0026-895X/00/061175-03$3.00/0
MOLECULAR PHARMACOLOGY
Copyright © 2000 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 58:1175–1177, 2000

Vol. 58, No. 6
652/873241
Printed in U.S.A.

PERSPECTIVE

Receptor Binding and Agonist Efficacy: New Insights from Mutants of the Thrombin Protease-Activated Receptor-1 (PAR-1)

MORLEY D. HOLLENBERG

Department of Pharmacology and Therapeutics and Department of Medicine, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada

The article by Blackhart et al. (2000), describing the differential activation of mutated thrombin protease-activated receptor-1 (PAR-1) either by the proteolytically revealed tethered ligand or by soluble receptor-activating peptides, adds yet another challenge to the theoretical understanding of receptor mechanisms. It is useful to view these new findings with the perspective offered by classical receptor theory. Stemming from the work of Ehrlich (1908) and Langley (1906) at the start of the century, the existence of specific "receptive substances" responsible for the actions of many toxins and drugs was clearly established ("agents must bind to act"). Subsequently, Clark (1926, 1927) set the stage for considering quantitatively the binding of a ligand to its receptor and the relationship between that binding and a biological effect (Clark, 1937). Clark was also the first to attempt to measure quantitatively (by bioassay!) the amount of an agonist (acetylcholine) that must be bound to its receptor to cause a response. It subsequently became apparent that certain substances could exhibit a "dual" antagonistic and stimulatory effect, ostensibly by acting on the same receptor. Ariëns (1954) dealt with this property of "dual action" by expanding on the concept of competitive antagonism to distinguish between the ability of an agonist to bind to a receptor (its affinity) and its ability to activate the receptor (its "intrinsic activity"). In his theoretical treatment of this issue, Ariëns envisioned that agents with different receptor affinities could have "intrinsic activities" ranging from zero (i.e., "pure antagonists") to unity (i.e., full agonists; see Fig. 1 in Ariëns, 1954). Stephenson (1956) soon enlarged upon this aspect of receptor theory to take into account the ability of drugs to cause the *same* response by occupying different proportions of the available receptor population. This property, termed drug "efficacy" does not, as can be mistakenly assumed, refer to the maximum effect a drug may have; rather, it relates to the effectiveness (or "bounce per ounce of bound ligand") of the drug-receptor combination to generate a signal. In theory, values for drug "efficacy" can range from zero (pure antagonists) to much greater than the value of 1.0 envisioned by Ariëns. What none of those dealing with classical receptor theory could have predicted was a receptor that brought with it its own "tethered" ligand.

The discovery of proteinase-activated receptors (PARs), heralded by the cloning of the G protein-coupled receptor for thrombin (PAR-1) (Rasmussen et al., 1991; Vu et al., 1991), has challenged a number of the classical concepts of receptor theory (outlined above) as well as challenging the precepts introduced in the receptor "grind-and bind" era, begun in the mid-1960s with the study of the binding of [^{3}H]atropine to the muscarinic receptor (Paton and Rang, 1965) and expanded by the study of the receptor binding of ^{125}I-labeled polypeptides, such as insulin (Cuatrecasas and Hollenberg, 1976). The unique feature of PARs, like the one for thrombin (there are now four family members: PARs 1 to 4: Dery et al., 1998; Hollenberg, 1999; Coughlin, 2000), relates to the proteolytic unmasking of a cryptic N-terminal receptor sequence that, remaining tethered, binds to and activates the receptor (Vu et al., 1991). Remarkably, synthetic peptides, modeled on the revealed receptor-activating sequence (e.g., SFLLRN. . . for human PAR-1) can in isolation trigger the receptor so as to mimic activation by the proteinase. Such PAR-activating peptides (PAR-APs) have proved to be of enormous utility in defining the potential physiological roles of PARs in vivo. Furthermore, structure-activity studies with the PAR-APs have resulted in the synthesis of receptor-selective peptide agonists and have suggested the existence of receptors that have yet to be cloned (Hollenberg et al., 1993; Tay-Uyboco et al., 1995; Vergnolle et al., 1998; Kawabata et al., 1999). Differences in the apparent efficacies of the PAR-APs have been noted in passing but have not been studied in any depth. Until recently, it has been assumed that the synthetic

ABBREVIATIONS: PAR, proteinase-activated receptor; PAR-AP, proteinase-activating receptor activating peptides.

PAR-APs, mimicking the action of the proteinases, interact with the receptor in the same way as the proteolytically revealed tethered ligand. It has been suggested that the relatively high concentrations of the free PAR-APs required to cause receptor activation (e.g., 10–20 μM) are necessary to compensate for their lack of being covalently held in place by tethering. Some researchers may have presumed (but did not explicitly state) that although the affinity of the soluble PAR-AP for the receptor is in the micromolar range, its "efficacy" would be comparable with that of the "tethered" ligand, which as a peptide domain could in theory have the same intrinsic affinity for the receptor as the free PAR-AP. However, being held in place (a loss of one degree of freedom), the tethered peptide domain would be thermodynamically favored over the free peptide.

Studies with the wild-type receptor led one to think that the free and tethered ligands acted the same. Importantly, however, work with the mutant receptors reveals that there are differences between the tethered and free ligands and that binding does not tell us about the intrinsic activity of the PAR-activating peptides. By combining a ligand binding approach with a study of the biological activity of either thrombin or PAR-1-activating peptides in site-mutated human PAR-1 receptor variants, the new work by Blackhart et al. (2000) has found that the PAR-AP SFLLRNP-NH_2 largely retained its receptor binding ability for the receptor mutants (only a 2- to 6-fold reduction in binding affinity; Table 6 in Blackhart et al., 2000) but was essentially biologically inactive in the mutated receptors (Blackhart et al., 2000; Table 2). Strikingly, the mutated receptors were otherwise still fully responsive to thrombin activation. These data indicate that the tethered ligand must differ from that of the free PAR-APs in terms of both its intrinsic efficacy and its docking mechanism. An essential element of the work described with the mutated PAR-1 receptors is the use of a ligand binding assay that meets the criteria for true "receptor" binding (Cuatrecasas and Hollenberg, 1976). Thus, as shown clearly in Table 4 and Fig. 3 of the new article by Blackhart et al. (2000), the binding affinities for a large number of agonists correlated extremely well with their biological activities in activating wild-type PAR-1. Thus, in contrast to the classical approach, wherein the agonist structure is altered to affect its "efficacy" at the receptor site, this new study has instead altered the receptor and demonstrated marked reductions in the efficacy of the same peptide agonist acting on a mutated receptor.

In the mutated receptors, the efficacy of the PAR-1-AP SFLLRNP-NH_2 has been reduced toward zero (without a loss of receptor affinity), whereas the efficacy of the same amino acid sequence as a tethered ligand seems little affected. Given that result, one would predict that the free peptide, SFLLRNP-NH_2, by binding with equal affinity but with low efficacy (e.g., in receptor mutant, DEC3; Tables 2 and 6), should be a good receptor antagonist, blocking receptor activation by the thrombin-revealed tethered ligand. The preliminary experiments designed to test this possibility found no inhibition of thrombin action by a high-affinity analog of SFLLRNP-NH_2 (Ser-pFPhe-Har-Leu-Har-Lys-Tyr-NH_2: compound C721–40, the binding probe) in such PAR-AP-insensitive mutants. This lack of inhibition can be taken to suggest that the site at which the thrombin-revealed tethered ligand docks differs from the site at which the free activating peptide binds. This possibility is supported by complementary work with the trypsin-activated PAR-2, wherein receptor mutation markedly reduces the biological activity of selected PAR-2-AP agonists, without markedly affecting the activity of the tethered ligand revealed by trypsin (Al-Ani et al., 1999). Collectively, these findings may have important implications for the design of selective nonpeptide PAR antagonists.

The new study by Blackhart et al. (2000) has just scratched the surface of the issue of possible differences in binding and intrinsic activity of the tethered ligand, as opposed to the soluble activating peptide. So far, this difference has been studied mainly for one peptide agonist (SFLLRNP-NH_2) and for just a few of the receptor mutants. It would be most interesting in future work to evaluate both binding and biological activity for a wider range of receptor mutants and with an expanded library of PAR-1-APs. It will be interesting to determine whether the same greater efficacy for the tethered receptor activating sequence (as opposed to the free peptide) can be observed not only in the context of the PARs but also for nonnatural self-activating receptor chimeras, such as the one recently described for the corticotropin-releasing factor receptor (Nielsen et al., 2000). In that chimera, with the corticotropin-releasing hormone activating peptide fused to the receptor N terminus, constitutive receptor activity was observed. However, issues of agonist efficacy (i.e., tethered versus free peptide) were not examined. What is clear is that the novel mechanism of PAR activation and the molecular pharmacology of PAR-activating peptides provide fertile ground for investigating basic issues regarding the relationships between receptor binding and efficacy.

References

Al-Ani B, Saifeddine M, Kawabata A and Hollenberg MD (1999) Proteinase activated receptor 2: Role of extracellular loop 2 for ligand-mediated activation. *Br J Pharmacol* **128:**1105–1113.

Ariëns EJ (1954) Affinity and intrinsic activity in the theory of competitive inhibition. *Arch Int Pharmacodyn* **99:**32–49.

Blackhart BD, Ruslim-Litrus L, Lu C-C, Alves VL, Teng W, Scarborough RM, Reynolds EE and Oksenberg D (2000) Extracellular mutations of PAR-1 results in differential activation by thrombin and thrombin receptor agonist peptide. *Mol Pharmacol* **58:**1178–1187.

Clark AJ (1926) The antagonism of acetylcholine by atropine. *J Physiol (Lond)* **61:**547–556.

Clark AJ (1927) The reaction between acetylcholine and muscle cells. Part II. *J Physiol (Lond)* **64**:123–143.

Clark AJ (1937) General Pharmacology. *Handbook of Experimental Pharmacology*, Vol 4, Springer Verlag, Berlin.

Coughlin SR (2000) Thrombin signalling and protease-activated receptors. *Nature (Lond)* **407:**258–264.

Cuatrecasas P and Hollenberg MD (1976) Membrane receptors and hormone action. *Adv Protein Chem* **30:**251–451.

Dery O, Corvera CU, Steinhoff M and Bunnett NW (1998) Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. *Am J Physiol* **274:** C1429–C1452.

Ehrlich P (1908) Nobel lecture on partial functions of the cell. *The Collected Papers of P. Ehrlich,* Vol III, (Himmelweit F, Marquardt M, Dale HH eds) pp 183–194, Oxford, Pergamon Press.

Hollenberg MD (1999) Proteinase-activated receptors: PAR_4 and counting how long is the course? *Trends Pharmacol Sci* **20:**271–273.

Hollenberg MD, Laniyonu AA, Saifeddine M and Moore GJ (1993) Role of the amino- and carboxyl-terminal domains of thrombin receptor-derived polypeptides in biological activity in vascular endothelium and gastric smooth muscle: Evidence for receptor subtypes. *Mol Pharmacol* **43:**921–930.

Kawabata A, Saifeddine M, Al-Ani B, Leblond L and Hollenberg MD (1999) Evaluation of proteinase-activated receptor -1 (PAR_1) Agonists and Antagonists using a cultured cell receptor desensitization assay: Activation of PAR_2 by PAR_1 targeted ligands. *J Pharmacol Exp Ther* **288:**358–370.

Langley JN (1906) On nerve endings and on special excitable substances in cells. *Proc R Soc B Biol Sci* **70:**170–194.

Nielsen SM, Nielsen LZ, Hjorth SA, Perrin MH and Vale WW (2000) Constitutive activation of tethered-peptide/corticotropin-releasing factor receptor chimeras. *Proc Natl Acad Sci USA* **18:**10277–81.

Paton WDM and Rang HP (1965) The uptake of atropine and related drugs by intestinal smooth muscle of the guinea pig in relation to acetylcholine receptors. *Proc R Soc B Biol Sci* **163:**1–44.

Rasmussen UB, Vouret-Craviari V, Jallat S, Schlesinger Y, Pages G, Pavirani A, Lecocq J-P, Pouyssegur J and Van Obberghen-Schilling E (1991) CDNA cloning and expression of a hamster α-thrombin receptor coupled to Ca^{2+} mobilization. *FEBS Lett* **288:**123–128.

Stephenson RP (1956) A modification of receptor theory. *Br J Pharmacol* **11:**379–393.

Tay-Uyboco J, Poon M-C, Ahmad S and Hollenberg MD (1995) Contractile actions of thrombin receptor-derived polypeptides in human umbilical and placental vasculature: Evidence for distinct receptor systems. *Br J Pharmacol* **115:**569–578.

Vergnolle N, MacNaughton WK, Al-Ani B, Saifeddine M, Wallace JL and Hollenberg MD (1998) Proteinase-activated receptor-2-activating peptides: Identification of a receptor distinct from PAR_2 that regulates intestinal transport. *Proc Natl Acad Sci USA* **95:**7766–7771.

Vu T-K, Hung DT, Wheaton VI and Coughlin SR (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64:**1057–1068.

Send reprint requests to: Dr. Morley D. Hollenberg, Department of Pharmacology & Therapeutics and Department of Medicine, University of Calgary, Faculty of Medicine, Calgary AB, Canada T2N 4N1. E-mail: mhollenb@ucalgary.ca