Interactions of Mast Cell Tryptase with Thrombin Receptors and PAR-2*

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Tryptase is a serine protease secreted by mast cells that is able to activate other cells. In the present studies we have tested whether these responses could be mediated by thrombin receptors or PAR-2, two G-protein-coupled receptors that are activated by proteolysis. When added to a peptide corresponding to the N terminus of PAR-2, tryptase cleaved the peptide at the activating site, but at higher concentrations it also cleaved downstream, as did trypsin, a known activator of PAR-2. Thrombin, factor Xa, plasmin, urokinase, plasma kallikrein, and tissue kallikrein had no effect. Tryptase also cleaved the analogous thrombin receptor peptide at the activating site but less efficiently. When added to COS-1 cells expressing either receptor, tryptase stimulated phosphoinositide hydrolysis. With PAR-2, this response was half-maximal at 1 nM tryptase and could be inhibited by the tryptase inhibitor, APC366, or by antibodies to tryptase and PAR-2. When added to human endothelial cells, which normally express PAR-2 and thrombin receptors, or keratinocytes, which express only PAR-2, tryptase caused an increase in cytosolic Ca²⁺. However, when added to platelets or CHRF-288 cells, which express thrombin receptors but not PAR-2, tryptase caused neither aggregation nor increased Ca²⁺. These results show that 1) tryptase has the potential to activate both PAR-2 and thrombin receptors; 2) for PAR-2, this potential is realized, although cleavage at secondary sites may limit activation, particularly at higher tryptase concentrations; and 3) in contrast, although tryptase clearly activates thrombin receptors in COS-1 cells, it does not appear to cleave endogenous thrombin receptors in platelets or CHRF-288 cells. These distinctions correlate with the observed differences in the rate of cleavage of the PAR-2 and thrombin receptor peptides by tryptase. Tryptase is the first protease other than trypsin that has been shown to activate human PAR-2. Its presence within mast cell granules places it in tissues where PAR-2 is expressed but trypsin is unlikely to reach.

Tryptase is a trypsin-like serine protease that is released by activated mast cells, leading to elevated levels in plasma, cerebrospinal fluid, and other sites (1–5). Once released, tryptase has been shown to trigger the degradation of extracellular matrix by activating matrix metalloproteinases (6). It has also been shown to stimulate DNA synthesis in fibroblasts (7–9) and tracheal smooth muscle (10) and to increase intercellular adhesion molecule 1 expression and granulocyte-macrophage colony-stimulating factor release by human umbilical vein endothelial cells (HUVEC) (11). Although it has been shown that tryptase must be proteolytically active to elicit these responses, little else is known about the mechanisms underlying its effects on cells. To begin to dissect this process, we have examined the interaction of tryptase with thrombin receptors and PAR-2, the only two known members of the family of protease-activated G-protein-coupled receptors. Thrombin receptors and PAR-2 are similar in structure to other G-protein-coupled receptors, but they are activated by a novel mechanism in which a protease binds to the N terminus of the receptor and cleaves it (12, 13). This exposes a new N terminus, the first 5 or 6 residues of which serves as a tethered ligand, activating the receptor by interacting with sites within the exofacial loops of the receptor (14, 15). One piece of evidence supporting this mechanism of activation is that synthetic peptides corresponding to the tethered ligand domains of the thrombin receptor and PAR-2 are able to act as full agonists on their respective receptors (12, 13).

Thrombin receptors were originally identified by their ability to respond to thrombin, but subsequent studies have shown that they can be activated by other proteases capable of exposing the tethered ligand, provided, of course, that the protease does not disable the receptor by cleaving it at additional sites. Tryptase is one example of a protease that can activate thrombin receptors by cleaving the N terminus at the same site as thrombin (12, 16). Cathepsin G, on the other hand, cleaves thrombin receptors at the normal thrombin site (Arg41-Ser42) but also at a second site downstream from the first (Phe55-Trp56). As a result, exposure of thrombin receptors to cathepsin G prevents a subsequent response to thrombin, and cathepsin G is able to activate human thrombin receptors on its own only when the second site is mutated or otherwise blocked (17).

Much less is known about the proteases that can activate...
PAR-2, although trypsin is clearly among them and thrombin is not (13, 18). Based upon the apparent tissue distribution of PAR-2 mRNA, it seems unlikely that trypsin is the sole protease capable of activating it, but others have not yet been described. Thrombin receptors and PAR-2 have a wide, but only partially overlapping, tissue distribution. Some cells, including platelets and several megakaryoblastic cell lines, express thrombin receptors (12, 19–22) but not PAR-2 (23, 24). Others, including keratinocytes, express PAR-2 but little, if any, thrombin receptor (18). Endothelial cells have been shown to express both (23, 25–28).

In the present studies, we examined the interaction of trypsin with human PAR-2 and thrombin receptors. It had been inferred previously that trypase does not activate thrombin receptors but may, if anything, disable them (7, 9). However, this had not been tested directly, and although no information was available about the interaction of trypase and PAR-2, the resemblance between the amino acid sequence surrounding the activating cleavage site in human PAR-2 and known peptide substrates for trypsin (29) suggested that PAR-2 might be a substrate for trypsin. The results that were obtained show that trypase can cleave synthetic peptides at the correct site for activation of both receptors, although the PAR-2 peptide was cleaved more efficiently. When added to intact receptors, trypase cleaved endogenous and transfected PAR-2. It also cleaved thrombin receptors overexpressed in COS-1 cells but did not activate endogenous thrombin receptors on platelets or megakaryoblastic CHRF-288 cells. This failure to activate endogenous thrombin receptors appears not to be due to a cathepsin G-like cleavage of a secondary site within the receptor N terminus but may reflect the relatively slower rate of hydrolysis suggested by peptide studies. Therefore, in addition to identifying the first protease other than trypsin that can activate PAR-2, these results suggest that factors in addition to receptor primary sequence limit the ability of proteases to evoke responses from this subfamily of G-protein-coupled receptors.

EXPERIMENTAL PROCEDURES

Materials—Highly purified α-thrombin was provided by Dr. J. Fenlon (New York State Department of Health, Albany, NY) and Calbiochem. Tryptase was obtained from Sigma and Athens Research and Technology (Athens, GA). Plasmin was obtained from Boehringer Mannheim. Urokinase was obtained from Calbiochem. The tissue kallikrein that was used as prepared was a recombinant protein at Arris Pharmaceutical Corp. Plasma kallikrein was obtained from Athens Research and Technology. Factor Xa was obtained from Hematologic Technologies, Vermont. The cDNA for human PAR-2 was generously provided by Dr. J. Sundelin (Lund University, Lund, Sweden). Antibody SAM11 is an IgG2a monoclonal antibody produced in mice immunized with the peptide SLIGKVDTGSHVTG, corresponding to residues 37–50 of the human PAR-2 sequence. Further details about this antibody will be provided elsewhere. Antibodies S16,38, ATAP2, and WEDE15 are peptide-directed monoclonal antibodies that recognize sites within the N terminus of the human thrombin receptor and have been described previously (22, 30). A neutralizing monoclonal anti-trypsin antibody (B12) was a gift from Dr. L. Schwartz (Virginia Commonwealth University, Richmond, VA). APC366 is a novel human trypsin inhibitor synthesized by the medicinal chemistry group at Arris Pharmaceutical Corp. (31).

Tryptase—The trypase used in the peptide hydrolysis and cell response studies was purified from an immunized human mast cell line (HMC-1) donated by Dr. J. Butterfield (Mayo Clinic, Rochester, MN) using an established protocol (32). The enzymatic activity of purified trypase was determined by spectrophotometrically measuring the hydrolysis of the substrate N-tosyl-Gly-Pro-Lys-pNA (Sigma) (33). The specific activity of individual batches of trypase ranged between 71 and 108/ml, 5-mmol at 37 °C. The specific activity of individual batches of trypase ranged between 71 and 108/ml, 5-mmol at 37 °C. The specific activity of individual batches of trypase ranged between 71 and 108/ml, 5-mmol at 37 °C. The specific activity of individual batches of trypase ranged between 71 and 108/ml, 5-mmol at 37 °C.

RESULTS

PAR-2 was thought to be a potential substrate for trypsin, because an earlier analysis of the P3P2P1 site preferences for trypsin in cleaving tripeptide nitroanilide substrates had revealed that the highest catalytic efficiency was for the tripeptide, Lys-Gly-Arg (−4 × 10^7 M^-1 s^-1) (29), which matches the P3P2P1 sequence at the activating cleavage site of human PAR-2 (Fig. 1). This sequence is different from the corresponding region of the human thrombin receptor. We and others have shown previously that longer peptides corresponding to sequences within the N terminus of the human thrombin receptor can be useful for predicting potential sites of cleavage within the intact receptor (17, 37). In preliminary studies, trypase was added to peptides whose sequences correspond to the regions around the activating sites of human thrombin receptors and PAR-2. In addition, trypsin and several other serine proteases were studied. Where distribution might reasonably be expected to bring them into contact with PAR-2, proteolytic fragments were identified by liquid chromatography and mass spectrometry.

At a final concentration of 1 mM, trypase cleaved a peptide corresponding to residues 32–45 in the human PAR-2 sequence at the N1 site (Arg36,Ser37) that would expose the receptor’s teth-

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ered ligand domain (Table I). At 100 nM, tryptase cleaved all of the starting material and, in addition, produced a further fragment by cleaving after the residue corresponding to Lys-Valoccurringat100nM(TableI).Neitherthrombin,which
does not activate PAR-2, urokinase, nor tissue kallikrein
cleaved the PAR-2 peptide. Plasmin, factor Xa, and plasma
kallikrein at 20 nM cleaved approximately 5% of the peptide
over a 30-min period during which tryptase and trypsin at the
same concentration cleaved >95% of the starting material.

When added to peptides based on the human thrombin recep-
tor sequence, tryptase cleaved at the site normally cleaved
by thrombin in the intact receptor (Arg-Ser41), producing
the fragments NATLDPR and SFLLR from the peptide
NATLDPR/SFLLRthatcorrespondstothethrombinreceptor
residues Asn35–Arg46. Thrombin cleaved the peptide at the same site
(Table I). In contrast, when thrombin and trypsin were added
to a second peptide, SFLLRNPNpndkyepf, corresponding to
thrombin receptor residues 42–55, tryptase cleaved the peptide
at two sites, Arg-Asn and Lys-Tyr, while thrombin, even at
1000 nM, had no effect.

Based upon these results, tryptase has the potential for
activating human thrombin receptors as well as human PAR-2.
However, it also has the potential to disable either receptor by
cleaving the N terminus at one or more sites downstream from
the activation site (Fig. 1). In the case of the PAR-2 peptide,
there was a clear concentration dependence in the cleavage at
the two sites, with only Arg-Ser cleaved at a tryptase concen-
tration (1 nM) that activates the intact receptor (see below).
Furthermore, trypsin, which is a known activator of PAR-2,
showed the same pattern of cleavage. In the case of the thom-
bin receptor peptides, there was a clear difference between
tryptase and thrombin. While thrombin cleaved the peptide only
at the activating site, tryptase also cleaved at two additional
sites, which, if they were to occur in the intact receptor, would
amputate the tethered ligand domain (Fig. 1). This suggests
that tryptase could activate PAR-2 but might disable thrombin
receivers in the manner previously seen with cathepsin G.

**Activation of Receptors Expressed in COS-1 Cells**—To test
the interaction of tryptase with intact PAR-2 and thrombin
receivers, the human forms of both receptors were individually
overexpressed in COS-1 cells. [3H]inositol phosphate formation
was used to detect receptor activation. PAR-2 expression was
detected using the monoclonal antibody SAM11, which was
produced in mice immunized with a peptide that includes the
PAR-2 tethered ligand domain (residues 37–50). Thrombin
receptor expression was detected with the monoclonal anti-
bodies: SPAN12, ATAP2, and WEDE15.

**TABLE I**

| Enzyme          | Concentration (nM) | Receptor (residues) Peptide Fragment(s) detected       |
|-----------------|--------------------|--------------------------------------------------------|
| Tryptase        | 1                  | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
|                 | 10                 | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
|                 | 10                 | huTR (32–45) NATLDPR/SFLLR                            |
|                 | 10                 | huTR (42–55) NATLDPR/SFLLR                            |
| Tryptase        | 1                  | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
|                 | 10                 | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
| Thrombin        | 20                 | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
|                 | 1000               | huTR (35–46) NATLDPR/SFLLR                            |
|                 | 1000               | huTR (42–55) NATLDPR/SFLLR                            |
| Plasmin, plasma | 20                 | huPAR2 (32–45) SSKGR/SLIGKVDGT                         |
| Kallikrein, Xa  |                    |                                                        |

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* huPAR2, human PAR-2.

* None of the parent peptide remained.

* huTR, human thrombin receptor.

* Comparatively little of the parent peptide was cleaved.

* From reference (17) and shown for comparison.

**FIG. 1.** Proteolysis of human PAR-2 (huPAR2) and thrombin receptor (huTR) peptides. The figure shows potential sites of cleavage
within the N terminus of human PAR-2 (top) and thrombin receptors (bottom) based upon the peptide hydrolysis studies shown in Table I.
The slashes indicate the sites of cleavage that in the intact receptors lead to exposure of the tethered ligand domain (the underlined resi-
dues) and receptor activation. The numbering refers to the sequence of the intact receptors. The hatched bars at the bottom show the approx-
imate locations of the epitopes for three thrombin receptor monoclonal antibodies: SPAN12, ATAP2, and WEDE15.
The results show that tryptase can activate human PAR-2 in a concentration-dependent manner. Peak inositol phosphate formation was 2.5-fold over base line and was half-maximal at 1 nM tryptase (Fig. 2). Tryptase had no effect on COS-1 cells transfected with the empty plasmid, pRK7, and increasing the tryptase concentration as high as 100 nM had no greater effect on the cells expressing human PAR-2 than was obtained with 10 nM tryptase (Fig. 3). This pattern of response was somewhat different from that observed with trypsin. At 1 nM, trypsin was, if anything, less potent than tryptase in stimulating phosphoinositide hydrolysis in the human PAR-2-expressing COS-1 cells, just as trypsin was somewhat less efficient than tryptase in cleaving the PAR-2 peptide that overlapped the activation site. However, at concentrations ≥10 nM, trypsin was more effective than tryptase in activating the human PAR-2-expressing COS-1 cells (Fig. 3). The dose-response curve for trypsin clearly continued to rise after the response to tryptase had become maximal. Tryptase also activated human thrombin receptors expressed in COS-1 cells. At 0.1, 1, and 10 nM, the response to trypsin in the human thrombin receptor-expressing cells was similar in magnitude to the response to tryptase in cells expressing PAR-2 (Fig. 4).

In order to demonstrate a requirement for active enzyme in tryptase-induced receptor activation and to further establish the specificity of this response, we examined the effects of several potential inhibitors with varying patterns of selectivity. The response of COS-1 cells expressing human PAR-2 to tryptase was eliminated by boiling the enzyme (Fig. 5A). It was also eliminated by adding leupeptin, an inhibitor of multiple serine proteases, and by APC366, a more selective inhibitor of tryptase (31). Soybean trypsin inhibitor, which completely blocked the response to 10 nM trypsin (not shown), had no effect on tryptase. Partial inhibition was seen when the transfected cells were incubated with the anti-PAR-2 monoclonal antibody, SAM11, or when tryptase was preincubated with an anti-trypsin antibody (Fig. 5A) but not with isotype-matched control antibodies (not shown). In the cells expressing human thrombin receptor, hirudin blocked the response to thrombin but had no effect on the response to trypase (Fig. 5B).

Taken together, the data in Figs. 2–5 show that tryptase is able to activate thrombin receptors and PAR-2 when these receptors are transiently expressed in COS-1 cells. By inference, cleavage at the secondary sites in each receptor suggested by the peptide studies does not occur, since that would prevent receptor activation, or it occurs only at higher tryptase concentrations and is responsible for the plateau in the tryptase dose-response curve at concentrations above 10 μM (Figs. 2 and 3).

**Activation of Endogenous Thrombin Receptors and PAR-2—** Different types of cells expressing the same receptor need not produce it at the same density nor subject it to precisely the same post-translational modifications. Therefore, we also examined the effects of tryptase on 1) HUVEC, which express thrombin receptors as well as PAR-2 (huPAR-2) or human thrombin receptors (huTR) were incubated for 45 min with tryptase. The results shown are the mean ± S.E. for four to seven studies in which each data point was measured in triplicate.

**Fig. 2.** Phosphoinositide hydrolysis in COS-1 cells. Tryptase at the concentrations shown was added to COS-1 cells expressing human PAR-2. The cells were prelabeled with [3H]inositol and incubated with the protease for 45 min. The results are expressed as a -fold increase in total [3H]inositol phosphate formation and are the mean ± S.E. for two to eight studies in which each data point was measured in triplicate.

**Fig. 3.** Comparison of tryptase and trypsin. COS-1 cells that had been transfected with either human PAR-2 (huPAR-2) (A) or empty vector (B) were incubated with each of the enzymes shown for 45 min. The results shown are the mean ± S.E. for two to six studies in which each data point was measured in triplicate.

**Fig. 4.** PAR-2 and thrombin receptor responses in COS-1 cells. COS-1 cells expressing either human PAR-2 (huPAR-2) or human thrombin receptors (huTR) were incubated for 45 min with tryptase. The results shown are the mean ± S.E. for four to seven studies in which each data point was measured in triplicate.
(18), respond to tryptase with a transient increase in cytosolic Ca\(^{2+}\). The data in Fig. 6 show that tryptase can also activate human umbilical vein endothelial cells. The size of this increase was notably smaller than the response to either the PAR-2 agonist peptide, SLIGKV, or to thrombin and was more variable from experiment to experiment. This variability did not appear to be due to the presence of an inhibitor of tryptase, since tryptase recovered after a 10-min incubation with HUVEC fully retained its ability to cleave a chromogenic substrate (not shown).

Since HUVEC express thrombin receptors as well as PAR-2, the response to tryptase shown in Fig. 6 could theoretically be due to activation of either receptor or both. In the experiment shown in Fig. 7, tryptase was added to washed human platelets reuspended in buffer containing Ca\(^{2+}\) and fibrinogen. Although the platelets aggregated promptly in response to the thrombin receptor peptide, SFLLRN, they showed no response to 10 nM tryptase. Increasing the tryptase concentration to 50 nM also had no effect; nor did repeating the experiment with platelets suspended in plasma (data not shown).

Tryptase was also tested with CHRF-288 cells, a human megakaryoblastic cell line that expressed thrombin receptors but not PAR-2. As previously reported, we found that thrombin causes an increase in the cytosolic Ca\(^{2+}\) concentration in Fura-2-loaded CHRF-288 cells. Tryptase, however, had no effect, and preincubation of the cells with tryptase did not block subsequent activation by thrombin (not shown). Finally, a comparison was also made of anti-thrombin receptor antibody binding to CHRF-288 cells before and after incubating the cells with 40 nM thrombin or tryptase (10 and 100 nM) for 10 min at 37 °C. The epitopes for the antibodies are shown in Fig. 1. Thrombin caused an 80% decrease in the binding of the two antibodies whose epitopes are C-terminal to the activation site, ATAP2 and WEDE15, and a >95% decrease in the binding of antibody SPAN12, whose epitope includes the activation site. We have previously shown that in these cells the loss of ATAP2 and WEDE15 sites is due to thrombin receptor internalization, while the loss of SPAN12 sites reflects receptor cleavage (22, 30). Tryptase, on the other hand, had no effect on the binding of any of the three antibodies (not shown).

These results suggest that tryptase can activate endogenous PAR-2, but it does not appear to activate endogenous thrombin receptors on platelets or CHRF-288 cells, and by implication, it appears not to activate thrombin receptors on HUVEC. The data also suggest that the failure to activate endogenous thrombin receptors is not due to cleavage of the receptor at a

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site immediately downstream from the activation site or at any other site that would disable the receptor.

**DISCUSSION**

At present, the family of G-protein-coupled receptors that respond to proteases consists of two members, thrombin receptors (sometimes referred to as PAR-1) and PAR-2. However, there is ample reason to believe that others exist, including the results of recent thrombin receptor knockout studies in mice (38, 42) as well as earlier observations that proteases other than thrombin and trypsin can evoke responses from cells that do not appear to be mediated by thrombin receptors or PAR-2. In theory, a protease can interact with thrombin receptors or PAR-2 in at least two different ways. If it can cleave the receptor at the specific site that exposes the tethered ligand domain, a protease has the potential for activating the receptor. If it cleaves downstream (i.e. C-terminal) to the activation site, the protease will disable the receptor for subsequent activation by an enabling protease, such as thrombin or trypsin. Depending on where the cleavage occurs, it may or may not prevent receptor activation by an agonist peptide. Cathepsin G is an example of a protease that cleaves thrombin receptors at two sites. One is the activation site. The other is a site further down in the N terminus that prevents receptor activation by thrombin but not by the peptide, SFLLRN (17). Plasmin may do the same (37).

Three proteases have been shown to activate thrombin receptors: thrombin (12), trypsin (12, 16), and granzyme A (39). Of these, only trypsin has been shown to activate PAR-2 (13). In the present studies, we have examined the interaction of thrombin receptors and PAR-2 with human mast cell tryptase. The results show that tryptase has the potential for activating both receptors. However, there are clearly differences in its ability to do so as well as reasons to believe that receptor activation by tryptase is subject to limitations that do not apply to thrombin and trypsin. When added to peptides whose sequence corresponds to the N terminus of human thrombin receptors, tryptase cleaved at the “activation site,” but unlike thrombin, tryptase was also able to cleave at least one site beyond this, a site that, were it to occur in the intact receptor, would amputate the tethered ligand domain. When added to a corresponding PAR-2 peptide, 1 nM tryptase cleaved only at the activation site, but 100 nM tryptase cleaved within the sequence corresponding to the tethered ligand domain, as did trypsin, which activates PAR-2 even at 100 nM. One nM was chosen because it proved to be the tryptase concentration that causes half-maximal [3H]inositol phosphate accumulation in COS-1 cells expressing PAR-2. One hundred nM tryptase is at least 10-fold greater than the tryptase concentration at which the COS-1 cell response is maximal. Notably, although detailed kinetic studies were not done, tryptase cleaved more of the PAR-2 peptide than thrombin receptor peptide, although the concentration of tryptase added to the thrombin receptor peptide was 10-fold greater. This suggests that the human PAR-2 sequence is a better substrate for tryptase than the thrombin receptor sequence.

The peptide cleavage studies help to explain some of the results that were obtained with the actual receptors. When added to COS-1 cells transiently overexpressing either PAR-2 or thrombin receptors, tryptase caused increased inositol phosphate formation via both receptors, indicating that phospholipase C had been activated. For PAR-2, this response was half-maximal at 1 nM and maximal by 10 nM. Interestingly, the response of the cells to trypsin was, if anything, somewhat less than tryptase at 1 nM, but it exceeded the tryptase response at 10 and 100 nM (Fig. 3). This difference may be due in part to the ability of trypsin to evoke a weak response from mock-transfected COS-1 cells, but it may also reflect differences between the two enzymes in their ability to cleave at a disabling site, perhaps the one suggested by the peptide studies, Arg31-Val42 (Fig. 1). Cleavage at the disabling site would limit the amount of PAR-2 activated by tryptase. This hypothesis was not tested directly; antibodies with epitopes spaced along the length of the receptor N terminus are available for thrombin receptors but not yet available for PAR-2.

Further differences between the two receptors emerged when tryptase was added to cells that express thrombin receptors and PAR-2 endogenously. Tryptase was able to activate PAR-2 in keratinocytes and HUVEC but appeared unable to activate thrombin receptors in platelets, CHRF-288 cells, and endothelial cells. This inability of tryptase to evoke a response through endogenous thrombin receptors could be due to cleavage of the endogenous receptors (but not the expressed receptors) at one or more of the sites downstream from the activation site suggested by the peptide studies. This possibility was suggested previously by Caughey and co-workers (9), who found that in rat aortic vascular smooth muscle cells, tryptase can inhibit thrombin-induced DNA synthesis while having no effect on the response to SFLLRN. However, unlike cathepsin G, which does disable thrombin receptors in this manner, we found no loss of thrombin responsiveness in platelets or CHRF-288 cells that had been preincubated with tryptase and no loss of binding sites for thrombin receptor antibodies directed downstream from the activating site. The explanation for the difference in tryptase’s ability to activate endogenous and transfected thrombin receptors is, therefore, still not entirely clear. In general, tryptase appears to be a less optimal activator of thrombin receptors than is thrombin, presumably reflecting differences in the substrate specificity of the two enzymes. Differences in thrombin receptor glycosylation within the N terminus in COS-1 cells, platelets, CHRF-288 cells, and endothelial cells might have a greater effect on tryptase than on thrombin. Similarly, since successful signaling through thrombin receptors requires multiple receptors to be activated in as short a time as possible (40), overexpression may make it possible for COS-1 cells to respond to tryptase via the thrombin receptor, while endogenous receptors do not. Of note, we observed that the response of HUVEC to tryptase was smaller in magnitude and much more variable than the response of the same cells to either trypsin or the peptide agonists. PAR-2 appears to be expressed at lower density on HUVEC than thrombin receptors (27). Variability caused by culture conditions or state of confluency might have a greater effect on tryptase than on the other agonists of PAR-2.

In conclusion, these results suggest that tryptase is able to activate human PAR-2 but probably not thrombin receptors. The activation of PAR-2 by tryptase causes phosphoinositide hydrolysis and an increase in cytosolic Ca2+, just as occurs when PAR-2 is activated by trypsin or an agonist peptide. The concentration required (EC50 = 1 nM) is compatible with a recent report by Schwartz et al. (5), who measured blood tryptase levels in normal subjects and patients with systemic mastocytosis, a disorder in which the number of mast cells is greatly increased. Normal subjects had a mean circulating tryptase concentration of 0.2 nM. The mastocytosis patients ranged from 1 to 7 nM. PAR-2 is known to be expressed in endothelial cells, vascular smooth muscle cells, and keratinocytes, areas in which mast cell degranulation can occur and in which even higher local concentrations of tryptase may be obtained. Studies of patients dying of myocardial infarction have demonstrated as much as a 50-fold increase in mast cells at or around the ruptured atherosclerotic plaque (41). Whether
PAR-2 activation by tryptase is responsible for some of the pathology that occurs in these areas remains to be seen.

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