus can regulate the passage of fluids and microorganisms across the gut mucosa. Organ bath studies with isolated tissue have shown that gastric smooth muscle PAR_1 and PAR_2 activation stimulate contraction (Saifeddine *et al*., 1996, 2001), whereas other studies have shown that elsewhere in the GI tract the activation of PAR_1 and PAR_2 can cause relaxation (Corvera *et al*., 1997; Cocks *et al*., 1999b; Mule *et al*., 2002). Studies done *in vivo* have demonstrated that PAR_1 and PAR_2 can enhance gastrointestinal transit, presumably due to a coordinated contraction–relaxation process (Kawabata *et al*., 2001b). PAR_4 activation, due to the administration of PAR_4-activating peptides *in vivo* has also been reported to cause contractile responses in colonic longitudinal muscle (Mule *et al*., 2004). Thus, PARs may mediate both contractile and relaxation responses in the gastrointestinal tract with the net effect of their actions being to enhance gastrointestinal transit. PARs also play an important role in mediating intestinal inflammation and indeed, important roles in diseases such as inflammatory bowel disease and colitis have been identified (Vergnolle *et al*., 2004; Cenac *et al*., 2005; Hansen *et al*., 2005). Investigation of PAR_1 and PAR_2 involvement *in vivo* with the use of models of intestinal inflammation has found that the intraluminal administration of PAR-APs results in a marked inflammatory response characterized by oedema and granulocyte infiltration (Cenac *et al*., 2002; Vergnolle *et al*., 2004). The PAR_2-mediated inflammatory response was shown further to be mediated by the activation of enteric nerves and the release of neuropeptides whereas the PAR_1-mediated response was independent of neuropeptide release.

PARs in the renal system

The initial studies cloning both PAR_1 (Rasmussen *et al*., 1991) and PAR_2 (Nystedt *et al*., 1995) reported abundant expression of mRNA for these receptors in the kidney. Further work has documented the presence of PARs 1, 2 and 4, widely distributed throughout the kidney (Kahn *et al*., 1998; Gui *et al*., 2003). In normal human kidney tissue, PAR_1 is expressed on endothelial, mesangial and epithelial cells (Grandaliano *et al*., 2000) and both PAR_1 and PAR_3 expression has been reported in primary human renal carcinoma cells (Kaufmann *et al*., 2002). Another study done *in vivo* has identified an important role for PAR_1 in a murine model of crescentic glomerular nephritis (Cunningham *et al*., 2000). PAR_1 activation enhanced glomerular crescent formation, T cell and macrophage infiltration, fibrin deposition and elevated serum creatinine, all of which are prominent features of glomerular nephritis. Exposure to hirudin, an inhibitor of thrombin, and studies in PAR_1-knockout mice showed attenuated responses with respect to all of the above indices of glomerular nephritis.

PAR_2 activation has been found to cause vasodilation in a perfused rat kidney model (Trottier *et al*., 2002; Gui *et al*., 2003). In this model, both trypsin and a PAR_2-AP increase renal perfusion flow, whereas the PAR_1-AP, TFLLR-NH_2 and thrombin cause a marked reduction in renal flow (Gui *et al*., 2003). These data suggest that in the renal vasculature, PAR_1 and PAR_2 mediate opposing effects in terms of regulating blood flow and glomerular filtration. These differences between the effects of PAR_1 and PAR_2 activation may be significant in the settings of disease where proteinases may activate one or the other receptor. In addition, a recent report has implicated PAR_2 activation by factor Xa in mediating mesangial cell proliferation (Tanaka *et al*., 2005).

Therapeutic implications: antagonizing proteinase-mediated signalling

Inhibiting PARs and their activating proteinases

Proteinases represent an important class of therapeutic targets, perhaps best typified by the development of the angiotensin converting enzyme inhibitors (Cushman *et al*., 1999). The success of the angiotensin converting enzyme inhibitors as a therapy for hypertension can be greatly tempered by the failure of metalloproteinase inhibitors to prove of value for cancer chemotherapy in humans, despite promising data obtained with these inhibitors in animal model systems (Coussens *et al*., 2002). Even the success of anticoagulant therapy with inhibitors of thrombin is qualified by the bleeding risk that accompanies the use of this approach (Hirsh, 2003; Weitz, 2003). Thus, in thinking of therapeutic approaches that target one of the PARs, efforts to develop receptor inhibitors rather than targeting the activating enzymes have taken priority. As outlined below, substantial success has been achieved to develop PAR_1 antagonists, and there has been some progress in developing inhibitors of PAR_4. PAR_2, however, has presented the greatest challenge, with very limited success in the development of potent receptor antagonists. Largely because of (1) the difficulty in synthesizing serine proteinase inhibitors that are enzyme-selective and (2) the ability of several serine proteinases to activate PARs 2 and 4, the receptor-activating proteinases themselves do not appear to be attractive

therapeutic targets for blocking the activation of these two PARs.

PAR antagonists

Targeting PAR_1. A number of peptide and peptidomimetic PAR_1 antagonists, for both experimental studies and pharmaceutical use in humans are currently available. Work focused on the development of selective and potent non-peptide PAR_1 antagonists has succeeded with the compounds described by the Johnson & Johnson team (RWJ-56110 and RWJ-58259) (Andrade-Gordon *et al.*, 1999; Zhang *et al.*, 2001; Maryanoff *et al.*, 2003) and by the Schering group (Chackalamannil *et al.*, 2005). The RWJ compounds, while effective in non-human models of thrombosis (Derian *et al.*, 2003) may not be clinically suitable as systemic antithrombotics, but may prove successful as local inhibitors of thrombosis for example for arterial stent adducts. The orally active PAR_1 antagonist developed by Schering (PAR_1 antagonist 55 or SCH-205831), which inhibits PAR_1 by competitively inhibiting TL-binding site (Chackalamannil *et al.*, 2005) has been found to be effective as an antithrombotic agent in humans. A recent report describes the successful completion of a phase-II clinical trial of a related PAR_1 antagonist, SCH5303048, providing hope for the availability of an effective orally available PAR_1-targeted antithrombotic compound (Camerer, 2007), (http://www.sch-plough.com/schering_plough/news/release.jsp?releaseID=977603). A number of other PAR_1 antagonists have also been previously described, but their use is limited by relatively low efficacies (Pakala *et al.*, 2000; Nantermet *et al.*, 2002; Kato *et al.*, 2003; Seiler and Bernatowicz, 2003).

Targeting PAR_2. PAR_2 has represented a substantial challenge in terms of developing a high potency receptor-selective antagonist. Some success has been achieved with antagonists based on modified PAR_2 TL peptides, but the potencies of these agents (for example FSLLRY-NH_2 and LSIGRL-NH_2) are too low to be considered for general systemic use (Al-Ani *et al.*, 2002). These peptides are able to inhibit trypsin activation of PAR_2 through possible interactions with the TL docking site, but are of limited use to block activation of PAR_2 by PAR-APs. The peptide, LIGK-NH_2, has also been found to block trypsin and PAR_2-AP activation of the receptor and a peptidomimetic based on this peptide sequence, ENMD-1068 (N1-3-methlybutyryl-N4-6-aminohexanoyl-piperazine), has proved of use in studies done *in vivo*, despite its very low potency (Kelso *et al.*, 2006). Although ENMD-1068 can serve as a 'lead' compound, the synthesis of high potency orally available PAR_2 antagonists is a goal that has yet to be reached. Notwithstanding, the use of PAR_2-targeted antibodies that block proteinase activation of the receptor may ultimately prove of therapeutic value. Proof of principle for the use of such antisera (for example a 'B5' polyclonal or SAM11 monoclonal antibody) has been demonstrated in a murine arthritis model in which both antisera blocked the development of joint swelling (Kelso *et al.*, 2006). That same study demonstrated the possibility of using PAR_2-targeted short interfering RNA as a mode of therapy, but neither the antibody nor the short interfering RNA approach is sufficiently developed to be considered for therapeutic use as of yet.

Targeting PAR_4. PAR_4 antagonism has been demonstrated using the peptide *trans*-cinnamoyl-YPGKF-NH_2 in rat and human platelets (Hollenberg and Saifeddine, 2001), but this peptide can have non-PAR actions that limit its use *in vivo* (Hollenberg *et al.*, 2004). A novel approach to receptor inhibition, through targeting the receptor intracellular loops with palmitoylated membrane penetrating peptides termed pepducins, has succeeded in developing a relatively high potency (micromolar range) PAR_4 antagonist (Covic *et al.*, 2002a, b; Kuliopulos *et al.*, 2003). Although not completely selective for PAR_4 and although the pepducins can have unusual non-PAR pharmacological actions (Hollenberg *et al.*, 2004), the pepducin, P4pal-10 has proved of utility to block PAR_4 activation both *in vivo* and *in vitro* (Covic *et al.*, 2002a, b).

Targeting the proteinases. As pointed out above, the PAR-activating proteinases are challenging therapeutic targets. That said, proteinase inhibition in a restricted environment may have value. For instance, the serine proteinase-dependent, PAR_2-mediated inflammation caused by *Citrobacter rodentium* in a murine model of colitis can be attenuated by the oral administration of the SERPIN, soya trypsin inhibitor. Further, the systemic administration of proteinase inhibitors in a murine model of joint inflammation is able to diminish joint swelling significantly (Kelso *et al.*, 2006). Thus, selective targeting of specific PAR-activating proteinases may have value in selected therapeutic settings.

Conclusions

Proteinases must now be considered as important hormone-like mediators that can signal to cells and tissues by a number of mechanisms including, but by no means restricted to the regulation of PARs, as outlined in Figure 3. Proteinases mediate a number of physiological and pathophysiological settings; and their target PARs are also now recognized for the many pathophysiological roles they can play. The complexity of this system, comprising so many proteinase agonists and their PAR and non-PAR mechanisms of signalling makes it a challenge to study because of the number of variables to be considered. The expression of proteinases and their inhibitory SERPINs, the expression of the PARs and the regulation of their expression by factors such as inflammatory mediators, as well as the specific proteinase-PAR combination present in a local environment, resulting in either receptor activation or silencing, can all contribute to protective or harmful responses *in vivo*. Further, while there is now a growing recognition of the signalling role of proteinases, the next frontier to face is developing an understanding of the biology and physiology of the proteinase inhibitors (for example SERPINs, or tissue inhibitors of metalloproteinases). Clearly, the proteinase–PAR combination that plays a number of roles in a variety of disease states ranging from inflammatory to neurodegenerative disorders, represents an attractive target for the design of new

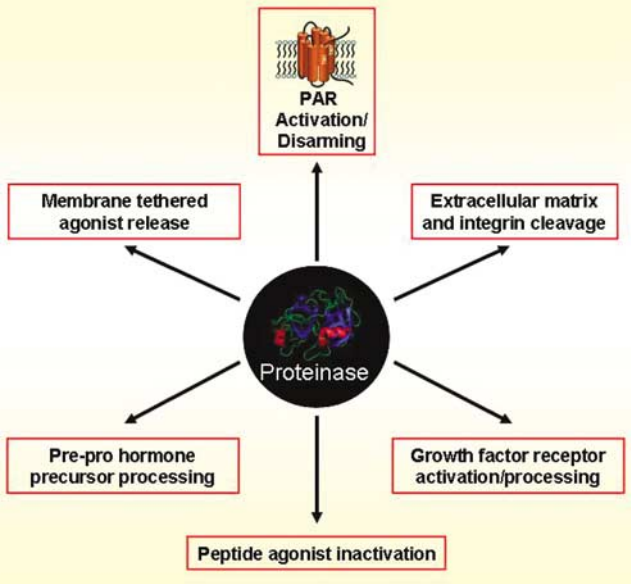

Figure 3 Signalling by proteinases: PARs and more. The scheme illustrates the main mechanisms that proteinases can use to regulate cell signalling in addition to targeting the PARs. The potential proteolytic targets for proteinase-mediated signalling include growth factor receptors, such as the one for insulin that can be activated by trypsin (Cuatrecasas, 1971; Shoelson *et al.*, 1988), membrane-tethered agonists like heparin-binding EGF that can be released by metalloproteinase action to activate the EGF receptor (Prenzel *et al.*, 1999), and either processing of peptide agonist precursors, like angiotensinogen, or inactivation of peptide agonists like neurokinins by membrane-tethered proteinases. Extracellular matrix–integrin interactions that regulate intracellular signalling are also shown as potential targets for enzymes, like the matrix metalloproteinases. Not shown is the ability of a proteinase like thrombin to regulate cell function via its non-catalytic domains (Bar-Shavit *et al.*, 1984, 1986; Glenn *et al.*, 1988). EGF, epidermal growth factor; PAR, proteinase-activated receptor.

therapeutic modalities. It is suggested that the reader 'stay tuned' for exciting developments in this area in the future.

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Conflict of interest

The authors state no conflict of interest.

References

Adam E, Hansen KK, Astudillo Fernandez O, Coulon L, Bex F, Duhant X *et al.* (2006). The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J Biol Chem* **281**: 6910–6923.

Ahlquist RP (1948). A study of the adrenotropic receptors. *American Journal of Physiology* **153**: 586–600.

Akers IA, Parsons M, Hill MR, Hollenberg MD, Sanjar S, Laurent GJ *et al.* (2000). Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2. *Am J Physiol Lung Cell Mol Physiol* **278**: L193–L201.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM *et al.* (2000). Inflammation and Alzheimer's disease. *Neurobiol Aging* **21**: 383–421.

Al-Ani B, Saifeddine M, Hollenberg MD (1995). Detection of functional receptors for the proteinase-activated-receptor-2-activating polypeptide, SLIGRL-NH2, in rat vascular and gastric smooth muscle. *Can J Physiol Pharmacol* **73**: 1203–1207.

Al-Ani B, Saifeddine M, Wijesuriya SJ, Hollenberg MD (2002). Modified proteinase-activated receptor-1 and -2 derived peptides inhibit proteinase-activated receptor-2 activation by trypsin. *J Pharmacol Exp Ther* **300**: 702–708.

Amadesi S, Nie J, Vergnolle N, Cottrell GS, Grady EF, Trevisani M *et al.* (2004). Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. *J Neurosci* **24**: 4300–4312.

Andrade-Gordon P, Maryanoff BE, Derian CK, Zhang HC, Addo MF, Darrow AL *et al.* (1999). Design, synthesis, and biological characterization of a peptide-mimetic antagonist for a tethered-ligand receptor. *Proc Natl Acad Sci USA* **96**: 12257–12262.

Antalis TM, Shea-Donohue T, Vogel SN, Sears C, Fasano A (2007). Mechanisms of disease: protease functions in intestinal mucosal pathobiology. *Nat Clin Pract Gastroenterol Hepatol* **4**: 393–402.

Aragay AM, Collins LR, Post GR, Watson AJ, Feramisco JR, Brown JH *et al.* (1995). G12 requirement for thrombin-stimulated gene expression and DNA synthesis in 1321N1 astrocytoma cells. *J Biol Chem* **270**: 20073–20077.

Asokananthan N, Graham PT, Fink J, Knight DA, Bakker AJ, Mcwilliam AS *et al.* (2002a). Activation of protease-activated receptor (PAR)-1, PAR-2, and PAR-4 stimulates IL-6, IL-8, and prostaglandin E2 release from human respiratory epithelial cells. *J Immunol* **168**: 3577–3585.

Asokananthan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ *et al.* (2002b). House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* **169**: 4572–4578.

Babich M, King KL, Nissenson RA (1990). Thrombin stimulates inositol phosphate production and intracellular free calcium by a pertussis toxin-insensitive mechanism in osteosarcoma cells. *Endocrinology* **126**: 948–954.

Baffy G, Yang L, Raj S, Manning DR, Williamson JR (1994). G protein coupling to the thrombin receptor in Chinese hamster lung fibroblasts. *J Biol Chem* **269**: 8483–8487.

Bar-Shavit R, Kahn AJ, Mann KG, Wilner GD (1986). Identification of a thrombin sequence with growth factor activity on macrophages. *Proc Natl Acad Sci USA* **83**: 976–980.

Bar-Shavit R, Kahn A, Fenton II JW, Wilner GD (1983a). Chemotactic response of monocytes to thrombin. *J Cell Biol* **96**: 282–285.

Bar-Shavit R, Kahn A, Mudd MS, Wilner GD, Mann KG, Fenton II JW (1984). Localization of a chemotactic domain in human thrombin. *Biochemistry* **23**: 397–400.

Bar-Shavit R, Kahn A, Wilner GD, Fenton II JW (1983b). Monocyte chemotaxis: stimulation by specific exosite region in thrombin. *Science* **220**: 728–731.

Bar-Shavit R, Maoz M, Yongjun Y, Groysman M, Dekel I, Katzav S (2002). Signalling pathways induced by protease-activated receptors and integrins in T cells. *Immunology* **105**: 35–46.

Belham CM, Tate RJ, Scott PH, Pemberton AD, Miller HR, Wadsworth RM *et al.* (1996). Trypsin stimulates proteinase-activated receptor-2-dependent and -independent activation of mitogen-activated protein kinases. *Biochem J* **320** (Pt 3): 939–946.

Belting M, Dorrell MI, Sandgren S, Aguilar E, Ahamed J, Dorfleutner A *et al.* (2004). Regulation of angiogenesis by tissue factor cytoplasmic domain signaling. *Nat Med* **10**: 502–509.

Berger P, Compton SJ, Molimard M, Walls AF, N'guyen C, Marthan R *et al.* (1999). Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. *Clin Exp Allergy* **29**: 804–812.

Berger P, Girodet PO, Begueret H, Ousova O, Perng DW, Marthan R *et al.* (2003). Tryptase-stimulated human airway smooth muscle

cells induce cytokine synthesis and mast cell chemotaxis. *Faseb J* **17**: 2139–2141.

Berger P, Perng DW, Thabrew H, Compton SJ, Cairns JA, Mceuen AR *et al*. (2001a). Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* **91**: 1372–1379.

Berger P, Tunon-De-Lara JM, Savineau JP, Marthan R (2001b). Selected contribution: tryptase-induced PAR-2-mediated Ca(2+) signaling in human airway smooth muscle cells. *J Appl Physiol* **91**: 995–1003.

Bilodeau ML, Hamm HE (2007). Regulation of Protease-Activated Receptor (PAR) 1 and PAR4 Signaling in Human Platelets by Compartmentalized Cyclic Nucleotide Actions. *J Pharmacol Exp Ther* **322**: 778–788.

Blanc-Brude OP, Archer F, Leoni P, Derian C, Bolsover S, Laurent GJ *et al*. (2005). Factor Xa stimulates fibroblast procollagen production, proliferation, and calcium signaling via PAR1 activation. *Exp Cell Res* **304**: 16–27.

Bohm SK, Kong W, Bromme D, Smeekens SP, Anderson DC, Connolly A *et al*. (1996). Molecular cloning, expression and potential functions of the human proteinase-activated receptor-2. *Biochem J* **314** (Pt 3): 1009–1016.

Boven LA, Vergnolle N, Henry SD, Silva C, Imai Y, Holden J *et al*. (2003). Up-regulation of proteinase-activated receptor 1 expression in astrocytes during HIV encephalitis. *J Immunol* **170**: 2638–2646.

Brass LF, Manning DR, Williams AG, Woolkalis MJ, Poncz M (1991). Receptor and G protein-mediated responses to thrombin in HEL cells. *J Biol Chem* **266**: 958–965.

Bretschneider E, Kaufmann R, Braun M, Nowak G, Glusa E, Schror K (2001). Evidence for functionally active protease-activated receptor-4 (PAR-4) in human vascular smooth muscle cells. *Br J Pharmacol* **132**: 1441–1446.

Bretschneider E, Kaufmann R, Braun M, Wittpoth M, Glusa E, Nowak G *et al*. (1999). Evidence for proteinase-activated receptor-2 (PAR-2)-mediated mitogenesis in coronary artery smooth muscle cells. *Br J Pharmacol* **126**: 1735–1740.

Bretschneider E, Spanbroek R, Lotzer K, Habenicht AJ, Schror K (2003). Evidence for functionally active protease-activated receptor-3 (PAR-3) in human vascular smooth muscle cells. *Thromb Haemost* **90**: 704–709.

Buddenkotte J, Stroh C, Engels IH, Moormann C, Shpacovitch VM, Seeliger S *et al*. (2005). Agonists of proteinase-activated receptor-2 stimulate upregulation of intercellular cell adhesion molecule-1 in primary human keratinocytes via activation of NF-kappa B. *J Invest Dermatol* **124**: 38–45.

Buresi MC, Buret AG, Hollenberg MD, Macnaughton WK (2002). Activation of proteinase-activated receptor 1 stimulates epithelial chloride secretion through a unique MAP kinase- and cyclo-oxygenase-dependent pathway. *Faseb J* **16**: 1515–1525.

Buresi MC, Schleihauf E, Vergnolle N, Buret A, Wallace JL, Hollenberg MD *et al*. (2001). Protease-activated receptor-1 stimulates Ca(2+)-dependent Cl(-) secretion in human intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* **281**: G323–G332.

Cairns JA, Walls AF (1996). Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J Immunol* **156**: 275–283.

Cairns JA (1998). Mast cell tryptase and its role in tissue remodelling. *Clin Exp Allergy* **28**: 1460–1463.

Camerer E (2007). Unchecked thrombin is bad news for troubled arteries. *J Clin Invest* **117**: 1486–1489.

Camerer E, Huang W, Coughlin SR (2000). Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc Natl Acad Sci USA* **97**: 5255–5260.

Camerer E, Kataoka H, Kahn M, Lease K, Coughlin SR (2002). Genetic evidence that protease-activated receptors mediate factor Xa signaling in endothelial cells. *J Biol Chem* **277**: 16081–16087.

Caughey GH (2007). Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* **217**: 141–154.

Cenac N, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P *et al*. (2007). Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* **117**: 636–647.

Cenac N, Cellars L, Steinhoff M, Andrade-Gordon P, Hollenberg MD, Wallace JL *et al*. (2005). Proteinase-activated receptor-1 is an anti-inflammatory signal for colitis mediated by a type 2 immune response. *Inflamm Bowel Dis* **11**: 792–798.

Cenac N, Chin AC, Garcia-Villar R, Salvador-Cartier C, Ferrier L, Vergnolle N *et al*. (2004). PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and -independent pathways. *J Physiol* **558**: 913–925.

Cenac N, Coelho AM, Nguyen C, Compton S, Andrade-Gordon P, Macnaughton WK *et al*. (2002). Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am J Pathol* **161**: 1903–1915.

Chackalamannil S, Xia Y, Greenlee WJ, Clasby M, Doller D, Tsai H *et al*. (2005). Discovery of potent orally active thrombin receptor (protease activated receptor 1) antagonists as novel antithrombotic agents. *J Med Chem* **48**: 5884–5887.

Chaikof EL, Caban R, Yan CN, Rao GN, Runge MS (1995). Growth-related responses in arterial smooth muscle cells are arrested by thrombin receptor antisense sequences. *J Biol Chem* **270**: 7431–7436.

Chambers RC, Dabbagh K, Mcanulty RJ, Gray AJ, Blanc-Brude OP, Laurent GJ (1998). Thrombin stimulates fibroblast procollagen production via proteolytic activation of protease-activated receptor 1. *Biochem J* **333** (Pt 1): 121–127.

Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ (2000). Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. *J Biol Chem* **275**: 35584–35591.

Chin AC, Vergnolle N, Macnaughton WK, Wallace JL, Hollenberg MD, Buret AG (2003). Proteinase-activated receptor 1 activation induces epithelial apoptosis and increases intestinal permeability. *Proc Natl Acad Sci USA* **100**: 11104–11109.

Choi BH, Kim RC, Vaughan PJ, Lau A, Van Nostrand WE, Cotman CW *et al*. (1995). Decreases in protease nexins in Alzheimer's disease brain. *Neurobiol Aging* **16**: 557–562.

Chow JM, Moffatt JD, Cocks TM (2000). Effect of protease-activated receptor (PAR)-1, -2 and -4-activating peptides, thrombin and trypsin in rat isolated airways. *Br J Pharmacol* **131**: 1584–1591.

Cicala C, Bucci M, De Dominicis G, Harriot P, Sorrentino L, Cirino G (1999). Bronchoconstrictor effect of thrombin and thrombin receptor activating peptide in guinea-pigs *in vivo*. *Br J Pharmacol* **126**: 478–484.

Cicala C, Morello S, Santagada V, Caliendo G, Sorrentino L, Cirino G (2001). Pharmacological dissection of vascular effects caused by activation of protease-activated receptors 1 and 2 in anesthetized rats. *Faseb J* **15**: 1433–1435.

Cocks TM, Moffatt JD (2001). Protease-activated receptor-2 (PAR2) in the airways. *Pulm Pharmacol Ther* **14**: 183–191.

Cocks TM, Fong B, Chow JM, Anderson GP, Frauman AG, Goldie RG *et al*. (1999a). A protective role for protease-activated receptors in the airways. *Nature* **398**: 156–160.

Cocks TM, Sozzi V, Moffatt JD, Selemidis S (1999b). Protease-activated receptors mediate apamin-sensitive relaxation of mouse and guinea pig gastrointestinal smooth muscle. *Gastroenterology* **116**: 586–592.

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

Colotta F, Sciacca FL, Sironi M, Luini W, Rabiet MJ, Mantovani A (1994). Expression of monocyte chemotactic protein-1 by monocytes and endothelial cells exposed to thrombin. *Am J Pathol* **144**: 975–985.

Compton SJ, Cairns JA, Holgate ST, Walls AF (1999). Interaction of human mast cell tryptase with endothelial cells to stimulate inflammatory cell recruitment. *Int Arch Allergy Immunol* **118**: 204–205.

Corvera CU, Dery O, Mcconalogue K, Bohm SK, Khitin LM, Caughey GH *et al*. (1997). Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. *J Clin Invest* **100**: 1383–1393.

Corvera CU, Dery O, Mcconalogue K, Gamp P, Thoma M, Al-Ani B *et al*. (1999). Thrombin and mast cell tryptase regulate guinea-pig myenteric neurons through proteinase-activated receptors-1 and -2. *J Physiol* **517** (Pt 3): 741–756.

Cottrell GS, Amadesi S, Grady EF, Bunnett NW (2004). Trypsin IV: a novel agonist of protease-activated receptors 2 and 4. *J Biol Chem* **279**: 13532–13539.

Coughlin SR (2005). Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost* **3**: 1800–1814.

Coussens LM, Fingleton B, Matrisian LM (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* **295**: 2387–2392.

Covic L, Gresser AL, Talavera J, Swift S, Kuliopulos A (2002a). Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl Acad Sci USA* **99**: 643–648.

Covic L, Misra M, Badar J, Singh C, Kuliopulos A (2002b). Pepducin-based intervention of thrombin-receptor signaling and systemic platelet activation. *Nat Med* **8**: 1161–1165.

Cuatrecasas P (1971). Properties of the insulin receptor of isolated fat cell membranes. *J Biol Chem* **246**: 7265–7274.

Cuffe JE, Bertog M, Velazquez-Rocha S, Dery O, Bunnett N, Korbmacher C (2002). Basolateral PAR-2 receptors mediate KCl secretion and inhibition of Na+ absorption in the mouse distal colon. *J Physiol* **539**: 209–222.

Cumashi A, Ansuini H, Celli N, De Blasi A, O'brien PJ, Brass LF *et al*. (2001). Neutrophil proteases can inactivate human PAR3 and abolish the co-receptor function of PAR3 on murine platelets. *Thromb Haemost* **85**: 533–538.

Cunningham MA, Rondeau E, Chen X, Coughlin SR, Holdsworth S, Tipping PG (2000). Protease-activated receptor 1 mediates thrombin-dependent, cell-mediated renal inflammation in crescentic glomerulonephritis. *J Exp Med* **191**: 455–462.

Cushman DW, Ondetti MA (1999). Design of angiotensin converting enzyme inhibitors. *Nat Med* **5**: 1110–1113.

Dai Y, Moriyama T, Higashi T, Togashi K, Kobayashi K, Yamanaka H *et al*. (2004). Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain. *J Neurosci* **24**: 4293–4299.

D'andrea MR, Derian CK, Leturcq D, Baker SM, Brunmark A, Ling P *et al*. (1998). Characterization of protease-activated receptor-2 immunoreactivity in normal human tissues. *J Histochem Cytochem* **46**: 157–164.

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

Daubie V, De Decker R, Nicaise C, Pochet R (2007). Osteosarcoma cell-calcium signaling through tissue factor-factor VIIa complex and factor Xa. *FEBS Lett* **581**: 2611–2615.

Davie EW, Ratnoff OD (1964). Waterfall sequence for intrinsic blood clotting. *Science* **145**: 1310–1312.

Debeir T, Benavides J, Vige X (1998). Involvement of protease-activated receptor-1 in the *in vitro* development of mesencephalic dopaminergic neurons. *Neuroscience* **82**: 739–752.

Derian CK, Damiano BP, Addo MF, Darrow AL, D'andrea MR, Nedelman M *et al*. (2003). Blockade of the thrombin receptor protease-activated receptor-1 with a small-molecule antagonist prevents thrombus formation and vascular occlusion in nonhuman primates. *J Pharmacol Exp Ther* **304**: 855–861.

Donovan FM, Pike CJ, Cotman CW, Cunningham DD (1997). Thrombin induces apoptosis in cultured neurons and astrocytes via a pathway requiring tyrosine kinase and RhoA activities. *J Neurosci* **17**: 5316–5326.

Dotti CG, Galvan C, Ledesma MD (2004). Plasmin deficiency in Alzheimer's disease brains: causal or casual? *Neurodegener Dis* **1**: 205–212.

Dulon S, Cande C, Bunnett NW, Hollenberg MD, Chignard M, Pidard D (2003). Proteinase-activated receptor-2 and human lung epithelial cells: disarming by neutrophil serine proteinases. *Am J Respir Cell Mol Biol* **28**: 339–346.

Dulon S, Leduc D, Cottrell GS, D'alayer J, Hansen KK, Bunnett NW *et al*. (2005). Pseudomonas aeruginosa elastase disables proteinase-activated receptor 2 in respiratory epithelial cells. *Am J Respir Cell Mol Biol* **32**: 411–419.

Ebeling C, Forsythe P, Ng J, Gordon JR, Hollenberg M, Vliagoftis H (2005). Proteinase-activated receptor 2 activation in the airways enhances antigen-mediated airway inflammation and airway hyperresponsiveness through different pathways. *J Allergy Clin Immunol* **115**: 623–630.

Esmon CT (2004). The impact of the inflammatory response on coagulation. *Thromb Res* **114**: 321–327.

Frungieri MB, Weidinger S, Meineke V, Kohn FM, Mayerhofer A (2002). Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARgamma: Possible relevance to human fibrotic disorders. *PNAS* **99**: 15072–15077.

Fujiwara M, Jin E, Ghazizadeh M, Kawanami O (2005). Activation of PAR4 induces a distinct actin fiber formation via p38 MAPK in human lung endothelial cells. *J Histochem Cytochem* **53**: 1121–1129.

Gingrich MB, Junge CE, Lyuboslavsky P, Traynelis SF (2000). Potentiation of NMDA receptor function by the serine protease thrombin. *J Neurosci* **20**: 4582–4595.

Glenn KC, Frost GH, Bergmann JS, Carney DH (1988). Synthetic peptides bind to high-affinity thrombin receptors and modulate thrombin mitogenesis. *Pept Res* **1**: 65–73.

Grandaliano G, Monno R, Ranieri E, Gesualdo L, Schena FP, Martino C *et al*. (2000). Regenerative and proinflammatory effects of thrombin on human proximal tubular cells. *J Am Soc Nephrol* **11**: 1016–1025.

Grant AD, Cottrell GS, Amadesi S, Trevisani M, Nicoletti P, Materazzi S *et al*. (2007). Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. *J Physiol* **578**: 715–733.

Green BT, Bunnett NW, Kulkarni-Narla A, Steinhoff M, Brown DR (2000). Intestinal type 2 proteinase-activated receptors: expression in opioid-sensitive secretomotor neural circuits that mediate epithelial ion transport. *J Pharmacol Exp Ther* **295**: 410–416.

Grishina Z, Ostrowska E, Halangk W, Sahin-Toth M, Reiser G (2005). Activity of recombinant trypsin isoforms on human proteinase-activated receptors (PAR): mesotrypsin cannot activate epithelial PAR-1, -2, but weakly activates brain PAR-1. *Br J Pharmacol* **146**: 990–999.

Gui Y, Loutzenhiser R, Hollenberg MD (2003). Bidirectional regulation of renal hemodynamics by activation of PAR1 and PAR2 in isolated perfused rat kidney. *Am J Physiol Renal Physiol* **285**: F95–F104.

Gurwitz D, Cunningham D (1990). Neurite outgrowth activity of protease nexin-1 on neuroblastoma cells requires thrombin inhibition. *J Cell Physiol* **142**: 155–162.

Hamilton JR, Moffatt JD, Frauman AG, Cocks TM (2001). Protease-activated receptor (PAR) 1 but not PAR2 or PAR4 mediates endothelium-dependent relaxation to thrombin and trypsin in human pulmonary arteries. *J Cardiovasc Pharmacol* **38**: 108–119.

Hamilton JR, Moffatt JD, Tatoulis J, Cocks TM (2002). Enzymatic activation of endothelial protease-activated receptors is dependent on artery diameter in human and porcine isolated coronary arteries. *Br J Pharmacol* **136**: 492–501.

Hansen KK, Saifeddine M, Hollenberg MD (2004). Tethered ligand-derived peptides of proteinase-activated receptor 3 (PAR3) activate PAR1 and PAR2 in Jurkat T cells. *Immunology* **112**: 183–190.

Hansen KK, Sherman PM, Cellars L, Andrade-Gordon P, Pan Z, Baruch A *et al*. (2005). A major role for proteolytic activity and proteinase-activated receptor-2 in the pathogenesis of infectious colitis. *Proc Natl Acad Sci USA* **102**: 8363–8368.

Hauck RW, Schulz C, Schomig A, Hoffman RK, Panettieri Jr RA (1999). alpha-Thrombin stimulates contraction of human bronchial rings by activation of protease-activated receptors. *Am J Physiol* **277**: L22–L29.

Hirsh J (2003). Current anticoagulant therapy–unmet clinical needs. *Thromb Res* **109** (Suppl 1): S1–S8.

Holinstat M, Voss B, Bilodeau ML, Hamm HE (2007). Protease-activated receptors differentially regulate human platelet activation through a phosphatidic acid-dependent pathway. *Mol Pharmacol* **71**: 686–694.

Hollenberg MD, Compton SJ (2002). International Union of Pharmacology. XXVIII. Proteinase-activated receptors. *Pharmacol Rev* **54**: 203–217.

Hollenberg MD, Saifeddine M (2001). Proteinase-activated receptor 4 (PAR4): activation and inhibition of rat platelet aggregation by PAR4-derived peptides. *Can J Physiol Pharmacol* **79**: 439–442.

Hollenberg MD, Saifeddine M, Al-Ani B, Gui Y (1999). Proteinase-activated receptor 4 (PAR4): action of PAR4-activating peptides in vascular and gastric tissue and lack of cross-reactivity with PAR1 and PAR2. *Can J Physiol Pharmacol* **77**: 458–464.

Hollenberg MD, Saifeddine M, Sandhu S, Houle S, Vergnolle N (2004). Proteinase-activated receptor-4: evaluation of tethered ligand-derived peptides as probes for receptor function and as inflammatory agonists *in vivo*. *Br J Pharmacol* **143**: 443–454.

Holzhausen M, Spolidorio LC, Ellen RP, Jobin MC, Steinhoff M, Andrade-Gordon P *et al.* (2006). Protease-activated receptor-2 activation: a major role in the pathogenesis of Porphyromonas gingivalis infection. *Am J Pathol* **168**: 1189–1199.

Howell DC, Goldsack NR, Marshall RP, Mcanulty RJ, Starke R, Purdy G *et al.* (2001). Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mRNA levels in bleomycin-induced pulmonary fibrosis. *Am J Pathol* **159**: 1383–1395.

Howell DC, Laurent GJ, Chambers RC (2002). Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. *Biochem Soc Trans* **30**: 211–216.

Howells GL, Macey MG, Chinni C, Hou L, Fox MT, Harriott P *et al.* (1997). Proteinase-activated receptor-2: expression by human neutrophils. *J Cell Sci* **110** (Pt 7): 881–887.

Hung DT, Vu TH, Nelken NA, Coughlin SR (1992a). Thrombin-induced events in non-platelet cells are mediated by the unique proteolytic mechanism established for the cloned platelet thrombin receptor. *J Cell Biol* **116**: 827–832.

Hung DT, Vu TK, Wheaton VI, Ishii K, Coughlin SR (1992b). Cloned platelet thrombin receptor is necessary for thrombin-induced platelet activation. *J Clin Invest* **89**: 1350–1353.

Hwa JJ, Ghibaudi L, Williams P, Chintala M, Zhang R, Chatterjee M *et al.* (1996). Evidence for the presence of a proteinase-activated receptor distinct from the thrombin receptor in vascular endothelial cells. *Circ Res* **78**: 581–588.

Ishihara H, Connolly AJ, Zeng D, Kahn ML, Zheng YW, Timmons C *et al.* (1997). Protease-activated receptor 3 is a second thrombin receptor in humans. *Nature* **386**: 502–506.

Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin S (1999). Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest* **103**: 879–887.

Kahn ML, Zheng YW, Huang W, Bigornia V, Zeng D, Moff S *et al.* (1998). A dual thrombin receptor system for platelet activation. *Nature* **394**: 690–694.

Kanke T, Macfarlane SR, Seatter MJ, Davenport E, Paul A, Mckenzie RC *et al.* (2001). Proteinase-activated receptor-2-mediated activation of stress-activated protein kinases and inhibitory kappa B kinases in NCTC 2544 keratinocytes. *J Biol Chem* **276**: 31657–31666.

Kanthou C, Kanse SM, Kakkar VV, Benzakour O (1996). Involvement of pertussis toxin-sensitive and -insensitive G proteins in alpha-thrombin signalling on cultured human vascular smooth muscle cells. *Cell Signal* **8**: 59–66.

Kataoka H, Hamilton JR, Mckemy DD, Camerer E, Zheng YW, Cheng A *et al.* (2003). Protease-activated receptors 1 and 4 mediate thrombin signaling in endothelial cells. *Blood* **102**: 3224–3231.

Kato Y, Kita Y, Hirasawa-Taniyama Y, Nishio M, Mihara K, Ito K *et al.* (2003). Inhibition of arterial thrombosis by a protease-activated receptor 1 antagonist, FR171113, in the guinea pig. *Eur J Pharmacol* **473**: 163–169.

Kaufmann R, Junker U, Nuske K, Westermann M, Henklein P, Scheele J *et al.* (2002). PAR-1- and PAR-3-type thrombin receptor expression in primary cultures of human renal cell carcinoma cells. *Int J Oncol* **20**: 177–180.

Kaufmann R, Patt S, Zieger M, Kraft R, Tausch S, Henklein P *et al.* (2000). The two-receptor system PAR-1/PAR-4 mediates alpha-thrombin-induced [Ca(2+)](i) mobilization in human astrocytoma cells. *J Cancer Res Clin Oncol* **126**: 91–94.

Kaufmann R, Schulze B, Krause G, Mayr LM, Settmacher U, Henklein P (2005). Proteinase-activated receptors (PARs)–the PAR3 Neo-N-terminal peptide TFRGAP interacts with PAR1. *Regul Pept* **125**: 61–66.

Kawabata A, Kinoshita M, Kuroda R, Kakehi K (2002). Capsazepine partially inhibits neurally mediated gastric mucus secretion following activation of protease-activated receptor 2. *Clin Exp Pharmacol Physiol* **29**: 360–361.

Kawabata A, Kinoshita M, Nishikawa H, Kuroda R, Nishida M, Araki H *et al.* (2001a). The protease-activated receptor-2 agonist induces gastric mucus secretion and mucosal cytoprotection. *J Clin Invest* **107**: 1443–1450.

Kawabata A, Kuroda R, Nagata N, Kawao N, Masuko T, Nishikawa H *et al.* (2001b). *In vivo* evidence that protease-activated receptors 1 and 2 modulate gastrointestinal transit in the mouse. *Br J Pharmacol* **133**: 1213–1218.

Kawabata A, Nishikawa H, Saitoh H, Nakaya Y, Hiramatsu K, Kubo S *et al.* (2004). A protective role of protease-activated receptor 1 in rat gastric mucosa. *Gastroenterology* **126**: 208–219.

Kelso EB, Lockhart JC, Hembrough T, Dunning L, Plevin R, Hollenberg MD *et al.* (2006). Therapeutic promise of proteinase-activated receptor-2 antagonism in joint inflammation. *J Pharmacol Exp Ther* **316**: 1017–1024.

Kim S, Foster C, Lecchi A, Quinton TM, Prosser DM, Jin J *et al.* (2002). Protease-activated receptors 1 and 4 do not stimulate G(i) signaling pathways in the absence of secreted ADP and cause human platelet aggregation independently of G(i) signaling. *Blood* **99**: 3629–3636.

Kimura M, Andersen TT, Fenton II JW, Bahou WF, Aviv A (1996). Plasmin-platelet interaction involves cleavage of functional thrombin receptor. *Am J Physiol* **271**: C54–C60.

Knecht W, Cottrell GS, Amadesi S, Mohlin J, Skaregarde A, Gedda K *et al.* (2007). Trypsin IV or mesotrypsin and p23 cleave protease-activated receptors 1 and 2 to induce inflammation and hyperalgesia. *J Biol Chem* **282**: 26089–26100.

Knight DA, Lim S, Scaffidi AK, Roche N, Chung KF, Stewart GA *et al.* (2001). Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. *J Allergy Clin Immunol* **108**: 797–803.

Kong W, Mcconalogue K, Khitin LM, Hollenberg MD, Payan DG, Bohm SK *et al.* (1997). Luminal trypsin may regulate enterocytes through proteinase-activated receptor 2. *Proc Natl Acad Sci USA* **94**: 8884–8889.

Kono T, Barham FW (1971). Insulin-like effects of trypsin on fat cells. Localization of the metabolic steps and the cellular site affected by the enzyme. *J Biol Chem* **246**: 6204–6209.

Koo BH, Chung KH, Hwang KC, Kim DS (2002). Factor Xa induces mitogenesis of coronary artery smooth muscle cell via activation of PAR-2. *FEBS Lett* **523**: 85–89.

Kuliopulos A, Covic L (2003). Blocking receptors on the inside: pepducin-based intervention of PAR signaling and thrombosis. *Life Sci* **74**: 255–262.

Kuliopulos A, Covic L, Seeley SK, Sheridan PJ, Helin J, Costello CE (1999). Plasmin desensitization of the PAR1 thrombin receptor: kinetics, sites of truncation, and implications for thrombolytic therapy. *Biochemistry* **38**: 4572–4585.

Lafleur MA, Hollenberg MD, Atkinson SJ, Knauper V, Murphy G, Edwards DR (2001). Activation of pro-(matrix metalloproteinase-2) (pro-MMP-2) by thrombin is membrane-type-MMP-dependent in human umbilical vein endothelial cells and generates a distinct 63 kDa active species. *Biochem J* **357**: 107–115.

Lamorte VJ, Kennedy ED, Collins LR, Goldstein D, Harootunian AT, Brown JH *et al.* (1993). A requirement for Ras protein function in thrombin-stimulated mitogenesis in astrocytoma cells. *J Biol Chem* **268**: 19411–19415.

Lan RS, Knight DA, Stewart GA, Henry PJ (2001). Role of PGE(2) in protease-activated receptor-1, -2 and -4 mediated relaxation in the mouse isolated trachea. *Br J Pharmacol* **132**: 93–100.

Lan RS, Stewart GA, Henry PJ (2000). Modulation of airway smooth muscle tone by protease activated receptor-1,-2,-3 and -4 in trachea isolated from influenza A virus-infected mice. *Br J Pharmacol* **129**: 63–70.

Lan RS, Stewart GA, Henry PJ (2002). Role of protease-activated receptors in airway function: a target for therapeutic intervention? *Pharmacol Ther* **95**: 239–257.

Laumonnier Y, Syrovets T, Burysek L, Simmet T (2006). Identification of the annexin A2 heterotetramer as a receptor for the plasmin-induced signaling in human peripheral monocytes. *Blood* **107**: 3342–3349.

Li Q, Laumonnier Y, Syrovets T, Simmet T (2007). Plasmin triggers cytokine induction in human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol* **27**: 1383–1389.

Lourbakos A, Chinni C, Thompson P, Potempa J, Travis J, Mackie EJ *et al*. (1998). Cleavage and activation of proteinase-activated receptor-2 on human neutrophils by gingipain-R from Porphyromonas gingivalis. *FEBS Lett* **435**: 45–48.

Lourbakos A, Potempa J, Travis J, D'andrea MR, Andrade-Gordon P, Santulli R *et al*. (2001). Arginine-specific protease from Porphyromonas gingivalis activates protease-activated receptors on human oral epithelial cells and induces interleukin-6 secretion. *Infect Immun* **69**: 5121–5130.

Ma L, Perini R, Mcknight W, Dicay M, Klein A, Hollenberg MD *et al*. (2005). Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets. *Proc Natl Acad Sci USA* **102**: 216–220.

Macfarlane RG (1964). An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature* **202**: 498–499.

Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R (2001). Proteinase-activated receptors. *Pharmacol Rev* **53**: 245–282.

Macfarlane SR, Sloss CM, Cameron P, Kanke T, Mckenzie RC, Plevin R (2005). The role of intracellular Ca2+ in the regulation of proteinase-activated receptor-2 mediated nuclear factor kappa B signalling in keratinocytes. *Br J Pharmacol* **145**: 535–544.

Malarkey K, Belham CM, Paul A, Graham A, Mclees A, Scott PH *et al*. (1995). The regulation of tyrosine kinase signalling pathways by growth factor and G-protein-coupled receptors. *Biochem J* **309** (Pt 2): 361–375.

Mari B, Guerin S, Far DF, Breitmayer JP, Belhacene N, Peyron JF *et al*. (1996). Thrombin and trypsin-induced Ca(2+) mobilization in human T cell lines through interaction with different protease-activated receptors. *Faseb J* **10**: 309–316.

Maryanoff BE, Zhang HC, Andrade-Gordon P, Derian CK (2003). Discovery of potent peptide-mimetic antagonists for the human thrombin receptor, protease-activated receptor-1 (PAR-1). *Curr Med Chem Cardiovasc Hematol Agents* **1**: 13–36.

Matsushima R, Takahashi A, Nakaya Y, Maezawa H, Miki M, Nakamura Y *et al*. (2005). Human airway trypsin-like protease stimulates human bronchial fibroblast proliferation in a protease-activated receptor-2 dependent pathway. *Am J Physiol Lung Cell Mol Physiol*.

Mcintosh KA, Plevin R, Ferrell WR, Lockhart JC (2007). The therapeutic potential of proteinase-activated receptors in arthritis. *Curr Opin Pharmacol* **7**: 334–338.

Mclaughlin JN, Patterson MM, Malik AB (2007). Protease-activated receptor-3 (PAR3) regulates PAR1 signaling by receptor dimerization. *Proc Natl Acad Sci USA* **104**: 5662–5667.

Mclean PG, Aston D, Sarkar D, Ahluwalia A (2002). Protease-activated receptor-2 activation causes EDHF-like coronary vasodilation: selective preservation in ischemia/reperfusion injury: involvement of lipoxygenase products, VR1 receptors, and C-fibers. *Circ Res* **90**: 465–472.

Mcnamara CA, Sarembock IJ, Gimple LW, Fenton II JW, Coughlin SR, Owens GK (1993). Thrombin stimulates proliferation of cultured rat aortic smooth muscle cells by a proteolytically activated receptor. *J Clin Invest* **91**: 94–98.

Mercer PF, Deng X, Chambers RC (2007). Signaling pathways involved in proteinase-activated receptor 1-induced proinflammatory and profibrotic mediator release following lung injury. *Ann NY Acad Sci* **1096**: 86–88.

Miike S, Mcwilliam AS, Kita H (2001). Trypsin induces activation and inflammatory mediator release from human eosinophils through protease-activated receptor-2. *J Immunol* **167**: 6615–6622.

Milia AF, Salis MB, Stacca T, Pinna A, Madeddu P, Trevisani M *et al*. (2002). Protease-activated receptor-2 stimulates angiogenesis and accelerates hemodynamic recovery in a mouse model of hindlimb ischemia. *Circ Res* **91**: 346–352.

Minn A, Schubert M, Neiss WF, Muller-Hill B (1998). Enhanced GFAP expression in astrocytes of transgenic mice expressing the human brain-specific trypsinogen IV. *Glia* **22**: 338–347.

Mirza H, Yatsula V, Bahou WF (1996). The proteinase activated receptor-2 (PAR-2) mediates mitogenic responses in human vascular endothelial cells. *J Clin Invest* **97**: 1705–1714.

Moffatt JD, Page CP, Laurent GJ (2004). Shooting for PARs in lung diseases. *Curr Opin Pharmacol* **4**: 221–229.

Molino M, Barnathan ES, Numerof R, Clark J, Dreyer M, Cumashi A *et al*. (1997). Interactions of mast cell tryptase with thrombin receptors and PAR-2. *J Biol Chem* **272**: 4043–4049.

Molino M, Blanchard N, Belmonte E, Tarver AP, Abrams C, Hoxie JA *et al*. (1995). Proteolysis of the human platelet and endothelial cell thrombin receptor by neutrophil-derived cathepsin G. *J Biol Chem* **270**: 11168–11175.

Molino M, Raghunath PN, Kuo A, Ahuja M, Hoxie JA, Brass LF *et al*. (1998). Differential expression of functional protease-activated receptor-2 (PAR-2) in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* **18**: 825–832.

Morris DR, Ding Y, Ricks TK, Gullapalli A, Wolfe BL, Trejo J (2006). Protease-activated receptor-2 is essential for factor VIIa and Xa-induced signaling, migration, and invasion of breast cancer cells. *Cancer Res* **66**: 307–314.

Mule F, Baffi MC, Cerra MC (2002). Dual effect mediated by protease-activated receptors on the mechanical activity of rat colon. *Br J Pharmacol* **136**: 367–374.

Mule F, Pizzuti R, Capparelli A, Vergnolle N (2004). Evidence for the presence of functional protease activated receptor 4 (PAR4) in the rat colon. *Gut* **53**: 229–234.

Nagai T, Ito M, Nakamichi N, Mizoguchi H, Kamei H, Fukakusa A *et al*. (2006). The rewards of nicotine: regulation by tissue plasminogen activator-plasmin system through protease activated receptor-1. *J Neurosci* **26**: 12374–12383.

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

Naldini A, Carney DH (1996). Thrombin modulation of natural killer activity in human peripheral lymphocytes. *Cell Immunol* **172**: 35–42.

Naldini A, Carney DH, Pucci A, Pasquali A, Carraro F (2000). Thrombin regulates the expression of proangiogenic cytokines via proteolytic activation of protease-activated receptor-1. *Gen Pharmacol* **35**: 255–259.

Nantermet PG, Barrow JC, Lundell GF, Pellicore JM, Rittle KE, Young M *et al*. (2002). Discovery of a nonpeptidic small molecule antagonist of the human platelet thrombin receptor (PAR-1). *Bioorg Med Chem Lett* **12**: 319–323.

Nguyen TD, Moody MW, Steinhoff M, Okolo C, Koh DS, Bunnett NW (1999). Trypsin activates pancreatic duct epithelial cell ion channels through proteinase-activated receptor-2. *J Clin Invest* **103**: 261–269.

Nicole O, Goldshmidt A, Hamill CE, Sorensen SD, Sastre A, Lyuboslavsky P *et al*. (2005). Activation of protease-activated receptor-1 triggers astrogliosis after brain injury. *J Neurosci* **25**: 4319–4329.

Noorbakhsh F, Tsutsui S, Vergnolle N, Boven LA, Shariat N, Vodjgani M *et al*. (2006). Proteinase-activated receptor 2 modulates neuroinflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. *J Exp Med* **203**: 425–435.

Noorbakhsh F, Vergnolle N, Hollenberg MD, Power C (2003). Proteinase-activated receptors in the nervous system. *Nat Rev Neurosci* **4**: 981–990.

Noorbakhsh F, Vergnolle N, Mcarthur JC, Silva C, Vodjgani M, Andrade-Gordon P *et al*. (2005). Proteinase-Activated Receptor-2 Induction by Neuroinflammation Prevents Neuronal Death during HIV Infection. *J Immunol* **174**: 7320–7329.

Nystedt S, Emilsson K, Larsson AK, Strombeck B, Sundelin J (1995). Molecular cloning and functional expression of the gene encoding the human proteinase-activated receptor 2. *Eur J Biochem* **232**: 84–89.

Offermanns S, Laugwitz KL, Spicher K, Schultz G (1994). G proteins of the G12 family are activated via thromboxane A2 and thrombin receptors in human platelets. *Proc Natl Acad Sci USA* **91**: 504–508.

Ogawa K, Yamada T, Tsujioka Y, Taguchi J, Takahashi M, Tsuboi Y *et al*. (2000). Localization of a novel type trypsin-like serine protease, neurosin, in brain tissues of Alzheimer's disease and Parkinson's disease. *Psychiatry Clin Neurosci* **54**: 419–426.

Ogino Y, Tanaka K, Shimizu N (1996). Direct evidence for two distinct G proteins coupling with thrombin receptors in human neuroblastoma SH-EP cells. *Eur J Pharmacol* **316**: 105–109.

Oikonomopoulou K, Hansen KK, Saifeddine M, Tea I, Blaber M, Blaber SI *et al*. (2006). Proteinase-activated receptors, targets for kallikrein signaling. *J Biol Chem* **281**: 32095–32112.

Okamoto T, Nishibori M, Sawada K, Iwagaki H, Nakaya N, Jikuhara A *et al*. (2001). The effects of stimulating protease-activated receptor-1 and -2 in A172 human glioblastoma. *J Neural Transm* **108**: 125–140.

Olianas MC, Dedoni S, Onali P (2007). Proteinase-activated receptors 1 and 2 in rat olfactory system: layer-specific regulation of multiple signaling pathways in the main olfactory bulb and induction of neurite retraction in olfactory sensory neurons. *Neuroscience* **146**: 1289–1301.

Pakala R, Liang CT, Benedict CR (2000). Inhibition of arterial thrombosis by a peptide ligand of the thrombin receptor. *Thromb Res* **100**: 89–96.

Pham CT (2006). Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol* **6**: 541–550.

Pike CJ, Vaughan PJ, Cunningham DD, Cotman CW (1996). Thrombin attenuates neuronal cell death and modulates astrocyte reactivity induced by beta-amyloid *in vitro*. *J Neurochem* **66**: 1374–1382.

Playford RJ, Hanby AM, Patel K, Calam J (1995). Influence of inflammatory bowel disease on the distribution and concentration of pancreatic secretory trypsin inhibitor within the colon. *Am J Pathol* **146**: 310–316.

Post GR, Collins LR, Kennedy ED, Moskowitz SA, Aragay AM, Goldstein D *et al*. (1996). Coupling of the thrombin receptor to G12 may account for selective effects of thrombin on gene expression and DNA synthesis in 1321N1 astrocytoma cells. *Mol Biol Cell* **7**: 1679–1690.

Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C *et al*. (1999). EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**: 884–888.

Puente XS, Sanchez LM, Overall CM, Lopez-Otin C (2003). Human and mouse proteases: a comparative genomic approach. *Nat Rev Genet* **4**: 544–558.

Quinton TM, Kim S, Derian CK, Jin J, Kunapuli SP (2004). Plasmin-mediated activation of platelets occurs by cleavage of protease-activated receptor 4. *J Biol Chem* **279**: 18434–18439.

Rahman A, True AL, Anwar KN, Ye RD, Voyno-Yasenetskaya TA, Malik AB (2002). Galpha(q) and Gbetagamma regulate PAR-1 signaling of thrombin-induced NF-kappaB activation and ICAM-1 transcription in endothelial cells. *Circ Res* **91**: 398–405.

Ramachandran R, Morice AH, Compton SJ (2006). Proteinase-activated receptor2 agonists upregulate granulocyte colony-stimulating factor, IL-8, and VCAM-1 expression in human bronchial fibroblasts. *Am J Respir Cell Mol Biol* **35**: 133–141.

Ramachandran R, Sadofsky LR, Xiao Y, Botham A, Cowen M, Morice AH *et al*. (2007). Inflammatory mediators modulate thrombin and cathepsin-G signaling in human bronchial fibroblasts by inducing expression of proteinase-activated receptor-4. *Am J Physiol Lung Cell Mol Physiol* **292**: L788–L798.

Rasmussen UB, Vouret-Craviari V, Jallat S, Schlesinger Y, Pages G, Pavirani A *et al*. (1991). cDNA cloning and expression of a hamster alpha-thrombin receptor coupled to Ca2+ mobilization. *FEBS Lett* **288**: 123–128.

Reed CE, Kita H (2004). The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol* **114**: 997–1008; quiz 1009.

Reed DE, Barajas-Lopez C, Cottrell G, Velazquez-Rocha S, Dery O, Grady EF *et al*. (2003). Mast cell tryptase and proteinase-activated receptor 2 induce hyperexcitability of guinea-pig submucosal neurons. *J Physiol* **547**: 531–542.

Renesto P, Si-Tahar M, Moniatte M, Balloy V, Van Dorsselaer A, Pidard D *et al*. (1997). Specific inhibition of thrombin-induced cell activation by the neutrophil proteinases elastase, cathepsin G, and proteinase 3: evidence for distinct cleavage sites within the aminoterminal domain of the thrombin receptor. *Blood* **89**: 1944–1953.

Ricciardolo FL, Steinhoff M, Amadesi S, Guerrini R, Tognetto M, Trevisani M *et al*. (2000). Presence and bronchomotor activity of protease-activated receptor-2 in guinea pig airways. *Am J Respir Crit Care Med* **161**: 1672–1680.

Rieser P, Rieser CH (1964). Anabolic Responses Of Diaphragm Muscle To Insulin And To Other Pancreatic Proteins. *Proc Soc Exp Biol Med* **116**: 669–671.

Rieser P (1967). The insulin-like action of pepsin and pepsinogen. *Acta Endocrinol (Copenh)* **54**: 375–379.

Riewald M, Ruf W (2001). Mechanistic coupling of protease signaling and initiation of coagulation by tissue factor. *Proc Natl Acad Sci USA* **98**: 7742–7747.

Riewald M, Ruf W (2005). Protease-activated receptor-1 signaling by activated protein C in cytokine-perturbed endothelial cells is distinct from thrombin signaling. *J Biol Chem* **280**: 19808–19814.

Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W (2002). Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* **296**: 1880–1882.

Robin J, Kharbanda R, Mclean P, Campbell R, Vallance P (2003). Protease-activated receptor 2-mediated vasodilatation in humans *in vivo*: role of nitric oxide and prostanoids. *Circulation* **107**: 954–959.

Roche N, Stirling RG, Lim S, Oliver BG, Chung KF (2003). Regulation of protease-activated receptor-1 in mononuclear cells by neutrophil proteases. *Respir Med* **97**: 228–233.

Rohatgi T, Sedehizade F, Reymann KG, Reiser G (2004). Protease-activated receptors in neuronal development, neurodegeneration, and neuroprotection: thrombin as signaling molecule in the brain. *Neuroscientist* **10**: 501–512.

Roznieckі JJ, Hauser SL, Stein M, Lincoln R, Theoharides TC (1995). Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. *Ann Neurol* **37**: 63–66.

Ruf W, Mueller BM (2006). Thrombin generation and the pathogenesis of cancer. *Semin Thromb Hemost* **32** (Suppl 1): 61–68.

Sabri A, Guo J, Elouardighi H, Darrow AL, Andrade-Gordon P, Steinberg SF (2003). Mechanisms of protease-activated receptor-4 actions in cardiomyocytes. Role of Src tyrosine kinase. *J Biol Chem* **278**: 11714–11720.

Sabri A, Muske G, Zhang H, Pak E, Darrow A, Andrade-Gordon P *et al*. (2000). Signaling properties and functions of two distinct cardiomyocyte protease-activated receptors. *Circ Res* **86**: 1054–1061.

Saifeddine M, Al-Ani B, Cheng CH, Wang L, Hollenberg MD (1996). Rat proteinase-activated receptor-2 (PAR-2): cDNA sequence and activity of receptor-derived peptides in gastric and vascular tissue. *Br J Pharmacol* **118**: 521–530.

Saifeddine M, Al-Ani B, Sandhu S, Wijesuriya SJ, Hollenberg MD (2001). Contractile actions of proteinase-activated receptor-derived polypeptides in guinea-pig gastric and lung parenchymal strips: evidence for distinct receptor systems. *Br J Pharmacol* **132**: 556–566.

Sambrano GR, Huang W, Faruqi T, Mahrus S, Craik C, Coughlin SR (2000). Cathepsin G activates protease-activated receptor-4 in human platelets. *J Biol Chem* **275**: 6819–6823.

Sambrano GR, Weiss EJ, Zheng YW, Huang W, Coughlin SR (2001). Role of thrombin signalling in platelets in haemostasis and thrombosis. *Nature* **413**: 74–78.

Scarborough RM, Naughton MA, Teng W, Hung DT, Rose J, Vu TK *et al*. (1992). Tethered ligand agonist peptides. Structural requirements for thrombin receptor activation reveal mechanism of proteolytic unmasking of agonist function. *J Biol Chem* **267**: 13146–13149.

Schechter NM, Brass LF, Lavker RM, Jensen PJ (1998). Reaction of mast cell proteases tryptase and chymase with protease activated receptors (PARs) on keratinocytes and fibroblasts. *J Cell Physiol* **176**: 365–373.

Schmidlin F, Amadesi S, Dabbagh K, Lewis DE, Knott P, Bunnett NW *et al*. (2002). Protease-activated receptor 2 mediates eosinophil infiltration and hyperreactivity in allergic inflammation of the airway. *J Immunol* **169**: 5315–5321.

Schmidlin F, Amadesi S, Vidil R, Trevisani M, Martinet N, Caughey G *et al*. (2001). Expression and function of proteinase-activated receptor 2 in human bronchial smooth muscle. *Am J Respir Crit Care Med* **164**: 1276–1281.

Schmidt VA, Nierman WC, Maglott DR, Cupit LD, Moskowitz KA, Wainer JA *et al*. (1998). The human proteinase-activated receptor-3 (PAR-3) gene. Identification within a Par gene cluster and characterization in vascular endothelial cells and platelets. *J Biol Chem* **273**: 15061–15068.

Seatter MJ, Drummond R, Kanke T, Macfarlane SR, Hollenberg MD, Plevin R (2004). The role of the C-terminal tail in protease-activated receptor-2-mediated Ca2+ signalling, proline-rich tyrosine kinase-2 activation, and mitogen-activated protein kinase activity. *Cell Signal* **16**: 21–29.

Seiberg M (2001). Keratinocyte-melanocyte interactions during melanosome transfer. *Pigment Cell Res* **14**: 236–242.

Seiler SM, Bernatowicz MS (2003). Peptide-derived protease-activated receptor-1 (PAR-1) antagonists. *Curr Med Chem Cardiovasc Hematol Agents* **1**: 1–11.

Seymour ML, Binion DG, Compton SJ, Hollenberg MD, Macnaughton WK (2005). Expression of proteinase-activated receptor 2 on human primary gastrointestinal myofibroblasts and stimulation of prostaglandin synthesis. *Can J Physiol Pharmacol* **83**: 605–616.

Seymour ML, Zaidi NF, Hollenberg MD, Macnaughton WK (2003). PAR1-dependent and independent increases in COX-2 and PGE2 in human colonic myofibroblasts stimulated by thrombin. *Am J Physiol Cell Physiol* **284**: C1185–C1192.

Shapiro MJ, Weiss EJ, Faruqi TR, Coughlin SR (2000). Protease-activated receptors 1 and 4 are shut off with distinct kinetics after activation by thrombin. *J Biol Chem* **275**: 25216–25221.

Shimizu S, Gabazza EC, Hayashi T, Ido M, Adachi Y, Suzuki K (2000). Thrombin stimulates the expression of PDGF in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* **279**: L503–L510.

Shiosaka S, Yoshida S (2000). Synaptic microenvironments–structural plasticity, adhesion molecules, proteases and their inhibitors. *Neurosci Res* **37**: 85–89.

Shoelson SE, White MF, Kahn CR (1988). Tryptic activation of the insulin receptor. Proteolytic truncation of the alpha-subunit releases the beta-subunit from inhibitory control. *J Biol Chem* **263**: 4852–4860.

Shpacovitch VM, Brzoska T, Buddenkotte J, Stroh C, Sommerhoff CP, Ansel JC *et al*. (2002). Agonists of proteinase-activated receptor 2 induce cytokine release and activation of nuclear transcription factor kappaB in human dermal microvascular endothelial cells. *J Invest Dermatol* **118**: 380–385.

Shpacovitch VM, Varga G, Strey A, Gunzer M, Mooren F, Buddenkotte J *et al*. (2004). Agonists of proteinase-activated receptor-2 modulate human neutrophil cytokine secretion, expression of cell adhesion molecules, and migration within 3-D collagen lattices. *J Leukoc Biol*.

Smirnova IV, Ma JY, Citron BA, Ratzlaff KT, Gregory EJ, Akaaboune M *et al*. (1996). Neural thrombin and protease nexin I kinetics after murine peripheral nerve injury. *J Neurochem* **67**: 2188–2199.

Soreide K, Janssen EA, Korner H, Baak JP (2006). Trypsin in colorectal cancer: molecular biological mechanisms of proliferation, invasion, and metastasis. *J Pathol* **209**: 147–156.

Steinhoff M, Buddenkotte J, Shpacovitch V, Rattenholl A, Moormann C, Vergnolle N *et al*. (2005). Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocr Rev* **26**: 1–43.

Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS *et al*. (2003). Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* **23**: 6176–6180.

Steinhoff M, Vergnolle N, Young SH, Tognetto M, Amadesi S, Ennes HS *et al*. (2000). Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. *Nat Med* **6**: 151–158.

Striggow F, Riek-Burchardt M, Kiesel A, Schmidt W, Henrich-Noack P, Breder J *et al*. (2001). 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* **14**: 595–608.

Suidan HS, Stone SR, Hemmings BA, Monard D (1992). Thrombin causes neurite retraction in neuronal cells through activation of cell surface receptors. *Neuron* **8**: 363–375.

Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S (2001). Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol* **167**: 1014–1021.

Suo Z, Wu M, Ameenuddin S, Anderson HE, Zoloty JE, Citron BA *et al*. (2002). Participation of protease-activated receptor-1 in thrombin-induced microglial activation. *J Neurochem* **80**: 655–666.

Suo Z, Wu M, Citron BA, Gao C, Festoff BW (2003a). Persistent protease-activated receptor 4 signaling mediates thrombin-induced microglial activation. *J Biol Chem* **278**: 31177–31183.

Suo Z, Wu M, Citron BA, Palazzo RE, Festoff BW (2003b). Rapid tau aggregation and delayed hippocampal neuronal death induced by persistent thrombin signaling. *J Biol Chem* **278**: 37681–37689.

Tanaka M, Arai H, Liu N, Nogaki F, Nomura K, Kasuno K *et al*. (2005). Role of coagulation factor Xa and protease-activated receptor 2 in human mesangial cell proliferation. *Kidney Int* **67**: 2123–2133.

Temkin V, Kantor B, Weg V, Hartman ML, Levi-Schaffer F (2002). Tryptase activates the mitogen-activated protein kinase/activator protein-1 pathway in human peripheral blood eosinophils, causing cytokine production and release. *J Immunol* **169**: 2662–2669.

Terayama R, Bando Y, Takahashi T, Yoshida S (2004). Differential expression of neuropsin and protease M/neurosin in oligodendrocytes after injury to the spinal cord. *Glia* **48**: 91–101.

Terayama R, Bando Y, Yamada M, Yoshida S (2005). Involvement of neuropsin in the pathogenesis of experimental autoimmune encephalomyelitis. *Glia* **52**: 108–118.

Tran T, Stewart AG (2003). Protease-activated receptor (PAR)-independent growth and pro-inflammatory actions of thrombin on human cultured airway smooth muscle. *Br J Pharmacol* **138**: 865–875.

Trejo J, Connolly AJ, Coughlin SR (1996). The cloned thrombin receptor is necessary and sufficient for activation of mitogen-activated protein kinase and mitogenesis in mouse lung fibroblasts. Loss of responses in fibroblasts from receptor knockout mice. *J Biol Chem* **271**: 21536–21541.

Trottier G, Hollenberg M, Wang X, Gui Y, Loutzenhiser K, Loutzenhiser R (2002). PAR-2 elicits afferent arteriolar vasodilation by NO-dependent and NO-independent actions. *Am J Physiol Renal Physiol* **282**: F891–F897.

Turgeon VL, Houenou LJ (1997). The role of thrombin-like (serine) proteases in the development, plasticity and pathology of the nervous system. *Brain Res Brain Res Rev* **25**: 85–95.

Ubl JJ, Grishina ZV, Sukhomlin T, Welte T, Sedehizade F, Reiser G (2002). Human bronchial epithelial cells express PAR-2 with different sensitivity to thermolysin. *Am J Physiol Lung Cell Mol Physiol* **282**: L1339–L1348.

Ubl JJ, Vohringer C, Reiser G (1998). Co-existence of two types of [Ca2+]i-inducing protease-activated receptors (PAR-1 and PAR-2) in rat astrocytes and C6 glioma cells. *Neuroscience* **86**: 597–609.

Uehara A, Muramoto K, Takada H, Sugawara S (2003). Neutrophil serine proteinases activate human nonepithelial cells to produce inflammatory cytokines through protease-activated receptor 2. *J Immunol* **170**: 5690–5696.

Uehara A, Sugawara S, Muramoto K, Takada H (2002). Activation of human oral epithelial cells by neutrophil proteinase 3 through protease-activated receptor-2. *J Immunol* **169**: 4594–4603.

Uusitalo-Jarvinen H, Kurokawa T, Mueller BM, Andrade-Gordon P, Friedlander M, Ruf W (2007). Role of protease activated receptor 1 and 2 signaling in hypoxia-induced angiogenesis. *Arterioscler Thromb Vasc Biol* **27**: 1456–1462.

Vanhauwe JF, Thomas TO, Minshall RD, Tiruppathi C, Li A, Gilchrist A *et al*. (2002). Thrombin receptors activate G(o) proteins in endothelial cells to regulate intracellular calcium and cell shape changes. *J Biol Chem* **277**: 34143–34149.

Vaughan PJ, Pike CJ, Cotman CW, Cunningham DD (1995). Thrombin receptor activation protects neurons and astrocytes from cell death produced by environmental insults. *J Neurosci* **15**: 5389–5401.

Vaughan PJ, Su J, Cotman CW, Cunningham DD (1994). Protease nexin-1, a potent thrombin inhibitor, is reduced around cerebral blood vessels in Alzheimer's disease. *Brain Res* **668**: 160–170.

Vergnolle N (2003). The enteric nervous system in inflammation and pain: the role of proteinase-activated receptors. *Can J Gastroenterol* **17**: 589–592.

Vergnolle N (2005a). Clinical relevance of proteinase activated receptors (pars) in the gut. *Gut* **54**: 867–874.

Vergnolle N (2005b). Protease-activated receptors and inflammatory hyperalgesia. *Mem Inst Oswaldo Cruz* **100** (Suppl 1): 173–176.

Vergnolle N, Bunnett NW, Sharkey KA, Brussee V, Compton SJ, Grady EF *et al*. (2001). Proteinase-activated receptor-2 and hyperalgesia: A novel pain pathway. *Nat Med* **7**: 821–826.

Vergnolle N, Cellars L, Mencarelli A, Rizzo G, Swaminathan S, Beck P *et al*. (2004). A role for proteinase-activated receptor-1 in inflammatory bowel diseases. *J Clin Invest* **114**: 1444–1456.

Vergnolle N, Derian CK, D'andrea MR, Steinhoff M, Andrade-Gordon P (2002). Characterization of thrombin-induced leukocyte rolling and adherence: a potential proinflammatory role for proteinase-activated receptor-4. *J Immunol* **169**: 1467–1473.

Vergnolle N, Ferazzini M, D'andrea MR, Buddenkotte J, Steinhoff M (2003). Proteinase-activated receptors: novel signals for peripheral nerves. *Trends Neurosci* **26**: 496–500.

Vergnolle N, Hollenberg MD, Wallace JL (1999b). Pro- and anti-inflammatory actions of thrombin: a distinct role for proteinase-activated receptor-1 (PAR1). *Br J Pharmacol* **126**: 1262–1268.

Vergnolle N, Hollenberg MD, Sharkey KA, Wallace JL (1999a). Characterization of the inflammatory response to proteinase-activated receptor-2 (PAR2)-activating peptides in the rat paw. *Br J Pharmacol* **127**: 1083–1090.

Vergnolle N, Macnaughton WK, Al-Ani B, Saifeddine M, Wallace JL, Hollenberg MD (1998). Proteinase-activated receptor 2 (PAR2)-activating peptides: identification of a receptor distinct from PAR2 that regulates intestinal transport. *Proc Natl Acad Sci USA* **95**: 7766–7771.

Vesey DA, Hooper JD, Gobe GC, Johnson DW (2007). Potential physiological and pathophysiological roles for protease-activated receptor-2 in the kidney. *Nephrology (Carlton)* **12**: 36–43.

Villegas-Mendez A, Montes R, Ambrose LR, Warrens AN, Laffan M, Lane DA (2007). Proteolysis of the endothelial cell protein C receptor by neutrophil proteinase 3. *J Thromb Haemost* **5**: 980–988.

Vliagoftis H, Befus AD, Hollenberg MD, Moqbel R (2001). Airway epithelial cells release eosinophil survival-promoting factors (GM-CSF) after stimulation of proteinase-activated receptor 2. *J Allergy Clin Immunol* **107**: 679–685.

Vliagoftis H, Schwingshackl A, Milne CD, Duszyk M, Hollenberg MD, Wallace JL *et al*. (2000). Proteinase-activated receptor-2-mediated matrix metalloproteinase-9 release from airway epithelial cells. *J Allergy Clin Immunol* **106**: 537–545.

Voss B, Mclaughlin JN, Holinstat M, Zent R, Hamm HE (2007). PAR1, but not PAR4, activates human platelets through a Gi/o/phosphoinositide-3 kinase signaling axis. *Mol Pharmacol* **71**: 1399–1406.

Vouret-Craviari V, Grall D, Van Obberghen-Schilling E (2003). Modulation of Rho GTPase activity in endothelial cells by selective proteinase-activated receptor (PAR) agonists. *J Thromb Haemost* **1**: 1103–1111.

Vouret-Craviari V, Van Obberghen-Schilling E, Scimeca JC, Van Obberghen E, Pouyssegur J (1993). Differential activation of p44mapk (ERK1) by alpha-thrombin and thrombin-receptor peptide agonist. *Biochem J* **289** (Pt 1): 209–214.

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

Walker TR, Cadwallader KA, Mackinnon A, Chilvers ER (2005). Thrombin induces DNA synthesis and phosphoinositide hydrolysis in airway smooth muscle by activation of distinct receptors. *Biochem Pharmacol* **70**: 959–967.

Wang H, Ubl JJ, Stricker R, Reiser G (2002). Thrombin (PAR-1)-induced proliferation in astrocytes via MAPK involves multiple signaling pathways. *Am J Physiol Cell Physiol* **283**: C1351–C1364.

Wang Y, Luo W, Wartmann T, Halangk W, Sahin-Toth M, Reiser G (2006). Mesotrypsin, a brain trypsin, activates selectively proteinase-activated receptor-1, but not proteinase-activated receptor-2, in rat astrocytes. *J Neurochem* **99**: 759–769.

Weitz JI (2003). A novel approach to thrombin inhibition. *Thromb Res* **109** (Suppl 1): S17–S22.

Weksler BB, Ley CW, Jaffe EA (1978). Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A 23187. *J Clin Invest* **62**: 923–930.

Wettschureck N, Offermanns S (2005). Mammalian G proteins and their cell type specific functions. *Physiol Rev* **85**: 1159–1204.

Xu WF, Andersen H, Whitmore TE, Presnell SR, Yee DP, Ching A *et al*. (1998). Cloning and characterization of human protease-activated receptor 4. *Proc Natl Acad Sci USA* **95**: 6642–6646.

Xue M, Hollenberg MD, Yong VW (2006). Combination of thrombin and matrix metalloproteinase-9 exacerbates neurotoxicity in cell culture and intracerebral hemorrhage in mice. *J Neurosci* **26**: 10281–10291.

Yang L, Bae JS, Manithody C, Rezaie AR (2007). Identification of a specific exosite on activated protein C for interaction with protease activated receptor 1. *J Biol Chem* **282**: 25493–25500.

Yasui H, Gabazza EC, Tamaki S, Kobayashi T, Hataji O, Yuda H *et al*. (2001). Intratracheal administration of activated protein C inhibits bleomycin-induced lung fibrosis in the mouse. *Am J Respir Crit Care Med* **163**: 1660–1668.

Yoshida S, Shiosaka S (1999). Plasticity-related serine proteases in the brain (review). *Int J Mol Med* **3**: 405–409.

Yousef GM, Kishi T, Diamandis EP (2003). Role of kallikrein enzymes in the central nervous system. *Clin Chim Acta* **329**: 1–8.

Zhang HC, Derian CK, Andrade-Gordon P, Hoekstra WJ, Mccomsey DF, White KB *et al*. (2001). Discovery and optimization of a novel series of thrombin receptor (par-1) antagonists: potent, selective peptide mimetics based on indole and indazole templates. *J Med Chem* **44**: 1021–1024.

Zoudilova M, Kumar P, Ge L, Wang P, Bokoch GM, Defea KA (2007). beta-Arrestin-dependent Regulation of the Cofilin Pathway Downstream of Protease-activated Receptor-2. *J Biol Chem* **282**: 20634–20646.