

## Minireview

# Proteinase-mediated cell signalling: targeting proteinase-activated receptors (PARs) by kallikreins and more\*

Katerina Oikonomopoulou<sup>1</sup>, Kristina K. Hansen<sup>2</sup>, Mahmoud Saifeddine<sup>2</sup>, Nathalie Vergnolle<sup>2</sup>, Illa Tea<sup>2</sup>, Eleftherios P. Diamandis<sup>1</sup> and Morley D. Hollenberg<sup>2,3,\*\*</sup>

<sup>1</sup>Departments of Laboratory Medicine and Pathobiology, and of Pathology and Laboratory Medicine, University of Toronto and Mount Sinai Hospital, Toronto M5G 1X5, ON, Canada

<sup>2</sup>Department of Pharmacology and Therapeutics, University of Calgary, Calgary T2N 4N1, AB, Canada

<sup>3</sup>Department of Medicine, University of Calgary, Calgary T2N 4N1, AB, Canada

\*\*Corresponding author  
e-mail: mhollenb@ucalgary.ca

## Abstract

Serine proteinases, like trypsin, can play a hormone-like role by triggering signal transduction pathways in target cells. In many respects these hormone-like actions of proteinases can now be understood in terms of the pharmacodynamics of the G protein-coupled 'receptor' responsible for the cellular actions of thrombin (proteinase-activated receptor-1, or PAR<sub>1</sub>). PAR<sub>1</sub>, like the other three members of this receptor family (PAR<sub>2</sub>, PAR<sub>3</sub> and PAR<sub>4</sub>), has a unique mechanism of activation involving the proteolytic unmasking of an N-terminally tethered sequence that can activate the receptor. The selective activation of each PAR by short synthetic peptides representing these sequences has demonstrated that PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub> play important roles in regulating physiological responses ranging from vasoregulation and cell growth to inflammation and nociception. We hypothesise that the tissue kallikreins may regulate signal transduction via the PARs. Although PARs can account for many of their biological actions, kallikreins may also cause effects by mechanisms not involving the PARs. For instance, trypsin activates the insulin receptor and thrombin can act via a mechanism involving its non-catalytic domains. Based on the data we summarise, we propose that the kallikreins, like thrombin and trypsin, must now be considered as important 'hormonal' regulators of tissue function.

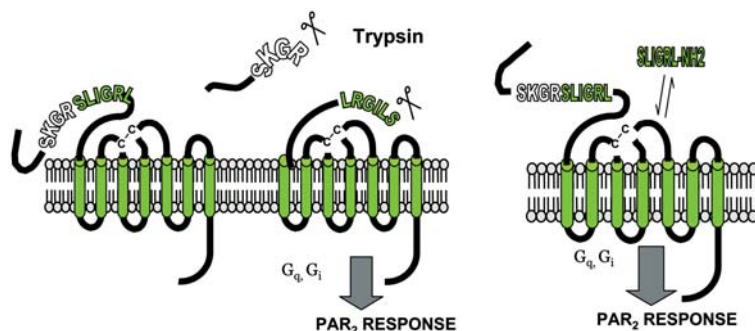
\*This article summarises information presented at the First International Symposium on Kallikreins, Lausanne/Switzerland, September 2–4, 2005.

Regarding a recommendation for future nomenclature of kallikrein gene-derived proteases, see the article 'A comprehensive nomenclature for serine proteases with homology to tissue kallikreins' by Lundwall et al., this issue pp. 637–641.

**Keywords:** hormone action; inflammation; kallikreins; protease-activated receptors; proteases; proteinases; receptors; signal transduction; trypsin.

## Introduction

Quite apart from their ability to generate active polypeptides from hormone precursors and to function as digestive enzymes, proteinases are now known to play a hormone-like role by triggering signal transduction pathways in target cells. For more than 40 years, serine proteinases such as trypsin have been known to trigger cellular hormone-like responses, in addition to their ability to convert inactive pro-hormone precursors to their active forms (e.g., pro-insulin to insulin). For instance, work in the mid-1960s documented the insulin-like actions of proteinases such as pepsin and chymotrypsin in a rat diaphragm preparation (Rieser and Rieser, 1964; Rieser, 1967). Subsequent work in the early 1970s showed that trypsin, like insulin, can both stimulate glucose oxidation and inhibit lipolysis in isolated adipocyte preparations (Kono and Barham, 1971). It is only over the past 15 years or so that the mechanisms for these cellular actions of proteinases have been elucidated in any detail. In part, the physiological actions of proteinases are mediated by 'proteinase-activated receptors' (PARs), as summarised in the following sections. Although, as outlined in some detail, the coagulation proteinase, thrombin, can be seen as a prototype physiological regulator of the PARs (signalling via PAR<sub>1</sub> and PAR<sub>4</sub>; see below), the candidate serine proteinases that regulate PARs *in vivo* have yet to be identified with confidence. Likely candidates as physiological PAR-regulating proteinases are trypsin, believed to activate intestinal epithelial PAR<sub>2</sub> (Kong et al., 1997) and human mast cell tryptase, which in certain settings (but no means all; Compton et al., 2002a,b) is another potential physiological regulator of PAR<sub>2</sub> (Mirza et al., 1997; Molino et al., 1997; Corvera et al., 1999). From our own perspective, we have put forward the hypothesis that tissue kallikreins, now known to comprise a large family of secreted serine proteinases with tryptic or chymotryptic activity, may represent important physiological regulators of the PARs (Borgono and Diamandis, 2004; Borgono et al., 2004). We and others have been able to show that PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub> play an important role in regulating a variety of physiological responses, ranging from vasoregulation, cell growth and cell motility to inflammation and nociception. That PARs may account for many of the biological actions of serine proteinases is now well accepted, but it is often overlooked that proteinases may regulate cell function by a number of mechanisms that



**Figure 1** Model for the activation of rat proteinase-activated receptor-2 (PAR<sub>2</sub>) either enzymatically by trypsin cleavage or via the action of a PAR-activating peptide (analogous to the PAR<sub>1</sub> model in Vu et al., 1991).

The image illustrates receptor activation either via exposure of the tethered ligand sequence (SLIGRL...) by trypsin (left-hand part of the Figure), or via the action of a PAR<sub>2</sub>-activating peptide (SLIGRL-NH<sub>2</sub>; right-hand part of the Figure), without the need for receptor proteolysis. Coupling via either G<sub>q</sub> or G<sub>i</sub> is shown to regulate PAR<sub>2</sub> signal transduction (adapted from Hollenberg and Compton, 2002).

do not involve PARs. For instance, trypsin has insulin-like activity because it activates the insulin receptor (Shoelson et al., 1988) and thrombin can regulate cell chemotaxis and mitogenesis via a mechanism involving domains apart from its catalytic site (Bar-Shavit et al., 1984, 1986; Glenn et al., 1988). Thus, although the PARs can account for many of the cellular effects of proteinases, there are many more mechanisms that merit exploration to explain the hormone-like actions of enzymes such as thrombin, trypsin and other serine proteinases like the kallikreins. The objectives of the sections that follow are: (1) to summarise both the PAR-related and -unrelated mechanisms by which serine proteinases can regulate cellular signal transduction; and (2) to summarise our preliminary findings pointing to a tissue-regulatory and inflammatory role for the tissue kallikreins. These actions of the kallikreins can most likely be attributed to their ability to regulate PAR activation. Our preliminary findings, reported elsewhere (Oikonomopoulou et al., 2006), support the working hypothesis that the human tissue kallikreins (hKs), similar to other serine proteinases, must now be considered as important 'hormonal' regulators of inflammation, nociception and cardiovascular function, thus representing new therapeutic targets for the treatment of vascular neoplastic and inflammatory diseases.

### Thrombin action and the discovery of G protein-coupled PARs

The search for the receptor on human platelets and hamster lung fibroblasts responsible for the ability of thrombin to trigger platelet aggregation and to stimulate cell division resulted in the cloning of a receptor that turned out to be a member of the G protein-coupled receptor superfamily (Rasmussen et al., 1991; Vu et al., 1991). The unique mechanism of thrombin action on platelets was discovered to involve the proteolytic unmasking of an N-terminal receptor sequence that then becomes a tethered ligand, binding to the body of the receptor and activating cellular signalling (Vu et al., 1991) (Figure 1). Based on this mechanism of activation, the receptor for thrombin (originally termed the thrombin receptor, or TR) is now referred to as a proteinase-activated receptor, assigned the acronym PAR by the International Union of Pharma-

cology (Hollenberg and Compton, 2002). Of key importance to understanding the potential pharmacology and physiology of PARs was the discovery that synthetic peptides with sequences matching those of the exposed 'tethered' ligand of the PAR could also activate the receptors in the absence of proteolysis (Vu et al., 1991). Thus, the synthetic peptide starting with the sequence of the human receptor, SFLLRN..., was found to be a surrogate activator of the receptor for thrombin in a variety of settings. The use of such peptides (initially termed thrombin receptor-activating peptides or TRAPs) to mimic thrombin action soon revealed that in certain cells, such as rodent platelets, the peptides did *not* cause a thrombin response (e.g., aggregation) (Kinlough-Rathbone et al., 1993). Unequivocally, these data indicated that the receptor(s) for thrombin on rodent platelets (now known to be PAR<sub>4</sub>; see below) differed from that on human platelets responding to SFLLRN. Other structure-activity studies with peptides based on the SFLLRN sequence also pointed to subtypes of the thrombin receptor in vascular and gastric tissues (Hollenberg et al., 1993). Since then, three other members of this intriguing receptor family have been identified (Coughlin, 2000; Macfarlane et al., 2001; Hollenberg and Compton, 2002; Ossovskaya and Bunnett, 2004; Hollenberg and Houle, 2005; Steinhoff et al., 2005) (Table 1). Now termed PARs 1–4, each receptor has a unique N-terminal tethered ligand sequence that is revealed by serine proteinase action (Table 1). PAR<sub>1</sub>, PAR<sub>3</sub> and PAR<sub>4</sub> have been found to be targets for thrombin, whereas PAR<sub>2</sub>, not readily activated by thrombin, can be activated by trypsin, tryptase and by other serine proteinase members of the clotting cascade apart from thrombin (e.g., the tissue factor VIIa-Xa complex) (Ruf et al., 2003; Ossovskaya and Bunnett, 2004). Although the signalling properties of PAR<sub>3</sub> are unclear, PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub> have all been found to signal via a G protein-coupled mechanism involving G<sub>q</sub> or G<sub>i</sub> (Macfarlane et al., 2001). Furthermore, based on the revealed tethered ligand sequences of PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub>, it has now been possible to design synthetic peptides (PAR-activating peptides, or PAR-APs) that can selectively activate each receptor. Appropriate standard inactive peptides, incapable of activating the PARs, are also known (Table 1). Although the PARs can be activated

**Table 1** The PAR family of G protein-coupled receptors.

IUPHAR receptor designation	Revealed tethered ligand sequence	Comments on activating peptides
PAR <sub>1</sub>	(h)SFLLRN... (r,m)SFLLRN...	Receptor-activating peptide, initially designated as TRAP, now PAR <sub>1</sub> AP SFLLRN... activates <i>both</i> PAR <sub>1</sub> and PAR <sub>2</sub> Standard PAR <sub>1</sub> -activating peptide: TFLLR-NH <sub>2</sub> Standard PAR <sub>1</sub> -inactive peptide: FTLLR-NH <sub>2</sub>
PAR <sub>2</sub>	(h)SLIGKV... (r,m)SLIGRL...	Designated PAR <sub>2</sub> AP Standard PAR <sub>2</sub> -activating peptide: SLIGRL-NH <sub>2</sub> Standard PAR <sub>2</sub> -inactive peptide: LSIGRL-NH <sub>2</sub> Selectively activates only PAR <sub>2</sub> ; murine and rat sequence more potent than human sequence
PAR <sub>3</sub>	(h)TFRGAP... (m)SFNGGP...	PAR <sub>3</sub> is not activated by PAR-APs PAR <sub>3</sub> -derived sequences, e.g., TFRGAP... or SFNGGP..., activate both PAR <sub>1</sub> and PAR <sub>2</sub>
PAR <sub>4</sub>	(h)GYPGQV... (m)GYPGKF... (r)GFPGKP...	PAR <sub>4</sub> AP sequences do not activate PAR <sub>1</sub> and PAR <sub>2</sub> , but are active via non-PAR <sub>4</sub> receptors in some bioassays Standard PAR <sub>4</sub> -activating peptide: AYPGKF-NH <sub>2</sub> Standard PAR <sub>4</sub> -inactive peptide: YAPGKF-NH <sub>2</sub>

Abbreviations: h, human; m, mouse; r, rat; IUPHAR, International Union of Pharmacology. The new N-terminal sequences revealed by serine proteinase cleavage are shown as 'tethered ligands'. These proteolytically revealed sequences activate signalling in PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub>, but not in PAR<sub>3</sub>.

by a variety of serine proteinases using the tethered-ligand mechanism outlined in Figure 1, it is also the case that cleavage of a PAR N-terminal sequence downstream of the tethered ligand portion would 'disarm' the receptor, preventing its subsequent activation by a proteinase. In some instances, a serine proteinase such as trypsin can, at relatively low concentrations, disarm one of the PARs (e.g., the disarming of PAR<sub>1</sub> towards thrombin activation) whilst activating another (e.g., PAR<sub>2</sub> activation over a concentration range that disarms PAR<sub>1</sub>) (Kawabata et al., 1999). Thus, the PARs can be said to have a variety of circulating agonists (i.e., serine proteinases that reveal the tethered ligand), as well as circulating functional 'antagonists' that can disarm the PARs downstream of their tethered ligands. That said, the proteolytically disarmed receptors would still be sensitive to activation by the PAR-APs that do not depend on the tethered ligand sequence for receptor activation. The unique features of the PARs are summarised in Table 2. As already pointed out, one of the key features of these receptors is their ability to be activated by the receptor-selective PAR-APs. These PAR-APs have been of considerable utility in determining the potential consequences of activating the PARs in bioassay systems *in vitro* or in inflammatory or other bioassay models *in vivo*. As summarised in the following sections, the PARs have been found to play an

important role in regulating cardiovascular function, as well as in triggering inflammatory processes, in part via a neurogenic mechanism (Vergnolle et al., 1999a,b, 2001a,b). For a more recent comprehensive collection of articles dealing with the PARs, the reader is invited to access the special issue volumes 59 (4) and 60 (1) of Drug Development Research to be found on <http://www.inflammation-calgary.com>.

### Discovering physiological roles for the PARs: a pharmacological approach

As mentioned above, structure-activity relationship (SAR) studies using peptides with sequences based on human PAR<sub>1</sub> revealed the presence of a receptor other than PAR<sub>1</sub> in an endothelium-dependent rat aorta relaxation assay (Hollenberg et al., 1993). That receptor, unknown at the time, turned out to be PAR<sub>2</sub> (al-Ani et al., 1995). The principle that led to the discovery of functional PAR<sub>2</sub> in the rat vascular endothelium was outlined some time ago by Ahlquist (1948) in defining the pharmacology of  $\alpha$ - and  $\beta$ -adrenoceptors. In essence, with only minor exceptions, a receptor can be typified for distinct responses in different tissues by the relative potencies (EC<sub>50</sub> or IC<sub>50</sub>) of a series of chemically related agonists and/or antago-

**Table 2** Unique features of proteinase-activated receptors.

Feature	Comment
Proteolytically activated by a number of serine proteinases via a revealed tethered ligand	Multiple circulating or local 'agonists' are possible
Can be disarmed by proteolytic cleavage downstream from the tethered ligand sequence, thereby preventing enzymatic receptor activation by agonist proteinases	Multiple circulating or secreted enzymes, such as neutrophil elastase, can act as functional 'antagonists' by disarming PARs
Proteinase activation (e.g., by trypsin) can be modulated by receptor glycosylation near the tethered ligand cleavage site	Trypsin does not activate fully glycosylated human PAR <sub>2</sub>
PAR-activating peptides can mimic signalling triggered by proteolytic activation	Receptors other than PARs may be activated, even by PAR-selective PAR-APs that do not activate other PARs

**Table 3** Potential tissue sites of pathophysiological roles for PARs.

Potential site of function	Comment
Platelet and leukocyte activation; haemostasis: thrombin-activated receptors (PAR <sub>1</sub> , PAR <sub>3</sub> , PAR <sub>4</sub> ) Endothelial cell function: (PARs 1, 2 and 4)	PARs regulate both secretion and aggregation; PAR <sub>1</sub> and PAR <sub>4</sub> can play separate roles PAR activation causes release of NO, von Willebrand factor; increased neutrophil adherence (not PAR <sub>1</sub> ); cell migration; gene induction
Vascular smooth muscle function Intestinal function: (PARs 1, 2 and 4)	Activation of contractility; angiogenesis Regulation of motility (GI smooth muscle) and secretion (GI epithelial cell); PAR activation induces colonic inflammation
Myenteric neuron function (PARs 1 and 2) Renal vascular function	Neuronal PARs can affect both motility and inflammatory responses Regulation of flow and afferent arteriolar function
CNS neuronal and astrocyte function Joint responses to injury or inflammation	Up-regulation of PARs in the setting of CNS inflammation PAR <sub>2</sub> -deficient mice do not develop adjuvant-induced arthritis

This Table is meant only as an introductory overview of the potentially broad tissue impact of PAR function. For more comprehensive overviews of the possible physiological roles PARs may play, see Ossovskaia and Bunnett (2004), Steinhoff et al. (2005), and the special issues of Drug Development Research [volumes 59 (4) and 60 (1)] that can be found on [www.inflammation-calgary.com](http://www.inflammation-calgary.com).

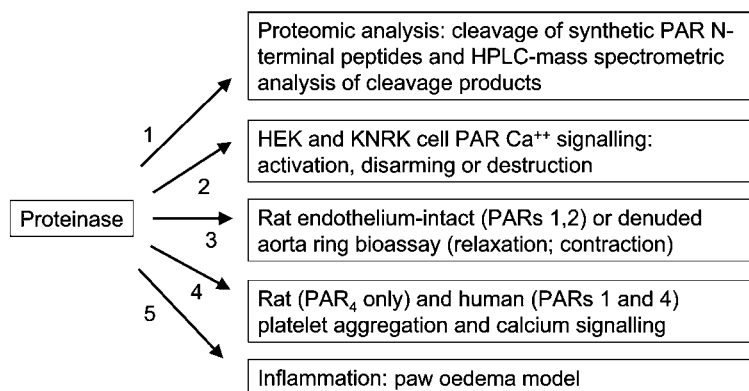
nists. The presence of distinct SAR relationships for the same set of compounds (e.g., agonists) in different tissue assays points to the existence of distinct receptors. This principle has been used to our advantage in studying potential PAR-mediated responses in different bioassay systems, employing, for example, a series of PAR<sub>1</sub> and PAR<sub>2</sub>APs, along with appropriate standard PAR-inactive 'control' peptides, as outlined in Table 1. It is through the judicious use of these PAR-APs that the potential physiological roles of distinct PARs are being elucidated (Steinhoff et al., 2005) (Table 3).

Although PAR<sub>1</sub> and PAR<sub>4</sub> were discovered primarily due to the complex actions of thrombin on mammalian platelets, the potential physiological role for PAR<sub>2</sub> was not known at the time of its discovery (Nystedt et al., 1994). However, the use of selective PAR<sub>2</sub>-activating peptides as probes for PAR<sub>2</sub> function quickly revealed a potential role for this receptor in regulating vascular and gastric smooth muscle tension (al-Ani et al., 1995; Hollenberg et al., 1996, 1997; Saifeddine et al., 1996). Acting either on the endothelial cells (PAR<sub>1</sub> and PAR<sub>2</sub>) or directly on the smooth muscle elements (PAR<sub>1</sub>), it is now known that PARs are involved in regulating the cardiovascular system. It was not expected, however, that functional PAR<sub>2</sub> as well as PAR<sub>1</sub> would be detected on neuronal elements (Corvera et al., 1999). Furthermore, it was also initially an unexpected finding that the activation of PAR<sub>1</sub> and PAR<sub>2</sub> in peripheral tissues, using selective PAR-activating peptides, would cause a marked inflammatory response (Vergnolle et al., 1999a,b). Notwithstanding, putting these two sets of seemingly unrelated observations together, it has now become evident that the inflammatory response triggered by PAR<sub>1</sub> and PAR<sub>2</sub> is mediated via both neurogenic and non-neurogenic mechanisms (Steinhoff et al., 2000; de Garavilla et al., 2001; Cenac et al., 2002; Nguyen et al., 2003; Vergnolle, 2004). It has also become clear that, in addition to triggering the inflammatory response, PAR<sub>1</sub> and PAR<sub>2</sub> also play a role in sensing pain (Vergnolle et al., 2001a,b; Asfaha et al., 2002; Vergnolle, 2004). Given the wide distribution of PARs on neurons in both the central and peripheral nervous systems and their presence on neuronal-associated cells such as astrocytes, it is to be expected that neu-

ronal PARs may play a widespread physiological role (Noorbakhsh et al., 2003). Examples are the upregulation of PAR<sub>1</sub> in astrocytes in the setting of HIV encephalitis (Boven et al., 2003) and the 'protective' induction of CNS PAR<sub>2</sub> in the neuroinflammatory setting of HIV (Noorbakhsh et al., 2005). Thus, in the CNS, PARs could be targets for serine proteinases such as thrombin and kallikreins, also known to be present in the same setting (Blaber et al., 2002, 2004; Boven et al., 2003; Noorbakhsh et al., 2006). The overarching working hypothesis that can be put forward is that PARs play a key role in the body's innate defence as a primary trigger of the inflammatory response due to tissue injury or remodelling caused by pathogenic processes. This hypothesis is strongly supported by: (i) the striking resistance of PAR<sub>2</sub>-deficient mice to adjuvant-induced arthritis (Ferrell et al., 2003); (ii) the effectiveness of PAR<sub>1</sub> antagonists in mitigating chronic inflammation in different animal models of inflammatory bowel disease; and (iii) the resistance of PAR<sub>1</sub>-deficient mice to inflammation in the same chronic models of inflammatory bowel disease (Vergnolle, 2004). Overall, a number of potential physiological roles that PARs may play are summarised in Table 3.

### Tissue kallikreins as potential PAR regulators

Human tissue kallikreins (hKs) represent a large family of secreted serine proteinases, the majority of which exhibit potent trypsin-like activity. In humans and a wide variety of mammals, these enzymes share a high degree of genomic and proteomic homology and are distributed throughout the body (Borgono and Diamandis, 2004; Borgono et al., 2004). The hKs, abundantly expressed in groups in many tissues, can be regulated in a sex-steroid hormone-dependent manner. These enzymes are upregulated in the local tumour area and in the circulation in many types of cancer (Diamandis et al., 2000; Kim et al., 2001; Tanimoto et al., 2001; Borgono et al., 2003; Yousef et al., 2003), as is widely recognised for PSA/hK3. Furthermore, sites of CNS inflammation also express kallikreins (Scarlsbrick et al., 2002; Blaber et al., 2002, 2004). In spite of the many organs in which kallikreins can be



**Figure 2** Paradigm for evaluating proteinase-mediated regulation of PARs.

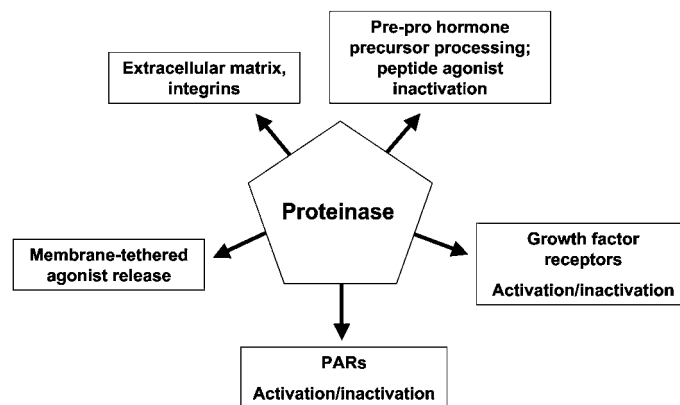
The Figure outlines five approaches for evaluating the ability of a given proteinase to regulate PAR activity. The regulation could involve: (1) proteolytic unmasking of the tethered ligand receptor-activating sequence; (2) disarming of the receptor by cleavage downstream of the tethered ligand sequence; or (3) disabling of the receptor by cleavage of extracellular loops essential for tethered ligand or PAR-activating peptide stimulation of PAR signalling. In principle, the approach can also determine which of PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub> are affected by a given proteinase.

found, the mechanisms by which this enzyme family regulates tissue function are not clear. Studies performed *in vitro* have identified a number of potential targets for kallikrein-mediated proteolysis: extracellular matrix components, pro-urokinase-plasminogen activator (pro-uPA), kininogens, growth factor precursors (and binding proteins), and other kallikreins. Proteolysis of these targets by kallikreins may play a role in cancer progression (Frenette et al., 1997; Takayama et al., 2001; Choong and Nadesapillai, 2003; Borgono and Diamandis, 2004; Borgono et al., 2004). Although cleavage of these targets may well explain some of the physiological actions of kallikreins, particularly in the setting of cancer, we suspect that because of their recognised tryptic activity, like trypsin, the kallikreins, can also potentially trigger signal transduction pathways by regulating PARs.

In work reported elsewhere (Oikonomopoulou et al., 2006) we tested our hypothesis that hKs can regulate tissue function by cleaving and activating one or more of PAR<sub>1</sub>, PAR<sub>2</sub> and PAR<sub>4</sub>. The general paradigm for evaluating proteinase-mediated regulation of PARs is outlined in Figure 2. The general approach involves: (1) analysis of the cleavage products yielded upon incubation of candidate proteinases, such as hK5, 6 and 14, with PAR N-terminal peptide sequences representing the cleavage/activation motifs of the different PARs; (2) a study of proteinase-mediated PAR-dependent calcium signalling responses in a number of target cells [e.g., human embryonic kidney (HEK) or Kirsten virus transformed normal rat kidney cells (KNRK)] expressing the several PARs, which identifies the ability of a proteinase to activate, disarm or disable a given PAR; (3) evaluation of the enzyme activity in a rat vascular bioassay system, in which relaxation is PAR-activated via an endothelium-dependent release of nitric oxide; (4) evaluation of the ability of the proteinase to cause PAR<sub>4</sub>- and PAR<sub>1</sub>-dependent aggregation and calcium signalling in isolated rat (PAR<sub>4</sub>-dependent) and human (PAR<sub>1</sub>- and PAR<sub>4</sub>-dependent) platelets; and finally (5) evaluation of the inflammatory action of a proteinase in a rat or murine paw oedema model, including the use of PAR-null mice to ascertain whether or not the proteinase-mediated inflammation is

indeed due to PAR activation. In our preliminary evaluation of the ability of kallikreins to regulate PARs, we used hK14 as a 'prototype' trypsin-like kallikrein because of its particularly wide tissue distribution. Given the well-recognised susceptibility of PAR<sub>2</sub> to tryptic activation, we suspected that this receptor would be a good potential target for hK14, as well as for the other kallikreins with tryptic activity (e.g., hK5, hK6). In addition to activating PAR<sub>2</sub>, trypsin is also known to both activate and disarm PAR<sub>1</sub> (Kawabata et al., 1999). Therefore, we also evaluated hK14 for its ability to regulate PAR<sub>1</sub> and investigated whether hK5 and 6 might, according to step 1 of the paradigm outlined in Figure 2, also be able to cleave peptides representing the tethered ligand sequences of human PAR<sub>1</sub> and PAR<sub>2</sub>. Our preliminary findings, described elsewhere (Oikonomopoulou et al., 2006), are summarised in the following paragraphs.

Taken together, our data based on the paradigm outlined in Figure 2 substantially support our working hypothesis that PARs can be regulated by the kallikreins, according to the following criteria (Oikonomopoulou et al., 2006): (1) human tissue kallikreins (hK5, 6 and 14) can cleave synthetic PAR-related tethered ligand peptide sequences either to yield PAR-activating tethered ligand sequences or to result in disarming of PAR<sub>1</sub> and PAR<sub>2</sub>; (2) in experiments using HEK-derived cells that constitutively express both PAR<sub>1</sub> and PAR<sub>2</sub> (but not PAR<sub>4</sub>) and KNRK cells expressing rat PAR<sub>2</sub>, hK5, 6 and 14 were able to trigger calcium signalling. Cross-desensitisation experiments using HEK and PAR<sub>2</sub>-expressing KNRK cells, in which PAR<sub>2</sub> was first pre-desensitised with the PAR<sub>2</sub>-activating peptide, SLIGRL-NH<sub>2</sub>, clearly demonstrated that the calcium signal generated by treating cells with hK6 and 14 was due to PAR<sub>2</sub> activation; (3) hK14 causes endothelium-dependent, nitric oxide-mediated relaxation in an endothelium-intact rat aorta preparation and does not affect vascular tension in an endothelium-denuded preparation (unpublished data and Oikonomopoulou et al., 2006); (4) in a PAR<sub>4</sub>-dependent rat platelet aggregation assay (Hollenberg and Saifeddine, 2001; Hollenberg et al., 2004), hK14 (but not hK5 or 6) is able to cause aggregation and hK14 is able to activate cal-



**Figure 3** Targets for proteinase-mediated signalling.

The diagram outlines the potential proteolytic targets for proteinase-mediated signalling including the PARs, which can be either activated or disarmed by a variety of proteinases, as discussed in the text. Other targets, as discussed, 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 such as heparin-binding EGF that can be released by metalloproteinase action to activate the EGF receptor (Prenzel et al., 1999), and either peptide agonist precursors, such as angiotensinogen, or peptide agonists, such as neurokinins, that are inactivated by membrane-tethered proteinases. Extracellular matrix-integrin interactions that regulate intracellular signalling are also shown as potential targets for enzymes such as the matrix metalloproteinases. Not shown is the ability of a proteinase such as thrombin to regulate cell function via its non-catalytic domains (Bar-Shavit et al., 1984, 1986; Glenn et al., 1988).

cium signalling; and finally, (5) hK14 triggers an inflammatory response equivalent to that of trypsin in a murine paw oedema model.

Taken together, our data support the hypothesis that hKs can signal via PARs (Oikonomopoulou et al., 2006), thereby adding these receptors to the list of kallikrein targets that may explain the signalling properties that this enzyme family has in many tissues. Via a PAR-activating mechanism, the kallikreins can potentially signal by a variety of G protein-coupled signalling pathways ( $G_q$ ,  $G_i$ ,  $G_{12/13}$ ) (Macfarlane et al., 2001). Although clearly able to regulate PAR activity *in vitro*, the next frontier to face is to determine whether or not kallikreins can also do so *in vivo*. In a specific tissue setting, the effects that kallikreins may have undoubtedly depend on the spectrum of kallikreins present and on the availability of PARs that may be expressed in the local environment. We propose the working hypothesis that, because of their wide tissue distribution, kallikreins, via PARs, very likely represent important endogenous local autocrine-paracrine cellular regulators that may be as important as thrombin for modulating tissue function. Based on our previous findings related to the potential pathophysiological roles of PARs (Vergnolle et al., 1999a,b, 2001a,b; Vergnolle, 2004; Noorbakhsh et al., 2003, 2005, 2006), we suggest that the kallikreins, via PAR-mediated signalling, may play a prominent role in a wide variety of pathologies, including inflammation, chronic pain, cardiovascular disease and cancer. In this regard, the kallikrein-PAR axis may be seen as a fruitful therapeutic target for a number of disease states.

In addition to these receptor-mediated signal pathways, serine proteinases such as the kallikreins can: (1) liberate novel receptor-activating agonists such as heparin-binding EGF from the cell surface; (2) inactivate agonist peptides such as bradykinin; and (3) potentially regulate signalling pathways via non-receptor mechanisms (e.g., modulating matrix-integrin interactions).

These actions of proteinases (Figure 3) can be added to their recognised ability to generate active polypeptides from pro-agonist protein precursors. The ability of the kallikreins to signal via PARs adds a novel dimension to the biological significance of this enzyme superfamily.

### Proteinase signalling by mechanisms other than PARs

#### Regulation of growth factor receptors

As mentioned above, one of the first indications that proteinases can activate cellular signals comparable to those of hormones came from the observations in the mid-1960s demonstrating that trypsin exhibits an insulin-like action in adipocytes and striated muscle tissue (Rieser and Rieser, 1964; Rieser, 1967). This hormone-like action of trypsin cannot be attributed to the activation of PARs, but is due rather to the effect of trypsin on the receptor for insulin. By cleaving at a di-basic residue of the insulin receptor  $\alpha$ -subunit, trypsin generates a truncated receptor that has intrinsic signalling activity (Shoelson et al., 1988). In principle, this type of action of serine proteinases, either activating or disarming growth factor receptors (e.g., at higher concentrations, trypsin can abolish the ability of the insulin receptor to bind insulin, thereby abrogating insulin signalling; Cuatrecasas, 1971), can modulate cell function in a variety of settings. There is every reason to expect that the kallikreins similarly affect the insulin receptor by these trypsin-like mechanisms.

#### Release of novel agonists from the cell surface

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 environ-

ment. For instance, the transactivation of the EGF receptor can result from the metalloproteinase-mediated release from the cell surface of a receptor agonist (heparin-binding EGF) (Prenzel et al., 1999). In this regard, thrombin, apart from signalling via PARs, can also yield chemotactic-mitogenic peptides from proteolytic processing of its non-catalytic domain (Bar-Shavit et al., 1984, 1986; Glenn et al., 1988). These thrombin-derived peptides cause their effects via receptors that are not PARs. Thrombin can also potentially cause its cellular effects via the activation of pro-metalloproteinases (Lafleur et al., 2001).

### Generation of novel agonists from extracellular or intracellular precursors

In addition to generating active peptide hormones from recognised pro-hormone precursors (e.g., pro-insulin) that in turn activate receptors, novel receptor-activating hormone-like agonists can be generated from precursors in the vicinity of target receptors. For instance, interleukin- $\beta$  is generated by the interleukin- $\beta$  converting enzyme (ICE), a cysteine proteinase that also plays a role intracellularly in the apoptotic process (Nicholson et al., 1995). Thus, proteinases in general and the kallikreins in particular may play a signalling role not only by receptor modulation and ligand generation, but also by regulating intracellular signalling pathways such as that responsible for the apoptotic response. Hence, as summarised in Figure 3, apart from activating or inactivating PARs, proteinases such as the kallikreins may play a hormone-like signalling role in a variety of cellular settings via non-PAR mechanisms. As already mentioned above, these types of actions of the kallikreins may involve regulating extracellular matrix-integrin signalling, generating kinins from kininogens, activating pro-uPA, generating growth factor precursors (and binding proteins), and triggering the activity of other kallikreins in a cascade manner akin to the coagulation cascade. This diversity of hormone-like roles played by proteinases is exceeded only by the diversity of the proteinase families themselves.

### Summary

We have discussed the various hormone-like roles that proteinases can play, not only by activating or silencing members of the G protein-coupled PAR family, but also by regulating the activity of growth factor receptors, like the one for insulin. In this regard, our preliminary data, demonstrating that the hKs can either activate or disarm PARs, add this novel G protein-coupled receptor family to the list of kallikrein targets. These actions may explain the signalling properties that this enzyme family has in many tissues, thereby contributing to the pathophysiology of a number of disease states. These signalling properties of proteinases such as thrombin, trypsin, tryptase and the kallikreins add a novel dimension to the biological significance of these enzyme superfamilies.

### Acknowledgments

Work by the authors summarised in this article has been supported in part by a Canadian Institutes of Health Research Pro-

teinases and Inflammation Network Group grant (M.D.H and N.V.), by a Servier International Alliance Project grant (M.D.H. and N.V.) and by operating grants from the Canadian Institutes of Health Research (M.D.H. and N.V.). Work in the laboratory of E.P.D. is supported by a University-Industry Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC and IBEX Technologies, Montreal, Canada). K.K.H. was supported by a post-doctoral fellowship from the Alberta Heritage Foundation for Medical Research. N.V. is an Alberta Heritage Foundation for Medical Research Scholar and a Canadian Institutes for Health Research Scientist.

### References

- Ahlquist, R.P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.* **153**, 586–600.
- al-Ani, B., Saifeddine, M., and Hollenberg, M.D. (1995). Detection of functional receptors for the proteinase-activated-receptor-2-activating polypeptide, SLIGRL-NH<sub>2</sub>, in rat vascular and gastric smooth muscle. *Can. J. Physiol. Pharmacol.* **73**, 1203–1207.
- Asfaha, S., Brussee, V., Chapman, K., Zochodne, D.W., and Vergnolle, N. (2002). Proteinase-activated receptor-1 agonists attenuate nociception in response to noxious stimuli. *Br. J. Pharmacol.* **135**, 1101–1106.
- Bar-Shavit, R., Kahn, A., Mudd, M.S., Wilner, G.D., Mann, K.G., and Fenton, J.W. II (1984). Localization of a chemotactic domain in human thrombin. *Biochemistry* **23**, 397–400.
- Bar-Shavit, R., Kahn, A.J., Mann, K.G., and Wilner, G.D. (1986). Identification of a thrombin sequence with growth factor activity on macrophages. *Proc. Natl. Acad. Sci. USA* **83**, 976–980.
- Blaber, S.I., Scarisbrick, I.A., Bennett, M.J., Dhanarajan, P., Seavy, M.A., Jin, Y., Schwartz, M.A., Rodriguez, M., and Blaber, M. (2002). Enzymatic properties of rat myelencephalon-specific protease. *Biochemistry* **41**, 1165–1173.
- Blaber, S.I., Ciric, B., Christophi, G.P., Bennett, M.J., Blaber, M., Rodriguez, M., and Scarisbrick, I.A. (2004). Targeting kallikrein 6 proteolysis attenuates CNS inflammatory disease. *FASEB J.* **18**, 920–922.
- Borgono, C.A. and Diamandis, E.P. (2004). The emerging roles of human tissue kallikreins in cancer. *Nat. Rev. Cancer* **4**, 876–890.
- Borgono, C.A., Grass, L., Soosaipillai, A., Yousef, G.M., Petraki, C.D., Howarth, D.H., Fracchioli, S., Katsaros, D., and Diamandis, E.P. (2003). Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. *Cancer Res.* **63**, 9032–9041.
- Borgono, C.A., Michael, I.P., and Diamandis, E.P. (2004). Human tissue kallikreins: physiologic roles and applications in cancer. *Mol. Cancer Res.* **2**, 257–280.
- Boven, L.A., Vergnolle, N., Henry, S.D., Silva, C., Imai, Y., Holden, J., Warren, K., Hollenberg, M.D., and Power, C. (2003). Up-regulation of proteinase-activated receptor 1 expression in astrocytes during HIV encephalitis. *J. Immunol.* **170**, 2638–2646.
- Cenac, N., Coelho, A.M., Nguyen, C., Compton, S., Andrade-Gordon, P., MacNaughton, W.K., Wallace, J.L., Hollenberg, M.D., Bunnett, N.W., Garcia-Villar, R., et al. (2002). Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am. J. Pathol.* **161**, 1903–1915.
- Choong, P.F. and Nadesapillai, A.P. (2003). Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. *Clin. Orthop. Relat. Res.* **415**, S46–58.
- Compton, S.J., McGuire, J.J., Saifeddine, M., and Hollenberg, M.D. (2002a). Restricted ability of human mast cell tryptase to activate proteinase-activated receptor-2 in rat aorta. *Can. J. Physiol. Pharmacol.* **80**, 987–992.

- Compton, S.J., Sandhu, S., Wijesuriya, S.J., and Hollenberg, M.D. (2002b). Glycosylation of human proteinase-activated receptor-2 (hPAR2): role in cell surface expression and signalling. *Biochem. J.* **368**, 495–505.
- Corvera, C.U., Dery, O., McConalogue, K., Gamp, P., Thoma, M., al-Ani, B., Caughey, G.H., Hollenberg, M.D., and Bunnett, N.W. (1999). Thrombin and mast cell tryptase regulate guinea-pig myenteric neurons through proteinase-activated receptors-1 and -2. *J. Physiol.* **517**, 741–756.
- Coughlin, S.R. (2000). Thrombin signalling and protease-activated receptors. *Nature* **407**, 258–264.
- Cuatrecasas, P. (1971). Perturbation of the insulin receptor of isolated fat cells with proteolytic enzymes. *J. Biol. Chem.* **246**, 6522–6531.
- de Garavilla, L., Vergnolle, N., Young, S.H., Ennes, H., Steinhoff, M., Ossovskaya, V.S., D'Andrea, M.R., Mayer, E.A., Wallace, J.L., Hollenberg, M.D., et al. (2001). Agonists of proteinase-activated receptor 1 induce plasma extravasation by a neurogenic mechanism. *Br. J. Pharmacol.* **133**, 975–987.
- Diamandis, E.P., Yousef, G.M., Soosaipillai, A.R., and Bunting, P. (2000). Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. *Clin. Biochem.* **33**, 579–583.
- Ferrell, W.R., Lockhart, J.C., Kelso, E.B., Dunning, L., Plevin, R., Meek, S.E., Smith, A.J., Hunter, G.D., McLean, J.S., McGarry, F., et al. (2003). Essential role for proteinase-activated receptor-2 in arthritis. *J. Clin. Invest.* **111**, 35–41.
- Frenette, G., Tremblay, R.R., Lazure, C., and Dube, J.Y. (1997). Prostatic kallikrein hK2, but not prostate-specific antigen (hK3), activates single-chain urokinase-type plasminogen activator. *Int. J. Cancer* **71**, 897–899.
- Glenn, K.C., Frost, G.H., Bergmann, J.S., and Carney, D.H. (1988). Synthetic peptides bind to high-affinity thrombin receptors and modulate thrombin mitogenesis. *Pept. Res.* **1**, 65–73.
- Hollenberg, M.D. and Compton, S.J. (2002). International Union of Pharmacology. XXVIII. Proteinase-activated receptors. *Pharmacol. Rev.* **54**, 203–217.
- Hollenberg, M.D. and Houle, S. (2005). Proteinases as hormone-like signal messengers. *Swiss Med. Wkly.* **135**, 425–432.
- Hollenberg, M.D. and 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, M.D., Laniyonu, A.A., Saifeddine, M., and Moore, G.J. (1993). Role of the amino- and carboxyl-terminal domains of thrombin receptor-derived polypeptides in biological activity in vascular endothelium and gastric smooth muscle: evidence for receptor subtypes. *Mol. Pharmacol.* **43**, 921–930.
- Hollenberg, M.D., Saifeddine, M., and al-Ani, B. (1996). Proteinase-activated receptor-2 in rat aorta: structural requirements for agonist activity of receptor-activating peptides. *Mol. Pharmacol.* **49**, 229–233.
- Hollenberg, M.D., Saifeddine, M., al-Ani, B., and Kawabata, A. (1997). Proteinase-activated receptors: structural requirements for activity, receptor cross-reactivity, and receptor selectivity of receptor-activating peptides. *Can. J. Physiol. Pharmacol.* **75**, 832–841.
- Hollenberg, M.D., Saifeddine, M., Sandhu, S., Houle, S., and 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.
- Kawabata, A., Saifeddine, M., Al-Ani, B., Leblond, L., and Hollenberg, M.D. (1999). Evaluation of proteinase-activated receptor-1 (PAR1) agonists and antagonists using a cultured cell receptor desensitization assay: activation of PAR2 by PAR1-targeted ligands. *J. Pharmacol. Exp. Ther.* **288**, 358–370.
- Kim, H., Scorilas, A., Katsaros, D., Yousef, G.M., Massobrio, M., Fracchioli, S., Piccinno, R., Gordini, G., and Diamandis, E.P. (2001). Human kallikrein gene 5 (*KLK5*) expression is an indicator of poor prognosis in ovarian cancer. *Br. J. Cancer* **84**, 643–650.
- Kinlough-Rathbone, R.L., Rand, M.L., and Packham, M.A. (1993). Rabbit and rat platelets do not respond to thrombin receptor peptides that activate human platelets. *Blood* **82**, 103–106.
- Kong, W., McConalogue, K., Khitin, L.M., Hollenberg, M.D., Payan, D.G., Bohm, S.K., and Bunnett, N.W. (1997). Luminal trypsin may regulate enterocytes through proteinase-activated receptor 2. *Proc. Natl. Acad. Sci. USA* **94**, 8884–8889.
- Kono, T. and Barham, F.W. (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.
- Lafleur, M.A., Hollenberg, M.D., Atkinson, S.J., Knauper, V., Murphy, G., and Edwards, D.R. (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.
- Macfarlane, S.R., Seatter, M.J., Kanke, T., Hunter, G.D., and Plevin, R. (2001). Proteinase-activated receptors. *Pharmacol. Rev.* **53**, 245–282.
- Mirza, H., Schmidt, V.A., Derian, C.K., Jesty, J., and Bahou, W.F. (1997). Mitogenic responses mediated through the proteinase-activated receptor-2 are induced by expressed forms of mast cell  $\alpha$ - or  $\beta$ -tryptases. *Blood* **90**, 3914–3922.
- Molino, M., Barnathan, E.S., Numerof, R., Clark, J., Dreyer, M., Cumashi, A., Hoxie, J.A., Schechter, N., Woolkalis, M., and Brass, L.F. (1997). Interactions of mast cell tryptase with thrombin receptors and PAR-2. *J. Biol. Chem.* **272**, 4043–4049.
- Nguyen, C., Coelho, A.M., Grady, E., Compton, S.J., Wallace, J.L., Hollenberg, M.D., Cenac, N., Garcia-Villar, R., Bueno, L., Steinhoff, M., et al. (2003). Colitis induced by proteinase-activated receptor-2 agonists is mediated by a neurogenic mechanism. *Can. J. Physiol. Pharmacol.* **81**, 920–927.
- Nicholson, D.W., Ali, A., Thornberry, N.A., Vaillancourt, J.P., Ding, C.K., Gallant, M., Gareau, Y., Griffin, P.R., Labelle, M., Lazebnik, Y.A., et al. (1995). Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* **376**, 37–43.
- Noorbakhsh, F., Vergnolle, N., Hollenberg, M.D., and Power, C. (2003). Proteinase-activated receptors in the nervous system. *Nat. Rev. Neurosci.* **4**, 981–990.
- Noorbakhsh, F., Vergnolle, N., McArthur, J.C., Silva, C., Vodjgani, M., Andrade-Gordon, P., Hollenberg, M.D., and Power, C. (2005). Proteinase-activated receptor-2 induction by neuroinflammation prevents neuronal death during HIV infection. *J. Immunol.* **174**, 7320–7329.
- Noorbakhsh, F., Tsutsui, S., Vergnolle, N., Boven, L.A., Shariat, N., Vodjgani, M., Warren, K.G., Andrade-Gordon, P., Hollenberg, M.D., and Power, C. (2006). Proteinase-activated receptor 2 modulates neuroinflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Exp. Med.* **203**, 425–435.
- Nystedt, S., Emilsson, K., Wahlestedt, C., and Sundelin, J. (1994). Molecular cloning of a potential proteinase activated receptor. *Proc. Natl. Acad. Sci. USA* **91**, 9208–9212.
- Oikonomopoulou, K., Hansen, K.K., Saifeddine, M., Vergnolle, N., Tea, I., Blaber, M., Blaber, S.I., Scarisbrick, I., Diamandis, E.P., and Hollenberg, M.D. (2006). Kallikrein-mediated cell signaling: targeting proteinase-activated receptors (PARs). *Biol. Chem.* **387**, 817–824.
- Ossovskaya, V.S. and Bunnett, N.W. (2004). Protease-activated receptors: contribution to physiology and disease. *Physiol. Rev.* **84**, 579–621.
- Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R., Wal-



- lasch, C., and Ullrich, A. (1999). EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**, 884–888.
- Rasmussen, U.B., Vouret-Craviari, V., Jallat, S., Schlesinger, Y., Pages, G., Pavirani, A., Lecocq, J.P., Pouyssegur, J., and Van Obberghen-Schilling, E. (1991). cDNA cloning and expression of a hamster alpha-thrombin receptor coupled to Ca<sup>2+</sup> mobilization. *FEBS Lett.* **288**, 123–128.
- Rieser, P. (1967). The insulin-like action of pepsin and pepsinogen. *Acta Endocrinol. (Copenh.)* **54**, 375–379.
- Rieser, P. and Rieser, C.H. (1964). Anabolic responses of diaphragm muscle to insulin and to other pancreatic proteins. *Proc. Soc. Exp. Biol. Med.* **116**, 669–671.
- Ruf, W., Dorfleutner, A., and Riewald, M. (2003). Specificity of coagulation factor signaling. *J. Thromb. Haemost.* **1**, 1495–1503.
- Saifeddine, M., al-Ani, B., Cheng, C.H., Wang, L., and Hollenberg, M.D. (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.
- Scarlsbrick, I.A., Blaber, S.I., Lucchinetti, C.F., Genain, C.P., Blaber, M., and Rodriguez, M. (2002). Activity of a newly identified serine protease in CNS demyelination. *Brain* **125**, 1283–1296.
- Shoelson, S.E., White, M.F., and Kahn, C.R. (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.
- Steinhoff, M., Vergnolle, N., Young, S.H., Tognetto, M., Amadesi, S., Ennes, H.S., Trevisani, M., Hollenberg, M.D., Wallace, J.L., Caughey, G.H., et al. (2000). Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. *Nat. Med.* **6**, 151–158.
- Steinhoff, M., Buddenkotte, J., Shpacovitch, V., Rattenholl, A., Moormann, C., Vergnolle, N., Luger, T.A., and Hollenberg, M.D. (2005). Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocr. Rev.* **26**, 1–43.
- Takayama, T.K., McMullen, B.A., Nelson, P.S., Matsumura, M., and Fujikawa, K. (2001). Characterization of hK4 (prostase), a prostate-specific serine protease: activation of the precursor of prostate specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase. *Biochemistry* **40**, 15341–15348.
- Tanimoto, H., Underwood, L.J., Shigemasa, K., Parmley, T.H., and O'Brien, T.J. (2001). Increased expression of protease M in ovarian tumors. *Tumour Biol.* **22**, 11–18.
- Vergnolle, N. (2004). Modulation of visceral pain and inflammation by protease-activated receptors. *Br. J. Pharmacol.* **141**, 1264–1274.
- Vergnolle, N., Hollenberg, M.D., and Wallace, J.L. (1999a). 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, M.D., Sharkey, K.A., and Wallace, J.L. (1999b). 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., Bunnett, N.W., Sharkey, K.A., Brussee, V., Compton, S.J., Grady, E.F., Cirino, G., Gerard, N., Basbaum, A.I., Andrade-Gordon, P., Hollenberg, M.D., and Wallace, J.L. (2001a). Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat. Med.* **7**, 821–826.
- Vergnolle, N., Wallace, J.L., Bunnett, N.W., and Hollenberg, M.D. (2001b). Protease-activated receptors in inflammation, neuronal signaling and pain. *Trends Pharmacol. Sci.* **22**, 146–152.
- Vu, T.K., Hung, D.T., Wheaton, V.I., and Coughlin, S.R. (1991). Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057–1068.
- Yousef, G.M., Polymeris, M.E., Grass, L., Soosaipillai, A., Chan, P.C., Scorilas, A., Borgono, C., Harbeck, N., Schmalfeldt, B., Dorn, J., et al. (2003). Human kallikrein 5: a potential novel serum biomarker for breast and ovarian cancer. *Cancer Res.* **63**, 3958–3965.