Characterization of a Functional Thrombin Receptor

Issues and Opportunities

Shaun R. Coughlin,*‡ Thien-Khai H. Vu,* David T. Hung,*‡ and Virginia I. Wheaton*
**Cardiovascular Research Institute, and ‡Department of Medicine, University of California, San Francisco, San Francisco, California 94143*

Introduction

How does the protease thrombin activate platelets and other cells? This question presents intriguing issues from both basic scientific and clinical perspectives. From a basic view, the fact that thrombin is a protease raises the possibility of a novel proteolytic mechanism of receptor activation. From a clinical view, thrombin regulates hemostasis and thrombosis. Moreover, a host of thrombin actions on cells have been defined in vitro; in vivo these actions may be critical for both normal responses to wounding and for pathological vascular events. An understanding of the mechanism of thrombin-induced cell activation promises reagents for identifying thrombin's role in cellular responses in vivo and possibly new therapeutics.

In this Perspectives, we review the recent cloning and characterization of a platelet thrombin receptor in our laboratory (1). Identification of the thrombin receptor did reveal a unique proteolytic mechanism of receptor activation, and led to the development of a novel agonist peptide which activates the thrombin receptor independent of thrombin and thrombin's protease activity. This agonist peptide is a useful new tool for addressing the role of thrombin receptor activation in cellular responses. It is our hope that having the cloned receptor in hand will also allow the development of thrombin receptor antagonists. Such reagents will define the role of thrombin receptor activation in normal and disease processes in vivo, and may provide the basis for a new class of antithrombotic or antiproliferative pharmaceuticals.

Cell-activating functions of thrombin

In addition to cleaving fibrinogen to fibrin, thrombin exhibits important "cell-activating" functions (Fig. 1; reviewed in ref. 2). First and foremost, thrombin is the most potent stimulator of platelet aggregation (3), a thrombin activity probably critical in arterial thrombosis (see below). A variety of other thrombin activities on cells has been defined, largely in vitro. Thrombin is chemotactic for monocytes (4), mitogenic for lymphocytes and for mesenchymal cells including vascular smooth muscle cells (5, 6), and has a number of effects upon the vascular endothelium. These include stimulating endothelial production of prostacyclin (7), platelet-activating factor (8), plasminogen activator-inhibitor (9), and the potent smooth muscle cell mitogen platelet-derived growth factor (10). Thrombin also induces neutrophil adherence to the vessel wall by an endothelial-dependent mechanism (11), probably by causing surface expression of GMP-140 (12). Teleologically, these multiple cell-activating functions of thrombin may be viewed as orchestrating the response to vascular injury, potentially mediating not only hemostatic but perhaps inflammatory and proliferative or reparative responses. Whether these disparate actions of thrombin are all important in vivo remains to be defined.

Thrombin activities that would not necessarily be associated with vascular injury have also been described. The surprising inhibitory effects of thrombin on neurite outgrowth (13, 14) and the apparent expression of prothrombin mRNA in brain (15) conjure up hypotheses regarding potential roles for thrombin or a related protease in neuronal plasticity or development, hypotheses which remain to be tested.

Recently developed potent and specific thrombin inhibitors such as recombinant hirudin, argatroban, and PPACK are defining the role of thrombin activity in vivo. These agents bind thrombin itself, blocking not only thrombin's ability to activate its receptor but all active site-dependent thrombin functions including thrombin's ability to cleave fibrinogen and to activate protein C and other zymogens. For this reason, they do not address whether thrombin's effects are mediated directly by thrombin receptor activation or indirectly via other actions of the thrombin protease. With these limitations, available antithrombins provide exciting support for the importance of thrombin in certain cellular responses in vivo. Animal studies with antithrombins do suggest a critical role for thrombin in platelet thrombus formation in several models of arterial thrombosis (16–20). In general, the importance of thrombin in mediating nonthrombotic responses in vivo remains to be defined. In particular, whether thrombin plays a significant role in inflammatory or proliferative responses to vascular injury remains to be tested. A recent study using recombinant hirudin does suggest a role for thrombin in mediating restenosis after angioplasty in one animal model (21). It is hoped that the characterization of the platelet thrombin receptor will permit the development of thrombin receptor blockers to directly address the importance of thrombin-induced cell activation in vivo.

Identification of a functional thrombin receptor

Previous theories of possible mechanisms by which thrombin activates platelets included classical receptor occupancy mechanisms, mechanisms involving proteolytic cleavage of the recep-

Address correspondence to S. R. Coughlin, Box 0524, University of California, San Francisco, San Francisco, CA 94143.
Received for publication 9 October 1991.

J. Clin. Invest.

0021-9738/92/02/0351/05 $2.00
Volume 89, February 1992, 351–355

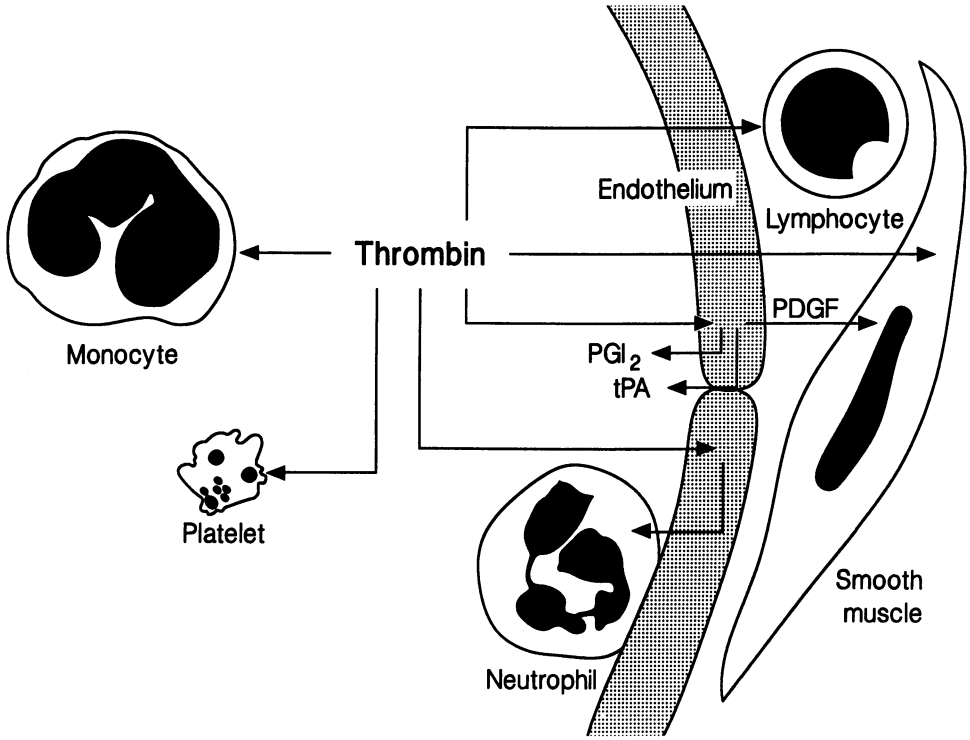

Figure 1. Cellular events stimulated by thrombin. Cellular targets for thrombin are depicted in the context of a blood vessel. Thrombin is the most potent stimulator of platelet aggregation, is chemotactic for monocytes and mitogenic for lymphocytes and mesenchymal cells. Thrombin also has a number of effects on the vascular endothelium, including stimulating expression of the neutrophil adhesive protein GMP-140 on the endothelial surface as well as the production of the potent smooth muscle cell mitogen, platelet-derived growth factor (*PDGF*). As discussed in the text, these multiple activating functions of thrombin may be viewed teleologically as orchestrators of hemostatic, inflammatory, and reparative responses to vascular injury.

tor, and combinations thereof (2, 22). Traditional ligand binding approaches to identify a functional thrombin receptor identified proteins which bind thrombin but have no apparent signalling function (23, 24). In fact, because modified thrombins that lack both agonist and antagonist activity do bind to platelets in a manner indistinguishable from wild type thrombin (25–29), it has not been possible to conclude that the sites identified in binding studies are related to the functional thrombin receptor. Because of difficulties with the classical binding approach and the undefined nature of thrombin-receptor interaction, we adopted an expression cloning approach which followed thrombin-induced responses in *Xenopus* oocytes expressing exogenous mRNA to isolate a functional human thrombin receptor cDNA (1). This strategy was based on studies by Masu and colleagues who had successfully used expression cloning in *Xenopus* oocytes to isolate a cDNA for the substance K receptor (30). The cloning of the thrombin receptor was described in detail elsewhere (1). The deduced amino acid sequence of this clone revealed a novel member of the seven transmembrane receptor family. Closest relatives included receptors for small peptides, substance P and substance K (1), suggesting that the thrombin receptor is peptide receptor-like. This analogy was a useful hint at the outset of the structure-function work described below.

Closer inspection of the receptor's amino acid sequence was revealing in the light of a large body of literature on thrombin action. Thrombin cleaves peptides after arginines (31). In hope of finding a receptor cleavage site, we examined sequences surrounding extracellular arginines in the receptor sequence. We noted that the receptor's relatively long (100 residue) extracellular amino-terminal extension contained the putative thrombin cleavage site LDPR/S (single letter amino acid code, / representing the point of cleavage after arginine 41 [R41] in the receptor, Figs. 2 and 3). This site resembled the known thrombin cleavage site found in the thrombin-activated zymogen protein C (LDPR/I) (32). Carboxyl to this putative cleavage site lies a receptor domain resembling the carboxyl tail of the polypeptide hirudin, a leech-derived anticoagulant which binds thrombin with remarkable avidity. To put these sequence observations into context, thrombin possesses an extended substrate binding surface that recognizes residues both amino and carboxyl to a substrate's cleavage site (33) (Fig. 2). Part of this extended substrate binding surface, the anion-binding exosite (Fig. 2), is important for thrombin's ability to activate its receptor (1, 34). Because the carboxyl tail of hirudin interacts with thrombin's anion-binding exosite (35), the presence of the hirudin-like sequence carboxyl to the putative thrombin cleavage site within the thrombin receptor's amino terminal extension suggested that receptor proteolysis at this cleavage site might be important in receptor activation (Fig. 2).

We evaluated the importance of the putative cleavage site at R41/S42 by changing the arginines in the receptor's amino terminal extension to alanines, a maneuver designed to render each site uncleavable by thrombin. These mutant receptors

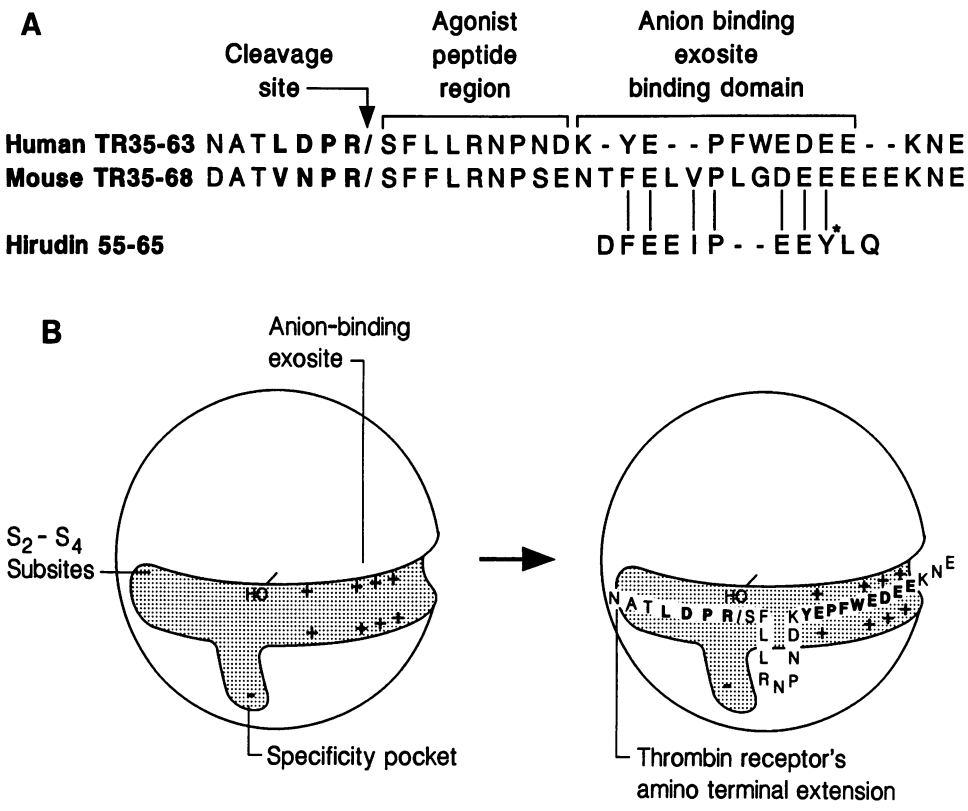

Figure 2. Thrombin-receptor interaction. Functional domains within the thrombin receptor's amino-terminal extension are depicted in *A*. The cleavage recognition sequence (LDPR), thrombin cleavage site, agonist peptide domain, and anion-binding exosite binding domain as defined by structure activity studies (38, 39) with the human receptor are shown. These are aligned with the murine thrombin receptor sequence and the anion-binding exosite binding sequence of the leech anticoagulant hirudin. A model for interaction of these domains with thrombin is shown in *B*. Thrombin has an extended substrate binding surface (represented by the canyon running laterally) which recognizes residues both amino and carboxyl to its substrate's cleavage site. Structure function studies (38, 39) suggest that the receptor's hirudin-like domain (YEPFWEDEE) interacts with thrombin's anion-binding exosite, while its cleavage site (LDPR/S) interacts with thrombin's S1-S4 subsites. This model has important implications for the development of blocking monoclonal antibodies and receptor-peptide based thrombin inhibitors, as discussed in the text (reprinted with permission from *Nature* [38]).

were expressed in *Xenopus* oocytes and their ability to respond to thrombin assessed. The putative cleavage site mutant (R41A) could not be activated by thrombin. The other mutants, R46A and R70A, were fully activatable. As an independent means of rendering the putative thrombin cleavage site uncleavable, serine 42 at the site's P1' position was changed to a proline. Like the R41A mutant, this S42P mutant receptor could not be activated by thrombin. These data strongly suggested that the receptor's putative thrombin cleavage site was critical for thrombin-induced receptor action (1).

How might proteolysis at this site lead to receptor activation? The favored hypothesis was that proteolysis at this site would unmask a new amino terminus that might serve as a peptide ligand for the receptor (1). Precedent for proteolytic unmasking of a "ligand" existed in trypsinogen activation to trypsin (36), and in fibrinogen cleavage to fibrin monomer (37). Indeed, a peptide mimicking the new receptor amino terminus that would be revealed by receptor cleavage at the R41 position was a full agonist for the cloned receptor expressed in oocytes. Not only could the wild type receptor be activated, the R41A receptor, unactivatable by thrombin, responded fully to the new amino terminus "agonist peptide." Thus the agonist peptide could bypass the requirement for proteolysis and activate the receptor directly (1). This agonist peptide not only activated the thrombin receptor expressed in oocytes; it was a full agonist for platelet activation (1). This suggests that activation of the cloned thrombin receptor or a highly related receptor is sufficient for platelet activation.

Model for thrombin receptor activation

The studies described above suggest that thrombin receptor activation proceeds by the novel mechanism depicted in Fig. 3. Thrombin cleaves its receptor after arginine 41 in the receptor's amino terminal extension, exposing a new amino terminus that functions as a tethered peptide ligand for the receptor. The new amino terminus then binds to an as yet undefined receptor site, effecting receptor activation (1).

Recent studies buttress this model (38, 39). Replacing the receptor's thrombin cleavage site (LDPR/S) with that for enterokinase (DDDDK/S) completely switched the receptor's specificity, creating a functional "enterokinase receptor" that was fully activatable by enterokinase but unresponsive to thrombin (38). These results strongly suggest that receptor proteolysis that unmasks the amino terminus beginning a position 42 (SFLL. . .) is sufficient for receptor activation (38). No special "hormone-like" properties of thrombin need to be invoked for activation of this receptor.

Additional studies have shown the receptor's hirudin-like sequence can indeed bind thrombin's anion-binding exosite (38, 39). Detailed mutagenesis studies have defined the specific residues important in this interaction and strengthened the analogy between the receptor's anion binding exosite binding

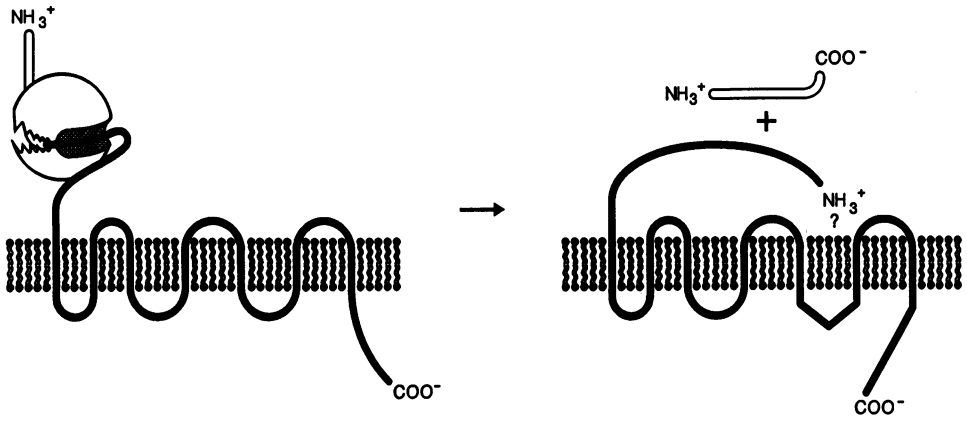

Figure 3. Model of thrombin receptor activation. Thrombin binds its receptor via the receptor's amino terminal extension as detailed in Fig. 2; whether additional receptor domains may participate in thrombin binding is unknown. After binding to the amino terminal extension, thrombin cleaves the receptor at the LDPR/S cleavage site (*junction between open and filled receptor segments*), releasing an inactive fragment of the receptor's amino terminus (*open fragment*) and exposing a new amino terminus. This newly unmasked amino terminus then functions as a tethered peptide ligand, binding to an as yet undefined pocket, thereby effecting receptor activation (1) (reprinted with permission from *Nature* [38]).

domain and hirudin's carboxyl tail (38). These relationships are cartooned in Fig. 2 *B*.

Issues and opportunities

The studies presented above raise provocative questions and present opportunities at both basic mechanistic and clinical levels. Some of these are discussed below.

From a basic perspective, we have learned a great deal about how the receptor senses thrombin in its environment, i.e., via the binding and proteolytic events described above. More detailed information regarding thrombin-receptor interaction will undoubtedly come from crystallographic studies. The rapid unravelling of the mechanism by which thrombin activates its receptor "fell out" of an analysis of the thrombin receptor's amino acid sequence. Existing literatures on thrombin structure/function and on seven transmembrane domain receptors allowed us to identify the receptor's thrombin cleavage site and binding domains and to frame the thrombin receptor as a "peptide receptor", leading naturally to the model depicted in Figs. 2 and 3.

Many questions remain unanswered. Where does the agonist peptide domain unmasked by receptor proteolysis bind, and how does this binding event lead to a transmembrane signal that allows the receptor to talk to intracellular signalling molecules (Fig. 3)? These intracellular signalling molecules are certainly G-proteins; the specific G-proteins remain to be defined.

The unique proteolytic mechanism by which the thrombin receptor becomes liganded prompts a number of questions. This mechanism produces a ligand that is tethered to the receptor and cannot diffuse away. How is the receptor turned off? We have shown that the agonist peptide can desensitize the receptor, thus at least one specific mechanism independent of proteolysis by thrombin exists to shut off this receptor. By analogy with other seven transmembrane receptors, it is probable that several mechanisms including receptor phosphorylation by a beta-adrenergic receptor kinase-like enzyme (40) and other protein kinases regulate thrombin receptor desensitization. Perhaps more interesting but entirely speculative is a possible second mechanism in which thrombin might cleave the receptor at a second site carboxyl to the R41/S42 activation site, relieving the receptor of its agonist peptide domain.

The unusual activation mechanism of thrombin receptor activation requires in essence a peptide receptor that contains its own ligand. Where did this mechanism come from? Was it an evolutionary specialization of a previous peptide receptor? Or is it representative of a more primitive heritage from which peptide receptors and ligands may have evolved?

Will this clone sire a family of thrombin or protease receptors? And how many of the assorted cell-activation functions of thrombin does this receptor account for? The agonist peptide described above, which activates the cloned thrombin receptor directly and independent of thrombin and its protease activity, will be useful in this regard. Specifically, thrombin responses mimicked by the agonist peptide are likely mediated by the unusual mechanism described above via the cloned receptor or a highly related receptor. Thrombin responses not mimicked by the agonist peptide may be mediated by a different mechanism.

What is the relationship of known thrombin binding proteins, in particular, platelet GPIb, to thrombin signalling and to this receptor? Bernard-Soulier syndrome platelets are deficient in GPIb and have a selective and partial defect in their responsiveness to thrombin (reviewed in 3, 22). Does GPIb promote activation of the cloned receptor by binding thrombin and localizing it to the cell surface (3, 20, 22)? A body of circumstantial evidence does support this model, but direct demonstration of an adjunctive role for GPIb in thrombin signalling using, for example, GPIb expressed in null cells is lacking at this time. As an alternative hypothesis, could GPIb serve as a sink for thrombin? Perhaps, at low ambient thrombin concentrations, GPIb might bind thrombin long enough to allow inactivation by antithrombin III. By preventing thrombin interaction with the receptor and receptor desensitization by trace levels of thrombin in the normal circulation, such a mechanism might also explain the defect in thrombin signalling seen in Bernard-Soulier. Lastly, it is possible that thrombin binding to GPIb serves an independent, as yet undefined function.

From the pathophysiologic and clinical perspective, these studies suggest a number of paths to novel reagents that will be useful in defining the role of thrombin receptor activation in vivo. Receptor-based peptides that bind and inhibit various thrombin functions have been produced (38, 39, 41). These are novel among thrombin inhibitor peptides (42) in that they are based on human sequence; whether this will translate into any practical clinical benefit over other agents in development is unknown.

More novel and useful would be thrombin receptor

blockers. Blocking monoclonal antibodies might be obtained in a number of ways. Antibodies to the receptor's hirudin-like sequence or thrombin cleavage site should inhibit thrombin-receptor interaction and receptor proteolysis. Antibodies binding these regions or the agonist peptide domain itself might also interfere with the "liganding" of the receptor after proteolysis. A similar function would be served by antibodies to receptor regions comprising or affecting the as yet undefined binding site for the agonist peptide. Whether nonantibody receptor blockers, i.e., competitive or noncompetitive antagonists of the agonist peptide, can be obtained remains to be explored. Such reagents will define the role of thrombin receptor activation in vivo, and may lead to the development of a new class of pharmaceuticals.

Acknowledgments

We thank Dr. Henry Bourne for critical review of this manuscript.

This work was supported in part by National Institutes of Health grants HL-44907 and HL-43821, and by University of California Tobacco-Related Disease Research Program grant 2RT-19, and was done during the tenure of an award to S. R. Coughlin from the American Heart Association and SmithKline Beecham.

References

1. Vu, T.-K. H., D. T. Hung, V. I. Wheaton, and S. R. Coughlin. 1991. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell.* 64:1057–1068.

2. Shuman, M. A. 1986. Thrombin-cellular interactions. *Ann. NY Acad. Sci.* 485:228–239.

3. Berndt, M. C., and D. R. Phillips. 1981. Platelet membrane proteins: composition and receptor function. *In* Platelets in Biology and Pathology. J. L. Gordon, editor. Elsevier/North Holland Biomedical Press, Amsterdam. 43–74.

4. Bar-Shavit, R., A. Kahn, G. D. Wilner, and J. W. Fenton II. 1983. Monocyte chemotaxis: stimulation by specific exosite region in thrombin. *Science (Wash. DC).* 220:728–731.

5. Chen, L. B., N. N. H. Teng, and J. M. Buchanan. 1976. Mitogenicity of thrombin and surface alterations on mouse splenocytes. *Exp. Cell Res.* 101:41–46.

6. Chen, L. B., and J. M. Buchanan. 1975. Mitogenic activity of blood components. I. Thrombin and prothrombin. *Proc. Natl. Acad. Sci. USA.* 72:131–135.

7. Weksler, B. B., C. W. Ley, and E. A. Jaffe. 1978. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A23187. *J. Clin. Invest.* 62:923–930.

8. Prescott, S. M., G. A. Zimmerman, and T. M. McIntyre. 1984. Human endothelial cells in culture produce platelet-activating factor when stimulated by thrombin. *Proc. Natl. Acad. Sci. USA.* 81:3534–3538.

9. Gelehrter, T. D., and R. Sznycer-Laszyk. 1986. Thrombin induction of plasminogen activator-inhibitor in cultured human endothelial cells. *J. Clin. Invest.* 77:165–169.

10. Daniel, T. O., V. C. Gibbs, D. F. Milfay, M. R. Garavoy, and L. T. Williams. 1986. Thrombin stimulates *c-sis* gene expression in microvascular endothelial cells. *J. Biol. Chem.* 261:9579–9582.

11. Zimmerman, G. A., T. M. McIntyre, and S. M. Prescott. 1986. Thrombin stimulates neutrophil adherence by an endothelial cell-dependent mechanism. *Ann. NY Acad. Sci.* 485:349–368.

12. Hattori, R., K. K. Hamilton, R. D. Fugate, R. P. McEver, and P. J. Sims. 1989. Stimulated secretion of endothelial vWF is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. *J. Biol. Chem.* 264:7768–7771.

13. Farmer, L., J. Sommer, and D. Monard. 1990. Glia-derived nexin potentiates neurite extension in hippocampal pyramidal cells in vitro. *Dev. Neurosci.* 12:73–80.

14. Gurwitz, D., and D. D. Cunningham. 1988. Thrombin modulates and reversed neuroblastoma neurite outgrowth. *Proc. Natl. Acad. Sci. USA.* 85:3440–3444.

15. Dihanich, M., M. Kaser, E. Reinhard, D. Cunningham, and D. Monard. 1991. Prothrombin mRNA is expressed by cells of the nervous system. *Neuron.* 6:575–581.

16. Eidt, J. F., P. Allison, S. Nobel, J. Ashton, P. Golino, J. McNatt, L. M. Buja, and J. T. Willerson. 1989. Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. *J. Clin. Invest.* 84:18–27.

17. Hansen, S. R., and L. A. Harker. 1988. Interruption of acute platelet-dependent thrombosis by the synthetic antithrombin PPACK. *Proc. Natl. Acad. Sci. USA.* 85:3184–3188.

18. Fitzgerald, D. J., and G. A. FitzGerald. 1989. Role of thrombin and thromboxane A2 in reocclusion following coronary thrombolysis. *Proc. Natl. Acad. Sci. USA.* 86:7585–7589.

19. Jang, I.-K., H. K. Gold, A. A. Ziskind, R. C. Leinbach, J. T. Fallon, and D. Collen. 1989. Prevention of platelet-rich arterial thrombosis by selective thrombin inhibition. *Circulation.* 81:219–225.

20. Heras, M., J. H. Chesebro, W. J. Penny, K. R. Bailey, L. Badimon, and V. Fuster. 1989. Effects of thrombin inhibition on the development of acute platelet-thrombus deposition during angioplasty in pigs. *Circulation.* 79:657–665.

21. Sarembok, I. J., S. D. Gertz, L. W. Gimple, R. M. Owen, E. R. Powers, and W. C. Roberts. 1992. Effectiveness of recombinant desulphatohirudin (CGP39393) in reducing restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. *Circulation.* In press.

22. Berndt, M. C., C. Gregory, G. Dowden, and P. A. Castaldi. 1985. Thrombin interactions with platelet membrane proteins. *Ann. NY Acad. Sci.* 485:374–386.

23. Gronke, R. S., B. L. Bergman, and J. B. Baker. 1987. Thrombin interaction with platelets: influence of a platelet protease nexin. *J. Biol. Chem.* 262:3030–3036.

24. Okamura, T., M. Hasitz, and G. A. Jamieson. 1978. Platelet glycocalicin: interaction with thrombin and role as thrombin receptor on the platelet surface. *J. Biol. Chem.* 253:3435–3443.

25. Davey, M. G., and E. F. Luscher. 1967. Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets. *Nature (Lond.).* 216:857–858.

26. Phillips, D. R. 1974. Thrombin interaction with human platelets: potentiation of thrombin-induced aggregation and release by inactivated thrombin. *Thromb. Diath. Haemorrh.* 32:207–215.

27. Martin, B. M., R. D. Feinman, and T. C. Detwiler. 1975. Platelet stimulation by thrombin and other proteases. *Biochemistry.* 14:1308–1314.

28. Tollefsen, D. M., J. R. Feagler, and P. W. Majerus. 1974. The binding of thrombin to the surface of platelets. *J. Biol. Chem.* 249:2646–2651.

29. Workman, E. F., Jr., G. C. White II, and R. L. Lundblad. 1977. Structure-function relationships in the interaction of alpha-thrombin with blood platelets. *J. Biol. Chem.* 252:7118–7123.

30. Masu, Y., K. Nakayama, H. Tamaki, Y. Harada, M. Kuno, and S. Nakanishi. 1987. cDNA cloning of bovine substance K receptor through oocyte expression system. *Nature (Lond.).* 329:836–840.

31. Muszbek, L., and K. Laki. 1984. Interaction of thrombin with proteins other than fibrinogen (thrombin-susceptible bonds). *In* The Thrombin. R. Machovich, editor. CRC Press, Boca Raton, FL. 83–90.

32. Stenflo, J., and P. Fernlund. 1982. Amino acid sequence of the heavy chain of bovine protein C. *J. Biol. Chem.* 257:12180–12190.

33. Bode, W., I. Mayr, U. Baumann, R. Huber, S. R. Stone, and J. Hofsteenge. 1989. The refined 1.9A crystal structure of human alpha-thrombin: interaction with D-Phe-Pro-Arg chloromethylketone and significance of the Tyr-Pro-Pro-Trp insertion segment. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3467–3475.

34. Jakubowski, J. A., and J. M. Maragonore. 1990. Inhibition of coagulation and thrombin-induced platelet activities by a synthetic dodecapeptide modeled on the carboxy terminus of hirudin. *Blood.* 75:399–406.

35. Rydel, T. J., K. G. Rabichandran, A. Tulinsky, W. Bode, R. Huber, C. Roitsch, and J. W. Fenton II. 1990. The structure of a complex of recombinant hirudin and human alpha-thrombin. *Science (Wash. DC).* 249:277–280.

36. Bode, W., P. Schwager, and R. Huber. 1978. The transition of bovine trypsinogen to a trypsin-like state upon strong ligand binding. *J. Mol. Biol.* 118:99–112.

37. Hantgan, R. R., C. W. Francis, H. A. Scheraga, and V. J. Marder. Fibrinogen structure and physiology. *In* Hemostasis and Thrombosis. R. W. Colman, J. Hirsch, V. J. Marder, and E. W. Salzman, editors. J. B. Lippincott Co., Philadelphia. 1987 269–288.

38. Vu, T.-K. H., V. I. Wheaton, D. T. Hung, I. Charo, and S. R. Coughlin. 1991. Domains defining thrombin-receptor interaction. *Nature (Lond.).* 353:674–677.

39. Liu, L.-W., T.-K. H. Vu, C. T. Esmon, and S. R. Coughlin. 1991. The region of the thrombin receptor resembling hirudin binds to thrombin and alters enzyme specificity. *J. Biol. Chem.* 266:16977–16980.

40. Lohse, M. J., R. J. Lefkowitz, M. G. Caron, and J. L. Benovic. 1989. Inhibition of beta-adrenergic receptor kinase prevents rapid homologous desensitization of beta2-adrenergic receptors. *Proc. Natl. Acad. Sci. USA.* 86:3011–3015.

41. D. T. Hung, T.-K. H. Vu, V. I. Wheaton, I. Charo, N. A. Nelken, N. Esmon, C. T. Esmon, and S. R. Coughlin. 1992. "Mirror image" antagonists of thrombin-induced platelet activation based on thrombin receptor structure. *J. Clin. Invest.* 89:444–450.

42. Maraganore, J. M., P. Bourdon, J. Jablonski, K. L. Ramachandran, and J. W. Fenton II. 1990. Design and characterization of hirulogs: a novel class of bivalent peptide inhibitors of thrombin. *Biochemistry.* 29:7095–7101.