Recently, a second member of the protease-activated receptor (PAR) family, named PAR-2, has been identified. Similar to the thrombin receptor, PAR-2 appears to be activated by proteolytic-mediated exposure of a “tethered ligand” sequence and can also be activated by the corresponding synthetic peptides. Similarities in the amino acid sequence of the receptors’ tethered ligand sequences suggest that their respective agonist peptides might not be absolutely specific for their particular receptors. To test this, the receptor specificity of each agonist has been determined by measuring the responses of Xenopus oocytes expressing the thrombin receptor or PAR-2 to agonist peptides or enzymes. Thrombin receptors responded to thrombin, the human thrombin receptor-activating peptide SFLLRNP-NH₂ (TRAP) (EC₅₀ = 0.1 μM), and Xenopus TRAP, TFRIFD-NH₂ (EC₅₀ = 1 μM), but did not show any increase in calcium efflux over control levels with trypsin (50 nM) or PAR-2 agonist peptides (100 μM). Human and murine PAR-2 receptors responded comparably to human and murine PAR-2 agonist peptides (SLIGKV and SLIGRL, respectively) (EC₅₀ = 0.5–20 μM) and trypsin, but not to thrombin. PAR-2 was also found to be responsive to TRAP (EC₅₀ = 1 μM) but was unresponsive to Xenopus TRAP (50 μM). Responses to additional peptide agonist analogs suggest that an amino-terminal serine is critical for PAR-2 agonist activity.

The G protein-coupled receptors constitute one of the largest families of cell surface receptors. They have the potential to activate several different signaling mechanisms via coupled heterotrimeric G protein complexes (1–3). The thrombin receptor, a member of this family, is unusual in its mode of activation (4). Thrombin, a proteolytic enzyme of the coagulation cascade, binds to the thrombin receptor and cleaves it at a specific site within the extracellular amino-terminal domain, exposing a new amino terminus, which is then thought to interact with a distal region of the receptor, leading to receptor activation. Recently, another receptor has been discovered which appears to share this novel mechanism of activation (5–7). A genomic clone of the murine protease-activated receptor (PAR)-2 was isolated using oligonucleotide probes based on the sequence of the substance K receptor. Subsequently human PAR-2 was also cloned and found to be >80% identical to the murine version of the receptor at the amino acid level (7). Comparison of the sequence of PAR-2 with known G protein-coupled receptor sequences revealed that it was most similar to the thrombin receptor (5). The overall amino acid identity between the two receptors is approximately 30%. However, in some regions such as the second extracellular loop the identity is significantly higher (~72%). The high degree of identity in this region of the human thrombin receptor is of interest since studies with the Xenopus thrombin receptor have suggested that the second extracellular loop of the thrombin receptor may be involved in “tethered ligand” interactions (8).

Examination of the structure of PAR-2 revealed the existence of a potential site for extracellular proteolytic cleavage and a possible tethered ligand agonist sequence (SLIGKVD or SLIGRL, murine or human PAR-2, respectively) which is similar to the thrombin receptor tethered ligand sequence (SFLLRN). Studies of murine PAR-2 expressed in Xenopus oocytes have demonstrated that the receptor can be activated by the protease trypsin and by the putative tethered ligand agonist peptides. Similarities in both amino acid sequence and functional characteristics of these two receptors, as well as the observation that the genes for both receptors reside in the long arm of chromosome 5, suggest that the receptors may be related evolutionarily (7, 9).

The existence of a protease receptor family and increasing evidence from studies with tissues and cells that thrombin and the thrombin receptor tethered ligand agonist peptides elicit different responses implicate the existence of potential thrombin receptor subtypes (10–15). One potentially simple explanation for some of these observations may reside in the similarities between the agonist peptide sequences for PAR-2 and thrombin receptor, and the possibility that the agonist peptides may display cross-reactivity among protease receptors. To explore this possibility, we have analyzed the structural features of the agonist peptides of the thrombin receptor and PAR-2 required for activation of their respective receptors and the ability of each to activate the heterodimerous receptors. Our results demonstrate that in addition to trypsin and the PAR-2 agonist peptide, PAR-2 can be activated fully by thrombin receptor agonist peptides. These results may explain some of the observations concerning disparate responses of cells and tissues to thrombin and TRAPs.

**Experimental Procedures**

**Materials**—Peptides were synthesized as described previously (16). All of the peptides except the Ala-scanning murine PAR-2 agonist peptides were synthesized as the carboxyl-terminal amide form. α-Thrombin was purchased from Hematologic Technologies (River Road Essex Junction, VT). Trypsin (bovine pancreatic type III, EC 3.4.21.4) and all other chemicals were purchased from Sigma.

**Expression Constructs**—The murine PAR-2 expression construct has
been described previously (5). A similar human PAR-2 construct was made by generating a polymerase chain reaction fragment of human PAR-2 containing the coding sequence flanked by BamHI cleavage sites (7). The fragment was cloned into the BamHI site of the vector pSP73 (Promega, Madison, WI). The thrombin receptor construct was made by isolating the Xho-EcoRI fragment from the expression construct, described previously (17), and inserting it into the XhoI and EcoRI sites of the vector pSP72 (Promega). The PAR-2 and thrombin receptor constructs were linearized with EcoRI or XbaI prior to use as in vitro transcription templates.

Oocyte Experiments—Xenopus laevis oocytes were prepared as described (18). The oocytes were injected with 50 nl of water or 10 ng of in vitro transcribed cRNA (Ambion message machine kit) generated from either human thrombin receptor or murine or human PAR-2 template in 50 nl of water. Approximately 44 h after injection the oocytes were either human thrombin receptor or murine or human PAR-2 templates transcribed (18). The oocytes were injected with 50 nl of water or 10 ng of PAR-2 cRNA (Ambion message machine kit) generated from either human thrombin receptor or murine or human PAR-2 template in 50 nl of water. Approximately 44 h after injection the oocytes were washed three times with the calcium-free medium OR-2 (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM HEPES, pH 7.5), then incubated in 2 ml of the same medium containing 0.05 μCi/ml ⁴⁵Ca²⁺ Cl₂ (10–40 μCi/mg of Ca²⁺⁺; Amersham Corp.) at room temperature for 2 h. The oocytes were washed repeatedly in ND-96 medium (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.5) until the level of radioactivity in the medium was reduced to stable background levels. Five oocytes were placed in wells of 48-well plates containing 200 μl of ND-96 medium.

Calcium Efflux Assay—Each concentration of agonist was tested on the pooled oocytes in triplicate. To initiate the assay the medium was removed from the well and replaced with 1 ml of medium containing the final concentration of agonist. A 200-μl aliquot was then removed immediately for the zero time point. An additional 200 μl was removed after 20 min, and the oocytes were then crushed in the remaining 600 μl of medium. The radioactivity of 100 μl of each was quantitated using a Wallac 1450 beta counter (Wallac Oy, Turku, Finland). The total cellular calcium was calculated from the values obtained for the 0- and 20-min time points and the crushed oocytes. The average total amount of radioactivity/oocyte was 1,000 cpm. The level of calcium efflux was calculated as the percentage of the total ⁴⁵Ca²⁺⁺ present in the oocytes. The maximum level of agonist-stimulated calcium efflux ranged from 25 to 40% of total calcium.

RESULTS

Murine PAR-2 expressed in Xenopus oocytes has previously been shown to be activated by trypsin but unresponsive to concentrations of thrombin up to 100 nM (5). Similar to these observations, we have found that thrombin is also unable to activate human PAR-2 expressed in Xenopus oocytes, but the protease trypsin is a potent activator of the human PAR-2, exhibiting an apparent submaximal EC₅₀ for the thrombin receptor (Fig. 1). It is difficult to determine an EC₅₀ for trypsin-mediated PAR-2 activation in oocytes since trypsin also elicits a calcium efflux. The EC₅₀ of the endogenous response of water-injected oocytes to trypsin was 1–3 nM. Treatment of oocytes expressing the thrombin receptor with trypsin did not produce a detectable increase in the level of trypsin-stimulated calcium efflux over that observed with water-injected oocytes (data not shown). The EC₅₀ of the endogenous response to trypsin in water-injected oocytes is severalfold lower than the EC₅₀ determined for trypsin activation of the thrombin receptor in other cell types, suggesting that thrombin receptor-specific responses in oocytes would be concealed by the endogenous response (19, 20). In contrast, oocytes expressing human thrombin receptor respond to α-thrombin with an EC₅₀ of 0.01–0.1 nM, indicating that when PAR-2 is expressed in oocytes it is at least 1,000-fold less responsive to thrombin than the human thrombin receptor.

The murine and the human PAR-2 agonist peptides, SLIGRL and SLIGKV, respectively, are quite similar, suggesting that cross-species responsiveness of receptors to agonist peptides was possible. To examine this, the responses of both murine and human PAR-2 to both agonist peptides were determined. Not surprisingly, a high degree of cross-reactivity was observed (Table I and Fig. 2). The EC₅₀ for the human agonist peptide SLIGKV is approximately 1–2 μM, and the EC₅₀ for the murine peptide SLIGRL is 0.2–1 μM with both receptors (Table 1).

The contribution of each residue in the TRAP peptides has been explored previously by replacement of each residue with alanine (16). We have conducted a similar analysis of the PAR-2 peptide SLIGRL by preparing an alanine scan series. The responses of murine PAR-2 to the modified peptides identified residues that are critical for PAR-2 agonist activity. Replacing the Leu² or Ile³ of the murine PAR-2 agonist peptide with alanine resulted in the most significant loss of potency as PAR-2 agonists (Fig. 3). Replacing the Ser¹ or Arg² with Ala also reduced the potency of the agonist peptides, whereas substitutions at the fourth and sixth positions had only a slight effect on the peptide’s ability to activate PAR-2.

The number of identical residues within TRAP and the PAR-2 agonist peptide is limited; however, the number of similar residues in the two peptides suggested the possibility that cross-reactivity of agonist peptides with both receptors might occur. The human thrombin receptor agonist heptapeptide SFLLRN (TRAP) activated human thrombin receptor expressed in oocytes with an EC₅₀ of ~0.1 μM. When tested for its ability to activate PAR-2 expressed in Xenopus oocytes, TRAP was found to be essentially equipotent to the PAR-2 activation...
peptides, having an EC50 of \(-0.5\) μM or less with both human and murine PAR-2 (Fig. 4A and Table I). Conversely, the human PAR-2 agonist peptide at concentrations up to 100 μM (Fig. 2) was not able to activate the thrombin receptor in oocytes.

The ability of the PAR-2 agonist peptide to activate endogenous thrombin receptor in thrombin receptor-expressing cells was also examined. The thrombin receptor is expressed in a number of cell types, including fibroblast, smooth muscle, endothelial, and platelets. In platelets, activation of the thrombin receptor initiates signal transduction, which results in platelet aggregation. TRAP (10 μM) induces a rapid aggregation of human platelets in platelet-rich plasma (16), whereas treatment of platelets with up to 1 mM human PAR-2 agonist peptide did not result in any detectable level of platelet aggregation or even platelet shape change (data not shown). Platelet GTPase activity has also been shown recently to be stimulated by TRAP, but it is unaffected by the PAR-2 agonist peptide (21). These observations confirm the lack of cross-reactivity of the PAR-2 agonist peptides with the human thrombin receptor and demonstrate that PAR-2 does not mediate platelet aggregation.

Several aspects of the species specificity of thrombin receptor agonist peptides have been reported by Gerszten et al. (8) who demonstrated that Xenopus TRAP (TFRIFD) is able to activate human thrombin receptor with an EC50 of 10 μM as well as the Xenopus thrombin receptor with an EC50 of 0.3 μM. However, the Xenopus thrombin receptor was shown to be unresponsive to up to 300 μM human TRAP. In our studies, oocytes expressing human thrombin receptor respond to TFRIFD with an EC50 of 1 μM; however, this peptide is unable to stimulate calcium efflux from oocytes expressing PAR-2 at concentrations up to 50 μM (Fig. 4B). These results provide for the possibility of using peptide agonists to activate each of the receptors specifically. The specificity of these peptides also provides a basis from which to probe further the structure-activity relationships of PAR agonist peptides and their receptors.

To this end, a series of analogs of the human and Xenopus TRAPs and PAR-2 agonist peptides were prepared in which one or more residues were substituted to determine which residues within the PAR-2 agonist peptides and human and Xenopus TRAP impart the observed receptor specificity (Table I). Each peptide was assayed for its effects on specific PARs by oocytes expressing PAR-2 or thrombin receptor, respectively. Panel A, ○, H2O; ●, human PAR-2; ▲, murine PAR-2. Panel B, ○, H2O; ●, thrombin receptor; ▲, human PAR-2; ▲, murine PAR-2.

**Table I**

| Peptide               | Thrombin receptor | PAR-2   |
|-----------------------|-------------------|---------|
|                      | EC50 μM           |         |
| SLIGRL-NH2            | ND*               | 0.5–2   |
| SLIGKVD-NH2           | >100              | 1–5     |
| SFLLRNPNH2            | ~0.1              | <0.5    |
| TFRIFD-NH2            | 0.5–2             | >50     |
| SLIGEL-NH2            | >50               | 0.5–2   |
| SLIGKVD-NH2           | >50               | 0.5–2   |
| SLIGKDD-NH2           | >50               | 5–10    |
| TFRKVD-NH2            | >50               | >50     |
| N-Acetyl-SLIGRL-NH2   | >50               | >50     |
| S[pFPhHarLRK-NH2      | 0.05–0.1          | 5–10    |
| SFLLRNIP-NH2          | 0.1–1             | 5–10    |
| SFLLNN-NH2            | 0.5–1             | 5–10    |
| SFLRNINH2             | 0.05–0.1          | 1–2     |
| SFLRNPNDKYE-NH2       | 0.05–0.5          | 5–10    |
| TFLRNPNDK-NH2         | 0.05–0.1          | >50     |
| T[pFPh]RIFD-NH2       | 0.5–2             | >50     |
| TFRIFRD-NH2           | 1–2               | >50     |
| TFRIFVY-NH2           | 0.05–0.5          | >50     |
| TFRIF-NH2             | 0.1–1             | >50     |
| SFRIFD-NH2            | 0.05–0.5          | *50     |
| TFRGFV-NH2            | *50               | *50     |
| SFRLF-NH2             | 5–10              | 5–10    |

* ND, not determined.
TRAP sequence also eliminated PAR-2 agonist activity of this peptide (Table I). Conversely, replacement of Ser1 for Thr1 in Xenopus TRAP yielded a peptide that activates PAR-2, albeit with a reduced efficacy relative to the human PAR-2 agonist peptide but significantly better than the native Xenopus TRAP. A concentration of 50 μM resulted in a level of calcium efflux which was only 50% of the maximum amount of efflux produced with SLIGKVD. This result suggests the importance of Ser1 for PAR-2 interactions with its agonists but also suggests that other residues in addition to the first residue within the Xenopus TRAP sequence may also modulate agonist peptide activity with PAR-2.

This possibility is supported by the results obtained with the TFRGFV and SFRGFV analogs of the Xenopus TRAP. These peptides, which contain the Gly4 substitution, and specifically the TFRGFV peptide, were found at a concentration of 50 μM to induce a PAR-2 response to 50% of that seen with SLIGKVD. However, human TRAP, which contains Leu at the fourth position, and a human TRAP analog with an Ile4 substitution display potent PAR-2 agonist activity, whereas the PAR-2 agonist peptide analog TLIGKVD was inactive, suggesting that glycine is not required, nor is it sufficient for agonist activity. The SFRGFV analog displayed agonist activity comparable to the human PAR-2 agonist peptide, indicating that the substitutions of Ser3 and Gly4 in the Xenopus TRAP have an additive effect. Both of the Xenopus TRAP analogs were less potent as thrombin agonist agonists than the wild type Xenopus TRAP peptide. The other modified Xenopus receptor TRAPs prepared in this study activated human thrombin receptor with a potency comparable to the native peptide. In contrast, the PAR-2 peptide analogs were uniformly inactive against thrombin receptor, most likely because they lack the critical Phe2 residue.

Additional modifications to agonist peptides were also examined for their effects on activation of protease receptors expressed in Xenopus oocytes (Table I). Included in these modifications were the acetylation of the amino terminus and replacement of Phe2 with p-fluoro-Phe2. As noted previously with the human thrombin receptor (16, 22), acetylation of the amino terminus of the murine PAR-2 agonist peptide eliminated agonist activity for PAR-2. In contrast to previous results and those of this study, where a p-fluoro-Phe2 substitution in human TRAP increased the activity of the peptide as a thrombin agonist (23), the p-fluoro-Phe2 substitution within TRAP does not appear to enhance the activity of the TRAP analog for PAR-2 activation significantly.

DISCUSSION

The recent discovery of PAR-2 has suggested the existence of a family of PARs. The thrombin receptor and PAR-2 are G protein-coupled receptors; they have a significant degree of amino acid residue identity, the genes for both receptors reside on the same chromosome, both receptors can be activated by a protease, and both are activated by peptides resembling the new amino terminus exposed by proteolytic cleavage of the receptor (5–7). The thrombin receptor can be activated by thrombin as well as other enzymes such as trypsin. PAR-2 is activated by trypsin, but not by thrombin as shown by the results of the work of Nystedt et al. (5) and this study. These results confirm previous observations that although PAR-2 is similar to the thrombin receptor at the amino acid level and can be activated by a protease, this receptor does not fulfill the requirements of a proposed thrombin receptor subtype (5, 10–11).

Comparison of the murine and human PAR-2 amino acid sequences reveals a high degree of similarity between the two species of receptors (5, 7). The tethered ligands are very similar; and, as shown in this study, PAR-2 agonist peptides of either species activate both species of receptors. An alanine scan series of the murine PAR-2 agonist peptide indicates that the potency of the agonist peptide is most negatively affected by alanine substitutions at the second and third positions. Replacing the Ser1 or Arg5 by alanine also resulted in an appreciable loss of activity, whereas substitutions at the fourth and sixth
positions had little effect on agonist activity. Alanine substitutions of the Leu\(^2\) and Lys\(^5\) of the murine PAR-2 agonist peptide have also been shown to reduce markedly PAR-2 activation in a rat aorta vascular tissue system (24). Alanine substitutions of TRAP have been shown previously to have the greatest negative effect on thrombin receptor agonist potency at the second, third, and fifth positions, whereas the agonist activity of agonist peptides substituted at the first, fourth, and sixth positions was relatively unaffected (16). Thus, the two receptor systems also show strong similarities with regard to the location of critical residues in their agonist peptides.

The observed similarities between the receptors and their agonist peptides prompted an examination of the ability of each agonist peptide of this family to activate the thrombin receptor. Most substitutions for Phe\(^2\) of TRAP have been found to result in a significant reduction of agonist activity (16, 22, 25–27), whereas a specific Leu for Phe\(^2\) substitution has been shown previously to eliminate agonist activity of TRAP (28, 29). In contrast, TRAP efficiently activates PAR-2 expressed in oocytes with a potency similar to that of the PAR-2 agonist peptides. The high potency exhibited by TRAP as an activator of PAR-2 was somewhat surprising since TRAP differs from the PAR-2 agonist peptide at residues that were determined by alanine scanning analysis of the murine PAR-2 agonist peptide to be important for activity (Fig. 3). The most notable functional group difference between the two PAR-2 agonist peptides and TRAP occurs at the second residue, Leu\(^2\) in the PAR-2 agonist peptide and Phe\(^2\) in TRAP. The ability of TRAP to activate PAR-2 indicates that there is a greater degree of tolerance for substitutions at the second position of the PAR-2 agonist peptides than has been observed for the thrombin receptor (16, 22, 25–27).

The Xenopus TRAP, TFRIFD, has been shown previously to activate the human thrombin receptor despite the differences in the amino acid sequences of the human and Xenopus TRAPs (8). We examined whether the ability of TRAPs to activate PAR-2 also included the Xenopus TRAP. We found that Xenopus TRAP could activate the human thrombin receptor with low micromolar EC\(_{50}\) but it was unable to activate PAR-2 at concentrations up to 50 \(\mu\)M. This result demonstrates that not all peptide agonists for the thrombin receptor display agonist activity at PAR-2 and that it should be possible to design agonist peptides that are specific for each of the PARs.

Analysis of the ability of various human and Xenopus TRAPs and PAR-2 agonist peptide analogs to activate the human thrombin receptor and PAR-2 have helped identify the residues responsible for the selectivity in receptor activation. An important difference in the agonist peptide requirements of the thrombin receptor and PAR-2 was found at the first position of the agonist peptides. Conservative substitutions of Thr or Ala for Ser\(^1\) are not well tolerated in PAR-2 agonist peptides, indicating that the structural requirements at this position for activation of PAR-2 by agonist peptides are moderately restrictive. This observation is in contrast to previous results showing that substitutions of Ser\(^1\) of TRAP by several other amino acid residues, including Thr and Ala, have minimal negative effects on thrombin receptor agonist activity of these analogs (16, 22, 25, 27). Gerszten et al. (8) have also reported that a Ser replacement of the Thr\(^1\) of Xenopus TRAP did not have a detectable effect on human or Xenopus thrombin receptor activation by this peptide.

We have also found that the specific functionality of the fourth residue of Xenopus TRAP also can affect the agonist peptide activity with the thrombin receptor and PAR-2. Two analogs with a Gly\(^4\) substitution of TFRIFD exhibited opposing effects on the ability of the peptides to activate PAR-2 and the thrombin receptor. Thrombin receptor agonist activity of these analogs was reduced compared with the wild type Xenopus TRAP, whereas agonist peptide activity with PAR-2 was enhanced. A Gly\(^4\)-substituted human TRAP has been shown previously to have reduced agonist activity for the thrombin receptor (27) and could have the same effect on Xenopus TRAP potency. How the Gly\(^4\) substitution improves the PAR-2 agonist potency of the Xenopus TRAP is unclear. Human TRAPs containing Ile\(^4\) and Leu\(^4\) were potent PAR-2 agonists, suggesting that Ile\(^4\) of the Xenopus TRAP should not have a negative effect on its PAR-2 agonist activity. Also, an Ala substitution at this position of the murine PAR-2 agonist peptide resulted in a minimal reduction of agonist potency, implying that the side chain of this residue does not have an important role in determining agonist peptide activity. The Gly\(^4\) substitution may exert its effect not by a direct interaction with the receptor, but rather by modulating the position of other important residue contacts of the agonist peptide with PAR-2.

These results demonstrate that the thrombin receptor and PAR-2 have overlapping but nonidentical requirements within their agonist peptide sequences. Structure-activity studies of human thrombin receptor agonist peptides have identified key residues required for retention or potentiation of agonist activity (16, 22, 25–27). These studies have demonstrated that many substitutions are tolerated at positions within TRAP except for the Phe\(^2\) residue, although the range of tolerated amino acid substitutions varied from position to position. Only the Phe\(^2\) residue was not amenable to any but the most conservative changes. The results of this study suggest that PAR-2 also is somewhat indiscriminate with regard to many of the residues of its agonist peptide sequence. In contrast to the thrombin receptor, the first residue appears to have the most stringent requirements, whereas the other positions can tolerate at least some conservative amino acid substitutions. It may be possible to use the differences in the specificity of agonist peptides of thrombin receptor and PAR-2 to identify residues within each receptor and agonist peptide which are critical for ligand-receptor interactions.

It is unlikely that TRAP-mediated activation of PAR-2 is physiologically relevant because the thrombin receptor agonist peptide that is exposed upon cleavage of the thrombin receptor by thrombin is tethered to the receptor, thus sterically inhibiting it to an intramolecular interaction. A recent study has reported that intermolecular signaling by the thrombin receptor tethered ligand is possible (30). However, the EC\(_{50}\) for thrombin receptor activation was found to be 1,000-fold higher than intramolecular signaling in the same system, thus suggesting that intermolecular signaling between homologous or heterologous receptors would be unlikely in vivo. Receptor specificity of the tethered peptides is not required because the specificity for activation of the receptor resides with the specific protease that is the physiological agonist for each receptor.

The present studies have demonstrated that PAR-2 is activated by TRAP peptides at concentrations similar to those required for thrombin receptor activation. This observation is especially important with respect to both in vivo and in vitro studies of thrombin receptor functions activated by TRAP peptides in cells or tissues that express these receptors. For example, PAR-2 is present on vascular endothelium (24), and its presence is consistent with observations that exposure of vascular tissue to PAR-2 agonist peptide produces an endothelium-dependent relaxation of vascular tone. This is quite similar to earlier observations in vascular tissues, which have been shown to be responsive to thrombin and TRAPs, presumably as...
a result of activation of the thrombin receptor present in these tissues \((10, 12, 31–33)\). Several studies have evaluated the responses of endothelial cells to thrombin and TRAPs, and although the responses to the two agonists in similar in most cases, some differences have been reported \((10–12, 32)\). A possible explanation for the differences in endothelial cell responses has been put forward which invokes the possibility of thrombin receptor subtypes that respond differently to the agonists. Differences in the responses to thrombin and TRAPs have also been reported in cell types other than endothelial cells. Recent studies in keratinocytes have revealed that even though these cells express both thrombin receptor and PAR-2, the responses to TRAP peptides appear to be mediated primarily by PAR-2 and not by thrombin receptors \((34)\). Keratinocytes respond well to SFLLRN and SLIGRL as well as trypsin, but the response to thrombin or TFRIFD was much weaker. An analysis of human neutrophil responses to thrombin and PAR-2 has suggested that a receptor other than the cloned thrombin receptor is present on these cells \((35)\). The observed cross-reactivity of agonist peptides within the PAR family suggests that a clear interpretation of cellular responses to these agonists may be difficult to achieve. Agonist peptides specific for each receptor need to be used to associate clearly the cellular responses to these agonists to the receptor cross-reactivity of agonist peptides within the PAR family suggests that a receptor other than the cloned thrombin receptor is present on these cells \((35)\). The observed cross-reactivity of agonist peptides within the PAR family suggests that a clear interpretation of cellular responses to these agonists may be difficult to achieve. Agonist peptides specific for each receptor need to be used to associate clearly the cellular responses with activation of specific receptors. In addition, the search for specific protease receptor antagonists may include an analysis of the potential for receptor cross-reactivity of prospective antagonists within the protease receptor family.

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