

Proteinase-Activated Receptors

Key References

Ahn, H.S., et al., Inhibition of cellular action of thrombin by N3-cyclopropyl-7-[[4-(1-methylethyl)phenyl]methyl]-7H-pyrrolo[3, 2-f]quinazoline-1,3-diamine (SCH 79797), a nonpeptide thrombin receptor antagonist., *Biochem Pharmacol.*, **60**, 1425-1434 (2000).

Andrade-Gordon, P., et al., Design, synthesis, and biological characterization of a peptide-mimetic antagonist for a tethered-ligand receptor., *Proc. Natl. Acad. Sci. USA*, **96**, 12257-12262 (1999).

Coughlin, S.R., Thrombin signalling and protease-activated receptors., *Nature*, **407**, 258-264 (2000).

Hollenberg, M.D. and Compton S.J., International Union of Pharmacology. XXVIII. Proteinase-activated receptors., *Pharmacol. Rev.*, **54**, 203-217 (2002).

Ishihara, H., et al., Protease-activated receptor 3 is a second thrombin receptor in humans., *Nature*, **386**, 502-506 (1997).

Kahn, M.L., et al., A dual thrombin receptor system for platelet activation., *Nature*, **394**, 690-694 (1999).

Nystedt, S., et al., Molecular cloning of a potential proteinase activated receptor., *Proc. Natl. Acad. Sci. USA*, **91**, 9208-9212 (1994).

Ossovskaya, V.S. and Bunnett, N.W., Protease-activated receptors: contribution to physiology and disease., *Physiol Rev.*, **84**, 579-621 (2004).

Rasmussen, U.B., et al., cDNA cloning and expression of a hamster alpha-thrombin receptor coupled to Ca²⁺ mobilization., *FEBS Lett.*, **288**, 123-128 (1991).

Vergnolle, N., et al., Proteinase-activated receptors in inflammation, neuronal signaling and pain., *Trends Pharmacol. Sci.*, **22**, 146-152 (2001).

Vu, T.K., et al., Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation., *Cell*, **64**, 1057-1068 (1991).

Xu, W.-F., et al., Cloning and characterization of human protease-activated receptor 4., *Proc. Natl. Acad. Sci. USA*, **95**, 6642-6646 (1998).

Overview

The search for a functional thrombin receptor, using expression cloning methods, led to the discovery of a G protein-coupled receptor that mediates the actions of thrombin on platelets and endothelial cells. The thrombin receptor, in contrast with other G protein-coupled receptors, lacks a circulating ligand. Rather, its unique mechanism of activation involves the proteolytic unmasking of an N-terminal receptor-triggering sequence buried in the "pro-receptor". The "revealed" N-terminal sequence acts as a "tethered" or anchored receptor-stimulating ligand. Remarkably, the Proteinase Activated Receptor for thrombin (now termed, PAR₁) can be activated in the absence of thrombin by relatively short peptides based on the sequence of the proteolytically revealed N-terminal domain (so-called Thrombin Receptor-Activating Polypeptides, or TRAPs). The TRAPs (now termed, PAR₁-Activating Peptides, or PAR₁APs) have been used as surrogate activators of PAR₁ to evaluate the potential actions of thrombin in tissues wherein the effects of the proteinase itself might be difficult to interpret. The TRAPs have also served as a basis for the development of receptor-selective PAR₁-targeted agonists and antagonists.

Results with the originally designed PAR₁APs, including peptide structure-activity studies, interspecies studies of platelet aggregation and the development of PAR₁-knockout mice, clearly pointed to the existence of other members of the PAR-family. Thus, the serendipitous discovery of a second G protein-coupled proteinase-activated receptor (PAR₂), that could be stimulated preferentially by trypsin in comparison with thrombin, was not entirely unexpected. Like PAR₁, PAR₂ is activated by a proteolytically-revealed tethered-ligand

mechanism. Also like PAR₁, PAR₂ can be activated by short peptides (e.g. SLIGRL... from rat PAR₂) based on the N-terminal trypsin-revealed "tethered-ligand" sequence. Whereas the PAR₂AP, SLIGRL-NH₂ cannot activate PAR₁, it was quickly realized that the originally described PAR₁APs (or TRAPs) could activate both PAR₂ and PAR₁; and that peptide structure-activity studies were required to design PAR₁APs that could selectively activate PAR₁, without activating PAR₂. Because of the cross-reactivity of PAR₁-targeted ligands with PAR₂, receptor-selective antagonists and binding probes have proved difficult to synthesize. For instance, the peptide PAR₁ antagonists that can block thrombin-mediated platelet activation have been shown to be agonists for PAR₂. Nonetheless, non-peptide PAR₁ antagonists (RWJ 56110; SCH 79797) have now been described that can block both thrombin- and peptide-mediated receptor activation.

The discovery of PAR₂ did not, however, explain the activity of thrombin in murine platelets that lack PAR₁. The absence of PAR₁ in murine platelets prompted a continued search for more thrombin receptors, resulting in the discovery of two more family members, PAR₃ and PAR₄, each of which has a unique thrombin-revealed tethered ligand. Unexpectedly, PAR₃ does not appear to signal itself, but rather acts as a cofactor for the activation of PAR₄. The physiological role for PAR₃ is still somewhat of an enigma. Synthetic peptides modeled on the thrombin-revealed sequence of PAR₃ (e.g. TFRGAP..) do not activate PAR₃, but are now known to activate both PAR₁ and PAR₂. The PAR₄ tethered ligands (murine-GYPGKF; human-GYPGQV) fail to activate other PARs, but are of low potencies (active in the 100-400 μM range). The

more potent PAR₄AP, AYPGKF-NH₂ (active in the 10 to 50 μM range), is more useful for physiological studies of PAR₄ function. In keeping with trans-cinnamoyl-substituted PAR₁-derived peptides that are PAR₁ antagonists, the peptide, trans-cinnamoyl-YPGKF-NH₂, is a selective PAR₄ antagonist that can be of use in studies done *in vitro*. Because of its relatively low potency, this trans-cinnamoyl derivative cannot be used *in vivo*, in contrast with the 'pepducin' PAR₄ antagonist, N-palmitoyl-SGRRYGHALR-NH₂ (P4pal-10).

In summary, four proteinase-activated receptors that are regulated by a proteolytically-revealed tethered ligand mechanism are now known. In addition to the recognized roles for PARs 1, 3 and 4 in regulating platelet and endothelial cell function, current data point to prominent roles for the PARs in physiological processes ranging from inflammation and pain sensation to the regulation of the vascular, pulmonary and gastrointestinal systems.

Proteinase-Activated Receptors

CURRENTLY ACCEPTED NAME	PAR ₁	PAR ₂	PAR ₃	PAR ₄
ALTERNATE NAMES	Thrombin receptor, PAR-1	PAR-2	Thrombin receptor, PAR-3	Thrombin receptor, PAR-4
STRUCTURAL INFORMATION	425 aa (human)	397 aa (human)	374 aa (human)	385 aa (human)
ACTIVATING PROTEINASES	Thrombin (T7513 (b), T1063 (h)) > Trypsin (T1426) >> Plasmin (P4895) cysteine proteinase (RgpB) produced by <i>Porphyromonas gingivalis</i> Factor Xa (F9302 (b))	Trypsin (T1426) ^a Tryptase (T7063) Trypsin-2, Trypsin IV Factor Xa TF/Factor VIIa Matriptase/MT-serine protease 1 Cysteine proteinase (RgpB) produced by <i>Porphyromonas gingivalis</i> Dust mite proteinases Der p3 And Der p 9	Thrombin (T7513 (b), T1063 (h)) >> Trypsin (T1426) > Factor Xa	Thrombin (T7513 (b), T1063 (h)) ~ Trypsin (T1426), Trypsin IV Cathepsin G (C4428), Factors VIIa/X
SUBTYPE SELECTIVE AGONISTS	TFRIFD, ^b TFLLR-NH ₂	SLIGRL-NH ₂ (S9317), 2-furoyl-LIGLRO-NH ₂	Thrombin cleaves, but does not activate	GYPGKF-NH ₂ , ^c GYPGQV-NH ₂ , AYPGKF-NH ₂ (A3227)
PUTATIVE ANTAGONISTS	BMS 200261, ^d Mercaptopropionyl-Phe-Cha-Arg-Lys-Pro-Lys-Pro-Asn-Asp-Lys-NH ₂ , ^e N-palmitoyl-RCLSSAVANRS (Pepducin P1pal 12), RWJ56110, SCH 79797	Not known	Not known	trans-cinnamoyl-YPGKF-NH ₂ (C7363), N-palmitoyl-SGRRYGHALR-NH ₂ , (Pepducin P4pal-10)
SIGNAL TRANSDUCTION MECHANISMS	G _{q/11} (increase IP ₃ /DAG) G _i (cAMP modulation) G _{12/13} (actin rearrangement)	G _{q/11} (increase IP ₃ /DAG) G _i (cAMP modulation)	Not known	G _{q/11} (increase IP ₃ /DAG)
TISSUE EXPRESSION	Platelets, vasculature (endothelium and smooth muscle), GI tract (neurons epithelia and smooth muscle), CNS (neurons and astrocytes), lung, kidney liver, leukocytes, heart	Vasculature (endothelium), GI tract (neurons, epithelia), CNS (neurons and astrocytes), lung, kidney, liver, leukocytes, heart	Platelets, vasculature (endothelium), liver, leukocytes	Platelets, vasculature (endothelium), GI tract (epithelia), leukocytes, lung, heart
PHYSIOLOGICAL FUNCTION	Platelet aggregation and secretion, vasoregulation, gastric motility, inflammation, nociception, neuronal regulation	Vasoregulation, gastric motility, inflammation, nociception, neuronal regulation	Not known	Platelet aggregation and secretion, inflammation
DISEASE RELEVANCE	Coronary thrombosis, inflammatory bowel disease, cancer	Arthritis, inflammatory, bowel disease, cancer, infectious colitis	Not known	Coronary thrombosis, inflammatory bowel disease, cancer

Abbreviation

BMS 200261: Transcinnamoyl-p-fluoroPhe-p-guanidinoPhe-Leu-Arg-NH₂

RWJ 56110: N-[(1S)-3-Amino-1-[[[(phenylmethyl)amino]carbonyl]propyl]-a-[[[1-(2,6-dichlorophenyl) methyl]-3-(1-pyrrolidinylmethyl)-1H-indol-6-yl]amino]carbonyl]amino]-3,-difluoro-benzenepropanamide

SCH 79797: N3-cyclopropyl-7-[[4-(1-methylethyl)phenyl]methyl]-7H-pyrrolo[3, 2-f]quinazoline-1,3-diamine

TF: Tissue factor **b:** bovine **h:** human

FOOTNOTES

a Thrombin is inactive.

b TFRIFD is the *Xenopus* thrombin receptor tethered-ligand domain (the human PAR₁ tethered-ligand domain sequence SFLLRN activates both PAR₁ and PAR₂ receptors).

c AYPGKF is 10-fold more potent than the natural human (GYPGQV) or murine (GYPGKF) tethered ligand sequences, which are also PAR₄ selective.

d May also act at PAR₂.

e Antagonizes PAR₁; acts as an agonist at PAR₂.