Minireview

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

Katerina Oikonomopoulou1, Kristina K. Hansen2, Mahmoud Saifeddine2, Nathalie Vergnolle2, Ilia Tea2, Eleftherios P. Diamandis1 and Morley D. Hollenberg2,3,**

1 Departments of Laboratory Medicine and Pathobiology, and of Pathology and Laboratory Medicine, University of Toronto and Mount Sinai Hospital, Toronto M5G 1X5, ON, Canada
2 Department of Pharmacology and Therapeutics, University of Calgary, Calgary T2N 4N1, AB, Canada
3 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 PAR1). PAR1, like the other three members of this receptor family (PAR2, PAR3, and PAR4), 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 PAR1, PAR2, and PAR3 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.

Keywords: hormone action; inflammation; kallikreins; protease-activated receptors; 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 PAR1 and PAR3; 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 PAR1 (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 PAR1 (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 PAR1, PAR2 and PAR4 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...
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 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 Pharmacology (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 PAR1; 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, PAR1, and PAR3 have been found to be targets for thrombin, whereas PAR2, not readily activated by thrombin, can be activated by trypsin, trypstatin and by other serine proteinase members of the clotting cascade apart from thrombin (e.g., the tissue factor Vila-Xa complex) (Ruf et al., 2003; Ossovskaya and Bunnett, 2004). Although the signalling properties of PAR2 are unclear, PAR2, PAR3, and PAR4 have all been found to signal via a G protein-coupled mechanism involving G, or Gs (Macfarlane et al., 2001). Furthermore, based on the revealed tethered ligand sequences of PAR1, PAR2 and PAR4, 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.

<table>
<thead>
<tr>
<th>IUPHAR receptor designation</th>
<th>Revealed tethered ligand sequence</th>
<th>Comments on activating peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR1 (h)SFLLRN... (r,m)SFFLRN...</td>
<td>Receptor-activating peptide, initially designated as TRAP, now PAR1AP</td>
<td>SFLLRN... activates both PAR1 and PAR2. Standard PAR1-activating peptide: TFLLR-NH2.</td>
</tr>
<tr>
<td>PAR2 (h)SLIGKV... (r,m)SLIGRL...</td>
<td>Designated PAR2AP</td>
<td>Selectively activates only PAR2; murine and rat sequence more potent than human sequence.</td>
</tr>
<tr>
<td>PAR3 (h)TFRGAP... (m)SFNGGP...</td>
<td>PAR3 is not activated by PAR-APs</td>
<td>Standard PAR3-derived sequences, e.g., TFRGAP... or SFNGGP..., activate both PAR1 and PAR2.</td>
</tr>
<tr>
<td>PAR4 (h)GYPGQV... (m)SFNGKF... (r)GFPGKP...</td>
<td>PAR-AP sequences do not activate PAR1 and PAR2, but are active via non-PAR3 receptors in some bioassays</td>
<td>Standard PAR3-activating peptide: AYPGKF-NH2. Standard PAR3-inactive peptide: YAPGKF-NH2.</td>
</tr>
</tbody>
</table>

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 PAR1, PAR2 and PAR4, but not in PAR3.

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 PAR1 towards thrombin activation) whilst activating another (e.g., PAR2 activation over a concentration range that disarms PAR3) (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, revealed the presence of a receptor other than PAR1, in an endothelium-dependent rat aorta relaxation assay (Hollenberg et al., 1993). That receptor, unknown at the time, turned out to be PAR2 (al-Ani et al., 1995). The principle that led to the discovery of functional PAR2 in the rat vascular endothelium was outlined some time ago by Ahlquist (1948) in defining the pharmacology of α- and β-adrenoceptors. In essence, with only minor exceptions, a receptor can be typified for distinct responses in different tissues by the relative potencies (EC50 or IC50) of a series of chemically related agonists and/or antago-

Table 2  Unique features of proteinase-activated receptors.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteolytically activated by a number of serine proteinases via a revealed tethered ligand</td>
<td>Multiple circulating or local ‘agonists’ are possible</td>
</tr>
<tr>
<td>Can be disarmed by proteolytic cleavage downstream from the tethered ligand sequence, thereby preventing enzymatic receptor activation by agonist proteinases</td>
<td>Multiple circulating or secreted enzymes, such as neutrophil elastase, can act as functional ‘antagonists’ by disarming PARs</td>
</tr>
<tr>
<td>Proteinase activation (e.g., by trypsin) can be modulated by receptor glycosylation near the tethered ligand cleavage site</td>
<td>Trypsin does not activate fully glycosylated human PAR3</td>
</tr>
<tr>
<td>PAR-activating peptides can mimic signalling triggered by proteolytic activation</td>
<td>Receptors other than PARs may be activated, even by PAR-selective PAR-APs that do not activate other PARs</td>
</tr>
</tbody>
</table>
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, and PARα,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, and PAR, were discovered primarily due to the complex actions of thrombin on mammalian platelets, the potential physiological role for PAR, was not known at the time of its discovery (Nystedt et al., 1994). However, the use of selective PAR, -activating peptides as probes for PAR, function quickly revealed a potential role for this receptor in regulating vascular and gastric smooth muscle tension (al-Ani et al., 1995; Hol- lenberg et al., 1996, 1997; Saieddine et al., 1996). Acting either on the endothelial cells (PAR, and PAR, ) or directly on the smooth muscle elements (PAR, ), it is now known that PARs are involved in regulating the cardiovascular system. It was not expected, however, that functional PAR, as well as PAR, would be found on neuronal elements (Corvera et al., 1999). Furthermore, it was also initially an unexpected finding that the activation of PAR, and PAR, 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, and PAR, 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, and PAR, 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 as astrocytes, it is to be expected that neuronal PARs may play a widespread physiological role (Noorbakhsh et al., 2003). Examples are the upregulation of PAR, in astrocytes in the setting of HIV encephalitis (Boven et al., 2003) and the ‘protective’ induction of CNS PAR, 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 kalli- kreins, 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,-deficient mice to adjuvant-induced arthritis (Ferrell et al., 2003); (ii) the effectiveness of PAR, antagonists in mitigating chronic inflammation in different animal models of inflammatory bowel disease; and (iii) the resistance of PAR,-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 kalli- Kreins (Scarisbrick et al., 2002; Blaber et al., 2002, 2004). In spite of the many organs in which kallikreins can be

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

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

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 Ossovskaya 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.
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 (Frengue 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 trypsin-like activity, like trypsin, the kallikreins, can also potentially trigger signal transduction pathways by regulating PARs.

In work reported elsewhere (Oikonomoupolou 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>1</sub>- and PAR<sub>4</sub>-dependent aggregation and calcium signalling in isolated rat (PAR<sub>1</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>1</sub> to trypsinic activation, we suspected that this receptor would be a good potential target for hK14, as well as for the other kallikreins with trypsinic activity (e.g., hK5, hK6). In addition to activating PAR<sub>1</sub>, trypsin is also known to both activate and disarm PARs, (Kawabata et al., 1999). Therefore, we also evaluated hK14 for its ability to regulate PARs, 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>4</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>4</sub>; (2) in experiments using HEK-derived cells that constitutively express both PAR<sub>1</sub> and PAR<sub>4</sub> (but not PAR<sub>2</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>2</sub>-dependent rat platelet aggregation assay (Hollenberg and Saijfeddine, 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 proteases, 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).

Figure 3  Targets for proteinase-mediated signalling.

- **Proteinase**
  - **Pre-pro hormone precursor processing:** peptide agonist inactivation
  - **Growth factor receptors**
    - **Activation/Inactivation**
  - **PARs**
    - **Activation/Inactivation**
  - **Extracellular matrix, integrins**
  - **Membrane-tethered agonist release**

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-β is generated by the interleukin-β 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, trypptase 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-

**References**


Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R., Wal-


