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Getting the message across: Pathophysiology and signaling via receptors for polypeptide hormones and proteinases

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Abstract

This article provides a personalized overview of the role of proteinases in generating hormone-like cell signals. Also outlined is the unexpected route of discovery that led one investigator over a four-decade time span, from early studies of the interactions of oxytocin and vasopressin with their neurophysin binding proteins to current studies of the tethered ligand activation mechanism that is unique for the G-protein-coupled family of proteinase-activated receptors (PARs). The focus is not only on the intriguing PAR receptor family, but also on alternative mechanisms whereby proteinases activate signal transduction pathways. Also summarized are the potential physiological and pathophysiological roles that PARs may play in the setting of inflammatory disorders ranging from arthritis to colitis. The therapeutic implications of considering PARs as drug targets are also discussed.

This overview aims to outline the field of hormone-like signaling by proteinases as I have come to know it. A second objective is to provide a perspective for the unexpected route that has led to my current research focus on proteinases as regulators of signal transduction. For instance, although my doctoral the-

sis could not have predicted the area of research I am now following, the thread of that work can be found in the approach to the projects currently in progress, long after the completion of my thesis project. Along the way, the themes have been consistent: (1) proteolytic generation of peptide hormones from precursor proteins, (2) structure-activity relationships for the interactions of peptide hormones with their binding proteins and receptors, (3) signal transduction mechanisms and the molecular pharmacology of polypeptide hormones and their receptors; and more recently, (4) signaling by proteolytic mechanisms.

My doctoral project, which dealt with the binding of oxytocin and vasopressin to their neurophysin storage proteins in the neurosecretory granules of the neurohypophysis,¹ involved the proteolytic generation of active polypeptide hormones from their precursors (see Figure 1). At that time, the mechanisms for the processing of proinsulin to insulin were just coming into view.² Work with the neurohypophysial hormones left me with several key perspectives that have directed the work that I have done since then: (1) the

FIGURE 1. Multiple mechanisms of proteinase-mediated signaling.

The scheme demonstrates the many ways in which proteinases can trigger hormone-like signals in target tissues. Adapted and re-drawn from reference 73

importance of proteinases as instrumental players in the generation of active polypeptides from precursor targets, (2) the importance of the structural basis for the interaction of peptide hormones with their binding targets (e.g., the binding of oxytocin and vasopressin either to their neurophysins or to their receptors) and (3) the importance of structure-activity profiles for understanding the interactions of ligands such as oxytocin and vasopressin with their receptors. For instance, the oxytocin and vasopressin receptors can bind both peptides, but with different selective affinities. As a consequence, oxytocin, at a sufficiently high concentrations, can cause antidiuresis via the vasopressin receptor; and reciprocally, vasopressin at relatively high concentrations can cause uterine contraction via the oxytocin receptor. Thus, my doctoral studies set the stage for my long-lasting interest in hor-

mone action, receptor mechanisms and the potential roles of proteinases in that regulatory process. These themes, developed in the course of my doctoral work, have been the compass that have led me to the projects done over the past 40 years or so. The following sections deal with these issues in greater detail, as reflected in part by the directions my work has taken since the late 1960s.

Proteinases and Signalling

The general overview of the many roles that proteinases can play in the process of signaling is summarized in Figure 1. It was already evident, prior to the late 1960s, that proteinases were responsible not only for generating active peptide hormones from precursors, but also for generating active peptides in the context of the complement and coagulation cascades in-

volving serine proteinases like complement C3 convertase and thrombin. Thus, the cartoon in Figure 1 depicts a very generalized overview showing the many sites at which proteinase activity can play key roles for polypeptide hormone function - ranging from agonist generation and turnover to cell activation. What was not fully appreciated was that proteinases could also regulate hormone receptor function directly. Thus, it was an eye-opener for me upon joining the Cuatrecasas laboratory in 1973 to learn that trypsin could either 'dis-arm' the insulin receptor, so as to reduce or abolish its response to insulin^{3,4} or in contrast, mimic the action of insulin. For instance, long exposure to trypsin 'disarms' the insulin receptor, preventing insulin binding and action, but very short exposure of adipocytes to limit trypsin proteolysis both stimulates glucose uptake/oxidation and inhibits lipolysis.⁵ This insulin-like action of proteinases had already been well documented in the literature by the Riesers,^{6,7} who observed the ability of serine proteinases to mimic the action of insulin (increased glycogen formation) in a rat diaphragm preparation. The insulin-like action of trypsin was subsequently discovered to be due to the dis-inhibition/activation of the insulin receptor by truncation of the insulin receptor's extracellular alpha-subunit.⁸

At the time, work was progressing to understand the mechanisms whereby the insulin receptor exerted its effects in target cells, and other hormones were being described in terms of their insulin-like actions, related to agonist-induced increases in protein synthesis, cell replication and the triggering of a number of 'anabolic' responses. In the Cuatrecasas lab during the early 1970s, I became involved in discussions that turned at one point to focus on the mitogenic and anabolic effects of trypsin and thrombin in cultured cell systems. Like insulin-Sepharose⁴, bead-bound trypsin or thrombin were observed to exhibit biological activity, presumably by acting via a membrane-localized receptor^{9,10}. The mitogenic effect of bead-bound trypsin and thrombin on cultured chicken fibroblasts was comparable to that of epidermal growth factor (EGF),

but the mechanism whereby these serine proteinases caused their anabolic, insulin-like effects was not known at the time. However, the same year as the mitogenic action of thrombin was demonstrated, it was observed that the addition of EGF to membrane preparations could stimulate the phosphorylation of membrane substrates on threonine and it was suggested that the EGF receptor might be a protein kinase¹¹. Shortly thereafter, it became apparent that activation of the EGF receptor resulted in phosphorylation of the receptor and its substrates on tyrosine,¹² as was subsequently found for the insulin receptor kinase and its substrates.^{13,14} Thus, it became possible to make a conceptual link between the anabolic or insulin-like actions of agonists like insulin, EGF and other hormones with the triggering of tyrosine kinase activity.

Non-Receptor Tyrosine Kinase Pathways and the Common Actions of EGF and Thrombin in Smooth Muscle: Insights for Proteinase-Activated Receptors (PARs).

Once the link was made between the activation of tyrosine kinase signaling pathways and anabolic responses in cells, it was hypothesised that agonists like vasopressin, angiotensin and thrombin, known to act via G-protein-coupled receptors to cause anabolic effects, might, like growth factors, also act via tyrosine kinase pathways. We had established that EGF regulates smooth muscle tension over a short time frame (minutes).^{15,16} This time course was much more rapid than the time required for EGF's transcriptional and translational effects. The contractile actions of EGF mimicked the contractile effects of vasopressin, angiotensin and thrombin, all of which, like EGF, are known to stimulate cell growth. Thus, when the G-protein-coupled receptor for thrombin was cloned (PAR₁),¹⁷ we were eager to determine if thrombin might mimic the actions of EGF in the smooth muscle assays we were studying. In many ways, the ability of thrombin to regulate vascular and gastric smooth muscle tension^{18,19} did reflect the actions of EGF. We

postulated that both EGF and thrombin might use common tyrosine kinase signaling pathways to cause an increase in smooth muscle tension. Indeed, with the use of selective non-receptor tyrosine kinase inhibitors, including tyrphostin 47/AG213 and the Src-selective inhibitor, PP1, we showed that the G-protein-coupled thrombin receptor (PAR₁) and EGF both used parallel signaling pathways to cause a contractile response involving as-yet-unidentified non-receptor tyrosine kinases.²⁰ This work was amongst the first to point to a role for the non-receptor tyrosine kinases, including MAPkinase-kinase (MEK), in regulating G-protein signaling in general and specifically in regulating intact vascular and gastric smooth muscle function.

It was at this point that the focus of my laboratory efforts turned to understanding in greater depth the mechanisms and roles whereby proteolytic enzymes could regulate tissue function in a manner that mimicked the actions of polypeptide hormones. At the time, it was clear that proteinases could act directly on the receptors for agonists like insulin and EGF either to activate or disarm the receptors, thereby either mimicking or blocking the actions of hormones; and intriguingly, the proteinases could act by cleaving and activating their own receptors (Proteinase-Activated Receptors, or PARs) to regulate G-protein-coupled processes. Moreover, these proteinase-triggered G-protein-coupled signal pathways could include both tyrosine-kinase-dependent and tyrosine kinase-independent signal pathways, with all the diversity of signaling that is now recognised for the G-protein-coupled receptor superfamily.

Multiple Mechanisms for Proteinase-Mediated Signaling

PARs rapidly became a major focus of my laboratory efforts in the early 1990s. Before dealing with the impact of PARs on cell and tissue function, it is important to place this unique mechanism for proteinase signaling into the context of the multiple ways by

which proteinases can signal (Figure 1). In addition to the signaling mediated by: (1) converting prohormones to active peptides, (2) regulating growth factor receptor signaling and (3) cleaving and activating PARs (Figures 1 and 2), proteinases can also signal via a number of other processes that involve both extracellular plasma membrane receptors and intracellular targets. For instance, by disrupting interactions between the extracellular matrix and cell surface integrins, the 'outside-in' signaling by integrins can be regulated. In this regard, the ability of thrombin to activate metalloproteinases, that in turn can lead to PAR-independent signaling via remodeling of the extracellular matrix, may be of importance in this regulation. In addition, the release from the cell surface of receptor agonists, like heparin-binding-EGF, can activate receptors via an autocrine/paracrine mechanism. This release of cell-surface agonists can, in principle, be triggered either by exogenous proteinases, or via an intracellular trans-activation process involving a cell membrane-localised metalloproteinase.²¹ A novel proteinase-mediated signaling process has been reported for plasmin, which, in addition to regulating PARs, can cleave cell surface annexin A2 to stimulate chemotaxis in human monocytes.²² Whether this annexin A2 mechanism can be activated by proteinases other than plasmin is an open question. In addition to signaling that involves the proteolytic activity of proteinases, there can be non-catalytic interactions between the enzyme domains and other receptors. For instance, within its structure, thrombin contains a sequence that on its own can cause mitogenic and chemotactic responses.^{23,24} These actions of thrombin via its non-catalytic domain appear to be due to a receptor that is not one of the PARs²⁵. The ability of proteinases to activate signaling at the cell surface is complemented by the intracellular proteolysis mechanisms like those triggered by caspases to regulate cellular apoptosis and the production of cytokines. Thus, in addition to their ability to signal via PARs, as will be described in the following sections, there is a diverse set of mechanisms whereby proteinases can trigger

FIGURE 2. Model of signaling by proteinase-activated receptors.

The scheme uses rat PAR₂ as a representative model. The putative cystine disulphide links between extracellular loops 1 and 2 are shown (C-C). The cartoon shows the tethered ligand domain in the intact receptor (left-hand panel: ...SKGR/SLIGRL...) that can be cleaved proteolytically by trypsin at the indicated target site (R/S) to release the N-terminal sequence (...SKGR) and reveal the receptor-activating tethered ligand (SLIGRL....: middle panel). The revealed tethered ligand promotes the interaction of PAR₂ with its signal-generating G-proteins (G_q, G_i, G_{12/13}) to cause a cellular response. Alternatively, in the absence of proteolysis (Right-hand panel), a PAR-activating peptide (SLIGRL-amide) can interact with the receptor to activate signaling via the same G-protein-coupled mechanisms. If the tethered ligand domain shown in the left-hand panel is cleaved (//) C-terminal to the tethered ligand domain (SKGR/SLIGRL ...//), the receptor will be disarmed and inaccessible for proteolytic activation by the revealed tethered ligand. Adapted and redrawn from reference 34.

cell signaling (Figure 1). This diversity is amplified by the many distinct proteinase families that can in principle activate signaling by both common and distinct mechanisms.

Par-Mediated Signaling: Activation by Proteinases or by Receptor-Derived Activating Peptides

The search for the receptor responsible for the actions of thrombin on platelets led unexpectedly to the cloning of a G-protein-coupled receptor that is the target for thrombin proteolysis and activation.^{17,26} As discovered by Coughlin and colleagues,¹⁷ the unique mechanism of receptor activation involves the proteolytic cleavage of the N-terminal receptor domain to unmask a tethered receptor-activating sequence (middle panel, Figure 2). As important as the discovery of this unique activation mechanism, was the observation by Coughlin and colleagues¹⁷ that a synthetic peptide

with a sequence in common with the revealed tethered ligand can activate the receptor without the need for proteolysis (right panel, Figure 2). We were intrigued by the ability to use the so-called receptor-activating peptides to mimic the actions of thrombin and immediately began to use these receptor-activating peptides to evaluate the actions of thrombin in vascular and gastric smooth muscle preparations.^{18,19,29} Structure-activity work with these receptor-activating peptides by us¹⁹ and by others^{27,28} quickly localised the receptor-activating amino acids within the first five residues of the proteolytically-revealed tethered ligand. In our bioassay systems, we noted significant differences in peptide potencies for triggering smooth muscle contraction, compared with endothelium-dependent NO-mediated vascular relaxation and we realized that the peptides might act via multiple receptors.^{29,30,31} At that time, restriction fragment gene

TABLE 1. PAR-activating peptides and their receptor-inactive control peptides[†]

Receptor	PAR-activating peptides and <i>standard inactive</i> control peptides
PAR ₁	TFLLR-NH ₂ <i>FTLLR-NH₂</i>
PAR ₂	SLIGRL-NH ₂ ; 2fuoyl-LIGRLO-NH ₂ <i>LSIGRL-NH₂</i>
PAR ₄	AYPGKF-NH ₂ <i>YAPGKF-NH₂</i>
PAR ₃	Does not activate via PAR-APs; its tethered ligand sequences activate PARs 1 and 2

[†]This table shows the peptide sequences of the receptor-selective PAR-activating peptides (upper case) along with the receptor-inactive control peptide sequences (Upper case *italics*) Amino acids are designated by their one-letter codes.

mapping suggested that there was only a single thrombin receptor. Turning to the same principles as those used by Ahlquist³² to identify alpha and beta adrenoceptors, we measured the relative potencies of the PAR-activating peptides in the vascular relaxation assays to identify receptor subtypes²⁹, well in advance of the cloning of the other three members of the PAR family.^{33,34} When the second member of the PAR family³⁵ was cloned in 1994, our understanding of the PAR-activating peptide structure-activity profiles for the vascular relaxant and gastric contractile bioassays positioned us well to show that both of these tissues can be regulated by PAR₂.³⁶ The cloning of PARs 3 and 4 were still in progress at that time and not published; but our structure-activity work with the receptor-activating peptides indicated that it would be possible to design receptor-selective agonists for each of the PARs (Table 1).

In summary, by the turn of this century, proteinases were known to be able to signal to cells by cleaving and activating proteinase-activated receptors. It was also evident that, in addition to the distinct receptors for thrombin on human and rodent platelets, PAR₂ represented another member of the family that could regulate vascular and gastric tissue. By 1995, structure-activity work with the PAR-activating peptides showed unequivocally that there was a receptor distinct from PAR₁ on rodent platelets³⁷; but PARs 3

and 4, which accounted for the observations of Kinlough-Rathbone and co-workers,³⁷ had not yet been cloned.³⁸ Nonetheless, the unusual features of the PARs were well-appreciated (Figure 2) in terms of: (1) their unique mechanism of activation, involving a tethered ligand mechanism (middle Panel, Figure 2), (2) their ability to be activated selectively by receptor-specific synthetic activating peptides, based on the sequences of the revealed tethered ligand (Right Panel, Figure 2), (3) their ability to be activated or inactivated/disarmed selectively by a variety of serine proteinases (e.g., thrombin activates PARs 1 and 4, but not PAR₂; trypsin at low concentrations can activate PAR₂ and PAR₄, but disarms PAR₁³⁹; elastase disarms PAR₂)⁴⁰ and (4) the ability of receptor glycosylation⁴¹ to regulate proteolytic activation of the PARs. Of note, a proteinase that cleaves C-terminal to the tethered ligand domain sequence (...//.. = cleavage) shown in the left panel of Figure 2 (...SKGRSLIGRL...//..) will disarm the PAR to prevent its activation by a second proteinase. Nonetheless, apart from the accepted physiological role for PARs 1 and 4 in the regulation of platelet and vascular function, and the observations *in vitro* that PAR₂ can regulate vascular and gastric smooth muscle function, it was not possible to predict the potential physiological roles that PARs might play *in vivo*. It was to this topic that my lab's attention now turned, with the use of the PAR-selective receptor-activating peptides that we had helped develop (Table 1).

Discovering The Pathophysiology of the PARs: Use of PAR-Selective Peptide Agonists to Evaluate Roles for Pars In Vitro and In Vivo

When discovered as the target for the action of thrombin, the platelet G-protein-coupled receptor now designated as PAR₁ was readily accepted as playing a role in signaling via the coagulation cascade. It was soon realized that this receptor is involved in the previously known action of thrombin on the vasculature, involving endothelium-dependent relaxation and endo-

TABLE 2. Tissue bioassays and disease models for evaluating PAR actions†

Assay	Reference
<i>In vitro tissue assays</i>	Summarized in 64, 73
Vascular relaxation/contraction (aorta, pulmonary, renal, mesenteric arteries)	18, 30, 34, 36
Gastric contraction (circular and longitudinal gastric smooth muscle)	19, 29, 31
Platelet aggregation/calcium signaling	65, 66
<i>In vivo inflammation assays</i>	Summarized in 64, 73
Hind paw edema	46, 47, 49, 67
Mechanical and thermal nociception	68
Colitis	48, 53, 63, 69
Adjuvant arthritis	54, 55
Allergic sensitization and lung function	56, 71, 72

†This table lists representative studies using the different bioassays done either *in vitro* or *in vivo* in which my laboratory has been involved to assess PAR function. The referenced articles are intended to be representative only and do not describe comparable work that is well described in the literature by others. Many of the assays are put into context in recent reviews^{64,73}.

thelium-independent vasoconstriction.^{42,43} Roles for PAR₁ and the other members of the PAR family, apart from those they played in the cardiovascular system (including platelet function), were essentially unknown at the time PARs were being cloned. In order to assess the roles of PARs *in vivo* and *in vitro*, we turned to a pharmacological approach.

Using PAR-selective receptor-activating peptides in wild-type and PAR-null mouse tissues.

To assess PAR function, we realized that the receptor-activating peptides would represent key tools. We, therefore, spent considerable energy developing receptor-selective peptide agonists for PARs 1, 2 and 4, along with suitable receptor-inactive, but closely related (control) peptides with comparable compositions but partial reverse sequences (Table 1). As a complement to the use of PAR-selective agonists (and antagonists, which have been a challenge to develop), the availability of PAR-null mice was of inestimable value for elucidating the physiological function of the PARs and validating the receptor-selectivity of the activating peptides. Our work showed that the use of the receptor-activating peptides to mimic PAR activation required a careful pharmacological evaluation, since some of the PAR-activating peptides turned out to have actions that are PAR-independent.⁴⁴ Thus, the

use of ‘control’ peptides, which cannot activate PARs, is an essential step in any study of PARs using the PAR-activating peptides (Table 1). Further, the availability of PAR-null mice enabled a test of the selectivity of the PAR-targeted activating peptides and alerted us to the possibility of non-PAR actions of the peptides. Thus, a thorough pharmacological approach using concentration-effect curves for the PAR-activating peptides, together with the appropriate control peptides, and using bioassays either *in vitro* or *in vivo* that permitted an accurate assessment of the peptide activities in PAR-null mice, allowed us to determine the involvement of PARs in a specific physiological process of interest.

Assessing PAR function in animal models in vivo.

Pivotal to the use of the receptor-selective activating peptides and the PAR-null mice was the availability of reliable *in vitro* bioassays and *in vivo* disease models in which the actions of the PARs could be monitored (Table 2). In the mid-1990s, using a rat intestinal ovalbumin sensitization protocol, it was discovered that intestinal mast cells could release rat mast cell proteinase II into the blood stream in response to ovalbumin exposure.⁴⁵ We, therefore, hypothesized that these released proteinases might have systemic

effects via PARs, either by targeting the vasculature or by triggering an inflammatory response. To test this hypothesis, we began using the PAR-activating peptides in several models of peripheral and intestinal inflammation.^{46,47,48} Perhaps surprisingly, the inflammatory action of PAR₂ activation in the paw involves not only an impact on the vasculature (both endothelium and smooth muscle), but also activation of sensory nerves, which play a key role in the process of PAR-induced swelling and neutrophil infiltration⁴⁹. In addition to the promotion of swelling and neurogenic inflammation, PAR₂ is now also recognised to play a role in the pain perception that is involved in the inflammatory response.⁵⁰ The next phase of study was to evaluate other *in vivo* disease models of inflammation and other smooth muscle tissues for the possible roles that PARs might play.

Roles for PARs In Vivo: Therapeutic Implications

PARs as therapeutic targets for inflammatory disease.

In our initial work, we established a number of cell culture models and *in vitro* tissue models (primarily vascular and gastric smooth muscle assays) in which to evaluate: (1) PAR agonist pharmacology and function (e.g., activation of calcium or MAPkinase signaling)^{39,51} and (2) the molecular pharmacology of the PARs as unique G-protein-coupled receptors.^{51,52} Based on the initial work with the Paw edema model, which identified the inflammatory role that PARs might play *in vivo*, we looked immediately for other *in vivo* disease models of inflammation, including infectious colitis⁵³, adjuvant-induced arthritis,^{54,55} allergic sensitization to inhaled antigens⁵⁶ and an experimental autoimmune encephalitis model of multiple sclerosis⁵⁷. Not only does PAR₂ appear to play a role in pulmonary function related to dust-mite and cockroach-induced asthma *in vivo*,^{58,59} but this receptor is also implicated in pulmonary infections ranging from pseudomonas⁶⁰ to viral H1N1/influenza-induced disease⁶¹. Our focus has been primarily on the potential role of PAR₂, which can be seen to play a key role

in all of the models we have evaluated to date. However, particularly in the models of infectious colitis⁵³ and adjuvant-induced arthritis,⁵⁵ the data are of clear therapeutic relevance. Of note, in the infectious colitis and adjuvant arthritis models, blocking the activation of PAR₂ can attenuate the inflammatory response.^{53,55} Although the development of high potency small molecule PAR₂ antagonists has proved less successful than the development of PAR₁ antagonists,⁶² several other approaches, in addition to using a receptor antagonist,⁵⁵ have proved of value: (1) neutralizing the activating proteinase,^{53,55} (2) blocking proteinase-triggered activation with a targeted antibody that restricts access to the cleavage/activation site^{53,55} and (3) down-regulating the receptor with the use of PAR₂-targeted siRNA.⁵⁵ Thus, PAR₂ appears as an attractive therapeutic target for dealing with inflammatory disease. That said, activation of PAR₂ may have both an inflammatory and anti-inflammatory impact.⁶³ In summary, targeting PAR₂ for therapeutic purposes may be able to take advantage of both agonists and antagonists in selected settings.

Conclusions and Looking to the Future

As a consequence of my pharmacological training related to structure-activity relationships for post-pituitary peptide hormones, and my early interest in the insulin-like actions of thrombin, my work has now come to focus on: (1) the proteinase-activated receptor (PAR) family of G-protein-coupled receptors and (2) the signaling mechanisms and hormone-like properties of proteinases. As outlined in Figure 1, the PAR system represents but one of the many mechanisms whereby proteinases can generate hormone-like signals. These actions of proteinases via the PARs and other processes, are implicated in a variety of pathophysiological states, particularly inflammatory processes. As outlined in Table 3, there are a number of disease settings in which PARs and proteinase-mediated signaling can be expected to play a role, and future work is aimed at evaluating those possibilities.

TABLE 3. Diseases in which PARs may play a role

Arthritis
Asthma and pulmonary infection
Intestinal function and intestinal inflammatory disease (Crohn's, Inflammatory Bowel Syndrome)
Cardiovascular disease (atherogenesis, angiogenesis)
Nephropathies (renal failure, fibrosis, graft rejection)
Neuropathies (neurodegeneration, neuropathic pain)
Tumorigenesis, cancer progression and metastasis

Important issues that future work will deal with relate to: (1) the receptor mechanisms and distinct signal pathways that are used for PAR signaling, (2) the identity of the physiological PAR-regulating proteinases that regulate both PAR- and non-PAR signaling *in vivo*, (3) receptors other than PARs that are activated or otherwise regulated by proteolysis and (4) the relevant endogenous inhibitors (e.g., serine proteinase inhibitors, or Serpins) that target the PAR-regulating proteinases *in vivo*. These issues relate directly to the main themes that have emerged from my work over the past decade or so, indicating that (1) proteinases can now be considered as signaling molecules that play hormone-like pathophysiological roles, particularly in the setting of inflammation, (2) proteinase-activated receptors can be seen to mediate many, but by no means all of the hormone-like actions of proteinases, and (3) PARs are widely expressed in most organs so as to play a potential role in the pathology of the vascular, gastrointestinal, skeletal and nervous systems (both peripheral and CNS). Thus, PARs and their signal-generating proteinases are an integral part of the body's innate defense system related to vascular, neuronal and rapidly deployed immune function. The disease states in which it is likely that PARs play a role are summarized in Table 3. The future looks particularly exciting in terms of the potential therapeutic impact that the understanding of this arm of the innate immune defense system will have.

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