Proteinase-Activated Receptors

Key References

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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 cellular actions of thrombin on platelets and endothelial cells. The distinguishing property of the thrombin receptor, compared with other G protein-coupled receptors, relates to its lack of a circulating agonist and its unique mechanism of stimulation that involves the proteolytic unmasking of an activating N-terminal sequence buried in the "pro-receptor". The "revealed" N-terminal sequence acts as a "tethered" or anchored receptor-stimulating ligand. Remarkably, it was discovered that the Proteinase Activated Receptor for thrombin (now termed, PAR_1) 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, PAR_1-Activating Peptides, or PAR_1APs) were used as surrogate activators of PAR_1 to evaluate the potential actions of thrombin in tissues wherein the effects of the proteinase itself might be difficult to interpret. The TRAPs also served as a basis for the development of PAR_1-targeted antagonists.

Results with the originally designed PAR_1-APs, including peptide structure-activity studies, interspecies studies of platelet aggregation and the development of PAR_1-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 that could be stimulated by trypsin, but not thrombin (PAR_2), was not entirely unexpected. In addition to having considerable homology with PAR_1, PAR_2 bore the hallmark of PAR_1 in terms of the tethered-ligand mechanism for its activation by trypsin. Like PAR_1, PAR_2 can be activated by short peptides (e.g. SLIGRL... from rat PAR_2) based on the N-terminal "tethered-ligand" sequence revealed by trypsin. Whereas the PAR_2AP, SLIGRL-NH_2 cannot activate PAR_1, it was quickly realized that the originally described PAR_1APs (or TRAPs) could activate PAR_2; and that peptide structure-activity studies were required to design PAR_1APs that could selectively activate PAR_1, without activating PAR_2. Because of the cross-reactivity of PAR_1-targeted ligands with PAR_2, receptor-selective antagonists, binding probes have proved difficult to synthesize. For instance, the peptide PAR_1 antagonists that can block thrombin-mediated platelet activation have been shown to be agonists for PAR_2. Nonetheless, a non-peptide PAR_1 antagonist (RWJ 56110) has recently been described that can block both thrombin- and peptide-mediated receptor activation.

The discovery of PAR_2 did not, however, explain the activity of thrombin in murine platelets that lack PAR_1. The absence of PAR_1 in murine platelets prompted a continued search for more thrombin receptors, resulting in the discovery of two more family members, PAR_3 and PAR_4, each of which has a unique thrombin-revealed tethered ligand. Unexpectedly, PAR_3 does not appear to signal itself, but rather acts as a cofactor for the activation of PAR_4. The physiological role for PAR_3 is at present somewhat of an enigma. Synthetic peptides modeled on the thrombin-revealed sequence of PAR_3 (e.g. TFRGAP...) do not activate PAR_3, but very likely would activate both PAR_1 and PAR_2. The PAR_4 tethered ligands (murine-GYPGKF; human-GYPGQV) fail to activate other PARs, but are of limited value as agonists for physiological studies because of their low potencies (active in the 100-400 μM range).

In summary, four proteinase-activated receptors that are regulated by a proteolytically-revealed tethered ligand mechanism are known at present. Apart from the recognized roles for PARs 1, 3 and 4 in regulating platelet and endothelial cell function, the precise physiological functions for these novel receptor systems have yet to be determined. Preliminary data point to prominent roles for the PARs in physiological processes ranging from inflammation to pain sensation and 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)
Agonist Proteinases	Thrombin (T 7513 (b), T 1063 (h)) > Trypsin (T 1426) >> Plasmin (P 4895)	Trypsin (T 1426) [a] Tryptase (T 7063) Trypsin-2 Matriptase/MT-serine protease 1 Factor Xa (F 2027)/TF/Factor VIIa	Thrombin (T 7513 (b), T 1063 (h)) >> Trypsin (T 1426) > Factor Xa (F 2027)	Thrombin (T 7513 (b), T 1063 (h)) ~ Trypsin (T 1426) Cathepsin G (C 4428); FactorsVIIa/X (F 4003)
Subtype Selective Agonists	TFRIFD [b] TFLLR-NH_2	SLIGRL-NH_2	Thrombin cleaves, but does not activate	GYPGKF-NH_2 [c] GYPGQV-NH_2 AYPGKF-NH_2
Putative Antagonists	BMS 200261 [d] Mercaptopropionyl-Phe-Cha-Arg-Lys-Pro-Lys-Pro-Asn-Asp-Lys-NH_2 [e] RWJ 56110			
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)		$G_{q/11}$ (increase IP_3/DAG)

Abbreviation
BMS 200261: Transcinnamoyl-p-fluoroPhe-p-guanidinoPhe-Leu-Arg-NH_2
RWJ 56110: N-[(1S)-3-Amino-1-[[(phenylmethyl)amino]carbonyl]propyl]-a-[[[[1-[(2,6-dichlorphenyl) methyl]-3-(1-pyrrolidinylmethyl]-1H-indol-6-yl]amino]carbonyl]amino]-3,-difluoro-benzenepropanamide
TF: Tissue factor

b: bovine
h: human

Footnotes

a Thrombin is inactive.
b TFRIFD is the *Xenopus* thrombin receptor tethered-ligand domain (the human PAR_1 tethered-ligand domain sequence SFLLRN activates both PAR_1 and PAR_2 receptors).
c AYPGKF is 10-fold more potent than the natural human (GYPGQV) or murine (GYPGKF) tethered ligand sequences, which are also PAR_4 selective.
d May also act at PAR_2.
e Antagonizes PAR_1; acts as an agonist at PAR_2.