Trypsin IV, a Novel Agonist of Protease-activated Receptors 2 and 4*

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Certain serine proteases signal to cells by cleaving protease-activated receptors (PARs) and thereby regulate hemostasis, inflammation, pain and healing. However, in many tissues the proteases that activate PARs are unknown. Although pancreatic trypsin may be a physiological agonist of PAR2 and PAR4 in the small intestine and pancreas, these receptors are expressed by cells not normally exposed pancreatic trypsin. We investigated whether extrapancreatic forms of trypsin are PAR agonists. Epithelial cells lines from prostate, colon, and airway and human colonic mucosa expressed mRNA encoding PARs, trypsinogen IV, and enteropeptidase, which activates the zymogen. Immunoreactive trypsinogen IV was detected in vesicles in these cells. Trypsinogen IV was cloned from PC-3 cells and expressed in CHO cells, where it was also localized to cytoplasmic vesicles. We expressed trypsinogen IV with an N-terminal IgG signal peptide to direct constitutive secretion and allow enzymatic characterization. Treatment of conditioned medium with enteropeptidase reduced the apparent molecular mass of trypsinogen IV from 36 to 30 kDa and generated enzymatic activity, consistent with formation of trypsin IV. In contrast to pancreatic trypsin, trypsin IV was completely resistant to inhibition by polypeptide inhibitors. Exposure of cell lines expressing PAR2 and PAR4 to trypsin IV increased [Ca2+]i, and strongly desensitized cells to PAR agonists, whereas there were no responses in cells lacking these receptors. Thus, trypsin IV is a potential agonist of PAR2 and PAR4 in epithelial tissues where its resistance to endogenous trypsin inhibitors may permit prolonged signaling.

Protease-activated receptors (PARs)† are a family of four G-protein-coupled receptors that control hemostasis, inflammation, pain, and healing (1–3). Proteases from the circulation, exocrine glands, and inflammatory cells cleave PARs to expose tethered ligand domains that bind and activate the cleaved receptors. Thrombin activates PAR1 (4), PAR2 (5), and PAR4 (6, 7), and coagulation factors VIIa and Xa activate PAR4 (8). Pancreatic trypsin is an agonist of PAR2 (9, 10) and PAR4 (6, 7). Mast cell trypsin activates PAR2 (11, 12), and cathepsin G from neutrophils is a PAR4 agonist (13). However, the proteases that activate PARs in many tissues are not known.

We investigated whether extrapancreatic trypsins are agonists of PAR2 and PAR4. PAR4 is present in the kidney, pancreas, stomach, intestine, airway, skin, bladder, and brain, where it is localized to epithelial and endothelial cells, myocytes, fibroblasts, immune cells, neurons, and glial cells (2, 3, 9, 10, 14). PAR4 is expressed in the liver, small intestine, pancreas, lung, placenta, thyroid, and prostate, and in megakaryocytes (2, 3, 6, 7, 15). Pancreatic trypsin may be a physiologically relevant agonist of PAR2 and PAR4 in the lumen of the small intestine, since trypsin can signal to enterocytes by cleaving PAR2 at the apical membrane (16), and in the inflamed pancreas, when there is premature activation of trypsinogen, which can signal to acinar or ductal cells (10, 17). However, given the widespread distribution of PAR2 and PAR4, it is unlikely that pancreatic trypsin is a physiologically relevant agonist in tissue other than the small intestine and pancreas.

Trypsinogens I, II, and mesotrypsinogen are the major trypsinogen genes in the human pancreas (18, 19). These genes encode proteins with a signal sequence, an activation peptide and a catalytic domain. Enteropeptidase cleaves (†) at the DDDDK site of the zymogens to release active trypsinogen (20, 21), which are highly homologous and share critical active site residues. Trypsinogens I and II are the principal forms of trypsin that are secreted in pancreatic juice, activated by enteropeptidase in the small intestine and participate in digestion. Mesotrypsinogen is a minor trypsinogen in human pancreatic juice (22). However, mesotrypsinogen is uniquely susceptible to activation by lysosomal cathepsin B and may thus be prematurely activated in the inflamed pancreas (23). Moreover, mesotrypsin is unaffected by polypeptide trypsin inhibitors such as soybean trypsin inhibitor (SBTI) and human pancreatic secretory trypsin inhibitor (hPSTI) (19) and efficiently degrades and thus inactivates these inhibitors (23). Trypsinogen IV was originally identified in human brain and may be a splice variant of mesotrypsinogen (24). Trypsinogen IV differs from mesotrypsinogen in exon 1 in that it lacks a recognizable signal sequence. However, trypsinogen IV contains three potential furin-processing sites (Arg(X)-X-Arg) and may be a secreted enzyme. Brain trypsinogen IV has been proposed to control expression of glial fibrillary acidic protein and to process the amyloid precursor protein, but its physiological function is unknown (25). Trypsinogens have also been identified in extrapancreatic tissues including endothelial cells, tumor cell lines and tissues, and neurons (26–30). However, the properties and functions of these extrapancreatic trypsinogens are not fully understood.
We characterized tryptosinogens expressed in human colonic mucosa and extrapancreatic epithelial cell lines and investigated whether they are agonists of PARs. Our aims were to: (a) determine if epithelial cell lines and tissues express tryptosinogens, enteropeptidase, and PARs; (b) clone, express, and characterize the major tryptosinogens in epithelial cells; (c) investigate whether extrapancreatic tryptosins activate PARs.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Oligonucleotides were from Sigma-Genosys (The Woodlands, TX). Enteropeptidase and Tgo DNA polymerase were from Roche Applied Science. Restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, MA). Mouse monoclonal antibody to human Myc was from Invitrogen (Carlsbad, CA). A rabbit polyclonal antibody to rat trypsin was a gift from Dr. Charles Craik (UCSF), and a mouse monoclonal antibody to human IgG was from Jackson ImmunoResearch Laboratories (West Grove, PA). Bovine pancreatic trypsin was from Worthington Biochemical Corporation (Lakewood, NJ), human hemoglobin was from Calbiochem (San Diego, CA). A peptide corresponding to the tethered ligand domain of human PAR, (SLGKV-NH2) and analogues of the tethered ligand of human PAR, (TFLLR-NH2) and PAR, (AYPGFK-NH2) were from Genemed Sciences, Inc. (South San Francisco, CA). Bovine pancreatic trypsin inhibitor (BPTI), soybean trypsin inhibitor (SBTI), and enteropeptidase inhibited trypsin activity, including proteinase K. Tosyl-glycine-proline-arginine-p-nitroanilide were from Sigma Chemical Co. E-64 and PPACK were from Calbiochem (San Diego, CA). Leupeptin was from Roche Applied Science.

**Cell Lines—**Tumor-derived human epithelial cell lines (PC-3, prostate; SW480, Caco2, colon; A549, airway; HPAC, pancreatic ductal) and CHO cells were obtained from American Tissue Type Collection. NCM460 cells are a non-transformed human colonic epithelial cell line (31) and were maintained in Ham's F12 with 20% fetal bovine serum containing hydrocortisone (0.4 μg/ml) and insulin (0.5 units/ml) (32). Cells were maintained in F12K medium (PC-3, A549), Dulbecco's modified Eagle's medium (SW480, HPAC), MEM (Caco2) or Ham's F12 (CHO) medium with 10% fetal bovine serum (PC-3, A549, SW480, HPAC, CHO) or 20% fetal bovine serum (Caco2) in 95% air, 5% CO2, at 37 °C. Sarcoma virus-transformed rat kidney epithelial cells (NRK) stably expressing human PAR, (N-terminal FLAG), human PAR, (N-terminal FLAG, C-terminal HA.11), and rat PAR, (N-terminal Myc) were generated as described.

**RT-PCR—**Total RNA from epithelial cell lines (PC-3, prostate; SW480, Caco2, colon; A549, airway; HPAC, pancreatic ductal) and CHO cells were obtained from American Tissue Type Collection. NCM460 cells are a non-transformed human colonic epithelial cell line (31) and were maintained in Ham's F12 with 20% fetal bovine serum containing hydrocortisone (0.4 μg/ml) and insulin (0.5 units/ml) (32). Cells were maintained in F12K medium (PC-3, A549), Dulbecco's modified Eagle's medium (SW480, HPAC), MEM (Caco2) or Ham's F12 (CHO) medium with 10% fetal bovine serum (PC-3, A549, SW480, HPAC, CHO) or 20% fetal bovine serum (Caco2) in 95% air, 5% CO2, at 37 °C. Sarcoma virus-transformed rat kidney epithelial cells (NRK) stably expressing human PAR, (N-terminal FLAG), human PAR, (N-terminal FLAG, C-terminal HA.11), and rat PAR, (N-terminal Myc) were generated as described.

**RESULTS**

**Expression of Trypsinogen, PARα, and Enteropeptidase in Epithelial Cell Lines—**We used RT-PCR to examine whether epithelial cell lines express trypsinogen, the trypsinogen activator enteropeptidase and the trypsin receptor PARα. PC-3, SW480, Caco2, A549, and NCM460 cells all expressed mRNA for the sizes predicted for trypsinogen (400 bp) and PARα (525 bp), and all cell lines except for Caco2 and NCM460 also expressed mRNA for enteropeptidase (568 bp) (Fig. 1A for PC-3; others not shown). The identity of PARα, trypsinogens, and enteropeptidase was confirmed by sequencing (2–8 sequencing reactions per gene per cell line). The primers for trypsinogen would amplify all known trypsinogen gene products. However, we only identified sequences corresponding to trypsinogen IV and mesotrypsinogen in all cell lines, except in NCM460 cells where we also found tryptosinogen I. These primers amplified products with sequences for trypsinogen I and II from the pancreatic cell line HPAC (not shown) and were thus able to amplify the other
forms of trypsinogen. We were unable to amplify full-length products using mesotrypsinogen-specific primers in any of the cell lines. However, primers specific to trypsinogen IV amplified a partial clone of trypsinogen IV. Thus, the extrapancreatic epithelial cell lines derived from several organs (prostate, colon, airway) express mRNA encoding trypsinogen IV and PAR2, and most also express enteropeptidase.

Expression of Trypsinogens in Normal Human Colonic Mucosa—To confirm that trypsinogens are expressed in normal human colonic mucosa, total RNA was analyzed by RT-PCR. A PCR product of the predicted size (400 bp) was obtained using the primers for trypsinogens (not shown). Products were subcloned and their identity confirmed by sequencing (21 sequencing reactions, 2 patients). All products were found to be identical to either trypsinogen IV or mesotrypsinogen. Using primers to distinguish between trypsinogen IV and mesotrypsinogen, a 474-bp product was amplified and identified as trypsinogen IV by sequencing (9 sequencing reactions, 1 patient). Thus, normal human colonic mucosa, which is known to express PAR2 (10), also expresses trypsinogen IV.

Localization of Trypsinogen Immunoreactivity in Epithelial Cell Lines—To confirm expression of trypsinogen at the protein level, PC-3, and SW480 cells were stained using a monoclonal antibody to human trypsin. This antibody would be expected to amplify full-length products using mesotrypsinogen-specific primers in any of the cell lines. However, primers specific to trypsinogen IV amplified a partial clone of trypsinogen IV. Thus, the extrapancreatic epithelial cell lines derived from several organs (prostate, colon, airway) express mRNA encoding trypsinogen IV and PAR2, and most also express enteropeptidase.

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matic assay. There was no detectable trypsin activity in conditioned medium from CHO-Ig\textsubscript{H9260}-TIV or CHO-vc cells. Treatment of conditioned medium from CHO-Ig\textsubscript{H9260}-TIV cells with enteropeptidase generated tryptic activity (29 nmol/min/mg total protein). However, there was no detectable activity in enteropeptidase-treated medium collected from CHO-vc cells. We compared the effects of various inhibitors on the activity of bovine pancreatic trypsin and trypsin IV. Pepstatin A (inhibits aspartyl proteases) and E-64D (inhibits cysteinyl proteases) had little or no effect on either forms of trypsin (Fig. 5). Synthetic, low molecular mass inhibitors such as leupeptin, PPACK, and benzamidine abolished or strongly inhibited the activity of the bovine trypsin, but had no effect on the activity of trypsin IV. Thus, trypsin IV

![Image](image1.png)

**Fig. 2.** Effects of graded concentrations of PAR\textsubscript{2} agonists on \([Ca^{2+}]_i\) PC-3 cells (A) and SW480 cells (B). Cells were exposed to a single concentration of agonists. Results are expressed as the change from basal level of the fluorescence ratio (340/380 nm). Each data point is from 59–74 cells (PC-3) and 58–98 cells (SW480) in 3–4 experiments.

![Image](image2.png)

**Fig. 3.** A, transient expression of trypsinogen IV in CHO cells assessed by Western blotting. Proteins were separated by SDS-PAGE and detected using a Myc antibody. Lane 1, CHO-vc lysate (30 \(\mu\)g); lane 2, CHO-TIV-myc lysate (30 \(\mu\)g); lane 3, CHO-vc medium (20 \(\mu\)g); lane 4, CHO-TIV-myc medium (20 \(\mu\)g). B–D, localization of trypsinogen in CHO-TIV-myc cells (B and D) and CHO-vc cells (C and E) by immunofluorescence using antibodies to Myc (B and C) and rat trypsin (D and E). Trypsinogen was localized to vesicles within the cytoplasm (arrows) of CHO-TIV-myc cells using both antibodies. CHO-vc cells were unstained. Scale bar, 10 \(\mu\)m.

![Image](image3.png)

**Fig. 4.** Expression and secretion of trypsinogen IV in CHO-Ig\textsubscript{H9260}-TIV cells. A, localization of trypsinogen IV by immunofluorescence using antibody to rat trypsin. Note the localization in granules (arrows). Scale bar = 10 \(\mu\)m. B, Western blot showing secretion of trypsinogen IV and generation of trypsin IV with enteropeptidase. Proteins were separated by SDS-PAGE and detected on Western blot using a rat trypsin antibody. Lane 1, CHO-vc lysate (30 \(\mu\)g); lane 2, CHO-vc medium (10 \(\mu\)g); lane 3, CHO-Ig\textsubscript{H9260}-TIV lysate (30 \(\mu\)g); lane 4, CHO-Ig\textsubscript{H9260}-TIV medium (10 \(\mu\)g); lane 5, CHO-Ig\textsubscript{H9260}-TIV medium + enteropeptidase (10 \(\mu\)g).
Trypsin IV Activates PAR$_2$ and PAR$_4$—To examine whether trypsin IV activates PAR$_2$, we measured [Ca$^{2+}$], in KNRK-PAR$_2$ cells. Enteropeptidase-activated conditioned medium from CHO-Ig$_x$-TIV cells stimulated Ca$^{2+}$ mobilization in KNRK-PAR$_2$ cells (Fig. 6A) but not in untransfected KNRK cells, which express PAR$_2$ at only very low levels (Fig. 6C). There was no response in KNRK-PAR$_2$ cells to enteropeptidase-activated conditioned medium from CHO-vc cells, or to conditioned medium from CHO-Ig$_x$-TIV cells that had not been treated with enteropeptidase. Thus, trypsin IV cleaves PAR$_2$ in KNRK-PAR$_2$ cells to activate the receptor and mobilize Ca$^{2+}$.

To confirm that trypsin IV cleaves PAR$_2$, we examined responses in KNRK-PAR$_2$ cells treated with bovine pancreatic trypsin to desensitize PAR$_2$. Challenge of KNRK-PAR$_2$ cells with 10 nM pancreatic trypsin stimulated a prompt increase in [Ca$^{2+}$], and caused strong desensitization to a second challenge with the trypsin 2 min later (Fig. 7A). Under these conditions of desensitization of PAR$_2$ by pancreatic trypsin, responses to trypsin IV were completely abolished. In a similar manner, trypsin IV stimulated an increase in [Ca$^{2+}$], and caused strong desensitization to a second challenge with the trypsin 2 min later. This treatment also desensitized the response to pancreatic trypsin by 56%, compared with cells treated with medium from vector control cells (Fig. 7A). These results confirm that trypsin IV activates the same receptor as pancreatic trypsin in KNRK-PAR$_2$ cells.

PAR$_4$ is a second receptor for trypsin. Therefore, we determined whether trypsin IV activates PAR$_4$. Activated medium from CHO-Ig$_x$-TIV cells stimulated Ca$^{2+}$ mobilization in KNRK-PAR$_4$ cells (Fig. 6B). There was no response in KNRK-PAR$_4$ cells to enteropeptidase-treated medium from CHO-vc cells, or to untreated medium from CHO-Ig$_x$-TIV cells. Thus, trypsin IV cleaves PAR$_4$ in KNRK-PAR$_4$ cells to activate the receptor and mobilize Ca$^{2+}$. Challenge of KNRK-PAR$_4$ cells with 1 units/ml thrombin stimulated a prompt increase in [Ca$^{2+}$], and caused strong desensitization to a second challenge with the same agonist 2 min later (Fig. 7B). Trypsin IV also increased [Ca$^{2+}$], and caused strong desensitization to a second challenge with the trypsin IV 2 min later. This treatment also desensitized the response to thrombin by 62%, compared with cells treated with medium from vector control cells (Fig. 7B). These results confirm that trypsin IV activates the same receptor as thrombin in KNRK-PAR$_4$ cells.

Pancreatic trypsin is able to cleave and activate PAR$_1$, albeit at higher concentrations of the enzyme (4). To determine if trypsin IV activates PAR$_1$, we exposed KNRK-PAR$_1$ cells to trypsin IV and measured [Ca$^{2+}$]. Trypsin IV had no effect on [Ca$^{2+}$], in these cells at concentrations that strongly activated PAR$_2$ and PAR$_4$ (Fig. 6D). In contrast, both thrombin (Fig. 6D) and an analogue of the PAR$_1$ activating peptide (TFLLR-NH$_2$) (not shown) increased [Ca$^{2+}$] in these cells. Thus, trypsin IV is not an agonist of PAR$_1$ at the concentration used.

To examine whether trypsin IV can activate PARs in epithelial cells naturally expressing these receptors, we measured [Ca$^{2+}$] in PC-3 and SW480 cells that express PAR$_2$ but not PAR$_4$. Enteropeptidase-activated medium from CHO-Ig$_x$-TIV caused a prompt increase in [Ca$^{2+}$], in PC-3 and SW480 cells (Fig. 8). In contrast, activated medium from CHO-vc did not affect [Ca$^{2+}$], in either cell line (data not shown). These results confirm that trypsin IV is able to cleave and activate PAR$_2$ in cells that naturally express this receptor.

**DISCUSSION**

Our results show that trypsinogen IV is co-expressed with PAR$_2$ in extrapancreatic epithelial cell lines and in normal colonic epithelium. Enteropeptidase, which is co-expressed with trypsinogen IV, cleaves and activates thezymogen, and trypsin IV is resistant to polypeptide inhibitors of pancreatic trypsins. Trypsin IV cleaves and activates both PAR$_2$ and PAR$_4$. Thus, trypsin IV may be a physiological agonist of PAR$_2$ and PAR$_4$ in epithelial tissues where it could activate these receptors in a paracrine or autocrine manner.

**Characterization of Trypsinogen IV**—We found that trypsinogen IV (a-form) is expressed in epithelial cells of the colon, prostate and lung. In support of these results, trypsinogen IV mRNA has been amplified from PC-3 cells (35). Although trypsinogen IV cDNA encodes a protein that lacks a recognizable signal peptide, there are potential cleavage sites for furin, which may process the full-length protein into secreted forms. Indeed, trypsinogen IV was detected in vesicles in transfected cell lines and in epithelial cells, and may be secreted from these vesicles. The regulation of secretion remains to be investigated.

Expression of trypsinogen IV with an Ig$\times$ signal sequence resulted in the constitutive secretion and allowed collection of sufficient quantities of the zymogen for characterization. Enteropeptidase treatment of trypsinogen IV generated active trypsin IV with an apparent molecular mass of 30 kDa, higher than the predicted size of 24 kDa. The additional mass of the expressed protein does not represent glycosylation (data not shown), and at present we have no explanation for the decreased electrophoretic mobility. We found that trypsin IV is resistant to polypeptide inhibitors of pancreatic trypsins I and II. In support of these results, mesotrypsin, a variant of trypsin IV, is also resistant to polypeptide inhibitors (19, 23). Sequence
alignments and crystallographic studies have revealed the basis for this resistance (36). A single base change from glycine to arginine in trypsin IV (Gly198 in trypsinogens I and II, Arg255 in trypsinogen IV) results in a steric interference between the arginine of the enzyme and the P2 side chain of the polypeptide inhibitor. Mesotrypsin is similarly resistant to SBTI and hPSTI by virtue of this Gly3Arg substitution since an R198G mutant is sensitive to polypeptide inhibitors (23). In addition, this substitution renders mesotrypsin more resistant to autocatalytic destruction (23). Moreover, mesotrypsin also degrades and inactivates polypeptide trypsin inhibitors such as SBTI and hPSTI (23). In view of the similarity between mesotrypsin and trypsin IV, it is probable that trypsin IV also degrades these inhibitors although we have not tested this possibility.

**Trypsin IV as a Potential Agonist of PAR2 and PAR4**—Several observations suggest that trypsin IV is a potential agonist of PAR2 and PAR4. First, enteropeptidase-treated conditioned medium from CHO-Igκ-TIV cells, which contained trypsin IV, increased [Ca2+]i in transfected cells expressing PAR2 or PAR4. Condensed medium from CHO-Igκ-TIV cells that was not treated with enteropeptidase, and enteropeptidase-treated conditioned medium from CHO-vc cells were inactive. Thus, the stimulatory effect of conditioned medium is due to the presence of trypsin IV. Second, trypsin IV did not signal to cells lacking PAR2 and PAR4, and responses to trypsin IV were abolished by desensitization of PAR2 or PAR4, which confirms that responses to trypsin IV are mediated by these receptors. Finally, trypsin IV also increased [Ca2+]i in epithelial cells lines that naturally express PAR2, confirming that trypsin IV can signal to cells expressing this receptor at physiological levels. To our knowledge, this is the first report that trypsin IV is an agonist of PAR2 and PAR4. We did not formally compare the potencies with which trypsin IV and pancreatic trypsins activate PARs due to the lack of pure enzyme. However, comparable activities of trypsin IV and pancreatic trypsin toward a synthetic substrate (Gly-Pro-Arg-pNA) generated similar Ca2+ signals in cell lines. In support of our results, other extrapancreatic trypsins have been reported to activate PAR2. Trypsin II from COLO-205 cells activates PAR2 in transfected cells (37), and trypsin I from colon cancer cells lines also activates PAR4 (38). However, in these studies the active enzyme was not expressed nor characterized, and the ability of the trypsins to activate other PARs was not examined.

**Physiological Roles of Trypsins as PAR Agonists**—Under physiological circumstances, pancreatic trypsins in the lumen of the small intestine can signal to enterocytes by cleaving PAR2 (16). During intestinal inflammation, when there is a loss of tight junctions in the intestinal epithelium, trypsin from the lumen could penetrate tissues and activate PAR2 and PAR4 on other cell types (39). In pancreatitis, trypsinogens are prematurely activated in the pancreas and may signal to acinar and ductal epithelial cells by cleaving PAR2 (10, 17). However,
PAR2 and PAR4 are widely expressed in epithelial tissues not normally exposed to pancreatic trypsins (2, 6, 7, 9, 10, 14, 15). We hypothesize that trypsin IV released from epithelial cells could signal in a paracrine or autocrine manner by cleaving and activating PAR2 and PAR4. Although most of the epithelial cell lines expressing trypsinogen IV also expressed enteropeptidase mRNA, and enteropeptidase can activate trypsinogen IV, the factors that regulate the release and activation of trypsinogen IV are unknown.

The observations that mesotrypsin is resistant to polypeptide inhibitors, can degrade these inhibitors and is also resistant to autocatalytic destruction by virtue of the Arg → Gly substitution has lead to new theories about the function of this enzyme (23). Under physiological conditions, mesotrypsin may degrade foods that are naturally rich in trypsin inhibitors. In the inflamed pancreas, mesotrypsin may degrade the naturally protective hPSTI and thus exacerbate pancreatitis. In view of its homology to trypsin IV, it is likely that mesotrypsin activates PAR2 and PAR4 in the pancreas, which may contribute to the genesis of inflammation. The possibility that trypsin IV also degrades endogenous trypsin inhibitors coupled with our observation that trypsin IV is resistant to these inhibitors may promote prolonged signaling of this enzyme in extrapancreatic tissues by cleavage and activation of PAR2 and PAR4.

Trypsinogen IV was originally identified in human brain (24) and, if released and activated, trypsin IV could be an agonist of PARs in neurons and glial cells. PAR2 and PAR4 are expressed throughout the nervous system (40–45), but the agonists of PARs in neural tissues are not characterized. Overexpression of trypsinogen IV in neurons in the mouse brain results in a marked up-regulation of expression of glial fibrillary acidic protein in astrocytes (25), which may be mediated by release of trypsinogen IV from neurons and subsequent signaling to astrocytes through PAR2 or PAR4.
Trypsin IV is the latest member of a growing number of proteases that can activate PAR2 and PAR4. Trypsin from mast cells activates PAR2 (11, 12) and tryptase may be a physiological agonist in cells that are in close proximity to mast cells and which could be exposed to high concentrations of this enzyme, such as sensory and enteric nerves (42, 45, 46). However, trypsin is several orders of magnitude less potent than trypsin for activation of PAR2, and its capacity to activate PAR2 is diminished by glycosylation of the receptor (47, 48). Coagulation factors VIIa and Xa may activate PAR2 in endothelial cells, but responses require that VIIa is anchored to the cell-surface in the vicinity of the receptor by tissue factor (8). A solubilized form of membrane-type serine protease 1, a cell-surface protease that is co-expressed with PAR2 in PC-3 cells and certain tissues, can also activate PAR2 and PAR4. 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